DGMS 2020 Münster

DGMS 2020

Book of Abstracts

53rd Annual Conference of the DGMS
Including 27th ICP-MS User's Meeting

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bioanalytik-muenster
TECHNOLOGY FOR THE LIFE SCIENCES

DGMS
Deutsche Gesellschaft für Massenspektrometrie

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Program and Abstracts

of the

53rd Annual Conference of the DGMS
including 27th ICP-MS User´s Meeting

held in Münster / Germany
from March 1st to March 4th 2020

Date: Sunday, 01/Mar/2020

12:00pm - 6:00pm Conference Office: Seminar Room O2	**Registration Day 1: Check-in at Conference Office**
2:00pm - 3:15pm Lecture Hall Building Chemistry: Hall O1	**WS01-1: Workshop 1, Part 1: FI, FD, LIFDI** Session Chair: **Mathias H. Linden** Session Chair: **H. Bernhard Linden** Session Chair: **Jürgen H. Gross**
2:00pm - 3:15pm Lecture Hall Building Physics: Hall HS1	**WS02-1: Workshop 2, Part 1: Mass Spectrometry Imaging** Session Chair: **Andreas Römpp** Session Chair: **Bernhard Spengler**
2:00pm - 3:15pm Lecture Hall Building Chemistry: Hall A1	**WS03-1: Workshop 3, Part 1: PTB: Metrological Infrastructure** Session Chair: **Claudia Swart** Session Chair: **Gavin O'Connor**
2:00pm - 3:15pm Lecture Hall Building Physics: Hall HS2	**WS04: Workshop 4: LC-MS** Session Chair: **Waldemar Hoffmann** Session Chair: **Michael Hoffmann** Session Chair: **Martin Penkert**
2:00pm - 3:15pm Lecture Hall Building Chemistry: Hall C2	**WS06-1: Workshop 6, Part 1: Protein Modification and Expression Analysis** Session Chair: **Simone König**
2:00pm - 3:15pm Lecture Hall Building Chemistry: Hall C1	**WS08-1: Workshop 8, Part 1: Applications of MS in Pharma Industry** Session Chair: **Jürgen Schäfer** Session Chair: **Nico Zinn**
3:15pm - 3:30pm	**Coffee Break 1: Coffee Break**
3:30pm - 4:45pm Lecture Hall Building Chemistry: Hall O1	**WS01-2: Workshop 1, Part 2: FI, FD, LIFDI** Session Chair: **Mathias H. Linden** Session Chair: **H. Bernhard Linden** Session Chair: **Jürgen H. Gross**
3:30pm - 4:45pm Lecture Hall Building Physics: Hall HS1	**WS02-2: Workshop 2, Part 2: Mass Spectrometry Imaging** Session Chair: **Andreas Römpp** Session Chair: **Bernhard Spengler**
3:30pm - 4:45pm Lecture Hall Building Chemistry: Hall A1	**WS03-2: Workshop 3, Part 2: PTB: Metrological Infrastructure** Session Chair: **Claudia Swart** Session Chair: **Gavin O'Connor**
3:30pm - 4:45pm Lecture Hall Building Physics: Hall HS2	**WS05: Workshop 5: ToF-SIMS** Session Chair: **Birgit Hagenhoff**
3:30pm - 4:45pm Lecture Hall Building Chemistry: Hall C2	**WS06-2: Workshop 6, Part 2: Protein Modification and Expression Analysis** Session Chair: **Simone König**
3:30pm - 4:45pm W043 (IAAC Building)	**WS07-1: Workshop 7: Core Facilities** Session Chair: **Christof Lenz**

3:30pm - 4:45pm Lecture Hall Building Chemistry: Hall C1	**WS08-2: Workshop 8, Part 2: Applications of MS in Pharma Industry** Session Chair: **Jürgen Schäfer** Session Chair: **Nico Zinn**
4:45pm - 5:00pm	**Coffee Break 2: Coffee Break**
5:00pm - 5:20pm Lecture Hall Building Chemistry: Hall C1	**Opening: Opening of DGMS and ICP-MS User Meeting 2020** Session Chair: **Andrea Sinz** Session Chair: **Uwe Karst** Session Chair: **Heiko Hayen**
5:20pm - 6:00pm Lecture Hall Building Chemistry: Hall C1	**PL 1: Plenary Lecture 1: T. Benter** Session Chair: **Heiko Hayen**
6:00pm - 6:15pm Lecture Hall Building Chemistry: Hall C1	**M.H. Award Session: Mattauch-Herzog Award Session** Session Chair: **Bernhard Spengler**
6:15pm - 7:15pm Lecture Hall Building Chemistry: Hall C1	**W.P. Lecture: Wolfgang Paul Lecture: A. Makarov** Session Chair: **Andrea Sinz**
7:15pm - 10:00pm	**Welcome Reception: Welcome Reception**

Date: Monday, 02/Mar/2020

8:00am - 4:00pm	**Registration Day 2: Check-in at Conference Office**
8:30am - 9:10am Lecture Hall Building Physics: Hall HS1	**PL 2: Plenary Lecture 2: Jörg Feldmann** Session Chair: **Uwe Karst**
9:10am - 9:35am Lecture Hall Building Physics: Hall HS1	**WPL 1: Wolfgang Paul Study Awards Session & Award Lecture** Session Chair: **Michael Mormann**
9:35am - 10:00am	**Coffee Break 3: Coffee Break, Exhibition, Posters**
10:00am - 10:30am Lecture Hall Building Chemistry: Hall C1	**SES-01-KN: Session 1: Proteomics-Basics; Keynote: Urner (143)** Session Chair: **Simone König**
10:00am - 10:30am Lecture Hall Building Physics: Hall HS1	**SES-02-KN: Session 2: Lipidomics I; Keynote: Heiles (242)** Session Chair: **Harald Köfeler**
10:00am - 10:30am Lecture Hall Building Physics: Hall HS2	**SES-03-KN: Session 3: ICP-MS: Elemental Imaging; Keynote: Clases (140)** Session Chair: **Daniel Pröfrock**
10:00am - 10:30am Lecture Hall Building Chemistry: Hall C2	**SES-04-KN: Session 4: Environmental MS; Keynote: T. Hoffmann** Session Chair: **Björn Meermann**
10:30am - 10:50am Lecture Hall Building Chemistry: Hall C1	**SES-01-O1: Session 1: Proteomics-Basics; Oral 1: Van Duijn (235)** Session Chair: **Simone König**
10:30am - 10:50am Lecture Hall Building Physics: Hall HS1	**SES-02-O1: Session 2: Lipidomics I; Oral 1: Niehaus (303)** Session Chair: **Harald Köfeler**
10:30am - 10:50am Lecture Hall Building	**SES-03-O1: Session 3: ICP-MS: Elemental Imaging; Oral 1: Bücker (233)** Session Chair: **Daniel Pröfrock**

Physics: Hall HS2	
10:30am - 10:50am Lecture Hall Building Chemistry: Hall C2	**SES-04-O1: Session 4: Environmental MS; Oral 1: Schade (243)** Session Chair: **Björn Meermann**
10:50am - 11:10am Lecture Hall Building Chemistry: Hall C1	**SES-01-O2: Session 1: Proteomics-Basics; Oral 2: Rzagalinski (310)** Session Chair: **Simone König**
10:50am - 11:10am Lecture Hall Building Physics: Hall HS1	**SES-02-O2: Session 2: Lipidomics I; Oral 2: Helmer (200)** Session Chair: **Harald Köfeler**
10:50am - 11:10am Lecture Hall Building Physics: Hall HS2	**SES-03-O2: Session 3: ICP-MS: Elemental Imaging; Oral 2: Nowak (206)** Session Chair: **Daniel Pröfrock**
10:50am - 11:10am Lecture Hall Building Chemistry: Hall C2	**SES-04-O2: Session 4: Environmental MS; Oral 2: Kuzmich (331)** Session Chair: **Björn Meermann**
11:10am - 11:30am Lecture Hall Building Chemistry: Hall C1	**SES-01-O3: Session 1: Proteomics-Basics; Oral 3: Tholey (106)** Session Chair: **Simone König**
11:10am - 11:30am Lecture Hall Building Physics: Hall HS1	**SES-02-O3: Session 2: Lipidomics I; Oral 3: Drotleff (146)** Session Chair: **Harald Köfeler**
11:10am - 11:30am Lecture Hall Building Physics: Hall HS2	**SES-03-O3: Session 3: ICP-MS: Elemental Imaging; Oral 3: Bleiner (188)** Session Chair: **Daniel Pröfrock**
11:10am - 11:30am Lecture Hall Building Chemistry: Hall C2	**SES-04-O3: Session 4: Environmental MS; Oral 3: Stadler (373)** Session Chair: **Björn Meermann**
11:30am - 11:50am Lecture Hall Building Chemistry: Hall C1	**SES-01-O4: Session 1: Proteomics-Basics; Oral 4: Koch (264)** Session Chair: **Simone König**
11:30am - 11:50am Lecture Hall Building Physics: Hall HS1	**SES-02-O4: Session 2: Lipidomics I; Oral 4: Schuhmann (248)** Session Chair: **Harald Köfeler**
11:30am - 11:50am Lecture Hall Building Physics: Hall HS2	**SES-03-O4: Session 3: ICP-MS: Elemental Imaging; Oral 4: Shaw** Session Chair: **Daniel Pröfrock**
11:30am - 11:50am Lecture Hall Building Chemistry: Hall C2	**SES-04-O4: Session 4: Environmental MS; Oral 4: Gehm** Session Chair: **Björn Meermann**
11:50am - 12:50pm	**Lunch 1: Lunch Break**
12:50pm - 1:50pm	**Vendor Seminars 1-5: Vendor Seminars 1-5: Agilent, Bruker, Shimadzu, Thermo, Waters** Vendor Session of the DGMS Sponsors
2:00pm - 2:30pm Lecture Hall Building Chemistry: Hall C1	**SES-05-KN: Session 5: Proteomics - Clinical and Biological Applications; Keynote: Winter (366)** Session Chair: **Hartmut Schlüter**
2:00pm - 2:30pm Lecture Hall Building Physics: Hall HS1	**SES-06-KN: Session 6: Imaging I; Keynote: Hagenhoff (348)** Session Chair: **Bernhard Spengler**
2:00pm - 2:30pm Lecture Hall Building Physics: Hall HS2	**SES-07-KN: Session 7: ICP-MS: Nano- / Bioanalysis; Keynote: Engelhard (176)** Session Chair: **Jörg Bettmer**

2:00pm - 2:30pm Lecture Hall Building Chemistry: Hall C2	**SES-08-KN: Session 8: Instrumentation I; Keynote: Cramer (175)** Session Chair: **Christopher Rüger**
2:00pm - 2:30pm Lecture Hall Building Chemistry: Hall A1	**Special Session (1): Science Management; Keynote: Mürtz** Session Chair: **Christof Lenz**
2:30pm - 2:50pm Lecture Hall Building Chemistry: Hall C1	**SES-05-O1: Session 5: Proteomics - Clinical and Biological Applications; Oral 1: Stolz 114** Session Chair: **Hartmut Schlüter**
2:30pm - 2:50pm Lecture Hall Building Physics: Hall HS1	**SES-06-O1: Session 6: Imaging I; Oral 1: Oetjen (163)** Session Chair: **Bernhard Spengler**
2:30pm - 2:50pm Lecture Hall Building Physics: Hall HS2	**SES-07-O1: Session 7: ICP-MS: Nano- / Oral 1: Buchholz (214)** Session Chair: **Jörg Bettmer**
2:30pm - 2:50pm Lecture Hall Building Chemistry: Hall C2	**SES-08-O1: Session 8: Instrumentation I; Oral 1: Wootton (376)** Session Chair: **Christopher Rüger**
2:30pm - 2:50pm Lecture Hall Building Chemistry: Hall A1	**Special Session (2): Science Management: Trevorrow** Session Chair: **Christof Lenz**
2:50pm - 3:10pm Lecture Hall Building Chemistry: Hall C1	**SES-05-O2: Session 5: Proteomics - Clinical and Biological Applications; Oral 2: Wenk (147)** Session Chair: **Hartmut Schlüter**
2:50pm - 3:10pm Lecture Hall Building Physics: Hall HS1	**SES-06-O2: Session 6: Imaging I; Oral 2: Müller (228)** Session Chair: **Bernhard Spengler**
2:50pm - 3:10pm Lecture Hall Building Physics: Hall HS2	**SES-07-O2: Session 7: ICP-MS: Nano- / Oral 2: Lemke (117)** Session Chair: **Jörg Bettmer**
2:50pm - 3:10pm Lecture Hall Building Chemistry: Hall C2	**SES-08-O2: Session 8: Instrumentation I; Oral 2: Papanastasiou (110)** Session Chair: **Christopher Rüger**
3:10pm - 3:30pm Lecture Hall Building Chemistry: Hall C1	**SES-05-O3: Session 5: Proteomics - Clinical and Biological Applications; Oral 3: Bräcker (294)** Session Chair: **Hartmut Schlüter**
3:10pm - 3:30pm Lecture Hall Building Physics: Hall HS1	**SES-06-O3: Session 6: Imaging I; Oral 3: Bookmeyer (352)** Session Chair: **Bernhard Spengler**
3:10pm - 3:30pm Lecture Hall Building Physics: Hall HS2	**SES-07-O3: Session 7: ICP-MS: Nano- / Oral 3: Retzmann (227)** Session Chair: **Jörg Bettmer**
3:10pm - 3:30pm Lecture Hall Building Chemistry: Hall C2	**SES-08-O3: Session 8: Instrumentation I; Oral 3: Gross (316)** Session Chair: **Christopher Rüger**
3:30pm - 3:50pm Lecture Hall Building Chemistry: Hall C1	**SES-05-O4: Session 5: Proteomics - Clinical and Biological Applications; Oral 4: Sickmann (367)** Session Chair: **Hartmut Schlüter**
3:30pm - 3:50pm Lecture Hall Building Physics: Hall HS1	**SES-06-O4: Session 6: Imaging I; Oral 4: Treu (372)** Session Chair: **Bernhard Spengler**
3:30pm - 3:50pm Lecture Hall Building Physics: Hall HS2	**SES-07-O4: Session 7: ICP-MS: Nano- / Oral 4: Von der Au** Session Chair: **Jörg Bettmer**

3:30pm - 3:50pm Lecture Hall Building Chemistry: Hall C2	**SES-08-O4: Session 8: Instrumentation I; Oral 4: Uteschil (276)** Session Chair: **Christopher Rüger**
3:50pm - 4:20pm	**Coffee Break 4: Coffee Break, Exhibition, Posters**
4:20pm - 5:00pm Lecture Hall Building Chemistry: Hall C1	**PL 3: Plenary Lecture 3: L. Konermann** Session Chair: **Kathrin Breuker**
5:10pm - 7:00pm Poster Areas in the Foyers of the Lecture Hall Buildings Chemistry and Physics	**Poster Session 1 (even numbers) and Exhibition**
6:00pm - 7:30pm	**DGMS Board Meeting: DGMS Board Meeting** Session Chair: **Andrea Sinz**

Date: Tuesday, 03/Mar/2020

8:00am - 4:00pm	**Registration Day 3: Check-in at Conference Office**
8:30am - 9:10am Lecture Hall Building Chemistry: Hall C1	**PL 4: Plenary Lecture 4: P. E. Barran** Session Chair: **Lars Konermann**
9:10am - 9:35am Lecture Hall Building Chemistry: Hall C1	**MS in BS Award: Mass Spectrometry in the Bio Sciences Award Session; Award Lecture** Session Chair: **Kathrin Breuker**
9:35am - 10:00am	**Coffee Break 5: Coffee Break, Exhibition, Posters**
10:00am - 10:30am Lecture Hall Building Chemistry: Hall C1	**SES-09-KN: Session 9: Imaging II; Keynote: Bhandari (285)** Session Chair: **Birgit Hagenhoff**
10:00am - 10:30am Lecture Hall Building Physics: Hall HS1	**SES-10-KN: Session 10: Lipidomics II; Keynote: Ahrends (375)** Session Chair: **Dominik Schwudke**
10:00am - 10:30am Lecture Hall Building Physics: Hall HS2	**SES-11-KN: Session 11: ICP-MS: Environment I; Keynote: Pröfrock (174)** Session Chair: **Carsten Engelhard**
10:00am - 10:30am Lecture Hall Building Chemistry: Hall C2	**SES-12-KN: Session 12: Affinity; Keynote: Glocker (122)** Session Chair: **Andreas Tholey**
10:30am - 10:50am Lecture Hall Building Chemistry: Hall C1	**SES-09-O1: Session 9: Imaging II; Oral 1: Pirkl (255)** Session Chair: **Birgit Hagenhoff**
10:30am - 10:50am Lecture Hall Building Physics: Hall HS1	**SES-10-O1: Session 10: Lipidomics II; Oral 1: Köfeler (154)** Session Chair: **Dominik Schwudke**
10:30am - 10:50am Lecture Hall Building Physics: Hall HS2	**SES-11-O1: Session 11: ICP-MS: Environment I; Oral 1: Krystek (207)** Session Chair: **Carsten Engelhard**
10:30am - 10:50am Lecture Hall Building Chemistry: Hall C2	**SES-12-O1: Session 12: Affinity; Oral 1: Falck (374)** Session Chair: **Andreas Tholey**

10:50am - 11:10am Lecture Hall Building Chemistry: Hall C1	**SES-09-O2: Session 9: Imaging II; Oral 2: Kim (403)** Session Chair: **Birgit Hagenhoff**
10:50am - 11:10am Lecture Hall Building Physics: Hall HS1	**SES-10-O2: Session 10: Lipidomics II; Oral 2: Al Machot (385)** Session Chair: **Dominik Schwudke**
10:50am - 11:10am Lecture Hall Building Physics: Hall HS2	**SES-11-O2: Session 11: ICP-MS: Environment I; Oral 2: Faßbender (108)** Session Chair: **Carsten Engelhard**
10:50am - 11:10am Lecture Hall Building Chemistry: Hall C2	**SES-12-O2: Session 12: Affinity; Oral 2: Lupu (130)** Session Chair: **Andreas Tholey**
11:10am - 11:30am Lecture Hall Building Chemistry: Hall C1	**SES-09-O3: Session 9: Imaging II; Oral 3: Heijs (323)** Session Chair: **Birgit Hagenhoff**
11:10am - 11:30am Lecture Hall Building Physics: Hall HS1	**SES-10-O3: Session 10: Lipidomics II; Oral 3: Fuchs (371)** Session Chair: **Dominik Schwudke**
11:10am - 11:30am Lecture Hall Building Physics: Hall HS2	**SES-11-O3: Session 11: ICP-MS: Environment I; Oral 3: Macke (312)** Session Chair: **Carsten Engelhard**
11:10am - 11:30am Lecture Hall Building Chemistry: Hall C2	**SES-12-O3: Session 12: Affinity; Oral 3: Wiegand (142)** Session Chair: **Andreas Tholey**
11:30am - 12:30pm	**Lunch 2: Lunch Break**
11:30am - 12:30pm	**Young Scientists: Young Scientists Meeting** Session Chair: **Jonas Maurice Will**
12:30pm - 1:30pm	**Vendor Seminars 6, 8: Vendor Seminars 6 and 8: ESI, Thermo (The Sciex Vendor Seminar had to be canceled)** Vendor Seminars of the DGMS Sponsors
1:30pm - 3:00pm Poster Areas in the Foyers of the Lecture Hall Buildings Chemistry and Physics	**Poster Session 2 (odd numbers) and Exhibition**
3:00pm - 3:30pm Lecture Hall Building Chemistry: Hall C1	**SES-13-KN: Session 13: Proteomics - Structural and Functional Proteomics; Keynote: Richter (297)** Session Chair: **Carla Schmidt**
3:00pm - 3:30pm Lecture Hall Building Physics: Hall HS1	**SES-14-KN: Session 14: Metabolomics; Keynote: Schmid (344)** Session Chair: **Anne Schnell** Session Chair: **Julica Folberth**
3:00pm - 3:30pm Lecture Hall Building Physics: Hall HS2	**SES-15-KN: Session 15: ICP-MS: Environment II; Keynote: Meermann (109)** Session Chair: **David Clases**
3:00pm - 3:30pm Lecture Hall Building Chemistry: Hall C2	**SES-16-KN: Session 16: Ions at Next Generation Lightsources; Keynote: Bari (394)** Session Chair: **Charlotte Uetrecht**
3:30pm - 3:50pm Lecture Hall Building Chemistry: Hall C1	**SES-13-O1: Session 13: Proteomics - Structural and Functional Proteomics; Oral 1: Piersimoni (150)** Session Chair: **Carla Schmidt**
3:30pm - 3:50pm Lecture Hall Building Physics: Hall HS1	**SES-14-O1: Session 14: Metabolomics; Oral 1: Fangmeyer** Session Chair: **Anne Schnell** Session Chair: **Julica Folberth**

3:30pm - 3:50pm Lecture Hall Building Physics: Hall HS2	**SES-15-O1: Session 15: ICP-MS: Environment II; Oral 1: Kautenburger (191)** Session Chair: **David Clases**
3:30pm - 3:50pm Lecture Hall Building Chemistry: Hall C2	**SES-16-O1: Session 16: Ions at Next Generation Lightsources; Oral 1: Marklund (400)** Session Chair: **Charlotte Uetrecht**
3:50pm - 4:10pm Lecture Hall Building Chemistry: Hall C1	**SES-13-O2: Session 13: Proteomics - Structural and Functional Proteomics; Oral 2: Singh (359)** Session Chair: **Carla Schmidt**
3:50pm - 4:10pm Lecture Hall Building Physics: Hall HS1	**SES-14-O2: Session 14: Metabolomics; Oral 2: Cakic (336)** Session Chair: **Anne Schnell** Session Chair: **Julica Folberth**
3:50pm - 4:10pm Lecture Hall Building Physics: Hall HS2	**SES-15-O2: Session 15: ICP-MS: Environment II; Oral 2: Zimmermann (213)** Session Chair: **David Clases**
3:50pm - 4:10pm Lecture Hall Building Chemistry: Hall C2	**SES-16-O2: Session 16: Ions at Next Generation Lightsources; Oral 2: Loru (363)** Session Chair: **Charlotte Uetrecht**
4:10pm - 4:30pm Lecture Hall Building Chemistry: Hall C1	**SES-13-O3: Session 13: Proteomics - Structural and Functional Proteomics; Oral 3: Kopicki (115)** Session Chair: **Carla Schmidt**
4:10pm - 4:30pm Lecture Hall Building Physics: Hall HS1	**SES-14-O3: Session 14: Metabolomics; Oral 3: Schöttler (266)** Session Chair: **Anne Schnell** Session Chair: **Julica Folberth**
4:10pm - 4:30pm Lecture Hall Building Physics: Hall HS2	**SES-15-O3: Session 15: ICP-MS: Environment II; Oral 3: Horstmann** Session Chair: **David Clases**
4:10pm - 4:30pm Lecture Hall Building Chemistry: Hall C2	**SES-16-O3: Session 16: Ions at Next Generation Lightsources; Oral 3: Poully (341)** Session Chair: **Charlotte Uetrecht**
4:30pm - 4:50pm Lecture Hall Building Chemistry: Hall C1	**SES-13-O4: Session 13: Proteomics - Structural and Functional Proteomics; Oral 4: CANCELED** Session Chair: **Carla Schmidt**
4:30pm - 4:50pm Lecture Hall Building Physics: Hall HS1	**SES-14-O4: Session 14: Metabolomics; Oral 4: Krüger (120)** Session Chair: **Anne Schnell** Session Chair: **Julica Folberth**
4:30pm - 4:50pm Lecture Hall Building Physics: Hall HS2	**SES-15-O4: Session 15: ICP-MS: Environment II; Oral 4: Brix 229** Session Chair: **David Clases**
4:30pm - 4:50pm Lecture Hall Building Chemistry: Hall C2	**SES-16-O4: Session 16: Ions at Next Generation Lightsources; Oral 4: Kirschbaum (343)** Session Chair: **Charlotte Uetrecht**
4:50pm - 5:20pm	**Coffee Break 6: Coffee Break, Exhibition, Posters**
5:20pm - 6:00pm Lecture Hall Building Physics: Hall HS1	**PL 5: Plenary Lecture 5: R. Boiteau** Session Chair: **Maria Montes-Bayón**
6:00pm - 7:15pm	**DGMS meeting: DGMS General Assembly Meeting** Session Chair: **Andrea Sinz**
7:15pm - 7:30pm	**Bus Transfer: Bus Transfer to the Conference Dinner Location**
7:30pm - 11:59pm	**Conference Dinner: Conference Dinner**

Date: Wednesday, 04/Mar/2020

8:00am - 2:00pm	**Registration Day 4: Check-in at Conference Office**
9:00am - 9:40am Lecture Hall Building Physics: Hall HS1	**PL 6: Plenary Lecture 6: Maria Montes Bayon** Session Chair: **Carsten Engelhard**
9:40am - 10:20am Lecture Hall Building Physics: Hall HS1	**PL 7: Plenary Lecture 7: Jana Roìthová** Session Chair: **Mathias Schäfer**
10:20am - 10:40am	**Coffee Break 7: Coffee Break, Exhibition, Posters**
10:40am - 11:10am Lecture Hall Building Chemistry: Hall C1	**SES-17-KN: Session 17: Metabolomics / Glycomics / Proteomics; Keynote: Frolov (104)** Session Chair: **Michael Mormann**
10:40am - 11:10am Lecture Hall Building Physics: Hall HS1	**SES-18-KN: Session 18: Ion Physics and Chemistry; Keynote: Schäfer** Session Chair: **Uwe Karst**
10:40am - 11:10am Lecture Hall Building Physics: Hall HS2	**SES-19-KN: Session 19: Forensic; Keynote: Putz (307)** Session Chair: **Sven Heiles**
10:40am - 11:10am Lecture Hall Building Chemistry: Hall C2	**SES-20-KN: Session 20: Instrumentation; Keynote: Peretzki** Session Chair: **Tim Esser**
11:10am - 11:30am Lecture Hall Building Chemistry: Hall C1	**SES-17-O1: Session 17: Metabolomics / Glycomics / Proteomics; Oral 1: Vakhrushev (342)** Session Chair: **Michael Mormann**
11:10am - 11:30am Lecture Hall Building Physics: Hall HS1	**SES-18-O1: Session 18: Ion Physics and Chemistry; Oral 1: Haack (182)** Session Chair: **Mathias Schäfer**
11:10am - 11:30am Lecture Hall Building Physics: Hall HS2	**SES-19-O1: Session 19: Forensic; Oral 1: Brungs 327** Session Chair: **Sven Heiles**
11:10am - 11:30am Lecture Hall Building Chemistry: Hall C2	**SES-20-O1: Session 20: Instrumentation; Oral 1: Foest (346)** Session Chair: **Tim Esser**
11:30am - 11:50am Lecture Hall Building Chemistry: Hall C1	**SES-17-O2: Session 17: Metabolomics / Glycomics / Proteomics; Oral 2: Wudy (180)** Session Chair: **Michael Mormann**
11:30am - 11:50am Lecture Hall Building Physics: Hall HS1	**SES-18-O2: Session 18: Ion Physics and Chemistry; Oral 2: Überschaar (123)** Session Chair: **Mathias Schäfer**
11:30am - 11:50am Lecture Hall Building Physics: Hall HS2	**SES-19-O2: Session 19: Forensic; Oral 2: Paßreiter (333)** Session Chair: **Sven Heiles**
11:30am - 11:50am Lecture Hall Building Chemistry: Hall C2	**SES-20-O2: Session 20: Instrumentation II; Oral 2: Rüger (389)** Session Chair: **Tim Esser**
11:50am - 12:10pm	**Coffee Break 8: Coffee Break, Exhibition, Posters**

12:10pm - 12:50pm Lecture Hall Building Chemistry: Hall C1	**PL 8: Plenary Lecture 8: Susan Richardson** Session Chair: **Uwe Karst**
12:50pm - 1:30pm Lecture Hall Building Chemistry: Hall C1	**Poster Awards: Poster Awards and Closing Ceremony** Session Chair: **Andrea Sinz** Session Chair: **Heiko Hayen** Session Chair: **Uwe Karst**
1:30pm - 2:30pm	**Farewell: Farewell Reception**

Abstracts of all Presentations of the DGMS & ICP-MS User´s Meeting 2020

Plenary Lecture 1: T. Benter

Time: Sunday, 01/Mar/2020: 5:20pm - 6:00pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Heiko Hayen

Charge Retention / Charge Depletion in ESI-MS

Marco Thinius, Christine Polaczek, Thorsten Benter
University of Wupertal, Germany

Even today, a comprehensive and congruent model for the electrospray ionization (ESI) process explaining all experimental observations is not established. This is certainly due to the fact that the ESI process does not only include the (liquid phase) ionization of a molecule but also the liquid-gas phase transfer - including fluid dynamics and electrochemistry. The formation and evolution of charged droplets, the release of ions from charged droplets, the transport of ions/droplets into the vacuum system of a mass spectrometer, ion activation, transformation (i.e., chemistry), and means of preparing a defined ion beam in the ion transfer stage all potentially impact on the observed mass spectrum.

A phenomenon coined in the literature as *supercharging* may yield new insights in the formation processes of multiply charged ions from ESI. Supercharging is used to generate highly charged ion species, which may then be subjected to selected fragmentation methods (e.g., ETD, ECD). There are two different approaches to achieve supercharging: The conventional way is adding supercharging agents (SCAs) to the sprayed analyte solution. In contrast, supercharging is also achieved by adding solvent vapor (e.g. acetonitrile) into the ion source.

This presentation focuses on the systematic investigation of the impact of liquid and gas phase modifiers on the observed ion population and changes of the average charge state of the peptide Substance P (SP, sequence: RPKPQQFFGLM). SP is used as a model analyte since it is well characterized with regard to its structure and ionization behavior. In addition, proxies of individual motives of SP, e.g., 1,5-diaminopentane, ethylenediamine, n-butylamine, were also investigated.

Results from experimental as well as theoretical work [1,2] are presented and discussed.

[1] Thinius, M. et al.; "*Charge Retention/Charge Depletion in ESI-MS – Experimental Evidence*"; J. Am. Chem. Soc. Mass Spectrom. DOI: 10.1021/jasms.9b00044.

[2] Haack, A. et al.; "*Charge Retention/Charge Depletion in ESI-MS – Theoretical Rationale*"; J. Am. Chem. Soc. Mass Spectrom. DOI: 10.1021/jasms.9b00045.

Plenary Lecture 2: Jörg Feldmann

Time: Monday, 02/Mar/2020: 8:30am - 9:10am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Uwe Karst

Environmental processes need the entire MS toolbox: imaging at cellular level and the combination of elemental and molecular mass spectrometry

Joerg Feldmann, Eva Krupp, Andrea Raab
University of Aberdeen, United Kingdom

The studies of pollutants in the environment make it necessary to have a highly sensitive and specific instrumentations since the matrix of environmental samples are highly complex and target analytes are often at the nM level.

We are mainly interested in environmental processes which contain a trace element for which a convincing explanation is missing. The commonly used analytical methods are revisited and nove approaches are developed which deliver more detailed information than the established methods. Here we will focus on speciation analysis which includes non-target molecular speciation of trace elements and the determination and characterisation of nanoparticles, e.g. metals, metalloids but also non-metals such as fluorine.

In this lecture we will give an overview of different types of studies by explaining the rationale for using parallel elemental and molecular mass spectrometry (HPLC-ICPMS/ESIMS) in addition to the use of complimentary methods such as XANES, flow-field flow fractionation, spICPMS, XRF, and NanoSIMS mapping and stable isotope ratio measurements of heavy elements using MC-ICPMS.

The case studies will include the fluorine, mercury and selenium accumulation in pilot whales stranded at the Scottish Coastline and in seaweed samples. This study includes the determination of HgSe naturally occurring nanoparticles, selenoprotein determination and fluorine speciation includes the determination of perfluorinated alkylated substances (PFAS), while another project identifies how arsenic occurs in seaweed at the subcellular level.

Session 1: Proteomics-Basics; Keynote: Urner

Time: Monday, 02/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Simone König

Oligoglycerol Detergents for Native Mass Spectrometry of Membrane Proteins

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Membrane proteins are targets for more than 50% of current drugs. Understanding their structure and interactions with native membranes is consequently of great interest in structural biology and drug discovery. Detergents enable the purification and analysis of membrane proteins. However, the challenge remains to identify guidelines that allow fine-tuning the structure of detergents for individual applications in membrane protein research. To address this shortcoming, here we introduce oligoglycerol detergents to membrane protein research. By means of native mass spectrometry we can identify how changing the structure of oligoglycerol detergents allows to control protein purification and the preservation of protein-lipid interactions during purification. In addition to a broad range of bacterial membrane proteins, oligoglycerol detergents enable the purification and analysis of a functional G-protein coupled receptor (GPCR). The GPCR family is currently intensively studied due to its substantial role in human health and disease. Oligoglycerol detergents represent therefore a significant advance for the investigation of membrane proteins and their interactions with lipids. Seen from a broader perspective, our studies also underline the potential of mass spectrometry techniques for researchers at the interface between supramolecular chemistry and structural biology.

Session 2: Lipidomics I; Keynote: Heiles

Time: Monday, 02/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Harald Köfeler

Reactive MALDI mass spectrometry imaging enabling Paternò-Büchi functionalization for C=C bond localization in isomeric phospholipids

Sven Heiles, Bernhard Spengler, Fabian Wäldchen
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Lipids are important for energy storage, cell signaling and cell membrane-formation. The molecular makeup of lipids is directly connected to their biochemical functions. Consequently, different cells and organs express characteristic lipid compositions and lipid isomer abundances. In multicellular organisms, this can result in local variations of e.g. phospholipids. In order to discern local phospholipid isomer abundance alterations, mass spectrometry imaging (MSI) is a powerful bioanalytical tool. However, C=C (DB) positions are not readily resolved in most MSI investigations. Therefore, we present benzophenone (BPh) as a novel reactive matrix-assisted laser desorption/ionization (MALDI) matrix enabling DB-positional-isomer-resolved MALDI-MS imaging.

For atmospheric-pressure MALDI MSI and tandem MSI (MS^2I) experiments, a BPh solution was pneumatically sprayed onto samples with a SMALDIPrep sprayer (TransMIT GmbH, Giessen, Germany). MALDI MS^2 / MS^2I experiments were performed, employing an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen, Germany) coupled to a Q Exactive HF (Thermo Fisher Scientific GmbH, Bremen, Germany).

Investigations commenced with positive-ion mode MSI of BPh-covered mouse brain tissue. Surprisingly, 343 nm MALDI laser irradiation of BPh-covered samples resulted in the immediate formation of BPh-attached phospholipid ions. Tandem MS of BPh-phospholipid adducts did not result in neutral loss of BPh. Instead, fragment ions consistent with retro-Paternò-Büchi (PB) reactions of oxetanes, formed between lipid DBs and BPh, were observed, suggesting that PB reactions occurred during MALDI operation. To corroborate this finding, lipid extracts of BPh-covered mouse brain were investigated via electrospray (ESI) MS. PB product ion yields were ~10,000 times lower in ESI than in MALDI mass spectra. Additionally, PB product ion yields increased when increasing the MALDI laser fluence. This combined evidence suggests that PB reactions occur during MALDI, enabling DB localization in MALDI-MS^2I experiments.

To showcase the capabilities of BPh for MALDI-based lipidomics, DBs were localized and relatively quantified using multiple authentic phospholipid standards and directly from tissue. MALDI-MS^2I of BPh-covered mouse-organ sections and intact *Schistosoma mansoni* parasite samples enabled visualization of phospholipid DB-positional-isomer distributions. The analytic sensitivity was demonstrated by identifying DB-position isomer abundance differences with pixel sizes down to 15 µm. For mammalian tissue these results indicate that DB-positional isomer variations are preserved down to the single cell-level. Reactive MALDI-MSI of *S. mansoni* surfaces unveiled the presence and the locally enhanced expression of unusual *n*-13 DB-positional isomers. This DB isomer is known to be uniquely formed in the parasitic organism, and our findings potentially hint to specific functions of *n*-13 isomers and/or associated enzymes.

Session 3: ICP-MS: Elemental Imaging; Keynote: Clases

Time: Monday, 02/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Daniel Pröfrock

Quantification strategies for elemental bio-imaging: progress, pitfalls and possibilities

David Clases, Raquel Gonzalez de Vega, Philip Doble
Atomic Medicine Initiative, University of Technology Sydney, Australia

The spatial determination of metals in heterogeneous and highly compartmentalised biological specimens requires selective and sensitive methods such as laser ablation – inductively coupled plasma – mass spectrometry (LA-ICP-MS). There are various quantification strategies attempting to overcome the inherent limitations of LA-ICP-MS such as standard and sample matrix matching, sensitivity fluctuations during long acquisitions, tissue density variability, whilst also enabling traceability and reproducibility.

This presentation will detail methods for preparing various matrix-matched standards, and critically evaluate their suitability to mimic the most important physical and biological characteristics of tissues in the context of instrumental and method limitations. Emphasis will be placed on novel matrix-matching materials for improved and facile preparation of repeatable standards. Consideration of developments of internal standardisation using isotope dilution analysis (IDA) to mitigate drifts in both the LA and ICP-MS systems will demonstrate that the latest techniques and materials may be combined for effective on-tissue IDA to improve quantification.

Further, recent advances in immunohistochemistry has extended the applicability of LA-ICP-MS and allows targeting and quantifying proteins in biological tissues. An overview of the necessary prerequisites and underlying aspects for quantitative imaging of proteins and potential future applications will also be presented.

Session 4: Environmental MS; Keynote: T. Hoffmann

Time: Monday, 02/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Björn Meermann

Development and application of mass spectrometric methods for atmospheric aerosol research

Thorsten Hoffmann
Johannes Gutenberg University, Germany

Atmospheric aerosol particles influence the radiation budget and climate of the Earth, directly by scattering sunlight back into space and indirectly by influencing the size and number of cloud droplets. Therefore, they play an important role for the global climate and the chemistry of the atmosphere. Furthermore, atmospheric aerosols influence our environment at local and regional level. Aerosols are now recognised as a major health problem, particularly with regard to respiratory diseases. The complexity of the organic and inorganic aerosol components, their chemical transformation during atmospheric transport, possible artifacts during sampling and the extremely low concentrations of some key compounds are the main reasons why the characterization of atmospheric aerosols remains an active and challenging field of analytical chemistry. From a mass spectrometric point of view, techniques for monitoring aerosol composition with high temporal resolution are of particular interest.

In recent years, various real-time mass spectrometric methods for particle analysis have been developed. In this contribution especially those methods that are particularly suitable for the determination of the organic composition of atmospheric aerosols will be discussed.

Proteomics-Basics; Oral 1: Van Duijn

Time: Monday, 02/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Simone König

On-line electrochemical reduction of inter- and intramolecular disulfide bonds for antibody analysis

Martijn VanDuijn[1], Pablo Sanz de la Torre[2], Jean Pierre Chervet[2], Theo Luider[1]
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In routine proteomics analysis, the reduction of disulfide bridges is achieved by chemical additives such as dithiothreitol (DTT), β-mercaptoethanol (β-ME) or tris(2-carboxyethyl)phosphine (TCEP). However, reduction by an on-line electrochemical method can offer several advantages. Until now, the cleavage by electrochemical methods of the disulfide bond between heavy and light chain components of antibodies was demonstrated in literature, but the reduction of additional internal disulfide bonds within these chains remained unsuccessful. We now demonstrate the full electrochemical reduction of the monoclonal antibody Avastin as it elutes from a chromatography system. The reduction is achieved in a flowcell (µPrepcell-SS) with a titanium working electrode and a platinum counter electrode. In this system, the intramolecular disulfides could successfully be reduced, as shown by the distribution of charge states, and also by detailed analysis of the top-down MS1 spectra. In addition, it was demonstrated that the electrochemical reduction permitted the acquisition of richer MS-MS spectra, which showed fragment signals that originated from the protein chain between the cysteine residues that are normally bridged. In a sample with closed disulfide bridges, signals from that region were suppressed in the data. This shows that complete protein reduction is important for MSMS-based sequence analysis. The electrochemical reduction conveniently provides this reduction, without a requirement for careful protein denaturation and alkylation as used in chemical reduction protocols for complete and durable reduction of protein chains. We will apply this methodology on the analysis of antibody mixtures, which can now be separated by chromatography as paired heavy and light chains, but analyzed by mass spectrometry as separate chains co-eluting from the flow-cell as cognate pairs.

Session 2: Lipidomics I; Oral 1: Niehaus

Time: Monday, 02/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Harald Köfeler

MALDI-2 at atmospheric pressure for postionization of glyco- and phospholipids in MALDI-MSI

Marcel Niehaus[1], Kenneth N. Robinson[1], Efstathios Elia[1], Rory T. Steven[1], Bin Yan[1], Alan M. Race[2], Teresa Murta[1], Josephine Bunch[1]

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Laser postionization was recently introduced in matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) for fine vacuum ion sources in the range of a few mbar [1]. The ion abundancies of several biomolecule classes are boosted by orders of magnitude via a MALDI-like gas-phase reaction process triggered by a second UV laser beam intercepting the developing MALDI plume a few hundred µm above the sample (therefore it was coined MALDI-2). MALDI-2 has proven highly effective for lipid imaging with both time-of-flight and Orbitrap mass spectrometers [1,2]. Due to the boost in ion intensity introduced by the second laser, MALDI-2-MSI experiments with pixel sizes as small as 600 nm were recently performed using a transmission mode MALDI-MSI setup [3].

Here, we present the first successful MALDI-2 experiments performed in a custom-build atmospheric pressure (AP) ion source coupled to an Orbitrap Elite instrument. Material was ejected with a UV laser at λ=349 nm (Explorer-349, Spectra Physics) using a transmission mode MALDI setup. The plume was intercepted by a second laser at λ=280 nm (NT230 OPO laser system, Ekspla) approximately 500 µm above the sample before entering a heated inlet (T ≈ 200 - 650°C) to transfer the ions to the mass spectrometer. We will show first results for lipid detection with MALDI-2 at AP conditions from DHB-coated tissue sections. The detection of similar glyco- and phospholipid species are boosted as seen previously in fine vacuum ion sources. Enhancements of about 1-2 orders of magnitude were achieved for galactosyl ceramides and protonated phosphatidylethanolamines and -cholines in positive ion mode. Further to the fundamental experiments, preliminary imaging results from mouse cerebellum with 20 µm pixel size will be presented.

[1] Soltwisch *et al*., 2015, Science

[2] Ellis *et al*., 2017, Chemical Communications

[3] Niehaus *et al*., 2019, Nature Methods

Session 3: ICP-MS: Elemental Imaging; Oral 1: Bücker

Time: Monday, 02/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Daniel Pröfrock

Gd and La in pharmaceuticals: New insights into their in-body deposition mechanism by means of laser ablation ICP-MS

Patrick Bücker[1], Henning Richter[2], Michael Sperling[1,3], Astrid Jeibmann[4], Uwe Karst[1]
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In the year 2000, the discovery of nephrogenic systemic fibrosis (NSF), a syndrome that involves the overproduction of connective tissue of the skin and organs, led to discussions about the application of gadolinium based contrast agents (GBCAs).[1] Especially linear GBCAs proved to have adverse health effects on patients with chronic kidney diseases, which resulted in linear GBCAs being highly regulated in Germany and Japan.

Despite the knowledge of GBCA-caused Gd deposition in different body parts including the brain, other lanthanide-based pharmaceuticals are used in modern medicine. This work focuses on lanthanum carbonate, which is used for the regulation of the serum phosphate concentration of patients with chronic kidney diseases via oral application. Even though most of the La is excreted via the bile, an absorption of a small portion of La in the gastrointestinal tract is presumed. However, its final deposition is mostly unknown but of high interest, especially regarding the adverse effects of the previously used aluminum-based serum phosphate regulators and the linear GBCAs as well as the high chemical similarity between La and Gd.

In this study, the deposition of La and Gd in different organs, including the brain, of patients with chronic kidney disease was investigated using the hyphenation of laser ablation (LA) and inductively coupled plasma-mass spectrometry (ICP-MS). This setup not only allows for spatially resolved results, but also for quantification of different analytes. To obtain the highest quality of 10 µm thin sections, paraffin-embedded samples were used. The thin sections were provided by the Institute of Neuropathology Münster. External calibration was performed using matrix-matched gelatin standards of La and Gd.

It was possible to optimize a method for the analysis of different human tissue samples using LA-ICP-MS hyphenation. The spatially resolved results enabled the detection of a spatial correlation between La and Gd deposition. Furthermore, the concentration ratio of La and Gd was calculated for each pixel of every organ, allowing for a comparison of the deposition behavior of both lanthanides. A similar ratio was found throughout most of the organs with exception of the brain, which showed an increase towards higher Gd deposition. Since La and Gd show high chemical similarity but are present in different chemical species, this results point towards a predominantly species dependent mechanism of lanthanide transportation into the brain.

Session 4: Environmental MS; Oral 1: Schade

Time: Monday, 02/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Björn Meermann

Resonance-Enhanced Detection of Metals in Aerosols using Single-Particle Mass Spectrometry

Julian Schade[1], Johannes Passig[1,2], Ellen Iva Rosewig[1], Robert Irsig[1,3], Thomas Kröger-Badge[1], Hendryk Czech[1,2], Martin Sklorz[2], Thorsten Streibel[1,2], Ralf Zimmermann[1,2]

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Natural and anthropogenic aerosols play a key role in global climate and biogeochemical cycles. While manifold investigations describe the effects of carbonaceous aerosols and sulfate on the global climate, the focus of this work lies on particle-bound transition metals such as Fe, Mn, or Zn. In addition to their health relevance, both natural dusts and anthropogenic particles are important sources of bioavailable metals to the marine environment, with complex effects on ecosystems and climate. Here we describe resonance effects in laser desorption/ionization (LDI) of single particles that increase the sensitivity and selectivity to transition metals in single-particle mass spectrometry (SPMS). Within the LDI ionization scheme, ablated metal atoms increase their ionization rate through resonant light absorption within a single laser pulse. Consequently, the corresponding laser wavelength can be tuned to substantially improve the detection efficiency for metals (Fe, Zn, Mn) in individual particles. We demonstrate that our KrF-excimer laser (248.3 nm wavelength) induces resonant ionization of particle-bound iron atoms by addressing a major Fe-absorption line. During a field study on ambient air, we performed an experiment to estimate the impact of resonant LDI on the metal detection efficiency in SPMS. In our new experimental setup of the bipolar SPMS system, two alternately firing excimer lasers were used (ArF at 193.3 nm and KrF at 248.3 nm) for ionization. The study revealed many more single-particle spectra with strong iron signals for resonant LDI at 248.3 nm compared to the ArF excimer laser at 193.3 nm. Moreover, the spectra from resonant ionization showed less dependency on the particle-matrix than conventional non-resonant LDI and featured a more universal detection scheme for particle-bound Fe. This gives us a powerful tool to detect traces of particle-bound metals, a health-relevant aerosol component and an important source of micronutrients to the surface oceans.

Session 1: Proteomics-Basics; Oral 2: Rzagalinski

Time: Monday, 02/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Simone König

Fast-tracking QconCAT approach for absolute protein quantification

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Max Planck Institute of Molecular Cell Biology and Genetics, Germany

QconCAT is a widely-used method for absolute protein quantification, in which a recombinant chimeric protein (CP), expressed from an artificial gene and composed of sequences of concatenated proteotypic peptides from target proteins, is used as a stable isotope-labeled internal standard. While concatenated peptides have some advantages over both the synthetic peptides and full-length protein standards, the proper and accurate certification of the chimeric protein concentration still remains a challenge and requires an extensive and difficult standard purification. In contrast to the original QconCAT concept, MS Western method (Kumar et al., 2018, MCP), recently developed by us, overcomes these limitations by integrating the CP's both purification (gel-based) and certification (in-sample, reference protein-based) steps into the course of the actual analysis. In this work, we aimed at further adapting the MS Western method to a simplified in-solution digestion protocol, and thereby at fast-tracking the whole QconCAT concept by direct using the crude (non-purified) cell extract of the CP as an internal standard.

First, three CPs of different MW were expressed in *E. coli* and analyzed with GeLC-MS/MS in order to investigate the purity of their full-length forms in comparison to truncated products. The obtained results clearly suggested a correlation between the length and the extent of the chimeric protein degradation/truncation, as well as revealed rather irregular (N- *vs.* C-terminus) degradation pattern. Second, as the chimeric protein is designed to generate a stoichiometric (equimolar) set of the labeled quantotypic peptides, and the molar amount of the CP is referenced to the known molar amount of the spiked-in reference protein standard (bovine serum albumin, BSA), we also investigated the influence of the reference peptides distribution along the CP's sequence on the final accuracy of the method. Finally, the newly proposed fast-track QconCAT approach, using an optimized-length CP standard in a combination with a fast and straightforward in-solution tryptic digestion protocol (with hot isopropanol protein precipitation) was cross-validated against the reference gel-based MS Western method.

Session 2: Lipidomics I; Oral 2: Helmer

Time: Monday, 02/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Harald Köfeler

Analysis of isomeric cardiolipin oxidation products by means of liquid chromatography and trapped ion mobility-mass spectrometry

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In recent years, ion mobility spectrometry (IMS) has become very popular in the field of lipid analysis. Lipids play key roles in many different biological processes. A major building block of many lipid classes, such as glycerolipids and glycerophospholipids, are fatty acids. To understand the function of a lipid, the analysis of lipid species and their structures is necessary.

An important lipid class is the phospholipid subclass of cardiolipin (CL) which is found in mitochondrial membranes and has specific properties in the biochemistry of organisms. CL are important for various functions of the respiratory chain and oxidized CL are known to be involved in apoptosis. Due to the dimeric structure of CL it is the only phospholipid, which consists of four fatty acyl moieties. The already high structural diversity due to differing length and degrees of saturation of the fatty acyl chains is further increased by oxidation. Hence, powerful separation techniques are required for the analysis of CL and their oxidation products. A separation of hydroxy and hydroperoxy species can be achieved by means of liquid chromatography. In this work a separation of those oxidation products is shown utilizing trapped ion mobility mass spectrometry (TIMS). Oxidized phospholipid standards especially for CL are not commercially available. For this reason, the influence of hydroxy and hydroperoxy groups on mobility was investigated by means of artificially oxidized standards and thus the general behavior was investigated.

After optimization of the TIMS separation, an artificially oxidized CL extract of bovine heart was analyzed by means of RP-LC-TIMS-ToF-MS. The main CL of heart with four linoleic acid moieties and its oxidation products have been separated utilizing a phenyl based RP phase. By the preceding LC separation followed by the separation utilizing TIMS it is possible to assign mobilities and collisional cross sections (CCS) values to the individual oxidation products. Isomeric oxidation products have been structurally characterized based on their mobility resolved MS/MS fragmentation patterns.

Session 3: ICP-MS: Elemental Imaging; Oral 2: Nowak

Time: Monday, 02/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Daniel Pröfrock

Qualitative and Quantitative Imaging of Transition Metal Deposition and Lithium Distribution Patterns in Lithium Ion Batteries

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The state of the art lithium ion battery (LIB) consists of a lithium transition metal oxide cathode, a graphitic anode, a separator and non-aqueous electrolyte, respectively. As the performance of the LIB technology is rising constantly, one of the major challenge remains in the form of capacity loss with continuous cycling, induced by various aging phenomena.

Among the most important processes is the formation of electrode surface films, for example the solid electrolyte interface (SEI). The SEI is essential for stable battery performance, as it ensures protective properties of the cell and inhibits further electrolyte decomposition. These protective properties can be deteriorated by the deposition of transition metals, which originate from dissolution (TMD) at the cathode. Furthermore, electrochemically active lithium can be “lost” during formation of interphases as well as during operation due to aging effects.

In this study, laser ablation-inductively coupled mass spectrometry (LA-ICP-MS) is applied as a technique in order to investigate TMD and subsequent deposition on the anode surface as well as the lithium distribution. Imaging *via* LA-ICP-MS allows for fast and easy investigation of deposition and distribution patterns on whole electrodes.

Session 4: Environmental MS; Oral 2: Kuzmich

Time: Monday, 02/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Björn Meermann

Crude oil weathering: formation of tar precursors under UV irradiation

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The modern industrial society is highly reliable on the energy consumption, while crude oil is considered as one of the most important sources of energy. The utilization of the petroleum can lead to accidents and subsequently to oil spills. The spilled crude oil penetrates water or soil, what can cause catastrophic harm to any ecosystem. The released petroleum undergoes a long term and complex transformation known as weathering. During this process, a number of products are being formed such as oxidized species, oil residue, surface residue and tar residue. The molecular mechanism of formation of the last one remains not addressed so far.

To investigate the weathering due to light irradiation, a light crude oil was irradiated for a long term range (10 months), using two custom-made photo reactors, simulating a sunlight spectrum. In the first setup, the crude oil was irradiated without any additives; in the second one it was mixed with water (1:1). The irradiated crude oil and the deposited material were characterized by ultra-high resolution mass spectrometry (UHRMS) with different ionization techniques, namely electrospray (ESI) and atmospheric pressure photoionization (APPI). The recorded mass range was m/z 150-1000 for APPI, and 150-1400 for ESI. Spectral stitching with mass window of 30 Da and 5Da overlap was used for all measurements. The acquired data were analyzed with Composer software.

In the pure oil it was observed that after 3 months of irradiation of the light crude oil, an orange-colored material deposited at the bottom of the vial. However, in the second setup a similar substance aggregated at the border of oil/water phases, followed by the formation of a spherical-shaped object. With UHRMS measurements it was established that the deposited compounds from both experimental setups consist mainly of oxidized hydrocarbons. Heteroatom classes from O_1 to O_{25} were assigned. In addition to this, for the hydrocarbons, detected as both radicals and protonated species, an interesting trend was observed where only the compounds with short side chains were found in the deposited substance. This can indicate the formation of oxygen-containing polymer and cleavage of side chains due to the oxidation. We believe that this can be the first step on the way to the formation of tar balls, with the formed high oxygen containing material acting as a precursor of tar residues.

Session 1: Proteomics-Basics; Oral 3: Tholey

Time: Monday, 02/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Simone König

Small droplets for high numbers – approaches for microfluidic supported LC-MS based proteomics

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Proteomics & Bioanalytics, Christian-Albrechts-Universität Kiel, Germany

While proteomics with high nanograms to micrograms of total protein has become routine, the analysis of samples derived from low cell numbers is challenged by factors such as sample losses, or difficulties encountered with the manual manipulation of small liquid volumes. We here describe the use of digital microfluidics (DMF) for the sample preparation of as little as 100 mammalian cells, allowing sensitive bottom-up proteome analysis by LC-MS. To this end, we developed effective cell lysis conditions optimized for DMF, as well as detergent-buffer systems compatible with downstream proteolytic digestion on DMF chips and subsequent LC-MS analysis. A major step was the introduction of the single-pot, solid-phase-enhanced sample preparation (SP3) approach on-chip, which allowed to remove salts and polymeric detergents, thus rendering sample preparation by DMF compatible with LC-MS-based proteome analysis. The efficiency of detergent removal by DMF-SP3 was demonstrated using a simple MALDI MS screen employing an ionic liquid matrix. Application of DMF-SP3 to the proteome analysis of Jurkat T cells led to the identification of up to 2,500 proteins from approximately 500 cells, and up to 1,200 proteins from approximately 100 cells on an Orbitrap mass spectrometer, emphasizing the high compatibility of SP3 with low protein input and minute volumes handled by DMF. Taken together, our findings show that the SP3 procedure can be conducted on a miniaturized low-cost microfluidic device, allowing the sensitive proteome analysis of limited biological material [1].

[1] Leipert J, Tholey A. *Miniaturized sample preparation on a digital microfluidics device for sensitive bottom-up microproteomics of mammalian cells using magnetic beads and mass spectrometry-compatible surfactants.* Lab Chip, 19: 3490-3498, (2019).

Session 2: Lipidomics I; Oral 3: Drotleff

Time: Monday, 02/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Harald Köfeler

Approaches towards large-scale lipid quantification in untargeted analysis by class-specific surrogate calibration

Bernhard Drotleff[1], Tomáš Pluháček[2], Michael Lämmerhofer[1]
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The hyphenation of liquid-chromatography (LC) and high-resolution tandem-mass spectrometry is the predominant analytical system that is currently utilized in untargeted lipidomics. Besides the general investigation of lipid profiles in various matrices, the principal goal of such studies is the generation or verification of hypotheses for novel biomarkers in health and disease. Although instrumentation, acquisition techniques and databases are steadily advancing for untargeted analysis in “omics” fields, the majority of methods still rely on the classic approach to elaborate relative fold changes of detected compounds between experimental groups. These relative results, however, have only limited potential for inter-assay or inter-batch comparability. In order to enhance the applicability of the gathered data, absolute quantification of as many features as achievable should be also pursued in untargeted analysis.

In general, absolute quantification requires preliminary calibration of the target analytes, preferably in the anticipated matrix and including internal standards to control for matrix effects and other variabilities in the instrument response. For biological samples and endogenous compounds like lipids, true blank matrices for the preparation of quantitative calibrant and quality control samples are usually not available. In consequence, alternative strategies, e.g. like surrogate calibration[1] via stable isotope labeled analyte analogues, had to be established to achieve accurate calibration.

Eventually, two untargeted lipidomics methods were developed, in which several labeled lipids were included as lipid class-specific surrogate calibrants for large-scale lipid quantification. The first method was based on reversed-phase LC and yielded a separation of lipid species. However, mainly due to differences in matrix effects and the composition of the mobile phase during elution, nonuniform ionization efficiencies of the separated lipid species had to be compensated by response factors to the corresponding surrogate calibrant. In a second method, using hydrophilic interaction chromatography, lipid class separation was achieved and differences in ionization efficiencies were reduced due to co-elution of target analytes and surrogate calibrants. The quantitative performance of both methods was indicated by validation experiments that complied with international guidelines.

[1] Li et al., Anal Chem., 2003 Nov., 1;75(21):5854-55859.

Session 3: ICP-MS: Elemental Imaging; Oral 3: Bleiner

Time: Monday, 02/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Daniel Pröfrock

Plasma-driven X-ray Laser for Nano-Scale Ablation and Desorption

Davide Bleiner
Empa, Switzerland

Laser-assisted microanalysis offers the advantage of spatially resolved mapping. Scanning a material with a sequence of sampling pulses, generating optical and/or mass spectra, one maps the local chemistry. However, the requirement to address smaller and smaller scales into the nano-scale, is limited by the optical diffraction and reduction of sensitivity, as explored also at beamlines, for which alternatives are needed[1].

Dramatic improvements are demonstrated utilizing a recently developed laser, operating in the soft X-ray region[2,3]. Firstly, a significant enhancement of spatial resolution is accomplished by one order of magnitude shorter wavelength, with respect to state-of-the-art laser lines, such as Nd:YAG. Reduction of operation wavelength is an alternative approach to the reduction of pulse duration (femtosecond laser) in order to obtain quasi non-destructive laser microsampling. Furthermore, the most interesting aspect, while utilizing "*ionizing radiation*", is the enhancement of ablation/desorption and sample ionization efficiency[4,5]. The high photon-energy (25-100eV) makes the ablation process essentially photo-chemicaln, with insignificant thermal contribution.

In the present talk, the basic information is summarized, in order to orient the audience that XUV/SXR is not just "one more wavelength" in the town, rather a disruptively different micro-sampling mechanism: i.e. by direct solid photo-ionization as opposed to classical collisional ablation. Further, a selection of applications in the depth profiling analysis of thin films (10-100nm thickness) is shown.

1. Arbelo, Y., Barbato, F. & Bleiner, D. He-doped pseudospark as a home-lab XUV source beyond the beamtime bottleneck. *Plasma Sources Sci. Technol.* **26**, 035005 (2017).

2. D. Bleiner et al. Eds. *Short Wavelength Laboratory Sources*. (Royal Society of Chemistry, 2014). doi:10.1039/9781849735018

3. Bleiner, D., Arbelo-Pena, Y., Masoudnia, L. & Ruiz-Lopez, M. Table-top x-ray lasers using a plasma gain-medium: limits and potentials. *Phys. Scr.* **T162**, 014050 (2014).

4. Müller, R. *et al.* Depth-Profiling Microanalysis of CoNCN Water-Oxidation Catalyst Using a λ = 46.9 nm Plasma Laser for Nano-Ionization Mass Spectrometry. *Anal. Chem.* **90**, 9234–9240 (2018).

5. Arbelo, Y. & Bleiner, D. Tabletop Extreme Ultraviolet Time-Of-Flight Spectrometry for Trace Analysis of High Ionization Energy Samples. *Rapid Commun. Mass Spectrom.* (2019). doi:10.1002/rcm.8463

Session 4: Environmental MS; Oral 3: Stadler

Time: Monday, 02/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Björn Meermann

Advanced Application of Desorption Electrospray Ionization Mass Spectrometry (DESI MS): Radionuclide Speciation in Plant Parts

Julia Stadler[1], Anna Kogiomtzidis[1], Michael Steppert[1], Moritz Schmidt[2], Clemens Walther[1]
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Americium-241 is an important radionuclide and currently the dominating *α*-emitter in the Chernobyl exclusion zone due to continuous buildup from its precursor Plutonium-241 ($t_{1/2}$ = 14.35 a) by beta decay. There is a high possibility, that americium transfers into plant parts via contaminated soil and eventually enters the human nutrition chain. In order to gain better understanding of the americium uptake mechanism on a microscopic scale, its chemical speciation has to be investigated. Bioavailability proves to differ for the various available compounds that can be formed in soil and during the uptake process. Therefore, the ambient *Desorption Electrospray Ionization Mass Spectrometry* (DESI MS) (Takáts et al., 2004) technique that couples a soft ionization procedure with a high resolution Orbitrap mass analyzer was introduced for the speciation directly in plant parts. In combination with *Time-Resolved Laser Fluorescence Spectroscopy* (TRLFS) (Fellows et al., 2013) and HPLC MS, it represents a promising method for chemical speciation of radionuclides.

First results were obtained for europium as non-radioactive homologue for trivalent actinides. Different crops are grown in a liquid Hoagland medium (Gupta et al., 2013), providing a controlled environment for plant growth experiments. Eu was quantified (ICP MS) and localized (REM EDX) in plant parts root, stem, leaf and fruit. Direct analysis with DESI MS confirms the uptake of Eu in form of its Hoagland species. These species were identified by comparison with previous ESI MS measurements from the contaminated nutrition medium. Furthermore, TRLFS analysis performed with *Phaseolus vulgaris L.* exhibits different species in the four plant parts and the results also indicate a possible tetragonal symmetry or crystallization of Eu species in leaf and fruit. Based on fluorescence lifetimes, Eu oxalate, phosphate and phytate were identified as potential species directly in the *Phaseolus vulgaris L.* plant parts.

In addition, we developed a method for a coupled HPLC Orbitrap technique that was implemented with a self-made algorithm for spectra evaluation. This techique should be able to detect available Eu species after sequential extraction, and promising measurements are currently in progress. First tests suggest several species, which will be identified in a next step in DESI mass spectra.

Finally, we are able to detect the taken europium hydroxide and nitrate species directly in the plant with DESI MS after contamination of the liquid nutrition medium (Hoagland) and showed some first results of additional analytical methods for chemical speciation with TRLFS and HPLC MS.

Session 1: Proteomics-Basics; Oral 4: Koch

Time: Monday, 02/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Simone König

diaPASEF: label-free quantification of highly complex proteomes

Scarlet Koch[1], Florian Meier[2], Andreas Brunner[2], Max Frank[3], Annie Ha[3], Stephanie Kaspar-Schoenefeld[1], Markus Lubeck[1], Oliver Raether[1], Ute Distler[4], Stefan Tenzer[4], Tejas Gandhi[5], Lukas Reiter[5], Hannes Roest[3], Ben Collins[6], Ruedi Aebersold[6], Matthias Mann[2]

[1]Bruker Daltonik GmbH, Germany; [2]Max Planck Institut für Biochemie, Germany; [3]Donnelly Centre for Cellular and Biomol. Research, Canada; [4]University of Mainz, Germany; [5]Biognosys, Switzerland; [6]ETH Zurich, Switzerland

Data-independent acquisition (DIA) promises reproducible and accurate protein quantification across large sample cohorts. The mass spectrometer typically cycles through many isolation windows covering a broader *m/z* range of interest. Current methods utilize only about 1-3% of all available ions. In principle, all ions could be utilized by parallel ion storage and sequential release from the TIMS device into a Q-TOF mass analyzer. Here, we asked if the PASEF principle could be transferred to DIA.

Whole-cell proteomes extracted from a human cancer cell line were analyzed via nanoLC coupled to a prototype timsTOF Pro (Bruker). We adapted the instrument firmware to perform data-independent isolation of multiple precursor windows within a single TIMS separation (100ms). We tested multiple schemes for precursor selection window size and placement in the *m/z*-ion mobility plane. Analysis of the four-dimensional data space has been incorporated into OpenSWATH and Spectronaut software (Biognosys). For ion mobility-aware targeted data extraction, we used a project-specific library from 48 high-pH reverse-phase peptide fractions acquired with PASEF.

As ion mobility and mass are correlated, a large proportion of the peptide ion current can be covered by scanning diagonal lines in the *m/z*-ion mobility space. We derived multiple diaPASEF acquisition schemes from the density distribution of about 130,000 precursors present in the library. TIMS provides highly precise measurements of collisional cross sections (CCS) with CVs << 1% in technical replicates. After linear alignment, CCS values extracted from the diaPASEF runs deviated < 2% from the library. In triplicate 120min runs of 200ng HeLa digest each, we quantified over 8000 proteins at a 1% FDR. Fragment ion-based quantification was very reproducible with a median CVs of 10% and a pairwise mean Pearson correlation >0.96.

The diaPASEF method captures and utilizes a very large proportion of the available ion current, approaching the ideal mass analyzer.

Session 2: Lipidomics I; Oral 4: Schuhmann

Time: Monday, 02/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Harald Köfeler

Accurate lipidome-wide quantification by Orbitrap FT MS/MS and fragment intensity adjustment

Kai Schuhmann[1], HongKee Moon[1], Henrik Thomas[1], Michael Groessl[2], Nicolai Wagner[1], Markus Kellmann[3], Andre Nadler[1], Andrej Shevchenko[1]

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The lipidome of a cell can comprise hundreds of structurally distinct molecular species of glycerophospholipids. They are identified and quantified by bottom-up shotgun lipidomics using Orbitrap FT MS/MS relying on the detection of carboxylate anion (CA) fragments of the molecular anion fatty acid (FA) moieties released during collision-induced dissociation. In shotgun lipidomics, lipids are quantified by a single reference standard with the same head group but a mass distinct from the analytes. For both, standard and quantified lipids, the same molar instrument response is thereby assumed. While the shotgun approach balances the ionization for different lipid species, the adjustment of instrument parameters and specifically a compensation for persisting analyte-specific fragmentation differences is nevertheless required.

To improve the accuracy and consistency of quantitative bottom-up lipidomics data, we have developed an approach that fills the gap between the existing "one standard per class" and the ideal yet not practically attainable "one standard - one analyte" approach. We developed a generic and portable CA fragment intensity model for fragmentation of glycerophospholipids based on HCD FTMS/MS reference data to balance the fragmentation differences and implemented it in the open source software LipidXte for automated correction and lipid quantification.

By adjusting the fragment intensities for analyte-specific differences, we identified a hidden instrument feature. Post-correction, intensities from FTMS/MS spectra showed a m/z dependent bias where precursor with lower m/z showed lower intensities and precursor with higher m/z had enhances intensities. The same was observed for t-SIM spectra acquired with narrow isolation windows, with a bias of -45% for lower m/z and +67% for higher m/z. This bias required additional adjustment for accurate quantification.

After correction for analyte-specific fragmentation differences and instrument induced biases we could quantify molecular species of GPL in complex biological samples. GPL isomers could be quantified directly from the MS/MS and the quantification accuracy was improved from -100% to +30% to +/- 10%. The fragmentation model validated by the analysing a standard lipid mixture on five QExactive Orbitrap instruments running under arbitrary settings.

Session 3: ICP-MS: Elemental Imaging; Oral 4: Shaw

Time: Monday, 02/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Daniel Pröfrock

Multi-element analysis with a time of flight ICP-MS capable of uninterrupted data acquisition at 1-30kHz readout speed with all argon and nitrogen interferences removed with novel segmented reaction cell interference removal device

Phil Shaw
Nu Instruments, United Kingdom

Time of Flight (TOF) ICP-MS is developing rapidly with the recent availability of very fast washout laser ablation cells for imaging and the growing requirement to characterise nanoparticles for multi-element content in individual particles. As TOF is essentially simultaneous technique, sampling the whole ion beam from the source in packets, there are several difficulties in using a TOF with the highly “bright” ICP source. This presentation will describe the ways in which the Nu Instrument Vitesse has overcome the classical difficulties of using a TOF in an ICP-MS and how the data acquisition system has been optimised for the most rigorous demands of nanoparticle and imaging analyses with application examples.

The single particle data acquisition technique has developed on quadrupole and high resolution ICP-MS with the start of the art data acquisition rates in the tens of kHz proving powerful at being able to differentiate nanoparticles from ionic background signals. The technique can accurately count particle numbers and sizes in a widening range of matrices. However, the information provided is incomplete as the analysis is single element which leaves many questions unanswered in terms of particle associations. Even when using field flow fractionation (FFF) to separate groups of particles of similar size, any associations of elements can only be inferred. Also the number concentrations of particles required for FFF can make the determination of particle associations in environmental samples difficult.

Attempts have been made to measure 2 elements in individual particles using fast jumping quadrupole ICP-MS but the results are qualitative at best and have severe limitations in reduction in sensitivity. The ideal is to use a fast data acquisition mass spectrometer such as a time of flight (TOF). Previous attempts have been limited by the technologies employed in the data acquisition as only a few milliseconds of data can be acquired at the highest data rate before a delay of several hundred milliseconds is needed to transfer data and restart the acquisition. This significantly reduced duty cycle makes the collection of statistically significant numbers of particles very time-consuming.

The latest laser ablation cells have washout times of only a few milliseconds allowing complete pixel separation of signals whilst ablating at repetition rates of 50-200Hz. This makes collecting a small spot-size, large area image a much faster task. However, a lot of information is lost if multiple elements can not be collected for each image and some samples such as cell tissues become completely consumed by the single ablation so all information must be collected for each shot. TOF is the optimal mass spectrometer for imaging as full spectra can be collected faster than the laser is ablating and so each pixel can be constructed from simultaneously acquired multi-element data. We will present the ways in which Vitesse is used with these new laser cells to obtain high density images quickly and easily.

Session 4: Environmental MS; Oral 4: Gehm

Time: Monday, 02/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Björn Meermann

Development and optimization of a membrane - inlet - photoionization mass spectrometer for fast analysis of (polycyclic)aromatic compounds in aquatic systems

Christian Gehm[1], Thorsten Streibel[1,2], Sven Ehlert[1,3], Detlef Schulz-Bull[4], Ralf Zimmermann[1,3]
[1]University of Rostock, Germany; [2]Helmholtz Zentrum München – German Research Center for Environmental Health GmbH, Germany; [3]Photonion GmbH, Germany; [4]Leibniz Institute for Baltic Sea Research Warnemünde, Germany

The development of sensitive analytical techniques for the real – time detection of aromatic (AH) and polycyclic aromatic hydrocarbons (PAH) is of high importance due to their impact on human health and the environment. However, low concentrations of these compounds in marine systems complicate their detection and monitoring. Common analytical strategies involve discrete sampling, followed by a wide variety of sample preparation techniques. Especially, the time consuming sample preparation steps are necessary to increase the concentrations up to a detectable level and to eliminate the water matrix. Hence, there is a need of new analytical systems for the direct detection of compounds in water. Herein, membrane introduction mass spectrometry (MIMS) in combination with resonance enhanced multiphoton ionization (REMPI) shows a high potential for fast and sensitive real time analysis of (P)AHs in aquatic systems. In MIMS, compounds are transported selectively into the vacuum region of the mass spectrometer through a semipermeable membrane, extracting the analytes from the water phase directly into the gas phase. With the aid of REMPI, sensitive determination of (polycyclic)aromatic species is possible due to the spectroscopic selectivity in ionization process.

Session 5: Proteomics - Clinical and Biological Applications; Keynote: Winter

Time: Monday, 02/Mar/2020: 2:00pm - 2:30pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Hartmut Schlüter

BirA* Mouse Lines for the Analysis of Cell Type-Specific Proteomes *in vivo*

Shiva Ahmadi[1], Elham Pourbarkhordariesfandabadi[1], Kenichi Kimura[2], Angela Egert[3], Martin Breitbach[2], Caroline Geissen[2], Michael Hesse[2], Bernd Fleischmann[2], Hubert Schorle[3], Volkmar Gieselmann[1], Dominic Winter[1]

[1]Institute for Biochemistry and Molecular Biology, University of Bonn, Germany; [2]Institute for Physiology 1, University of Bonn, Germany; [3]Institute for Developmental pathology, University of Bonn, Germany

The analysis of cell type-specific proteomes in tissues and organs is a major challenge, which is mainly due to their cellular heterogeneity. To date, approaches addressing this issue mainly rely on in vitro cultivation of the desired cell types, or the isolation of intact cells/structures. These techniques come with certain limitations, which are related to e.g. artificial growth environments, harsh conditions for tissue dissociation, or isolation of mixed populations.

To develop a method which is able to capture the physiological state of cell type-specific proteomes with higher efficiency, we generated two mouse lines. They are based on the cell type-specific expression of the R118G mutated E. coli biotin ligase BirA*, which catalyzes the biotinylation of proteins in the cytosol and nucleus. In these animals, BirA* is either expressed from the ROSA26 locus, allowing for control of expression by Cre recombinase, or in the Col1A1 locus, where it can be controlled by both Cre recombinase and administration of doxycycline.

After breeding of both mouse lines with ubiquitous driver lines, theoretically resulting in BirA* expression in all tissues, we evaluated the presence and activity of BirA* with Western blot and immunohistochemistry experiments. Using pancreas, lung, and liver tissues, we developed mass spectrometry workflows to facilitate the identification of cell type-specific biotinylated proteins. These methods are based on streptavidin enrichment, on-bead digestion, dimethyl labeling, offgel fractionation, and mass spectrometric analysis on Orbitrap Velos and Fusion Lumos mass spectrometers. For the determination of specifically enriched – and therefore biotinylated – proteins, we developed a novel approach based on the distribution behavior of binding proteins.

We applied this approach to the analysis of the pancreatic beta cell proteome under resting and drug-perturbed conditions. We generated Ins1-Cre-BirA* mice which result in exclusive expression of BirA* in pancreatic beta cells. Using these samples, we established a TMT 6plex/10plex strategy in combination with basic reversed phase fractionation and synchronous precursor selection analysis on the Orbitrap Lumos. This resulted in a first draft of the in vivo pancreatic beta cell proteome, covering ~4100 proteins groups in total of which ~3000 were determined to be biotinylated and therefore putative beta cell proteins. Finally, we investigated the effect of the pro-diabetic drug Streptozotocin and the anti-diabetic drug Harmine on beta cells. Analysis of regulated proteins by gene ontology (GO) revealed regulation of pathways that had been previously connected to the effects of these drugs, as well as novel pathways.

Session 6: Imaging I; Keynote: Hagenhoff

Time: Monday, 02/Mar/2020: 2:00pm - 2:30pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Bernhard Spengler

Surface Mass Spectrometry (SIMS): Where We Are and Where We Need to Go

Birgit Hagenhoff
Tascon GmbH, Germany

The chemical composition of surfaces and interfaces at the micro- and nanoscale plays an important role with respect to the macroscopic behaviour of materials and products. Therefore, detailed knowledge about main matrix as well as trace components is required along the complete production chain, from research and development along production to after sales and failure analysis.

Generally, the analytical question in this context contains three main questions: What (identification) is sitting where (localisation) and how much is it (quantification). These questions need to be answered for the complete surface near three-dimensional volume, i.e. with sufficient lateral and depth resolution. In addition, both, the elemental and molecular composition needs to be probed with high sensitivity.

In this respect, ToF-SIMS (Time-of-Flight Secondary Ion Mass Spectrometry has matured into a versatile mass spectrometry for and directly at surfaces and interfaces. The talk will discuss the current status and future challenges in ToF-SIMS along its merits for localisation (lateral resolution 50 nm, depth resolution 1 nm), identification (cluster ion bomabrdment, multivariate approaches, MS/MS options) as well as quantification (e.g. suited reference materials or reference technques).

Session 7: ICP-MS: Nano- / Bioanalysis; Keynote: Engelhard

Time: Monday, 02/Mar/2020: 2:00pm - 2:30pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Jörg Bettmer

ADVANCES IN SINGLE PARTICLE ICP-MS: DEAD TIME CORRECTION INCREASES LINEAR RESPONSE AND PARTICLE SIZE COVERAGE

Carsten Engelhard[1], Ingo Strenge[1], Darya Mozhayeva[1], Annika Schardt[1], Antonio Montoro Bustos[2], Karen Murphy[2]
[1]University of Siegen, Germany; [2]National Institute of Standards and Technology, USA

In the first part of the presentation, recent advances in the field of single particle inductively coupled plasma mass spectrometry (spICP-MS) will be briefly reviewed with a focus on the characterization of inorganic nanoparticles (NPs), instrumental advances, and applications. Both, the number of publications of stand-alone spICP-MS as well as spICP-MS coupled to separation and fractionation techniques is steadily increasing. Data acquisition was typically performed with millisecond dwell times in the past while a time resolution of hundreds of microseconds has been used more often in the last five years with quadrupole-type mass spectrometers.

In the second part of the presentation, our recent efforts to further push the limits in spICP-MS will be discussed. While the fundamental concepts of spICP-MS were established long ago, off-the-shelf mass spectrometers are still limited in hardware and software capabilities for ideal spICP-MS nanoanalysis. Specifically, the instruments' hardware is typically limited in data acquisition speed and maximum measurement time. We have shown earlier with a custom-built high-speed data acquisition (DAQ) unit that signals from discrete ion clouds in the ICP can be acquired continuously with a time resolution of five microseconds and 100% duty cycle. Fully time-resolved temporal profiles of individual single droplet or particle events can be recorded and signal artifacts due to particle coincidence and split-events are virtually eliminated. In addition, our data processing approach allows the quantification of both the silver NP size distribution and the concentration of dissolved silver ions in mixtures. Poisson statistics are used to determine thresholds to identify the beginning and end of NP signal events. In doing so, NPs can be detected even in the presence of a significant concentration of ionic background (e.g. $^{107}Ag^{+}$ up to 1.000,000 cps).

Finally, we report a dead time correction approach for spICP-MS. Detection of small NPs usually requires utilization of the pulse counting signal, which entails the risk of nonlinear response for larger NPs. The use of dead time correction to microsecond time-resolved (µsTR) spICP-MS data allowed us to increase the maximum number of counts tolerated per particle. Further, a unique combination of microsecond time-resolved pulse counting data and microsecond time-resolved analog data resulted in linear response for AuNP ranging from 30 – 250 nm. Our findings support the hypothesis that dead time related count losses are the main reason for nonlinear response in pulse counting spICP-MS.

Session 8: Instrumentation I; Keynote: Cramer

Time: Monday, 02/Mar/2020: 2:00pm - 2:30pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Christopher Rüger

Liquid AP-MALDI MS and its potential in high-speed sample analysis

Henriette Krenkel[1], Cristian Piras[1], Jeffery Brown[1,2], Michael Morris[2], Rainer Cramer[1]
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Liquid AP-MALDI MS, in comparison to conventional solid-state vacuum MALDI MS, provides many additional analytical benefits such as the production of multiply charged analyte ions, homogenous analyte distribution in the sample droplet, stable ion yield and fast sample introduction. In particular with respect to the latter aspects, liquid AP-MALDI MS is well suited for extremely high analysis speed and sample throughput. In this presentation, we will demonstrate how liquid AP-MALDI MS on a high-performing hybrid QTOF mass spectrometer (Synapt G2-Si) can be applied to various analytical questions, from peptide analysis to enzymatic assay read-outs and complex biofluid profiling, at a speed of more than 3 samples per second. We will discuss further improvements of our current set-up, ultimately paving the way to an analytical speed of more than 10 samples per second with a theoretical limitation of 100 samples per second due to the practical limitations of the generated ion beam and its analysis on the specific type of QTOF instrument used so far. In combination with other advantages such as good tolerance to a variety of sample additives/contaminants and on-line reaction monitoring, this newly developed MALDI MS approach has the potential to add substantially to the tool box for large-scale compound screening and assay development in the biopharmaceutical field.

Special Session (1): Science Management; Keynote: Mürtz

Time: Monday, 02/Mar/2020: 2:00pm - 2:30pm · *Location:* Lecture Hall Building Chemistry: Hall A1
Session Chair: Christof Lenz

DFG recommendations for proposals and operation of mass spectrometers at universities

Manfred Mürtz
Deutsche Forschungsgemeinschaft, Germany

The guidelines of the Deutsche Forschungsgemeinschaft for proposals and operation of MS instruments are presented. The DFG funding programme 'Core Facilities' is highlighted as well as the possibilities for funding of user fees in DFG individual projects and grants.

Session 5: Proteomics - Clinical and Biological Applications; Oral 1: Stolz

Time: Monday, 02/Mar/2020: 2:30pm - 2:50pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Hartmut Schlüter

In-depth Characterization of Glycated and Carbamylated Hemoglobin in Clinical Samples by Top-down Capillary Electrophoresis-Tandem Mass Spectrometry

Alexander Stolz[1], Liesa Salzer[1], Laurent Leclercq[2], Ylva Hedeland[3], Hervé Cottet[2], Jonas Bergquist[4], Christian Neusüß[1]
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Hemoglobin (Hb) is an important oxygen transport protein in nearly all vertebrates. Due to its high abundance in blood, several non-enzymatic reactions with blood constituencies are known. Some of these protein modifications such as glycation and carbamylation serve as important biomarkers for diabetes and renal failure. In the clinical context, analysis of glycated and carbamylated Hb is performed by ion exchange chromatography or capillary electrophoresis with optical detection. Alternatively, immunological methods such as enzyme-linked immunosorbent assay (ELISA) are often applied. However, these methods are prone to interferences by Hb sequence variants or other modified Hb species. Therefore, the combination of sophisticated separation methods with mass spectrometry (MS) is aspired for unambiguous characterization and identification of Hb species.

We developed a capillary electrophoresis-mass spectrometry (CE-MS) method using successive multiple ionic-polymer layer (SMIL) coated capillaries for the separation and characterization of glycated and carbamylated human Hb. The 5-layer SMIL coatings used in this study result in a reversal of the electroosmotic flow, enabling the separation of proteins in reversed polarity mode with high separation efficiency. Hence, it is possible to separate the α- and β-chain of hemoglobin and their glycated and carbamylated variants. The method was tested and optimized with chemically glycated human Hb standard and subsequently applied to the analysis of dried blood spot (DBS) samples of patients with diabetes and kidney failure. Besides detecting and quantifying glycated and carbamylated Hb, it was also possible to quantify fetal Hb (HbF) in a single run.

To characterize the glycated and carbamylated Hb species, top-down MS was applied. Combination of electron-transfer dissociation (ETD) and higher-energy collisional dissociation (HCD) yielded fragmentation coverages of up to 70% for glycated α and β chains. With ETD fragmentation, it was possible to pinpoint the glycosylation site. We discovered the presence of several glycation sites, including N-terminal and not N-terminal glycated species. By tracing diagnostic fragment ions (C_8 ion) for the N-terminal and not N-terminal glycated species, we were able to show partial separation of these positional isomers.

To demonstrate the high versatility of the CE-MS method, we also analyzed cat and dog DBS samples and identified a variety of species including glycated and carbamylated Hb as well as several α and β sequence variants.

Session 6: Imaging I; Oral 1: Oetjen

Time: Monday, 02/Mar/2020: 2:30pm - 2:50pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Bernhard Spengler

Proteomic profiles of breast cancer tumor subpopulations defined by MALDI guided SpatialOMx on a timsTOF fleX

Janina Oetjen[1], Annika Koch[1], Romano Hebeler[1], Frédéric Dewez[2], Corinna Henkel[1], Benjamin Balluff[2], Ron Heeren[2]
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Since tissue and disease are correlated, SpatialOMx provides the unique opportunity to combine regiospecific information of MALDI Imaging with deep proteomic coverage for biomarker discovery and molecular characterization. Here, we present SpatialOMx for microproteomic characterization of tumor subpopulations in breast cancer. In this efficient workflow, unsupervised segmentation of MALDI Imaging data with SCiLS Lab is used to define ROIs. Image processing strategies provide boundary information of segments of tumor subpopulations for laser capture microdissection. Protein extraction and tryptic digestion of small microdissected material is followed by proteomic analysis using PASEF. Fresh-frozen breast tumor sections were cut at 12 µm thickness and mounted on PEN (polyethylene naphthalate) membrane slides. After drying, the slides were coated with Norharmane matrix (7 mg/mL in $CHCl_3$:MeOH, 2:1, v:v) using a TM-sprayer (HTX Technologies) and measured on a timsTOF fleX. After defining ROIs from lipid MALDI Imaging analysis in SCiLS lab, the data where further processed in Matlab. Areas containing approximately 2000 cells were dissected from each segmented tumor subpopulation with a Leica LMD 7000 and directly transferred to reaction tubes for microextraction and tryptic digestion. Microextracted peptide samples were LC-separated and ran on the same instrument using PASEF. SpatialOMx is a new workflow for in-situ characterization of tissue subtypes. Lipid imaging on the timsTOF fleX from tumor sections was used to define ROIs by segmenting intratumor heterogeneity for laser microdissection (LMD) based microproteomics. timsTOF fleX allows for in-depth proteomic analysis of small microdissected tissue pieces. In average, about 3500 protein IDs were obtained from the three microextractions to provide a deep insight into the proteome from just 160 µg (estimated) of sample. Interestingly, the proteomic profiles of the tumor subpopulation differed largely. The gene ontology terms “Biological regulation” and “Developmental processes” were underrepresented in subpopulations 1 and 2, respectively, while “cellular component organization” was overrepresented in tumor subpopulation. In summary, SpatialOMx provides guidance to specific regions of interest for microextraction and in-depth proteomics analysis to characterize regiospecific molecular changes in detail.

Session 7: ICP-MS: Nano- / Oral 1: Buchholz

Time: Monday, 02/Mar/2020: 2:30pm - 2:50pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Jörg Bettmer

Investigating the biodistribution and long-term fate of ^{57}Fe-enriched iron oxide nanoparticles by means of LA-ICP-MS

Rebecca Buchholz[1], Max Masthoff[2], Andre Beuker[2], Katharina Kronenberg[1], Moritz Wildgruber[2], Cornelius Faber[2], Uwe Karst[1]

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Magnetic resonance imaging (MRI) is already established since the early 1980´s and is an inherent part of medical imaging techniques. In case of insufficient contrast of different tissues, the usage of various contrast agents is possible. Especially in the last few years, contrast agents based on gadolinium (GBCA) gained unfortunate publicity due to recent research results indicating the persistence of these contrast agents in the brain. Contrast agents based on iron oxide nanoparticles (ION) are alternatively used substances to enhance the contrast during an MRI examination.

In this study, the biodistribution of ION contrast agents was analyzed. Because of the high natural iron background, ^{57}Fe-enriched IONs were used to distinguish between the natural iron and iron from the nanoparticles. Various organs of healthy mice (liver, kidney, spleen and brain) were scanned at various time points after injection of the contrast agents with MRI (*in vivo*) first and were then analyzed with laser ablation coupled to inductively coupled plasma - mass spectrometry (LA-ICP-MS, *ex vivo*). For cell tracking experiments inflammation-inducing pellets were inserted under the skin. ^{57}Fe-ION uptake by cells of the anti-inflammatory system were analyzed with LA-ICP-MS.

The quantification of iron was performed by external calibration based on matrix-matched gelatin standards. In this project the two isotopes ^{56}Fe and ^{57}Fe were quantified specifically in consideration of the natural isotopic ratio and mass bias of these isotopes.

In addition to the spatially resolved results gained from the LA-ICP-MS experiments, homogenized organ samples were analyzed by means of ICP-MS to determine statistically relevant results from all various sample types.

Enrichment of the ^{57}Fe IONs was detected in all analyzed organs by quantification and by changes in the ratio from ^{57}Fe to ^{56}Fe in comparison to naive organs. Highest concentrations were found in spleen and liver. The distribution patterns changed over the different time points especially in the spleen samples. High 57Fe concentrations in border regions of the inserted pellets indicate the uptake by cells of the anti-inflammatory system.

Session 8: Instrumentation I; Oral 1: Wootton

Time: Monday, 02/Mar/2020: 2:30pm - 2:50pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Christopher Rüger

New developments in Two-Dimensional Mass Spectrometry (2DMS)

Christopher A. Wootton, Tomos E. Morgan, Bryan P. Marzullo, Yuko P.Y. Lam, Alina Theisen, Dianna C. Palacio Lozano, Anisha Haris, Mark P. Barrow, Peter B. O'Connor
University of Warwick, United Kingdom

Aims / Introduction:

Two dimensional mass spectrometry is a truly data independent MSMS technique allowing fragmentation of all species in a sample and correlation of fragments for every precursor ion observed without ion isolation or data dependent parameters. 2DMS is able to provide a second dimension to MSMS analysis where precursors are not separated in bins as is for standard quadrupole-isolated MSMS/DDA/SWATH, instead 2DMS provides a true continuum in the second dimension of MS allowing high precursor resolving power and still retaining the ultra-high resolving power and mass accuracy of the FTMS mass analyser in the fragment dimension. Herein we present the latest developments in 2DMS methodology and analysis to analyse samples of ever-increasing complexity including; IRMPD/ECD/EID fragmentation modes, ppb mass calibration, 3D peak picking algorithms, automated proteomic searching, mDa precursor resolution, and combining UVPD with 2DMS.

With these capabilities; 2DMS is now in a position to tackle wide varieties of complex samples. Examples shown to showcase these capabilities are agrochemical mixtures, bio-compatible polymers, bottom-up proteomic digests, and petroleum mixtures. A 2DMS spectrum of each sample shows fragments for each individual precursor species m/z down to mDa precision and allows a truly DIA acquisition methodology to study complex and/or unknown samples.

Experimental and Results:

Two dimensional mass spectra were acquired using a modified pulse-delay-pulse Gaumann-style excitation profile on a Bruker 12T SolariX FT-ICR MS. IRMPD, ECD, EID, and UVPD at 193nm were used as fragmentation modes for 2DMS analysis. IRMPD was achieved via a continuous wave CO2 laser. ECD/EID was achieved via a hollow dispenser cathode. UVPD was achieved using a pulsed ArF excimer laser (193nm) operating on the same instrument. Proteomic, agrochemical, and polymer samples were ionised using nano-ESI, whereas petroleum samples were ionised using APPI via a 10.6eV krypton lamp. 2DMS spectra were processed using SPIKE to conduct the 2D-FFT and demodulation operations. Data was analysed using T2D software developed in house.FT-ICR 2DMS data was able to resolve precursor species <=1mDa apart and provide ppb mass assignments for fragments and precursors observed. EID 2DMS showed remarkably high intensity fragments for small molecule analysis compared to standard EID MSMS. IRMPD 2DMS data showed widespread applicability and high fragmentation efficiency for many species, whereas UVPD explores new avenues of complementary fragmentation pathways and complex fragment patterns requiring the performance of high-resolution accurate mass MS to elucidate and assign.

Special Session (2): Science Management: Trevorrow

Time: Monday, 02/Mar/2020: 2:30pm - 2:50pm · *Location:* Lecture Hall Building Chemistry: Hall A1
Session Chair: Christof Lenz

From Open Access to Publish and Read models. The evolution of journal access.

Paul E Trevorrow
Wiley, United Kingdom

In this Science Management session Paul E. Trevorrow will reflect on the evolution process of journal access.

Session 5: Proteomics - Clinical and Biological Applications; Oral 2: Wenk

Time: Monday, 02/Mar/2020: 2:50pm - 3:10pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Hartmut Schlüter

Investigation of the impact of functionally selective dopamine receptor D2 ligands on the cellular protein expression by untargeted nano-LC-MS/MS

Deborah Wenk[1], Vladimir Ignatchenko[2], Andrew Macklin[2], Harald Hübner[3], Peter Gmeiner[3], Monika Pischetsrieder[1], Thomas Kislinger[2]

[1]Food Chemistry Unit, Friedrich-Alexander Universität Erlangen-Nürnberg, Germany; [2]Princess Margaret Cancer Centre, University Health Network, Canada; [3]Medicinal Chemistry Unit, Friedrich-Alexander Universität Erlangen-Nürnberg, Germany

Introduction:

The dopamine receptor D2 (D2R) is an important drug target. D2R agonists, for example, are used for the treatment of Parkinson´s disease. These compounds, however, can induce severe side effects like hallucinations and somnolence. The development of functionally selective ligands is a promising approach towards drugs with custom tailored activity and less side effects. These ligands bind to the same target receptor with similar affinity but trigger different cellular reactions. Binding to the D2R, for example, can selectively activate G-protein or β-arrestin pathways. So far, functionally selective ligands are mostly evaluated based on their primary cellular signaling. This, however, gives only limited insight into their impact on cells. For a more detailed evaluation of functionally selective D2R ligands we performed a comprehensive analysis of cellular protein expression upon their receptor binding.

Experimental: HEK 293T cells transiently transfected with D2R were stimulated with one of five different D2R ligands selected from the classes of unbiased agonist, G-protein selective agonist, β-arrestin selective agonist and inverse agonist. Quantitative changes of intracellular proteins were then determined via untargeted nano-LC-MS/MS. Briefly, tryptic peptides derived from trifluoroethanol-based protein extracts were purified by a C18 solid phase extraction and measured on an EASY-nLC-QExactive HF system in a top 25 untargeted mode. The data was then analyzed by MaxQuant and statistically evaluated in R.

Results: In total, 5290 protein groups (PGs) were detected by proteomic analysis. ANOVA ($p \leq 5$ %) revealed 1462 differentially expressed PGs. Between 47 (β-arrestin selective agonist) and 128 (unbiased agonist) PGs showed statistically (Tukey´s HSD, $p \leq 5$ %) and biologically (expression change ≥ 1.5) significant changes to the control. Unsupervised hierarchical clustering based on treatment demonstrated a smaller impact of the functionally selective ligands on the overall cellular protein composition as compared to the unbiased agonists, indicating that functionally selective ligands influence cellular processes more selectively. Based on this data, functional analysis of differential proteins will be performed to evaluate physiological consequences of biased signaling. Thus, it could be demonstrated that untargeted LC-MS/MS based protein expression profiling can be useful to understand the physiological consequences of receptor-ligand interaction.

Session 6: Imaging I; Oral 2: Müller

Time: Monday, 02/Mar/2020: 2:50pm - 3:10pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Bernhard Spengler

High resolution atmospheric-pressure mass spectrometry imaging of biological samples using a matrix-free ionization-assisting DIUTHAME foil

Max Alexander Müller[1], Dhaka Bhandari[1], Kerstin Strupat[2], Bernhard Spengler[1]
[1]Justus Liebig University, Giessen, Germany; [2]Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany

Since its introduction, mass spectrometry imaging (MSI) has proven to be a valuable tool for spatially resolved chemical analysis of surfaces. Amongst a variety of ionization methods, focused laser beams are advantageously used, providing high spatial resolution and soft-ionization mechanisms, further improved by ionization-assisting substrates or coatings.

As a widely used technique, matrix-assisted laser desorption/ionization (MALDI) MSI relies on covering the sample surface with a thin matrix layer to assist analyte volatilization and ionization, complicating sample preparation.

Besides matrices, nanostructured surfaces can also assist ionization. Due to the possibility of automated premanufacturing, such substrates promise to be easier and faster to apply than MALDI matrices. This opens up the field for untrained personnel and high-throughput applications. Among such substrates, DIUTHAME foils (Hamamatsu Photonics, Hamamatsu, Japan) have shown great potential, consisting of a 5 µm thin alumina membrane with densely packed 200 nm wide holes. Due to its homogenous surface, the foil is potentially suitable for high spatial resolution MSI.

MSI measurements of thin sections from various biological samples were performed using an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen, Germany) coupled to an orbital trapping mass spectrometer (Q Exactive HF, Thermo Fisher Scientific, Bremen, Germany). MSI results from DIUTHAME and MALDI were compared. For that, different areas of consecutive mouse brain sections were spray-coated with α-cyano-4-hydroxycinnamic acid using a pneumatic sprayer (SMALDIPrep, TransMIT GmbH) or covered with DIUTHAME foil and measured at 5 µm lateral resolution.

DIUTHAME and MALDI both showed a well-resolved analyte distribution that correlated well with microscopic images. Under high-resolution conditions, DIUTHAME showed an increase in image sharpness, contrast and authenticity, compared to MALDI, due to the fact that DIUTHAME does not require solvents and is highly homogeneous.

Nevertheless, MALDI MSI experiments resulted in a significantly higher number of useful images, due to higher signal intensities compared to DIUTHAME. MALDI yielded about 20-fold higher ion intensities than DIUTHAME experiments at 5 µm ablation spots. Consequently, it was possible to detect and image more analytes with MALDI than with DIUTHAME, such as low-abundant markers of Purkinje cells in mouse brain sections.

DIUTHAME MSI experiments were performed on various tissue types including mouse brain, mouse kidney, insect larvae or rapeseed sections at different lateral resolutions (between 5-30 µm). Analyte classes detectable by DIUTHAME MSI included phospholipids, triglycerides, enzyme cofactors and small metabolites, discriminating different tissue-specific regions and making it a promising and easy-to-use supplement to MALDI MSI.

Session 7: ICP-MS: Nano- / Oral 2: Lemke

Time: Monday, 02/Mar/2020: 2:50pm - 3:10pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Jörg Bettmer

Sulphur isotope dilution ICP-MS for traceable protein quantification: application on an Alzheimer's biomarker

Nora Lemke[1,2], Ahmed H. El-Khatib[1], Franz Theuring[2], Norbert Jakubowski[3], Jochen Vogl[1]
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Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful method for the matrix-independent quantitative analysis of target elements. Developed for the use in inorganic trace analysis, ICP-MS is nowadays a valuable tool for bioanalytical questions. Especially the use of ICP-MS for quantitative proteomics by measuring heteroatoms has gained recognition in the last decade, considering that established quantification methods like organic mass spectrometry depend on labelling of the target protein or the existence of matched protein and peptide standards. The need for reliable quantification of proteins is continuously growing, but only a limited number of well-characterized and quantified protein standards are available so far. Accurately quantified, traceable protein standards are necessary to ensure comparability of measurements between laboratories, not only in basic research but also in a clinical context. One example of this is the Alzheimer's disease biomarker tau protein. However, existing tau standards lack comparability, emphasizing the need for a well-quantified protein standard.

Therefore, we developed a method for the quantification of pure proteins via sulfur isotope dilution ICP-MS (IDMS). As sulfur is present in two amino acids, cysteine and methionine, it exists in nearly all proteins and can be used for the quantification of proteins of known stoichiometry. We employed simple offline strategies for the separation of non-protein bound sulfur species. Quantification of these contaminations by IDMS allows for correction of the protein content and enables reliable protein quantification. We report the protein mass fractions of a standard reference material and commercially available proteins determined by sulfur IDMS, including the expanded uncertainties. The developed method can be applied for the reliable and traceable quantification of pure proteins for use as in-house standards. Here, we successfully used this method for the quantification of the tau protein.

Session 8: Instrumentation I; Oral 2: Papanastasiou

Time: Monday, 02/Mar/2020: 2:50pm - 3:10pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Christopher Rüger

Multiple Stage Tandem Mass Spectrometry of Protein Ions in the Omnitrap Platform Involving Variable Energy Electrons and Hydrogen Atoms.

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The versatile design and diverse ion activation network available in the omnitrap platform allows for complex multiple-stage tandem mass spectrometry experiments to be performed with high efficiency. A series of novel MS3 experiments for processing ions in the gas phase are presented here. The first experiment involves interactions between trapped protein ions and energetic electrons to form multiply charged hydrogen deficient radical species, which are subsequently subjected to slow heating collision induced dissociation. Different protonated charge states of ubiquitin ions extending from the lower charge states corresponding to their native-like structure to the highest charge state achieved under denaturing conditions are meta-ionized by fast electrons. The top-down spectra produced by subsequent CID of the meta-ionized species are analyzed and new fragmentation channels producing new series of radical fragment ions are reported for the different charge states examined. The second experiment involves attachment of hydrogen atoms to heme proteins to form hydrogen enriched multiply protonated ions. The enriched species are subjected to slow heating CID and isotopic shifts of specific fragment ions are monitored and reported. It is shown that the free heme ion can accommodate at least 12 hydrogen atoms with the estimated maximum uptake most likely determined by the number of double bonds present in the molecule. It is shown that the hydrogen atom uptake and corresponding isotopic distribution shifts observed for the free heme ions produced by CID of cytochrome c and of myoglobin exhibit significant differences, presumably due to the different oxidation state of iron. Superior signal-to-noise MS3 experiments are generated by operating the omnitrap in ion accumulation mode where multiple fragment populations are superimposed before ejection of ions to the mass analyzer. These novel experiments highlight the advanced capabilities of the omintrap platform afforded by its unique design.

Session 5: Proteomics - Clinical and Biological Applications; Oral 3: Bräcker

Time: Monday, 02/Mar/2020: 3:10pm - 3:30pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Hartmut Schlüter

Analysis of Wheat Proteins by LC-HRMS/MS to elucidate the Human Gastrointestinal Metabolism in an *in-vitro* Model

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Wheat is one of the eight major allergenic foods that can cause severe reactions and additionally adverse reactions such as celiac disease. But little is known so far about the fate of wheat proteins during gastrointestinal digestion. The aim of this project is to simulate the human gastrointestinal digestion of bread in an *in-vitro* model. This allows to observe time dependent degradation of highly relevant wheat proteins and formation of potential immunogenic degradation products.

Human gastrointestinal digestion was simulated with a standardized *in-vitro* digestion model (COST Infogest) [1]. While time dependent degradation of wheat proteins was analyzed by SDS-PAGE, identification of the highly complex set of degradation products was performed by LC-HRMS/MS on a Q-TOF instrument in a software assisted proteomics approach. Degradation rates of the formed peptides were estimated by label-free quantification.

Highly complex mixtures of low-molecular weight products were identified for several relevant wheat proteins in terms of immunogenic reactions, for example α-amylase inhibitors and gluten proteins. Gastrointestinal digestion of these proteins seems to follow a multi-step mechanism in repetitive patterns regarding time dependent formation and degradation kinetics of peptides with different length. Investigation of digestion kinetics by label-free quantification allows the conclusion that some proteins are degraded into larger peptides at an early digestion state, whereas others are degraded at later stages of the gastric phase. Regions in the amino acid sequence overlapping with previously identified epitopes could be determined, suggesting that these peptides can trigger immunogenic reactions after absorption by the intestinal mucosa. In total about 4000 peptides resulting from the digestion of wheat proteins by gastrointestinal proteases could be identified. In case of gluten proteins 25 peptides were found after 120 minutes of intestinal digestion covering known epitopes and thus having immunogenic potential.

Altogether, this approach allows to delineate the mode of action of the formation of already proven as well as potential immunogenic wheat protein fragments that contribute to the pathogenesis of different immunological disorders known to be caused by wheat.

[1] Brodkorb, André, et al. "INFOGEST static in vitro simulation of gastrointestinal food digestion." *Nature protocols* 14.4 (2019): 991-1014.

Session 6: Imaging I; Oral 3: Bookmeyer

Time: Monday, 02/Mar/2020: 3:10pm - 3:30pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Bernhard Spengler

SPICIng up your MALDI image: Soft and versatile post-ionization for enhanced ion yields of numerous analyte classes in MALDI-Single-Photon-Induced-Chemical Ionization-MSI

Christoph Bookmeyer[1], Jens Soltwisch[1,2], Ulrich Röhling[3], Klaus Dreisewerd[1,3]
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Matrix-assisted laser desorption/ionization MS imaging (MALDI-MSI) is increasingly used to visualize distributions of biomolecules in tissue, but is restricted by low ionization efficiencies to some analyte classes. In this study, we 'simplified' our recently introduced laser-based MALDI-2 post-ionization scheme by integrating pulsed VUV lamps into a fine-vacuum ion funnel. Crucial parameters like electric potentials in the ion source region, buffer gas pressure, energy of the desorption laser, type of dopant, VUV pulse energy and burst width, and the delay relative to the laser pulse were evaluated for an optimal Single-Photon-Induced Chemical Ionization (SPICI) process.

Kr-PID-lamps (PKR106-6-14, Heraeus; emission: 117/124 nm, operated at 13.56 MHz) were placed ~1 mm to the sample plane of a dual-ion funnel MALDI/ESI injector. Custom-made electronics enabled pulsed emission with adjustable burst width. The in-source N_2 buffer gas pressure was adjusted to ~8 mbar. Optionally, dopants (e.g., acetone, toluene) were introduced via a capillary to affect the ionization pathways. Release of intact biomolecules into the gas phase was achieved with a Q-switched Nd:YLF-laser (349 nm; 7 ns; focal spot diameter, ~18 µm). Samples were analyzed by direct LDI or by MALDI-Orbitrap-MS imaging of microtome sections at R=280,000 (@*m/z* 200).

A set of chemically or physiologically relevant test systems was selected to explore the performance characteristics and application ranges of our LDI/MALDI-SPICI method: (i) In MALDI-MS imaging of tissue sections, e.g., many classes of glycerophospho- and sphingolipids exhibit a strong boost in signal intensity by up to three orders of magnitude by SPICI in both ion modes, an effect similar to laser-based MALDI-2-MSI. However, a few differences between the two techniques are notable and will be discussed.

(ii) Lipid standard samples were tested to monitor the degree of fragmentation in MALDI-SPICI, which was shown to be low compared to standard APPI.

(iii) Using deuterated matrix, dopant or D_2O vapor gave rise to an understanding of the proton transfer mechanism.

(iv) High sensitivity gains were also found for highly nonpolar analytes (b-carotene, polycyclic aromatic hydrocarbons), or of (semi-)volatile organic compounds like free fatty acids.

Depending on chemical class, the generation of singly-charged ions as radical or protonated species could be adjusted by different dopants. This indicates a high degree of physiochemical reactions to take place under the micro-reactor conditions of our ion source.

In summary, SPICI is a powerful tool to post-ionize numerous analyte classes in an elevated-pressure environment and could constitute a low-cost alternative to laser-based MALDI-2-MSI.

Session 7: ICP-MS: Nano- / Oral 3: Retzmann

Time: Monday, 02/Mar/2020: 3:10pm - 3:30pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Jörg Bettmer

Pushing the limits – Low level Ca isotopic analysis using DS TIMS in biological tissues

Anika Retzmann[1], Dorothy Walls[2], Kerri A. Miller[2], Johanna Irrgeher[1,2], Thomas Prohaska[1,2], Michael Wieser[2]
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Introduction: Previous studies have shown that biological processes like biomineralization and (human) Ca homeostasis can result in a significant fractionation of Ca isotopes. The analysis of Ca isotopes in biological tissue, including human bone, blood and urine, has great potential as a diagnostic tool for monitoring changes in bone mineral balance and for bone diseases like osteoporosis. The extent of natural variations of stable Ca isotopes is relatively small, with ca. 4 ‰ – 5 ‰ in the $\delta(^{44}Ca/^{40}Ca)$ ratio, and therefore reliable measurement of calcium isotopic composition has remained very challenging. Precise techniques are a prerequisite to resolve the small variations among natural samples, especially considering the often limited amounts of material available for analysis and significant blank levels.

Ca isotope amount ratios in biological samples are typically analysed by either by thermal ionization mass spectrometry (TIMS) or multiple collector inductively coupled plasma mass spectrometry (MC ICP-MS). The number and large mass dispersion of (stable) Ca isotopes (40, 42, 43, 44, 46, 48), instrumental isotopic fractionation, isobaric interferences and blank contribution are the major analytical challenges – all increasing the uncertainty of the method.

Method: In this study, Ca purification was performed using the DGA resin, optimized for low procedural blanks and matrix separation from K, Mg, Ti and Fe. A ^{42}Ca–^{48}Ca double spike (DS) was applied to correct for isotopic fractionation occurring during Ca purification and the measurement. Ca isotope analysis was performed using a Triton TIMS (ThermoFisher Scientific, Bremen, Germany), operated in dynamic mode with two lines. Data reduction of the measured Ca isotope ratios was performed using in-house developed software solving the DS algorithm.

Results: The developed DS TIMS method enabled processing of total Ca amounts of 1000 ng, with total procedural blank of <10 ng. The reproducibility of NIST SRM 915a was found to be $\delta(^{44}Ca/^{40}Ca)_{NISTSRM915a}$ -0.01 ‰ ± 0.13 ‰ (2 *SD*, *n* = 25). The determined $\delta(^{44}Ca/^{40}Ca)_{NIST\ SRM915a}$ and $\delta(^{44}Ca/^{42}Ca)_{NIST\ SRM915a}$ values of the reference material NIST SRM 1400 (bone ash), NIST SRM 1486 (bone meal) and IAPSO (seawater) were in good agreement with literature data. Data on additional reference materials for biological tissues (blood, bovine liver, hair) will be presented.

Session 8: Instrumentation I; Oral 3: Gross

Time: Monday, 02/Mar/2020: 3:10pm - 3:30pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Christopher Rüger

Gas Chromatography-Field Ionization Coupling with Acquisition-Synchronized Emitter Flash Heating on an Orthogonal Acceleration Time-of-Flight Instrument

Mathias H. Linden[1], H. Bernhard Linden[1], Norbert Nieth[2], Jürgen H. Gross[2]
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Field ionization (FI) achieves soft ionization mainly yielding molecular ions while fragmentation is virtually absent. This renders FI superior to electron ionization (EI) when molecular ion identification is of highest relevance. In FI-MS, samples are supplied in gaseous form, and thus, reservoir inlets, gas chromatographs or the transfer capillary of liquid injection field desorption/ionization (LIFDI) sources can be used.

We describe GC-FI operation of a new combined FI/FD/LIFDI source, particularly designed for JEOL AccuTOF GC orthogonal-acceleration time-of-flight (oaTOF) mass spectrometers. Acquisition-synchronized emitter flash heating is implemented to reactivate the emitter periodically in order to preserve ionization efficiency of the emitters.

A FI/FD/LIFDI source designed for the JEOL AccuTOF GC series of oaTOF instruments (Jeol, Tokyo, Japan) has been employed. All FI/FD/LIFDI components were manufactured by Linden CMS (Weyhe, Germany). Emitter high voltage (–10 kV) and emitter heating current (0–120 mA) were controlled using the LIFDI-700 electronics and control software. Activated tungsten wire emitters, either 13 µm FD or 10 µm wire FI emitters, were used. The counter electrode was attached to the probe tip. The ion source was heated to 120–150 °C while the GC interface was set to 250 °C. The instrument was tuned using the molecular ion of toluene. External mass calibration was performed in LIFDI mode by measuring a mixture of polystyrenes.

Acquisition-synchronized emitter flash heating is of key importance for GC-FI coupling to maintain constant sensitivity for the duration of the analysis. The parameters to be optimized are inter-acquisition delay (IAD, 0.003–0.100 s), ratio of IAD to actual emitter heating time (10–90 %), and emitter heating current (0–120 mA). Once suitable settings are established, GC operation can be performed as in EI mode, which is demonstrated on fatty acid methyl esters (FAMEs, C_4–C_{24}).

Next, the position of the emitter wire with respect to exit of the GC column needs optimization. With a new flat counter electrode it becomes feasible to insert the probe by 2–2.5 mm deeper into the ion source block than with our previous round-rod electrode. Overall, we increased the intensity of GC-FI spectra by a factor of 5–6 as compared to the starting conditions.

Finally we explore the capability of the present setup for accurate mass measurements. Introduction of a mass calibrant during the entire GC-FI analysis is not feasible whereas one known analyte for lock mass calibration of all components presents an option.

Session 5: Proteomics - Clinical and Biological Applications; Oral 4: Sickmann

Time: Monday, 02/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Hartmut Schlüter

Excellent sensitivity through excellent recovery – ERLIC for absolute quantification of low abundant protein phosphorylation events in cancer patient tissue

Albert Sickmann, Stefan Loroch
ISAS, Germany

Quantification of low abundant phosphorylation events from minute amounts of sample is a prerequisite for establishing phosphoprotoemics workflows in the clinics circumventing costly generation of antibodies. However, workflows need excellent recovery to overcome problems with low phosphoprotein stoichiometry and the limitations of sample amount (e.g. biopsies). Here we demonstrate that Electrostatic repulsion-hydrophilic interaction chromatography (ERLIC) allows improved analyte recovery and grants access to nearly all phosphopeptides in a digest. Consequently, we employed ERLIC enrichment of low abundant protein phosphorylation events in minute amounts of tissue from colon cancer patients.

To assess quantitative losses during enrichment, we spiked a “heavy”-labeled phosphopeptide-enriched fraction into an unlabeled-digest followed by another round of enrichment. Phosphopeptide ratios reflected the recovery of each phosphopeptide.

In case of TiO2-affinity purification, quantification of 1,800 different phosphopeptides revealed an average recovery of 38 ± 2% with poor recovery rates (< 10%) for a large portion of short and basic phosphopeptides. In contrast, ERLIC demonstrated a 1.7-fold higher recovery with 65 ± 15%, as determined by quantification of 1,100 different phosphopeptides. Most notably, recovery was independent of physicochemical peptide properties rendering ERLIC an excellent method for efficient purification of nearly any phosphopeptide from a digest. Since antibody-based detection repeatedly failed to work, we applied an ERLIC-based targeted assay to quantify S125 phosphorylation levels of PHD2 (mediating Hif1α degradation) in healthy and cancerous tissue of 10 colon cancer patients. PRM analysis revealed fairly stable PHD2 expression levels (358 ± 154 amol/µg) with phosphorylation levels of 2.5 ± 0.9% in all healthy tissue samples. Surprisingly, we detected diminished S125 phosphorylation levels in cancerous tissue of all 10 patients down to 0.9 ± 0.4% ($p \leq 0.001$), even though total PHD2 expression remained unchanged. Subsequent, biochemical validation revealed a concomitant elevation of Hif1α expression levels rendering S125 an “on/off-switch” for PHD2 activity.

Session 6: Imaging I; Oral 4: Treu

Time: Monday, 02/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Bernhard Spengler

Integrating high resolution MALDI imaging into the development pipeline of anti-Tuberculosis drugs

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Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacteria. TB is the leading cause of death from a single infectious disease, before HIV/AIDS or Malaria. TB leads to the formation of centrally necrotic granuloma in the lung. To treat TB, anti-TB drugs must penetrate into the bacilli rich centres of TB granuloma in sufficient concentrations. The penetration behaviour is highly drug specific, which presents a major challenge in drug development. In order to support the development of future anti-TB drugs, we used high-resolution MALDI imaging and a dedicated data analysis tool to visualize the penetration of anti-TB drugs into granuloma in lung tissue of a novel IL-13^{tg} mouse model, which closely resembles human TB pathology.

Methods

MALDI imaging of drugs and lipids on γ-ray sterilised mouse lung tissue was performed using a QExactive™ HF mass spectrometer (Thermo Fisher Scientific), coupled to an AP-SMALDI10 ion source (TransMIT GmbH) with high mass accuracy (< 1 ppm) and a mass resolution of 240k @ *m/z* 200. Measurements were acquired with 10-30 µm step size and imzML files were used for data analysis. The developed semiautomatic penetration tool (MATLAB) consists of two parts: 1) Lipid based edge detection and 2) calculation of penetration plots.

Results

We developed a high-resolution MALDI imaging method and data analysis tool to visualise distribution and study the penetration of anti TB-drugs at therapeutic concentrations into granuloma of IL-13^{tg} mice. Our workflow was established for all current first line anti-TB drugs (Pyrazinamide, Rifampicin, Isoniazid, Ethambutol) as well as Moxifloxacin and Clofazimine. Characteristic lipid species detected in different tissue regions were used for image segmentation and edge detection. Based on the lipid pattern it was possible to visualise penetration of the anti-TB drugs Pyrazinamide, Clofazimine and Rifampicin into TB granuloma. PZA is detected inside the granuloma region with a near homogenous distribution across the tissue, while the detected intensities of Clofazimine and Rifampicin show a sharp decline towards the necrotic centre. This behaviour is in accordance with results from human studies and shows the suitability of the IL-13^{tg} model for pre-clinical drug trials. At 10 µm resolution, the highest reported spatial resolution for MS imaging of anti-TB drugs, it was possible to distinguish different granuloma regions and obtain information about the drug distribution into these regions. Within the development pipeline of novel anti-TB drugs, our workflow will accelerate the development of future anti-TB drugs.

Session 7: ICP-MS: Nano- / Oral 4: Von der Au

Time: Monday, 02/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Jörg Bettmer

HR-CS-GF-MAS as a new screening method for emerging pollutants - per- and polyfluorinated substances in the environment

Marcus von der Au[1], Lennart Gehrenkemper[1], Matthias Metzger[2], Björn Meermann[1]
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The introduction of fluorine in organic molecules leads to new chemical/physical properties. Especially in the field of pharmaceuticals, fluorinated organic molecules are becoming more and more popular and at present amount up to 25% of market share, with an upward trend. The main benefits of fluorinated pharmaceuticals are: (i) enhanced fat solubility; (ii) enhanced interaction of catalytic-center of enzymes with fluorine-drugs; (iii) a delayed metabolism within the human body. Highly fluorinated organic substances are also used in technical applications (e.g. coatings, fire-extinguishing agents).

Due to the broad variety of fluorinated substances and increasing production volumes numerous and up to date unknown fluorine-species are most likely to be present in the (aquatic) environment. Analytical methods to assess the degree of contamination of surface waters with organically bound fluorine are highly needed and up to now only combustion ion chromatography based method is available, which is relatively laborious.

Since a few years' high resolution-continuum source-graphite furnace atomic absorption spectrometers (HR-CS-GFAAS) are commercially available from Analytik Jena. By means of this technique, the detection of high resolution molecular absorption spectra (MAS) is enabled. Thus, fluoride is detectable upon the addition of a modifier and the formation of a diatomic molecule (e.g. GaF). Just recently, we applied this technique for total fluorine (mainly dissolved fluoride) analysis in river water samples.

In the present work a HR-CS-GFMAS method for extractable organically bound fluorine (EOF) analysis in surface water samples was developed by us. The method is based on SPE extraction of organically bound fluorine even in the presence of high fluoride concentrations followed by HR-CS-GFMAS analysis upon elution. Due to high enrichment factors, LODs in the low ng/L range were achieved. We successfully applied our SPE HR-CS-GFMAS method to Rhine water samples and EOF in the range of about 50-300 ng/L was detectable.

Session 8: Instrumentation I; Oral 4: Uteschil

Time: Monday, 02/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Christopher Rüger

Thermogravimetry atmospheric pressure photoionization mass spectrometry (TG-APPI-MS): Development and Applications

Florian Uteschil, Dominik Brecht, Prof. Oliver J. Schmitz
University Duisburg-Essen, Applied Analytical Chemistry, Germany

Plasticizers of the ortho phthalic pose an emerging problem due to the increased production and consumption of polymers. The restriction of hazardous substances regulates the use of plasticizers in plastics and electronic devices. In this context, an analytical platform that uses thermal analysis of the plastic and mass spectrometry of the evolved gas is proposed. This coupling is characterized by the reduced sample preparation and easy identification of degradation products with the help of the mass spectrometer. An instrumental coupling was developed to connect a thermogravimetry to a mass spectrometer that is equipped with a photoionization ion source.

A method to analyse plasticizers in solution by evolved gas analysis was developed. The reliable performance of the instrumental coupling will be shown on different phthalates as well as on the example of a certified reference material.[1]

Nowadays, the use of falsified drugs is a growing problem in the industrial countries. Here, the introduced coupling can help to identify falsified drugs or can be used in the product control of pharmaceuticals. It can also be shown which companies belong to each other by the analysis of commercial available tablets.[2, 3]

The analysis of purchased acetylsalicylic acid tablets of different manufacturers is shown with the described coupling. The sample preparation was very easy and fast because the tablets were homogenized in a mortar and directly analyzed. Besides the detection of the active substance in every sample, the unique formulation of a drug was found.[1]

The outlook for applications for TG-APPI-MS will be presented on additional pharmaceutical drugs as well as on the example of a complex herbal mixture.

Literature:

1. Brecht, D.; Uteschil, F.; Schmitz, O. J., Thermogravimetry coupled to an atmospheric pressure photo ionization quadrupole mass spectrometry for the product control of pharmaceutical formulations and the analysis of plasticizers in polymers. *Talanta* **2019,** *198*, 440-446.

2. Kovacs, S.; Hawes, S. E.; Maley, S. N.; Mosites, E.; Wong, L.; Stergachis, A., Technologies for detecting falsified and substandard drugs in low and middle-income countries. *PloS one* **2014,** *9* (3).

3. Johansson, M.; Fransson, D.; Rundlöf, T.; Huynh, N.-H.; Arvidsson, T., A general analytical platform and strategy in search for illegal drugs. *Journal of pharmaceutical and biomedical analysis* **2014,** *100*, 215-229.

Plenary Lecture 3: L. Konermann

Time: Monday, 02/Mar/2020: 4:20pm - 5:00pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Kathrin Breuker

Exploring the Journey of Proteins from Solution into the Gas Phase during ESI

Lars Konermann, Haidy Metwally, Quentin Duez, Insa Peters, Leanne M. Martin
The University of Western Ontario, Canada

Electrospray ionization (ESI) is an essential technique for transferring proteins from solution into the gas phase for mass spectrometry and ion mobility spectrometry. The mechanisms whereby $[M + zH]^{z+}$ protein ions are released from charged nanodroplets during ESI have been controversial for many years. This presentation will discuss recent computational and experimental studies that have shed light on many of the mysteries in this area. Four types of protein ESI experiments can be distinguished, each of which appears to be associated with a specific mechanism. (i) Native ESI proceeds according to the charged residue model (CRM) that entails droplet evaporation to dryness, generating compact protein ions in low charge states. (ii) Native ESI supercharging is also a CRM process, but the dried-out proteins accumulate additional charge because supercharging agents such as sulfolane interfere with the ejection of small ions (Na^+, NH_4^+, etc.) from the shrinking droplets. (iii) Denaturing ESI follows the chain ejection model (CEM), where protein ions are gradually expelled from the droplet surface. H^+ equilibration between the droplets and the protruding chains culminates in highly charged gaseous proteins, analogously to the collision-induced dissociation of multi-protein complexes. (iv) Denatured ESI supercharging also generates protein ions via the CEM. Supercharging agents stabilize protonated sites on the protein tail via charge-dipole interactions, causing the chain to acquire additional charge. There will likely be scenarios that fall outside of these four models, but it appears that the framework outlined here covers most of the experimentally relevant conditions.

References

“Enhancing Protein Electrospray Charge States by Multivalent Metal Ions: Mechanistic Insights from MD Simulations and MS Experiments" L. M. Martin and L. Konermann *JASMS 31* 25-33 (2020).

"Charging and Supercharging of Proteins for Mass Spectrometry: Recent Insights into the Mechanisms of Electrospray Ionization" L. Konermann, H. Metwally, Q. Duez, and I. Peters ***Analyst*** *144*, 6157 (2019).

"Mechanism of ESI Supercharging for Unfolded Proteins: Solvent-Mediated Stabilization of Protonated Sites During Chain Ejection" I. Peters, H. Metwally, and L. Konermann ***Anal. Chem.*** *91*, 6943 (2019).

"Chain Ejection Model for ESI of Unfolded Proteins: Evidence from Atomistic Simulations and Ion Mobility Spectrometry" H. Metwally, Q. Duez, and L. Konermann ***Anal. Chem.*** *90*, 10069 (2018).

"Crown Ether Effects on the Location of Charge Carriers in ESI Droplets: Implications for the Mechanism of Protein Charging and Supercharging" H. Metwally and L. Konermann ***Anal. Chem.*** *90*, 4126 (2018).

Poster Session 1 (even poster numbers)

Affinity Mass Spectrometry

AFF-02: Characterization of Primary structure and microheterogeneity of polypeptide of Paracelsin A and Trichotoxin A by MALDI-MS

Rana Boustani[1], Loredana Lupu[1], Thomas Degenklob[2], Hans Brückner[2], Michael Pryzbzlski[1]

[1]Steinbeis Center for Biopolymer Analysis and Biomedical Mass Spectrometry, Germany; [2]Interdisciplinary Research Center for Bio Systems,Land Use and Nutrition ,Department of Applied Entomology and Food Science

Peptaibols are a naturally occurring family of linear polypeptide antibiotic isolated from soil fungi [1]. Peptaibols vary in size from 15-20 amino acids, with the shortest peptaibols discovered in 1998 by ESI-MS and has only 5 amino acids (peptaibolin) [2]. These peptides have unique antibiotic properties, they are resulting of non-ribosomal biosynthesis and are composed of non-standard amino-acids. Structurally they are comprised mostly of α-aminoisobutyric acid (Aib), the C-terminus is hydroxylated to an amino alcohol and the N-terminus is acetylated.

Here we present the MALDI-MS analyses of Paracelsin A and Tricotoxin A, two peptaibol grope extracted from Trichoderma. Paracelsin consist of 20 amino-acid, a molecular mass of 1907 Da, whereas for Trichotoxin we identified two different peptaibols Trichotoxin A-40 and Trichotoxin A-50, with the sequence difference comprised of a single Glu / Gln .

MALDI was found to be useful for molecular weight and microheterogeneity analysis of Polypeptaibols. The Sequence determination are obtained by MALDI CID MS/MS. Similar results were obtained in previous studies using FAB and FD-MS [3].

[1] César de la Fuente-Núñez, L. Whitmore, B.A. Wallace, Handbook of Biologically Active Peptides , 2nd ed., Chap.22 – Peptaibols (Editor(s): Abba J. Kastin) , Academic Press, 2013, Pp 150-156

[2] Hülsmann H, Heinze S, Ritzau M, Schlegel B, Gräfe U. Isolation and structure of peptaibolin, a new peptaibol from Sepedonium strains. J Antibiot 1998;51:1055–8.

[3] M.Przybylski , H.Brückner ,Institut für Organische Chemie , Universität Mainz J.J Becher Weg 18-20,D-6500 Mainz, FRG

AFF-04: Novel analytical approach for food allergen detection: Aptamer-based affinity purification of peptide biomarkers before analysis via tandem mass spectrometry

Marion Fresch, Jens Brockmeyer

Institute of Biochemistry and Technical Biochemistry, Department of Food Chemistry, University of Stuttgart

Recent reports have shown that the number of food allergenic patients is slowly but constantly growing, emphasizing the fact that food allergy is a significant health problem. Sensitized consumers are often instructed to strictly avoid consumption of the allergenic food, being difficult when hidden allergens are present due to cross-contamination during food production. Sensitive and efficient analytical methods must therefore be developed to ensure clear and reliable food labeling.

Mass-spectrometric methods rely on the detection of peptide biomarkers resulting from a tryptic digest of the allergenic proteins. For several years, those techniques have been more and more enhanced, but allergen detection in processed foodstuffs remains a big issue. In this work, a new approach based on affinity purification prior MS analysis was implemented in order to separate peptide biomarkers from matrix components and therefore increase the sensitivity.

Aptamers are synthetic single-stranded DNA oligonucleotides with proper secondary structures, can be easily modified for immobilizing onto a surface and bind with high affinity to specific target molecules. All those facts make them ideal as affinity ligands.

The goal of this exhibition is to present an overview on the establishment of such a method, which essentially include the identification of allergen peptide biomarkers and the selection of peptide-specific aptamers.

AFF-06: Epitope identification and affinity quantification of protein- ligand interactions using online SPR-biosensor- ESIMS

Loredana-Mirela Lupu[1], Pascal Wiegand[1], Delia Mihoc[1], Oliver Müller[1,2], Friedemann Völklein[2], Frederik Barka[3], Günes Barka[3], Michael Przybylski[1]

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Bioaffinity analysis using biosensors has become an established technique for detection and quantification of biomolecular interactions. However, a principal limitation of biosensors is their lack of providing chemical structure information of affinity-bound ligands. Proteolytic excision/extraction (Protex-MS), hydrogen-deuterium exchange (HDX-MS) of peptide backbone hydrogens, and Fast- Photochemical Oxidation (FPOP) are major techniques for mass spectrometry based elucidation of protein-ligand interactions, but none of these tools alone provide quantitative affinity data. Using a surface plasmon resonance (SPR) biosensor, we have developed a continuous online biosensor-MS combination with electrospray ionization mass spectrometry that enables the simultaneous affinity isolation, structure identification and affinity quantification of biopolymer ligands from a protein- ligand complex immobilized on a gold chip. Key tool of the online biosensor-MS epitope analyzer is a new integrated, automated interface that provides sample concentration and in-situ desalting for the direct MS analysis of the ligand eluate [1]. ESI-MS systems from all major manufacturers can be coupled, using a newly developed software.

The broad application potential of the online-SPR-MS epitope analyzer will be shown by recent studies of an unusual mixed-disulfide antibody epitope of the rheumatoic target protein, HLA-B27; and the interaction site identification of chaperone

complexes of lysosomal enzymes [2, 3] as well as for the interaction of protein-antibody complexes of horse heart myoglobin and anti-monoclonal antibody against myoglobin. Interaction epitopes as diverse as antigen-antibody and lectin- carbohydrate complexes [4], and affinity binding constants (K_D) from milli- to nanomolar ranges can be directly analysed. Applications amenable with the online-SPR-MS epitope analyzer include affinity-based biomarker identification, identification of protein and peptide epitopes, precise antibody affinity determinations, and direct label-free antigen quantification.

[1] Lupu L, et al. (2019) ChemMedChem. 10.1002.

[2] Iurascu, M. et al. (2016) J. Am. Soc. Mass Spectrom., 27, 1105-1112.

[3] Moise A., et al. (2016) J. Am. Soc. Mass Spectrom., 27,1071-1078.

[4] Moise, A., et al., (2011) J. Am. Chem. Soc. *133*, 14844-14847.

Environmental and Forensic Mass Spectrometry

ENV-02: Development of an ETV/ICP-MS method as a powerful and complementary tool for single organism metal analysis in ecotoxicological tests

Marcus von der Au[1], Hannah Karbach[2], Björn Meermann[1]

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To assess metal-pollution within environmental samples ecotoxicological tests are performed. E.g. for the assessment of sediment toxicology due to heavy metal pollution, *Corophium volutator* is a common test organism.

However, according to norm ISO 16712 [1] only mortality and re-digging abilities are recorded - no further (instrumental) analytical investigations were performed to, e.g. evaluate “real” organisms´ metal uptake. But, to interconnect common ecotoxicological benchmarks (e.g. EC50) with “real” metal-concentrations instrumental methods are required.

One conventional approach relies on acid-digestion of the organisms via microwave systems followed by the analysis via ICP-based methods. One benefit of this approach is averaging a huge number of organisms accounting for natural variability. However, this approach also comprises several disadvantages: (i) sample preparation is relatively time consuming as well as prone to sample contamination; (ii) especially with regard to small organisms large numbers are needed; (iii) due to pooling of organisms single organism information is getting lost.

To meet these challenges, we developed a method to analyze single-organisms via electrothermal vaporization coupled on-line to ICP-MS (ETV-ICP-MS) [2, 3]. This approach turned out being suitable for highly sensitive ultra-trace metal analysis in single organisms. We established the method via incubation of a test organism *Corophium volutator* with relevant aquatic environmental metal contaminants, such as Cu, Zn, Pd, Cd. For validation purposes an appropriate CRM material was used. Valid single-organism quantification on basis of liquid standards was achieved. Absolute limits of detections for the elements investigated are in the pg-range.

This approach enables the investigation of natural variability on single organism basis as well as bridges material ó environmental sciences with ecotoxicological test systems.

[1] ISO 16712, Amphipodentest. [2] Vanhaecke F., Resano M., De Loos-Vollebregt M., *J. Anal. At. Spectrom.* **2008**, 23, 1450-1475. [3] Aramendia M., Resano M., Vanhaecke F., *Anal. Chim. Acta* **2009**, 648, 23-44.

ENV-04: Microplastic Analyzed by Secondary Ion Mass Spectrometry

Karsten Lamann[1,2], Daniel Breitenstein[1], Elke Tallarek[1], Uwe Karst[2], Birgit Hagenhoff[1]

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Microplastic is defined as plastic with a length below 5 mm. Nano- or microplastic particles are used in a number of application forms, including cosmetic and pharmaceutic formulations. Furthermore, microplastic particles result from the degradation of polymeric materials.

Today, microplastic is mainly discussed as a pollutant of the environment, e.g. of sea water. Microplastic is expected to be hazardous due to two mechanisms: on the one hand the uptake of microplastic may cause severe damage to the uptaking organisms. On the other hand, it is expected that the adsorbtion of ubiquitous substances onto microplastic may cause an enrichment of these substances on the surface of microplastic particles. This may lead to an increased uptake of these substances into organisms.

Goal of this study was to analyze microplastic particles by Time-of-Flight Secondary Ion Mass Spectrometry.

ENV-06: online uSPE forensics

Michaela Schmidt[2], Arnd Ingendoh[1], Marina Schumacher[1], Birgit Schneider[1], Laura M. Huppertz[3], Jürgen Kempf[3]

[1]Bruker Daltonik GmbH, Germany; [2]Faculty Medical and Life Sciences, Furtwangen University, Schwenningen, Germany; [3]Institute of Forensic Medicine, Forensic Toxicology, Medical Center - University of Freiburg, Germany

Systematic toxicological analysis (STA) is a major part of everyday work in forensic toxicology and immunological screening offers great advantages in automation of sample preparation and reporting of results. During the last decade, LC-MS has become a key technique in STA, but in contrast to immunoassays an appropriate sample preparation is crucial for screening of body fluids. The aim of this project was to implement an online µSPE to an existing LC-MS method to achieve a fully automated LC-MS screening of urine samples Three different types of cartridges, UCT C18 endcapped cartridges 10 mg (C18-10), UCT C18 endcapped cartridges 30 mg (C18-30) and UCT DAU cartridges 10 mg (DAU) (ITSP Solutions, Inc, Hartwell, GA, US) were compared according to their S/N ratios at low, med, and high concentration in pooled urine. Reproducibility of the complete extraction process was tested by tenfold preparation of pooled urine and Recovery (RE) and matrix effects (ME) were evaluated using a protocol adapted from Matuszewski et al. Adjusting the cleaning steps after the different extraction steps led to no detectable carry-over caused by the µSPE system. Using DAU cartridges for sample preparation led to higher S/N ratios for six analytes (nordiazepam, fentanyl, buprenorphine, morphine and morphine-glucuronide). The C18-30 cartridge showed low absolute peak areas and was excluded from further evaluation studies. The RSD for the complete extraction process (10 fold extraction) ranged from 5.6 to 10.9% (C18-10) and 10.0 to 14.9% (DAU) at low concentrations.

ENV-08: Characterization and Quantification of organic and inorganic compounds from Chinese and Iranian Aerosol Filter Samples by MS Imaging and ICP-MS

Christof Barth, Klaus-Peter Hinz, Bernhard Spengler

Justus Liebig University, Germany

A huge variety of organic and inorganic substances are ubiquitously present in urban aerosols, of which polyaromatic hydrocarbons (PAH) and heavy metals are of special interest. Both substance classes are responsible for adverse health effects after strong aerosol exposure, typically known from strongly polluted megacities. Detection and quantification of such compounds is hardly possible if only a very small amount of particulate matter (PM) is available, and preparation of the filter samples is often complex and time consuming. Here we present a combined method based on direct introduction of aerosol particles into an inductively coupled plasma mass spectrometer (ICP-MS) and atmospheric-pressure high-resolution scanning laser desorption ionization mass spectrometry (AP-LDI-MS). In order to compare data from two strongly polluted megacities, particles were sampled in Tehran (Iran) and Hangzhou (China) in February 2018 on quartz filters. Direct introduction of the resuspended filter-deposited particles via an optical particle counter into the ICP-MS allowed for a quantification of 46 elements without any need for sample preparation. High-resolution MS imaging of the filter surfaces with a pixel size of 50 µm and a mass resolution of 240000 in positive- and negative-ion mode were employed to assign more than 2300 sum formulas and allowed for a statistical evaluation of co-localized substances within particles on the filter samples. This enabled a quasi particle-based classification of different particle types on the filters. Additionally a quantification of various PAH species was carried out via standard addition method. Especially the samples from Tehran showed elevated amounts of PAHs. In summary, a much higher number of sulphurous inorganic and organic compounds in particles was detected in Tehran samples compared to Hangzhou samples, possibly resulting from increasing efforts of the Chinese government to reduce sulphur emissions in the last years. Furthermore, NO_2 emissions increased due to enhanced traffic, represented by an increasing amount of nitrate on the Chinese filters. Sample pre-treatment was reduced to a minimum with this method, and only small numbers of particles were necessary to create a comprehensive picture for a given filter sample. Results allow for a better assessment of air pollution in China and Iran.

Acknowledgement

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ENV-10: Mass spectrometric investigation of the interactions of Gd^{3+}-ions with biomolecules

Karolin Sommer, Uwe Karst

University Münster, Germany

Gadolinium based contrast agents (GBCA) are widely applied during magnetic resonance imaging and therefore contributed to improving medical diagnostics over the past years. These metallopharmaceuticals contain a Gd^{3+}-ion complexed with various organic ligands, generating stable complexes, which is necessary due to the high toxicity of the free Gd^{3+}-ions. After application, these contrast agents are rapidly excreted mainly as intact complexes via the kidneys and were therefore considered a safe class of drugs for a long time. Nevertheless, recent research has shown that Gd can accumulate in many parts of the body like brain, skin or bones. Since the toxicity of chelated and dechelated Gd may be different, species information on the deposition in the body is of high interest.

Due to the broad application of GBCAs, large amounts are released into the environment via the waste water, leading to enriched Gd-concentrations in river and surface water. Similar to the depositions in the body, dechelation of the complexes and interaction of Gd-ions with different biomolecules in the environment is possible.

In the body and the environment, a large number of possible binding partners for Gd is present. Therefore, model incubation studies of free Gd^{3+}-ions with different biomolecules were carried out. The interactions were investigated using ESI-MS and LC-ESI-MS to rule out the possibility of adduct formation in the gas phase of the ESI source. Furthermore, the hyphenation of LC and the element specific ICP-MS allowed the detection of adducts difficult to ionize via ESI.

ENV-12: Using Free, High-Performance, Computer Modeling Software to Simulate Gas Chromatographic Separations

Jaap de Zeeuw[1], Chris Rattray[1], Chris Nelson[1], Scott Adams[1], Kristi Sellers[1], Christian Weyer[2]

[1]Restek Corporation, Bellefonte, PA, USA; [2]Restek GmbH, Bad Homburg, Germany

Our recently introduced modeling software is a selectivity tool that relies on a pre-loaded library of thermodynamic retention indices. This makes it possible to predict retention times and optimize chromatographic methods without the need to analyze compound sets under many different conditions. The program allows the user to select the stationary phase and simultaneously adjust: film thickness, temperature, column length, column internal diameter and flow. Users can enter each compound or cut/paste large lists of compounds into the program. Since its introduction there have been thousands of searches across a broad range of compound classes. The program outputs: compound retention time, resolution and peak width along with the column conditions and dimensions. A model chromatogram is provided to illustrate retention, peak width and resolution. Users have the option to view compound mass spectral data with the added benefit of overlaying mass spectra for coeluting analytes. Specific searches can be saved and accessed at a later date. Examples of these features will be presented with a focus on challenging separations. The program allows user to simulate GC separations using a laptop. It's the most easy and cost-effective way to optimize separations in the shortest time. It only needs a registration. You can access it at: https://www.restek.com/proezgc

ENV-14: A Novel, and Versatile Hybrid HILIC and Ion Exchange Column for the Separation of a Wide Range of Polar Compounds

Connor Flannery[1], Xiaoning Lu[1], Vernon C. Bartlett[1], Ahren Green[1], Terry S. Reid[1], Christian Weyer[2]

[1]Restek Corporation, Bellefonte, PA, USA; [2]Restek GmbH, Bad Homburg, DE

Introduction:
Hydrophilic interaction chromatography (HILIC) paired with mass spectrometry (MS) has increasingly become a powerful analytical tool because of its ability to retain and separate a wide variety of challenging polar analytes. Where reversed-phase chromatography struggles, HILIC offers new solutions for many challenging assays. HILIC separations, operated under high organic conditions, are compatible with MS detection and facilitate increased ionization efficiency; resulting in improved signal response when compared to more aqueous conditions.
We have developed a versatile hybrid HILIC/ion exchange column and have demonstrated its ability to not only retain and separate polar anions including glyphosate and its metabolites, organic acids, and water-soluble vitamins, but also neutral and positive analytes such as amino acids, carbohydrates and peptides. Methods:
Several challenging applications were chosen to exhibit the unique selectivity and characterize the versatility of this new stationary phase across different classes of polar analytes including polar anions (glyphosate, and water-soluble vitamins), as well as, neutral and cationic analytes (amino acids, carbohydrates and peptides). Preliminary Data:
There are a variety of HILIC phase chemistries that currently exist on the market including bare silica, polyols, aminos, zwitterionic, and mixed-mode phases, however, difficulties with method development and method robustness have brought challenges to the adoption of HILIC. In addition, the sufficient retention of highly polar anions such as glyphosate and its metabolites is still difficult to achieve with the current HILIC phases on the market. Several difficult applications were chosen to highlight the ease of use, robustness, and selectivity advantages of this novel phase. Through these applications, the various retention mechanisms are demonstrated while maintaining MS compatibility through the use of MS friendly mobile phases. Retention, selectivity, and symmetrical peak shapes are achieved for the polar anionic herbicide glyphosate and its metabolites. Water soluble vitamins including Thiamine and its metabolite TPP are also well retained on this column. An Amino Acid application exhibits selectivity for difficult to resolve isobars leucine and isoleucine, while also showing balanced retention and gaussian peak shape for neutral, acidic, and basic amino acids. Novel Aspect:
This novel hybrid stationary phase provides highly effective solutions across a wide array of challenging assays.

ENV-16: Analysis of Ultrashort-Chain and Alternative PFAS: LC-MS/MS Method Development and Application to Water Samples

Shun-Hsin Liang[1], Justin Steimling[1], Mike Chang[1], Paul Connolly[1], Christian Weyer[2]

[1]Restek Corporation, Bellefonte, PA, USA; [2]Restek GmbH, Bad Homburg, DE

LC-MS/MS methods for the analysis of legacy short-chain (C4, C5) and long-chain (>C5) per- and polyfluoroalkyl substances (PFAS) have been well-developed based on reversed-phase (RP) chromatography. With proper modification, these typical RP methods can be applied to the analysis of emerging PFAS alternatives such as GenX and ADONA, which are perfluoroalkyl ether carboxylic acids used as PFOA substitutes. F-53B is a China-produced PFOS alternative containing two polyfluoroalkyl ether sulfonate components, 9Cl-PF3ONS and 11Cl-PF3OUdS, which are included as analytes in the updated EPA 537.1 method. Current LC methods, however, may not be suitable for the analysis of newly trending ultrashort-chain (C2, C3) PFAS mainly due to their insufficient retention on typical RP columns. While the use of short-chain PFAS (PFBA and PFBS) is intentional, more and more studies have shown the ubiquitous occurrence of C2 and C3 PFAS in aqueous environmental samples. These include trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), perfluoroethane sulfonate (PFEtS), and perfluoropropane sulfonate (PFPrS). It was shown that PFPrA is the predominant PFAS (up to 45% of total detectable PFAS) in the rain and snow samples collected from USA, France, and Japan. To date, there are not many studies showing the contamination sources and levels for these ultrashort-chain PFAS. A recent study showed the detection of PFEtS and PFPrS in aqueous film-forming foams (AFFFs) and ground waters from 11 military sites in the US, indicating AFFF firefighting foam may be one of the sources of the ultrashort-chain PFAS. This presentation will discuss the LC-MS/MS method development for simultaneous quantification of C3, C4, C8, and alternative PFAS in a variety water samples.

ENV-18: Development and optimization of a membrane - inlet - photoionization mass spectrometer for fast analysis of (polycyclic)aromatic compounds in aquatic systems

Christian Gehm[1], Thorsten Streibel[1,2], Sven Ehlert[1,3], Detlef Schulz-Bull[4], Ralf Zimmermann[1,3]

[1]University of Rostock, Germany; [2]Helmholtz Zentrum München – German Research Center for Environmental Health GmbH, Germany; [3]Photonion GmbH, Germany; [4]Leibniz Institute for Baltic Sea Research Warnemünde, Germany

The development of sensitive analytical techniques for the real – time detection of aromatic (AH) and polycyclic aromatic hydrocarbons (PAH) is of high importance due to their impact on human health and the environment. However, low concentrations of these compounds in marine systems complicate their detection and monitoring. Common analytical strategies involve discrete sampling, followed by a wide variety of sample preparation techniques. Especially, the time consuming sample preparation steps are necessary to increase the concentrations up to a detectable level and to eliminate the water matrix. Hence, there is a need of new analytical systems for the direct detection of compounds in water. Herein, membrane introduction mass spectrometry (MIMS) in combination with resonance enhanced multiphoton ionization (REMPI) shows a high potential for fast and sensitive real time analysis of (P)AHs in aquatic systems. In MIMS, compounds are transported selectively into the vacuum region of the mass spectrometer through a semipermeable membrane, extracting the analytes from the water phase directly into the gas phase. With the aid of REMPI, sensitive determination of (polycyclic)aromatic species is possible due to the spectroscopic selectivity in ionization process.

In our study, we present a homebuilt REMPI – MIMS system equipped with external membrane inlets for sheet or hollow fiber membranes. With these early – stage systems, measurements of low concentrations of selected (P)AHs in different simulated (tap water, artificial sea water) and real world water samples are carried out without any sample preparation.

For the sheet membrane assembly, the membrane is heated indirectly by tempering the inflowing water. With increasing water temperature improvement of response times and signal heights can be achieved. For volatile compounds response times from 30 seconds up to 2.5 min were measured, while semi-volatile substances show increasing response times up to 15 min. Limits of detection of several tens of ng/L for selected (P)AHs were determined.

For the hollow fiber membrane setup, trap-and-release technique can be utilized for direct determination of the selected (P)AHs in water samples down to ng/L-range in less than 5 minutes. Here, the pervaporating compounds are trapped inside of the membrane tube for one minute and are released as a sharp peak into the MS by a fast heating of the membrane.

The presented REMPI-MIMS systems show promising results with respect to the sensitive real time determination of selected (polycyclic)aromatic compounds in artificial laboratory samples, environmental samples as well as for process monitoring.

ENV-20: Phospholipid Removal from Protein Precipitated Plasma UsingIn-Line Sample Preparation (ILSP-PR)

Sharon Lupo[1], Randy Romesberg[1], Xiaoning Lu[1], Frances Carroll[1], Christian Weyer[2]

[1]Restek Corporation, Bellefonte, PA, USA; [2]Restek GmbH, Bad Homburg, DE

Phospholipids are major constituents of plasma membranes and have been shown to cause severe ion suppression or enhancement in the analysis of biological samples by LC-ESI-MS. Additionally, phospholipids tend to accumulate on reversed-phase columns, causing a decrease in column performance and reduced life time.

In this study we have developed an innovative inline sample preparation (ILSP) technique to remove phospholipids from protein precipitated plasma samples.

Protein precipitation of human plasma was performed by adding acetonitrile containing 1% formic acid to blank or fortified plasma in a (3:1) ratio. Samples were vortexed for 30sec at 3000rpm, and centrifuged for 10min at 4000rpm/10°C. All data was collected on a Shimadzu Nexera UHPLC equipped with an additional binary pump and 6-port switching valve. Analysis was conducted using a 5x2.1 mm ILSP cartridge followed by a Raptor Biphenyl 2.7 µm, 100 x 2.1 mm analytical column. Phospholipids and target analytes were monitored in SIM and scan modes using a Shimadzu 2020 MS with ESI+ ionization.

Accurately timed valve switching is critical for a successful inline phospholipid removal method to facilitate washing of phospholipids from the ILSP cartridge while maintaining target analytes. Timing is dependent on the degree of phospholipid retention on the ILSP cartridge in conjunction with the hydrophilic or hydrophobic properties of the analytes. Once the timing of the valve switch is determined, an analytical column is added and the gradient is optimized for speed. For initial experiments, the Raptor Biphenyl 2.7 µm, 100 x 2.1 mm column was chosen. The time program was optimized to allow for simultaneous flushing of phospholipids from the ILSP cartridge, while gradient analysis proceeds on the analytical column. The final analysis with phospholipid removal was completed in <7 minutes. Utilizing this method, lifetime of the ILSP cartridge was demonstrated by successfully performing 500 consecutive injections of protein precipitated fortified human plasma with consistent analyte response (≤2.2% RSD) and retention time (≤0.24% RSD).

Phospholipid removal by ILSP results in very clean sample extracts and improved signal to noise. A comparison study was performed where plasma samples were fortified with ketoprofen, sulfadiazine, amphetamine, methadone, nortriptyline, and prednisolone. Samples were extracted using 36 wells of a representative 96-well plate offline phospholipid removal product and compared to 36 ILSP injections following protein precipitation. Average analyte signal to noise ratios increased by 15–189% on average for the ILSP technique, with the exception of amphetamine, which showed a marginal decreased of 23%.

ENV-22: Mass spectrometric detection of doping agents in simulated exhaled breath aerosol using sampling devices equipped with an electret membrane

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Currently, only blood and urine samples are routinely used for sports drug testing, but the interest in alternative matrices that can potentially be advantageous with regard to the invasiveness, intrusiveness, and duration of the sampling procedure, the risk for sample manipulation, the analyte stability, and the effort and costs for sample transportation and storage continuously increased over the last years. Recent studies demonstrated that exhaled breath (EB) represents a promising complementary matrix for doping control purposes.

In this study, a multi-analyte liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to analyze model compounds representing various classes of substances prohibited in sports by the World Anti-Doping Agency (WADA) in EB samples and different analytical characteristics were determined. EB collection devices equipped with an electret-based filter unit supplied by SensAbues® were used to generate test specimens. A system was established, which can be used to simulate EB aerosol containing doping agents, as the artificial EB samples used for earlier method developments and validations had to be prepared by spiking the analytes directly onto the filter of the sampling device. SensAbues® sampling devices were characterized in terms of the retention efficacy by measuring the amounts of substance breakthrough (via the exit of the collection device), and the analyte stability was addressed by analyzing multiple preparations of drug-containing artificial EB samples stored for 0-28 days at different temperatures (-20 °C, 4 °C, and RT).

All model compounds were successfully detected via LC-MS/MS, however, polyethylene glycol originating from the electret membrane was detectable in the extracts obtained from the cartridges, affecting the mass spectrometric results. No detectable amounts passed through the device at the conditions used for ventilation. When the membrane and cartridge housing of artificial EB samples were extracted separately, the ratios "$Extraction_{Membrane}/Extraction_{Cartridge}$" varied from 57-98% depending on the analyte, indicating that the analytes adsorb to both the electrostatic membrane and the container housing. Independent from the storage temperature, all model substances were detectable after a storage period of 28 days.

EB is a promising alternative test matrix for doping controls, however, further authentic post-administrations are required to provide evidence that relevant drugs or diagnostic metabolites are excreted/exhaled into the collected matrix.

ENV-24: Fully automated dried blood spot sample preparation enables the detection of lower molecular mass peptide and non-peptide doping agents

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The added value of dried blood spot (DBS) samples complementing the information obtained from commonly routine doping control matrices is continuously increasing in sports drug testing. In this project, a robotic-assisted non-destructive hematocrit measurement from dried blood spots by near-infrared spectroscopy followed by a fully automated sample preparation including strong cation exchange solid phase extraction and evaporation enabled the detection of 46 lower molecular mass (< 2 kDa) peptide and non-peptide drugs and drug candidates by means of LC-HRMS. The target analytes included, amongst others, agonists of the gonadotropin-releasing hormone receptor, the ghrelin receptor, the human growth hormone receptor, and the antidiuretic hormone receptor. Furthermore, several glycine derivatives of growth hormone releasing peptides (GHRPs), arguably designed to undermine current anti-doping testing approaches, were implemented to the presented detection method. The initial testing assay was validated according to the World Anti-Doping Agency guidelines with estimated LODs between 0.5 and 20 ng/mL. As a proof-of-concept, authentic post-administration specimens containing GHRP-2 and GHRP-6 were successfully analyzed. Furthermore, DBS obtained from a sampling device operating with microneedles for blood collection from the upper arm were analyzed and the matrix was cross-validated for selected parameters. The introduction of the hematocrit measurement method can be of great value for doping analysis as it allows for quantitative DBS applications by managing the well-recognized 'hematocrit effect'.

ENV-26: Structural elucidation of compounds in supercomplex mixtures using a two-dimensional chromatographic approach with high-resolution mass spectrometry

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In modern society the usage of crude oil serves multiple purposes and forms the basis for many products. However, the global energy consumption has nearly exhausted conventional oil resources. This led to the exploitation of heavier accessible feedstocks which cause problems during their processing. In order to guarantee the continuous quality of products, deeper knowledge about this new type of feedstock is required. Structural information of constituents suit well to characterize the certain raw materials and adapt industrial processes to them. Thus, the structural elucidation of individual compounds in heavy crude oil fractions was focused during this study. However, the complexity of crude oil makes a simplification of the sample imperative.

Chromatography was performed using an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany). Offline analysis of samples was performed on a research-type Orbitrap Elite mass spectrometer (Thermo Fisher, Bremen, Germany) with a resolving power of R = 480000 at m/z 400 (FWHM) using the spectral stitching method (30 Da windows, 5 Da overlap). Online measurements of the SEC fractions using argentation chromatography were performed on a 7T LTQ FT Ultra FT-ICR mass spectrometer (Thermo Fisher, Bremen, Germany) at a resolving power of R = 200000 at m/z 400 (FWHM). Mass spectra were recorded in positive mode within a scan range of m/z 200-1000 using APPI as ionization technique.

Structural elucidation using mass spectrometric techniques generally involves collision induced dissociation of isolated precursor ions. The major problem for structural elucidation of individual compounds in complex samples is the high amount of precursor ions per mass unit. Therefore, a two-dimensional chromatographic approach was developed to simplify the

asphaltene fraction from a heavy crude oil prior to analysis. As first dimension, size-exclusion chromatography was successfully utilized in an offline application for the separation of the asphaltenes fraction. In the second dimension, a ligand-exchange chromatography (argentation chromatography) was implemented as online application. As it turned out, the silver-(I)-mercaptopropyl silica based stationary phase is capable of separating NOS-compounds according to their heteroatom content besides separation by the strength of the π-system. The online HRMS analysis enables the possibility to perform fragmentation experiments (MS^2). The 2D chromatographic approach provides rather clean isolation windows. The fragmentation studies successfully revealed structural features of individual compounds present in the asphaltenic fraction.

The technical setup combines a two-dimensional chromatographic approach with HRMS to investigate individual compound structures in heavy crude oil by utilizing tandem mass spectrometry (MS/MS).

ENV-28: Passive Sampling Analysis of PAH Contaminated Soils using Gas Chromatography – Atmospheric Pressure Laser Ionization – Mass Spectrometry (GC-APLI-MS)

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Soils in urban and industrial areas often contain increased concentrations of petrogenic and/or pyrogenic polycyclic aromatic hydrocarbons (PAH) resulting from anthropogenic substrate admixtures such as coals, ashes, soots or tailings [1]. Based on these carbonaceous geosorbents, potential risks of toxic PAH in soils mostly depend on their bioavailability and freely dissolved concentrations ($c_{PAHfree}$). In bituminous coals or soots, $c_{PAHfree}$ and bioavailability are low despite increased native PAH contents. As it is difficult to differentiate between particle-bound and freely dissolved PAH, passive sampling was developed to estimate $c_{PAHfree}$ [2, 3].

In this study, $c_{PAHfree}$ from nine petrogenic and pyrogenic samples have been investigated using a new approach including silicone-coated amber glass vials for equilibrium passive sampling combined with analysis by gas chromatography – atmospheric pressure laser ionization – mass spectrometry (GC-APLI-MS). Studied samples include three tailings from bituminous coal mining, one bituminous coal (petrogenic), three urban soils (primarily pyrogenic) and two floodplain soils (mixed PAH sources).

APLI is highly selective for aromatic structures since ionization is performed in a two-step absorption process of photons (1+1 REMPI) emitted by an excimer laser (KrF, λ = 248 nm) at a resonance energy of 5 eV that is below the ionization potential of most other molecules. Moreover, APLI is sensitive (LODs between 5 and 50 fg on column) since the ionization process is highly efficient and in addition nearly no fragmentation of target ions occurs. [4] Hence, GC-APLI-MS is an optimal analytical method if PAH extract concentrations are low and/or sample amount is limited, which is the case in the performed passive sampling experiments.

Results show that equilibrium $c_{PAHfree}$ from tailings (total concentrations of 91 - 157 mg/kg ∑55 PAH) were considerably lower at 0.13 µg/L ∑55 PAH (mean) compared to those from samples assigned to predominantly pyrogenic sources (total concentrations of 23 - 120 mg/kg ∑55 PAH) which were at 0.79 µg/L ∑55 PAH (mean). In the mixed soil samples, a low content of coal particles is concluded to be responsible for observed reduced $c_{PAHfree}$ compared to the pyrogenic samples. $c_{PAHfree}$ of all tailing samples were in the same range, whereas those from the urban soils differed widely.

[1] Hindersmann & Achten, Envpol., 2018, 242: 1217–1225.

[2] Jahnke et al., Environ. Sci. Technol., 2012, 46: 10114–10122.

[3] Smedes et al., Environ. Sci. Technol., 2013, 47: 510–517.

[4] Große Brinkhaus et al., Anal Bioanal Chem., 2017, 409(11): 2801–2812.

ICP-MS User´s Meeting: Analysis of Nanomaterial

ICP-02: Investigating the in vivo gold nanoparticle translocation – Quantitative bioimaging and single particle analysis by means of LA-ICP-MS

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In the past decades, nanoparticles have become an important part of our everyday lives, for example, in form of food additives or in sportswear. Simultaneously, medical applications employing nanoparticles have been under development. Hand in hand with the fast-growing range of applications, possible health and environmental hazards of nanoparticles have attracted the attention of researchers worldwide. A major research topic is the biodistribution of nanoparticles in living organisms after exposition. Their in vivo behaviour not only depends on their size but also on their chemical composition, shape, surface modification and chemical stability, leaving a tremendous amount of variables that need to be taken into account for possible applications and for risk assessment.

The localization of nanoparticles in specific functional areas of organs can give a first impression of the distribution pathway of the particles. This can be investigated by using spatially resolved bioimaging techniques. Laser ablation hyphenated with inductively coupled plasma-mass spectrometry (LA-ICP-MS) is an established method for the investigation of elemental distributions in solid samples. In comparison to microscopic techniques, LA-ICP-MS allows to map larger sample areas in a shorter amount of time while also delivering multi-elemental information.

A fairly new technique for nanoparticle analysis is the use of the single particle approach for LA-ICP-MS. By using short dwell times (e.g., 1 ms or shorter) a signal caused by a single nanoparticle ionized in the plasma can be detected. This results in a direct correlation between the signal intensity and particle size, thus allowing to determine particle size distributions in solid samples.

In this study, several extrapulmonary organ and blood samples of rats treated with gold nanoparticles via intratracheal instillation were investigated for their gold content by means of LA-ICP-MS. For spleen and kidney samples the localization of gold signals in specific functional areas of the organs could be shown. Furthermore, a method for particle size distribution analysis by LA-spICP-MS was developed and successfully employed to determine the species of translocated gold.

ICP-04: SINGLE CELL ANALYSIS WITH ICP-TQ-MS DETECTION TO CHARACTERIZE SELENIZED YEAST

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Selenium is an essential trace element for humans that is present in proteins and enzymes, which play critical roles in very relevant processes such as the antioxidant defence. Additionally, at high doses it has been used in cancer prevention treatments. Thus, adequate dietary intake of Se is essential and can be complemented using selenium supplements. One of the most commonly used supplements is based on selenized yeast (Se-yeast) that is produced by growing different strains of *Saccharomyces cerevisiae* on Se-containing media and can accumulate up to 3000 µg g^{-1} of Se [1]. In this regard, the optimization of Se incorporation during Se-yeast production and the characterization of the amount of Se and its chemical forms present in the final products require the development of suitable analytical methods.

In this work, we propose the development and optimization of an analytical methodology based on single cell inductively coupled plasma mass spectrometry (SC-ICP-MS). The instrument was fitted with a microflow nebulizer and a total consumption spray chamber to permit individual cell introduction with high transport efficiency. Additionally, the use of triple-quadrupole detection, makes possible the monitoring of constitutive elements like phosphorus useful as cell markers. Analytical figures of merit are presented, and the methodology is applied to the analysis of this food supplements in order to determine important parameters like the percentage of selenium-containing cells and the Se amount per cell. Complementary techniques such as HPLC-ICP-MS and TEM were used for the identification of the biogenically formed selenium-containing nanoparticles present in the yeast samples after an appropriate cell lysis.

[1] G.N. Schrauzer, Pure Appl. Chem, 78 (2006) 105-109.

[2] R. Álvarez-Fernández García, M. Corte-Rodríguez, M. Macke, K.L. LeBlanc, Z. Mester, M. Montes-Bayón, J. Bettmer, Analyst, (2020) DOI: 10.1039/C9AN01565E.

ICP-06: Investigation of Transition Metal Species in Lithium Ion Batteries by Means of CE/ICP-MS

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In state-of-the-art lithium ion batteries (LIB), several transition metals (TM) like iron, manganese, cobalt and nickel are commonly used as lithium TM oxides for cathode materials. Furthermore, copper is applied as current collector for the negative anode. The dissolution of these TMs into the electrolyte and their incorporation into the protective layer on the negative electrode are strongly linked to capacity fading and reduced cell-life, due to accelerated electrolyte decomposition and loss of active lithium.[1,2] However, the dissolution mechanisms and the impact of the TMs on the cell performance are not fully understood and controversial discussed. For a better understanding of the dissolution mechanisms in LIBs, investigations regarding the oxidation states of dissolved TM are essential, but only few references can be found in literature. Previous

investigations on the oxidation states of dissolved manganese in electrolytes and on anodes were inconclusive, resulting either in dissolved Mn^{2+} or Mn^{3+}.[3-5] And up to now, no investigation on the oxidation states of dissolved iron, cobalt and copper were performed for LIBs, why further investigations are necessary.

To investigate the dissolved TM species in electrolytes more detailed, it is important to stabilize and separate these species for quantification. CE is the ideal technique for their investigation, due to the wide range of running buffers and complexing agents, which allow optimal stabilization of the TM species during separation. Moreover, the hyphenation of the CE and the ICP-MS is a powerful tool for quantification of these TM species, due to the species independent detection. In this work, different CE/ICP-MS methods were developed to separate and quantify these dissolved TMs in LIB electrolytes regarding their oxidation states, using various complexing agents for stabilization.

[1] S. Komaba *et al.*, *Electrochim. Acta*, **2002**, *47*, 1229-1239.

[2] C. Zhan *et al.*, *Nat. Commun.* **2013**, *4*, 2437.

[3] Y. Shilina *et al.*, *Anal. Chem*. **2016**, *88*, 4440-4447.

[4] Y. Terada *et al.*, *J. Power Sources* **2001**, *97-98*, 420-422.

[5] Z. Li *et al.*, *J. Power Sources* **2014**, *272*, 1134-1141.

ICP-08: Bioimaging of heavy metal hyperaccumulating plants by means of LA-ICP-MS

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In recent years, bioimaging of animal or human tissues has become an important analytical procedure to answer biological and medicinal problems, and well-established protocols have been generated. For plants, however, only few such protocols are available.
Laser ablation-inductive coupled plasma-mass spectrometry (LA-ICP-MS) is a powerful method to uncover the lateral distribution of elements within a sample. Using a laser, the sample is ablated and turned into a fine aerosol. Via gas flow, the sample is introduced into the ICP-MS system where it is ionized and detected. By screening the sample and enabling time-dependent acquisition for each line, an image is generated. It contains information about the lateral localization and absolute intensity of all elements of interest.
In this work, freshly harvested leaves were glued onto a sample plate and analyzed via LA-ICP-MS. Due to the heterogeneous thickness and topology of the samples, the instrumental parametrization proved to be much more complex compared to evenly cut thin slices that are usually analyzed via LA-ICP-MS. Increasing the energy of the laser led to blockage of the transfer lines, while energies set too low did not ablate properly. Additionally, since the leaves were not dried, complete adherence to the sample plate was of utmost importance. Any motion or detachment of the sample would disturb the lateral accuracy and lead to loss of local information.

Using the biopolymer hydroxyethyl cellulose (HEC), the leaves could be glued onto the sample plate as flat as possible. By focusing the laser beam on topologically lower regions of the sample, superjacent regions could still be ablated to a satisfactory level. Thus, images generated in this study were able to show the distribution of naturally abundant as well as artificially added elements in the samples up to spot sizes of 50 µm.

Comparison of genetically modified leaves with the wild type revealed vast differences in metal distribution due to influenced metal homeostasis. It can be shown that these leaves can be identified and distinguished via LA-ICP-MS analysis.

ICP-10: Coupling of an ICP-QQQ-MS and a Laser Ablation for Elemental Mapping of Environmental Samples

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We coupled our Triple-Quadrupole-Mass spectrometer 8900 from Agilent with the nanosecond laser ablation unit LSX-213 G2+ from Teledyne Cetac for the mapping of different elements. This coupling has the typical advances of a spatial resolution of the concentrations over the sample areas. In this study natural inorganic stomatolites as well as organic components like carrots plants and shitake mushrooms were investigated. The last two were contaminated with additional radionuclide solutions for the investigation of the uptake.

Stromatolites as biogenic rocks can be used as monitor for marine changes in a long time history. We analysed the deposition of nearly 20 main and rare earth elements in the thin layer structure of this rocks. We observe a correlation between some cooperative deposited elements the different layers. Mass ratios were semiquantified by the NIST 610 glass standard and in the area of ppm for the rare earth elements.

The carrot plants and shitake mushrooms were selected due to their importance in the human diet. Carrots were spiked with strong beta decaying long-living Tc-99 and mushrooms with dominant gamma emitting Cs-137/Ag-108m. Both matrices have truly incooperated the anthropogenic radionuclide in their compartments due to the signals inside of the cross section.

We successfully coupled both system and measured various kinds of matrices. In the future, we want to extend this possibility to use the build-in reaction cell of the mass spectrometer to improve the system for more difficult to measure elements/radionuclides.

ICP-12: Quantitative determination of potential biomarkers for Alzheimer's disease by ID-ICP-MS

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According to estimates over 6 million people are affected by neurodegenerative diseases in the European Union which pose a big challenge to the health care systems. With about 70 % the most common form of dementia is Alzheimer's disease (AD). In an ageing population the diagnosis and treatment of these diseases are gaining more and more importance. Determination of the biomarkers for AD (most established are β-amyloid peptide and τ-protein) is generally performed using immunoassays or optical methods which often lead to incomparable results. Besides the established biomarkers, metalloproteins are under discussion as potential clinical markers. For example, ferritin (FER), ceruloplasmin (CER) or superoxide dismutase (SOD) levels in cerebrospinal fluid (CSF) or serum alter in patients affected by AD compared to healthy individuals [1,2]. A promising approach for the quantitative determination of these proteins is species-specific isotope dilution inductively coupled plasma mass spectrometry (SS-ID-ICP-MS), as it is a primary measurement method and gives results that are traceable to the International System of Units (SI). Various methods for certain metalloproteins have been developed and are presented.

To perform SS-ID-ICP-MS, a well-characterized protein reference with natural isotopic abundance and an adequate spike material containing the metal in an isotopically enriched form are needed. As there are very few protein reference materials commercially available, pure native proteins have been characterized in-house for the use as reference. Isotopically enriched protein spike materials have been produced recombinantly or in-house by demetallation and remetallation procedures some of which are presented here.

Acknowledgement: This project has received funding from the EMPIR programme co-financed by the Participating-States and from the European Union's Horizon 2020 research and innovation programme.

[1]: Squitti R., *Front. Biosci.*, **2012**, *17*, 451

[2]: Ayton S. et al., *Nat. Commun.*, **2014**, *6*, 6760

ICP-14: Determination of heteroatom-containing, non-evaporable impurities in OLED and polymeric substances

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Devices such as flat-screen TVs, tablet PCs and smartphones are now a natural part of our everyday life. As the world market and technology leader in liquid crystals, Merck accelerates future innovation to advance display technologies. Especially, organic light emitting diodes (OLEDs) enable new kinds of application beyond traditional displays and lighting. OLED displays are extremely thin and lightweight and deliver a perfect image from every viewing angle with outstanding color brilliance and high contrast. As OLEDs can be applied on a lot of different substrates, it will be possible to turn virtually every surface into a display or light source in future.

In order to ensure the flawless functionality of the costumers' high-performance products, it is essential to guarantee an exceedingly high quality of the materials used for production. Merck puts a lot of effort into fulfilling the customers' requirements by permanent analytical monitoring of intermediates and final products. Additionally, if the product performance does not match the expectations, fingerprinting analytics are in high demand. Gas chromatography (GC) coupled to inductively coupled plasma-mass spectrometry (ICP-MS) is a promising and very sensitive analytical technique which provides information regarding the elemental composition of the sample including the quantification of heteroatoms, such as P, S, Cl, Br and I. To determine the impurities in OLED and other polymeric materials – which often have too high molecular masses for GC separation – a pyrolysis sample preparation step is applied for thermal fragmentation of the sample at temperatures of up to 1000 °C. In this study, the suitability and reproducibility of this method for the determination and quantification of heteroatom-containing impurities in different materials was tested.

ICP-16: TQ ICP-MS with brineFAST:A Strong Tool to Detect Ultratrace Elemental Impurities in 25% Brine for Chlor AlkaliMembrane Electrolysis

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The Chlor-Alkali (CA) industry produces chlorine, sodium/potassium hydroxide and hydrogen by the electrolysis of brine. Since chlorine and sodium hydroxide find usage in a broad variety of processes and applications, this energy intensive process displays the basis for approximately 55% of the chemical industry in the EU-27 and EFTA countries[1]. The state-of-the-art membrane cell technology represents more than 60% of the total European capacity[1]. Although the membrane technique has been labelled as the best available technology for CA production, its main drawback is the intensive energy demand. Therefore, technology developments in the CA process are mainly aimed to increase the operational current densities in membranes, involving higher fluxes through membranes and higher brine quality requirements to maintain the membranes' lifetime.

The control and optimization of the brine purification system has therefore a significant impact on the performance, environmental profile and economy of the process. Undesirable components are - amongst others - calcium, magnesium, aluminium and iron, which can precipitate inside the membrane. Therefore, the development and setup of a suitable and sensitive analytical system essential for the CA membrane electrolysis.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is commonly used and established in the CA plant laboratories, but is limited by the achievable limits of detection. Other trace elemental techniques like Single Quadrupole Inductively Coupled Plasma Mass Spectroscopy (SQ ICP-MS) system proved to fail as it could not overcome molecular interferences. Newly introduced Triple Quadrupole ICP-MS (TQ ICP-MS) systems reopened this field of investigations, as this technique is able to overcome the system inherent limitations of SQ ICP-MS. Anyhow, matrix effects and total dissolved solids (TDS) are still problems to overcome.

Whereas High Resolution (HR) ICP-MS is suitable with its detection power, this work describes a new approach by combining an ESI brineFAST system and a Thermo Fisher Scientific iCAP TQ ICP-MS. By using this powerful configuration, matrix elements like Na and K can be efficiently removed and 25% brine can be measured directly, leading to a significant increase of respective limits of quantification. Results of 25% brine analyses comparing ICP-OES, TQ ICP-MS with brineFAST as well as HR ICP-MS are presented, discussing the advantages and disadvantages of the systems with respect to their ease of use, affordability and limits of detection.

[1] Isabel Garcia-Herrero et al., Life Cycle Assessment model for the chlor-alkali process: A comprehensive review of resources and available technologies; Sustainable Production and Consumption 12 (2017); 44-58

Instrumentation - Ion Sources and Analyzers

IIS-02: Aging of lithium ion battery electrolyte – Accessing potentially toxic organophosphorus compounds using GC-MS and GC-ICP-SF-MS

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Since established in many both mobile and stationary applications, lithium ion batteries (LIBs) have to meet challenging demands. On the one hand, high power, high capacity and long lifetime have to be accomplished, while on the other hand, safety properties cannot be neglected. Therefore, comprehensive knowledge of degradation mechanisms of battery components during cell operation is important. Today's state-of-the-art LIBs use mixtures of cyclic and linear carbonates as solvents with a combination of lithium hexafluorophosphate ($LiPF_6$) as conductive salt. Since this salt is highly hygroscopic, trace-amounts of water are always present in the battery system. Furthermore, the dissolved salt is in constant equilibrium with LiF and PF_5. The latter can undergo decomposition reactions with water and organic carbonates forming organophosphorus compounds and hydrofluoric acid in a diverse decomposition cascade.

These organophosphates show structural similarity to chemical warfare agents and therefore have a supposedly high toxicity due to a similar metabolism in the human body. Even though only present in relatively low concentrations in laboratory scale cells, the quantity in mobile and stationary applications becomes significant when larger amounts of electrolyte are implemented.[1] In previous investigations, a large variety of organophosphates could be qualified in different electrolyte systems (*e.g.*, electric vehicles).[2] A major drawback is the lack of commercially available standards for those compounds which makes quantification using molecular investigations difficult. In this study, an inductively coupled plasma-sector field-mass spectrometer (ICP-SF-MS) is used to quantify organophosphorus compounds of the LIB system.[3] Speciation of non-acidic compounds was performed *via* gas chromatography (GC). Challenges regarding difficult matrices and mass resolution dependency in plasma-based phosphorus detection are discussed. Identification of the $LiPF_6$ decomposition products was performed using GC-MS in order to obtain molecular information. Using spectral database comparison with battery-specific decomposition products and their respective fragmentation pattern, identification of compounds was accomplished.

1. Henschel, J.; Horsthemke, F.; Stenzel, Y.P.; Evertz, M.; Girod, S.; Lürenbaum, C.; Kösters, K.; Wiemers-Meyer, S.; Winter, M.; Nowak, S. Lithium ion battery electrolyte degradation of field-tested electric vehicle battery cells–A comprehensive analytical study. *Journal of Power Sources* **2020**, *447*, 227370.

2. Stenzel, Y.P.; Horsthemke, F.; Winter, M.; Nowak, S. Chromatographic Techniques in the Research Area of Lithium Ion Batteries: Current State-of-the-Art. *Separations* **2019**, *6*, 26.

3. Stenzel, Y.P.; Winter, M.; Nowak, S. Evaluation of different plasma conditions and resolutions for understanding elemental organophosphorus analysis via GC-ICP-SF-MS. *Journal of Analytical Atomic Spectrometry* **2018**, *33*, 1041-1048.

IIS-04: MALDI with laser-induced post-ionisation (MALDI-2): Effect of the post-ionisation laser pulse width on the lipid ion yields

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MALDI with laser-induced post-ionisation (PI), also named MALDI-2, can crucially boost the ion yields for numerous classes of lipids, metabolites, and oligosaccharides in MS imaging applications. The current working thesis for describing the PI-MALDI-2 effect is the resonant two-photon ionisation of matrix molecules within a confined particle plume, which is followed by ample, presumably collision-mediated charge transfer reactions. Previous studies showed that both the yields of radical matrix ions and those of protonated/deprotonated analyte molecules are strongly effected by a set of experimental parameters, especially the N_2 buffer gas pressure in the ion source, delay between the ablation and PI laser, and their pulse energies.

Here we probed the MALDI-2 ionisation mechanisms by conducting experiments with two PI lasers of 28 ps (frequency-quadrupled Nd:YAG laser) and 6 ns (OPO tuned to 266 nm) pulse widths, respectively. Life times of electronically excited states in MALDI matrices have been reported to fall into the low ns-range, hence the elevated photon flux of the ps-laser could be expected to increase the ion yields substantially, if a two-photon process was the predominant primary ionisation channel.

A MALDI-2 Synapt G2-S QTOF (Waters) equipped with a N_2 laser (337 nm) for desorption/ablation of material was employed as mass spectrometer. Samples were prepared by spraying a polar lipid extract onto a clean glass slide and subsequent coating with a homogeneous layer of 2,5-Dihydroxybenzoic acid (DHB) matrix.

For each of the PI lasers multiple mass spectra were recorded on the sample while increasing the PIL pulse energy incrementally from 0 µJ to 250 µJ for the nanosecond laser and from 0 µJ to 150 µJ for the picosecond system. The remaining

parameters were held constant: delay between AL and PIL 10 µs, pressure in the ion source 2 mbar, distance between sample surface and PIL 500 µm, beam diameter of the PIL at the intersection with the MALDI Plume 100 µm. For data analysis, ion signal intensities of characteristic lipids are plotted as a function of employed laser pulse energy for the respective laser systems.

Results show that laser pulse energies can be reduced by a factor of 3 when using picosecond pulses as compared to nanosecond long irradiation in order to produce similar post-ionisation efficiencies. Based on the assumed ionisation process, this indicates that the transition state of DHB resonantly excited in the MALDI-2 process has a lifetime shorter than the pulse duration of the nanosecond laser.

IIS-06: Characterization of quadrupole mass filters regarding elevated entrance ion currents

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Quadrupole mass filters are commonly used as high-or band pass filters. In most cases the former “RF only” mode is used to transport ions to a second mass selective device (e.g. a TOF) with the option to suppress abundant (low-mass) ions and thus to increase the overall dynamic range of the tandem system. Our studies suggest though that strongly elevated ion currents directed through an RF only quadrupole used as high-pass filter significantly impact on the filtering characteristics of such a device.

A commercially available RGA system QMG 422 (Inficon, Bad Ragaz, Switzerland) consisting of a closed EI source, quadrupole filter (20 cm rod length), off-axis SEM as well as Faraday cup is used for all studies.

Earlier results from our lab motivated the present study: An order of magnitude baseline shift has been observed in an RGA system in filter mode at ion currents reaching the µA range. Several hypotheses have been constructed to explain this behavior. These hypotheses include i) generation of photons as a result of electron acceleration and subsequently emitted Bremsstrahlung, which could reach the SEM and lead to an increase in the output signal; ii) high ion currents favoring space charge effects in a way that ions shield each other from the RF and traverse the quadrupole unfiltered; iii) upon filtering ions may hit the quadrupole rods and electrons are emitted by ion impact. These electrons are then accelerated towards the rods having positive half period RF voltage and a fraction of them could ionize neutral gas molecules. This mechanism can occur throughout the length of the quadrupole while ions generated at the end of the rod system can pass without being filtered out. Experiments conducted on an EI-CTOF system (Tofwerk, Thun, Switzerland) equipped with a high-pass filter quadrupole demonstrated that ions are quantitatively filtered out at low ion currents according to the stability diagram. However these ions are detected in the orthogonally accelerating TOF instrument as ion currents increase by orders of magnitude. Trajectory simulations regarding the cut-off voltage are in good accord with data obtained from experiments for lower ion currents but show a markedly different behavior at higher ion currents. It appears as if ions “break-through” the quadrupole mass filter despite their respective cut-off behavior in the simulations. Experiments towards this effect were conducted with nitrogen, helium and hydrogen as carrier gas, and dodecane as well as noble gases as analyte gas.

IIS-08: LILBID-MS enables detection of UV-light induced MHC-I protein complex formation

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Laser induced liquid bead ion desorption (LILBID) is a native and soft ionisation technique and a versatile tool to analyze large proteins and non-covalently bound protein complexes as well as their assembly itself. In this ionisation technique ions are released from aqueous microdroplets (30 µm) by irradiation with an IR-laser operating at the absorption wavelength of water. Additional UV-laser-light (360 nm) irraditaion of the sample-containing microdroplets within the ion-source right before releasing the ions by IR-irradiation allows, for the first time, observation of fast light induced reactions with LILBID-MS. For example, uncaging based reactions can be performed right before MS-analysis as well as photocleaving of peptides and light induced protein-complex formation.

For instance, we are able to show for the first time the successful formation of an UV-light induced complex involving the human major histocompatibility complex class I (MHC-I). The MHC-I heterodimer, consisting of a heavy chain and b2m, can be refolded in the presence of a small peptide containing a photocleavable amino acid. This peptide in turn blocks the binding-pocket at the MHC-I at which a specific chaperone can dock. With our method we can demonstrate a general photocleaving of the peptide alone as well as the release of the photocleavable peptide from the binding-pocket due to UV-irradiation, which results in the formation of a new complex. Besides this, we furthermore are able to show differences in the efficiency of releasing the photocleavable peptide from the binding pocket of different MHC-I allomorphs.

IIS-10: Visualization of the MALDI-2 plume development via ultrafast shadowgraphy technique

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To further advance MALDI-2, a powerful technique in which a pulsed UV-LASER interacts with the MALDI particle plume and initiates numerous secondary ionization processes, a deep understanding of the expansion dynamics is crucial. Both plume composition and expansion dynamics will generally depend on several relevant “input” parameters, in particular buffer gas pressure, focal spot size of the MALDI Laser, LASER fluences, and type of MALDI matrix. Here we developed a prototype vacuum chamber for a shadowgraphy technique to visualize these dynamics at the example of the solid state matrix 2,5-dihydroxybenzoic acid. By precise alignment of nanosecond-illumination and CCD-Camera, we achieved a spatio-temporal resolution in the low nanosecond and nanometer ranges, respectively, across an about 1 mm^2-wide square image plane.

Following subtraction of the background via a differential data acquisition protocol (N_2 MALDI-LASER on/off), this high precision critically supported revealing fine features of the plume development under elevated MALDI-2 pressure (a few mbar of N_2) and at ambient pressure conditions. After showing fluence- and pressure dependences of plumes with larger spot diameters of ~150 µm and fluences up to 700 J/m² we achieved reducing adjustable spot diameters down to ~15 µm. This results in the possibility to prove the spot size and fluence dependent coexistence of two ablation/desorption mechanisms shown by Niehaus *et al.* [1]. after first investigations we are able to distinguish the dynamics between the desorption/ablation of macroscopic clusters and the microscopic particle plume.

[1](SCIENTIFIC REPORTS | (2018) 8:7755 | DOI:10.1038/s41598-018-25946-z)

IIS-12: Increasing the Ease of Use and Robustness of Nanoflow with Plug and Play Low Flow Source

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When performing electrospray ionization mass spectrometry, reducing chromatographic flow rates can improve sampling efficiency and therefore increase sensitivity. When operating in the nanoflow regime (50-500 nL/min) very high sampling efficiencies are possible. However, nanoflow ionization can require significant user expertise and advanced tuning to get best performance. Significant research was done to determine whether most source parameters could be optimized and locked in, to take out most of the user interactions. This led to the development of a plug and play low flow source, that would cover the full spectrum of low flow rates for high sensitivity LC-MS analysis with high usability. Here the source was tested in a variety of proteomics applications.

Using Design of Experiments, x,y,z positioning, tip protrusion, ionization voltage, nebulization gas and other parameters were examined. Spray electrodes were developed for maximal robustness. This led to the development of the OptiFlow Source for nanoflow and microflow applications. The source performance was evaluated relative to a highly tuned NanoSpray® Source III on both the TripleTOF 6600+ system and the QTRAP 6500+ system. Equivalent chromatographic performance was observed comparing peak width and area. 30 day testing was performed to check spray electrode robustness and minimal change in backpressure was observed. The SWATH® Acquisition Performance kit was run with both sources and very equivalent performance was again observed, within 5% in IDA and SWATH acquisition modes.

Next, a complete nanoflow study of over 500 injections of complex samples was run with a single column and electrode. The sample set was an immunopeptidomics study which requires high sensitivity due to the difficulty to obtain HLA bound peptides.

Very good raw peak area reproducibility was observed across the dataset when monitoring the signals from the standard peptides spiked into each sample, the average CV of 21.5%. The signal intensity stayed relatively constant over the runs with only a small decrease in signal across the study. LC system pressure remained constant across the study indicating no column or tip occlusion occurred and visualization of tip before and after study showed minimal change.

This data highlights the reproducibility of the electrode design with the OptiFlow Source, providing long-lasting spray tip lifetime and good reproducibility enabling much larger studies to be run using nanoflow chromatography.

Instrumentation - New Developments

IND-02: Aroma profiling of brewing hops by a prototype HS-GC-MS/IMS setup and machine learning strategies

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Due to its high sensitivity, its selectivity and its rugged design, gas chromatography ion mobility spectrometry (GC-IMS) gains increasing attention for the analysis of volatile organic compounds (VOCs) in fields of breath analysis, food quality and process control [1]. Depending on mass, charge and structure-related collision cross section (CCS) ions are separated along the drift tube at ambient pressure under the influence of an electrical field. In this study, an IMS cell was coupled to a headspace GC mass spectrometry (HS-GC-MS) system as orthogonal detection method for the analysis of the complex aroma profiles of different hop cultivars. These consist of a variety of different VOCs, such as aldehydes, ketones, terpenes and terpenoids [2]. The resulting two-dimensional IMS and MS aroma fingerprints of the investigated hop species were classified according to similarities in their VOC profile. The aim of this approach was to group typically used hop cultivars from all over the world with similar aroma profiles in order to facilitate substitution with regard to the partially limited availability. In addition, specific substance classes could better be distinguished by IMS in comparison to MS by the characteristic drift times of their ions, even when coelution occurred. Interestingly, some terpene and terpenoid ions featured similar drift behavior, indicating a complex gas phase ion chemistry occurring in the ionization process. With the help of the temperature-ramped, high-resolving HS-GC-MS/IMS setup used in this study, together with modern signal processing and data analysis strategies, the characteristic drift time ion patterns of hop aroma profiles obtained are extensively described for the first time. Furthermore, clustering according to their aroma profiles was accomplished using specifically developed machine learning tools. This indicates that HS-GC-MS/IMS or even HS-GC-IMS on its own could serve as easy to handle and cost-effective profiling technique for aroma analysis.

References

[1] R.Cumeras, E. Figueras, C.E. Davis, J.I. Baumbach and I. Gràcia, Review on Ion Mobility Spectrometry. Part 1: current instrumentation, Analyst, 2015,140,1376-1390.

[2] N. Rettberg, M. Biendl and L.-A. Garbe, Hop Aroma and Hoppy Beer Flavor: Chemical Backgrounds and Analytical Tools - A Review, Journal of the American Society of Brewing Chemists, 2018, 76, 1-20.

IND-04: Orbitrap-SIMS: New Analytical Options Demonstrated on Highlighter Inks

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A new Hybrid-SIMS instrument combines an Orbitrap™-based Q Exactive™ HF mass analyzer (Thermo Fisher Scientific) with a high-end ToF-SIMS system (IONTOF GmbH). Especially the use of an orbitrap™-analyzer within a SIMS experiment is a new technological option. The obtainable high mass resolving power and mass accuracy is a key advantage when using this type of analyzer in a SIMS experiment. Based on these features an unambiguous identification of the chemical composition of each detected secondary ion species is often possible. Furthermore, due to the high mass resolution, this analyzer can resolve mass interferences which are unresolvable for standard TOF analyzers. As a consequence, the resulting spectra contain even more peaks than normal TOF-SIMS spectra.

The large number of signals is a challenge in the data evaluation process. In this respect, Multivariate Analysis is a valuable tool to either simplify the data evaluation or to generate an initial hypothesis for the fast identification of unknown components.

In the current study six different highlighter inks were analyzed in SIMS experiments using either the Time-of-Flight or the orbitrap based analyzer system for separation and detection. Although the composition of the inks was unknown before the experiment, it was possible to identify all the dyes and a number of others components of the inks. Furthermore, the results indicate that the color system of the six inks is based on mixtures of only a limited number of different dyes.

The findings – derived from surface spectrometric measurements on a 400x400 μm^2 field of analysis – were in line with results obtained by two different chromatographic techniques. However, for chromatography not the surface of an applied ink but extracts from the highlighter refills were analyzed.

All-in-all identifying unknown sample components becomes feasible and more simple with the unique capabilities of the novel Hybrid SIMS instrument. However, elaborated statistical tools and experienced data evaluation strategies are still very important to deal with the large amount of data generated.

IND-06: A software platform for the quality control of synthetic oligonucleotides

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The routine quality control (QC) of synthetic oligonucleotides currently faces challenges by the increasing demand for oligos in research or diagnostic and therapeutic applications. MS can address these needs, though with limitations: MALDI-MS can easily provide for the analysis of several 1000 samples per day, but its analysis success rate drops beyond the size of 30mers. ESI-MS can provide good quality data beyond the size of 100mers, but its throughput is limited to a few hundred samples per day. We have developed and applied a software platform for routine oligo QC that can be applied to both ESI and MALDI. Oligonucleotides were obtained by solid phase synthesis. MALDI samples were prepared in 3-HPA/DAHC matrix using robotics on a 384 AnchorChip target and analyzed on a autoflex MALDI (Bruker). ESI samples were on-line desalted using a C18 precolumn and DIEA as ion pairing agent. They were then eluted using acetonitrile to a microTOF-Q III QTOF (Bruker). Data were analyzed in BioPharma Compass 3.1 (BPC, Bruker), which provides a multi-attribute traffic light overview of each analysis which evaluates mass accuracy and sample purity. A common software platform was developed and validated for the QC of synthetic oligonucleotides, both for MALDITOF as well as ESI-QTOF analysis. Together, high throughput and success rate of QC analysis were achieved, which is not conceivable with one ionization technique alone. The traffic light reporting icons allow to speed up the validation of large sample numbers. CFR 21 part 11 compliant features available.

IND-08: Gangliosides associated to human brain development and aging: a high resolution mass spectrometry study

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Gangliosides (GGs), a particular sialylated class of glycolipids, are present and concentrated on cell surfaces, with the two lipid chains of the ceramide moiety embedded in the plasma membrane and the oligosaccharide chain located on the extracellular surface as point of recognition for extracellular molecules or neighboring cell surfaces. Their expression, distribution and structure change with brain development, maturation and aging. In this study we have developed a high resolution (HR) MS approach based on nanoelectrospray ionization (nanoESI) Orbitrap MS and multistage MS fragmentation (MS^n) for monitoring the changes occurring with development and aging in the composition and structure of cerebellar gangliosidome.

The native ganglioside mixtures analyzed in this study were purified from histopathologically-defined healthy cerebellar biopsies originating from fetuses in different intrauterine developmental stages, *i.e.* 15^{th} (denoted C15gw) and 40^{th} (C40gw) gestational weeks and from a 65 years old male (C65y). For MS analysis the samples were dissolved in methanol up to final concentration of 10 pmol/µL and subjected to nanoESI Orbitrap MS in the negative ion mode. After only 2 minutes of signal acquisition were identified no less than 159 ions in the three spectra, which, based on mass calculation, were assigned to 120 distinct ganglioside species differing in the composition of their glycan core and/or lipid aglycone as follows: 90 species in C15gw, 101 in C40gw and 63 in C65y. The species containing the highest number of Neu5Ac residues, heptasialo GS1 (t18:0/20:0), was detected solely in C15gw sample as a $[M\text{-}5H^+\text{+}2Na^+]^{3-}$ at *m/z* 1126.4804. No less than 91 species bearing more than one sialic acid were detected in C40gw *vs.* 72 in C15gw and 54 in C65gw. Further, (-) nanoESI HR high energy collision-induced dissociation (HCD) MS^n acquired at variable collision energy within 30-65 eV range provided the detailed structural characterization of the following precursor ions: i) the $[M\text{-}2H^+]^{2-}$, which, according to the MS^n results corresponds to

GD3(d18:1/18:0), a disialylated species characterized by a shorter glycan chain and ii) the $[M-4H^{+}+Na^{+}]^{3-}$ identified at *m/z* 812.7035 by MS screening. Following the MS^{n} results, this ion was attributed to the *d* isomer of the tetrasialylated tetraose GQ1 (d18:1/18:0).

The comparative assay fetal *vs.* aged cerebellum disclosed significant differences in the expressed ganglioside species, which are attributable to the neurodevelopmental processes and might be used accordingly as markers of cerebellum development and aging.

IND-10: LILBID-MS based method for the quantitative assessment of dsDNA binding affinities

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Recent work in native mass spectrometry has sought to extend its applicability from primarily analyzing the stoichiometries of noncovalent biomolecular complexes to also reliably quantify their binding affinities. Such methods face several challenges, including accounting for dissociation in the spectrometer and achieving sufficient reproducibility through consistent ion generation. This study introduces a new LILBID-MS based method that addresses these concerns, presents data from twelve dsDNA sequences, and validates the method with comparisons to UV melting curve and ITC results.

In the LILBID ion source, microdroplets of aqueous sample are irradiated with infrared laser light, leading to desorption of analyte ions. The amount of laser energy transfer to sample droplets controls both sample response factors and sample dissociation. More laser energy transfer results in more sample dissociation. For each sample, we varied the amount of laser energy transferred to each of several hundred droplets, imaging each droplet's response to IR radiation and recording the respective mass spectra. The shape of the droplet's explosive expansion can be taken as a proxy for laser energy transfer. By plotting droplet explosion width against percent dissociated DNA, we could achieve laser dissociation curves analogous to melting curves.

Results obtained from the LILBID laser energy dissociation curves were strongly correlated with results from solution phase methods. This correlation between the LILBID and solution phase results was not influenced by DNA length or GC content. The method shows good reproducibility and could be used to successfully predict the T_m and K_d of three DNA sequences. This study thus introduces LILBID-based laser dissociation curves as a promising native MS method for determining the binding affinities of noncovalent complexes, greatly improving on the time and sample consumption required for melting curve- or titration-based studies.

IND-12: Coupling Microfluidic Free-Flow Electrophoresis to Mass Spectrometry for the Separation of Biomolecules

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We present a novel method to couple microfluidic free-flow electrophoresis (µFFE) with mass spectrometry. µFFE is a tool for the continuous separation of electrically charged analytes. With this novel setup it is possible to continuously fractionate and collect compounds while simultaneously monitoring the process online with MS. The variable connection of multiple microchip outlets to ESI-MS is automated using a multiposition valve. To demonstrate the functionality of the method, the biomolecules AMP, ATP and CoA, which are fundamental for numerous biochemical processes in every organism, were separated and identified.

IND-14: Characterization of an Integrated Low Pressure Gas Dynamics and Ion Migration Simulation Method within the SPARTA-DSMC Framework

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Direct Simulation Monte Carlo (DSMC) is a well-established method to investigate the dynamics in low density gases with a particle based numerical approach. For the consideration of neutral gas particles, SPARTA (Stochastic PArallel Rarefied-gas Time-accurate Analyzer), is a popular open-source DSMC code. SPARTA allows basic chemical reactions and arbitrary surface geometry in the simulation domain. However, albeit charged particles (charged droplets, molecular ions) are implemented in SPARTA, their interaction with external electric fields is currently not. To widen the applicability of the code, we present a new module in SPARTA, which allows the simultaneous determination of the electric field force and the interaction between charged and neutral particles by the e.g. soft-sphere collision model.

From the experience gained in previous simulation projects regarding the behavior of charged particles over the past couple of years it is concluded that SPARTA calculations lead to valid results. This conclusion is also supported by comparison with literature data. DSMC is particularly well suited for the calculation of pressure distributions and gas flow profiles in complex mass spectrometer inlet stages. It was shown that depending on the simulation problem and the available compute resources, two-dimensional axisymmetric or even full three-dimensional simulations are required.

In order to do a first step towards the integrated description of the transport of charged particles in gas flows in mass spectrometers or ion-mobility spectrometers (IMS), uniform electric fields were recently implemented in SPARTA using a custom module (“fix”). This allows to determine the force acting on the ions caused by the electric field and the resulting change of the ion velocity. The collisions between the ions and neutral particles are also taken into account by the soft sphere collision model provided in SPARTA. The module allows a more comprehensive description of gas dynamics in low pressure regimes, e.g., upon considering the impulse transport from ions to neutral gas particles. For a first approach a generic IMS is used as benchmark system for code validation. It will be used to characterize the new fix in respect of e.g. the computing time or usability for IMS simulations.

IND-16: European Network of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Centres (EU_FT-ICR_MS)

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High resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) enables due to its high resolving power and mass accuracy the calculation of sum formulae from the mass defect of the measured m/z values and MS-MS experiments allow structural insights as well. The ultra-high resolution provides the investigation of high complex mixtures and therefore, the application fields reach from Proteomics, Lipidomics, Glycomics to Cultural heritage, Medicine and environmental application as well as Petroleomics, but also physical chemistry and a variety of other fields are covered.

The EU FT-ICR MS project is a Horizon 2020 INFRAIA Project which aims at establishing a European wide network of 11 FT-ICR MS centres with different specialisations in association with a SME software company. The project aims to fulfil 4 main goals to promote the use and development of FT-ICR MS. First, the TransNational Access provides EU academia, SMEs and industrial communities free access to world-class FT-ICR MS centres. Second, training and education build an EU community of end-users and FT-ICR MS scientists. Third, an open data & e-infrastructure program aims to develop open-source software and open access to the EU FT-ICR MS network results, and fourthly, joint research activities strengthen the FT-ICR MS application fields by promoting innovative and cooperative research between European FT-ICR MS academic scientists and private companies (instrumentation and software).

To get free access to the newest and most FT-ICR advanced tools: http://www.eu-fticr-ms.eu/

IND-18: LC-MS^2 based method development for therapeutic drug monitoring

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In 2018 there were an estimated 10 million cases of tuberculosis (TB) [1] reported. TB is caused by *Mycobacterium tuberculosis* and mostly affects the lung of patients but can also cause systemic infection. Due to an increased incidence of multidrug resistant tuberculosis (MDR TB) worldwide there is a massive requirement to develop and apply combination regimens with up to 5-7 antibiotics. Such antibiotic therapy may last a prolonged period of time up to 20 months. Lack of adherence or incorrect dosages can lead to development of further resistances during treatment, while high doses could lead to increased side effects. The assessment of pharmacokinetics- (PK)/ pharmacodynamics (PD) for combinations of antibiotics used for TB treatment are difficult because of their diverse chemical nature. Single drug quantitation is the current status quo but are time consuming to that extent that personalized drug monitoring is impossible.

Therefore, the aim of this study was to develop a high-performance liquid chromatography-mass spectrometry (HPLC-MS^2)-based multi-analyte assay, which enables to quantify 20 antibiotics in all medically applied combination regimens in one HPLC run. Measurements of antibiotics are performed on an Agilent 1100 Series HPLC system utilizing a Milipore SeQuant ZIC-HILIC column which was coupled to a Quattro Premier XE triple quadrupole MS applying electro spray ionization. This LC-MS^2-based multi-analyte assay is based on multiple reaction monitoring (MRM) for 20 antibiotics and five internal standards with a runtime of only 20 minutes.

This established LC-MS^2-based assay allows us to determine the drug concentration in murine lung tissue (lung homogenates/lung cryosections) of new antibiotic compositions and to measure PK/PD profiles in TB patient's plasma. Furthermore, the method can be customized to quantify the drug concentration of new antibiotic formulations.

[1] WHO Report 2019

IND-20: QCloud: The First Community System for Automated Daily Quality Control at MS Facilities

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Introduction

MS-Facilities are the centers of data generation for academic and clinical projects that provide access to complex and expensive instrumentation. Therefore, accurate quality control (QC) of instrument performance is vital for them. Usage of lab-own standards and protocols, which is a common QC-practice, complicates comparison of acquired data and their reproducibility. Proteomics Working Group of the strategic alliance Core for Life (https://coreforlife.eu/) endeavored to set up generic community QC-system based on ***QCloud*** – an automated quality control pipeline. The Group performed longitudinal harmonization study involving eight Proteomics Facilities across Europe and evaluated performance of seventeen LC-MSMS systems. Systematic analysis of common standards and interlaboratory comparison enabled participants to set reference for individual performance.

Material and Methods

The study included 4 Orbitrap Fusion Lumos, 7 QExactive HF and 6 QExactive HF-X instruments coupled with different chromatographic systems located at eight institutions. All instruments were involved in routine facility work. Fixed amount of commercially available "QC4L" standard (Promega)– a high-complexity protein mixture spiked with 6 isotopological peptides – was regularly analyzed over 6 monthes. Participating laboratories used common acquisition methods for each type of

instruments, while chromatrographic settings varied across the sites. For Orbitrap Fusion Lumos instruments, a generic acquisition method was compiled for all types of fragmentation. Obtained raw data were on-line submitted and centralized processed by QCloud pipeline. Extracted values were than compared between sites.

Results and Discussion

Altogether, over 250 standard experiments were acquired in course of the study until now. In the first blind phase, the participants had access only to own data. The obtained results outlined individual performance for each instrument and enabled monitoring of instrument stability over the long time span. Furthermore, the dataset revealed a few clear cases of irregular drop in performance of individual instruments. Detailed analysis suggested possible reasons for this misbehavior thus helping the following troubleshooting. For example, the parameters extracted by QCloud pipeline allowed detection of chromatography-related problems and early signs for cleaning of the instrument.

In the following phase of the study, the participants were granted access to the data from all sites and could do interlaboratory comparison. It enabled first of all identifying of systematically outperforming and underperforming outliers within each group of instruments. These cases trigged optimization of chromatography settings and refinement of common parameter in acquisition methods. Furthermore, multiintrumental dataset also allowed rough comparison between different types of instrumental setups.

Instrumentation and Application of MS Imaging

IMA-02: Novel MALDI imaging solution empowered by a dual-source Q-TOF and a dedicated bioinformatics pipeline for identification of peaks from tissue

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MALDI Mass Spectrometry Imaging (MALDI-MSI) has emerged as a technique with a broad range of applications in Omics research. However, a gap exists between desired mass resolution capabilities and the acquisition speed of current instrumentation. We present initial results from the timsTOF flex system; a timsTOF Pro QTOF mounted with a high-throughput, high spatial resolution MALDI source and stage. The instrument allows for the robust, high speed acquisition of both MALDI and LC-MS/MS data. When combined with a software processing pipeline for automatically annotating measured ions, this enabled generation of annotated images from MALDI-imaging data. A timsTOF Pro was mounted with a MALDI source and 10 kHz smartBeam 3D laser featuring electronically controlled spot positioning and imaging beam profile. ESI mode performance was evaluated by analyzing a commercially available HeLa digest (Pierce) using DDA PASEF. For MALDI-MSI experiments, tissue sections were mounted on conductive glass slides, and coated with matrix using standard protocols (TM Sprayer; HTX Technologies) or a specialised sublimation device. Ion mobility imaging experiments were acquired at 150 1/K0 mobility resolution. SCiLS Lab was used for visualising and statistical analysis of MALDI-MSI data. MetaboScape was used for annotations, using the T-ReX2 algorithm for feature extraction, de-isotoping and ion deconvolution. High spectral quality MALDI Imaging data could be acquired at a rate of up to 15 pixels/second in both positive and negative mode using standard parameters (m/z range 300-1000, 20µm pitch, 10kHz laser repetition rate). Using these parameters, measurement of a sagittal rat brain section produced an image consisting of approximately 370,000 pixels and took ~5 hours acquisition time. For standard operation MALDI-MSI experiments, spatial resolution of 20 µm was confirmed by matching ion signals to specific cells and structures in rat brain. Rat testis sections measured at 10µm zoom-mode showed different seminiferous tubule structures. In experiments designed to stress the system, 20 hours of image acquisition or ~1.5 million pixels showed no decline in imaging dataset quality and a mass deviation of RMS 2.06 without lock mass. Trapped ion mobility imaging measurements removed isobaric interferences in lipid imaging.
Proteomics analysis was used to assess if the dual source design and MALDI Imaging experiments affected LC-MS/MS performance. Injections of 200ng HeLa revealed over 5000 protein groups identified; this figure is maintained over the course of measuring 20 million MALDI pixels.
MetaboScape was used for lipid annotation using the customizable analyte list tool.

IMA-04: Combination of Thin Layer Chromatography and Secondary Ion Mass Spectrometry for the Analysis of Complex Sample Systems

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Thin Layer Chromatography (TLC) is a well established technique for the separation of analytes. Although its separation power is typically inferior when compared to hyphenated techniques such as High Pressure Liquid Chromatography (HPLC), still a number of applications for TLC are currently state-of-the art. Whereas the detection of the resulting TLC-spots is possible (e.g. directly or after staining of the TLC plate), the exact identification of the analyte is difficult.

In recent years, a number of mass spectrometric techniques were combined with the TLC separation process. The identification of the separated analytes is typically aspired by this technique combination. Examples for combined approaches are the use of Electrospray Ionisation Mass Spectrometry (ESI-MS; after elution of the analyte from the TLC plate) or Matrix Assisted Laser Desorption and Ionization Mass Spectrometry MALDI-MS (applied directly on the TLC plate).

In this study the use of Secondary Ion Mass Spectrometry (SIMS) for the identification of analytes on TLC plates is demonstrated. The technological developments in both the TLC and the SIMS technology make it meanwhile possible to

establish the respective technique combination. Within the study the use of two different mass analysers within such experiments was tested. On the one hand, a Time-of-Flight mass analyser and on the other hand an Orbitrap mass analyser was used for analysis.

In summary, a TLC-SIMS combination was meanwhile successfully established.

IMA-06: Electrochemistry meets Imaging – Electrode surface analysis by means of MALDI-ToF-MS

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Minimization of side reactions is a crucial step during the development of a typical organic synthesis route to maximize product yield and reduce educt consumption. In the last decades, electrochemistry has emerged to be a powerful tool in organic chemistry, since it enables easier access to several moieties via single-electron transfer. The efficacy of electrochemical reactions strongly depends on parameters such as electrolyte composition and electrode condition. Electrochemical side reactions, e.g. polymerization, can cause deposition of substrate material, thus limiting the activity of the electrode's surface. This process is predominantly known as "electrode fouling" and is a crucial factor not only in electroorganic synthesis, but also in the design and functionality of modern battery developments.

In this work, matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) is presented as valuable tool for the surface analysis of commonly used boron-doped diamond (BDD) electrodes. Three different organic compounds with common functional groups, including aniline and phenol, have been investigated. A thin-layer flow-through cell was used to perform oxidative polymerization experiments. The treated BDD electrodes were subsequently analyzed by MALDI-ToF-MS to visualize the resulting deposition of oligomers on the electrode's surface.

All three compounds show significant adsorption when polymerized via electrochemical oxidation with different localization on the surface depending on the respective oligomer size. Additionally, MALDI imaging reveals a dependency of oligomer size and the flow direction and therefore the mean contact time of the compounds.

IMA-08: Optimized MS imaging workflow for on-tissue digestion of proteins for high-resolution MALDI mass spectrometry imaging

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MALDI-mass spectrometry imaging (MALDI-MSI) is a unique analytical *in-situ* technique which has a variety of applications in bioanalytical sciences including the investigation of proteins. Here, the bottom-up approach in MSI, namely tryptic digestion of proteins on tissue and MS imaging of tryptic peptides, is the method of choice when protein identification is necessary. However, on-tissue digestion of proteins is one of the most challenging approaches in MSI. Due to the requirement of protein digestion directly *in-situ*, numerous experimental parameters have to be considered and controlled if high-resolution imaging is desired. This project is focused on the optimization of the dedicated MS imaging workflow to achieve high resolution MALDI-MSI of tryptic peptides as well as obtain experimental reproducibility.

Coronal mouse brain sections (20 µm thickness) were used for imaging experiments. After storage at -80°C, sections were dried in a desiccator. Lipids and salts were removed from tissue by washing the sections in a series of ethanol and water. Protein digestion was performed by spraying trypsin on tissue using a pneumatic spraying device. After enzyme application, the tissue was placed 10-15 minutes inside a digestion chamber at 37°C and high humidity to ensure proper conditions for enzymatic digestion. This procedure was repeated several times. 2.5-DHB matrix was applied using a pneumatic spraying device. MS imaging experiments were performed using an atmospheric pressure MALDI imaging source (AP-SMALDI10) attached to an orbital trapping mass spectrometer (Q Exactive HF ™).

Several sample preparation steps including trypsin application/digestion, tissue washing and measurement settings were optimized in this project. Our workflow allows reproducible imaging of tryptic peptides in mouse brain sections at 50 µm pixel size with high mass resolution (R > 100000 FWHM) and mass accuracy (RMSE < 3 ppm). Furthermore, the applicability of the workflow is demonstrated with high-resolution measurements at 20 µm pixel size. Here, MALDI imaging revealed detailed histological structures of tryptic peptides corresponding to a variety of proteins in the mouse brain. In addition to the improved imaging workflow, peptide identification based on on-tissue MS/MS under imaging conditions is demonstrated.

In this study, we further investigated the bottom-up workflow in MSI with respect to experimental reproducibility at 50 µm step size in mouse brain sections and showed applicability of this workflow at higher spatial resolution. Furthermore, MALDI-MS/MS lead to identification of peptides corresponding to different proteins in the mouse brain.

[1] Huber,K.,Khamehgir-Silz,P.,Schramm,T.,Gorshkov,V.,Spengler,B.,Römpp,A.(2018). Anal.Bioanal.Chem. *410*, 5825–5837.

[2] Schober,Y.,Guenther,S.,Spengler,B.,Römpp,A.(2012). RapidCommun.MassSpectrom.26,1141–1146.

[3] Enthaler,B.,Bussmann,T.,Pruns,J.K.,Rapp,C.,Fischer,M.,Vietzke,J.-P.(2013). RapidCommun. MassSpectrom.27,878–884.

IMA-10: Towards elemental bioimaging by means of an in-house developed visual light-based laser ablation system

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The spatially resolved analysis of metals in biological samples is crucial to investigate fundamental problems in biomedical research, such as the development of pathologies like Alzheimer's and Parkinson's disease. Therefore, elemental bioimaging (EBI) is a growing field in Analytical Chemistry, which aims to visualize the elemental distribution in biological samples. For this purpose, laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) was established as a suitable technique since the first application in 2003.

Originally, LA-ICP-MS was designed to approach geological problems, such as the investigation of the elemental composition of rock samples and their absolute age determination. As these applications continue to dominate the field of LA-ICP-MS, the commercial laser ablation systems are still designed for a geological purpose. Due to the transparency of most inorganic minerals to visual light, ultraviolet laser wavelengths down to 193 nm are used since the light absorption is crucial for the ablation process. Because all elements in the optical path need to be transparent to the laser beam, commercial systems require high cost equipment, in acquisition as well as maintenance. Also, due to the particularly long analysis times in LA-ICP-MS, many components become wearing parts and cause additional maintenance costs.

Due to several chromophores in biological samples, a visual light laser beam can be applied in order to ablate the sample. This approach promises a significant increase in maintenance as well as acquisition costs and a much easier handling. In this study, we suggest an alternative laser ablation system, based on a 405 nm laser diode. Because of the commercial use in optical storage media, these laser diodes can be obtained very easily and cost-efficiently. In this study, the suitability of a 405 nm laser diode was investigated regarding the applied voltages, pulse frequency and laser scan rate. The ablation process was assessed by the mass spectrometric detection of the ablated material and morphological investigations of the sample after the ablation.

IMA-12: Optimization of sample preparation parameters to enhance the detection limit of Statins in a MALDI-2-MSI approach

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Statins belong to the most often prescribed drugs worldwide. In addition to lowering cholesterol levels, they are believed to have a variety of pleiotropic effects. Unwanted side effects are more often detected in patients taking lipophilic rather than hydrophilic statins. Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) could be a useful tool to visualize the distribution of statins in dosed tissue in order to investigate differences between these two subgroups of compounds. Limits of detection (LOD) however, are often critical parameters and strongly depend on physico-chemical properties of analyte, MALDI-matrix, biological matrix and sample preparation. We here present how LODs of four common statins can be lowered by optimized sample preparation and the use of laser post-ionization (MALDI-2).

Two lipophilic statins (simvastatin, atorvastatin) and two hydrophilic statins (pravastatin, rosuvastatin) were investigated. To determine LODs, porcine liver tissue was homogenized using a blender, spiked with three different concentrations of the respective statin at concentrations of $4{\cdot}10^{-5}$ M, $2{\cdot}10^{-4}$ M, and $1{\cdot}10^{-3}$ M and homogenized further using a dismembrator. Following a protocol proposed by Barry *et al.* (*Protocol Exchange,* 2019), mimetic tissue arrays were constructed, sectioned, and mounted onto indium-tin-oxide coated glass slides. Matrix was applied using an ultrasonic spray robot. MS-images were recorded on a timsTOF fleX-MS (Bruker) modified in-house with a Q-switched, frequency-quadrupled Nd:YAG-laser (NL 204-1k-FH, EKSPLA) to allow for MALDI-2 at 266 nm and 1 kHz repetition rate. Data analysis was performed using SCiLS Lab software.

Cryo-sections of mimetic tissue constructed of four sections that contained one of the statins each were used to optimize a number of different parameters regarding sample preparation including matrix concentrations and solvent compositions. Three matrices commonly used in drug analysis were employed (4-nitroaniline, norharmane, 2,5-dihydroxyacetophenone). Proper extraction conditions for each statin compound and measurement parameters, e.g., pulse energies of the two lasers, were optimized. To determine the LODs, mimetic tissue models of decreasing concentration of the drug were constructed for each statin separately.

Under optimized conditions LODs were improved from values between $1.1{\cdot}10^{-4}$ M for pravastatin and $4.9{\cdot}10^{-4}$ for simvastatin to values between $2.5{\cdot}10^{-5}$ M and $3.6{\cdot}10^{-4}$ M. Statin concentrations from animal models are reported to be in the order of $2.2{\cdot}10^{-4}$ M in plasma and $2.0{\cdot}10^{-3}$ M in the liver of mice that were administered with the statins. LODs achievable with MALDI-2-MSI are therefore adequate and sufficient for dosed tissue analysis and the technique may serve as valuable tool in forthcoming animal studies.

IMA-14: MALDI-TIMS-MS based analysis of cannabis plant samples

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The over 100 different cannabinoids found in *Cannabis sativa L.* show wide variations in their psychotropic and pharmacological activities. Among them are various isomeric species like the psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC) and its non-psychoactive isomers cannabidiol (CBD) and cannabichromene (CBC). Due to its high antipsychotic, anxiolytic, anti-epileptic and anti-inflammatory activity, CBD is the isomer of highest interest for pharmaceutical applications and is emerging in commercial consumables. Thus, the differentiation between isomeric cannabinoids is a major challenge in the fields of pharmaceutical and forensic analysis. Ion mobility spectrometry (IMS) is establishing as a post-ionization separation technique for isomeric species. Trapped IMS (TIMS) offers high mobility resolution by applying an electric potential against a constant gas stream and a serial elution of the trapped ions. The integration of TIMS with matrix-assisted laser desorption/ionization - mass spectrometry (MALDI-TIMS-MS) enables the differentiation between isomeric species. Dried plant sample material of different cannabis varieties were analyzed using a MALDI-TIMS-qToF setup.

The direct MALDI-MS analysis of the cannabis samples led to the identification of various signals related to different cannabinoids. The most intense signals were found for the corresponding carboxylic acids, as these are the predominant species in cannabis plants. The main signal was found for *m/z* 357.2071, which could refer to different isomers like cannabidiolic acid (CBDa), tetrahydrocannabinolic acid (THCa) or cannabichromenic acid (CBCa). By adding IMS as additional separation dimension, multiple mobility signals were found for different *m/z*, indicating the presence of isomeric cannabinoids. Two mobility signals were separated for *m/z* 357.2071. These signals can be correlated with the high THCa and CBDa contents of the analyzed cannabis varieties bedrocan, bediol and industrial hemp. While the mobility separation alone does not

guarantee an unequivocal distinction between CBDa and THCa, the mobility resolved fragmentation (TIMS-MS/MS) of the isomers enables their identification based on their different fragmentation pathways. Imaging experiments for a mixture of bedrocan, bediol and industrial hemp proved that MALDI-TIMS-MS based imaging (MALDI-TIMS-MSI) allows to depict the lateral distribution for the different isomeric cannabinoids. Furthermore, it was found that the three cannabis varieties can be identified within the cannabis mixture based on the distinct ratios of their CBDa and THCa signal intensities.

IMA-16: Host-parasite interaction of *S. mansoni* eggs in hamster liver investigated by high-resolution AP-SMALDI MSI

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The parasitic trematode *Schistosoma mansoni* is prevalent in distinct areas of the tropics and subtropics, causing schistosomiasis, one of the most important neglected tropical diseases worldwide. Approximately 200 million people are affected, and there is currently only one available drug, praziquantel. Male and female adult worms live in constant pairing contact in the mesenteric veins of the host, shedding around 300 eggs per day. The eggs can enter the bowel lumen from where a fraction is excreted into the environment but a large part is retained in the venous system and is transported mainly to the host liver. Here, they can cause severe impairment of the tissue surrounding the eggs, accompanied by inflammatory processes and granuloma formation.

Inflammation of the host liver is the most critical symptom of schistosomiasis, and if untreated, chronic infection can lead to liver cirrhosis and necrosis. We have analyzed liver tissue of an established rodent infection model, *Mesocricetus auratus* (Syrian hamster), using MALDI MS Imaging to compare zones of egg deposition, granuloma formation and supposedly unaffected liver tissue.

8 µm sections of liver tissue were produced using a cryomicrotome (HM 525, Thermo Fisher Scientific, Bremen) and coated with 90 µL DHB matrix (30 mg/mL in H_2O/Acetone 1:1 v/v, 0.1% TFA) in positive-ion mode using a pneumatic sprayer (SMALDIprep, TransMIT GmbH, Giessen). Matrix for negative-ion-mode experiments was 150 µL diaminonaphtalene (DAN, 3 mg/mL in Ethanol/H_2O 1:1 v/v). MS Images were recorded using an AP-SMALDI[5] AF ion source (TransMIT GmbH, Giessen) coupled to a Q Exactive high resolution orbital trapping mass spectrometer (Thermo Fisher Scientific, Bremen) with 5 - 20 µm pixel size and a mass resolution of R = 240,000 at *m/z* 200.

Data analysis revealed specific metabolites for deposited eggs, for zones of granuloma formation as well as for surrounding hepatic tissue. In positive-ion mode, phosphatidylcholine and sphingomyelin lipids were especially suited to depict the different areas of inflamed liver tissue. In negative-ion mode, phosphatidylserine and phosphatidylinositol lipids were detected and used to assign areas of interest in correlation to optical images. Statistical analysis revealed further lipids and lipid classes that showed high intensity in one of the above-mentioned liver parts. We evaluated our findings with regard to biological pathways known to play a role in lipid metabolism during inflammation processes, for example an increase in saturated fatty acids and decrease in unsaturated fatty acids. LOEWE project DRUID and DFG INST162/500-1FUGG are gratefully acknowledged

IMA-18: UV- and IR-MALDI-2-MS imaging to visualize the metabolic exchange between competing gram-negative and positive bacterial cultures

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MALDI mass spectrometry imaging (MALDI-MSI) is increasingly used to analyze the molecular make-up of microbial communities in a spatially-resolved manner. We recently introduced an advanced membrane-based workflow for improved microbial MALDI-2-MSI (Brockmann *et al.*, *Anal. Chem.*, 2019). Using a rapid heat-inactivation step, our workflow allows to safely inactivate non-sporulating gram-negative and –positive bacteria (including pathogens) while, at the same time, conserving the metabolic situation within the colony. Laser-induced postionization (MALDI-2), adapted to a Waters Synapt G2-S mass spectrometer, enabled a crucial boost in the analytical sensitivity and chemical coverage for a wide class of bacterial signaling molecules and structural phospholipids. Next to discussing the principles/potentials of our method, we here present several recent improvements.

In particular, we achieved a higher sensitivity for UV-MALDI-2-MSI of gram-positive bacteria (especially *Staphylococcus aureus* colonies) upon adding 10% TFA to our matrix solution. With relevance to the pressing need for development of new antibacterial compounds, we exploited our advanced UV-MALDI-2 methodology to analyze the interaction of *S. aureus* with *S. lugdunensis* at about 60 µm pixel size; here, the focus was on mapping the distribution of lugdunin, a cyclic peptide containing a thiazolidine group, that was recently shown to exhibit pronounced inhibitory effects on the growth of co-growing pathogenic *S. aureus* strains (Zipperer *et al.*, *Nature*, 2016).

Secondly, we demonstrate the potential of infrared (IR) laser for the matrix (background-) free LDI-2-MSI analysis of numerous signaling molecules (e.g., a series of 2-alkylquinolones of *Pseudomonas aeruginosa*) and numerous further low molecular weight secondary bacterial metabolites, such as nucleotides, directly from bacterial colonies. To avoid disadventageous interaction of IR radiation with the membrane substrate, for IR-LDI-2-MSI, bacterial cultures were grown on nylon membranes instead of the otherwise used mixed cellulose ester. Due to the reduced focusability of the IR-laser, in this case a pixel size of 150 µm was employed.

Our described methodological/instrumental advances could assist microbiologists in obtaining deeper insights into the chemical communication in and between microbial communities.

IMA-20: Identifying transition metal deposition patterns on aged graphite anodes by means of LA-ICP-MS imaging

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A comprehensive understanding of electrode-electrolyte interphases in lithium ion batteries, namely the solid- (SEI) and cathode electrolyte interphase (CEI), with regards to formation and evolution is crucial to the development of improved cell chemistries as their properties drastically affect battery performance. One predominant aging phenomenon impeding the SEI properties is the deposition of transition metals on the anode surface, originating from degradation of commonly used transition metal oxide-based cathodes.

For this purpose, a wide variety of surface sensitive techniques, such as time of flight-secondary ion mass spectrometry (TOF-SIMS), glow discharge-optical emission spectroscopy (GD-OES), X-Ray photoelectron spectroscopy (XPS) and energy-dispersive X-ray spectroscopy (EDX) are commonly used in battery research, in order to investigate electrode degradation. However, small spot sizes, insufficient limits of detection or the inability to provide spatial resolved information limit the applicability of these techniques. Especially the investigation of large sample areas of several cm^2 (*e.g.* pouch bag electrodes) poses a significant problem.

Laser ablation–inductively coupled plasma-mass spectrometry (LA-ICP-MS) offers a unique set of advantages to address these shortcomings, allowing for analysis of whole electrodes in reasonable time frames. In addition, both spatially as well as depth resolved elemental information can be obtained by subsequent ablation runs.

This study showcases the applicability of LA-ICP-MS to visualize transition metal deposition on graphite anodes and their use as indicators for locally accelerated aging within battery cells. Qualitative analysis of long term cycled NCM111/graphite pouch bag cells revealed different deposition behaviour of nickel, manganese and cobalt, respectively. Based on their varying correlation with the lithium signal, two different types of deposition sites could be identified as well as possible origins could be postulated.

IMA-22: IR-MALDI MSI of apolar metabolites

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Polar biomolecules such as peptides or proteins are readily ionized with matrix-assisted laser desorption/ionization (MALDI), whereas detection apolar compounds including metabolites and lipids suffer from low ionization efficiencies. A possibility for efficient ionization of apolar analytes are plasma-based ionization methods, such as low-temperature plasma (LTP), which, however, cannot efficiently desorb compounds from biological tissue. One MALDI modality that does not require application of a crystalline matrix is IR-MALDI, only requiring water as a matrix. It offers efficient sample desorption. By combining IR-MALDI with LTP for post-ionization, the advantages of both techniques can be extended. Herein, we show that apolar analytes, including sterols, can directly be visualized by LTP IR MALDI mass spectrometry imaging (MSI) without sample preparation or the use of chemicals. The spatial resolution is thereby determined by the laser focus.

A homebuilt atmospheric pressure IR-MALDI source was modified. It is equipped with an optical parametrical oscillator (OPO, GWU Lasertechnik GmbH, Erftstadt, Germany), pumped by an externally triggered Nd:YAG laser (SpitLight 400, InnoLas Laser GmbH, Krailling, Germany) and emitting a wavelength of 2.94 µm. The laser was focused, after attenuation, through a centrally bored objective lens onto the sample surface. The sample was cooled with a Peltier element on a moveable stage. The LTP probe was integrated in a way that the active species from the plasma stream could interact with the desorbed analytes while minimizing the shading of the laser beam. Helium was used as a discharge gas. Ions were analysed with a LTQ mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany) in positive-ion mode.

Different groups of authentic standards were dissolved and dropped on glass slides. After drying, the samples were transferred into the ion source and water ice was deposited on the surface. The surface was scanned by IR-MALDI with and without the LTP probe operating. Several compounds showed an up to 100-fold increase in signal intensity or were only detectable when the LTP was running. To demonstrate the suitability for MALDI MSI, cryosections of biological tissues were frozen with the Peltier element and measured with a pixel size of 100 µm. Metabolites, including cholesterol detected as $[M - H_2O + H]^+$, were visualized.

An atmospheric pressure IR-MALDI ion source was extended with a LTP probe for post-ionization of desorbed analytes. In first proof-of-concept experiments authentic standards of apolar metabolites and tissues were investigated with post-ionization IR-MALDI MSI, demonstrating the potential of this methodology.

IMA-24: MALDI imaging in Tuberculosis research - Characterization of lipid profiles and drug detection in mouse lung tissue

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Tuberculosis (TB) remains one of the most significant causes of death from infectious diseases. The rise of multi drug-resistant *Mycobacterium tuberculosis* strains creates an ever increasing need for novel anti-TB drugs. Tuberculosis (TB) is characterized by necrotic granulomas, with bacilli rich centers not being reached in sufficient concentrations by standard anti-TB drugs.

Therefore, we established a MALDI imaging workflow to map the distribution of phospholipids and multiple anti-TB drugs in lung sections of a novel mouse model.

MS imaging experiments were carried out on a Q-Exactive™ HF Hybrid-Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen), coupled to the AP-SMALDI10 ion source (TransMIT GmbH, Gießen). Mass spectra with high mass accuracy (< 2 ppm) and high mass resolution (R=240,000 @m/z 200) were acquired. Measurements were performed on lung tissue of BALB/c mice and infected (interleukin (IL)-13-overexpressing (tg)) mice treated with the anti-TB drugs Clofazimine

(CFZ), Pyrazinamide (PZA) and Rifampicin (RIF). Mtb infected lung sections were decontaminated using γ-irradiation to be able to investigate them in the mass spectrometry lab. Different matrices for measurements in positive and negative ion mode were applied using a pneumatic sprayer system. Measurements were performed with 10-40 µm step size.

Sample preparation including sectioning and matrix application was optimized for the detection of the anti-TB drugs Clofazimine (CFZ), Pyrazinamide (PZA) and Rifampicin (RIF) in mouse lung sections. A decontamination protocol was established to be able to investigate Mtb infected lung sections in the mass spectrometry lab and the effect of the irradiation process on the drug distribution was investigated. With the established workflow, the distribution of CFZ, PZA and RIF could be observed in control and infected lung tissue sections.

To investigate drug penetration into the necrotic granulomas, lipid profiles of sterilized infected lung tissue sections were acquired in both polarities. A modified graph cuts clustering algorithm was developed to perform clustering on the positive and negative mode data at the same time. Using the chemically unique regions identified by the clustering, lipids characteristic for the granuloma region and surrounding tissue were identified. These phospholipid signatures are an ideal basis for investigating drug penetration into lesions. PZA could be detected in the granuloma region but CFZ and RIF were not detected in the granuloma. These findings are in agreement with human studies and show therefore the suitability of the novel IL-13tg mouse model and the developed MSI method for studying the penetration of novel anti-TB drugs into lesions.

Ion Physics and Ion Chemistry - Applications

IPC-02: Characterization of artificially oversulfated glycosaminoglycan-like oligosaccharides by electrospray ionization ion trap (ESI-IT) mass spectrometry

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Glycosaminoglycans (GAG) as important building blocks of proteoglycans are major components of the extracellular matrix (ECM). GAG vary not only in the length and monosaccharide composition, but as well in the type of glycosidic linkages and the number and position of sulfate residues along the chains. These long, unbranched polysaccharides do not only define the structure and biophysical properties of the respective tissue, but also affect the embedded cells via their surface receptors and the interaction with different signaling molecules such as interleukins and growth factors. These effects are significantly depending on the sulfation pattern of the applied GAG: the recent "heparin contamination crises" has tremendously emphasized this aspect and pointed out that the chemical and biochemical characterization of GAGs is extremely important.

Oligosaccharides from natural GAGs with a moderate extent of sulfation can be routinely analyzed by either matrix-assisted laser desorption/ionization time-of-flight or electrospray ionization mass spectrometry. However, oversulfated GAGs are much more refractive to MS analysis due to the small ion yields and the loss of the sulfate residues in the gas phase. The effect of unwanted sulfate loss is less pronounced for deprotonated sulfate residues or residues capable of ion paring to a stabilizing cation. Even using electrospray as one of the softer ionization methods, this effect is a serious problem for the characterization of unknown samples since sulfate loss cannot be neglected even with optimized measuring conditions.

In the case of deprotonated, sulfated residues a strongly sulfated oligosaccharide with more than four sulfate residues requires high-resolution MS measurements since otherwise these ions cannot be adequately characterized. In this work, we concentrated on the second possibility, i.e. the use of stabilizing cations, tested different desalination methods and optimized the measuring conditions using a commercially available model compound: Fondaparinux is a synthetic pentasaccharide with eight sulfate residues, which is already in clinical use.

We investigated the influence of different desalination protocols on the ESI mass spectra and the achievable sensitivity. Besides sodium chloride, we also used volatile, quaternary ammonium salts as alternative counter ions as described before. After optimization of the measuring conditions for the positive and negative ion mode, we could establish a gentle and fast method for the MS characterization of artificially sulfated GAG oligosaccharides. Our protocol enabled us to obtain reliable mass spectra in both ion modes even for highly negatively charged GAG derivatives with up to sixteen sulfate residues.

IPC-04: Simulations of Charged Nanodroplets in MS Transfer Stages

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Electrospray Ionization (ESI) is among the most common ionization techniques for many classes of polar substances, particularly fragile macromolecules.

Current experimental evidence supports the notion that ESI generates small, highly charged droplets (nanodroplets) which are able to penetrate deeply into a mass spectrometer inlet stage. This potentially affects the analytical performance of an instruments and can lead to contamination in the inlet stage region. The physical and chemical dynamics of such charged nanodroplets under transfer stage conditions, characterized by significantly increased collision energies due to the present electric fields and the relatively low background pressure, is mostly unknown currently.

Charged nanodroplets generated by ESI can be simulated with different approaches: One promising method is classical molecular dynamics simulation. With such simulations, the collision induced energy take up and the resulting evaporation processes of nanodroplets can be examined. Results from such calculations are then used as input parameters for more high level trajectory simulations of evaporating droplets. Thus, these tools in combination allow to investigate the motion of charged nanodroplets in the ion source and MS inlet stage.

Previous simulations demonstrated that nanodroplets surrounded by a background gas can be successfully simulated with MD simulations. The droplets consisted of mixtures of different typical solvents and small ions. Argon was chosen as the background gas. The size of the droplets is about 5 nm.

An increase of the charge density within the nanodroplets results in destabilization and fission of the droplet, which is in accordance with the Rayleigh limit. Further simulations were done with a charge density of 0.6 %, which is well below the Rayleigh limit, to avoid random fission events in studies of collision induced droplet heating. The background temperature and electric field were varied in the calculations and had expected effects.

The collisions between droplets and background gas particles is modeled by deliberately shooting individual “impactor” particles on droplets, due to the comparably short simulated time frame of classical MD simulations. The internal energy distribution and heating of the droplets under those particle impacts is analyzed. In addition, collisions between droplets and droplets and walls, e.g. electrode surfaces, are simulated. From these MD simulations input parameters for high level particle trajectory simulations can be extracted from the MD calculations, to investigate the transport of charged nanodroplets through an MS inlet stage in detail.

IPC-06: Fragmentation Mechanisms of Metal-Lipid Complexes in the Gas Phase

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Glycerophospholipids (GPs) are essential physiological molecules. Cellular membranes, protein-protein interactions and molecular signaling are influenced by GPs. One phospholipid class, phosphatidylcholines (PCs), are the major components of most eukaryotic cell membranes and have been associated with multiple biochemical processes. Discrimination of the fatty acid (FA) identity and positions on the glycerol backbone is crucial to understand PC associated biochemical events.

Mass spectrometry (MS) is arguably the bioanalytical method of choice to study PC structures. Sum formulae are derived from accurate mass measurements while tandem MS (MS^2) is used to identify head groups and FA composition. Many researchers investigated PC fragmentation using collision-induced dissociation (CID) MS^2 and deduced empirical fragmentation mechanisms that are consistent with experimental fragment ion masses. These previous studies showed that charge carriers influence PC fragment ion identities. In positive ion mode CID of PCs results in headgroup or FA loss if protons or divalent metals are attached. Proposed mechanisms suggest that dioxolane or dioxane derivatives are formed upon headgroup loss, whereas five- and six-membered phosphodiester structures are discussed as a result of FA loss. Our study intends to identify precursor and fragment ion structures of PCs as a function of charge carrier in order to rationalize PC ion fragmentation mechanisms.

Investigations of the PC-cation complexes were carried out using electrospray ionization MS, structurally probing gas-phase ions by infrared multiple photon dissociation (IRMPD) spectroscopy at the Free Electron Lasers for Infrared eXperiments (FELIX) facility. PC adducts with H^+, Na^+, K^+, Fe^{2+} and corresponding fragment ions were formed upon CID prior to IRMPD. IR-photons were spectroscopically interrogated in the range of 1850 to 650 cm^{-1}. Qualitative analysis of the IRMPD spectra was performed by comparing spectra of PCs with IRMPD results for synthesized authentic standards. For detailed spectrum interpretation, conformational analysis of the PC-cation complexes was performed on the GFN2-xTB level of theory. Energetically most stable structures finally were further optimized using PBE0-D3(BJ) on a def2-SVP level of theory. By comparing experimental with theoretical results and IRMPD spectra of authentic standards, we were able to assign structures to most PC adducts and corresponding fragment ions. These structures are in line with proposed fragmentation pathways of gas-phase PC adducts and hint to predominate five-membered ring formation in investigated fragment ions.

IPC-08: Product study in the interaction of selected metal and semiconductor surfaces with H_2 plasma generated species

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Plasmas can be utilized to initialize chemical and physical processes through the interaction between plasma constituents and solid/gaseous matter. The reactivity of excited hydrogen and metals is known in the literature for decades. The nature of the involved heterogeneous chemistry and the deeper understanding of the corresponding processes recently gained substantial interest with regard to high-energy hydrogen plasmas in the presence of metals, especially tin.

In a dedicated plasma chamber, a low-pressure (0.01 to 5 mbar) hydrogen RF plasma is used to generate the metal derived products. The RF-plasma is powered by an RFG-13-100 RF-generator (Barthel HF-Technik) with 13.56 MHz at 100 W and a MatchingCube i-300 with 40.68 MHz at 300 W. For detection of neutral species, the plasma chamber is directly coupled to a MAT95XP double-focusing sector field mass spectrometer with electron ionization (Thermo Finnigan). Ionic species are identified by a C-TOF time of flight mass spectrometer (Tofwerk AG); this instrument, also allows to detect neutral species via EI.

The hydrogen RF plasma stage primarily generates abundant H^+ ions, which are detected as H_3^+. Hydrogen plasma experiments with elemental tin or other relevant pure metals show besides the Sn^+ (in general M^+) also ionic tin hydrides of the type SnH_x^+. These species are likely generated through direct surface interaction between plasma constituents (mainly H_3^+). To a lesser extent oxygenated tin-hydrogen compounds of the type $OSnH_x^+$are present in the plasma effluent as well. The oxygen source is either an oxide layer present on the metal surface residual gas phase oxygen. The rich isotopic pattern of Sn is fully reproduced and thus used for compound identification. Since the mass spectrum of the ionic species detected in the TOF without the EI source turned on is identical to the spectrum obtained by EI of solely neutral species, it is assumed that the formation of all SnH_x^+ species takes places via formation of the neutral stannane SnH_4. The thermodynamic instability of neutral metal hydrides and their spontaneous dissociation into the elements is experimentally well known. This is further supported here by ab initio calculations. In addition, some metal surfaces have a catalytic effect on the decomposition reactions. It is expected that the employed experimental setups will lead a to much better understanding of the chemical behavior of tin and other relevant metals in the presence of reactive hydrogen/surfaces.

IPC-10: Influence of the aromatic moiety on gas phase reactions of heptamethine cyanine dyes using femtosecond-laser-pulse induced photodissociation

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Introduction

Heptamethine cyanine dyes with indole moieties are widely used for medical purposes, e.g. the dye indocyanine green (ICG) is used as a fluorophore for in vivo imaging and photodynamic therapy. In previous experiments, ICG showed different stability for protonated ions and sodium ion adducts. Subsequently, the stability of different alkali metal adducts was examined. Furthermore, the photodissociation (PD) reactions of another cyanine dye (IR 746) – with indole as aromatic structure instead of benzo[e]indole - were compared with the PD reactions of ICG.

Methods

The ionization was performed via electrospray ionization with different alkali metal salts. The trapped ions of cyanine dyes were analysed using an Apex III FT-ICR mass spectrometer (7.05 T) (Bruker Daltonik, Germany) by means of femtosecond-laser-pulses (NIR region) (Ti-Light, Quantronix, USA). The laser system (150 fs pulse duration) was operated at ca. 790 nm, a Ti:sa laser was acting as seed laser (Ti-Light, Quantronix, USA) pumped by a cw-Nd:YAG laser system (Opus 5W, Laser Quantum Inc., USA). A home-built shutter was used to control the irradiation time. The shutter opening time was set to 1 s.

Results

The spectrum IR 746 shows less intense fragmentation than ICG due to weaker absorption of the applied wavelength. The absorption maximum of IR 746 in methanol is at 746 nm in contrast to approx. 810 nm for ICG.

Two types of fragments have been identified. The first type contains two metal cations and shows higher intensities than the second type, which contains only one metal cation.

One notable fragmentation path of the alkali metal ion adducts is the loss of the butane-1-sulfonate chain, presumably as a cyclic sulfonic ester 1,4-butanesultone.

The fragmentation pattern is similar for different metal adducts in most cases. Therefore, the aromatic moieties (indole in IR 746 and benzo[e]indole in ICG) do not lead to major differences in the fragmentation products, but to different intensity distributions. The abundance of a certain fragment (containing two metal cations) of ICG is nearly equal to the intensity of the main fragment mentioned above. The intensity of the analogue fragment of IR 746 is quite low, which indicates, that the coordination of two cations depends on the size of the aromatic moiety.

The same applies to the fragments with one metal cation. The size of the cation impact the intensity of the observed fragment. Intensities of peaks with same molecular formula are higher for adducts with larger metal cations.

IPC-12: Investigation of Non-Covalent Clusters of Various Aniline-Derivatives via REMPI-Spectroscopy

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In previous works on non-covalent intermolecular interactions, anisole-clusters proved to show only a single electronic ground state in REMPI spectra. This differs from the behaviour observed in similar benzene-phenol-clusters. In addition, the influence of structural features as well as polarity on cluster formation has been investigated.

To complement these studies, additional experiments were carried out employing aniline-derivatives. This was done to introduce amino-groups, another feature present in structures like DNA base pairs.

Experiments were carried out on a modified custom-build ReToF-MS using Argon as a carrier gas (4 – 5 bar). The molecules were ionized in 1c2p process using a tunable dye-laser (Lambda Physik) pumped by the 2nd harmonic (355 nm) of a Nd:YAG laser (Spectra-Physics). Coumarin 153, rhodamine 6G and rhodamine B were used as laser dyes. Using a BBO crystal for the SHG process led to effective spectral ranges of 266 – 285 nm (coumarin) and 290 – 310 nm (rhodamines) respectively.

The spectra only share some characteristics with those previously recorded, including a red-shift relative to the anisole-monomer. The extent of this shift appears to differ depending on the clustering partner. For clusters containing anisole and aniline or diethyl-*o*-toluidine it is similar to shift in the anisole-dimer. Employing ethyl-*o*-aniline, ethyl-*o*-toluidine, diethyl-*o*-aniline diethyl-*o*-toluidine or dimethyl-*o*-aniline as clustering partners for the anisole led to an increased red-shift, which was highest for diethyl-*o*-aniline.

In addition, the clusters containing substituted toluidines yielded spectra more closely resembling the spectrum of the anisole-dimer while spectra of those containing the substituted anilines displayed additional features, thus indicating substitution on the ring system has a bigger influence then substitution on the amino-group.

Due to the large difference in the electronic origin between anisole (36371 cm^{-1}) and the investigated aniline-derivatives (the highest being 34071 cm^{-1} for aniline), two separate electronic origins are to be expected for the clusters. Thus far, only transitions close to the electronic origin of the anisole-monomer could be observed for the respective clusters. Clustering behaviour between the different aniline-derivatives (excluding the anisole) is subject of current investigations.

Quantum-chemical calculations are pending to gain deeper insight into the structure of these newly formed clusters.

IPC-14: Fragmentierung von 3,4-Dimethoxy-4'-Dimethylamino-azobenzol mittels CID und PD

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Einleitung

Azobenzole spielen in der Farbstoffindustrie immer noch eine große Rolle. Im Rahmen dieser Arbeit wurde die Fragmentierung eines Azobenzols mit einer benachbarten Dimethoxygruppe mittels sustained off-resonance irradiation collision-induced dissociation (SORI-CID) und Photodissoziation (PD) untersucht. Es zeigte sich ein ungewöhnliches Fragmentierungsverhalten der Dimethoxygruppe in Abhängigkeit von der verwendeten Wellenlänge. Mit Hilfe von kinetischen Messreihen wurde diese Fragmentierung weiter untersucht, sowie die Zahl der absorbierten Photonen und kinetische Konstanten ermittelt.

Experimentalteil

Das verwendete Azobenzol wurde mittels Azokupplung hergestellt. Die Messungen erfolgten an einem 9,4 T APEX-IV-Qe FT-ICR Massenspektrometer (Bruker, Bremen). Als ESI-Lösungsmittel diente eine Mischung aus Ethanol, Wasser und Ameisensäure (50/50/0,2). Der Molekülpeak wurde isoliert und die Aktivierung der Ionen erfolgte zum einem mittels SORI-CID unter Verwendung von Argon als Stoßgas, zum anderen mittels PD. Dazu wurde ein cw-Argon-Ionen-Laser (Innova 70C, Coherent, Santa Clara, USA) in die ICR-Zelle eingekoppelt. Mit Hilfe eines Shutters konnte die Einstrahlzeit zwischen 0,2 s und 2 s verändert werden. Die Messungen wurden im Multiline- sowie im Singleline-Modus durchgeführt.

Ergebnisse und Diskussion

Im CID- und PD-Spektrum treten neben den erwarteten Methylabspaltungen auch Fragmente auf, bei denen das Molekül an der Azogruppe gespalten wird. Beim intensivsten Fragment handelt es sich um das Dimethoxyphenyl-Kation.

Die Laserleistung wurde variiert und darüber die Anzahl der benötigten Photonen bestimmt. Die Abspaltung des Dimethoxyphenyl-Kations benötigt bei 514 nm ein Photonen. Bei 476 nm wird hingegen ein Zweiphotonenprozess beobachtet. Dies könnte mit einem anders verlaufenden Reaktionsmechanismus begründet werden. Der kombinierte Verlust der Anilingruppe und des Stickstoffes (N_2) ist eine mögliche Erklärung für den Zweiphotonenprozess. Das Zwischenprodukt wird im ICR in niedriger Intensität beobachtet. Dieses Fragment weist darauf hin, dass die Bindung zur Azogruppe gebrochen wird und deutet darauf hin, dass die Protonierung am Stickstoffatom erfolgt.

Zudem wurde die Einstrahlzeit variiert und somit die Kinetik der Fragmentierung untersucht. Mit den erhaltenen Ratenkonstanten kann eine genauere Erklärung des ablaufenden Reaktionspfads erfolgen. 3,4-Dimethoxy-4'-Dimethylamino-azobenzol zeigt viele Fragmentierungen und im PD-Spektrum hat die Weglänge einen großen Einfluss auf die Bildung der Fragmente.

IPC-16: Oxidation of Tetraphenyldihydrodiazapentacene Studied by ESI-MS(/MS)

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For mechanistic and kinetic studies, spectrophotometric methods are well established. In the last decades, Electrospray Ionisation Mass Spectrometry (ESI-MS) has become a promising method for these investigations as well.[1] We report on the oxidation of tetraphenyldihydrodiazapentacene (TPDAP-H_2) to tetraphenyldiazapentacene (TPDAP) using silver(I) in solution. This system is particularly interesting for ESI-MS analysis, because silver(I) cations are both involved in the oxidation reaction in solution and additionally act as a charge tag for the molecules under study by complexation. The silver adducts of the TPDAP-H_2 reactant and the oxidized TPDAP product show distinctly different energy requirements for dissociation in energy-resolved collision-induced dissociation (CID) experiments. A breakdown graph with both species present, leads to a distinct plateau. The analysis of this plateau was used to monitor the reaction progress. Additionally, the fraction of TPDAP-H_2 and TPDAP was calculated from the isotope pattern of the MS^1.

MS and MS/MS data were recorded as a function of the reaction time using q-ToF instrumentation, with the initial TPDAP-H_2 complexes gradually decreasing and turning into TPDAP complexes. We monitored the fragmentation of the dimeric- and monomeric complexes of the molecules (M) with silver(I) ions, i.e. MAg^+ and MAg^+M. The measurements were performed under the influence of light as well as in the dark. In day light, the oxidation reaction was completed within seven hours, while in the dark the complete conversion of TPDAP-H2 into TPDAP took nine days.

The poster discusses mechanistic aspects of the silver(I)-induced oxidation of dihydrodiazapentacenes and provides insight into the kinetics of the reaction. The data obtained by MS/MS (energy-resolved CID breakdown graph) are carefully compared with those obtained in MS^1 mode by isotope pattern analysis. While the MS/MS data were in reasonable agreement with each other, the data obtained by MS^1 isotope pattern analysis deviated more strongly.

References:

[1] D. Schröder, *Acc. Chem. Res.* **2012**, *45*, 1521–1532.

Lipidomics: Techniques and Applications

LIP-02: Investigation of Unsaturated γ-Lactones Found in Riboflavin Fermentation Broths Using Paternò-Büchi Functionalization

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Riboflavin, also known as vitamin B_2, is a common additive for food. It is produced industrially from the fungi *Ashbya gossypii* and *Bacillus subtilis* whose fermentation broths emit an intense fruity flavor. This flavor mainly stems from lactones and is influenced by their chemical structure and, in particular, by their C=C double bond (DB) position. Therefore, these lactones are used as natural food additives, meeting the increasing demand of the industry and customers. Currently, no industrial process for the microbial production of certain lactone compounds is available. For this reason, using culture broths arising from riboflavin fermentation as a source for natural flavor compounds would be advantageous.

The aim of this work was to develop a mass spectrometry (MS) based workflow in order to differentiate DB positions in unsaturated γ-lactones directly in the *A. gossypii* fermentation broths using Paternò-Büchi mass spectrometry (PB-MS). MS experiments including collision-induced dissociation of lactones mainly resulted in water losses from protonated precursor ions but no fragments specific for the DB position were detected. To overcome this issue, we applied a PB functionalization nano-electrospray ionization (nanoESI) workflow employing 3-acetylpyridine (3-acpy) for DB position determination that did not require lactone separation prior MS analysis.

3-Acpy was mixed with fermentation broth extracts and the acidified sample solution was loaded into nanoESI capillaries. A voltage of about +800 V with respect to the mass spectrometer's inlet was applied to the sample solution using a platinum wire to generate protonated analyte ions. For PB functionalization, the capillary was irradiated with 254 nm UV light from a pencil lamp in order to initiate PB functionalization between 3-acpy and unsaturated lactones. In the resulting mass spectra, *m/z* values consistent with the addition of protonated 3-acpy to unsaturated lactones were detected. Subsequent tandem MS experiments of photoproduct ions yielded retro-PB fragments consistent with the formation of oxetanes upon UV irradiation. Retro-PB fragment ions were used to pinpoint DBs in five structurally diverse unsaturated γ-lactones. The results were in

accordance with experimental data obtained from gas chromatography experiments of synthesized lactone standards and PB-MS of authentic standards.

In this work, we extended the PB functionalization workflow to metabolites found in *A. gossypii* fermentation broths. The method is suitable for shotgun MS experiments and requires no tedious sample preparation or separation steps for fast screening of unsaturated metabolites.

LIP-04: Lipid profiling of *Pseudomonas* bacteria using heart-cut two-dimensional chromatography coupled to HRMS

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Biosurfactants are a more eco-friendly and biodegradable alternative to commonly used petrochemical-based surfactants. Possible producers of biosurfactants are *Pseudomonas* bacteria, which produce rhamnolipids. Genomic adaptation made it possible to use the non-pathogenic *Pseudomonas* species *Pseudomonas putida* (*P. putida*). For tailored applications, the understanding of lipid metabolism in *P. putida* is important. Since rhamnolipids possess amphiphilic properties, an influence on membrane fluidity is suspected. The fluidity of cell membranes, which are mainly composed of amphiphilic phospholipids, is influenced by the comprising lipids' structure, i.e. the chain length of the fatty acyls and their double bond number as well as double bond configuration. This work aims to analyse lipid changes induced in *P. putida* during rhamnolipid production by high resolution mass spectrometry (HRMS).

As a main component of *P. putida* cell membranes, a targeted analysis of phosphatidyl-ethanolamines (PE) using heart-cut two-dimensional liquid chromatography was performed. In the first dimension phospholipids are separated based on their polar head group using hydrophilic interaction liquid chromatography (HILIC). After heart-cutting of the PE fraction, a further separation based on hydrophobic interactions was achieved on a C18 reversed phase. This second dimension can also give information on isomeric lipid species, i.e. *cis* and *trans* isomers. Accurate mass and MS/MS experiments enabled identification of the lipids.

The application of the developed method to *P. putida* with and without rhamnolipid production shows altered PE levels between the two groups. In addition, isomeric species can be separated, indicating the presence of *cis*/*trans* isomers or methyl-branched lipid species. A complementary GC-MS analysis gives further information on the fatty acid profile of a specific lipid class and subsequent hydrolysis and derivatization to the corresponding fatty acid methyl esters.

LIP-06: CCSPredict: Using a Machine Learning Approach for Higher Confidence in Lipid Identification

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Trapped ion mobility (TIMS) MS offer new options for higher confidence in annotations of target molecules. First, with the additional separation dimension compounds co-eluting from LC columns can be separated. This can result in cleaner MS/MS spectra which are crucial for any ID in small molecule workflows. Second, TIMS enables the determination of collisional cross sections (CCS). These are specific properties for any ion species and provide increased confidence in compound identification if compared to references or to predicted values as generated by machine learning (ML) algorithms. Lipid Standards of the Lipidomix® Kit (Avanti Polar Lipids, Inc.) were dissolved in MeOH:DCM (9:1). Milk samples were extracted by a modified Bligh & Dyer method. A Bruker Elute UHPLC system (20 min gradient program) was coupled to the Bruker timsTOF pro with and without TIMS separation in ESI positive and negative autoMS/MS modes. CCS calibration was performed using TuneMix (322 - 1221 m/z). Data sets were processed by MetaboScape (Bruker) using the Time aligned Region complete eXtraction (T-ReXTM) algorithm.Statistical analysis, molecular formula annotation, MS/MS spectral library queries using LipidBlast [2, 3, 4] and prediction of CCS values were conducted in the same integrated client/server software solution. timsTOF Pro instruments provide highly reproducible CCS values (0.17% RSD). MetaboScape enables prediction of lipid CCS values based on machine learning. CCS values increase confidence in lipid annotation as orthogonal information in addition to accurate mass, true isotopic pattern, MS/MS and retention time information. MetaboScape provides a fully integrated solution for confident lipid annotation.

LIP-08: Investigation of Lipid A species in gram-negative bacteria by LC-MS/MS

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The lipopolysaccharide layer is a main component of the outer membrane from gram-negative bacteria. It consists of three regions: an O-polysaccharide, which extends outwards from the cell surface, an oligosaccharide core, and the membrane-anchored Lipid A moiety. Lipid A is the part that can be sensed by humans and animals to detect the presence of gram-negative bacteria in their tissues and plays a key role in the pathogenesis of bacterial infections.

Lipid A features a β(1,6)-linked glucosamine backbone, having fatty acids substituted at the 2, 3, 2', and 3' positions and zero, one or two polar groups like phosphate groups linked to the 1 and 4' positions. The toxicity of Lipid A is strongly influenced by its primary structure, speaking of the number and length of the linked fatty acids, as well as the level of phosphorylation. A great degree of structural diversity is not only observed between distinct bacteria but also within individual species. These dynamic modifications help the bacteria to survive under various conditions, such as different temperatures or growing media compositions. Therefore, several Lipid A structures can be observed in a single organism. The environmental factors can be manipulated to engineer specific Lipid A molecules and use these for the development of therapeutics such as vaccine adjuvants.

In this work, we present a new method to distinguish and characterize various Lipid A species originating from different gram-negative bacteria. Cells of *Escherichia coli* (*E. coli*), *Pseudomonas putida* (*P. putida*) and *Pseudomonas taiwanensis* (*P. taiwanensis*) have been extracted and analysed by LC-MS/MS. The Lipid A species were identified using high-resolution mass spectrometry and tandem-MS experiments.

Eight Lipid A species could be identified in the *E. coli* extract. They vary in their bound fatty acids and their degree of phosphorylation. Four of these structures were confirmed via MS/MS fragmentation. In *P. taiwanensis* 17 different Lipid A molecules have been detected, with ten of these also occurring in *P. putida*. 13 and six, respectively, of these structures were observed for the first time. Detailed structural elucidation by tandem-MS and confirmation of the bound fatty acids via GC-MS are carried out.

LIP-10: Differences in the lipid patterns during maturation of 3T3-L1 adipocytes investigated by thin-layer chromatography, gas chromatography and mass spectrometric approaches

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Populations of industrialized countries have registered a dramatically increasing prevalence in obesity since many years. According to the WHO, obesity and related diseases such as stroke, heart attack and even cancer are among the most important causes of death in the industrialized countries. In addition, the related medical treatments result in enormous costs. Despite continuous research, mechanisms involved in the storage of chemical energy and utilization in adipocytes are still poorly understood. Adipocytes have the task to store excessive energy in the form of triacylglycerols (TG) and it is already well-known that the fatty acyl (FA) composition of TG is largely determined by the composition of the consumed diet. In contrast to TG, the composition of adipocyte phospholipids was less comprehensively investigated.

In this study the compositions of the most abundant phospholipid classes of 3T3-L1 undifferentiated (preadipocytes) and differentiated cells (adipocytes) were determined. The lipid fractions were isolated by normal phase high performance thin-layer chromatography (HPTLC) and subsequently analyzed by electrospray ionization mass spectrometry (ESI-MS). Additionally, the FA compositions were determined by gas chromatography (GC). The positions of the FA residues were further confirmed by phospholipase A_2 digestion of the respective isolated phospholipid classes. It will be shown that undifferentiated 3T3-L1 and mature adipocytes differ extremely regarding their compositions. This goes along with an increase in odd chain fatty acids which indicates the use of propionyl-CoA instead of acetyl-CoA for the fatty acids synthesis.

In addition to the biological background, we also addressed the question which analytical method (ESI-MS, HPTLC and GC) is most suitable to assess the lipid composition of complex samples.

LIP-12: Leishmania contain phosphatidylserine, possibly responsible for host-invasion mechanism

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Leishmaniasis is a diverse vector-borne parasitic disease caused by different species of the protozoan genus Leishmania. Most prominent examples are *L. major,* causing cutaneous *leishmaniasis* and *L. donovani,* leading to visceral *leishmaniasis*. The infection of a host upon a bite of infected female phlebotomine sandfly is a crucial step in the life cycle of the parasite. While in the sandfly vector, *leishmania* are present as promastigotes, they transform into amastigotes inside the host. One possible invading mechanism involves uptake of living and apoptotic promastigotes by the host organism. The apoptotic promastigotes with phosphatidylserines on their outer membrane leaflet trigger an “eat-me” signal, recognized by macrophages, thus living and apoptotic promastigotes can invade the host. In literature, Annexin V was typically used to validate PS on the outer leaflet on the membrane. Annexin V is, however, not specifically binding to PS. While Pomorski *et al*. claim that there are no PS species in the promastigote stage, we can show the opposite for *Leishmania major* promastigotes. First, after MTBE extraction of apoptotic promastigotes, the lipid extract was analyzed by ultra-high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). For HPLC analysis, a C_{18} reversed-phase column was used, coupled to a high resolution orbitrap mass spectrometer (Q Exactive HF-X, Thermo Fisher Scientific). Additional matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) measurements of the parasite prior to extraction were done. In a second step, living promastigotes of the logarithmic growth phase (0% binding to Annexin V) and apoptotic ones of the stationary phase (about 80% binding to Annexin V) were analyzed separately by HPLC-MS/MS with addition of PS(16:0/18:1) as internal standard. In the first experiments in total four PS species were identified for apoptotic promastigotes by negative-ion HPLC-MS. The MALDI MS experiments confirmed the presence of PS by accurate mass. MS/MS during HPLC-MS/MS allowed determination of the fatty acid composition of PS species. Next, comparison of living promastigotes in the logarithmic growth phase and apoptotic ones of the stationary phase showed that PS(18:1_22:5), PS(18:2_22:5) and PS(18:3_22:5) were present in both samples. However, the species PS(22:5_22:5) and PS(22:5_22:6) were exclusively detected in the stationary phase. As control no PS was present in the culture medium. The results indicate the presence of PS species in the stationary phase as well as in the logarithmic growth phase, thus supporting the hypothesis that hosts take up apoptotic promastigotes.

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LIP-14: Analysis of Lipid Signaling Class Analytes Using a Travelling Wave Cyclic Ion Mobility Separator (cIM)

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Lipid signaling analytes represent a diverse group of biomolecules that have essential roles in structural, storage and signaling processes in living systems. Class separation is readily achieved using chromatographic and MS based identification techniques; however, the analysis remains challenging due to the chemical structure diversity and isobaric nature of these types of compounds. The addition of IMS enhances system peak capacity and improves isomer resolution. IM separation was achieved using a multi-pass cyclic IM-device, where increasing the number of passes around the device allows of the increase in mobility resolution. MS and CID fragmentation data were obtained on precursor IM separated analytes followed by ToF detection.
Unsaturated free fatty acids (FA), differing in chain length and number of cis/trans configurations, steroid hormones, isomers differing in the positon of a functional group, and isobaric lipid mediators (prostaglandins) were chosen to determine the degree of IM separation required to separate isomers and isobars. Data were collected on a cyclic ion mobility-enabled QToF (Q-cIM-oaToF). In all experiments, the isomeric and isobaric compounds were introduced as two or three component mixtures by direct infusion. Both precursor and product ion CCS data were collected, facilitated by a multifunction region within the cIM device.

In all direct infusion cIM-MS measurements, cis oriented FAs were found to be more compact than those with trans-orientations. A different number of cycles through the cIM -device, thereby increasing the effective path length/resolution, were required to achieve a similar degree of IM separation for mono-unsaturated FAs of differing chain length. The required IM resolution (Ω/ΔΩ) values typically ranged from 100 to 350. Unsaturated FAs with two or more double bonds, separated by two mid-chain carbons, could not be distinguished. Shorter, structurally more rigid and compact FAs were discriminated at reduced resolution, as could longer chain mono-unsaturated FAs as a result of partial chain back-folding. Following IM separation, isomeric FAs were successfully CID fragmented and identified.

The analysis of 17-hydroxyprogesterone and 21-hydroxyprogesterone indicated that an IM resolution (Ω/ΔΩ) of at least 200 was required to achieve baseline separation. Following IM separation, nearly identical, but individual product ion spectra were detected, arguing the need for the separation of these types of compounds. A three compound isomer steroid mixture consisting of 11-deoxycortisol, 21-deoxycortisol, and corticosterone was partially resolved. The IM separation was sufficient to extract product ion spectra for the three individual components and establish their identity using informatics methodology.

LIP-16: Similarities of lung tissue lipidomes: what are the main physiological determinants of the human lung lipidome?

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Across species, the mammalian lung is largely conserved in its anatomy and physiology. The functionality of the lungs is accompanied by a specific lipid composition, which must be retained by maintaining homeostatic conditions. However, the biological boundaries of how the lung lipidome can vary to retain a functional state are still largely unexplored. Within the framework of a systems biology approach, we investigate how environmental factors and diseases, but also physiological parameters such as age, gender and BMI influence the overall composition of the lipidomes.

In a cross-species meta-analysis we investigated differences in the lipidome between resected lung tissues of 30 male human adenocarcinoma patients, 16 male sheep of similar age and weight with the breed “German Blackheaded Mutton”, 8 female mice of similar age and weight of the breed “BALB/c” and 20 pig lipidomes of similar age and weight and of breeds “German Large White” and “German Landrace”. Human samples were split into cancerous tissue and cancer-free tissue. Tissue of humans, mice, pigs and sheep were histopathologically characterized and parameters of emphysema grade, fibrosis and inflammation identified.

We catalogued the lung lipidomes of the animal models and human samples using the shotgun lipidomics approach in positive and negative ESI mode. More than 500 lipid species from 16 lipid classes were quantified, with a core lipidome of 150 lipids present in at least 80% of all specimen, representing the categories of glycerolipids, glycerophospholipids, sphingolipids and sterol lipids.

We present the results of hierarchical clustering, volcano plots and OPLSR as well as dimensionality reduction methods PCA and UMAP to define and cluster biological species and data sets according to the similarities of the quantified lipid species. In this study we were able to clearly separate different biological species and found that the differences between species clearly outweighed the intrinsic variability in one biological species.

LIP-18: Combined lipid- and transcriptomic profiling in inflammatory processes

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Combined lipid- and transcriptomic profiling in inflammatory processes

Mass Spectrometry in Physics

PHY-02: Investigation of the catalytic effect of manganese (II) on lithium ion battery electrolytes *via* ion chromatography hyphenated to mass spectrometry

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Since the first lithium ion battery (LIB) was implemented by Sony in 1991, it became the first choice for portable electronical devices, energy storage systems and electric vehicles. The state-of-the-art LIB consists of a transition metal-based cathode, a graphitic anode, a carbonate-based electrolyte with dissolved lithium salt, a separator and current collectors. The lithium-nickel-cobalt-manganese oxide (NCM) cathode material shows several performance advantages. However, lifetime and capacity fading in LIBs are mainly affected by detrimental reactions of LIB components, the so-called aging. The resulting transition metal dissolution (TMD) from NCM due to impurities in the host lattice, particle cracking and phase transformation leads to the growth of protection layers at the anode/electrolyte interface, which results in an increased active lithium loss, capacity fading, and less specific energy of the battery.[1] Furthermore, the LIB electrolyte is able to react with battery components leading to safety problems and capacity loss. Due to chemical and thermal instability of lithium hexafluorophosphate, the conducting salt decomposes and is able to react with carbonates to immense variety of aging compounds, which are potential toxic.[2]

While numerous studies deal with causes of TMD and effect on anode, an effect of dissolved transition metals on electrolyte decomposition has been neglected so far. Therefore, the catalytic influence of manganese (II) ions on electrolyte aging mechanisms by means of ion chromatography hyphenated to mass spectrometry are shown in this work. It could be shown, that manganese (II) destabilize carbonate-based electrolytes, resulting in a stronger electrolyte decomposition and increased formation of ionic decomposition products.

References:

[1] Evertz, M., F. Horsthemke, J. Kasnatscheew, M. Börner, M. Winter, and S. Nowak, *Journal of Power Sources*, **2016**, *329*, 364-371.

[2] Hammami, A., N. Raymond, and M. Armand, *Nature*, **2003**, *424*, (6949), 635-636.

PHY-04: Radionuclides from Space

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The determination of long-lived radionuclides by means of accelerator mass spectrometry (AMS) is usually outstandingly successful when an interdisciplinary team comes together. The “heart” of AMS research is of course an accelerator equipped with sophisticated ion sources, analytical tools and detectors run by experienced and ambitious physicists. Setting-up and further developing AMS systems is one of the most interesting and challenging topics.

Another essential part in AMS research is the radiochemical sample preparation preceding the measurement where the goals are: 1.) Enrichment of nuclides of interest by reduction of the matrix. 2.) Depletion of isobars. 3.) Production of a thermally stable chemical compound such as AgCl, AgI, Al_2O_3, BeO, CaF_2, Fe_2O_3, MnO_2 etc. of relatively high purity.

One of the most interesting applications of AMS is the analysis of extraterrestrial material such as meteorites. While being at the surface of their so-called parent body (asteroids, Moon, Mars,…) and again while travelling through space as a so-called meteoroid, these unique pieces are bombarded by high-energy particles from the cosmic radiation. Long-lived radionuclides are produced in the material by nuclear reactions in both stages potentially until saturation. However, they start decaying in a third stage, when meteorites have landed on Earth because the cosmic radiation is shielded by the Earth’s atmosphere and magnetic field. Hence, the concentrations of radionuclides are records of all three stages allowing the reconstruction of the exposure history (duration, shielding, size,…) of the individual meteorite and the cosmic radiation itself.

Meteorite projects and projects with artificially-irradiated targets are also well-suited to develop measurements of “new” AMS radionuclides as the isotopic ratios are at much higher levels (up to 10^{-10} radioactive/stable) than e.g. in terrestrial natural samples (10^{-14}-10^{-16}). The AMS community is very open to any input and questions from “outside”. The DREsden AMS (DREAMS) and other European AMS facilities offer researchers from academia free measurements via a Trans-National-Access proposal program (www.ionbeamcenters.eu) and also national access (www.dresden-ams.de; DREAMS only).

Metabolomics: Techniques and Applications

MET-02: Clarification of decomposition pathways in a state-of-the-art lithium ion battery electrolyte through ^{13}C-labeling and LC-HRMS^2

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In recent years, enormous amounts of information were gained for research fields with highly complex issues (*e.g.*, lipidomic, proteomic and metabolomics) by means of liquid chromatography (LC) hyphenated to high resolution mass spectrometry with fragmentation possibilities (HRMS^2). In this work, LC-HRMS^2 capabilities were used for the clarification of degradation pathways of different electrolyte decomposition classes with >300 species identified in lithium ion batteries (LIBs).[1,2]

The electrolyte degradation is one of many aging phenomena in LIBs and can be initiated by redox reactions at the electrode surfaces, thermal strain as well as protic impurities.[3] The coherence of electrolyte decomposition resulting in capacity loss is

an emerging research topic. In particular, the formation of electrode-electrolyte interphases (preventing further electrolyte decomposition) is a crucial process for the life-time of the whole battery system. The exact composition of these interphases is still under investigation and only some inorganic and organic components are known; which are electrolyte decomposition products. Therefore, the LC-HRMS^2-based identification of electrolyte soluble decomposition species can contribute to obtain conclusions about interphase compositions. Consequently, knowledge of the origin of decomposition products can be used to modify electrolyte formulations for improved interphase formation, hence advanced battery performance.

Since state-of-the-art electrolytes commonly comprise a conducting salt (lithium hexafluorophosphate), cyclic organic carbonates (*e.g.*, ethylene carbonate) as well as linear organic carbonates (*e.g.*, diethyl carbonate), the origin of decomposition products can be deciphered *via* ^{13}C-labeling of electrolyte components.$^{13}C_3$-labeled ethylene carbonate was applied in LIB electrolytes and the occurring decomposition products were investigated after two charge/discharge cycles as well as after 500 cycles by means of LC-HRMS^2.

References:

[1] J. Henschel *et al.*, *Chem. Mater.* **2019**, 31 (24), 9970-9976.

[2] J. Henschel *et al.*, *Chem. Mater.* **2019**, 31 (24), 9977-9983.

[3] S. Nowak *et al.*, *J. Electrochem. Soc.,* **2015**, 162, A2500-2508.

MET-04: Metabolomics-based identification of specific biomarkers for bell pepper intake in human urine

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Nutritional biomarkers are food constituents or their metabolites which are detectable in urine or blood after consumption of a particular food. Ideally, these biomarkers allow for the objective and quantitative determination of the human food intake and can potentially be used as a complementary tool to possibly biased nutrition diaries or questionnaires. The combination of both methods facilitates and improves the assessment and evaluation of the individual nutritional status.

The present study aimed at the identification of potential urinary biomarkers for bell pepper intake. Bell peppers belong to the top five fresh vegetables consumed in Germany with a significant proportion of 8,3% of the total fresh vegetable consumption [1]. In a preliminary study with a total of 14 volunteers, urine samples were collected after several days of abstinence from bell peppers and pepper products (control group) and after consumption of a defined amount of fresh bell peppers (case group). To compare the samples of both groups, they were analyzed by high-performance liquid chromatography coupled with high-resolution mass spectrometry. After raw data processing with MZmine 2 [2], discriminating features, depending on the *m*/*z*, the retention time and the intensities of the peaks, were identified by statistical analysis using MetaboAnalyst [3] to find potential biomarkers.

The data evaluation of the preliminary study for the determination of biomarkers in urine after bell pepper consumption showed significant differences in the urine metabolome of the control and the case group. Based on principal component analysis, the urine metabolome of both groups was clearly distinguishable even 20-24 h after bell pepper intake. By comparison of the MS data several features were revealed as potential biomarkers for the consumption of bell peppers, many of which represent glucuronides according to their mass spectrometric fragmentation. These unknown glucuronides are currently being identified based on their exact mass, isotopic pattern, MS/MS data and database comparison, and will be subsequently investigated regarding their suitability as valid biomarkers for bell pepper consumption.

[1] BMEL, Statista, **2018**, https://de.statista.com/statistik/daten/studie/695032/umfrage/anteile-der-gemuesesorten-am-gemuese-absatz-in-deutschland/ (accessed 25.06.2019).

[2] T. Pluskal, S. Castillo, A. Villar-Briones, M. Oresic, *BMC Bioinform.* **2010**, 11:395.

[3] J. Chong, O. Soufan, C. Li, I. Caraus, S. Li, G. Bourque, D.S. Wishart, J. Xia, *Nucleic Acids Res.* **2018**, 46, W486-494

MET-06: Instrument comparison of non-targeted UHPLC-HRMS analysis for wine authentication

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Non-targeted, mass spectrometry-based analytical approaches are increasingly used in various fields of science, e. g. food authentication. In combination with multivariate data analysis, they enable the comprehensive characterization of a product and are therefore suitable for investigating all kinds of authenticity questions (e. g. variety, origin, adulteration). However, a number of requirements must be fulfilled prior to the implementation of such non-targeted analytical approaches in official control. The most important requirement, especially with regards to jointly used databases, is the comparability between instruments and laboratories.

In this study, using the example of grape variety differentiation in wine, the comparability of two identically constructed mass spectrometers was investigated. Ultra high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) was used to analyze a sample set of 201 monovarietal red and white wines in two laboratories following the same protocol. First results regarding the differences in chromatograms and the individual data evaluation using Principal Component Analysis and supervised classification models will be presented.

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MET-08: In vitro metabolism of *Arnica* sesquiterpene lactones

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Arnica montana preperations are used for topical treatment of injuries and inflammations, as well as rheumatic muscle and joint complaints [1]. In recent studies, an ethanolic tincture of *A. montana* flowers effectively cured cutaneous leishmaniasis (CL) in a golden hamster model. Leishmaniasis is one of the 20 communicable diseases currently classified by WHO as neglected tropical diseases [2]. To use *Arnica* preparations on open wounds such as CL lesions, absorption, distribution, metabolism and excretion of *Arnica* constituents have to be analysed. Therefore the analytes must be quantified with UHPLC-Qq-TOF MS and GC-Qq-TOF MS in urine, plasma, feces and skin of infected and treated golden hamsters. In vitro metabolism experiments with rat liver microsomes showed conjugation reactions with glutathione (GSH) and indicate, besides GSH-conjugates, the formation of a thioglycolate. Further experiments with rat liver microsomes instead of the S9 mix are in progress. To enable highly sensitive UHPLC-Qq-TOF MS and GC-Qq-TOF MS measurements, salts, proteins and phospholipids need to be removed from biological matrices. Different sample preparation techniques, e.g. salting out assisted liquid liquid extraction (SALLE) and hydrophilic lipophilic solid phase extraction (HLB-SPE), for the analysis of *Arnica* sesquiterpene lactones in biofluids were investigated. With all techniques, samples of *Arnica* tincture (10% in urine, corresponding to 50 µg/mL sesquiterpene lactone (STL) content) showed the typical STL pattern which is composed of helenalin as well as 11,13-dihydrohelenalin and their esters. However, sample preparation methods differed in their suitability for the processing of samples with low analyte concentrations. Rodent excretion, tissue and blood plasma are ready to be analyzed for the unchanged STLs and their metabolites after dermal application of *Arnica* tincture. Furthermore, other matrices like gauzes used during rodent treatment are intended to be analyzed.

[1] Schmidt TJ, et al. (2000). Planta Medica 66(7): 678-681.

[2] Robledo SM, et al. (2018). Molecules 23(1): 150.

MET-10: A comprehensive metabolomics and lipidomics profile of ischemic stroke

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Ischemic stroke is one of the leading causes of mortality and adult disability worldwide. Although there has been great progress over the last years the underlying metabolic alterations and processes related to stroke are not yet fully understood. Therefore, we performed an untargeted metabolomics and lipidomics analysis using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to determine metabolic alterations between ischemic stroke patients and healthy controls.

LC-MS/MS is the method of choice for untargeted analysis due to outstanding sensitivity and high-confidence identification based on specific fragmentation patterns and prior separation via LC. Serum samples from 141 stroke patients were analyzed with two different approaches using a high-resolution accurate mass (HRAM) platform. Reversed-phase (RP-) LC and Hydrophilic interaction LC (HILIC) was conducted for lipidomics and metabolomics, respectively. Data-dependent data acquisition was performed using a hybrid quadrupole-Orbitrap mass spectrometer (Q-Exactive). Software based data analysis (TraceFinder, Compound Discoverer) included compound identification supported by an MS^2 in-house library.

We were able to detect and confirm 174 lipid species out of 11 lipid classes as well as 94 metabolites related to several metabolic pathways. Particularly noteworthy are the statistical differences observed in relation to the phospholipid metabolism, including alterations in numerous phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs) and phosphatidylethanolamines (PEs).

The combination of metabolomics and lipidomics analysis allows for the detection of a broad range of substances. Without hypothesis driven restrictions the untargeted approach enables the discovery of new biomarkers and compounds of interest. Earlier metabolomics or lipidomics studies of stroke have reported contradictory findings especially related to the phospholipid metabolism. Here we were able to detect a variety of different phospholipid species which benefits the more detailed interpretation of processes.

Due to the high number of identified compounds, comprehensive additional data - e.g. mRS/mRS90 and NIHSS scores - and follow-up sampling over four days after stroke for several patients, our data not only provide insights into acute effects following ischemic stroke but also into early prediction of post-stroke impairment and correlations to age, obesity or diabetes. These findings contribute to a better understanding of ischemic stroke and the underlying molecular and metabolic processes.

MET-12: AP-SMALDI MSI of *Besnoitia besnoiti* cysts in cattle

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Besnoitia besnoiti is an obligate intracellular, cyst-forming apicomplexan parasite which causes bovine besnoitiosis, an infectious disease that is currently considered as emerging in Europe. Bovine besnoitiosis leads to considerable economic losses in cattle industry and causes animal welfare impairment. Acute infections are characterized by unspecific signs, such as fever and anorexia, and are caused by tachyzoite stages. During the chronic phase of infection, large tissue cysts containing bradyzoites are mainly formed in the skin. As such, most prominent chronic symptoms are immense skin thickening (elephant skin), swelling of joints and chronic orchitis leading to sterility of the bulls. Currently, no treatment is available.

Sections (20 µm thick) of skin tissue were prepared using a cryomicrotome (HM 525, Thermo Fisher Scientific, Bremen) and coated with 100 µL DHB matrix (30 mg/mL in H_2O/Acetone 1:1 v/v, 0.1% TFA) for positive-ion mode using a pneumatic sprayer (SMALDIprep, TransMIT GmbH, Giessen). MS images were recorded using an AP-SMALDI^5-AF ion source (TransMIT GmbH) coupled to a Q Exactive high resolution orbital trapping mass spectrometer (Thermo Fisher Scientific) with 5 - 20 µm pixel size

and a mass resolution of R = 240,000 at *m/z* 200. LC-MS-based identification of lipid signals was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific).

Applying both, LC-MS and MALDI MS, it was possible to identify markers to distinguish *B. besnoiti*-infected from non-infected cells. In addition, stage-specific markers were identified for tachyzoites and bradyzoites in cell lines and skin sections, respectively. Some bradyzoite markers were assigned to specific parasite structures within the tissue, such as cyst wall or cyst content. For preliminary results, bradyzoite-related marker signals were partially identified by both, LC-MS and database search. In future, a more comprehensive molecular identification via LC-MS and further optimization of sample preparation process for measurements in negative-ion mode will be performed. Since cholesterol plays an important role in many metabolic pathways and given that *B. besnoiti* cannot synthesize cholesterol *de novo* but has to scavenge it from its host cell, cholesterol presence in parasitized skin was analyzed. Overall, cholesterol accumulation around the cysts was detected in skin samples, but these findings are preliminary and have to be confirmed in further biological replicates.

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MET-14: Probing carbonyl metabolome by liquid chromatography-mass spectrometry (LC-MS)

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Lipid peroxidation is one of the main sources of oxidative and lipoxidative modifications in cellular proteins. In plant tissues, abiotic stress, in particular, drought, results in enhanced generation of reactive oxygen species (ROS).It leads to increased production of reactive carbonyl compounds (RCC), i.e. aldehydes, ketones and keto acids. These products readily interact with cellular proteins, resulting in their covalent modification, the role of which in plant physiology is still unknown. On the other hand, in mammals, free radicals are among the major factors behind the pathogenesis of some chronic diseases, neurodegenerative disorders and aging. In this context, characterization of the reactive carbonyl metabolome in human plasma is the key to the identification of prospective biomarkers of metabolic diseases like type 2 diabetes mellitus (T2DM).

To address the dynamics of plant carbonyl metabolome, seven-week old *A. thaliana* plants were exposed to experimental drought for three–ten days. Afterwards, the leaves were harvested, frozen, ground and extracted with methanol. The RCCs, present in methanol extracts, were derivatized with 7-(diethylamino)-coumarin-3-carbohydrazide (CHH). After incubation, derivatives were extracted with methyl tert-butyl ether (MTBE) and analysed by ultrahigh-performance liquid chromatography-electrospray ionization-linear ion trap-Orbitrap mass spectrometry (ESI-LIT-Orbitrap-MS). To address the differences in carbonyl contents related to a metabolic disease, blood plasma samples were obtained from 20 type 2 diabetes mellitus (T2DM) patients and from 20 nondiabetic individuals. The 10 µL aliquots of each blood plasma sample was used for derivatization of RCCs with CHH and further extraction with MTBE. Pooled samples from plant extracts and those from human plasma were used as quality controls, i.e. were repeatedly analysed within the sample batch. For Arabidopsis carbonyl metabolome, based on their degradation kinetics, all carbonyl derivatives could be distributed in five clusters. As a result, more than 600 HRCCs were annotated and characteristic profiles of their dynamics were described. To compare the abundances of individual RCCs in the control and experimental groups, label-free quantification was employed. The statistical analysis includes variance stabilizing normalization, Welch's t-test, followed by Benjamini and Hochberg correction for multiple comparisons. It was shown that general content of RCCs in control plants is higher in comparison to the drought-treated ones. For example, 16 from 50 the most abandunt carbonyl compounds showed significant difference between the stress and control groups. Thereby, only six of them demonstrated an abundance increase in response to stress.

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MET-16: Differentiation of dihydroxylated vitamin D_3 isomers using tandem mass spectrometry

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Vitamin D compounds are a group of fat-soluble secosteroids, which are vital for maintaining bone health in humans. In particular, recent studies have shown that the dihydroxylated vitamin D_3 compounds: 1,25-dihydroxyvitamin D_3 (active form of vitamin D) and 24,25-dihydroxyvitamin D_3 (inactive form) have significant biological effects, playing a role in diseases such as osteoporosis.

Identification and differentiation of the isomers by mass spectrometry can be challenging due to the zero mass difference and minor structural differences between them. The isomers may require separation by liquid chromatography (LC) with the presence of a derivitization agent, which can add extra complexity to the spectra and tandem mass spectrometry using collisional activated dissociation (CAD) also shows no significant differences in the spectra of the two isomers.

Here, we investigated the use of alternative fragmentation methods such as electron induced dissociation (EID) and ultraviolet photodissociation (UVPD), available on a Bruker 12T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). EID uses high energy electrons generated from a hollow dispenser cathode to fragment the ions. UVPD is implemented using a 193 nm ArF excimer laser, which has shown to result in extensive fragmentation of peptides, proteins, lipids and other small molecules.

Isomer specific fragments were observed for 1,25-dihydroxyvitamin D_3, which were clearly absent in the 24,25-dihydroxyvitamin D_3 EID and UVPD spectra and vice versa. The fragments generated due to cleavage of the C-6/C-7 bond in the 1,25-dihydroxyvitamin D_3 compound demonstrate that the OH groups were retained during EID and UVPD MS/MS.

In summary, a number of diagnostic fragments were observed, enabling quick and easy differentiation between the two dihydroxylated vitamin D_3 isomers without the need for prior chromatographic separation.

Natural Product Mass Spectrometry

NAT-02: On the structural diversity of Shiga toxin-binding glycosphingolipids of porcine kidney epithelial cells determined by immunochemical detection and mass spectrometry

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Introduction: The Shiga toxin (Stx) of subtype Stx2e, released by certain swine-pathogenic Stx-producing *Escherichia coli* (STEC) strains, cause the edema disease in piglets shortly after weaning. Stx2e damages mainly endothelial cells of the kidney, whereas the involvement of epithelial cells in the disease is largely unknown. Stx2e binds to the glycosphingolipids (GSLs) globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer). The detailed structural characterization of Stx2e-binding GSLs of porcine epithelial cells of the kidney has been so far understudied.

Objective: The aim of the study was a comprehensive structural characterization of Stx2e-binding GSLs of porcine renal epithelial cells by means of immunochemical detection combined with mass spectrometry.

Materials and Methods: GSLs were isolated from the porcine kidney epithelial cell line PK-15, which was cultivated *in vitro* under serum-free conditions. Gb3Cer and Gb4Cer were detected by thin-layer chromatography (TLC) overlay analysis using anti-Gb3Cer and anti-Gb4Cer antibodies as well as Stx2e together with an anti-Stx2e antibody. The antibody-positive GSLs were extracted from the silica gel of the TLC plates, and their precise structures were elucidated by nano electrospray ionization mass spectrometry (nanoESI MS) using a SYNAPT G2-S mass spectrometer equipped with a Z-spray source in the positive ion sensitivity mode.

Results: The PK15 cells were found to exhibit the two Stx receptors Gb3Cer and Gb4Cer occurring in different lipoforms. Their structural heterogeneity was found to derive from various ceramides composed of the dihydroxylated monounsaturated long-chain aminoalcohol sphingosine (d18:1) as the invariable portion and a variable fatty acid with an alkyl chain ranging from C16 to C26. Furthermore, hydroxylation of the fatty acid contributed to the structural diversity of the Stx2e-binding globo-series GSLs. In PK15 cells the Gb4Cer species clearly dominated over those of Gb3Cer as deduced from TLC binding strengths and differing ion intensities detected by nanoESI MS. In addition, a novel Stx2e-binding GSL with an uncommon Gal_2Cer structure was identified.

Conclusion: In this study we provide first data on the structural diversity of the globo-series GSLs Gb3Cer and Gb4Cer acting as major Stx2e receptors of porcine kidney epithelial cells. Our data suggests involvement of renal epithelial cells in the swine edema disease.

NAT-04: Progress in polyphenol analysis: Multi-step analysis of diol LC-ESI-HRMS data reveals proanthocyanidin composition of complex plant extracts

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Proanthocyanidins (PACs) are complex phenolic biopolymers composed of flavan-3-ol building blocks, belonging to the class of tannins. They are natural products of a wide range of medicinally used plants and exhibit various functional bioactivities. The analytical methodology for PACs with low degree of polymerisation (DP) is well established, but there is still a lack of methods for the analysis of higher oligomers and polymers [1]. In this study, proanthocyanidin-rich extracts from Tilia spp. flowers, Crataegus spp. leaves and flowers, *Wisteria floribunda* fruits and *Rumex acetosa* herb were examined by HPLC on diol stationary phase using fluorescence and qTOF-MS detection. PACs were separated by DP on a diol stationary phase. Therefore, fluorescence detection and qTOF-MS contour plots [$t_R \rightarrow m/z$] provided an overview on the respective PAC distribution of the extracts. Subsequently, high resolution mass spectrometry data was used for Kendrick mass defect (KMD) analysis, with (epi)catechin, the predominantly occurring flavan-3-ol unit in PACs, as reference unit. The resulting KMD plots enabled detailed elucidation of polymer chain composition with regard to the respective PAC derivatives and modifications. Finally, analysis of MS/MS fragmentation patterns of PAC-polymers confirmed the structural features obtained from the KMD plot. Whereas Tilia spp. flowers contain oligomeric A-type and B-type PACs composed of only (epi)catechin as building block, *Wisteria floribunda* fruits contain PACs consisting of variably hydroxylated building blocks. Galloylated PAC polymers were apparent in *Rumex acetosa* and cinchonains, A-, and B-type PACs were detected in Crataegus spp. leaves and flowers. Multi-step analysis of LC-ESI-qTOF-HRMS data has proven to be a fast and powerful method for assessing the complex PAC-composition of plant extracts. This method provides information regarding the respective DP cluster distribution, as well as detailed composition of each DP cluster, with special respect to structural modifications of polymer chains.

[1] Neilson AP, O'Keefe SF, Bolling BW. High-Molecular-Weight Proanthocyanidins in Foods: Overcoming Analytical Challenges in Pursuit of Novel Dietary Bioactive Components. Annu Rev Food Sci Technol 2016; 7:43–64

NAT-06: Thermochemical and Thermophysical Mapping of Burning Superslim and Kingsize Cigarettes

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After presenting and publishing the proof of principle of the in-situ thermochemical and thermophysical mapping inside a burning Super Slim (SS) cigarette, we now extended the study scope to other cigarette formats and smoking regimes [1].

A Chinese Virginia-style Super Slim and the respective King Size (KS) cigarette were investigated under ISO as well as Health Canada Intense (HCI) smoking regime.

A micrometre sampling bench was used to accurately position the in-situ sampling probes for insertion into the cigarettes. An array of 0.254-mm thermocouples (for gas-phase temperature determination) and multiple 0.35-mm diameter quartz tubes connected to transducers (for pressure determination) were inserted into the SS cigarette. For chemical analysis, a single heated 0.5-mm chemical sampling microprobe was also inserted and coupled to a single-photon soft ionisation (SPI) mass spectrometer through a heated transfer line. The different measurement techniques were synchronised by mapping two probes at one point in time (e.g. temperature/pressure or temperature/chemistry).

The mapping approach provides complex as well as dynamic variating data that allows the comprehensive description of the main thermophysical and thermochemical phenomena. Due to the different geometric parameters of SS and KS cigarettes, differences between the SS and KS cigarettes in the chemical fingerprint patterns during a puff were observed. In addition, the higher intensity of the puff under HCI in comparison to the ISO regime alters the thermophysical and thermochemical profile inside the burning cigarette.

The spectrum of the generated information ranges from simple process understanding, such as the spatially resolved thermal desorption of nicotine or the thermal degradation of nicotine, to more complex mechanistic physical-chemical understanding inside a burning cigarette.

References:

1. Huapeng Cui, Sven Ehlert, Fuwei Xie, Jan Heide, Nan Deng, Bin Li, Chuan Liu, Kevin McAdam, Andreas Walte, Ralf Zimmermann; Integration of time and spatially resolved in-situ temperature and pressure measurements with soft ionisation mass spectrometry inside a burning superslim cigarette, Journal of Analytical and Applied Pyrolysis, Volume 135, 2018, Pages 310-318

NAT-08: HPLC-MS/MS Analysis of Cell Culture Samples: *In vitro* Studies on Biotransformation and Transport of Nutritional Relevant Alkaloids across Cellular Barriers

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According to a general definition, alkaloids are naturally occurring secondary metabolites that primarily protect plants from external influences. These heterocyclic, nitrogen-containing alkaline substances are present in various plants and thus widely distributed over the human food chain. As of today, more than 20000 alkaloids have been described, which exhibit diverse biological activities, including beneficial and adverse effects.

As part of a human dietary-intervention study, Hövelmann et al. recently identified novel imidazole alkaloids structurally based on amides of histamine or histidinol and fatty acids as potential specific intake markers for tomatoes.[1] Similarly, the glucose-derived β-carboline alkaloids tangutorid E and F as well as their 3′-dehydroxy-derivatives were detected. Another example of an alkaloid in the human diet is hordenine occurring in germinated barley. This phenethylamine alkaloid was recently identified as a selective dopamine D2 receptor agonist contributing potentially to the rewarding effects of beer. Due to the occurrence of the mentioned alkaloids in the human diet, and limited data on potential biological activities, further investigations on their bioavailability and transfer properties across the intestinal and the blood-brain barrier are required.

To study the transfer across cellular barrier, two *in vitro* models based on the cultivation of Caco-2 cells (intestinal) and primary porcine brain capillary endothelial cells (PBCEC, blood-brain barrier) on Transwell® filter inserts were used. After the application of the compounds in either the apical and/or basolateral compartments, samples were withdrawn up to 48 h and the alkaloids were quantified using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to determine passive and active transport rates. Furthermore, biotransformation of alkaloids and its metabolites were analyzed by coupling HPLC with high-resolution mass spectrometry (HRMS). In the course of the transport studies, a sensitive and reliable HPLC-MS/MS method was an essential tool for the quantification of all compounds in cell culture media, whereas the simultaneous determination of small, polar compounds and moderate polar analytes was challenging.

In conclusion, the intestinal absorption of the imidazole and ß-carboline alkaloids as wells as hordenine indicated that all test compounds exhibit moderate bioavailability. In addition, a metabolic conversion of a number of alkaloids in Caco-2 cells was observed. On the basis of the effective transport of all tested alkaloids across the blood-brain barrier, further studies should investigate the effects on cerebral cells, including health-promoting or neurotoxic potential.

[1] Hövelmann et al. (2019), J. Agr. Food Chem. 67 (13).

Proteome Analysis - Basic Research

PRB-02: Analysis of recombinant erythropoietin by mass spectrometry

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Erythropoietin (EPO) is a naturally occurring red blood cell stimulating hormone produced in the kidney and was one of the first therapeutic recombinant protein. In order to get a high peptide sequence coverage, we used trypsin and chymotrypsin to digest. The resulting peptides were measured with nanoLC-MS/MS. The peptide identification was performed by pFind software. 62% and 54.8% of peptide sequence was identified by trypsin and chymotrypsin digestion respectively. For the glycopeptides, we also used the two proteases to digestion and enriched by zwitterioic hydrophilic interaction liquid chromatography. After measured with nanoLC-MS/MS, the glycopeptide was analyzed with the pGlyco. Combined with these data, we can get the information of the peptide sequence, the composition of *N*-glycans and the site-localizations of *N*-glycans.

PRB-04: Protein glycation and oxidation sites in raw milk and flavored milk drinks

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To ensure the microbiological safety and a longer shelf-life of milk, different heat treatments are applied, such as pasteurization (72 °C, 15 s) and ultra-high temperature (UHT, 135 – 150 °C, 1 - 4 s) treatment. However, these conditions favor physicochemical changes of milk constituents, e.g., by Maillard reactions and oxidations, leading to the formation of Amadori products, advanced glycation end products (AGEs), and oxidized (including carbonylated) proteins. These modifications may reduce the nutritional value of milk (blockage of lysine residues), trigger an immune response, or form potentially toxic compounds. AGEs may also promote oxidative stress and inflammation. Therefore, it is important to identify all modified proteins and the modification sites in processed milk to understand their effects and determine which processing steps affect the modification degrees the most.

Here, changes in the glycated and oxidized/carbonylated proteome were studied in four differently flavored milk drinks (chocolate, strawberry, vanilla, and cocoa) including samples collected before (raw milks), during (pasteurization, mixing with flavorings, final heat treatment) and after processing (commercial available drink). Twenty-six different glycation- and oxidation-derived modification types were targeted by nRPC-ESI-MS/MS (DDA, CID and ETD modes) after excluding all unmodified peptides identified before by data-independent acquisition (MS^E). Overall 67 Amadori, 82 AGE-modified, and 37 oxidized or carbonylated peptides from 95 proteins were identified. Lactosyl-, formyl-, and carboxymethyl-lysine were the most common modification types. Thermal processing, especially UHT treatment, increased the number and relative quantities of lactosylated peptides in all flavored milk drinks, whereas processing did not significantly influence the number of detected AGE-modified, oxidized, and carbonylated positions in milk proteins.

PRB-06: Complementarity of different SDS-PAGE gel staining methods for the identification of sORF-encoded peptides in *Methanosarcina mazei* by in-gel digestion

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The analysis of short open reading frame-encoded peptides (SEPs) is important for the understanding of the molecular processes in the cell. SEPs have been identified across all domains of life and are predicted to be involved in many important biochemical processes, however, for the vast majority of SEPs there biological function is still unknown. Special methodologies have to be used for the mass spectrometric analysis of SEPs, because traditional methods of bottom-up proteomics have a bias against small proteins. Here, we investigated the influence of different staining methods for SDS-PAGE gels (negative, Coomassie and without staining) and different enzymes in in-gel digestion following LC-MS/MS analysis for the identification of SEPs in the Archaeon *Methanosarcina mazei*. In total, 91 SEPs with at least one proteotypic peptide (FDR<1 %), and 48 SEPs using strict criteria (five consecutive b- or y-ions in the MS2-spectrum), were identified. With this method, significantly more SEPs with greater sequence coverage were identified compared to previously described methods (GELFrEE, 2D-LC, Acetonitrile-precipitation). The staining methods provided complementary data that allowed for a range of different SEPs to be identified. The highest number of SEPs were identified in the samples stained with Coomassie brilliant blue. However, the highest quality of the identified SEPs (highest number of PSMs, highest average Sequest Xcorr) were achieved in the samples without staining. Digestion with the enzymes chymotrypsin, GluC and LysArgiNase showed that the sequence coverage of the identified SEPs can be increased when using different proteases. The GluC digest increased the sequence coverage of 13 SEPs by an average of (28 ± 8) % compared to the trypsin digest data. These data demonstrate that in-gel digestion is amenable and well suited for the identification of SEPs, and that through the incorporation of minor variations, this classical technique may provide further insights into the world of small proteins and SEPs.

PRB-08: UVPD-FTICR-2DMS: Expanding the toolbox for biomolecule analysis

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UVPD has gained a lot of interest in recent years for applications involving biomolecules such as top-down proteomics where UVPD affords very good sequence coverage without charge limitations, and complementary information to other fragmentation methods. Due to the complexity of UVPD fragmentation resulting in all fragment types which may be very close in mass, it is an ideal partner for ultra-high resolution mass spectrometry, especially for 2DMS experiments which allow the fast and simultaneous characterization of all analytes in complex samples. In this work, we couple 213 nm and 193 nm UVPD to a Bruker solariX 12T FTICR-MS.

A 213 nm laser beam (Nd:YAG, 10 Hz, Litron UK) or a 193 nm laser beam (ArF Excimer, 500 Hz, ExciStar, Coherent) were introduced into the back of the infinity cell of the instrument through a BaF_2 window and ions stored in the cell were irradiated with 1 to 12 laser shots at varying pulse energies. No hardware modifications were required due to the pre-existing IRMPD setup. Resulting UVPD spectra were analysed using DataAnalysis 4.4 (Bruker) and fragments were assigned both manually and using software developed in-house with an error of <2 ppm.

Performance of the setup was tested initially using model systems ubiquitin and cytochrome c and compared to CID, IRMPD and ECD. UVPD at both wavelengths gave the highest cleavage coverage for all measured protein charge states compared to other fragmentation methods, presenting a/a+1/b/c/x/y/y-1/z fragments. Using a single shot, 193 nm provided the best yield and was further implemented as a fragmentation method for 2DMS, optimized using LeuEnk peptide and promising first results were obtained for BSA digest and ubiquitin where good fragmentation of multiple precursors in a mixture was achieved.

We further applied UVPD-FTICR-MS to the analysis of a cyclopeptide-polymer conjugate, a novel type of biomolecule which has garnered interest in the pharmaceutical industry as a potential drug delivery system. Unlike ECD which was limited to

fragmentation in the polymer only, UVPD generated complete cleavage coverage of both polymer and cyclopeptide parts of the molecule allowing full chemical characterization and achieving the highest level of detail of any analytical method.

In conclusion, UVPD has provided additional information on all systems studied and proved a valuable addition to the MS/MS toolbox for both 1D and 2D modes.

PRB-10: Multi-omics analysis of a PS1-E280A mutation of familiar Alzheimer's disease brain tissue

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Early onset familial Alzheimer's disease (FAD) is commonly caused by mutations of the presenilin 1 (PS1) gene. Residing in Antioquia, Colombia, near 700 members of a large family carry the PS1-E280A mutation and is therefore the largest known population of early onset FAD. This population presents a wide phenotypic variability regarding age of onset and clinical manifestation, being therefore highly suitable for clinical trials and the study of molecular mechanisms of Alzheimer's disease. In an effort to explore the association of clinical and pathological phenotypes with high-throughput data analysis, 23 PS1E280A postmortem brains have been selected for deep phenotyping and multi-omics analysis. Using data independent acquisition (DIA) LC-MS/MS, 2691 proteins were relatively quantified. In this dataset, 14 out of 93 known gamma secretase targets were identified. Apart from APP, CD44 was found to be significantly upregulated in PS1-E280A brain tissue compared to control brains, indicating abnormal gamma-secretase activity in PS1-E280A mutants towards CD44. Using Phyre2 and Chimera structural homology of the CD44 intra cellular domain (ICD) and the A-beta peptide was observed, corroborating the findings that the protease specificity of the gamma-secretase complex depends on the substrate's three-dimensional structure, rather then its amino acid sequence. Next an unsupervised approach will be used, with principal component and clustering analyses in genomic, transcriptomic and proteomic data to be associated with clinical and pathological findings in these patients. Aiming to build a machine learning predictive model that could help in therapy design.

PRB-12: Enhancement in sensitivity and reliability of identification of protein carbonylation sites

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Irreversible oxidative post-translational modifications (oxPTMs), especially protein carbonylation, are clinically significant biomarkers for pathologies underlined with a chronic oxidative distress. However, the structural diversity and low contents of carbonylation sites challenges the confident identification and quantitation. Owing to its sensitivity and high-throughput capabilities, LC-MS based bottom up proteomics relying on hybrid fast and high-resolution mass spectrometers such as Q-TOF instruments is commonly used for broad profiling of modification sites. However, due to the pulsed acquisition nature of Q-TOF instruments, only a fraction of the product ions is actually detected. This aspect becomes critical for low abundant peptides containing the PTMs of interest.

Here, oxidized human serum albumin (OxHSA) was derivatized with aldehyde reactive probe (ARP), a carbonyl specific biotin-tag, and digested with trypsin. Non-derivatized peptides were depleted by selectively trapping peptides with ARP using avidin affinity chromatography. The depleted mixtures were analyzed by nRPC-ESI-MS/MS using CID in traditional DDA mode and HD-DDA with Wideband Enhancement®, which improves significantly the sensitivity for fragment ions. Considering tag specific reporter ions and modification specific neutral losses increased the reliability of PTM site localization. Thus, 126 ARP-labelled peptides were identified by HD-DDA using Wideband Enhancement® versus 93 ARP-labelled peptides identified in DDA mode, i.e., a 33% higher number of annotated ARP-peptides.

PRB-14: A novel robust LCMS proteomics approach using micro pillar array columns (µPAC™)

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Bottom-up proteomics using 50 to 100 µm C18 packed capillaries coupled to high resolution mass spectrometers is used to analyze protein samples from tissues, body fluids or cell lysates. Typically, micrograms of samples are separated in 30 to 240 min nano LC gradients. However, ease-of-use and reproducibility of nanoflow LCMS using packed capillaries does not yet allow novice and routine use.

PharmaFluidics' µPAC™ technology (micro Pillar Array Column) is a unique and novel approach to a chromatographic support structure and builds upon micromachining chromatographic separation beds into silicon, with exceptional properties that result in excellent chromatographic performance with high resolution and high sensitivity.

The performance of PharmaFluidics' µPAC™ columns for sensitive analysis of limited copy number samples has been demonstrated in nanoflow mode, coupling a 200 cm µPAC™ column via a nanoflex source with a 10 µm silica emitter to a Thermo EasynLC 1200 pump and a Thermo Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer. However, due to the high permeability of the µPAC™, high throughput operation at capillary flow is also possible. Here we demonstrate this by coupling the µPAC™ via a Thermo EasySpray source to a Thermo Ultimate RSLC nano 3000 and a Thermo Q-Exactive HF-X MS under capillary flow conditions with a flow rate of 1 µl/min in short 30 to 90 minute gradient runs.

Capillary flow conditions in proteomics are used for throughput and robustness reasons in quantitative analyses. A HeLa cell digest dilution series was used to investigate the effect of injected sample amount on the output in terms of protein identifications. For the highest concentration of 1000 ng/µl, over 4800 proteins could be identified in a single 90 minute gradient separation (120 minute run time). For the lowest concentration of 2 ng/µl, approximately 600 proteins could be identified in a

single 30 minute gradient separation, once more highlighting the potential of µPAC™ Orbitrap LCMS workflows for limited sample proteomics experiments.

This set of experiments proves that a single µPAC™ column can be used over a wide range of flow rates, both nano and capillary flow can easily be applied on the same column. Consequently, it is a viable alternative to packed fused silica columns in proteomics experiments for both qualitative and quantitative analysis.

PRB-16: Mass spectrometric approaches towards the differentiation between glucose- and fructose-derived glycation products

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From dietary perspective, fructose has superior properties than glucose, as it is sweeter and does not trigger an insulin response. Among all caloric sweeteners, fructose and fructose-based sugars, especially in form of high fructose corn syrup (HFCS) are commonly used. Although fructose was considered as source of "empty calories" in the past, recently its excessive consumption could be associated to metabolic syndrome (MetS) and type 2 diabetes (T2D). As reducing sugars, both glucose and fructose contribute to protein glycation yielding Amadori (APs) and Heyns (HPs) products, respectively. Nevertheless, there is evidence, that long-term fructose is more extensively involved in glycoxidation favoring the formation of advanced glycation endproducts (AGEs). These heterogeneous compounds interact with receptors, such as RAGE, contributing to inflammatory diseases. While the formation of glucose-derived Amadori products was studied extensively, the in-vivo effects of fructosylation are largely unknown. In particular, the differentiation between the isomeric glycation products is challenging.

Therefore, we studied the fragmentation pattern of Amadori- and Heyns-modified lysine and specifically glycated synthetic peptides. Tandem mass spectra were acquired on MALDI-TOF/TOF and ESI-QTOF instruments using collision induced dissociation (CID) modes. The tandem mass spectra were dominated by characteristic neutral loss patterns resulting from pyrylium and furylium ions. This lead to consecutive losses of up to three water molecules (18 Da, 36 Da, and 54 Da) and a loss of three water and one formaldehyde molecules (84 Da) for both glucosylated and fructosylated substances. Additionally, all Heyns-products displayed an additional loss corresponding to 96 Da, which was presumed to be specific. However, it appears that this mass loss is sequence dependent and that even Amadori-peptides show this mass loss indicating that the identification of Heyns-peptides based on this mass loss is misleading. Thus, we studied the fragmentation behavior of glycated peptides also using electron transfer dissociation (ETD) and tested travelling wave ion mobility spectrometry as a possible separation technique to differentiate glucosylated and fructosylated products.

Proteome Research - Applications in Biology and Biochemistry

PRA-02: Structural Insights into Full-Length Retinal Guanylyl Cyclase 1 (ROS-GC1) by Cross-linking/Mass Spectrometry and Homology Modeling

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The retinal guanylyl cyclase 1 (ROS-GC1) is a transmembrane protein that is regulated by guanylyl cyclase-activating proteins (GCAPs) on the intracellular site in response to changes in intracellular Ca^{2+} concentration. Dysfunction and mutations in ROS-GC1 correlate with different retinal diseases, such as Leber's congenital amourosis (LCA) and Cone-Rod-Dystrophies (CORD), which often lead to blindness.

To date, no structural data are available for ROS-GC1.To obtain first 3D-structural information of full-length ROS-GC1, we now conduct cross-linking/MS studies of cell lysates containing ROS-GC1. So far, we have obtained cross-linking data of the ROS-GC1 intracellular domain using different cross-linking principles. The cross-links identified are located within the kinase homology domain (KHD) and between the KHD and the catalytic domain of ROS-GC1, providing evidence that the catalytic domain is located in close neighborhood to the KHD. Moreover, the cross-links identified give strong hints on the existence of a ROS-GC1 homodimer in its natural cellular environment. Based on the cross-linking constraints, structural models of the intracellular domain of ROS-GC1 are currently being established via integrative structural biology methods.

PRA-04: Quantification of isoforms of citrate synthase in *Desulfurella acetivorans* that are involved in citrate cleavage during inorganic carbon fixation

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Biological CO_2 fixation proceeds through a number of autotrophic pathways varying in energetic efficiency and oxygen tolerance, with the reversed oxidative TCA (roTCA) cycle being energetically the most efficient pathway. The roTCA cycle has been discovered recently in *Desulfurella acetivorans* [1] and *Thermosulfidibacter takaii* [2] and represents a variant of the reductive TCA cycle that uses citrate synthase (CS) instead of ATP-citrate lyase for citrate cleavage. The usage of this cycle requires unusually high activity of CS (~40 µmole min^{-1} mg^{-1} protein in *D. acetivorans* [1]), suggesting high abundance of CS in

this bacterium. The genome of *D. acetivorans* possesses three CS genes, and quantification of the corresponding key enzymes using target mass spectrometry (MS) and their characterization is necessary to understand the functioning of this pathway.

Isoforms were heterologously produced and used as standards for quantification. Proteins were separated using 1D-PAGE and used for MS method development after tryptic digestion. Pseudo-MRM experiments were set-up both for the isoforms individually and their mixture (Synapt G2 Si, M-Class; Waters Corp.). Analyses were performed using total cell lysates and gel bands (Skyline). Relative quantification of the cell lysate proteomes was achieved using high-definition protein expression analysis following filter-based tryptic digestion.

CS DESACE_08345 was shown to be one of the most abundant proteins in the cell lysates, contributing 7% of the total protein content; it had the highest of three enzymes CS activity with k_{cat} of 128 s^{-1}. The other two isoforms were present at less than 1% with DESACE_06860 being twice as abundant as DESACE_09325, which is a promiscuous citrate/methylcitrate synthase that is probably involved in propionyl-CoA oxidation. Quantification with the standard mixture was not useful because of the very high concentrations of DESACE_08345 and the low concentrations of the other isoforms. Interestingly, the K_M value of DESACE_08345 to citrate (1 mM) was very close to the intracellular citrate concentration in autotrophically grown cells (1.4 mM) [1], suggesting that the protein was adapted to the performed function in the roTCA cycle (K_M of porcine CS to citrate is 8.2 mM [1]). Our data show that the abundance of CS may be used as indication for the functioning of the roTCA cycle. Furthermore, the data contradict experiments [2] that showed more than 100 times lower K_M of the roTCA-cycle-CS of *T. takaii* to citrate.

[1] Mall et al. 2018, Science 359: 563; [2] Nunoura et al. 2018, Science 359 : 559.

PRA-06: Generating an assembly pathway for the Na^+ F_1F_O ATP synthase of *Acetobacterium woodii* using LILBID-MS

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F_1F_O ATP synthases are macromolecular key enzymes composed of two separated coupled generators (F_1 and F_O) providing adenosine triphosphate (ATP) as the main source of energy of living cells and is used for a variety of energy-consuming biological processes, such as nerve conduction, muscle contraction and transport across membranes.

However, many details of the precise assembly of bacterial F_1F_O-ATP synthases are still unclear. We are investigating the *in vitro* assembly of separately purified subunits (α, β, δ, γε and ε) into the soluble part F_1, followed by the coupling with the membrane-embedded c-ring. We investigate the requirements for the different assembly steps. To analyze the subunit assembly in the Na^+ F_1F_O ATP synthase of *Acetobacterium woodii*, where we employ *Escherichia coli* (*E.coli*) as a model system, we use *Laser Induced Liquid Bead Ion Desorption Mass Spectrometry* (*LILBID-MS*), negative stain electron microscopy (EM) and High Performance Liquid Chromatography (HPLC).

In LILBID-MS a droplet generator generates droplets of an aqueous sample. These are transferred into vacuum and irradiated with an IR laser, leading to an explosive expansion of the droplets, ionization and detection by a time-of-flight analyzer.

Employing LILBID we finally propose a model of the F_1 assembly pathwayfrom single subunits and show preliminary results of the interaction with the c-ring.

A relevant question is, if assembly is sufficient to guarantee enzymatic activity. Therefore, we are using an enzyme coupled activity assay to probe ATPase activity of the *in vitro* assembled F_1 (sub)-complexes and compare these results to those from *in vivo* experiments.

PRA-08: Proteome and Phosphoproteome Mapping of Proteins Specificity in Different Subcellular Membrane Fractions of Rat Brain

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Membranes contain important proteins that are capable of initiating signals and interacting with specific substrate molecules which play important roles in various physiological and pathological processes of brain. The functional role of signal transduction in brain is accomplished by a specific protein-protein/protein-metabolic interaction. The characterization of proteins specificity which is associate with membrane is, therefore, essential for a better understanding of brain development, function and diseases and thus helpful to pharmaceutical discovery. However deep profiling of these proteins in a proper way is particularly challenging due to their low abundance and immature membrane enrichment technology.

Here, we present a streamlined modified workflow that focuses on two major goals: first, to monitor the efficiency of subcellular separation workflow which has established by our collaborator; second, to clarify the proteins specificity in different subcellular membrane fractions. A TMT based quantitative approach was applied to three different membrane fractions isolated by sucrose gradient ultra-centrifugation: crude membrane, synaptosomes and synaptic junction. In total more than 5000 proteins and 13477 phosphopeptides of 3250 proteins were quantified by us. Among them, 3500 proteins and 9260 phosphopeptides differentially expressed among the three parts were further analyzed by statistics analysis and GO functional annotation. The proteins like shank family, magusk family and glutamate receptors which are associated with synaptic function were highly enriched and proteins which are related with cytokceleton, cytosol and myelin sheath deeply depleted respectively towards to synaptic junction, which confirmed the good separation efficiency during the sample preparation. 91.4% of phosphopeptides of transmembrane proteins and 69.6% of phosphopeptides of kinase were highly enriched in the synaptic junction, which also comfirmed that synaptic junction plays more important roles in signal transduction than the other parts of brain.

Our work presents the specificity of proteins and phosphoproteins of three different membrane part, from which could later help us to explain the mechanisms behind regulation of lipid composition with its corresponding lipidomics data. This modified workflow also can be extend as a QC tool to monitor the efficiency of sample purification in future researches.

PRA-10: Identification of low affinity SUMO interaction partners by photo-inducible crosslinking

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Posttranslational modifications are an important mechanism to regulate cellular events including signal transduction and protein interactions by influencing protein activity, stability and conformation. Apart from the modification with small chemical moieties like phosphate or acetyl groups, target proteins can be covalently linked with small proteins like ubiquitin or SUMO (*small ubiquitin-related modifier*). The SUMOylation of target proteins provides a new binding site mediating a non-covalent interaction with proteins that contain a SUMO interaction motif (SIM). Thousands of proteins are SUMOylated in the cell and misregulation of the SUMO pathway is, among others, related to neurodegenerative diseases.

We aim to identify SIM-mediated SUMO interaction partners with relative low binding affinities by a novel covalent capture strategy. To this end, we have established chemically modified SUMO probes, which stabilize the transient SUMO-SIM recognition by photo-crosslinking. A genetically encoded photo-inducible crosslinker is incorporated in the SIM-binding interface of SUMO such that after UV irradiation SUMO-SIM interaction partners are covalently linked. It was shown that SUMO-SIM interactions are captured using purified proteins as well as in the presence of a complex cellular environment of mammalian cell extracts.[1]

In this work, our photo-crosslinking approach is applied to the enrichment and identification of to date unknown SUMO binding partners from mammalian cell extracts by tandem mass spectrometry. Due to the covalent capture of interacting proteins, low affinity binders can be identified and the direct mapping of the interaction site is feasible. Furthermore, using SUMO chain mimics we are currently targeting multi-SIM containing proteins.

Reference: [1]: K. F. Taupitz, W. Dörner, H. D. Mootz, Covalent capturing of transient SUMO-SIM interactions using unnatural amino acid mutagenesis and photocrosslinking. *Chem. Eur. J.* **2017**, 23, 5978-5982.

PRA-12: AN INVESTIGATION INTO THE USE OF CYCLIC ION MOBILITY FOR THE SEPARATION OF BIOPHARMACEUTICAL PEPTIDE AND PROTEIN MODIFICATIONS

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As biotherapeutics become more complex, ever more sophisticated tools are being investigated to provide in-depth detailed molecular characterisation. Such studies focus on acquiring knowledge of the post-translational modifications (PTMs) including glycosylation, oxidation and deamidation present in the protein product, with control of these being paramount. Mass spectrometry (MS) is a central technique in biopharmaceutical characterization due to its ability to report on such a wide range of attributes. However, the presence of isobaric PTMs with differing biological properties can often be refractory to traditional LC-MS workflows even when chromatographically separated. In this work we investigate cyclic ion mobility technology as a means to distinguish isomeric PTMs to improve biotherapeutic characterization.

PRA-14: Identification and Quantitation of Phosphopeptide Positional Isomers using Trapped Ion Mobility Spectrometry and PASEF

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Deep characterization and quantitative analysis of proteins and their modifications as they occur post-translationally is critical to understand signaling pathways and aberrant disease states. PTMs exist as a common chemical moiety that selectively modify a small repertoire of amino acids. As the detection of PTMs by MS is often trivial, the site localization and quantitation is not. Although common to identify 10,000s phosphopeptides in a single shot LCMS run, the percentage of those occurring as positional isomers is unknown. Here we show that Trapped Ion Mobility Spectrometry uniquely enables the site localization and quantitation of 100s phosphopeptide positional isomers that would otherwise remain uncharacterized. Enrichments of digested human cell lysates were performed by both IMAC and CSTPScan Phospho-Tyrosine Rabbit mAb(P-Tyr-1000). A Bruker nanoElute using an IonOpticks 25cm C18 column at a flow rate of 400nL/min was coupled to a timsTOFPro to acquire data in a parallel accumulation serial fragmentation (PASEF) workflow. All data was searched using PEAKS X, which has the unique capabilities to decipher chimeric MS/MS spectra and to determine a positional fragmention for irrefutable assignment of phospho-groups. Single shot 90-minute gradients of IMAC enriched and pY-1000 enriched lysates identified more than 18,500 and 1,100 unique PTM modified peptides, respectively. The percentage of phosphorylated forms between these pools was greater than 90%. For the IMAC enriched dataset some 6315 were identified as positional isomers and 4902 had the required positional determinate fragmentions.

PRA-16: New workflow for the identification of global marker peptides using a LC-MS/MS approach

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Food adulteration affecting foods of animal origin has been raised consumers' awareness due to recent incidences. Especially substitution of meat and fish ingredients with other species of lower value is commonly used to increase profit. Due to economic, religious or health reasons consumers must be able to rely on correct labeling of food products calling for effective and sensitive analytical methods for species identification.

Mass spectrometry has become a promising alternative to routinely used protein- and DNA based methods in the field of authenticity control. Emphasis has been placed on the identification of species-specific marker peptides which have been successfully applied to highly processed food products. However, species-specific markers are lacking for numerous species relevant for human consumption. In addition, unknown or unexpected adulterations cannot be detected following a targeted proteomics approach.

We therefore implemented a new workflow for the identification of global marker peptides representing the taxonomic classes of birds, fish and mammals. A shotgun proteomics approach was applied for the database-independent identification of tryptic peptides. Specificity and sensitivity validation of potential marker peptides were achieved subsequently using targeted analysis.

More than 100 animal species belonging to the taxonomic groups of vertebrates and invertebrates were included in the workflow resulting in 7 bird-, 2 fish- and 8 mammal-specific marker peptides. Consequently, unexpected adulterations across vertebrate classes can be detected without the need for species-specific marker peptides. Furthermore, the identified marker peptides are suitable to confirm the presence of a bird, fish or mammal species in processed meat and fish products. It is aimed to use global markers in combination with species-specific markers in routine analysis to guarantee food quality and safety to the consumers.

PRA-18: Replacing Immunoassays with MS-based Technology: Quantitative Proteomics Assays Enabling Deep Molecular Phenotyping of the Mouse

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Mass spectrometry (MS) based proteomics is an indispensable technology for biological and biomedical research. The unbiased, 'discovery' proteomics analysis (e.g., 'shotgun' proteomics) can now provide genome-scale coverage and quantification of both proteins and posttranslational modifications. However, verification of these discovery findings requires higher specificity, higher precision, and accuracy in quantification and higher sample throughput. Targeted proteomic assays are an ideal choice and a good alternative for antibody-based verifications as well as for large-scale clinical studies. The present project includes an approach for rapid quantitative analysis of 3000 mouse proteins, i.e., one capable of accessing a significant portion (~20%) of the mouse proteome. The list of selected proteins contains insulin signaling, glycolysis, oxidative phosphorylation, pentose phosphate pathway, and other key metabolic and signaling pathways. The development of targeted assays includes the creation of tissue- and cell-specific spectral libraries (i), selection and synthesis of surrogate stable isotope-labeled standard (SIL) peptide (ii), calibration of these peptides (iii) and development of PRM assays for determination of reference values for tissues of three main mouse laboratory strains (iv). Finally, all data will be deposited to the Mouse Proteome Atlas: Publically available interactive quantitative map of mouse proteome.

PRA-20: Sample matrix effects in Walnut proteomics: Pellicle components induce high protein oxidation and chemical modifications

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Food authenticity is nowadays highly relevant since food fraud of expansive foods is a common problem. Differential proteomics approaches are powerful tools in this regard. However, it is essential that the sample preparation is highly reproducible to avoid false quantitation and subsequent false classification. Therefore, sample matrix effects can be a reason for poor reproducibility and have to be evaluated.

Proteome measurements of walnut kernels revealed an elevated protein oxidation rate. In addition, oxidation of other amino acids as methionine was observed frequently. Therefore, the effect of the walnut pellicle, a thin skin surrounding the kernel, was investigated in terms of peptide oxidation rate, protein identification and chemical modifications.

To access the effect of the pellicle, walnut kernels were cut in half. One half was peeled while the other half remained non-peeled. Nutmeat of both halves was grinded and ~ 30 mg were boiled in sodium deoxycholate (SDC) containing buffer for protein extraction. Proteins were precipitated with acetonitrile (ACN), subsequently digested with trypsin and analyzed by LC-MS/MS.

The oxidation rate was accessed as the percentage of oxidized peptides. Peeled samples had a peptide oxidation rate of 6.13 % ± 0.36 % while non-peeled samples showed an oxidation rate of 19.9 % ± 1.85 %. 532 protein groups were identified on average in peeled samples while only 358 protein groups were identified in non-peeled samples. The decreasing protein identification rate indicated chemical modifications introduced during sample preparation. Therefore, a subsequent experiment was conducted in which the model protein bovine serum albumin (BSA) was incubated with pellicle components which were extracted using 60 % methanol, 1 % formic acid in water. The incubated BSA sample and a negative control were subsequently digested with trypsin and analyzed by LC-MS/MS.

The resulting chromatograms displayed differences in certain RT windows indicating the peptide modification. These modifications could be well observed for the peptide TVMENFVAFVDK. Since the chemical modification takes place at the N-terminus most of the y-ions are unchanged. This allows the identification of chemical modified versions of this peptide via

pattern matching of the fragment ions. Besides the oxidized peptide several differentially modified versions of this peptide were detected, displaying modifications of up to several hundred Daltons.

These results clearly demonstrate matrix effects for proteomics experiments with walnuts which originate from pellicle components. The pellicle component which is responsible for these matrix effects is yet unknown and has to be further investigated.

PRA-22: TMT-based quantitative proteomics of lung samples from cytomegalovirus infected and non-infected mice

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The Cytomegalovirus (CMV) is a widespread pathogen infecting most of the world's population. [1,2] CMV infection is usually asymptomatic in healthy individuals but infection of immunocompromised adults may result in significant morbidity and mortality. [1,2,3] Typical symptoms that occur in these individuals are spiking fever, leukopenia, malaise, hepatitis, pneumonia, gastrointestinal disease and retinitis. In addition, CMV is the most common viral cause of birth defects often leading to deafness and mental retardation in the fetus if a woman is infected during pregnancy. [1] CMV has been implicated as a possible cofactor in the development of inflammatory and proliferative diseases including certain cardiovascular diseases and cancer. [1,2] There is currently no effective vaccine against CMV and the available antivirals improve the survival and quality of life of immunosuppressed patients however emergence of resistance and potentially serious side effects limit their use. [1,3] Therefore, it is important to conduct further studies to identify proteins involved in processes associated with CMV infection in order to develop novel drugs.

Here, quantitative proteomics was used to compare lung samples from neonatal and adult mice at different days post CMV infection with non-infected age matched mice. For the relative quantitation, isobaric labeling with 10-plex tandem mass tags (TMT) was performed. The labeled samples are analyzed by an Orbitrap Tribrid mass spectrometer before data analysis to identify peptides and quantify reporter ion relative abundance.

The results obtained from this analysis indicated substantial differences of the lungs based on the age of the mice with about 111 proteins showing significant protein abundance differences (q-value 0.05, fold change greater 2-fold). We also observed infected vs non-infected differences, with most protein changes (65%) similar in the different age groups but also protein abundance changes unique to the neonatal or adult mice groups. Further, several proteins in both age groups showed temporal changes correlating with the time point post infection. In addition, when comparing the protein level of neonatal infected and non-infected mice, differences in the abundance of several proteins can be observed.

[1] Tomtishen J. P., Virology Journal, 9:22, (2012).

[2] Crawford L. B. et al.,Current Opinion in Virology, 13, 86–92, (2015).

[3] Bravo F. J. et al., Antiviral Research, 76, 104–110, (2007).

PRA-24: The mechanisms of formation of the resistance of seeds of *Brassica napus L.* to oxidative stress during storage.

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Almost three quarters of the food we get from seeds, so getting high quality seeds is the basis of the country's food safety. Sowing qualities of seeds are determined by a combination of properties characterizing the degree of their suitability for sowing and storage. The nutritional quality of seeds is determined by their suitability for human and animal nutrition. Accordingly, high-quality seeds should be able to be stored for a long time without compromising functional activity and nutritional value. Despite the fact that the conditions are optimized in agricultural production, during long-term storage, seeds gradually accumulate structural and metabolic damage and, ultimately, lose their viability. This process is called seed aging. Under optimal storage conditions, damage accumulates slowly, and seeds can maintain viability for years. With increased humidity and temperature, aging takes place in several days (accelerated aging).

Damage leading to deterioration of seed quality during storage is based on protein glycation, lipid peroxidation, and generation of reactive oxygen species and free radicals. In this work, an analysis of accelerated and natural aging of seeds was carried out. Accelerated seed aging was carried out by short-term incubation at elevated temperature and humidity. First, the seeds were kept in a desiccator over a saturated KCl solution, creating 86% equilibrium air humidity. Then the seeds were placed in airtight bags with an aluminum base, hermetically packed and incubated in an thermostat at 40 ° C for 1 and 7 days. Control seeds were stored for 2 years, with a germination rate of 99%. After 4 years of storage and 1 day, the seed germination rate decreased to 91%. After 9 years of storage and 7 days of accelerated ageing seed germination decreased to 46%.

The total protein fraction from the harvested seeds was isolated by phenol extraction, and the resulted digests were analyzed by nanoLC-ESI-Q- and LIT-Orbitrap-MS in a data-dependent acquisition (DDA) mode. Database search was based on the SEQUEST algorithm using TAIR proteins, whereas quantification relied on the label-free strategy. Differential expression analysis was performed using limma package in R programming environment with the following cluster analysis within the groups of natural and accelerated ageing. Enrichment analysis of protein functions and localizations were carried using *WebGestalt* tool. Proteomics results were examined in the context of metabolomics GC MS.

PRA-26: The role of the c-ring in the assembly of bacterial F-type ATP-synthases analyzed by LILBID-MS

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ATP synthases are rotary machines that use an electrochemical H^+ or Na^+ gradient to produce ATP and consist of several subunits. They are divided into three distinct classes: The F-type, V-type and A-type ATPases. In general, ATPases have two structurally and functionally distinct domains, of which one domain (A_O, V_O, F_O) is anchored in the cytoplasmic membrane and connected to the soluble cytoplasmic domain (A_1, V_1, F_1) via stems. The H^+or Na^+transport occurs at the c-ring of the membrane-bound domain. The Na^+ F_1F_O ATP synthase of the *Acetobacterium woodii* consists of an unusual membrane-bound F_OV_O hybrid rotor, because its c-ring is constructed from nine copies of the F_O-like subunit (8 kDa) and one copy of the V_O-like subunit (18 kDa).

In a previous study we expressed the subunits of the soluble F_1 part of the ATP synthase in *E.coli* and applied LILBID-MS to study the assembly of the soluble F_1 subkomplex *in vitro.*

In this study we want to determine how the membrane-bound F_O subcomplex integrates into the soluble F_1 part.

Therefore we purified the c-ring from the heterologous produced Na^+ F_1F_O ATP synthase of *A. woodii* in *E.coli*. With the help of the LILBID-MS we were able to confirm the identity of the c-ring and are working to determine the coupling into the soluble F_1 part and whether any substrates, such as chaperone, are necessary for the coupling with F_1.

LILBID-MS has a droplet generator which generates droplets of an aqueous sample and then transfers them into vacuum. There, the droplets are irradiated with an IR laser leading to the explosive expansion of the droplets, setting the solvated ions free. These can then be detected using a time-of-flight analyzer.

PRA-28: Investigation of the Post-translation Modification Isoforms of the Transcription Factor EB (TFEB)

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Post-translational modifications (PTMs), such as phosphorylation, acetylation, and methylation, are important for the structure and function of proteins, and can influence their subcellular localization. One heavily post-translationally modified protein is the transcription factor EB (TFEB). TFEB is a member of the microphthalmia (MiT) family of transcription factors, and is often described as the master regulator of autophagy and lysosomal biogenesis. Lysosomes are the main degradative organelles of cells, and have recently been shown to act as a central point for metabolic signaling. A major event identified in this context is the nutrient-dependent PTM of TFEB, which regulates its transcriptional activity. Several TFEB phosphorylation sites have been thoroughly studied and it was shown that, e.g., phosphorylation of S142 and S211 leads to the cytoplasmic retention of TFEB, resulting in an impaired transcriptional activity. Furthermore, it has been shown that TFEB activity can be regulated by modulating its stability. It is therefore of great interest to further investigate the role of TFEB PTMs, their distribution on individual isoforms of the protein, and to identify previously unknown modification sites.

In this study, TFEB PTMs and their response to cellular signals were further investigated using both human and murine samples. After optimizing the experimental setup for both expression and immunoprecipitation of TFEB, it was enriched from overexpressing NIH/3T3 and HeLa cells. To maximize the sequence coverage, trypsin, chymotrypsin, and AspN were used for the in-gel digestion of TFEB, resulting in an overall coverage of 91 %. Mass spectrometric (MS) analyses of unfractionated and phosphopeptide-enriched digests measured with an Orbitrap Fusion Lumos/LTQ Orbitrap Velos, and PTM analyses with both standard Mascot and error-tolerant searches, revealed a total of 36 TFEB PTMs. Employing 2D gel electrophoresis, the transcription factor was then separated into its isoforms, >30 of which could be detected. To facilitate reproducible and sensitive investigation of the isoforms by quantification of the TFEB PTMs, a targeted MS approach utilizing parallel reaction monitoring (PRM) was established on the Orbitrap Fusion Lumos. In its current version, the PRM assay, comprising 206 targets, covers a total of 24 phosphorylation sites. Furthermore, in order to allow determination of PTM specific stoichiometry and PRM assay verification, an unmodified TFEB standard was generated in *E. coli*. Taken together, the combination of classical molecular biology methods and state-of-the-art MS approaches resulted in the identification of 36 PTMs and over 30 isoforms, some of which are novel and previously not published.

PRA-30: Development of a multiple-reaction monitoring (MRM) LC-MS/MS method for detection of microbial transglutaminase from *Streptomyces Mobaraensis*

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The so-called “meat glue enzyme” transglutaminase is used by the meat industry to add value to meat by gluing together smaller scraps into a larger chunk. And it’s not just used to make fake steak—Transglutaminase can be used to cross-link smaller pieces of any type of meat, fish, or meat product, producing large virtually intact looking meat or fish. This has raised food safety concerns in many different aspects. The transglutaminase enzyme is the target antigen of autoantibodies found in the serum of patients suffering from coeliac disease. A rapid and robust analytical method is necessary to ensure the protein content is correctly determined and consumers can be confident in the origin of their food.

Characteristic tryptic peptide markers were selected from Microbial Transglutaminase (MTG) from Streptomyces mobaraensis (JIRA 2017). Target MRM transitions were optimized using DiscoveryQuant™ Software and scheduled using the Scheduled MRM™ Algorithm Pro to maximize acquisition timings. An MRM-based assay for qualitative detection of MTG in meat and meat products was performed using SCIEX QTRAP® 6500+ LC-MS/MS system operated in low mass mode equipped with Turbo V™ source. Assay sensitivities were determined using a dilution series of the synthesized peptide markers. All measurements were performed in triplicate and data processed using MultiQuant™ Software to perform quantification and statistical analysis.

This analytical method provides a reproduceable and robust measurement of peptide targets found in MTG from Streptomyces mobaraensis. The total HPLC run time was reduced to 15 minutes for high throughput analysis without loss of performance. Selectivity and sensitivity of the MRM method was confirmed for Mass Spec measurement of MTG using a dilution series of peptide targets. Multiple peptides were monitored and two transitions per peptide are used for identification and quantitation. Peptide detection was confirmed both mass transitions had a signal-to-noise ratio of at least 3:1. Calibration plots using standards spiked into 0.1% formic acid in water were also constructed and fitted with linear regression, R^2>0.99.

Proteome Research - Clinical Applications

PRC-02: Intact Transition Epitope Mapping - Targeted High-Energy Rupture of Extracted Epitopes (ITEM-THREE) of the anti-pfMSP1$_{19}$ Antibody – Towards Malaria Screening

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Malaria is a life-threatening disease. The estimated number of malaria deaths stood at 435,000 in 2017, with 93 % from Africa, which is basically among children under 5 years. Rapid diagnostic tests (RDTs) are desired first line diagnostic tests as they are suitable for use in even rural communities. Diagnostic tests ought to apply antibodies which target at conserved malaria-specific antigens, such as MSP1$_{19}$. To ascertain assay precision, antibody – antigen interaction is studied in molecular detail. The goal of this project is to develop and test mass spectrometric epitope mapping methods that enable to precisely characterize antibody-antigen interactions.

An MBP-pfMSP1$_{19}$ fusion protein including a His-tag is applied as model system. The MBP-pfMSP1$_{19}$ protein was expressed in *E. coli* to obtain a fusion protein which was enriched from background *E. coli* proteins by amylose-affinity columns via binding to the MBP protein part. Enriched and purified MBP-pfMSP1$_{19}$ was structurally and functionally characterized using SDS-PAGE, Western blot, and MALDI-ToF-MS before and after high pressure-assisted tryptic digestion or GluC digestion. ITEM-THREE (Intact Transition Epitope Mapping - Targeted High-Energy Rupture of Extracted Epitopes) was carried out to map the epitopes of both, anti-His-tag antibody and 'malaria disease-specific' anti-MSP1$_{19}$ antibody.

Over-expression of MBP-pfMSP1$_{19}$ was successful and by amylose affinity chromatography were enriched MBP-pfMSP1$_{19}$ together with endogenous MBP from *E. coli*; the latter of which is proteolysis resistant. Western blotting showed that the anti-His-tag antibody recognized MBP-*pf*MSP1$_{19}$. By contrast, MBP-pfMSP1$_{19}$ and the pfMSP1$_{19}$ fragment therefrom were immuno-positive to the anti-pfMSP1$_{19}$ antibody. The pfMSP1$_{19}$ fragment was obtained by high pressure-assisted tryptic digestion of MBP-pfMSP1$_{19}$. Upon GluC digestion of MBP-*pf*MSP1$_{19}$ the antiHis-tag antibody recognized from the peptide mixture two C-terminal His-tag epitope carrying peptides. Peptides ^{476}GIFCSHHHHHH^{486} and ^{465}CTKPDSYPLFDGIFCSHHHHHH^{486} were selectively bound in solution, the immune complexes were translated into the gas phase by nanoESI, and the doubly and/or triply protonated epitope peptide ions were released upon collision induced dissociation in the gas phase to yield ion signals at m/z 703.32 and 910.43, respectively. Similarly, the anti-pfMSP1$_{19}$ antibody recognized three epitope carrying peptides: ^{415}CKCLLNYKQE^{424}, ^{386}GRNISQHQCVKKQCPQNSGCFRHLDE^{411}, and ^{386}GRNISQHQCVKKQCPQNSGCFRHLDEREE^{414}. They were selectively bound by the antibody, carried through the quadrupole into the mass spectrometer´s collision cell, and were released as ions with signals at m/z 678.33, 796.94, and 900.22, respectively, upon collision induced dissociation. While the anti-His-tag antibody epitope is a consecutive epitope, the anti-pfMSP1$_{19}$ antibody epitope is assembled and comprises amino acid residues from different regions of the antigen.

PRC-04: Comparative DIA analysis of Zika virus positive and negative human serum proteomes

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The Zika virus (ZIKV) infections occurs in tropical and subtropical regions of all continents. It manifests in fever, headache, conjunctivitis and rarely in neurological symptoms. A more significant issue is the infection during pregnancy leading to abnormalities of the fetus such as evolving microcephaly [1]. This complication is mainly observed in Latin America [2]. Therefore, it is of interest to investigate possible markers for microcephaly and its progression in offspring. The created study consists of serum samples of 296 donors including ZIKV positive and negative pregnant woman and their offspring from the Latin America region. The donors are assigned to the groups: pregnant woman 1st Trimester ZIKV positive (25 samples) and negative (25 samples), their offspring 1st Trimester ZIKV positive (25 samples) and negative (25 samples), pregnant woman 3rd Trimester ZIKV positive (50 samples) and negative (48 samples) and their offspring 3rd Trimester ZIKV positive (50 samples) and negative (48 samples). We already expect significant differences with groups of at least 25 donors.

We present the tailored development of a workflow for the deep proteome analysis of serum including "our novel semi-automated sample processing platform for filter aided-sample preparation" (FASP) [3][4] and data-independent acquisition (DIA) [5]. The application of DIA and a project-specific library to the complex serum proteome enables the opportunity to analyse even low abundant proteins, which are lost in the common data-dependant acquisition (DDA). Therefore, it is important to create an appropriate library based analysis of a pH8-RPLC fractionated serum pool. The measurement of the library fractions and samples is performed with a RPLC-Q Exactive HF. For the generation of the library and the DIA analysis Spectronaut software is used.

[1] Anfasa, Fatih, et al. MSphere. 2017

[2] Ferguson, Neil M., et al. Science. 2016

[3] Citate: Loroch, Schumbrutzki, Sickmann et al. submitted 2020

[4] Manza et al. Proteomics. 2005 and Wisniewski et al., Nat Methods. 2009

[5] Bruderer, Roland, et al. Molecular & Cellular Proteomics. 2017

Structural and Functional Proteomics

PRS-02: Structural Characterization of α-Synuclein Liquid-Liquid Phase Separation by Cross-linking/Mass Spectrometry

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α-Synuclein is well known for being involved in several neurodegenerative diseases called synucleinopathies where Parkinson´s Disease (PD) is one of the main types. α-Synuclein is an intrinsically disordered protein (IDP) that undergoes fibrillation and lastly forms insoluble intracellular aggregates in neurons, called Lewy´s bodies, that are the disease hallmark. However, it has been reported that neurotoxicity precedes the formation of the aggregates, and even removing them does not slow down neuronal death and the pursuance of the pathology.

α-Synuclein has recently been reported to undergo liquid-liquid phase separation (LLPS), both *in-vitro* and *in-vivo*. This phenomenon drives the protein to form liquid droplets, in which their concentration is highly increased. Such a highly concentrated protein environment can trigger the fibrillation process and the liquid-solid phase separation that occurs in PD. Insights into the molecular mechanisms of formation of these proteinaceous particles are crucial to better understand this pathology.

To obtain structural insights about α-synuclein LLPS, we will take advantage of cross-linking/mass spectrometry (XL-MS). XL-MS is a well-established technique for structural elucidation of proteins and their complexes. XL-MS employs bifunctional reagents that bridge specific amino acid residues located in a specific range of distance, defined by the length of the cross-linker. After the chemical reaction, the protein sample is digested and analyzed by liquid chromatography coupled to tandem mass spectrometry (nano-HPLC coupled to nano-ESI-Orbitrap-MS/MS) to map the cross-linking sites in α-synuclein and retrieve structural and interactional informations that can be used for computational modelling.

PD is only one of the diseases that are characterized by the aggregation of an IDP, which may be triggered by LLPS. TDP43 for Multiple Sclerosis, FUS for Amyotrophic Lateral Sclerosis, and Tau for Alzheimer Disease are just a few additional examples. Setting up the right protocol to study the early stages of these pathogeneses and their triggering factors is essential to find effective therapeutic approaches.

PRS-04: Chemical modification of proteins for structure elucidation and analysis of protein orientation in lipid bilayer

Marie Barth, Julian Bender, Til Kundlacz, Carla Schmidt

Interdisciplinary Research Center HALOmem, Charles Tanford Protein Center, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany

Structural mass spectrometry (MS) includes a variety of techniques, which are advantageous when traditional methods like protein crystallography or nuclear magnetic resonance prove difficult. One such technique is covalent labelling which identifies solvent accessible residues on the surface of proteins. In this study, we evaluate two chemical labelling strategies using N-hydroxysuccinimidyl acetate (NHS-Acetate) and diethylpyrocarbonate (DEPC) as labelling reagents. We further explore the applicability of the established workflow to determine the orientation of membrane proteins in liposomes.

Alcohol dehydrogenase (ADH) and rabbit pyruvate kinase (PK) were used as model proteins to establish a (quantitative) labelling workflow for investigating protein dynamics under different conditions. Labelling was performed using (i) NHS-Acetate, acetylating lysine, tyrosine, serine and threonine, and (ii) DEPC, modifying histidine, lysine, tyrosine, serine, threonine and cysteine. The proteins were then digested with trypsin and generated peptides were analysed by LC-MS/MS using a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer. Acquired raw data were analysed using MaxQuant software and an R script to identify and quantify modified residues. Using NHS-Acetate, we identified 49 and 85 acetylated residues in ADH and PK, respectively. Of these, 43 (ADH) and 79 (PK) sites were identified in all three replicates confirming a high reproducibility. Similar results were obtained for DEPC-labelling. Close inspection of the mass spectra obtained from DEPC-labelling revealed neutral loss of the label from histidine, lysine, serine and threonine residues. Including these neutral losses in the database search increased identification scores. The identified modified residues were mostly localised at the solvent accessible surface of the proteins and showed quantitative differences in labelling efficiencies depending on their localisation in the protein structure.
Using ion mobility-MS, we assessed labelling-induced structural changes of ADH that might occur due to "over-labelling". NHS-Acetate and low DEPC concentrations do not alter protein structure and do not affect complex stability. We therefore conclude that both, NHS-Ac and DEPC, are promising labelling agents for structural analysis of proteins and their complexes.

Currently, we are extending our labelling workflow to investigate the orientation of membrane proteins in liposomes (i.e. right-side-out *versus* inside-out orientation). Usually, this proves difficult and requires purification tags or fluorescence labels. We utilise membrane permeable NHS-Acetate and non-membrane permeable Sulfo-NHS-Acetate and with the use of our quantitative labelling workflow. The labelling ratio of the modified residues using the two reagents provides insights into the orientation of membrane proteins in the liposome membrane and allows a quantitative description.

PRS-06: Establishing proteoliposomes for the analysis of membrane proteins

Melissa Frick, Julian Bender, Carla Schmidt
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Liposomes are phospholipid bilayer vesicles which resemble cellular organelles and membranes. Due to their variability in size, composition and amphiphilic character they are promising mimetics of natural membranes. However, due to their heterogeneity and the ability of lipids to form large clusters in the gas phase, liposomes were not employed for mass spectrometric analysis to-date. To analyze proteins in a native-like membrane environment, we explored liposomes for the mass spectrometric analysis of lipids and proteins directly from phospholipid bilayers. We envision that, in the gas-phase of the mass spectrometer, proteins can be released intact from lipid bilayers allowing their structural analysis.

To reach this goal we first analysed “empty” liposomes under denaturing and non-denaturing conditions. For this we prepared liposomes varying in size, composition and concentration. Mass spectrometric analysis showed that liposomes dissociate independent of their size, composition or concentration. Using shotgun lipidomics we were able to identify and quantify different phospholipid species from various classes directly from the lipid bilayer. Employing a mass spectrometer modified for transmission of high masses revealed the presence of lipid clusters, which dissociate at higher collisional voltages. Next, we mixed proteins and liposomes in solution and analysed them together by denaturing and non-denaturing mass spectrometry. We obtained mass spectra showing both proteins and lipids. Finally, we reconstituted transmembrane proteins and encapsulated soluble proteins into liposomes. We are currently optimizing their structural analysis by mass spectrometry. Using both, denaturing and non-denaturing conditions, we aim to release soluble and transmembrane proteins from the liposomes to study their structures in the gas phase of the mass spectrometer.

PRS-08: HybG and its Role in Diatomic Ligand Biosynthesis of NiFe-Hydrogenases studied by native mass spectrometry.

Christian Arlt, Kerstin Nutschan, Gary Sawers, Andrea Sinz
Martin-Luther-Universität Halle-Wittenberg, Germany

[NiFe]-hydrogenases have a bimetallic active-site cofactor in which the iron ion carries a CO and two CN^- as diatomic ligands ($Fe(CN)_2CO$). While biosynthesis of the CN^- ligands seems to be clear, the biosynthesis of the CO ligand remains unresolved. Six Hyp proteins are involved in cofactor biosynthesis, but only the FeS-cluster-containing HypD protein is redox-active. HypD is assumed to be required to transfer the CN^- ligands from the HypE protein to the iron, and circumstantial evidence also suggests that the CO ligand is generated by HypD from endogenous CO_2 already bound to an iron ion on a HypD-HybG (HypC paralogue) sub-complex. The assembly of the $Fe(CN)_2CO$ co-factor was known to happen in the HybG/HypD complex where HypD acts as a scaffold for assembly. The interaction sites for the CN^-, Fe and CO_2 within the HybG/HypD complex remained elusive. We were able to determine not only the binding site for the CN^--ligand but also the dependency of CN^--binding for CO_2 attachment by utilizing native mass spectrometry. We discovered that HybG binds the CN^- ligand at position C2 by analyzing a C2A mutant of HybG. Impairing this interaction also hinders CO_2 binding of HybG which is required to form the CO-ligand for the assembly of $Fe(CN)_2CO$. A further analysis of HybG derived from a HypE deficient *E.coli* strain yielded identical result. This finding indicates an alternative source for the CN^- ligand besides the assumed HypE/F maturation factors. These investigations give new insights to understand the mechanisms of the maturation process of [NiFe]-hydrogenases.

PRS-10: Determination of Protein Complex Stoichiometries using LILBID-MS and Crosslinking

Nils Hellwig[1], Siyuan Sima[2], Klaus Richter[2], Nina Morgner[1]
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LILBID-MS is a native-MS ionization technique that has been shown in the past to complement other native-MS methods like nESI well, especially when analyzing membrane proteins or working with buffers not well suited for other native-MS methods.[1]

In this work use LILBID to determine the formerly unknown stoichiometry of a heat-shock complex from *c. elegans*, a soluble heterocomplex. The complex containing four different soluble proteins was investigated using a stepwise build-up of the complex to reveal its composition. This stoichiometry was also confirmed by a cross-linking experiment.

The investigated complex contains the molecular chaperone Hsp90, the kinase specific cofactor Cdc37 and the kinase-domain of B-RAf as client protein. In addition the large PPIase and Hsp90-cofactor FKB-6 is attached. This cofactor was found to stabilize the Hsp90-cdc37-sBRaf complex by cooperative interactions. We were able to determine this fixed stoichiometry to be Hsp90:cdc37:sBRaf:FKB-6 2:1:1:1.

All measurements were performed in a 25 mM Tris, 20 mM KCl, pH 7.5 buffer. For the crosslinked protein complex sample BS^3 was used as a crosslinker with all proteins present in equimolar ratios.

LILBID-MS (Laser-Induced Liquid Bead Ion Desorption) spectra were obtained on an in-house-build re-TOF instrument. In the LILBID source, micro droplets of the sample solution are introduced into the ion source with a frequency of 10 Hz and are irradiated with mid-IR laser pulses at a wavelength of 2.94 µm. The subsequent explosive evaporation of the micro droplets leads to the release of the sample ions from the solution. Detection of the low-charged protein complex ions is done by a Daly-type high mass detector.

Plenary Lecture 4: P. E. Barran

Time: Tuesday, 03/Mar/2020: 8:30am - 9:10am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Lars Konermann

Adventures with Dynamic and Disordered Systems and Joy

Perdita Barran
The University of Manchester, United Kingdom

In the last ten years mass spectrometry (MS) coupled with electrospray ionisation (ESI) has been extensively applied to identify proteins and elucidate stoichiometry of protein complexes, often without the need for labels. Because desolvated species are affected by solvent conditions such as pH, buffer strength and concentration, ESI-MS is an appropriate method by which to consider the range of conformational states that proteins may occupy including natively folded, disordered, denatured and amyloid.

Rotationally averaged collision cross sections of the ionised forms of proteins, provided by the combination of mass spectrometry and ion mobility (IM-MS), are also instructive in exploring conformational landscapes in the absence of solvent. The mass and conformer selected ions can also be subjected to dissociation (CAD, ECD, SID, UVPD) which can delineate the structure further. This presentation will present recent results from our group in this area.

Sometimes working in one area can lead science in a radically different direction, and so it came to pass. The latter part on the talk will concentrate on how we have developed the first chemical test for Parkinson's Disease and how Joy Milne helped us to do that.

Session 9: Imaging II; Keynote: Bhandari

Time: Tuesday, 03/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Birgit Hagenhoff

Molecular signatures during plant-pathogen interaction.

Dhaka Ram Bhandari[1], Laura Righetti[2], Sven Gottwald[1], Chiara Dall'Asta[2], Bernhard Spengler[1]
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Fungal infections are among the main diseases of grain crops such as wheat and maize. It is essential to understand molecular interactions during infection, in order to develop possible strategies to combat fungal infections in plants. In order to understand the local regulatory networks responsible for selected traits, it is crucial to visualize the metabolite distributions in plants. Mass spectrometry imaging enables visualization of complex biological processes. The technique provides label-free visualization with chemical specificity and unprecedented details of metabolic biology from thin tissue sections. Such information is essential to gain new insights into complex plant-pathogen interactions. Here, we present atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) to visualize the topography of metabolites related to fungal (*Fusarium* spp. and *Aspergillus* spp.) and mycotoxin treatment in wheat and maize. Experiments were done using a high-resolution MALDI imaging source (AP-SMALDI5 AF, TransMIT GmbH, Giessen, Germany), coupled to an orbital trapping mass analyzer (Q Exactive HF, Thermo Fisher Scientific GmbH, Bremen, Germany). Our results provide new insights into the mechanism of plant defense against fungal infection, as well as into the systematic or local effects of mycotoxin-induced stress. For fungal infection, MS imaging disclosed metabolic changes over time (10, 14, 21 days after root inoculation), related to linoleic acid and α-linoleic acid metabolism, which showed tissue sites of enhanced oxylipin as well as oxidative stress formation. In mycotoxin-treated samples, several lipids (i.e. galactolipids, diacylglycerols) and metabolites (i.e. hydroxycinnamic acids) differentially accumulated with respect to the control samples. The obtained information on tissue-specific compound accumulation enables a 'location-dictates-function' strategy to interpret the roles of detected plant metabolites and fungal mycotoxins.

Acknowledgment

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Session 10: Lipidomics II; Keynote: Ahrends

Time: Tuesday, 03/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Dominik Schwudke

Lipidomics informatics for life-science

Nils Hoffmann[1], Fadi Al Machot[2], Jacobo Miranda Ackermann[3], Andrej Shevchenko[4], Dominik Schwudke[5], Robert Ahrends[6]

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Lipidomics encompasses analytical approaches that aim to identify and quantify the complete set of lipids, defined as lipidome in a given cell, tissue or organism. Most of the lipidomics workflows are based on mass spectrometry and have been proven as a powerful tool in system biology in concert with other Omics techniques. However, bioinformatics infrastructure for this relatively young discipline is limited to some specialists only. Tools developed and restructured by the 'Lipidomics Informatics for Life-Science' (LIFS) are offered to provide standardized bioinformatics pipelines. Here, we present different pipeline parts that allow for lipid identification and quantification (LipidXplorer, LipidCreator), optimization of lipid detection (LipidCreator), the visualization of lipidomics results and chemical space (Clover, Luxscore) and the comparison of quantitative lipidomics data (LipidCompass). The platform aims to support biomedical challenges such as large-scale comparisons of clinical samples in which lipid metabolic alterations can be causal and where diagnostics can be improved. LIFS is a member of the 'German Network for Bioinformatics' (de.NBI) and our mission is to provide software solutions and consulting services that will foster capacity building for biomedical research on lipids.

Session 11: ICP-MS: Environment I; Keynote: Pröfrock

Time: Tuesday, 03/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Carsten Engelhard

Application of multiple ICP-MS based approaches to decipher the chemical Anthropocene – from legacy pollution to new inorganic emerging contaminants

Daniel Pröfrock[1], Anna Reese[1,2], Lars Hildebrandt[1,2], Tristan Zimmermann[1], Fenna Nack[1,3], Ole Klein[1,2], Claudia Elena Schmidt[1,4]

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Legacy heavy metal and species contamination still represents a major adverse threat for many aquatic and marine ecosystems within highly populated catchment areas because of their inherent toxicity, vast sources and persistence.

Despite the ongoing reduction of emissions of such contaminants into the environment, the coastal zones of the North Sea still belong to the most impacted ecosystems worldwide. In particular, the ongoing evolution of coastal zones into industrialized areas, e.g. due to extensive shipping or the construction of offshore wind parks within the framework of the ongoing energy transition in Europe even boosted the release of either known, but also of various new contaminants into the marine environment. Nowadays, due to changing industrial processes and product portfolios also other elements such as the REE beside PGEs and TCEs and also new element species indicate an increasing release into the environment beside other threats such as nano materials, micro plastic or organic contaminants. The accurate analysis of such contaminants is in particular of significance for public health concerns beside the overall future sustainable development and management of the coastal zones as required by EU wide legislation.

This contribution will focus on some recent developments covering the field of ICP-MS based elemental and element speciation analysis and their application within the context of large scale environmental studies on the interactions of entire river catchments and coastal zones. The focus of this lecture will be in particular on the recent possibilities arising from the application of ICP-MS/MS for interference handling as well as on the role of new hyphenation approaches for routine ultra trace analysis of emerging contaminants at ng/L levels, in order to evaluate systematically the emission load caused by anthropogenic activities within river systems and the coastal zone as well as to develop suitable future tracers and proxys for these processes.

Session 12: Affinity; Keynote: Glocker

Time: Tuesday, 03/Mar/2020: 10:00am - 10:30am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Andreas Tholey

Unraveling the Three-dimensional Molecular Recognition Codes of Experimental and Diagnostic Antibodies by Mass Spectrometry

Bright D. Danquah[1], Claudia Röwer[1], Kwabena F.M. Opuni[2], Reham A. El-Kased[3], Cornelia Koy[1], Michael O. Glocker[1]
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Monoclonal antibodies have become the work-horses as sophisticated laboratory tools, in clinical diagnostics, and as cutting-edge therapeutics. Despite their unparalleled success, their epitopes, i.e. the antibodies´ antigenic determinants, are mostly unknown. Moreover, even when knowing the epitope region on an antigen it remains in most cases indistinct which of the surface amino acid residues execute intermolecular binding forces and which remain passive bystanders, merely required for scaffolding. The three-dimensional positioning of those amino acid residues which are important for binding i.e. the “specificity-determining positions (SDPs)”, defines the “three-dimensional molecular recognition code” that is recognized by the antibody.

The Proteome Center Rostock has developed mass spectrometry-based methods to identify and characterize epitopes on protein antigens, termed “Intact Transition Epitope Mapping (ITEM)”. Its main feature: epitopes are identified by determining the accurate masses of complex-released peptides. With “Intact Transition Epitope Mapping – Targeted High-Energy Rupture of Extracted Epitopes (ITEM-THREE)” we recently have introduced a method which identifies epitope peptides by partial amino acid sequencing in a rapid and accurate fashion.

Most recently, we reported on a novel analytical concept through which quasi-thermodynamic information was obtained on desolvated and multiply charged and accelerated protein - protein complex ions in the gas phase. Applied to Immune complexes this procedure enables to investigate antibody - peptide complexes and, thus, is named “Intact Transition Epitope Mapping - Thermodynamic Weak-force Order (ITEM-TWO)”. “ITEM-TWO” enables to determine affinity, i.e. binding strength, of antibodies to antigens in a straightforward gas phase experiment. By design “ITEM-TWO” is capable to efficiently probe the importance of single amino acid residues of the epitope on an antigen which is recognized by a specific antibody, hence “ITEM-TWO” allows to identify “specificity-determining positions (SDPs)”. Knowing the “three-dimensional molecular recognition code” of an antibody places scientists and clinicians into positions to better choose and pick their immuno-analytical tools and/or their therapeutic agents.

In this presentation methodological principles for unraveling “three-dimensional molecular recognition codes” of antibodies by mass spectrometry are described and application examples are given including “point-of-care”-suitable antibody – antigen pairs.

References:

Yefremova et al., **Anal. Bioanal. Chem.**, *409*, 6549-6558 (2017).

Yefremova et al., **J. Am. Soc. Mass Spectrom.**, *28*, 1612-1622 (2017).

Danquah et al., **Mol. Cell. Proteom.**, *18*, 1543-1555 (2019).

Danquah et al., **J. Proteom.**, *212*, 103572 (2020). doi: 10.1016/j.jprot.2019.103572.

Session 9: Imaging II; Oral 1: Pirkl

Time: Tuesday, 03/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Birgit Hagenhoff

Sub-micron 3D imaging combined with automated, high mass resolution MS/MS

Alexander Pirkl[1], Henrik Arlinghaus[1], Daniel Breitenstein[2], Karsten Lamann[1,3], Elke Tallarek[2], Birgit Hagenhoff[2], Ewald Niehuis[1]

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Secondary ion mass spectrometry (SIMS) offers the possibility to perform mass spectrometry imaging with submicron spatial resolution on inorganic and organic samples. While new ion sources expanded the usability of SIMS instruments for wider applications, e.g. organic depth profiling and organic 3D imaging, only recently SIMS analysers emerged with the required mass resolution, mass accuracy and MS/MS capabilities for the thorough investigation of more complex materials. Here we present a method for using a recently developed SIMS instrument, which combines 3D imaging with automated MS/MS using an Orbitrap™ mass analyser.

3D mass spectrometry imaging was performed on a Hybrid SIMS (IONTOF GmbH, prototype described in Nature Methods volume 14, pages 1175–1183 (2017)), a combined ToF-SIMS/Orbitrap mass spectrometer. Bi_3 ions (30 keV) were used for sub-micron surface imaging in combination with the ToF analyser, while singly-charged Ar_n clusters (20 keV, 1000<n<4000) served as primary ions for the Orbitrap analyser (Q Exactive™ HF, Thermo Fisher Scientific™) and to sputter through the sample at the same time. A 3D dataset was acquired by alternating these two phases. Acquisition was controlled by SurfaceLab 7.1 (IONTOF) employing Thermo's Exactive API.

Additionally, ions from the sputter phase, analysed in the Q Exactive HF mass spectrometer, can be automatically subjected to MS/MS analysis, controlled by an in-house developed algorithm. Thereby, from the sample area, imaged at sub-micron resolution, high mass resolution MS(/MS) spectra (R=240 000 @ m/z 200) could be acquired. Based on the spectral data of the latest Orbitrap MS scan, the developed algorithm can select a fixed number of precursors and fragments these in the subsequent sputter scans. The algorithm chooses an ion signal as precursor based on its signal intensity, but suppresses peaks that already occurred as fragments in previous MS/MS spectra. Thus, only the major components or their largest fragments are subjected to MS/MS analysis, reducing measurement time and sample consumption significantly.

We will present 3D data from dyes in text marker lines with a lateral resolution below 1 µm, using the apparatus and method described above.

Session 10: Lipidomics II; Oral 1: Köfeler

Time: Tuesday, 03/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Dominik Schwudke

Lipid Data Analyzer: Identification of lipids by decision rule sets

Harald Köfeler[1], Jürgen Hartler[2]
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Lipid Data Analyzer (LDA) is a decision rule set based software, which enables automated and reliable annotation of lipid species and their molecular structures in high-throughput fashion from chromatography-coupled tandem mass spectrometry data. The key strengths of this software compared to conventional spectral library approaches are: i) reliability; ii) ability of identifying novel lipid molecular species; iii) easy extendibility; iv) capability of unambiguously detecting coeluting species. The instrument independence of LDA was proven in many mass spectrometric experiments, running at various collision energies on various Orbitraps, Q-Exactive, Q-TOF and QTrap instruments. In a benchmark to other software, LDA proved to be superior in terms of positive predictive value and sensitivity.

Session 11: ICP-MS: Environment I; Oral 1: Krystek

Time: Tuesday, 03/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Carsten Engelhard

EMERGING ENVIRONMENTAL CONTAMINANTS - Niches for research based on ICP-MS -

Petra Krystek[1,2]

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Since millennia, research about emerging contaminants and issues about water, soil or air contamination have been around. Emerging contaminants are moving targets as new chemical compounds and materials are continuously being produced and science continuously improves its understanding of current and past contaminants. Potential contaminants remain “emerging” as long as there is a scarcity of knowledge about their behavior in the environment or on their toxic effects on human health.

During our current decade, the attention for emerging contaminants is attracted by the smallest (nano)particles which are often engineered, metallic nanomaterials, and plastics; possibly recycled or degraded as micro or nano-plastics. By linking ICP-MS research to recent emerging contaminants; bottlenecks are often the related validations and quality criteria.

Various niche applications will be presented:

- The determination of engineered nanoparticles and their derivatives, especially nano-silver, in different types of water:

ICP-MS is applied as key analytical technique while different approaches like total quantification by ICPMS, single particle (sp)-ICP-MS or hyphenated to separation techniques like asymmetric flow field flow fractionation (AF4) are exploited as well as other relevant complementary techniques.

- In monitoring studies of heavy metals in surface water, the sampling steps as well as the sample conservation (by acidification) and the sample pretreatment (by filtration) are of great influence on the achievable results. These effects are studied more closely and the most significant differences are found for the metals zinc and copper.

- Plastic seems to be the most urgent contaminant at this moment; Recycling shows an enormous added value in the circular economy. The measurement of complex material compositions in challenging recycling processes and the robust processing of the resulting data in operationally relevant information create valuable importance in the product loop of recycling.

During the production of polymers and during recycling processes, pigments can be added for different purposes, e.g. as coloring agent of the polymeric product but also as tracer for tracking process development and control in the final recyclates *versus* possible by-products.

An analytical method for tracking the pigment Solvent Blue 15 in input materials, in intermediates as well as in recycled polyethylene is developed by tracing and quantifying an indicator metal. First test results on relevant process samples from a field-lab circular plastics were analysed and the results will be discussed.

Session 12: Affinity; Oral 1: Falck

Time: Tuesday, 03/Mar/2020: 10:30am - 10:50am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Andreas Tholey

FcɣR affinity chromatography – MS for structure-function analysis of proteoforms of therapeutic antibodies

Lippold Steffen[1], Simone Nicolardi[1], Elena Dominguéz-Vega[1], Gestur Viddarsson[2], Manfred Wuhrer[1], David Falck[1]
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Affinity chromatography (AC) is an excellent way to study interactions between biomolecules from mixtures. Hyphenation of AC to mass spectrometry (MS) has demonstrated its potential in dealing with the significant proteoform complexity observed in therapeutic monoclonal antibodies (mAbs). Furthermore, AC-MS can be operated under native conditions. Recently, AC was introduced for studying the interaction of immunoglobulin G monoclonal antibodies (mAbs) with the Fc gamma receptor IIIa (FcɣRIIIa). This is a key interaction in the mechanism-of-action of many therapeutic mAbs. AC-UV analysis necessitates the laborious synthesis of highly proteoform-enriched mAbs, because of the impact of mAb glycosylation on FcɣRIIIa affinity. This is overcome by applying AC-MS which yields an in-depth structure-function relation.

We developed an FcɣRIIIa AC-MS platform which allows the direct structure and affinity assessment of many proteoforms of a therapeutic mAb. AC relied on an analytical scale separation with FcɣRIIIa coupled Sepharose beads. MS analysis was performed on a 15 T solariX XR FT-ICR – MS (Brucker) in an *m/z* range of 506-20,000. Our initial approach measured intact/folded antibodies at good sensitivity (50 µg injected). Combining separation and mass analysis allowed us to differentiate the lowest expected mass difference with respect to glycosylation: 16 Da difference between a hexose and a deoxyhexose (fucose). Consequently, we could demonstrate that singly afucosylated antibodies had lower affinity than doubly afucosylated antibodies. This is generally not resolved in widely-used surface plasmon resonance approaches. In total, 21 glycoforms could be distinguished structurally and their relative affinity measured.

However, therapeutic mAb formats are increasingly complex, for example, they may contain antigen-binding fragment (Fab) glycosylation. These antibodies are too heterogeneous to confidently assign proteoforms by only MS analysis. Therefore, we optimized a limited proteolytic cleavage, to disconnect the fragment crystallisable (Fc) and the Fab while retaining full FcɣRIIIa affinity of the Fc fragment. The Fab fragment eluted in the flow-through allowing the characterization of 11 Fab glycoforms of the therapeutic mAb Cetuximab. Since the affinity was fully preserved, the Fc proteoforms could be identified analogous to the intact Fc-only glycosylated mAbs. 20 proteoforms could be distinguished including lysine clipping variants. Importantly, this Fc fragment analysis (middle-up approach) featured higher resolution in the separation and the MS analysis compared to analyzing the intact antibody. Consequently, we observed the (partial) separation of isomeric antibody proteoforms differing only in the branching of the glycan with respect to the position of the terminal hexose (galactose).

Session 9: Imaging II; Oral 2: Kim

Time: Tuesday, 03/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Birgit Hagenhoff

Probing accelerated ageing of pea (*Pisum sativum*) root nodules by mass spectrometric techniques

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Pea (*Pisum sativum*) is one of the most important legume crops in the modern agriculture. Therefore, understanding of the molecular mechanisms behind the changes in the root nodule metabolite patterns during their ageing could be crucial for building new strategies aiming improvement of crop productivity. In this context, mutants, lacking nitrogen fixation might represent an efficient tool for dissecting these mechanisms. Therefore, here we employed FIX-7 mutants, defect by the gene sym27 and characterized by early degradation of bacteroids and nodules. Thus, in this study, we characterized the age-related changes in the metabolism of pea (*Pisum sativum*) root nodules using the combination of gas chromatography mass spectrometry (GC-MS) and matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI). Analysis of primary metabolites relied on a well-established GC-MS-based workflow. To ensure sufficient volatility of analytes, and to provide their reliable identification, a two-step derivatization procedure, comprising methoximation followed with thrimethylsilalation was employed. The spectra were deconvoluted with AMDIS (Automated Mass Spectral Deconvolution and Identification System) software and identified by spectral similarity search against NIST (National Institute of Standards and Technology) library. For MALDI-MSI, nodules were immediately embedded in 2 % (w/v) carboxymethyl cellulose (CMC) and the 20 µm thick tissue sections were prepared by cryostat. Thereafter, 2,5-dihydroxybenzoic acid (DHB) for positive ion mode and 9-aminoacridine for negative ion mode were applied to glass slides by SunCollect sprayer. Finally, mass spectrometry imaging was performed on MALDI-TOF-MS (Bruker Daltomic Ultraflex III). The processing of the acquired data relied on SCiLS 2016b software tool. Individual metabolites were annotated by their exact mass (mass tolerance of 1 ppm) and the annotation was confirmed bytandem MS fragmentation using a Q-Exactive Plus mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with AP-MALDI-Orbitrap-MS. The quantitative results obtained for the changes of metabolite in GC-MS and MSI were subjected to principle component analysis (PCA), which revealed clear difference for all four groups. Moreover, we demonstrate differences in the changes of metabolite distribution between wild type and FIX-7 mutant nodules using MSI. These results were in agreement with our data on age-related changes in root nodule metabolome and finally it might indicate the suppression of biosynthetic processes by the sym27 mutation.

Session 10: Lipidomics II; Oral 2: Al Machot

Time: Tuesday, 03/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Dominik Schwudke

A LUX Score-Based Lipidome Comparison of Lung Tissue of Humans, Mice, Pig and Sheep Using a Dedicated Template SMILES Generator

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Cellular lipidomes represent the integral status of genetic and epigenetics of an organism and they help to investigate complex differences between lung tissue of humans and other mammals. The goal of this study is to compare the lipidomes of multiple species aiming at system biological assessment of phenotypes and physiological parameters and its impact of lipid metabolic processes.

To provide such an association, we apply the LUX Score [1] as homology metric. The LUX Score computes the structural differences in lipidomes based on lipid structure representation as Template SMILES that are generated according to a dedicated database model. This database contains building blocks for each lipid class, e.g., fatty acids, head groups, and sphingosines. All Template SMILES are generated according to a specific molecular starting point to form a well-defined and compact chemical space model of any lipidome. As bench-mark test, we compared Template SMILES to SMILES generated by Lipid Maps [2] and Swiss Lipids [3] on lipidome data sets of yeast [4] and the fruit fly [5]. Best consistency in the chemical space models was found for the Template SMILES generation process.

We analysed the differences in lung tissue lipidomes between 30 human, 16 sheep, 8 mice and 20 pig samples including 16 lipid classes in glycerolipid, glycerophospholipid, sphingolipid and sterol lipid categories. The overall number of unique lipids was 729 of which 710 Template SMILES were computed on basis of a conservative building block selection for mammalian lipidomes. Hierarchical clustering based upon the LUX Score metric revealed an intrinsic signature within the lung lipidomes of the different species that was decisive for the grouping behavior.

[1] Marella et al. (2015) pLos computational Biology 11 (9), e1004511

[2] www.lipidmaps.org

[3] www.swisslipids.org

[4] ejsing et al. (2009) pnas February 106 (7) 2136-2141

[5] carvalho et al. (2012) molecular systems biology 8 (1) 60

Session 11: ICP-MS: Environment I; Oral 2: Faßbender

Time: Tuesday, 03/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Carsten Engelhard

On-line hyphenation of CE with multicollector-ICP-MS for species-specific isotopic analysis of sulfur

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In many scientific fields, isotopic analysis can offer valuable information, e.g., for tracing the origin of food products, environmental contaminants, forensic and archaeological samples (provenance determination), for age determination of minerals (geochronological dating) or for elucidating chemical processes. Up to date, typically bulk analysis is aimed at measuring the isotopic composition of the entire elemental content of the sample. However, the analyte element is usually present under the form of different species.[1] Thus, separating species of interest from one another and from matrix components prior to isotope ratio measurements can provide species-specific isotopic information,[2,3] which could be used for tracing the origin of environmental pollutants and elucidation of (environmental) speciation. Using on-line hyphenations of separation techniques with multicollector-ICP-MS (MC-ICP-MS) can save time and effort and enables the analysis of different species during a single measurement.

In this work, we developed an on-line hyphenation of CE with multicollector-ICP-MS (CE/MC-ICP-MS) for isotopic analysis of sulfur species. With this method, the isotopic composition of sulfur in sulfate originating from river water could be analyzed without sample preparation. The results were compared with data from off-line analysis of the same samples to ensure accuracy. The precision of the results of the on-line measurements was high enough to distinguish the rivers from one another by the isotopic signature of the river water sulfate. Next to environmental applications, a future field could be species-specific isotopic analysis of biomolecules, as sulfur is the only covalently bound constituent of proteins which can be analyzed by MC-ICP-MS.

References

[1] P. Rodriguez-Gonzalez, V.N. Epov, et al., *Mass Spectrom. Rev.* **2012,** *31*, 504-521.

[2] M. Moldovan, E.M. Krupp, et al., *J. Anal. At. Spectrom.* **2004,** *19*, 815-822.

[3] V.N. Epov, S. Berail, et al., *Anal. Chem.* **2010,** *82*, 5652-5662.

Session 12: Affinity; Oral 2: Lupu

Time: Tuesday, 03/Mar/2020: 10:50am - 11:10am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Andreas Tholey

Molecular Epitope Determination of Aptamer Complexes of the Multi-domain Protein C-Met by Proteolytic Affinity- Mass Spectrometry

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C-Met is a glycosylated receptor tyrosine kinase of the hepatocyte growth factor [1]. Upon ligand binding and autophosphorylation, C-Met transmits intercellular signals by its unique multi-substrate docking site, which are essential for tissue repair, embryonic development, wound healing and liver regeneration [2]. However, pathophysiological activation of the C-Met pathway leads to tumorigenesis, schizophrenia and cardiomyocytes death [3,4].

Using a combination of affinity mass spectrometry approaches with surface plasmon resonance methods we herby report the interaction of the C-Met protein with two DNA aptamers. Nucleic acid-based aptamers have very similar properties with monoclonal antibodies. In comparison with antibodies, they are chemically synthesized [6].

This study shows that both aptamers (CLN0003; 60 bases and CLN0004; 64 bases) have high affinities when interacting with the C-Met protein. The SPR results were determined by comparing and confirming the KD calculations of different experiments. In the first case the C-met protein immobilized and the dilution series of aptamers was run and in the other cases the aptamers where immobilized and a dilution series was analyzed. KD determinations for the CLN0003 aptamer interaction with C-Met provided KDs of 2 x 10^{-6} M (KD1) and 3.8 x 10^{-8} M (KD2). Whereas for the interaction of the CLN0004 aptamer comparable KDs were obtained (1 x 10^{-7} M, KD1; and 5 x 10^{-8}, KD2).

In order to determine the structure for the interaction between the C-Met protein and each aptamer epitope extraction method was used. The elution from of the epitope for the CLN0003 aptamer showed that the aptamer interacts with the protein in two different sites: sequence 524-543 SEECLSGTWTQQICLPAIYK and 567-576 NNKFDLKK. In comparison, the interaction of the CLN0004 aptamer with the C-Met protein reviled only one epitope 381-393 NSSGCEARRDEYR.

Session 9: Imaging II; Oral 3: Heijs

Time: Tuesday, 03/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Birgit Hagenhoff

Laser-induced post-ionization for the enhanced MALDI-2-MS analysis of *N*-glycans

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The analysis of *N-linked glyc*ans has garnered a lot of interest in the biomedical and clinical research community. *N*-glycans are involved in numerous cellular processes, including cell-cell interactions, and signaling. Aberrant glycosylation patterns have been associated with disease, including auto-immune diseases, and multiple cancer types. One of the most recent analysis platforms for *N*-glycans is matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). Laser-induced post-ionization (PI)-MALDI-MS, or MALDI-2-MS, has recently been shown to drastically enhance ion yields and measurement sensitivity for the MALDI-MS-based analysis of (glyco-)lipids. Here we aim to investigate the possible benefits of MALDI-2-MS for the analysis of polysaccharides, to ultimately improve the MALDI-MS imaging-based analysis of *N*-glycans.

Clean glass microscope slides were spray-coated with maltoheptaose (DP7) and 7 mg/mL norharmane in 70:30 acetonitrile:H_2O (%v/v; 33.3 pmol DP7/mm^2). A series of semi-automated measurements, with changing ablation and PI-laser energies, as well as cooling-gas-pressure and inter-laser-delay-time, was performed to identify optimal PI-conditions for MALDI-2 of polysaccharides. A sprayed DP7 dilution series (33.3 pmol/mm^2 – 3.33 fmol/mm^2) was measured in negative ion-mode after spray coating with 7 mg/mL norharmane matrix in 70:30 (%v/v) acetonitrile:H_2O and in positive ion-mode after spray coating with 25 mg/mL 2,5-dihydroxybenzoic acid in 50:50 (%v/v) acetonitrile:H_2O. MALDI-2-MSI analyses of native and derivatized *N*-glycans were performed on formalin-fixed, paraffin-embedded human brain and colon tissue sections after on-tissue digestion with PNGase F.

In MALDI-MS applications, polysaccharides are commonly detected in positive ion-mode as alkali metal adducts, and in negative ion-mode as deprotonated species. However, the ion yields of polysaccharides are inferior in comparison to peptides or also polar lipids. Here we have applied MALDI-2-MS in both positive and negative ion-mode to study the effects of laser-induced PI for the analysis of polysaccharides. No benefit for the analysis of the alkali metal-adducts was observed in positive ion-mode, which corroborates earlier findings for MALDI-2-MS of lipids. In contrast, negative ion-mode detection of deprotonated polysaccharides was enhanced substantially with a gain by three orders of magnitude compared to negative ion-mode without PI, and one order of magnitude compared to positive ion-mode analysis. Common polysaccharide fragmentation in negative ion-mode did occur, however the formation of higher-energy cross-ring fragments was substantially more pronounced under MALDI- compared to MALDI-2-conditions. The feasibility of negative-mode MALDI-2-MSI was tested on a set of human brain and colon tissues. Incorporation of a DP7 internal standard showed to be beneficial for removal of ionization bias, and enhancement of *N*-glycan distributions.

Session 10: Lipidomics II; Oral 3: Fuchs

Time: Tuesday, 03/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Dominik Schwudke

Lipidomic analysis of muscle and adipose tissue from lambs fed diets supplemented with microalgae

Beate Fuchs[1], Dirk Dannenberger[1], Susana Alves[2]
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Increasing the content of very long chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA) in meat is a highly desirable production target, due to the well-established health promoting effects of those fatty acids. However, the constraints to n-3 LC-PUFA enrichment in ruminant meat are mostly associated to the extensive rumen biohydrogenation of PUFA, the low elongation and desaturation of 18:3n-3 into n-3 LC-PUFA, and the capacity of muscle lipids to incorporate n-3 LC-PUFA. The first two constraints could be supplanted by using *microalgae* rich in n-3 LC-PUFA that is also resistant to ruminal biohydrogenation. Nevertheless, the factors that influence the deposition of n-3 LC-PUFA in muscle and adipose tissue neutral lipids are not yet fully understood.

An experiment with 21 Merino branco lambs was conducted in INIAV, Portugal, during 4 weeks. Animals were randomly allocated to one of three diets: Control diet; control diet with 2.5% of freeze dried *microalgae* biomass; and control diet with 1 % of oil extracted from *microalgae* and samples were collected from the 21 animals. For lipid analysis, samples from muscle and adipose tissue were chosen. Lipids were extracted by a modified Bligh & Dyer method and analyzed by high-resolution LC-MS/MS (Vanquish-Q Exactive Plus).

Dietary n-3 LC-PUFA enrichment strategies lead to an increase of n-3 LC-PUFA in muscle not only of polar lipids but also in triacylglycerols.

Session 11: ICP-MS: Environment I; Oral 3: Macke

Time: Tuesday, 03/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Carsten Engelhard

Automated speciation analysis of gadolinium-based contrast agents in surface and drinking waters

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In the recent decades, magnetic resonance imaging (MRI) has emerged as an important and frequently applied tool in clinical diagnosis. The improvement of MR signal and image contrast often requires the intravenous administration of gadolinium-based contrast agents (GBCA). Due to the acute toxicity of free Gd^{3+}, polyaminocarboxylic acid ligands are used to create thermodynamically stable chelate complexes. [1] The highly polar Gd complexes are known to be excreted fast and unmetabolized via the kidneys and end up in wastewater treatment plants. Since these compounds pass through wastewater treatment plants almost unaffected, there is a significant release of Gd species into the environment. [2] Nevertheless, the long-term behaviour and ecotoxicological effects of GBCA in aqueous environments are largely unknown. [3]

To investigate the distribution and fate of GBCA in water samples, powerful methods of speciation analysis are required. In this work, a fully automated single platform system for total metal analysis and syringe-driven chromatography in combination with inductively coupled plasma-mass spectrometry (ICP-MS) was used to identify and quantify several contrast agents in environmental samples. A method based on anion-exchange chromatography (IC) was developed to achieve the separation of the polar to ionic substances, whereas the hyphenation to quadrupole-based ICP-MS led to a highly sensitive element specific detection. Furthermore, the use of an automated inline-dilution function allowed a fast-external calibration from single stock standards to determine total Gd and GBCA concentrations.

The developed IC/ICP-MS method enables a fast separation of several commonly administered contrast agents in less than seven minutes, which is a significant improvement of analysis time in comparison to previously published methods. Limits of detection between 45 and 80 pmol/L turned out to be sufficient for the detection and quantification of GBCA in environmental samples without prior sample enrichment. To investigate the release and distribution of GBCA into the environment, the method was finally applied for speciation analysis of water samples obtained from different surface and drinking waters around the City of Münster.

Literature:

[1] J. Wahsner, E. M. Gale, A. Rodríguez-Rodríguez, P. Caravan, *Chem. Rev.* **2019**, 119, 957-1057. [2] L. Telgmann, M. Sperling, U. Karst, *Anal. Chim. Acta* **2013**, 764, 1-16. [3] J. Rogowska, E. Olkowska, W. Ratajczyk, L. Wolska, *Environ. Toxicol. Chem.* **2018**, 37, 1523-1534.

Session 12: Affinity; Oral 3: Wiegand

Time: Tuesday, 03/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Andreas Tholey

Epitope Identification and Affinity Determination of an Inhibiting Human Antibody to Interleukin 8 (IL8) by SPR-Biosensor-Mass Spectrometry Combination

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The chemokine Interleukin-8 (IL8) is a 72 amino acid biomarker for inflammation and is involved in lung diseases, rheumatoid arthritis and cancer. Previous studies have shown that the interaction between IL8 and its natural receptors CXCR1 and CXCR2 is critical in these diseases. Antibodies against IL8 and its receptors CXCR1 and CXCR2 have been used to identify its role in several diseases. Furthermore, antibodies have been used to study the receptor IL8 interaction, but the epitope binding site(s) of inhibitory antibodies are unknown. A monoclonal anti-human IL8 antibody, which inhibits the IL8 receptor binding, has been investigated for its epitope and affinity to IL8. Characterization of the IL8-antibody interaction was carried out by MALDI mass spectrometry (MALDI MS) and a surface plasmon resonance (SPR) based biosensor. First SPR measurements with immobilized antibody revealed a high affinity for the protein antibody interaction (K_D, 82.2 nM). For epitope identification the epitope extraction method was used. This method includes the digestion of the protein (IL8), immobilization of the ligand (antibody) to a column and extraction of the epitope peptides from the digestion with the affinity column. Digestions were performed under normal and high pressure for a optimization of the digestion conditions. Eluted fractions from the affinity column were analyzed by MALDI MS and revealed an assembled (discontinuous) epitope comprising the two specific peptides, IL8 [12-20] and IL8 [55-60]. Identical epitope peptides were identified from eluted peptide fractions from the immobilized anti-IL8 antibody on a SPR chip by MALDI MS. For epitope confirmation the peptides were synthesized and characterized by SPR measurements, too. The epitope peptides showed binding affinities (K_D) in the µM range which indicates that they are part of the binding site. However, the affinities are lower than for the full length protein which indicates that it needs both peptides present in a specific confirmation for high affinity binding. Lastly, the reported finding that the anti-IL8 antibody is inhibiting the IL8−CXCR1 interaction is well consistent with the overlapping region of the identified epitope peptides.

Poster Session 2 (odd poster numbers)

Affinity Mass Spectrometry

AFF-01: Epitope Identification and Affinity Characterization of Myoglobin by online combination of SPR biosensor analysis and ESI Mass Spectrometry

Delia Mihoc[1], Loredana Lupu[1], Pascal Wiegand[1], Michael Przybylski[1], Oliver Müller[2], Friedemann Völklein[2], Michael Glocker[3], Frederik Barka[4], Günes Barka[4]

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Myoglobin (MG) is a biomarker for muscle injury making it a potential target protein for early detection of myocardial infarct. Elevated myoglobin levels alone have low specificity for acute myocardial infarction (AMI), but in combination with cardiac troponin T determination have been considered highly efficient biomarkers for diagnosis. Myoglobin is a monomeric heme protein with a molecular weight of 17 kDa that is found in skeletal and cardiac tissue as intracellular storage unit of oxygen. Myoglobin consists in 8 α- helices connected by loops and a heme group responsible for oxygen binding. Monoclonal antibodies are widely used analytical tools in biomedical research, and have been used for immunoanalytical detection of MG; however the epitope(s) recognized by antibodies are unknown. Precise identification and molecular knowledge of the corresponding epitopes recognized by antibodies is of key importance for the development of MG as a diagnostic biomarker. As major tools for epitope identification and affinity characterization, proteolytic epitope excision and extraction- affinity methods in combination with mass spectrometry and surface plasmon resonance (SPR) biosensor analysis have been developed in our laboratory. In this study epitope identification was performed by immobilization of an MG antibody, followed by affinity incubation of a tryptic digestion mixture and elution of the epitope, using an SPR-MS interface. The SPR-MS combination was developed in a model study using horse heart myoglobin and commercially available monoclonal antibodies. The mass spectrometric results established the epitope sequence to be specifically located in the C-terminal sequence [146-153] [YKELGFQG] of MG. SPR kinetic evaluation provided an affinity binding constant KD of 450 nM for MG; the high specificity and affinity of the epitope was ascertained by synthesis and characterization of the epitope peptide.

AFF-03: Immunepitope: New Approaches for Enzyme Replacement Therapy of Lysosomal Diseases with high Immunogenicity by Mass Spectrometric Identification of Antibody Epitopes

Pascal Wiegand[1], Stefan Maeser[1], Zdenek Kukacka[1], Loredana Lupu[1], Christina Uth[2], Sascha Knauer[2], Andreas Hahn[3], Julia B. Hennermann[4], Michael Przybylski[1]

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Introduction

Enzyme replacement therapies (ERT) have been successfully developed for different Lysosomal Storage Diseases (LSDs) such as Gaucher (GD), Fabry (FD), and Pompe (PD) Disease. While effective for several LSDs, substantial problems are caused by development of antibodies. Patients who develop antibodies upon ERT can have allergic reactions, from mild symptoms to life threatening events. IgG antibodies may neutralize the infused lysosomal enzyme and prevent successful treatment. Here we report new therapeutic approaches by identification and affinity characterization of antibody epitopes upon ERT, using synthetic epitope peptides that block antibodies directed against the infused enzyme. It is expected that the application of epitopes to block neutralizing antibodies will substantially improve efficiency and safety of ERT in LSD patients.

For epitope identification, blood from FD and PD patients who had developed antibodies was analyzed using proteolytic excision- MS and surface plasmon resonance (SPR) biosensor combined with electrospray (ESI) mass spectrometry. SPR-MS provided the concomitant structural identification and affinity quantification of epitopes. The epitope from FD patients was identified with a human anti-alpha-galactosidase antibody immobilized on an activated-sepharose microcolumn by incubation for 2 hrs at 37°C. The tryptic peptide mixture resulting from digestion (12 h, 37°C, protein:trypsin 1:30) was loaded onto the SPR-MS via the SPR injector. After washing out the nonbinding peptides, epitopes were eluted with 0.1 % TFA into the MS interface equipped with a C4-desalting microcolumn; ESIMS was performed with a Waters Quattro Ultima instrument.

The SPRMS combination was successfully applied to the epitope elucidation and affinity characterization of antibodies against alpha-galactosidase in 3 FD patients, and provided identical peptide sequences, αGal (309-332). The epitope (309-332) was synthesized by solid phase peptide synthesis (SPPS), and purified by reversed phase HPLC. SPR Analysis provided high affinity to the antibody (K_D, 39 nM) [1]. For cell culture studies skin fibroblasts were used to evaluate the epitope peptides on the uptake of lysosomal enzymes in the presence of the patient´s antibodies. The results showed that antibodies were blocked by tight binding to the epitope peptide, thus opening a new concept to reconstituting therapeutic efficiency of ERT.

Innovative aspects

Epitope identification is opening a new therapeutic concept to hyposensitizing patients from immunogenicity and reconstituting therapeutic efficiency of ERT. Individual epitopes of patient antibodies basis for personalized medicinal approaches Epitopes and affinities are concomitantly identified by SPR-MS.

AFF- 05: Intact Transition Epitope Mapping - Thermodynamic Weak-force Order (ITEM-TWO) applied to Troponin I antigen epitopes with single amino-acid residue mutations.

Claudia Röwer[1], Bright D. Danquah[1], Kwabena F.M. Opuni[2], Cornelia Koy[1], Michael O. Glocker[1], Reham F. El-Kased[3]

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Heart failure is a leading cause of mortality in industrialized countries and about 16 million myocardial infarctions occurred world-wide in 2015. Early and sensitive diagnosis is crucial to limit myocardial damage and to preserve cardiac function. Human cardiac troponin I (hcTropoI) is a highly specific serum marker of myocardial infarction. Diagnostic assay accuracy depends on immune complex formation which itself is dependent on precise epitope – paratope interaction. Single amino-acid polymorphisms (SAPs) on hcTropoI are known to be present in patients with hereditary cardiomyopathies. Yet, it has not been studied whether presence of SAPs shall have an effect on diagnostic test outcome. Here we examined six epitope peptides, five of them carrying single amino-acid exchanges in or next to the epitope region of the anti-hcTropoI antibody, modelling SAPs of the human population.

Intact Transition Epitope Mapping - Thermodynamic Weak-force Order (ITEM-TWO) was applied to examine relative binding strength differences between the tested epitope peptides. Micro-molar solutions of immune complexes consisting of anti-hcTropoI antibody and epitope peptides with and without single amino-acid exchanges were nano-electrosprayed one after the other. In all experiments, multiply charged ions were translated into the gas phase from volatile buffer solutions at neutral pH. Then, the intact immune-complex ions were separated from unbound peptide ions by quadrupole filtering. Increasing the voltage differences in a subsequently aligned collision cell resulted in collision induced dissociation of the immune complexes by which bound peptide ions were released. Step-wise increase of collision cell voltage differences changed the intensity ratios (i) of surviving immune complex ions, (ii) of released peptide ions, and (iii) of free antibody ions. At high collision cell voltage differences ion signals of antibody fragments were observed as well. From all ion signals´ intensity changes were calculated apparent gas phase dissociation constants of the antibody – epitope peptide complexes. ITEM-TWO revealed an order of apparent gas phase dissociation constants for all tested peptides with single amino-acid mutations in relation to that of the “wild-type” epitope peptide. Next shall be evaluated in-solution binding strength differences of epitope peptides with and without mutations using surface acoustic wave biosensor experiments.

ITEM-TWO shall ultimately be applied to the study of point-of-care diagnostic antibodies. The goal is to improve myocardial infarction detection of individuals with (i) familial incidence of heart failures and (ii) being at high risk because of carrying mutations in the epitope region of a diagnostic antibody.

Environmental and Forensic Mass Spectrometry

ENV-01: HR-CS-GF-MAS as a new screening method for emerging pollutants - per- and polyfluorinated substances in the environment

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The introduction of fluorine in organic molecules leads to new chemical/physical properties. Especially in the field of pharmaceuticals, fluorinated organic molecules are becoming more and more popular and at present amount up to 25% of market share, with an upward trend. The main benefits of fluorinated pharmaceuticals are: (i) enhanced fat solubility; (ii) enhanced interaction of catalytic-center of enzymes with fluorine-drugs; (iii) a delayed metabolism within the human body. Highly fluorinated organic substances are also used in technical applications (e.g. coatings, fire-extinguishing agents).

Due to the broad variety of fluorinated substances and increasing production volumes numerous and up to date unknown fluorine-species are most likely to be present in the (aquatic) environment. Analytical methods to assess the degree of contamination of surface waters with organically bound fluorine are highly needed and up to now only combustion ion chromatography based method is available, which is relatively laborious.

Since a few years’ high resolution-continuum source-graphite furnace atomic absorption spectrometers (HR-CS-GFAAS) are commercially available from Analytik Jena. By means of this technique, the detection of high resolution molecular absorption spectra (MAS) is enabled. Thus, fluoride is detectable upon the addition of a modifier and the formation of a diatomic molecule (e.g. GaF). Just recently, we applied this technique for total fluorine (mainly dissolved fluoride) analysis in river water samples.

In the present work a HR-CS-GFMAS method for extractable organically bound fluorine (EOF) analysis in surface water samples was developed by us. The method is based on SPE extraction of organically bound fluorine even in the presence of high fluoride concentrations followed by HR-CS-GFMAS analysis upon elution. Due to high enrichment factors, LODs in the low ng/L range were achieved. We successfully applied our SPE HR-CS-GFMAS method to Rhine water samples and EOF in the range of about 50-300 ng/L was detectable.

ENV-03: Lysergic Acid Diethylamide (LSD) Analogues Investigated by ToF-SIMS and Orbitrap-SIMS

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A typical dosage form of the hallucinogenic drug Lysergic Acid Diethylamide (LSD) is the use of “LSD blotting paper”. For production a preprinted blotting paper is incubated with a solution of the drug. The uptake of the drug by the consumer is performed either via sucking or via swallowing of individual pieces (“trips”) of the LSD blotting paper.

The possession as well as the consumption of LSD is illegal in most countries. However, a number of chemical analogues of LSD exist that are legally unregulated. Examples for analogues are ETH-LAD[1], 52-ALD[2] and PRO-LAD[3]. The identification of the respective analogues as well as the identification of potential contamination is of interest for the legal authorities, once a confiscation of the material has been performed. Furthermore, the identification of the concentration and the evaluation of the homogeneity of the distribution of the respective drug on the blotter paper should be revealed. This information allows to percept the harm that derives from an individual trip to the potential consumer (e.g. the risk of overdosing).

In this study a number approaches to identify LSD analogues and their distribution on confiscated blotting papers are shown. The samples were analyzed by ToF-SIMS and Orbitrap-SIMS.

[1]N-Ethyl-nor-lysergic acid diethylamide

[2]Acetyl lysergic acid diethylamide

[3]6-propyl- 6-nor-lysergic acid diethylamide

ENV-05: Cleaning the River Clun: targeted and non-targeted screening

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Many pollutants are ubiquitous in surface waters because of continuous discharges from municipal wastewater treatment plants. Investigative monitoring of water bodies failing ecological standards as set out in the Water Framework Directive is now a requirement of all European Union member states. In this study we test the combination of a passive sampling device in combination with targeted and non-targeted workflows on a QTOF MS for monitoring surface water. Chemcatcher® passive samplers were deployed for four weeks in the River Clun, Wales, UK, whose WFD status is classified as poor, to determine which chemicals may be responsible. The extracts obtained were analyzed using an impact II (Bruker) LC-QTOF-MS followed by targeted and non-targeted processing. Samples were acquired from July 2017 until April 2018 from 8 different places of the River Clun. For each site there was a time course of 10 data points covering 10 months. With the passive sampling unit every sample is an average of the pollution flowing past the sampling device during the 4 weeks it is submerged in the river. As expected, pollution in the surface water due to discharges from municipal wastewater treatment plants varies significantly depending on the location and season. With the targeted approach between 5 and 93 compounds per sample could be identified with high confidence; in average about 50 compounds per sample.

ENV-07: Metabolism Simulation of Designer Drugs by means of Electrochemistry/Mass Spectrometry

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Why are designer drugs a matter of rising concern? The rapid variation, distribution and the lack of control procedures result an increasing consumption that can only be counteracted with appropriate analysis and detection methods. Unfortunately, current analysis procedures are complex, cost-intensive and time-consuming. The high diversity of the drugs complicates the analysis and due to the biotransformation after ingestion, the detection of the parent substances is often not possible. In order to establish suitable screening methods, knowledge of the metabolism is necessary since the substances are mostly excreted in form of their metabolites.

In order to investigate the metabolic pathway, conventional experiments with liver cells or animal testing are commonly used. However, the purely instrumental setup of electrochemistry (EC) coupled with mass spectrometry (MS) provides another possibility to simulate the metabolism. This tool enables the formation and detection of possible intermediates and products without disturbances by biological matrices. So far, a high degree of correlation was achieved by comparing the electrochemically generated products with metabolites from *in vivo* or *in vitro* experiments. Although many enzymatic-catalyzed reactions can be simulated, EC/MS cannot replace the well-established model systems, but it can be considered as a complementary method for metabolism elucidation.

In this work, the designer drug 3',4'-methylenedioxy-α-pyrrolidinohexiophenone (MDPHP) was investigated due to the lack of knowledge about pharmacology, toxicity and metabolism. MDPHP was electrochemically transformed in a thin-layer cell equipped with a boron-doped diamond working electrode. The electrochemical transformation provides oxidation and reduction products detected online by ToF-MS. The mass spectra are plotted in dependence on different potentials forming a three-dimensional waterfall diagram, a so-called mass voltammogram (MV). The MV visualizes the entire transformation process of the drug. The first oxidation step of MDPHP shows the elimination of hydrogen. In the reductive mode, the keto-group of MDPHP is transformed to an alcohol. Furthermore, hydroxylation was observed. Possible isomers of the hydroxylated species were identified using HPLC and trapped ion mobility spectrometry (TIMS)-qToF-MS. Accurate masses and fragmentation experiments were used in order to elucidate the respective structures. In summary, this work has shown that EC/MS can be valuable in the field of forensics and may contribute to counteract the growing consumption of designer drugs.

ENV-09: C-IRMS concept development for online GC investigations for industrial utilisation of low concentrated methane

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Low-concentrated methane (CH_4) from landfills and biogas plants should be recycled in an environmentally friendly, safe and biological process [1]. The biological utilisation was investigated using high performance GC. Using a special combination of columns and an application-adapted valve switching technology, molecules such as hydrogen sulphide (H_2S) and carbon dioxide (CO_2) are separated and detected independently from smaller molecules such as CH_4, oxygen (O_2), nitrogen (N_2) and hydrogen (H_2). With this two-channel GC technique, online headspace volume analyses from reactors for denitrification and

biogas production could be investigated. The presented results show that CH_4 is eliminated with activated sludge of denitrification, although it is poorly soluble in water. However, it must be investigated whether methane utilisation takes place with the help of dissolved O_2 or O_2 from nitrate (NO_3^-) [2]. It should also be investigated under which conditions the microbiocenosis uses the CH_4 for energy production or for building biomass. These questions will be answered using a combustion isotope ratio mass spectroscopy (C-IRMS) using ^{13}C-labelled CH_4 [3] and ^{18}O-labelled NO_3^- [4] as markers. If only ^{13}C is found in the metabolic product CO_2 and no altered isotope ratio for O_2 is found, then it is not certain that it originates predominantly from the NO_3^- to be eliminated. If ^{18}O is also found in CO_2, methane oxidation is nitrate-dependent. This poster should contribute to the critical discussion of our proposal to extend the existing online GC by a C-IRMS [5]. The C-IRMS extension should also be used to investigate the methane mass balance of biogas plants. The question is whether methane oxidation occurs in case of substrate deficiency, which would reduce the efficiency of biogas plants.

ENV-11: Analysis of mycotoxins in indoor samples using UHPLC-MS/MS

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Mould is ubiquitously spread in the environment and can contaminate not only food but also interiors. In indoor environments the growth of mould takes place especially after water damages and is generally promoted by an increased room air humidity. In Germany, visible mould infestation occurs in almost every tenth flat. Due to the fact, that people in industrial countries spend an average of 80-90 % of their time in closed rooms, indoor environments can thus represent an important source of exposure to mould. People living in affected housing show an increased incidence of diseases, especially of the respiratory tract. Thus, it can be assumed, that mould and in particular its toxic secondary metabolites (mycotoxins) evoke or contribute to these illnesses.

A reliable quantification of indoor mycotoxin exposure is not yet possible. The aim of the investigations at the Institute of Food Chemistry of the Westfälische Wilhelms-Universität Münster is therefore the development of a universal screening approach for the qualitative and quantitative detection of mycotoxins in interior samples. The methods used for this purpose are based on the separation of analytes by high-performance liquid chromatography in combination with detection by quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) respectively tandem mass spectrometry (UHPLC-MS/MS). The determined mycotoxin contents in the samples contribute to the assessment of indoor exposure to mycotoxins.

House dust collected in vacuum cleaners represents a good medium for the investigation of indoor contaminants and pollutants with moderate to low volatility and was therefore selected as sample material. However, its complex and abundant matrix components are challenging for sample preparation prior to UHPLC-MS/MS analysis and for the mass spectrometric analysis itself.

ENV-13: Quantitation of Mycotoxins in Four Food Matrices Comparing Stable Isotope Dilution Assay (SIDA) with Matrix Matched Calibration Methods by LC-MS/MS

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Mycotoxins are secondary fungal metabolites produced by mold that may be found in food or feed. They can cause severe health problems in humans and animals, and can result in significant economic losses. Among the hundreds of toxic mycotoxins, aflatoxins, fumonisins, deoxynivalenol, ochratoxin A, HT-2 toxin, zearalenone, and T-2 toxin are considered as a major concern for corn, wheat, peanuts and other agricultural products. LC-MS has become the standard and is now widely used for routine mycotoxin analysis and identification. One of the challenges faced by LC-MS techniques is the matrix effects caused by the use of electro-spray ionization (ESI). Generally, sample preparation, chromatographic and calibration techniques are the common strategies for reducing the negative effects of matrix effects. Standard addition, matrix matching, and stable isotope dilution assay (SIDA) are all possible calibration solutions. In this work, a quick “dilute-filter-shoot” method was used for sample preparation. A seven-minute LC-MS/MS method using a biphenyl phase column was developed and verified for quantifying twelve mycotoxins in four commodities: corn, peanut butter, brown rice, and corn & wheat mixed. Both SIDA and matrix matched calibration methods were applied, compared, and evaluated in terms of recovery, efficiency, advantages, and limitations.

ENV-15: The Analysis of Acrylamide Using an Aqueous Compatible Reversed-Phase Column by LC-MS/MS Detection

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Acrylamide is formed when substances containing asparagine and aldehyde sugars (i.e. glucose, fructose etc.) are roasted, fried or baked at temperatures above 120 °C. The foods with the most acrylamide are roasted coffee and starchy foods such as potato chips, toasted bread, and cereal. Acrylamide is also found in drinking water that has been treated with polyacrylamide as a flocculating agent. Laboratory studies performed on mice show that high level exposure can cause reproductive harm, neurological defects and cancer. Accordingly, many methods have been developed to determine the amount of acrylamide present in foods, tobacco and water. Multiple sample preparation techniques are effective at extracting acrylamide from matrix, but it is important to determine how much time should be invested on sample preparation and what stress the sample will place on your analytical system. QuEChERS-based methods tend to be quicker than SPE-based methods, but typically have more matrix components co-extracted which can lead to worse detection levels and more stress on your detector. SPE-based methods, result in a cleaner sample, but take a lot of time to prepare. LC columns used to quantify acrylamide often suffer from irreproducibility and poor column lifetimes. This results in longer turnaround times, less instrument uptime, and poor data quality. The Allure Acrylamide column addresses these pain points. This silica-based, aqueous compatible, reversed-phase column is part of a reproducible, retentive, and robust solution. The benefits of this column will be discussed showing examples

of acrylamide separation from difficult matrices such as coffee and potato products. Particular emphasis on the role varying degrees of sample preparation will be discussed.

ENV-17: Proven consistency in PFAS analytical workflow with extended compound list

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Per- or polyfluorinated alkyl substances (PFAS) have been used in various consumer products and industrial applications including clothing, food packaging, kitchenware, and aqueous film forming foams (AFFFs), which are critical for firefighting practice and widely used globally in many military bases and airports. The production of these chemicals began in late 1940's and toxicity information on many PFAS is still unknown except for the two with the longest production history and usage: perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), which are C8 based. With known toxicity of traditional C8 PFAS, the industry trend has been moving toward various alkyl chain length – typically shorter than C8 – as well as functional group modifications. While its unique chemical and physical properties (water resistance, persistence, and surface tension lowering) have provided benefits in many applications, appearances of these compounds are not limited to the regions where manufacturers of these compounds are located. Their ubiquitous use and the stability of these compounds make them present everywhere from the Arctic environment [1] to the bloodstream of the majority of human populations including even the most remote one at measurable concentrations [2, 3]. The analytical workflows for the low level detection of PFAS requires an LC-MS/MS (liquid chromatography tandem mass spectrometry) system. Often many components in the system (degasser, LC pump, mobile phase bottle lining, etc.) contain various fluoropolymer materials such as PTFE (polytetrafluoroethylene). Slow leaching of those materials can interfere with accurate quantitation of target PFAS. The most recent findings in PFAS analysis and research are presented here with an extended list of target PFAS in various environmental samples. The LC-MS/MS system was equipped with a delay column to selectively retain system-related PFAS interferences for accurate quantitation of the target PFAS in the actual samples.

1. Enviro Sci Technol 15;41 (10): 3455-3461, Young CJ et al.

2. Chemosphere, 88 (11) 1269-1275, Christian H et al.

3. https://www.ewg.org/research/pfcs-global-contaminants/pfoa-pervasive-pollutant-human-blood-are-other-pfcs

ENV-19: Rapid Profiling and Quantification of 17 Bile Acids in Human Plasma by LC-MS/MS

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Bile acids are a group of major catabolic products of cholesterol. They are important biomarkers for signaling potential harmful side effects for new drug development. Quantitation of bile acids in matrices proves to be very challenging due to a number of factors, including, the similarity of structures, varying polarities and stereochemistries, limited fragmentation for unconjugated bile acids in mass spectrometer, high endogenous levels, and matrix effects caused by phospholipids or triglycerides. In this study, a rapid, robust, selective and reliable LC-MS/MS method was established and validated in human plasma using a Raptor C18 column with baseline separation of 17 bile acids in 6 minutes.

ENV-21: Derivatisation causes peptide formation: Considerations for the analysis of prebiotic oligomerisation

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Peptide formation is considered the most crucial prerequisite for the origin of life [1]. Low level amino acid oligomerisation (<10%) is thus of interest and often studied based on HPLC-separation. Amino acids are difficult analytes due to their high polarity, low volatility and lack of a chromophore group and are therefore often studied using post- and pre-column derivatisation [2,3]. However, when we investigated salt-induced dipeptide formation [3] with glycine and valine using Marfey's reagent for derivatisation we noticed products already at the starting time point.

In order to avoid artifacts generated by this agent we switched to dabsyl chloride. Both chemicals modify the amino acids and dipeptides at the N-terminus and increase their size and hydrophobicity so that they can be detected by RP-LC-MS/MS. A method targeting di-and tripeptide formation was set up using MClass UPLC / Synapt G2 Si (Waters Corp.). Mixed di- and tripeptides were observed at levels up to 8.5% when G and V standards in aqueous solution were dabsylated. Interestingly, tripeptides were also detected when the pure dipeptide standards were measured following dabsylation. Apparently, the addition of a modification agent, even at gentle reaction conditions (1 h incubation in 90 mM $NaHCO_3$, 90% acetonitrile, 37°C) caused activation of the otherwise very stable - due to the tendency to form a resonating structure [4] - amide bond, and peptide reassembly.

Consequently, analyte derivatisation is not an option in research of primordial anabolism. This factor severely limits the use of mass spectrometry, although the use of ion-pairing agents or amide-based columns show some promise.

[1] Rode & Suwannachot (1999) Coordination Chemistry Reviews 190-192:1085.

[2] Rutherford & Gilani (2009) Current Protocols in Protein Science 11.9.1-11.9.37.

[3] Schwendinger & Rode (1992) Origins of Life and Evolution of the Biosphere 22:349.

[4] Mahesh et al. (2018) Molecules 23102615.

ENV-23: Characterization of gasoline and diesel range fuels derived from plastics using GC-EI-Orbitrap

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Plastics have been widely applied for usage in our daily life, such as packages, bottles. In return, the usage of plastic productions also generates a large amount of household and industrial plastic waste. An estimated 55% of the global plastics were discarded, 25% was incinerated and only 20% was used for recycling in 2015. The common way of discarded plastics is to dump them into oceans or landfillings. One big problem behind this strategy is that it takes decades to hundreds of years' for plastics' natural degradation, which harms environment and living creatures as well. However, plastics are carbon rich materials, which make them a useful energy resource. Here, we introduce a new approach for a final utilization of plastic waste in a conversion to a fuel.

Fuels for individual plastics and corresponding mixtures were prepared by fast pyrolysis at a temperature of 500°C and then condensed by two times ice/water and dry ice/acetone bath. For analysis, plastic fuels and corresponding fractions were analyzed by using GC-EI-Orbitrap at a resolution of 120,000 at *m/z* =200. The temperature program for GC was performed by increasing from 35 to 320 °C at a heating rate of 10 °C/min and then hold at 320 °C for 5 min.

For the complete conversion of plastic materials into fuels a pyrolysis reactor was developed and tested using individual materials such as polyethylene and polypropylene. The different fractions of the fuel were collected and analyzed by GC-HRMS. The results show, that for these materials a highly efficient transformation of plastics to fuel was achieved. To make a high quality liquid fuel, distillation optimization was successfully performed with a difference of around 70 °C between actual cutting temperature and theoretical distillation temperature indicated by GC-EI-Orbitrap investigation for specific molecules inside plastic fuels. This temperature difference was corrected and applied for distillation separation of gasoline and diesel fuel in plastic fuels. The molecular carbon number distribution was summarized for each gasoline and diesel fuel.

New aspects:

Plastic to fuels is a solution for waste management and alterative partial substitution of fossil fuel.

ENV-25: Investigation of biodiesel degradation products via FT-MS

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One approach to reduce CO_2 emissions of petro-based fuels is by blending them with biofuels. A major biofuel is biodiesel which main constituents are fatty acid methyl esters (FAMEs) that are prone to degradation processes and therefore may be heavily transformed during storage periods. Precipitates, possible products of degradation, can lead to blockage of filter systems and jets, and other serious problems. Fundamental basics of these processes are yet to be uncovered. To develop a better understanding of the emerging products, as well as the underlying processes, the aging of biodiesel in a long term storage simulation is investigated.

Measurements have been made with an Orbitrap Elite prototype. Spectra were gained via spectral stitching method with 30Da windows and a 5Da overlap in the mass range of *m/z* 135-1000 and a resolution of 480000 at *m/z* 400.

A major challenge in the investigation of aging effects in blends of petro- and bio-based fuels are the huge differences between the two kinds of fuels. Petro-based diesel is a mixture of several thousands of non-polar compounds, whereas biodiesel mainly consists of five different polar esters. This imbalance is the key problem in the non-target analysis of the blends. The methods of analysis of polar or non-polar compounds vary greatly. APCI is mostly used for non-polar compounds, whereas ESI is the method of choice for polar samples. In ESI non-polar compounds are suppressed by little concentrations of polar molecules. This is further complicated by the emerging degradation products, which primarily are oxygenated species and therefore even more polar.

During the first months of storage, there was a significant increase of oxygen atoms in the detected compounds. The oxygen was incorporated into the structures of the FAMEs, thus we were able to detect FAME derivatives with up to eight oxygen atoms. After three months of storage, the relative intensities of dimers of the FAMEs began to rise. Dimeric structures of FAMEs are possible precursors of precipitates. First MS/MS measurements of these dimers yield interesting findings about their molecular configuration.

New Aspect

The new aspect of this work is the non-target analysis of biodiesel degradation products via FT-MS, as well as the comparison of these products with the findings of irradiation experiments.

ENV-27: Feature-based molecular networking: a powerful tool for the identification of transformation products in environmental water samples

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Due to growing concern about organic micropollutants and their transformation products, the reliable identification of unknowns in our surface and drinking waters is of increasing interest. Non-target analysis with liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) enables the prioritization and identification of unknown compounds in environmental samples without prior knowledge or the need of reference standards. By applying this approach to water samples from four riverbank filtration sites with different redox conditions, we prioritized drinking water relevant and seasonally dependent compounds. Detected features (defined by mass-to-charge ratio (*m/z*), retention time and intensity) were subsequently processed with the feature-based molecular networking (FBMN) tool of the Global Natural Products Social

Molecular Networking platform (GNPS, gnps.ucsd.edu/). Features were connected based on their MS/MS spectral similarity score (cosine > 0.7) and numerous networks were formed. Additionally, every spectrum was compared to the GNPS spectral database. After filtering all networks containing at least one database-identified node, we selected a network containing the antihypertensive drugs sartans and their transformation products. With chromatographic and spectral similarity information, the identification of four transformation products (dealkylated valsartan, dealkylated irbesartan, IRB_442_C and C1) and olmesartan up to confidence level two (Schymanski et al., 2015) was enabled. The identification of the sartans was confirmed with reference standards, proving the method to be well applicable to identification of organic micropollutants in environmental samples. The sartans were investigated regarding their removal behavior under different redox conditions and seasons. Antihypertensives were grouped into compounds being well removed during riverbank filtration, compounds being primarily removed under anoxic conditions and rather persistent compounds. Seasonal variations were mainly limited to varying river water concentrations. Feature-based molecular networking therefore proved to be a powerful tool for the identification of previously unknown or unexpected compounds and their transformation products in environmental water samples and the direct assessment of their appearance in defined groups or processes.

ENV-29: Method development for speciation analysis of MRI contrast agents in river and seawater of Sydney (Australia) by µSPE and HILIC-ICP-MS

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Gd-based contrast agents (GdCAs) are often used to enhance contrasts in magnetic resonance imaging (MRI). Following MRI examination, they are excreted by patients and enter surface and seawater via effluents of local wastewater treatment plants and the frequent application led to an anthropogenic anomaly in the environment, which is particularly pronounced in areas with developed healthcare. The balancing of total Gd levels against those of other lanthanide analogues is the most common way to investigate this anomaly. However, data on Gd species are relatively scarce and so far, not available for any location in Australia. Greater Sydney is the largest metropolitan region (12,400 km^2) of Australia producing more than 1.3 billion litres of wastewater every day and discharging more than 90% into the South Pacific Ocean via deep ocean outfalls. This complicates sampling and confronts speciation analysis with very low concentrations and complex matrices.

In this work hydrophilic interaction liquid chromatography (HILIC) and inductively coupled plasma mass spectrometry (ICP-MS) are used to target individual Gd species in surface and seawaters obtained from the Greater Sydney region. To address low levels of Gd, figures of merit were improved by increasing ion transmission. This was accomplished by employing hard extraction conditions and operating the quadrupole in band pass mode. Furthermore, complex saltwater matrices require efficient matrix elimination and species preconcentration. This was achieved with a novel automated micro-solid phase extraction (µSPE) method.

The increase in ion transmission and the operation of a quadrupole in band pass mode translated in improved figures of merit for the analysis of commonly administered GdCAs (Gd-DTPA, Gd-DOTA, Gd-DO3A-butrol, Gd-DTPA-BMA). The automated µSPE method allowed matrix elimination and analyte preconcentration with quantitative recoveries from river and seawater. Collectively, this decreased limits of detection below 1 ng/L, which was crucial for targeting individual species in the Greater Sydney region.

ICP-MS User´s Meeting: Analysis of Nanomaterial

ICP-01: Detection of Silver and Titanium Dioxide Nanoparticles in Waste Waters and Natural Water *via* Single-Particle ICP-MS with Microsecond Time Resolution

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Engineered nanomaterials (ENP) are increasingly released into the environment due to their implementation in manifold industrial and consumer products. One important pathway of ENP distribution is *via* waste water treatment plants (WWTP). Although ENP possess many interesting properties, the exposure of nanomaterials to the environment is a potential threat towards humans and animals. Specialized methods are required to detect nanoparticles (NP) in a variety of matrices (*e.g.*, surface water, sewage sludge, and waste water) in order to analyse their fate in the aquatic environment and effect on organisms and ecosystems.

Single-particle inductively-coupled plasma mass spectrometry (spICP-MS) is an emerging technique for the detection, quantification, and sizing of inorganic NP. Nevertheless, sizing of particles in samples with complex matrices and high content of dissolved analytes still presents a significant challenge to off-the-shelf mass spectrometers due to limitations in hardware and software. The ICP-MS instrument that was used in this work was operated in combination with a home-built data acquisition unit that records transient signals with microsecond time resolution for single-particle detection. Low size-based limits of detection can be achieved with this system, which is crucial for the analysis of particles in the low nanometre range.[1]

As part of an extensive investigation on the fate and effect of NPs in water of a WWTP with a discharge in an Austrian lake on their aquatic ecosystem (FENOMENO project), water from the influent and effluent of the WWTP and the lake was sampled over the course of two years. Goal of the project was to detect silver and titanium dioxide NP in the samples and to analyse their particle number concentration and size distribution *via* spICPMS. In the study, a dedicated particle sampling protocol, spICP-MS method, and particle data processing were carefully developed and optimized for environmental water analysis. Silver and titanium containing nanoparticles (size range: 18–98 nm and 16–129 nm, respectively) were successfully detected in some samples.[2] A seasonal variation of titanium dioxide NP size distribution in WWTP influent and lake water was observed, while sizes of detected silver-containing NP were found to not vary significantly.

References:

[1] I. Strenge, C. Engelhard, *J. Anal. At. Spectrom*. **2016**, *31*, 135 – 144.

[2] R. Vogt, D. Mozhayeva, B. Steinhoff, A. Schardt, B. T. F. Spelz, A. Philippe, S. Kurtz, G. E Schaumann, C. Engelhard, H. Schönherr, D. K. Lamatsch, J. Wanzenböck, *Sci. Total Environ.*, **2019**, *696*, 134034.

ICP-03: A statistical model fit to extrapolate signal durations in the inductively coupled plasma-mass spectrometry in single nanoparticle mode

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Nanoscale materials with a size of 1 to 100 nanometers in at least one spatial dimension are a key technology of the 21st century due to their unique physical and chemical properties. Considering their widespread distribution, diverse applications and compositions, versatile analytical methods are needed to assess a potential environmental and toxicological impact. Among standard techniques such as electron microscopy and light scattering, the inductively coupled plasma-mass spectrometry (ICP-MS) has proven to be a valuable tool for the analysis of single nanoparticles (sp). Using fast time-resolved data acquisition, spICP-MS enables the determination of the elemental composition, particle size and number concentration of a nanoparticle suspension. Its benefits include high matrix tolerance, straightforward liquid sample preparation and the opportunity to acquire quantitative information on both nanoparticles and dissolved species. Nonetheless, recent publications have highlighted the necessity of adequate measurement condition control and data treatment strategies to harness the full potential of spICP-MS applications.[1]

In this study, quadrupole ICP-MS measurements of metal-based nanoparticles with detector dwell times (DTs) in the low milliseconds range were conducted to develop a mathematical model of the underlying counting statistics. Since a proper estimate of the event duration complements the correct choice of measurement conditions, the main goal was to characterize mean event durations without the need for sampling entire ion cloud profiles. Thereby, we aimed for a substantial data reduction and enabled the great fraction of ICP-mass spectrometers with slower detector systems to provide information on the signal duration. The central result of this study is a model fit that allows for a quick estimate of the nanoparticle signal duration using millisecond DT. For a set of gold nanoparticles with low background level, typical durations of several hundred microseconds were calculated from the model and validated using microsecond DT. In a second experiment, the fitting approach was applied to determine the signal elongation due to collision/reaction gases in a triple quadrupole (TQ) setup. Since many common nanomaterials contain elements with plasma interferences (e.g., silicon, titanium or iron) especially TQ experiments are expected to benefit from the new model.

Reference: [1] M. D. Montaño, J. W. Olesik, A. G. Barber, K. Challis, J. F. Ranville, *Anal. Bioanal. Chem*. **2016**, 408, 5053–5074.

ICP-05: Quantification of Organo(fluoro)phosphates by means of HPLC-ICP-MS from field tested electric vehicles

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Nowadays, state-of-the-art lithium ion battery (LIB) electrolytes consist of a mixture of linear and cyclic organic carbonates as well as the conducting salt $LiPF_6$. However, due to chemical and thermal instability, the electrolyte decomposes during operation and, in presence of trace amounts of water, an immense variety of organo(fluoro)phosphates (O(F)Ps) are formed. The qualitative and quantitative analysis of LIB electrolyte decomposition products is not only relevant for understanding the aging mechanism but needs also a classification of potential toxic hazards.[1] The formed O(F)Ps could have potential toxic effects, comparable to chemical warfare agents such as sarin.[1- 3]

In this study, two different liquid-chromatography inductively coupled plasma-mass spectrometry HPLC-ICP-MS methods were successfully developed for the separation and quantification of O(F)Ps. ICP-MS hyphenated to liquid chromatography is a promising tool to quantify ionic and polar as well as non-acidic phosphorus-based electrolyte decomposition products. Due to similarities to chemical warfare agents, only few standards are available, and structural independent quantification *via* phosphorus signal by means of ICP-MS is necessary. Due structural similarities and compound variety of the formed decomposition products, gradient elution was applied to separate the non-polar O(F)Ps. Changing temperature and carbon building, which occur during the change of organic content in the mobile phase, interfere the quantification with ICP-MS. Therefore, a counter gradient was applied, to lower the organic solvent alteration entering the ICP-MS and compensate the plasma fluctuations. The polar and ionic O(F)Ps were separated isocratically, why a counter gradient was not required. The structural elucidation of the quantified O(F)Ps was performed on an ion trap-time of flight-mass spectrometer (LC-MS-IT-TOF).

With this study, the quantification and identification of O(F)Ps from field-tested electric vehicles battery electrolytes was conducted for the first time using HPLC-ICP-MS. The knowledge of the concentration of the decomposition products is necessary for further investigations regarding toxicology of the formed substances.

References:

[1] S. Nowak, M. Winter, *J. Electrochem. Soc.* 2015, *162*, A2500–A2508.

[2] F. M. Raushel, *Nature* 2011, *469*, 310–311.

[3] J. Henschel *et al.*, *Anal. Chem.*, 2019, 91, 3980-3988.

ICP-07: Comparison of hydrodesulfurization products from different catalysts by LEC-FTMS and LEC-ICP-MS/MS

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Strong legal requirements demand that sulfur is to be removed almost entirely from fossil feedstocks before they can be used as transportation fuels. This removal is based on the hydrodesulfurization process during which sulfur is removed from given compounds as H_2S. The process involves high temperature and high pressure of hydrogen gas. In all industrial scale applications the reaction is performed using a heterogenous (solid state) catalyst that will be partially altered and poisoned during the reaction.

Depending on the sulfur atoms chemical surrounding, certain compounds are more recalcitrant towards the desulfurization process and are typically found as remaining compounds. The outcome of such a reaction depends on many factors, including the type of catalyst that was used.

We recently introduced an analytic approach employing both qualitative high resolution mass spectrometry and quantitative ICP-MS/MS for the analysis of different types of sulfur containing compounds in fossil material. The method comprises the separation of a feedstock according to the type of sulfur containing components by ligand exchange chromatography on a Pd(II) stationary phase. The chromatography separates sulfur containing compounds into non-condensed thiophenes, condensed thiophenes (benzothiophenes or higher) and sulfidic compounds. The classification into these three groups is useful for the evaluation of desulfurization reactions as the reaction efficiency is supposed to change along the boundary lines between these compound classes. The online combination of the chromatographic technique with an analysis by ICP-MS/MS offers a fast and quantitative approach to evaluating the outcome of such a reaction. This was enabled by using a recently introduced triple-quadrupole ICP mass spectrometer in a way that uses MS/MS not as a means for fragmentation but for building up a larger reaction product. Oxygen as collision gas leads to the partial formation of SO^+ from S^+ ions so that sulfur can be detected without isobaric interferences using its most abundant isotope.

Here we use this method to evaluate the performance of three different catalysts that have been used for the hydrodesulfurization of a synthetic crude oil mixture. Differences between the catalysts lead to different outcomes in the distribution of remaining sulfur containing compounds that are discussed in detail.

ICP-09: Trace element mapping of high-pressure, high-temperature experimental samples with LA-ICP-TOFMS – Illuminating melt-rock reactions in the lithospheric mantle

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Laser ablation inductively coupled plasma time-of-flight mass spectrometry (LA-ICP-TOFMS) is a fast, relatively high-spatial resolution method which allows to measure and visualize major, minor and trace elements in fine-grained samples. Thus, it is a promising tool for the investigation of high-pressure, high-temperature (HP-HT) reaction experiments that typically produce small grain sizes. Here, we apply LA-ICP-TOFMS mapping to HP-HT reaction experiments by Gervasoni et al. 2017 between a) hydrous eclogite and fertile peridotite, which simulates metasomatism in subduction zones, and b) ultramafic silicate-carbonate melt and peridotite, which simulates metasomatism at the base of cratonic lithospheric mantle. A spatial resolution (i.e., laser spot size) of 5 µm and an acquisition rate of 20 multi-element pixels per second (i.e., laser repetition rate of 20 Hz) were used for mapping of the experimental samples. We demonstrate the applicability of LA-ICP-TOFMS by comparison of our results to maps created with electron probe micro-analysis (EPMA) and elemental concentrations measured by conventional (i.e., crater-drilling) LA-ICP-MS. Our new data suggest that metasomatism in subduction zones is expected to result in the formation of Al–Ti-rich amphiboles that strongly fractionate the rare earth elements (REE) and high-field strength elements (HFSE) from other trace elements. In contrast, metasomatism of the cratonic lithosphere involves fractionation in the light and middle REE, large ion lithophile elements (LILE) and HFSE, leaving Ni and heavy REE behind in olivine and garnet, respectively. The trace element enrichment patterns of the resulting melt bear a general resemblance to those of natural Group I kimberlites.

ICP-11: Retention Behavior of Radionuclides on Calcium Silicate Hydrate (C-S-H-) Phases: A Kinetic Study under Highly Saline and Hyperalkaline Conditions

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The safe disposal of radioactive waste and resulting hazardous substances is one of the major challenges of our generation. For long term disposal, the most appropriate solution seems to be deep geological repositories. The radioactive waste is supposed to be stored several hundred meters in the underground in a geologically impermeable and stable formation surrounded by an engineered barrier. Most of the site investigations focusing on the host rock as natural barrier. However, it is necessary to analyze backfill materials in order to assess the safety and the technical capabilities of deep geological repositories. Cement -based backfill and construction material will be used to provide an engineered barrier to radionuclide release over a long period of time. Calcium silicate hydrate (C-S-H-) phases are a major part of hardened cement materials and aim to prevent hazardous substances entering into the environment originated from a leakage in a waste disposal. For Germany, generic repository site models considering clay as host rock for northern and southern Germany are being developed to test the applicability of the long-term safety case. In northern Germany, high salinity host rock pore waters up to 5 M are assumed in a possible deep geological repository for radioactive waste. The interaction of host rock pore water and cements could potentially lead to cement corrosion. Consequently, hyperalkaline pH values (pH > 12) of the pore water can be achieved due to alteration processes of C-S-H-phases.

The presentation will demonstrate the combination of kinetic retention experiments with systematic Inductively coupled plasma mass spectrometry (ICP-MS) investigations. Kinetic studies of the UO_2^{2+}, I^-, Cs^+ and Eu^{3+} retention as waste cocktail on C-S-H-phases have been carried out in a highly saline reference pore water solution (2.6 M) under hyperalkaline conditions (pH 12.5–13), to reconstruct realistic conditions in a deep geological repository in northern Germany. While the retention behavior of U(VI), I^-, Cs^+ and Eu(III) was investigated individually on C-S-H phases in literature, retention kinetics as mixture of these elements are largely unknown. Based on realistic assumptions hazardous substances will enter surroundings as waste cocktail. Our research is required to gain out new results for kinetic retention processes of radionuclides as waste cocktail under highly saline and hyperalkaline conditions on C-S-H-phases as major part of cement based backfill material.

We sincerely thank the German Federal Ministry for Economic Affairs and Energy (BMWi) and the Project Management Agency Karlsruhe (PTKA-WTE) for funding (project: 02E11415D)

ICP-13: Speed up Your Productivity – High-Throughput Measurement of Drinking Water by PlasmaQuant MS Q

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For labs routinely measuring a large number of samples, sample throughput and the cost per sample are highly important. The unmatched sensitivity of the PQMS Elite allows to use shorter measurement times and still achieving a very competitive precision. Here, we report a high throughput method analyzing >80 drinking water samples per hour according to the US EPA 200.8 which specifies the criteria for drinking water analysis by means of ICP-MS. In this study, 21 elements (+3 internal standards) were measured over 7 hours with an average RSD of 2.2 %. Interferences were removed using the patented iCRC technology and quality control samples were measured to verify accuracy, precision, robustness of the method. The recovery rates of the quality control samples were between 90-105 % during the entire measurement and were evaluated fully automatically by the AspectMS software. The user friendly software, the lowest argon consumption on the market and the unmatched sensitivity result in the highest sample throughput and lowest cost per sample making the PQMS the ideal solution for labs measuring routinely a large number of samples.

ICP-15: Calibration of Mg isotope amount ratios and delta values

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In the past, $\delta^{26/24}$Mg measurements were referenced to NIST SRM 980, the initial zero of the $\delta^{26/24}$Mg scale. With the development of MC-ICPMS, the detection of small but measurable isotopic differences in different chips of SRM 980 became apparent. To solve this problem a suite of magnesium isotope reference materials, ERM-AE143, -AE144 and -AE145, has been certified in a first study by applying an *ab initio* calibration for absolute Mg isotope ratios without any *a priori* assumptions, a procedure which fulfils all requirements of a primary method of measurement. We could achieve for the first time measurement uncertainties for isotope amount ratios close to the typical precision of magnesium delta values, $\delta^{26/24}$Mg, which are at the 0.1 ‰ level (2SD). In addition, it was demonstrated that commonly used fractionation laws are invalid for correcting Mg isotope ratios in multi-collector ICPMS as they result in a bias which is not covered by its associated uncertainty. Depending on their type, fractionation laws create a bias up to several per mil, with the exponential law showing the smallest bias between 0.1 ‰ to 0.7 ‰.

With these isotope reference materials, it is possible to establish SI-traceability for magnesium delta measurements. To realize this, we organized a second study within which five expert laboratories participated to cross-calibrate all available magnesium isotope standards, which are NIST SRM 980, IRMM-009, ERM-AE143, ERM-AE144, ERM-AE145 and the standards DSM3 and Cambridge-1. The mean $\delta^{26/24}$Mg values for the individual iRMs, calculated from the laboratory means show 2 SD reproducibilities varying between 0.025 and 0.093 ‰. Propagated measurement uncertainties suggest a standard uncertainty of about 0.1‰ for $\delta^{26/24}$Mg determinations (2SD). Thus, SI traceability for magnesium isotope amount ratios and delta values is demonstrated to be established.

ICP-17: Development of a calibration strategy for LA-ICP-MS based on dried droplet residues of individual picoliter droplets

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Laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) is a powerful technique for the analysis of solid samples due to fast, quasi-nondestructive analysis of small samples and detection or determination of elements up to the ultra-trace range. Elemental fractionation and strong matrix effects necessitate the use of matrix matched standards for external calibration. Commercial reference materials with a certain matrix, sufficient homogeneity and the required elements are rare, which is the major limitation of LA-ICP-MS. Hence, the development of calibration strategies without matrix matched standards is a topic of current research.

The use of dried droplet residues as calibration standard is a matrix independent approach for quantitative LA-ICP-MS analysis. It was utilized for quantitative analysis of different materials e.g. glass[1] and polymers[2] via standard addition as well as for quantitative analysis of single cells[3] via external calibration. The dosing process has to be highly reproducible regarding the impact location as well as the droplet size and consequently the transferred analyte mass. For single particle analysis and

spatially resolved analysis of materials the droplets should be in the low pL-range so that residues in the low µm-range are formed.

In this poster, a *drop-on-demand* (DOD) system[4–6] for the generation of single pL-droplets will be presented, which is based on the thermal inkjet technology and fulfills all the above-mentioned requirements. A fundamental characterization of this unique system concerning the dosing precision will be outlined. Results for the quantification of thin layered materials using the dried droplet standard addition approach will be discussed critically.

[1] Á. Villaseñor, C. Greatti, M. Boccongelli, J. L. Todolí, *J. Anal. At. Spectrom.* **2017**, 32, 587.

[2] Á. Villaseñor, M. Boccongelli, J. L. Todolí, *J. Anal. At. Spectrom.* **2018**, 33, 1173.

[3] M. Wang, L. N. Zheng, B. Wang, H. Q. Chen, Y. L. Zhao, Z. F. Chai, H. J. Reid, B. L. Sharp, W. Y. Feng, *Anal. Chem.* **2014**, 86, 10252.

[4] J. O. Orlandini v. Niessen, J. N. Schaper, J. H. Petersen, N. H. Bings, *J. Anal. At. Spectrom*. **2011**, 26, 1781.

[5] J. O. Orlandini v. Niessen, K. M. Krone, N. H. Bings, *Spectrochim. Acta B* **2014**, 92, 51.

[6] N. H. Bings, J. O. Orlandini v. Niessen, J. N. Schaper, *Spectrochim. Acta B* **2014**, 100, 14.

Instrumentation - Ion Sources and Analyzers

IIS-01: Determining the Influence of Pre-Lithiation Techniques on the Lithium Distribution in Graphitic Electrodes for Lithium Ion Batteries

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The lack of appropriate energy storage technologies restrains the reliability of renewable energy production for a constant power supply. Facilitating the change from conventional towards greener technologies, energy storage systems experience a growing market and increasing research interest. In the field of electrochemical energy storage systems, the lithium ion battery (LIB) is the most promising and dominating technique. State-of-the-art LIBs consist of a carbonaceous negative electrode and lithium transition metal oxides ($LiMO_2$: M = Ni_x, Co_y, Mn_z (x+y+z=1)) as positive counterpart, as well as an electrolyte allowing ionic conductivity. This cell chemistry is commercialized in electric vehicles and grid storage systems.[1]

However, LIBs are still under investigation and continuous development to overcome disadvantages concerning battery cell aging, hence performance fading. One effect examined by different research groups is the loss of active lithium. In particular, during the first charge/discharge cycle the formation of a *solid electrolyte interphase* on the negative and a *cathode electrolyte interphase* on the positive electrode, consume active lithium but are crucial for long-term performance. One compensation strategy to reduce the loss of active lithium is the addition of supplementary lithium to the lithium content of the electrodes prior to cell assembly.[2] This so-called pre-lithiation can overcome decreased coulombic efficiencies in the formation process and reduced cell capacities. Nonetheless, surface and depth characterization of pre-lithiated electrodes are rarely described in literature, although lithium gradients in the bulk are known to influence the performance of LIBs.[3]

Glow discharge-sector field-mass spectrometry (GD-SF-MS) was applied to investigate the influence of pre-lithiation techniques on the lithium depth-distribution. In comparison to surface sensitive techniques like X-ray photoelectron spectroscopy (XPS) or scanning electron microscope-energy dispersive x-ray analysis (SEM-EDX), which are commonly used in the research field of LIBs, GD-SF-MS offers the possibility of a fast depth-profiling and semi-quantification without standards. In this work, three different pre-lithiation techniques are compared, the required amount of supplementary lithium is estimated based on the lithium content found in cycled cells, and pre-lithiation techniques are optimized to compensate this lithium loss.

References:

[1] G. E. Blomgren, *J. Electrochem. Soc.* **2017**, *164*, A5019-A5025.

[2] F. Holtstiege, P. Bärmann, R. Nölle, M. Winter, T. Placke, *Batteries* **2018**, *4*, 4.

[3] K. P. C. Yao, J. S. Okasinski, K. Kalaga, I. A. Shkrob, D. P. Abraham, *Energy Environ. Sci.* **2019**, *12*, 656.

IIS-03: Identification of Decomposition Products in Pyrrolidinium-based Ionic Liquid Electrolytes in Lithium Ion Batteries by means of GC/APCI-Q-TOF

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Ionic liquids (ILs) are a promising alternative electrolyte solvent for lithium ion batteries (LIBs) and lithium metal batteries (LMBs). ILs are salts with a melting point below 100 °C. In contrast to conventional carbonate-based electrolyte solvent, ILs benefit from their low flammability and a negligible vapor pressure. Pyrrolidinium-based ILs in combination with lithium bis(trifluoromethanesulfonyl)imide (LiTFSI) or lithium bis(fluoromethanesulfonyl)imide (LiFSI) as conducting salt are most studied IL electrolytes for application in LIBs and LMBs. Although ILs have a high thermal stability as well as an electrochemical stability window (ESW) up to 5.5 V, they still decompose during thermal treatment and long-term cycling. While a big variety of aging investigations of carbonate-based systems is reported in literature, data addressing the aging of ILs-based systems is rare. In the work of Pyschik *et al*. cation decomposition species were identified in pyrrolidinium- and imidazolium-based ILs after thermal aging experiments by capillary electrophoresis/electrospray ionization-mass spectrometry (CE/ESI-MS).[1] However, this technique is only targeting ionic species. Furthermore, there are only a few studies indicating the presence of tertiary amines as degradation products generated in pyrrolidinium based ILs, such as *N*-butyl-*N*-methylpyrrolidinium bis(trifluoromethanesulfonyl)imide (Pyr_{14}TFSI).[2,3]

In this study, a method for the identification of amine-based aging products of $Pyr_{14}TFSI$ after thermal aging in direct contact with lithium metal by gas chromatography/atmospheric pressure chemical ionization-quadrupole-time of flight mass spectrometry (GC/APCI-Q-TOF) was developed. In contrast to electron impact ionization (EI), which is commercially established, the APCI results in less fragmentation of the analytes. Furthermore, we introduce water into the source to promote the formation of $[M+H]^+$ enabling the detection of the molecular mass and simplifying fragmentation experiments for structure elucidation.

[1] M. Pyschik, M. Winter and S. Nowak, *J. Chromatogr. A*, **2017**, 1485, 131–141.

[2] M. C. Kroon, W. Buijs, C. J. Peters and G. J. Witkamp, in *Green Chemistry*, Royal Society of Chemistry, **2006**, vol. 8, pp. 241–245.

[3] E. Markevich, R. Sharabi, V. Borgel, H. Gottlieb, G. Salitra, D. Aurbach, G. Semrau and M. A. Schmidt, *Electrochim. Acta*, **2010**, **55**, 2687–2696.

IIS-05: Dynamics of desorption from a microdroplet with IR laser in LILBID-MS

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Laser induced liquid bead ion desorption (LILBID) is a soft ionization method that allows the analysis of thermally labile molecules and non-covalent complexes by mass spectrometry. This soft ionization method is used in native mass spectrometry (MS), which is an important tool in structural biology. A piezo droplet generator is used to form microdoplets (Ø 30-50 µm) of the sample solution. They are transferred to high vacuum and are irradiated by a mid-IR (infrared) laser. The laser is tuned to 2.9 µm, which corresponds to the symmetric and asymmetric H-O stretching vibrational mode of water. The absorption of this energy by the droplet leads to an explosive expansion, ejecting solvated ions and allowing them to be analyzed by the time of flight (TOF) analyzer.

With the purpose of understanding the LILBID desorption process, we have developed a method to study the initial velocity of the ions produced by IR laser desorption of microdroplets. The ion current was detected with a linear TOF analyzer and the IR laser desorption of the microdroplets was recorded via time-resolved imaging by a CMOS sensor.

For this work, we have used a 500µM solution of vancomycin (MW: 1485.7g/mol), and the ion detection was done in negative ion mode. The droplet explosion occurs in a Wiley-MacLaren type accelerator, consisting of a repellor and an extractor, which are set to a field free environment for the droplet during laser irradiation. At different delay times after IR irradiation we switch on the extraction field, which only accelerates the ions that had passed the extractor. Only these ions are detected, while the other ions are accelerated in the opposite direction to prevent them from being detected. Thus, based on geometry and the delay time used, we can assume that detected ions had a certain minimal velocity leaving the explosion. We determined that the initial velocity of the ions is around 600 m/s. With the method developed, using the images recorded of the shape of the explosion, we will study the dependence of the ions´ velocity distribution on how the droplet was irradiated by the IR laser, whether it was completely or partially illuminated.

Understanding the ion desorption processes is important to help us develop new quantitative and qualitative methods for the study of biomolecules with LILBID-MS.

IIS-07: What determines the post-ionisation efficiency in MALDI-2: A combined soft-/hardware-based set-up to characterise the role of relevant input parameters

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MALDI with laser-induced post-ionisation (PI), also named MALDI-2, increases the ion yields for numerous classes of lipids, metabolites and carbohydrates in MALDI-MS imaging experiments. Here we present a semi-automatic protocol to investigate and optimise the efficiency of the PI process in dependence of five relevant input parameters: pulse energies of the two lasers, PI laser wavelength, delay between the laser pulses, and buffer gas pressure in the ion source. Our experiments crucially assist in identifying optimal MALDI-2 settings and provide valuable insight into the MALDI plume expansion dynamics and MALDI-2 ionisation mechanisms.

All experiments were conducted with a modified MALDI-2 Synapt G2-S HDMS mass spectrometer (Waters). A N_2 laser (337 nm) served for desorption/ablation of material at ≥10 µm spot size. A wavelength-tuneable optical parametric oscillator (OPO) laser was used for PI at 260 nm or 280 nm (sample to beam waist distance ~500 µm, pulse duration ~6 ns). The investigated MALDI matrices were: 2,5-Dihydroxybenzoic acid (DHB, pos. ion mode, +), 2,5-Dihydroxyacetophenone (DHAP, +), α-Cyano-4-hydroxycinnamic acid (CHCA, +), Norharmane (Nor, neg. ion mode, -), and 1,5-Diaminonapthalene (DAN, -). A mixture of polar phospholipids (PL) from porcine brain was used as lipid standard. In order to obtain uniform coatings, in all cases MALDI matrix and analyte were sprayed separately onto a glass slide.

At the core of our study stood a newly developed set-up that combines hardware components and LabVIEW VI-based software for control and read-out of all of the above defined input parameters. In this way, during a MALDI imaging run with a homogenous sample, up to four (out of the overall five studied) input parameters could be systematically varied and mass spectra recorded simultaneously.

In line with our initial MALDI-2 studies, in particular PLs that are less well ionizing from complex mixtures under standard MALDI-MSI conditions were the greatest beneficiaries of the PI process. In the positive ion mode, a maximum ion boost for the generated $[M + H]^+$ species was found with a N_2 buffer gas pressure of ~2 mbar and a delay between the laser emissions of about 10 µs. In the negative ion mode, higher optimal delay times of several 10 µs were registered for the two studied matrices and generation of $[M – H]^-$ species of the PLs.

Our comprehensive data cube provides valuable insight into the mechanisms underlying the MALDI and MALDI-2 processes and could help to further optimize this emerging technique.

IIS-09: Advancing ionization of analytes using Low-Temperature Plasma Mass Spectrometry

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Introduction

Dopants have been used in different ionization techniques [1]. Dopants are chemicals to assist ionization, which means they get ionized easily and transfer a proton to the analyte. Therefore, the dopants need to have low proton affinity compare to the analyte. In this work, the dopant was used to enhance the ionization of analytes by using Low-Temperature Plasma (LTP) source.

Another enhancement method was studied using photocatalytic nanoparticles (NP) [2]. So we are planning to study this method for higher molecular weight compounds (500-900g/mol). And also, compare helium versus argon as discharge gas for the signal intensity of compounds in the presence of photocatalytic NPs.

Experimental part

LTP source, a self-made experiment based on the design by Nørgaard et al. [3] was coupled with FT-ICR APEX (IV) mass spectrometer (Bruker Daltonics) which has 9.4 T superconducting magnet. Plasma voltage was 12.0 ± 0.9 kV used. Argon or Helium (99.999%) used as the discharge gas. The analyte is introduced through a capillary spray in front of LTP.

Toluene was used as a first dopant. As photocatalytic nanoparticles (NP) TiO_2 (mean size 23 nm) was engaged.

Results and Discussion

LTP was driven using Argon as a discharge gas up to now. Due to the energy of the lowest excited metastable state in argon is 11.5 eV [5], the ionization yield was quite low. There are serval pathways to overcome. One might be using TiO_2 (NP), which was already engaged successfully using helium gas (energy of the metastable state is 19.8 eV) [2]. Our study will compare the use of TiO_2 in argon or helium plasma.

A different way to increase ionization yield is to use dopants like toluene which enhanced the $[M+H]^+$ signal of acridine already in our experiment, even using argon plasma. In a further study, other dopants will be compared with other analytes.

References

[1] D. B. Robb, T. R. Covey, A. P. Bruins, *Anal. Chem.* **2000**, 72, 15, 3653.

[2] Dan Bee Kim, Sohee Yoon, *J.A.S.M.S.* **2018**, 29, 12, 2333-2340.

[3] A. W. Nørgaard, A. Vibenholt, M. Benassi, P. A. Clausen, P. Wolkoff, *J.A.S.M.S.* 2013, *24* (7), 1090,

[4] Bell, K. L., Dalgarno, A., Kingston, A. E., J. Phys. B At. Mol. Phys., 1, 1968, 18.

[5] Hebner, Journal of Applied Physics, **1996**, 80, 2624.

IIS-11: Paperspray mass spectrometry – A potential novel technique for the detection of polarcompounds in sports drug testing?

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High performance liquid chromatography coupled to mass spectrometry (LC-MS) is widely used in sports drug testing. With the development of increasingly powerful instrumentation, a tendency towards simplified assays has been observed in doping control, where sample pretreatment has been reduced to a minimum, leading eventually to so called “dilute-and-inject” assays. However, extraordinary hydrophilic compounds still represent a challenging task in LC-MS due.

In this pilot study, paperspray mass spectrometry (PS-MS) was investigated as an alternative technique for the initial testing of highly polar compounds that are considered as prohibited in sports. The technique utilizes a strategy that allows for ionizing analytes from complex sample matrices spotted on a paper strip leading to mass spectrometric results in less than two minutes.

Experiments were carried out using the VeriSpray™ ion source on a TSQ Altis™ triple quadrupole mass spectrometer. The paperspray workflow typically involves the following steps: 1. Spotting the sample, 2. Drying the sample, 3. Applying a rewet solvent, 4. Sample strip slide out / applying a spray solvent, and 5. Applying the spray voltage to induce electrospray ionization of the analytes.

As model compounds of this proof-of-concept study meldonium, metformine, bemitil, p-hydroxyamphetamine, oxilofrine, octopamine and its sulfoconjugate, 5-aminoimidazole-4-

carboxamide-1-β-D-ribofuranoside (AICAR), myo-inositol trispyrophosphate (ITPP), ethyl glucuronide (ETG), ethyl sulfate (ETS), tramadol, and cyanocobalamine were chosen.

Except ITPP, all of the aforementioned hydrophilic model compounds were successfully determined in human urine using PS-MS at relevant concentrations. The correlation coefficient for each calibration curve was greater than 0.98, indicating good linearity with detection limits meeting the criteria of sports drug testing.

For meldonium, ETG and ETS, ion mobility provided by a FAIMS Pro™ Interface coupled to the VeriSpray™ ion source proved particularly valuable. In brief, high field asymmetric ion mobility spectrometry (FAIMS) is an ion separation technique based on

differences in ion mobility by applying alternating low and high electric fields between two cylindric electrodes. FAIMS provides a significant increase in selectivity by effectively reducing the background noise, which led to 5-10-fold improved detection limits of meldonium.

In comparison to conventional LC-MS approaches, the key benefits of this technique are the enormous cost- and time-savings due to minimal sample pretreatment and omission of chromatographic separation. All investigated model compounds fulfilled WADA requirements regarding the applicable minimum required performance limits for initial testing procedures. The combination of the VeriSpray™ ion source and the FAIMS Pro interface adds additional selectivity and sensitivity to the assay.

Instrumentation - New Development

IND-01: Characterization of Isomeric Glycolipids by Cryogenic Gas-Phase Infrared Spectroscopy

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Glycolipids occur in all organisms ranging from bacteria to men. They are composed of a glycan and a lipid moiety, which can both exhibit different kinds of isomerism. Minute structural alterations are often crucial for the biological functionality of the glycolipid; however, no standalone analytical technique can provide a comprehensive structure elucidation of complex glycolipids to date. Moreover, insufficient tools to distinguish isomers have led to the falsification of research results in the past.

Cryogenic gas-phase infrared (IR) spectroscopy in superfluid helium droplets was recently demonstrated to be a powerful complement to existing analytical workflows for glycolipid analysis. In this technique, mass-to-charge selected glycolipid ions are captured in helium nanodroplets and interrogated by a tunable IR free electron laser. Upon sequential absorption of multiple IR photons, the ion is released from the droplet and detected by mass spectrometry. The resulting IR spectra are highly resolved and can be compared with theoretical calculations.

Cryogenic gas-phase IR spectroscopy was employed for a systematic investigation of isomeric glycosphingo- and glycoglycerolipids The comprehensive sample sets allowed probing the influence of the glycan, the lipid and the configuration of the interconnecting glycosidic bond on the vibrational spectra. All kinds of isomerism could be unambiguously resolved by diagnostic IR fingerprints. Furthermore, sphingolipids with different double bond positions and configurations were investigated. Due to an interaction between the protonated amine and the double bond, the isomers could be distinguished by characteristic N-H vibrations. Overall, the results underpin the exceptional power of IR spectroscopy to distinguish (glyco-)lipid isomers.

IND-03: Utilization of mass defects and mass remainders in LC/HRMS data of polymers for simplified interpretation of multiply charged ions

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The mass spectrometric data obtained from complex polymer formulations contains a vast amount of signals, which arise from different homo- and co-polymer series. In contrast to MALDI, which leads to singly charged ion species, ESI further complicates the spectra since it leads to a distribution of multiply charged ion species. The manual assignment of the signals is time consuming and challenging due to the vast number of signals and occurring spectral overlap.

In the last few years, several approaches for the interpretation of polymer one-shot MALDI-MS and ESI-MS spectra, based on Kendrick mass defect (KMD) and mass remainder (MR) analysis, were published. While they are readily applicable to MALDI-MS data, the interpretation of KMD and MARA plots of ESI-MS data is still time consuming, due to the isotopic split and misalignment of multiply charged ion species. These splits and misalignments can be corrected for, but only for one charge state at a time, spreading the other charge states over the plot and making it not readily accessible.

A new charge independent KMD and MARA analysis approach for the interpretation of LC/ESI-MS data is proposed. In contrast to one-shot data, the use of a short chromatographic separation to circumvent spectral overlap was applied. After chromatogram building as well as peak detection and deconvolution, a list of peaks and their m/z values is obtained. By comparing peak shape and retention time, those peaks which form an isotope pattern are grouped together. The thereby determined charge state and isotope pattern is saved, while only the peaks with monoisotopic m/z values remain in the peak list.

Based on this peak list, charge independent KMD and MARA plots were obtained by correcting each peaks m/z value according to its charge state and charge carrier. Instead of m/z, a charge state corrected value being $m/z*z-m_{cc}$, with z being the charge state and m_{cc} being the exact mass of the charge carrier, is used for KMD and MARA calculation.

With this approach oligomeric species line up horizontally in the KMD and MARA plots independent from their charge states, making the identification of homo- and alternating co-polymer series with different head groups by LC/ESI-MS easily accessible.

IND-05: Identification of Molecular Indicator Paper Components by Orbitrap-SIMS and Multivariate Analysis

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Indicator papers are often used as quick tests for the analysis of liquid solutions. An example for an indicator paper is “universal indicator paper” which is used for the pH-determination of a solution.

Typically an indicator paper consists of a cellulose matrix that is incubated with one or several indicator substances.

Goal of this study was the unambiguous identification of molecular pH paper components by Orbitrap-SIMS and Tandem-MS. The complex Orbitrap-SIMS spectra of different indicator papers were evaluated with help of multivariate analysis. In summary, it was possible to determine the identity as well as the SIMS fragmentation pattern of the indicator substances.

Overall the approach is a feasibility study for the identification of a priori unknown sample components in a set of self-similar samples.

IND-07: Instrumentation developments in omnitrap technology for advanced processing of gas phase ions

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The omnitrap platform is a linear arrangement of ion trap segments supporting a diverse ion activation network to enable multiple-stage tandem mass spectrometry experiments for in-depth characterization of gas phase ions. The segments of the linear ion trap are driven by a pair of anti-phase rectangular RF waveforms providing access to an extended m/z range through variations in the RF frequency. The rectangular waves allow for external injection of electrons and low mass ions with precise kinetic energy. The stability conditions of trapped ions are investigated by scanning the RF and resolving DC components applied to the omnitrap under variable space charge loads. Space-charge resonances are measured with high precision across the entire a-q parameter space revealing the space charge capacity of the design. Multiple-stage tandem experiments can be performed with high efficiency in ion accumulation mode, which is an essential feature to maintain sufficient levels of signal-to-noise for spectra interpretation. Despite reducing the duty cycle, this advanced operation allows for increasing ion population at different stages of an MSn experiment where the number of accumulated ions is ultimately determined by the space-charge limit of the design. Additional features explored on the omnitrap platform involve beam type collision induced dissociation enabled by fast switching of DC potentials applied for axial ion acceleration. The formation of immonium species is demonstrated experimentally for multiply protonated ubiquitin ions. A new electron source is simulated under space charge conditions including a system of steering lenses to maximize electron injection into the trap. Optimum conditions are identified from the low energy range applied in electron capture dissociation to the higher energy range employed in electron ionization and electron induced dissociation.

IND-09: Automated chiral analysis of amino acids by trapped ion mobility-mass spectrometry

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The role of D-amino acids in organisms was underestimated for decades, while L-amino acids were commonly known as the building blocks of life. However, recent studies on D-amino acids revealed specific biological functions and also indicated that abnormal D-amino acid levels, for example in the brain, can be linked to several diseases such as autism or Alzheimer’s disease. In this context, D-amino acids have become a research area of growing interest, for example in the fields of biomedicine or nutrition.[1] The development of novel separation techniques for chiral analysis is therefore an attractive field in the life sciences. Automation of these processes could also enable high throughput analysis, which is relevant for industrial applications.

In this study, a fast and fully automated method for the chiral analysis of amino acids was developed. A syringe-driven chromatography system was used for automated inline chiral derivatization and chromatographic pre-separation within five minutes. The system was directly coupled to the electrospray interface of a trapped ion mobility-mass spectrometer for post-ionization separation of the formed amino acid diastereomers. D/L amino acid ratios in fermented food samples were determined to demonstrate applicability of the method.

The use of chiral acyl chlorides, for example (*S*)-naproxen chloride, as derivatization agents under optimized reaction conditions proved to be most efficient concerning reactivity and thus overall conversion and speed. Using a strong cation-exchange column in ion-exclusion mode enabled a fast pre-separation and thus prevented ion suppression effects in the electrospray interface. After ionization, a fast separation based on different gas-phase mobilities of the diastereomers could be achieved for alanine and several other amino acids. Quantitative information on the enantiomeric ratio could be directly obtained from the resulting mobilograms and the detection of diastereomers was possible down to the lower nanomolar range. The final analysis of fermented food samples revealed a high D-alanine content in soy sauces, which originates from bacterial activity during food processing.

[1] Grishin, D. V.; Zhdanov, D. D.; Pokrovskaya, M. V.; Sokolov, N. N. D-amino acids in nature, agriculture and biomedicine. *Frontiers in Life Science* **2019**, *13*, 11-22.

IND-11: Increasing resolving power in a High Field Cassinian ion trap by comparison of simulated a measured mass spectra

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The Cassinian ion trap [1] is a variation of the Kingdon trap. These static ion traps are well known for their high resolving power. In this work we have calculated ion trajectories for different Cassinian ion traps to optimize the resolving power of a special high field trap. The electric potential difference applied between the electrodes of this high field version is at around 8000 V. We used different numerical methods. Electric fields were calculated solving the Laplace Equation using a commercial Fenite-Element-Method tool (COMSOL v.4.3b). The induced signals were determined using the Shockley-Ramo theorem and the corresponding mass spectra were calculated via FFT.

For comparison with experimental results, data were recorded on the Bruker Twin-Trap experiment [3]. However, this experiment is currently only equipped with a low field variant of the Cassinian trap. Hence, we have also calculated signals for this variant in previous work [2]. A low field electric potential in this case is at around 4000 V.

By numerically solving the Newtonian Equations of Motion we were able to increase the resolving power by 60.75 % for the simulated spectra.

References (3)

[1] C. Köster, The concept of electrostatic non-orbital harmonic ion trapping. International Journal of Mass Spectrometry **2009, *287***, 114-118 URL https://doi.org/10.1016/j.ijms.2009.01.014.

[2] B. Raupers, H. Medhat, F. Gunzer, J. Grotemeyer, Influence of the trap length on the performance of Cassinian ion traps: A simulation study, International Journal of Mass Spectrometry **2018**, *438*, 55-62 URL http://dx.doi.org/10.1016/j.ijms.2018.12.017.

[3] C. Köster, Twin Trap or Hyphenation of a 3D Paul- and Cassinian Ion Trap, *Journal of The American Society for Mass Spectrometry* **2015**, *26* (3), 390 URL http://dx.doi.org/10.1007/s13361-014-1050-5.

IND-13: Description of polymers and composite materials with thermal analysis hyphenated to photoionization mass spectrometry

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Introduction: High-performance materials, such as specially designed polymers, are of high importance for modern society and can be found in a variety of products from consumer goods to industrial applications. In this respect, the recycling of modern composite materials is of rising relevance in particular for larger infrastructure. Wind turbines for the production of renewable energy have gained substantial interest over the last decades. Therefore, thousands have been installed in Germany. Technological developments and the limited service life, force the industry to dismantle a substantial proportion in the coming years. In this respect, the material of the rotor blades, mainly consisting of fibreglass and epoxy-resins, is envisioned to be recycling, *e.g.* via pyrolysis. Nonetheless, even the pyrolysis of simple polymers, such as polyethene, is far from understood. Hyphenation of powerful analytical instrumentation can help to overcome this aspect.

Methods: In this study thermal analysis methods were coupled to mass spectrometry (MS) equipped with selective and sensitive photoionization schemes. Flash-pyrolysis gas chromatography (Pyr-GC) with mass spectrometric detection serve as routine techniques for validation. For detailed insight a direct inlet probe ion mobility high-resolution MS platform were used. Various polyethene samples (simple polymeric training set) and different real-world composite materials from a wind turbine rotor blade were investigated.

Results: First, the results of the polyethene pyrolysis will be discussed. For the single-photon ionization (SPI, 118nm) mass spectrometry setup, the polyethene decomposition pattern (380-420°C) revealed a complex spectrum dominated by alkanes, alkenes and dienes. A wide mass range from C_3 (*m/z* 42) up to *m/z* 500 was found. Statistical analysis of the different polyethene samples revealed diagnostic species. Bulk parameter, such as crystallinity, can be correlated to ratios of diagnostic species. Interestingly, low molecular weight species could be shown to be essential to describe the differences between the polyethene samples. Pyr-GC was not able to specify those light compounds with high reliability and repeatability. The more complex real-world composite materials showed a drastically different pyrolysis pattern. Homologue patterns are less pronounced, and the dominant species were assigned to monomer structures, *e.g.* phenol derivatives. First attempts unravelling structural motives, in rotor blades, allowed the tentative assignment of Bisphenol-A as core structure.

Conclusion: Sensitive, soft, and universal mass spectrometric ionization schemes coupled to thermal analysis were able to describe the pyrolysis pattern of simple polymers and composite materials. Knowledge of the pyrolysis behaviour of those materials might help in the future for designing recycling strategies.

IND-15: Autarkic DESI platform with integrated liquid extraction pen for on-site analysis of consumer goods

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In the field of ambient mass spectrometry, a clear lack of portable systems exists. While some small mass spectrometers were developed in the last decades, ion sources which are available in a portable version are still rare. Especially for on-site analysis, a simple measurement procedure without time consuming sample preparation is desirable.

In this context we present an autarkic working platform which provides desorption electrospray ionization (DESI) and a liquid extraction pen (LEP) in a laboratory-independent workspace. The portable device is adaptable to any kind of mass spectrometer which is equipped with an atmospheric pressure interface. A more user-friendly sampling procedure is realized for DESI through a novel sprayer geometry. The new arrangement provides for a further reduction of sample preparation and allows for a higher sample throughput of individually shaped samples. The handheld liquid extraction pen allows for flexible sampling. A capillary system guides solvent through the pen to the sample surface. After analyte extraction, the solvent-analyte mixture is guided to an electrospray ionization source which is located in front of the mass spectrometer. The platform delivers a precise and adjustable solvent flow rate in the microliter per minute range for DESI and LEP. Filtered and dried air is used as nebulizing gas for both methods. Together with a portable mass spectrometer the ion source can operate continuously for more than three hours, powered by an internal battery. Depending on the instrumental requirements of the mass spectrometer, switching between both methods takes just a few seconds without any further instrumental changes.

We show, as a proof of principle, potential application fields for the portable device, using the ambient ionization methods DESI and LEP. Both methods can easily be performed by untrained operators. Tested target compounds include pesticides, plasticizers and drugs, each measured from representative daily consumer goods using a high-performance lab-based mass spectrometer and a miniaturized portable device. The fully autarkic DESI/LEP MS system could for example be utilized in the future at border controls to screen imported goods for potentially harmful or restricted compounds before they enter the market. Further applicational fields in homeland security or medical investigations are conceivable.

IND-17: Setup of an orthogonal acceleration time-of-flight system (oa-tof) with simultaneously on-line vacuum single- and multi-photon ionization.

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The development of oa-tof technology is driven forward due to higher mass accuracy than conventional tof instruments. This is achieved by a better definition of starting energies and coordinates of ions by spatial separation of ion generation and ion extraction. Consequently, oa-tof instruments lose the ability for covering the whole spectrum without scanning in one extraction. Therefore, for high duty-cycles and consequently high temporal resolution, continuous ion sources are favored. We have combined an oa-tof with deuterium lamp single-photon ionization (SPI) as a continuous ion source together with a pulsed 2000 Hz excimer (KrF) laser for resonance-enhanced multi-photon ionization (REMPI). These two ionization techniques can be used simultaneously in one experiment. Due to the characteristic oa-tof setup and high laser frequency, REMPI can be used for covering the whole mass range or targeted on-line monitoring of one or several smaller mass ranges. For demonstration of this novel simultaneously vacuum SPI/REMPI-oa-tof technique, two examples for on-line monitoring of coffee roast gas and e-cigarette vapor are shown.
This tool is the first of its kind, for the best of our knowledge. The combination of an unselective soft broadband ionization such as SPI in combination with a very sensitive and selective soft ionization such as REMPI is an ideal tool for monitoring of highly dynamic processes and serves promising results.

IND-19: Comparison of UHPLC-MS Solvents with High-resolution MS

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In the past years, the sensitivity of mass spectrometers increased rapidly. In theory, increasing sensitivity is the key to achieve lower detection limits. However, there are limitations.
One important factor is the solvent purity. Considering the ionization process in the hyphenation of UHPLC and MS, the solvent concentration is clearly higher as the analyte concentration. In addition, UHPLC-instruments allow higher solvent flow rates than former HPLC-instruments due to their ultra-high pressure stability.
The ratio between solvent and analyte is well known and lead to the development of high purity solvents that are specified for (U)HPLC-MS applications. Today, UHPLC-MS grade solvents are essential to achieve the requirements of a UHPLC-MS system.
Nevertheless, the specifications of different UHPLC-MS grade solvents are not equal, as there are various impurities that influence the ionization process (i.e. trace metals ions, leachables and extractables from packaging, particle size and concentration).
We analyzed state-of-the-art UHPLC-MS grade solvents with a highly sensitive mass spectrometer and compared overall performance by means of background and common impurities.

Instrumentation and Application of MS Imaging

IMA-01: AP-SMALDI MSI of *Cryptosporidium parvum* and *Neospora caninum*-infected cells and tissues

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Parasites and resulting diseases pose health and economical threats worldwide. Some parasites of phylum Apicomplexa, i.e. *Neospora caninum, Eimeria bovis* or *Cryptosporidium parvum*) have not been studied extensively in a biochemical context.

Therefore, mass spectrometry (MS) and MS imaging (MSI) were used, combined with high performance liquid chromatography (HPLC) or matrix-assisted laser desorption/ionisation (MALDI), respectively. The aim of the study was to identify molecular biomarkers for parasitic infections of host cells and, if possible, to clarify their function. With MALDI MS(I), infected and non-infected cell pellet samples were investigated in order to detect possible markers. A Q Exactive™ HF orbital trapping mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) in combination with a AP-SMALDI[5] AF imaging ion source (TransMIT GmbH, Giessen, Germany) was used for this purpose (Mass resolution R = 240,000 @ m/z 200; pixel size: ≥ 5 µm). Primary host cells consisted of bovine umbilical vein endothelial cells (BUVEC) or bovine small intestine cells (BSIC) which are highly immunoreactive and relate well to the cell types to be infected *in vivo*. In comparison, immortalized cell lines were used as an additional, simplified infection model. Monolayers of both cell types were suitable for MSI analysis. This allowed for depicting marker compounds in parasite-infected single host cells in comparison to non-infected controls. In case of cryptosporidiosis, *C. parvum*-infected bovine intestinal biopsy samples were additionally examined by MALDI MSI, thereby mimicking *in vivo* situation. The software Mirion (TransMIT GmbH) in combination with the Perseus software platform (MPI of Biochemistry, Martinsried, Germany) was used to find potential biomarkers.HPLC-MS/MS (Dionex UltiMate 3000 RSLC-System, Thermo Fisher Scientific, Dreieich, Germany) experiments were employed for structural identification of detected molecular markers. Ions in purified and chromatographically pre-separated fractions of cell pellet extracts were fragmented by HCD. The software LipidMatch (SECIM, Gainesville, USA) was used for preliminary identification of detected molecular markers. It was found that the lipid class of phosphatidylcholines in particular was highly abundant. The current state of marker detection for *N. caninum* and *C. parvum* will be presented here. In case of *N. caninum*, we focused on the identification of the detected marker signals, while for *C. parvum*, the comparison of metabolic profiles in cell culture models and bovine intestinal biopsies was the most challenging task.

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IMA-03: Different Mass Spectrometry Imaging Applications on a timsTOF fleX MALDI-Q-TOF Instrument

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MALDI Imaging can detect a broad range of molecules in their native histological location and has emerged to a powerful technique for biomedical and pharmaceutical research. The timsTOF fleX is a novel MALDI-QTOF system consisting of a high spatial resolution MALDI source mounted on a timsTOF Pro. This combination allows for the fast data acquisition at high mass resolution. We examined different MALDI-MSI applications. covering different mass ranges, sample preparation methods and polarities. Samples were prepared by standard MALDI imaging protocols.
For glycan imaging, FFPE tissues underwent deparaffinization, rehydration and antigen retrieval, sprayed with PNGaseF enzyme by a HTX sprayer, and digested in humid conditions. CHCA matrix was deposited using the same spraying device. For drug and lipid imaging, frozen kidney sections from dosed rats were dried and sprayed with DHB. For endogenous metabolite and lipid imaging, whole mouse eyeball sections (20 µm) embedded in supercryo embedding medium (Section-Lab Ltd., Japan) were coated with 1,8-bis(pyrrolidinyl)naphthalene.
All data were collected on a timsTOF fleX with 10-50 µm lateral resolution. Human carcinoma sections were prepared and analyzed for N-glycan content and compared against a master list of 61 N-linked glycans generated using a 7T solariX. Samples consistently showed a difference in expression between glycans that were present in the tumor vs non-tumor regions. Kidneys from untreated rats and rats that had been treated with substance Factor Xa antagonist were analyzed for compound distribution, compound metabolites and reactive lipid changes (n = 3/group). A number of lipids were found to be either upregulated or downregulated in response to compound treatment across the samples. The MALDI-MSI of endogenous small molecules is often performed using extremely high mass resolving instruments due to high interference from isobaric matrix ions. We detected a number of fatty acids and lipids corresponding to distinct layers in the retina

IMA-05: Analysis of Tattooed Human Skin Samples by ToF-SIMS and Orbitrap-SIMS

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Within recent years tattooing has become more and more popular in a number of countries, including Germany. Technically, the colored skin is the result of the injection of inorganic as well as organic pigments into the dermis during the process of tattooing.

One risk of tattooing is the inflammation of the skin after application of the ink formulations. In extreme cases a removal of the skin by surgery has to be performed.

Within this study, inflamed human skin samples were analyzed by ToF-SIMS as well as Orbitrap-SIMS. Goal of the study was to determine the lateral distribution of tattoo ink components within the sample. Furthermore, the identification of the inorganic as well as organic pigments was aspired. Finally, the SIMS-results were compared with respective results obtained by Laser Desorption Ionization Mass Spectrometry (LDI-MS) as well as Micro X-Ray Fluorescence Spectroscopy (µXRF).

In summary, the combined use of ToF-SIMS and Orbitrap-SIMS is a valuable tool for the localization and identification of inorganic as well as organic pigments in tattooed skin samples.

IMA-07: MS Imaging of processed food of plant origin exemplified by the contaminant acrylamide in gingerbread

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In food analysis, MS Imaging has so far been successfully used for analysing endogenous compounds in non-processed food, such as raw fish, wheat grain or grape [1,2,3]. Recent advances aim at MS Imaging of processed food. For instance, Maslov et al. recently investigated the peptide distribution in dry-cured ham [4]. Because of the heterogeneous composition of most processed food, the sample preparation protocol for MS Imaging purposes differs substantially compared to unprocessed food matrices. In this study, we present a sample-preparation protocol for traditional German gingerbread as an example for highly processed food of plant origin.

Due to its heterogeneous composition, sections of the frozen sample were prepared using an electric micro saw. After pneumatic application of 2,5-DHB matrix, MS Imaging measurements were performed on an AP-SMALDI10 ion source (TransMIT GmbH, Giessen, Germany) coupled to a Q Exactive HF Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). All measurements were performed in positive ion mode with a mass resolution of 240,000 at *m/z* 200 and a lateral step size of 200 µm.

Using the developed sample-preparation protocol, we detected both hydrophilic and hydrophobic endogenous compounds in gingerbread, such as disaccharides $[M+NH_4]^+$ or trioleate $[M+K]^+$. By alternating between the two mass ranges *m/z* 50 - 165 and *m/z* 250 - 1000, we could simultaneously analyse the distribution of the carcinogenic process contaminant acrylamide $[M+H]^+$ at *m/z* 72.04439 as well as its precursors asparagine $[M+H]^+$ at *m/z* 133.06077 and monosaccharides $[M+H]^+$ at *m/z* 163.06010. The identification of acrylamide was confirmed in tandem MS experiments showing its typical fragment ion at *m/z* 55.01841. For both the full MS and the tandem MS measurements the mass accuracy was lower than 1 ppm (RMSE). Acrylamide is formed from the naturally occurring constituents asparagine and reducing sugars in certain foods when prepared at temperatures higher than 120 °C and low moisture. With respect to reduction strategies in foodstuffs containing its precursors, acrylamide is under current discussion since recently the new Regulation (EU) No. 2017/2158 establishing mitigation measures and benchmark levels entered into force.

This is the first MS Imaging analysis of a contaminant's distribution in processed food. Furthermore, we established a sample-preparation protocol for MS Imaging of highly-processed food of plant origin for the first time. This method may be the basis for further MS Imaging studies of minor components in various processed food.

[1] N.Goto-Inoue,T.Sato,M.Morisasa,Y.Igarashi,T.Mori,*J.Agric.FoodChem.* **2019,67,125**.

[2] DR.Bhandari,Q.Wang,W.Friedt,B.Spengler,S.Gottwald,A.Römpp,*Analyst*. **2015,140,22.**

[3] A.Berisha,S.Dold,S.Guenther,N.Desbenoit,Z.Takats,B.Spengler,A.Römpp,*RapidCommun.MassSpectrom.* **2014,28,14.**

[4] DR.Maslov,A.Svirkova,G.Allmaier,M.Marchetti-Deschamann,S.Kraljević-Pavelić,*FoodChem.* **2019,283.**

IMA-09: Novel approaches for mass spectrometry imaging of single cells

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Cells form the main building block of all living organisms. Even within the same cell line single cells can exhibit highly differential metabolic expression profiles. MALDI mass spectrometry imaging is a tool capable of recording the metabolic profiles of cell classes within a composite such as an organ. However, due to the lack of spatial resolution and instrumental sensitivity, it generally cannot resolve this information on a single cell level. We here report first results of our recently introduced transmission (t-)MALDI-2-MSI method (Niehaus *et al.*, Nat. Meth. 2019) in an attempt to analyze metabolic profile within cell cultures at 1-2 µm pixel size. MALDI-MSI experiments were furthermore conducted with a timsTOF fleX in "top-illumination" at 5-10 µm pixel size.

We used a well-established approach to directly cultivate cells on 8-well glass chamber slides or indium tin-oxide (ITO-)coated slides overnight. As model cell lines we selected Vero B4 cells, Lewis lung carcinoma (LLC) cells and human fibroblasts. Cells were washed with PBS and iso-osmotic ammonium acetate to remove cell culture media and experiments were carried out with untreated and with formalin-fixed cells for comparison. 2,5-dihydroxyactophenone (DHAP) was used as the MALDI matrix and prepared by either sublimation or by spray-coating. In both our top-illumination and t-MALDI-2-MSI approaches sublimated DHAP matrix provided rich chemical information and high spatial resolution. In particular, with MALDI-2, the overall glycerophospholipid composition could be monitored well in both ion modes. Using an orbitrap mass analyzer, numerous major and minor lipoforms of the glycosphingolipids globotriaosyl (Gb3Cer) and globotetraosylceramide (Gb4Cer) were moreover detected from the fibroblasts. Not unexpectedly, to obtain artefact-free MALDI-images at high resolution, for all tested cell lines the optimization of sample preparation protocols turned out as a particularly critical. Drying of unfixed cells was found to lead to morphology changes of the cells while spray-coated matrix preparation led to lysis, leakage, and delocalization of the cytosol. Brief fixation with formalin and sublimation of the matrix was found to reduce these effects and to preserve the overall cell morphology. Using high resolutiont-MALDI-2 data to optimize sample preparation enabled "low resolution" experiments on the timsTOF at 10 µm pixel size at high acquisition speed. This high-throughput screening tool can then be used to evaluate cell-to-cell heterogeneity on a large scale. Correction by the effectively measured pixel-area is furthermore necessary to compare the averaged pixel results of low-resolution with the "true" ones of the high-resolution measurements.

IMA-11: Detection of metabolites and lipids using nanospray desorption electrospray ionization mass spectrometry imaging (nano-DESI MSI)

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Nanospray desorption electrospray ionization combines liquid extraction on the sample surface with electrospray ionization in front of the MS inlet capillary. The highly localized and defined liquid extraction allows for imaging of biomolecules, including

small metabolites and lipids, from a variety of sample surfaces. Nano-DESI is a potentially non-destructive and soft ionization technique that allows further sample processing such as matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI).

The achievable lateral resolution is affected by various parameters, such as capillary diameters and their positioning, solvent flow rate and sampling speed. Mass spectrometry imaging of standard mouse brain was used for optimization. Different solvent mixtures (ACN:H_2O; MeOH:H_2O) and sampling speeds were tested for signal optimization. Stage speed and flow rate combinations were optimized for minimal signal spreading along the scan direction. The achieved modifications finally led to square-shaped pixels and a lateral resolution in the range of 30 µm. Nano-DESI MSI and MALDI MSI data were sampled on a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany). 2,5-Dihydroxybenzoic acid was applied as matrix for MALDI MSI measurements in positive-ion mode, following nano-DESI MSI. The signal intensity of biomolecules was sufficient to generate adequate ion images.

The sampled nano-DESI MSI data were compared to MALDI MSI data with the same lateral resolution, to confirm that the local information of nano-DESI MSI is consistent. LC/MS data from a neighboring mouse brain sample were used as a reference library for supporting lipid assignments with nano-DESI MSI. The collected data were also evaluated with online databases such as Metaspace – annotation platform, showing a high consistency.

Nano-DESI ion images showed molecular distributions in agreement with reference data obtained with MALDI MSI. The achieved improvements make nano-DESI a reproducible extraction-based method for soft and non-destructive, ambient MSI of large sample areas with reasonable lateral resolution.

Additionally, the optimal setting of the solvent flow rate results in a constant extraction-droplet shape and less solvent spreading along the scan direction. Optimization of the nano-DESI ion source resulted in an increased lateral resolution, a better signal stability and in extended maximum measurement times. The accessible mass range allows for simultaneous data acquisition of amino acids, steroids and lipids.

IMA-13: Venom gland mass spectrometry imaging of *Hottentotta saulcyi* (Scorpiones: Buthidae) at high lateral resolution

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The scorpion venom gland is the place for synthesis, storage and secretion of complex mixtures of components including lipids, salts, amino acids, mucopolysaccharides, nucleotides and polypeptides used by scorpions as a venom. There are approximately more than 2000 species of scorpions described in the world. However, there is still little histological knowledge about scorpion venom glands. In addition, information about the cellular secretion and production processes of the gland as well as about venom components is adequately incomplete and has so far been mostly neglected by the omics disciplines. Here, for the first time, we studied liquid chromatography mass spectrometry (LC-MS) based top-down venom proteomics and lipidomics of venom gland from *Hottentotta saulcyi*. Additionally, we used high-resolution atmospheric-pressure matrix-assisted laser desorption/ionization (AP-SMALDI) mass spectrometry imaging (MSI) to investigate lateral distributions of endogenous biomolecules in the venom gland. Initially, several sample preparation approaches were tested, namely fresh-frozen, glutaraldehyde and formaldehyde fixation with gelatin and carboxymethylcellulose as embedding media, to obtain model species venom gland sections. High-resolution MSI was performed on sections at a spatial resolution of 10 µm, and small metabolites and polypeptides were assigned by database search.

IMA-15: Immuno-Mass Spectrometry Imaging of Manganese Transporters in Cancers

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The underlying biological mechanisms of widespread radioresistance of many human tumours remain elusive despite decades of investigations. Research efforts have largely focussed on the genomics/proteomics-based enzymology of DNA repair and free radical scavenging enzymes such as the superoxide dismutases. A recent novel hypothesis is that radiation resistance is predominantly underpinned by non-enzymatic complexes of manganese and small molecular metabolites[1]. These complexes are thought to act as free radical scavenges which provide metabolic radioprotection that render cells variably resistant to the products of ionising radiation.

Multiple efflux and influx metal transporters are involved in manganese homeostasis and are potentially differentially expressed on the surface of cancer cells, leading to variable concentrations of manganese within tumours. This poster explores the feasibility of applying concomitant immuno-mass spectrometry imaging (iMSI) and elemental bioimaging (EBI) to spatially determine transporter proteins and transition elements to provide insights of the transporter pathway in human melanomas.

This proof of principle study targeted the ZIP8 protein which is a known manganese, zinc and iron influx transporter. A tissue microarray consisting of representative melanoma samples was imaged by iMSI for ZIP8 following immunohistochemical staining with europium labelled anti-ZIP8. Manganese, copper, zinc and iron were imaged by conventional EBI on a consecutive section of the microarray and co-localised with the ZIP8 expression. The results show that transition elements were variably correlated with ZIP8 demonstrating a viable method of interrogation of the complex interplay between metals and their respective transporters.

(1) Doble, P. A.; Miklos, G. L. G. Distributions of Manganese in Diverse Human Cancers Provide Insights into Tumour Radioresistance. Metallomics 2018. https://doi.org/10.1039/c8mt00110c.

IMA-17: Mapping of imatinib in cryo-sections of drug-treated *Schistosoma mansoni* via high-resolution AP-SMALDI MSI

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The parasitic flatworm *Schistosoma mansoni* causes schistosomiasis, which affects about 200 million people worldwide and is classified as a neglected tropical disease by WHO. The female produces around 300 eggs per day, which cause pathology in humans. Currently, praziquantel is the only approved drug bearing the inherent risk of resistance formation. Therefore, alternatives are investigated such as the cancer drug imatinib, which showed efficacy *in vitro*. Besides the gonads, imatinib also affects the intestine in male and female worms. The uptake and distribution of imatinib in *S. mansoni* was studied in the present investigation.

Paired adult *S. mansoni* were examined by atmospheric-pressure scanning microprobe MALDI MS imaging (AP-SMALDI MSI) after different exposure times to imatinib (5 min, 20 min, 1 h, 4 h). 40 µm thick sections were prepared on a cryotome (HM525, Thermo Fisher Scientific). Imaging experiments were performed on a Q Exactive HF Orbitrap equipped with an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen). Pixel sizes between 5 and 9 µm were set and pixelwise autofocusing was used. The m/z range was 250 to 1000 in positive-ion mode, and 2,5-dihydroxybenzoic acid was used as matrix.

S. mansoni cryo-sections were optically assessed after optimization of sample preparation. Characteristic anatomical structures (oral and ventral sucker, ovary and gut) were identified microscopically. Distribution of imatinib within the sections was easily traceable by AP-SMALDI MSI. Also, changes in distribution with increased exposure time was followed. At early time points of treatment (5 min, 20 min), little imatinib was detected in female worms, whereas imatinib was found mainly in the tegument and on the ventral side of males. This might be explained by the paired state of the worms, exposing mainly male tegument to surrounding medium while the female is enveloped by the male's body. After one hour of exposure, in the female, imatinib was mainly present in the digestive tract while intensity was low in the ovary. After 4 h of exposure, the drug was found in the esophagus and the intestine of both sexes. Optically observed regions of tissue damage correlated well with the localization of imatinib. Sex-specific lipid markers were identified. Furthermore, anatomical features like the ovaries were visualized by specific lipid markers. MALDI MSI of drugs is useful to study kinetics of drug accumulation in parasitic worms. Acknowledgement: Financial support by the LOEWE Center DRUID (State of Hesse) and by DFG Sp314/13-1 and INST 162/500-1 FUGG is gratefully acknowledged.

IMA-19: Chemical topography of metal-associates allergens on non-planar everyday items

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Metals are essential to almost every aspect of our life. They are commonly used in jewelry and coins. Trace amounts of metals and chemical compounds containing metal ions can affect human health, i.e., cause allergy or inflammation. The human skin covers most of the surface of the body. Therefore, it is a major entrance point for a wide range of metal-associated allergens. As a consequence, skin allergies caused by everyday items have appeared. The best-known example is allergic contact dermatitis upon contact with nickel-containing jewelry. When a metal-containing material comes into contact with the skin, the material surface is affected by sweat, present on the skin, potentially resulting in allergic hypersensitivity, a form of eczema. However, details of this metal-induced allergic reaction are not well understood. Complexes of nickel, copper and zinc are known to be easily formed through contact of metal alloys with ubiquitously available organic compounds such as triglycerides, amino acids and fatty acids found in skin secretions. Thus, an analytical technique is required to characterize and identify metal complexes formed on metallic everyday products, ideally offering a chemically specific, laterally resolved view of allergy-causing processes. In order to characterize metal-organic compounds formed on the surface of jewelry or coins, we here present an analytical workflow and first results for compounds formed on everyday items, using 3D-surface autofocusing LDI mass spectrometry imaging. In first experiments, chemically cleaned everyday metal objects were investigated. These experiments allowed to reconstruct 3D-topographic maps of jewelry and coins before contact with human skin. Subsequently, these objects were brought into contact with skin and analyzed with 3D-LDI-MSI. Metal-containing ions, only found after contact with skin, were assigned based on accurate mass measurements and isotopic distributions. Future measurements will investigate how these metal compounds affect the skin.

Acknowledgement

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IMA-21: First Insights into Tracing the Lithium Ion Movement During the Formation Process of Lithium Ion Batteries

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Since their invention at the end of the last century, lithium ion batteries (LIBs) became the most prominent power storage technology for mobile as well as stationary applications. Nevertheless, the continuous loss of capacity – so called aging – remains a major drawback of state-of-the-art LIBs. In the first electrochemical cycles of a battery, passivation layers are formed which are of greatly importance for an acceptable operational lifetime[1]. The formation of a passivation layer on both electrode surfaces inhibits extended electrolyte decomposition and therefore further capacity loss. Understanding these processes and the composition in more detail will help to reduce initial capacity loss and improve lifetime.

In general, electrochemical cycling includes the reversible migration and storage of lithium ions, whereas the deposited lithium species in the passivation layers are considered to be irreversibly lost for the electrochemical process[2]. This study applies an isotopically enriched cathode material in order to distinguish between cathodic and electrolytic lithium and by this, trace the

lithium migration in LIBs. Investigations by means of time of flight – secondary ion mass spectrometry (ToF-SIMS) allow visualizing lithium ion migration on particle scale. Therefore, cross sections of electrodes are prepared using a focused ion beam (FIB). Afterwards, the intersections are visualized in the high lateral resolution mode of ToF-SIMS.

References

(1) Aurbach, D.; Markovsky, B.; Weissman, I.; Levi, E.; Ein-Eli, Y. On the correlation between surface chemistry and performance of graphite negative electrodes for Li ion batteries. *Electrochimica Acta* **1999**, *45*, 67–86.

(2) Agubra, V.; Fergus, J. Lithium Ion Battery Anode Aging Mechanisms. *Materials* **2013**, *6*, 1310–1325.

IMA-23: The Role of Matrix Layer Thickness in ME-SIMS Ion Yields for Selected Biomolecules

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The major unsolved problem in high lateral resolution secondary ion mass spectrometry (SIMS) imaging of biomolecules is the efficient ionization of sputtered molecules. Although ion sources can be focused to less than 50 nm, the low ionization efficiency of many relevant biomolecules limits the practical lateral resolution to a few micrometers. One promising approach for increasing ion yields for biological imaging is matrix-enhanced SIMS (ME-SIMS). In ME-SIMS, molecular ion signals are increased by chemically modifying the surface using a matrix that aids in intact molecule sputtering and ion formation. Although ME-SIMS has been known for many years, results have been variable. Better understanding of the fundamentals of the enhancement process is needed for reproducible high spatial resolution ME-SIMS imaging. In this work we have looked at molecular ion yield enhancement for a variety of biomolecules using α-Cyano-4-hydroxycinnamic acid (CHCA), a matrix commonly used in MALDI-MSI. Sublimation/deposition, a solvent-free sample preparation technique, was used to deposit controlled thickness layers of CHCA on samples of selected analytes. ToF-SIMS spectra of both coated and uncoated analyte samples were collected using a Bi_3^+ primary ion source. Enhancement of the molecular ion signal was a function both of the analyte chemistry as well as the thickness of the matrix overlayer. Significant enhancement was observed for some biomolecules even when the overlayer was many times the SIMS sampling depth, indicating that migration of the analyte into the overlayer is a critical factor. To better understand this effect, the distribution of the analyte through the depth of the overlayer has been studied as a function of time and sample storage conditions. The data indicate that solubility and mobility of the analyte in the matrix play an important role in the matrix enhancement process. The results provide new insight into the role of matrix coating in both ME-SIMS imaging and MALDI-MSI.

Ion Physics and Ion Chemistry - Applications

IPC-01: Investigations of Isotope Labeled Lithium Ion Battery Electrolytes *via* GC-MS-based Techniques

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State-of-the-art lithium ion battery (LIB) electrolytes consist of a conducting salt (*e.g.*, lithium hexaflourophosphate dissolved in mixtures of cyclic (*e.g.*, ethylene carbonate) and linear organic carbonates (*e.g.*, dimethyl carbonate). Especially cyclic carbonates are electrochemically reduced during the first cycles and form a mandatory interphase to protect the electrolyte from ongoing decomposition at the anode surface during long term cycling. Resulting aging products range from oligo- and polymeric carbonate structures over (organo)phosphate moieties to inorganic lithium salts.[1] Within ongoing investigations of aging phenomena, mass spectrometry coupled to gas chromatography (GC-MS) has proven to be a powerful technique for electrolyte screening as well as for identification of volatile decomposition species.[2]

However, postulating possible intermediates and decomposition pathways starting from the pristine electrolyte formulations are limited to reasoned assumptions based on observed decomposition products. To get a more reasonable basis for reaction pathway presumptions, isotope labeling can be applied to enable atom origin tracing of different electrolyte components.[3]

In this work $^{13}C_3$ labeled ethylene carbonate-based electrolytes were analyzed after two cycles, to get insights into solvent molecule participation during crucial electrode-electrolyte interphase formation. Within this context different (high resolution) mass analyzers and ionization techniques coupled to GC separation were utilized to trace carbon atom origins of dissolved and volatile decomposition products. Having differentiated linear and cyclic solvent molecular decomposition, proposed reaction mechanism can be addressed and electrolyte formulations can be improved regarding their interphase formation ability.

References:

[1] J. Henschel *et al.*, *Chem. Mater*. **2019**, 31 (24), 9977 9983.

[2] Y. Stenzel *et al.*, *Separations,* **2019**, 6(2), 26.

[3] A. Michan *et al.*, *Chem. Mater.* **2016**, 28, 1, 385-398.

IPC-03: Fully quantum mechanically calculated EI and CID mass spectra using the QCEIMS program

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We present EI and CID mass spectra which are fully quantum mechanically calculated with the QCEIMS program. The methodology underlying these computations is basically first-principle and does not depend on any experimental results. QCEIMS provides a detailed insight into the reactions occurring in mass spectrometry experiments and can unveil fragmentation procedures of bond breaking and structural rearrangements. Based on the good results obtained for electron ionization mass spectra, we have extended the program to simulate the mechanisms occurring in collision-induced dissociation events. With our low-cost tight-binding methods GFN1-xTB and GFN2-xTB implemented into the program, QCEIMS is independent of any third party software and free for academic use.

IPC-05: Spectroscopic investigations of the first excited state and ionic ground state of non-deuterated and deuterated *m*-chloro- and *m*-fluoropyridine

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Introduction

Pyridine is the backbone of many biochemical relevant compounds like nicotine, niacin and pyridoxine. The reactivity of these compounds depends on their geometrical and electronic structure. Upon electronic excitation, a common activation step in biochemical reactions, these structures experience great changes. Consequently, a characterization of the electron distribution and geometrical properties in electronically excited states and in the ionic ground state is highly desirable.

Pyrdine and some substituted derivatives have already been studied by means of Resonance-Enhanced-Multi-Photon-Ionization(REMPI) and Mass-Analyzed-Threshold-Ionization(MATI) spectroscopy. In contrast, little information is available on *m*-chloro- and *m*-fluoropyridine. Hence, the first excited state $S_1(n\rightarrow\pi^*)$ and the ionic ground state D_0 of these (non-)deuterated compounds were characterized to identify the influence of the halogen substitution upon the vibronic structure.

Experimental

The measurements were conducted with two dye lasers (LAS/Lambda Physics) and a home-built time-of-flight mass spectrometer. Subsequent to an adiabatic supersonic expansion, the molecules were electronically excited by the first and ionized by the second laser. The REMPI spectra were obtained by tuning the first dye laser while the MATI spectra were measured by tuning the second laser. After ionization, the ions were accelerated towards the detector.

Results

The excitation energies of *m*-chloropyridine-d_0 and -d_4 were determined to be 34840 ± 3 cm^{-1} and 35009 ± 3 cm^{-1}, respectively. For *m*-fluoropyridine-d_0, -d_3 and -d_4 the excitation energies are 35064 ± 3 cm^{-1}, 35212 ± 3 cm^{-1} and 35240 ± 3 cm^{-1}, respectively. The deuteration shifts the electronic states' energy to higher values and lowers the excitation energy of most vibrations. In addition, the Franck-Condon factor for several $S_1\leftarrow S_0$ transitions is altered by the introduction of deuterium into the system. The $n\rightarrow\pi^*$ transition causes a boat distortion of both pyridine derivatives that is very sensitive to changes in the substituents. For *m*-chloropyridine the distortion is more pronounced than for *m*-fluoropyridine.

The adiabatic ionization energies of *m*-chloropyridine-d_0 and -d_4, *m*-fluoropyridine-d_0, -d_3 and -d_4 were determined to be 75879 ± 7 cm^{-1}, 76015 ± 7 cm^{-1}, 76607 ± 7 cm^{-1}, 76677 ± 7 cm^{-1} and 76704 ± 7 cm^{-1}, respectively. The influence of the deuteration on the adiabatic ionization energy is smaller compared to the effect on the S_1 excitation energy. Furthermore, the Franck-Condon factors for the $D_0\leftarrow S_1$ transition do not experience a significant change by the deuteration.

Novel aspects

The influence of deuteration upon the energy levels of the S_1 and D_0 states of *m*-chloro- and *m*-fluoropyridine is characterized.

IPC-07: Mechanistic investigations of a dual-activated gold/or organocatalytic cyclization

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The group of *Jørgensen*[1]reported a cyclization reaction of *α*,*β*-unsaturated aldehydes and an alkyne-tethered nucleophile. While the alkyne moiety is activated by a Lewis acid gold(I) catalyst, an l-proline-derived Lewis base catalyst activates the aldehyde group and additionally induces chirality into the products. Since our group is experienced in mechanistic studies of ll-proline-catalyzed reactions,ha[2,3] we aim of illuminating this catalytic system by means of ESI-MS. Based on previous experiments, there are two possible mechanisms for the 5-*exo*-*dig* cyclization of the alkyne function with *α*,*β*-unsaturated aldehydes. The first cycle proceeds by formation of an enamine species, while in the second cycle, the amine solely functions as a base and the gold catalyst binds at the aldehyde's *β*-position. In both cases the gold catalyst coordinates to the formyl alkyne and forms a covalent bond after the cyclization has occurred. Finally, the released vinyl product isomerizes to the cyclopentene carbaldehyde and the catalysts are reobtained.

By means of ESI-MS, we were able to detect an enamine species with and without gold as well as solely gold-tethered intermediates. In most cases, two gold cores were bound to the substrate, albeit one single gold species could be observed. Since it was neither possible to detect the substrates nor the product with ESI-MS, we are presently utilizing GC-MS to explore the reaction's kinetics.

[1] K. L. Jensen, P. T. Franke, C. Arróniz, S. Kobbelgaard, K. A. Jørgensen, *Chem. Eur. J.* **2010**, *16*, 1750–1753.

[2] J. A. Willms, R. Beel, M. L. Schmidt, C. Mundt, M. Engeser, *Beilstein J. Org. Chem.* **2014,** *10*, 2027–2037.

[3] A. Schnell, J. A. Willms, S. Nozinovic, M. Engeser, *Beilstein J. Org. Chem.* **2019,** *15*, 30–43.

IPC-09: Adduct Suppression and Enhanced Fragment Intensity of Oligosaccharides by Means of SORI-CID Technique

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Introduction

Oligosaccharides are important for many biochemical processes, such as cell differentiation and immune response. Therefore, they have already been extensively studied. Among other techniques mass spectrometry combined with sustained off-resonance irradiation - collisional induced dissociation (SORI-CID) can be used for characterization of oligosaccharides by analyzing their fragmentation patterns. The influence on collisional induced fragmentation mechanisms of oligosaccharides by alkali metal adduction is a well-known effect [1]. In addition, the protonated species can provide structure information as well. However, usually the adduct signals exhibit way higher intensities compared to the protonated species. Thus, the suppression of the adduct signals is essential. Furthermore, the effect of different SORI parameters on the resulting intensities is already investigated [2] and these results were applied on oligosaccharides. Subsequently, the presence and the intensities of the fragment signals dependent on the SORI-CID parameters were investigated.

Methods

The experiments were performed on an APEX III (7.05 T) and an APEX IV (9.4 T) Fourier-Transform-Ion-Cyclotron-Resonance mass spectrometer (FT-ICR-MS, *Bruker Daltonik, Germany*). Maltopentaose, Maltotetraose and Isomaltotetraose (*Carbosynth, UK*) were dissolved in MeOH/H_2O (50:50) and were ionized using an Apollo I ESI-source. Ammonium acetate was added to the samples in different concentrations to suppress the adduction of alkali metals. Fragmentations of the molecular cations were achieved by SORI-CID with argon as collision gas. The SORI-CID was performed with different values for the ion activation duration and for the offset frequency.

Preliminary data

Several spectra of the analytes with different concentrations of NH_4Ac were recorded. The intensity of the protonated species could be enhanced by the factor of almost seven for every analyte due to the addition of ammonium acetate. Even though a complete suppression could not be achieved, the comparison of the fragmentation behavior for different SORI-CID parameters could be realized. We observed a high dependency of the fragment intensities on the variation of the activation duration. A rise of intensity occurred for ¼, ½ and ¾ of the resulting SORI cycle. Moreover, the protonated Maltopentaose exhibits a crossring-cleavage, whose presence also is dependent on the activation duration. In contrast, the spectra of the fragmentation of the adducted specie does not show this specific crossring-cleavage.

Refrences

[1] M. Cancilla, A. Wong, L. Voss, C. Lebrilla, *Analytical Chemistry* **1999**, *71* (15), 3206.

DOI: 10.1021/ac9813484

[2] D. Mortensen, C. Jones, D. Dearden, *International Journal of Mass Spectrometry* **2012**,

330-332, 241. **DOI:** 10.1016/j.ijms.2012.08.026

IPC-11: New fragmentation pathways of ortho-, meta-, and para-methylbenzalacetone

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Introduction

Ionized benzalacetones are known to form resonance-stabilized 2-methylbenzopyrylium ions [1]. A first scheme for the fragmentation pathways of methylbenzalacetones has already been developed [2]. However, the origin of some fragments is still uncertain. Hence, ortho-, meta- and para-methylbenzalacetone were first synthesized and then investigated using a double-focussing sector field mass spectrometer and different fragmentation conditions.

Experimental

All measurements were performed on a Micromass ZAB-2F (Vacuum Generators). The analytes were evaporated and ionized by electron ionization. Afterwards, mass spectra were recorded and all signals with significant intensities were analyzed by means of Mass-Analyzed-Ion-Kinetic-Energy (MIKE) spectrometry with and without collisional activation using argon gas.

Results and discussion

As previously reported, the analyzed methylbenzalacetons exhibit almost identical mass spectra [2]. It was possible to reproduce and confirm these results. However, we observed several differences among the three isomers in the MIKE-spectra under collisional activation conditions. The loss of CO (-28 Da) from the 2-methylbenzopyrylium ion (m/z 145) was already reported. Yet, using argon as collisional gas reveals a previously unnoticed loss of 30 Da which corresponds to the loss of formaldehyde (CH_2O). This loss results in a significant peak in the mass spectra at m/z 115.

Another significant loss for the ortho derivate was the elimination of H_2O that occured after the loss of one methyl group (m/z 145). In contrast, the loss of H_2O is significantly less distinct in the meta and para derivate. Deuteration of the side chain revealed that the origin of the consecutive H_2O (HDO, respectively) elimination is the preceding loss of the side chain methyl group. This results in a significant peak at m/z 127 in the mass spectrum of the ortho isomer.

Novel aspects

The scheme for the fragmentation pathway of ortho-, meta- and para-methylbenzalacetone has been extended and the loss of previously unreported fragments was reported.

References

[1] J. Ronayne, D.H. Williams and J.H. Bowie, J. Am. Chem. Soc., **1966**, Vol. *88*, (21), 4980.

[2] B. Schaldach, B. Grotemeyer, J. Grotemeyer and H.F. Grützmacher, Org. Mass Spectrom., **1981,** Vol. *16*, (9), 410.

IPC-13: X-raying protein molecular ions: small steps to large fragments. An update.

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In current mass spectrometric techniques – especially when applied to proteins or peptides – deliberate fragmentation is an indispensable tool to confirm analyte identity by comparison of theoretically expected and experimentally observed fragment ions and to gain additional information about the precursor ion's composition. This is all the more essential in measurements of natively electro-sprayed protein complexes, the spectra of which typically lack the high resolution seen for instance with proteins or peptides sprayed from denatured solutions.

In nowadays native top-down MS, the most common way to activate a selected precursor ion is its acceleration and collision with a stationary gas, followed by vibrational heating and final dissociation (CID). Apart from this and other time-honoured methods, direct activation of the analytes' own electrons by exposure to photons with high energy is increasingly in the focus of development. By choosing the wavelength of the light, i.e. its energy, one can excite either the precursor ion's valence electrons (UVMPD) or, by using X-rays, even its inner electrons.

This latter approach is one subject of our efforts: we further customized a Q-ToF mass spectrometer already modified for high-mass measurements and coupled it to different branches of the Petra III P04 beamline at the DESY campus in Hamburg. Varied settings of the synchrotron source were tested as were different interaction regions within the mass spectrometer. During our three "beamtimes", we measured a multitude of proteins and protein complexes, natively sprayed and from denatured solutions with most emphasis placed on the first and covering a wide range of mass and complex stoichiometry. Furthermore, we chose well-known and lesser known proteins; the most telling being those that deviate from strict CID pattern.

Although not always as efficient as anticipated, the X(-ray)-factor was observed throughout. With the technique of X-raying proteins still at the beginning of its development, many details, especially those concerning possible reaction mechanisms, are still unknown or under debate. We are confident that our results will provide valuable input to this discussion and will contribute to the understanding of "enlighted" protein molecular ions.

IPC-15: Experimental Determination of Bond Dissociation Energies of Silver(I)-Helicene Adducts in the Gas-Phase by ESI-MS/MS

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[n]Helicenes (n = 6 and 7) are known to form 1:1 complexes with silver cations, where the helicene acts as a molecular tweezer, attaching to Ag^+ in a bidentate fashion. The binding energies of these complexes have been recently examined in a combination of DFT calculation and ESI-MS/MS experiment.[1] [2] However, controversy arose since the mass spectra indicated the loss of a neutral silver atom rather than the silver cation from the complex. Therefore, a correct procedure would have to consider the charge transfer from Ag^+ to the helicene.[3] The present study intends to solve the dilemma by producing reliable values for the bond energies. Our MS/MS experiments confirm the charge transfer from the Ag^+ to the [n]helicene during CID (collision induced dissociation). This observation is consistent with the fact that [n]helicenes possess lower ionization energies than silver. Furthermore, the bond dissociation energy of [8]helicene could be measured in experiments by calibration of the energy scale with calculated energy values of Ag^+ complexes with [6] and [7]helicene.[1] [2] [3] [4]

The helicene radical cations are normally not accessible by ESI, but the observed charge transfer formation enables the investigation of the CID behavior of these ions. The [n]helicenes$^{+\bullet}$ (n = 6 - 8) do not only feature the dissociation behavior known from other PAHs (i.e. the loss of small C_nH_m units), but also the abundant formation of the coronene radical cation as a fragment. The fragmentation mechanism of the coronene formation has been elucidated by MS^n experiments.

References:

[1] E. Makrlík, J. J. Dytrtová, P. Vaňura, J. Sýkorad, V. Církvad, J. Storch, *Chem. Phys. Lett.* **2015**, *633*, 105-108.

[2] E. Makrlík, B. Klepetářová, D. Sýkora, S. Böhm, P. Vaňura, J. Storch, *Chem. Phys. Lett.* **2015**, *635*, 355-359.

[3] J. F. Gal, *Chem. Phys. Lett.* **2017**, *680*, 28-30.

[4] J. Míšek, M. Tichý, I. G. Stará, I. Starý, D. Schroeder, *Collect. Czech. Chem. Commun.* **2009**, 74, 323-333.

IPC-17: Mechanistic Investigations of Unidirectional Hydrogen Rearrangement Reactions in EI-MS

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Kuck and Filges reported an unidirectional triple-hydrogen (3H) rearrangement in standard electron ionization (EI) mass spectra of *trans*-2-(4'-dimethylaminobenzyl)-1-indanol (**1**) and *trans*-2-(4'-methoxybenzyl)-1-indanol (**2**).[1,2] The observed 3H rearrangements are very rare in EI-MS of organic ions, and still not completely understood. Kuck *et al.* proposed a mechanism for the 3H rearrangement based on EI-MS, deuterium labeling and mass-analyzed ion kinetic energy (MIKE) experiments.[1,2] Remarkably, only the trans-isomers exhibit the peculiar 3H rearrangement. The proposed mechanism involves the transient formation and rearrangement of distonic ions and also ion/neutral complexes as crucial intermediates. Ultimately, a distonic radical ion at *m/z* 123 for **1** (and *m/z* 110 for **2**) is generated from the complex dissociation reactions of the molecular ion. However, even though the mechanism is based on an extended set of experimental evidence and profound considerations, the authors clearly point out that structural and mechanistic assumptions remain tentative and further verification is appreciated. Besides the ion structures proposed by Kuck *et al.* there are also alternative ion structures thinkable that would match the

compositions of the 3H rearrangement products. It was our intention to shed more light onto this unique fragmentation reaction by explicit structure elucidation of the rearrangement product *m/z* 123 of analyte **1** (and *m/z* 110 of **2**) in the gas-phase by IRPD spectroscopy and theory.[3] An EI-MS ion source is necessary to generate the open-shell molecular ion $[M]^{+\bullet}$, which in turn dissociates to form the distonic 3H rearrangement product ions. The ions of interest are in the mass range of the FELion instrument built and run by S. Schlemmer and S. Brünken at the FELIX Laboratory.[3] DFT computations at the B3LYP/cc-pVDZ level of theory of the proposed ion structure at *m/z* 123 show characteristic absorptions in the wavenumber range provided by the free electron laser (FEL) of the FELIX Laboratory.

Aside from preliminary results such as the synthesis and derivatization of **1** and **2,** also a novel crystal structure and DFT computed IR spectra will be presented. Mass spectra of **1** taken at the FELion instrument will be shown, as well as neon-tagging experiment results and OPO laser measurements of **1**. Due to the low volatility of **1** and the still inefficient sample introduction into the ion source, a gas-phase FEL-IR spectrum of *m/z* 123 could not yet be obtained.

Lipidomics: Techniques and Applications

LIP-01: High speed untargeted 4D-lipidomics LC-MS/MS workflows with Parallel Accumulation Serial Fragmentation (PASEF)

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The search for new and validated biomarkers is of particular interest in clinical areas like oncology or neurology. As lipids play an important role in many diseases, the area of lipidomics has become central for clinical research. Clinically-oriented projects often demand a high-throughput for large sample cohorts. Therefore, a short cycle time per sample is necessary to realize research projects with hundreds or even thousands of samples in a reasonable time frame. This is realized by the PASEF mode on the timsTOFPro system. Lipids from NIST SRM1950 reference plasma were extracted based on a known protocol. RP-LC was performed on an Elute UHPLC system (Bruker) with a YMC Triart C18 column (100 x 2.1mm, 1.9µm). Run times were 6, 10 and 20 min. The MS data was acquired in positive ESI mode using a timsTOF Pro instrument (Bruker) in PASEF MSMS mode. The transfer parameters were optimized for 100- 1500 m/z, precursors were fragmented from 300-1500 m/z. The PASEF technology enables to increase the sample throughput using 4D lipid profiling by ca. 4x. Even at reduced LC run times, the ion mobility separates co-eluting isobaric or isomeric compounds and provides accurate and reproducible CCS values for high confident lipid ID. Complementary to an in-depth “ID as many as possible” approach, PASEF enables a very fast lipid profiling based on MS/MS spectra

LIP-03: Mass spectrometric analysis of liamocin biosurfactants

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Biosurfactants are more eco-friendly than conventional petrochemical surfactants because of their lower toxicity and good biodegradability with comparable effects regarding physical and chemical properties like surface activity or critical micelle concentration. Additionally, antimicrobial and antiproliferative activities are an advantage compared to their synthetic counterpart. Thus, the applications of biosurfactants cover many different industrial fields like medicine, foods, cleaning materials or cosmetics.

Liamocins are biosurfactants secreted as heavy oils by the fungi *Aureobasidium pullulans* and belong to the glycolipid class. Their structural diversity is based on a polar polyol head group and a polyester tail consisting of up to five 3,5-dihydroxydecanoic ester groups. The structure of the polyol head group depends on the conditions of the culture medium.

Structural characterization of liamocins is up to now limited to MALDI-MS and nuclear magnetic resonance spectroscopy. These methods have a common trait in that they require time-consuming sample handling and separation steps. Therefore, different chromatographic techniques were hyphenated to mass spectrometry to separate the individual liamocin species produced by *Aureobasidium pullulans* in a complex mixture without time-consuming sample preparation. It was possible to detect ten different liamocin species as well as structural related compounds. The structure elucidation was based on accurate masses obtained by high-resolution mass spectrometry and by data dependent MS/MS experiments of the sodium adduct and the chlorine adduct, respectively.

LIP-05: Monitoring (phospho-)lipid biosynthesis in human multipotent stromal cells by isotopic labelling and MALDI TOF MS – What’s up with sphingomyelin?

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Musculoskeletal diseases are extremely widespread and a significant burden on the health systems of the industrialized countries. The use of mesenchymal stem cells is a promising approach to cure cartilage and tendon injuries which often also occur in younger people as consequences of sport accidents. Although particular interest is on the collagen and the glycosaminoglycan composition of the tendon and potential alterations compared to healthy tissue, there is nowadays also increasing evidence that some selected phospholipids (PL) are potential mediators of tissue regeneration. Therefore, PL (and

potential changes thereof) attracts increasing interest in this field. We have used positive and negative ion MALDI-TOF mass spectrometry to elucidate the lipid compositions of human mesenchymal stem cells in dependence on the composition of the cell culture medium and the cultivation time. The *de novo* biosynthesis of PL was monitored by adding ^{13}C labeled glucose to the cells and the incorporation of ^{13}C into the different PL classes was investigated by MS. It is remarkable that all PL classes (for instance, phosphatidylcholine and -inositol) exhibited ^{13}C incorporation - but not the sphingomyelin (SM) which is the most abundant sphingolipid in the majority of human tissues and body fluids. Using suitable internal standards it could be shown, that only ^{12}C-containing SM is *de novo* generated while no ^{13}C labeled SM could be monitored - independent of the cultivation time which was varied between 7 and 28 days. SM impurities stemming from the cell culture medium and the used MALDI matrix compounds (2,5-dihydroxybenzoic acid (DHB) or 9-aminoacridine (9-AA)) could be definitely ruled out. Therefore, it is suggested that there must exist another, so far unknown SM biosynthesis pathway. This pathway does not make use of glucose but is based on the use of other molecules as energy sources. Potential pathways to explain the experimental observations are discussed.

LIP-07: Analysis of glycolipid-based surfactants by supercritical fluid chromatography-mass spectrometry

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In response to the growing demand for sustainable products, biosurfactants are increasingly attracting industry's attention as an alternative to petrochemically produced surfactants. Due to their amphiphilic structure, which consists of a sugar moiety and long-chain hydroxy fatty acids, glycolipidic rhamnolipids and sophorolipids represent promising biosurfactants. They are produced by various microorganisms (e.g. *Pseudomonas spp*. or *Starmerella bombicola*) based on renewable raw materials and additionally exhibit significantly better degradability. Rhamnolipids (RL) are among the most intensively studied and characterized biosurfactants, but their use in industry is currently failing due to lack of efficient production processes. In contrast, sophorolipids (SL) are already produced in industrial scale and commercialized worldwide by various companies.

Currently high performance liquid chromatography (HPLC) is used in research and development, but also for quality control of biosurfactants. In order to reduce the usage of environmentally harmful organic solvents, a method based on supercritical fluid chromatography has been developed. With this technique organic solvents can be replaced by supercritical CO_2 as part of the mobile phase. For method development, different stationary phases have been evaluated. The separation method has further been optimized with respect to different parameters, for example, temperature, back pressure and mobile phase composition. The hyphenation with mass spectrometry (MS) enables the identification and structural characterization of the analytes through accurate mass and data-dependent MS/MS-experiments.

The optimized SFC-MS-methods enable the separation of SL congeners depending on the degree of acetylation of the sophorose unit and their presence in the free acid or lactone form. Moreover they facilitate the separation of mono-RL (one L-rhamnose unit), di-RL (two L-rhamnose units) and their precursors 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAA) within 3.5 minutes. In a technical mixture of SL, 30 congeners have been identified of which congeners with an octadecenoic acid chain form the main components. Furthermore 29 RL-species have been identified in the examined fermentation supernatant of *Pseudomonas putida,* whereby species with two saturated fatty acyl chains with in total 20 carbon atoms predominate. Due to the coelution and the resulting similar ionization conditions, the relative concentrations of the RL species within a subclass could be quantified.

LIP-09: Analysis of trans-Fatty Acids in Food Products Using Various GC Columns

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Partially hydrogenated oils, which are the main source of artificial trans-fatty acids (TFAs), have been phased out in Europe and the United States due to concerns of their negative effect on human health. Unfortunately, artificial TFAs remain present in the food industry of the rest of the world. Moreover, certain food product have been exempted from the ban, such as frosting. In order to analyze the presence of TFA in US food products we evaluated several fat-containing products: margarine, shortening, butter flavored popcorn and chocolate frosting. The fatty acids were trans-esterified using sodium methoxide and analyzed on multiple Restek columns, namely Rtx-2330, Rt-2560, and FAMEWAX using GC-FID and GC-MS. With the exception of frosting, none of the studied products contained TFAs. However, there a tradeoff. The elimination of partially hydrogenated oils lead to significant increase of saturated acid content in margarine and shortening as compared to previously reported values by USDA. In terms of column selection, the Rt-2560 was the best choice for the separation of C18:1 isomers extracted from chocolate frosting.

LIP-11: Lipid Compass

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With the advent of high-throughput lipidomics platforms based on high-resolution mass spectrometry during the last decade, the need for a central, well connected and comprehensive resource for both experimental and computational scientists has increased.

With Lipid Compass, we want to simplify the exploration of the lipidomics datasets from different angles, following the structural hierarchy, as established by LipidMaps and the proposed extension to further levels based on high-resolution MS technologies, as proposed by Liebisch *et al*. [1], which has been implemented in the LipidHome database for theoretical lipids [2] and in

Swiss Lipids [3]. Based on quantitative evidence for lipid species sourced from the literature and publicly available data sets, LipidCompass provides a unique overview to browse, search and compare the structural hierarchy and quantitative variability of lipid profiles within and between studies.

As a first proof-of-concept, we have imported multiple, human plasma-related quantitative lipidomics datasets from a global ring trial [4] and show initial results that illustrate the usefulness of the database and its visualizations for comprehensive and comparative applications. Cross-linked lipid entries within the database enable crosscutting analysis and queries, together with external links to PubChem, Chebi, or other supporting evidence and relevant resources. Lipids are annotated with their level of confidence and support from literature, complemented by both qualitative and quantitative data. Query and comparison functions are also available as a machine readable API to support integration into bioinformatics workflows.

Lipid Compass will furthermore be an integration point for multiple lipid-related web services, such as LUX Score and LipidXplorer as part of the Lipidomics informatics for life-science (LIFS) project [5].

[1] G. Liebisch *et al.*, "Shorthand notation for lipid structures derived from mass spectrometry," *J. Lipid Res.*, vol. 54, no. 6, pp. 1523–1530, Jun. 2013, doi: 10.1194/jlr.M033506.

[2] J. M. Foster *et al.*, "LipidHome: a database of theoretical lipids optimized for high throughput mass spectrometry lipidomics," *PloS One*, vol. 8, no. 5, p. e61951, 2013, doi: 10.1371/journal.pone.0061951.

[3] L. Aimo *et al.*, "The SwissLipids knowledgebase for lipid biology," *Bioinformatics*, vol. 31, no. 17, pp. 2860–2866, Sep. 2015, doi: 10.1093/bioinformatics/btv285.

[4] J. A. Bowden *et al.*, "Harmonizing Lipidomics: NIST Interlaboratory Comparison Exercise for Lipidomics using Standard Reference Material 1950 Metabolites in Frozen Human Plasma," *J. Lipid Res.*, p. jlr.M079012, Oct. 2017, doi: 10.1194/jlr.M079012.

[5] D. Schwudke, A. Shevchenko, N. Hoffmann, and R. Ahrends, "Lipidomics informatics for life-science," *J. Biotechnol.*, vol. 261, pp. 131–136, Nov. 2017, doi: 10.1016/j.jbiotec.2017.08.010.

LIP-13: Investigating reactivity trends of Paternò-Büchi functionalization for C=C localization in ESI and MALDI lipidomics studies

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The Paternò-Büchi (PB) reaction is a well-known organic photoreaction between a carbonyl and a C=C double bond (DB). Ma and Xia showed that solution-phase UV-light-induced functionalization of DBs in PB reactions allows to pinpoint DB positions in fatty-acyl residues by nano-electrospray ionization (nESI) tandem mass spectrometry (MSn) experiments. We recently demonstrated that PB functionalization for lipid DB localization also occurs during matrix-assisted laser desorption/ionization (MALDI), enabling visualization of local DB-position isomers in mass spectrometry imaging. Here, we investigated if the underlying principles and the mechanism of PB functionalization, initiated during nESI and MALDI, are the same or if differences exist between functionalization mechanisms, wavelength dependences and reactivity trends of these two ionization modalities.
For atmospheric-pressure MALDI MS, benzophenone-based compounds (BzBs) were applied onto samples by sublimation with a homebuilt setup. MALDI MS experiments were performed employing an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen) coupled to a Q Exactive HF (Thermo Fisher Scientific GmbH, Bremen). All nESI-MS experiments were done with 10-4 mol/L solutions in 9:1 acetonitrile/water and a homebuilt nESI source coupled to a LTQ FT Ultra (Thermo Fisher Scientific GmbH). Two UV pencil lamps (Edmund Optics, York, UK) were used to irradiate the nESI capillary with 254 nm (4.5 mWcm-2) and 365 nm (2.5 mWcm-2) UV light in nESI-PB experiments. For UV/Vis spectra, 10-4 mol/L solutions in acetonitrile were analyzed by a Lambda20 instrument (PerkinElmer, Buckinghamshire, UK). Benzophenone, 4-fluoro-, 4-chlorido-, 4-bromo- and 4-iodo-benzophenone were used for the functionalization of a phosphatidylcholine standard to correlate PB reactivity changes with the systematically changed electronic structure of the PB compounds. For MALDI and nESI, lower electron density due to electron-withdrawing inductive effects facilitates PB reactions, whereas the electron-donating mesomeric effects of larger halogens lower PB yields. Each BzB was tested with various phospholipid standards. For both ionization modalities, no evident trend of a PB-yield dependence on varying lipid headgroups could be inferred but increasing PB yields were observed when increasing the number of DBs. To investigate effects of the excitation wavelength on PB yields, nESI-PB MS of benzophenone and a phosphatidylcholine standard were performed with 365 nm and 254 nm light. Our results suggest that PB reactions proceed with higher yields at 365 nm, which is also in line with 343 nm MALDI results. Our work indicates that PB reactions during nESI and MALDI follow the same reactivity trends and reactions most likely proceed with similar reactive intermediates and excited electronic states.

LIP-15: Proof of principle study for the quantitative analysis of lipid mediators of sputum samples of Tuberculosis patients

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Tuberculosis, caused by *Mycobacterium tuberculosis* is the most prevalent bacterial infection worldwide. To advance TB therapy monitoring by molecular markers, we tested the applicability of an analytical workflow for lipid mediators (LM) from sample collection until mass spectrometric analysis. We aim to correlate individual LM concentrations and treatment outcome to offer a better diagnostics in personalized TB therapy.

We employed liquid-chromatography tandem mass spectrometry (LC-MS/MS) using a Q Exactive Plus mass spectrometer (Thermo ScientificTM, Bremen, Germany) coupled to an Agilent 1100 series HPLC-system for LM analysis.[1] For the profiling

of glycerolipids, glycerophospholipids, sphingolipids, cardiolipins and cholesterol derivatives a shotgun lipidomics approach was applied.[1]

Special attention was given to the mass spectrometric compatible sample preparation to reduce chemical background and enhance sensitivity. We tested the applicability of an integrated workflow of a lipidomics shotgun approach and a LC-MS/MS analysis of LM omitting any usage plastic consumables and determined sensitivity and calibration ranges for PGE2-d9, RvD2-d5, LTB4-d4, 5-(S)-HETE-d8 and AA-d11. While sputum seems at first a well suited sample type for TB patients, the inter- and intraindividual variability is very high. We critically discuss possible normalization strategies to make LM and lipid analysis of sputum a useful diagnostic tool. To keep sample treatment as uniform as possible, all samples were immediately homogenized, diluted in methanol containing butyl-hydroxy-toluol and stored at -80 °C to block metabolic activity and inactivate Mtb.

Recent analysis of sputum from four different TB patients before and during therapy allowed quantification of 21 LMs from total lipid extracts by LC-MS/MS. Since the study lacked an optimized reproducible workflow, we intended to develop a more stable one to analyze sputum from three TB patients under treatment and sampled on a weekly basis until culture conversion as a proof of principle.

[1] A. Wutkowski, M. Krajewski, N. Bagwan, M. Schäfer, B.R. Paudyal, U. E. Schaible, D. Schwudke. Analytica Chimica Acta (2018). doi:10.1016/j.aca.2018.01.044

LIP-17: Lipidomic study to investigate the incorporation of *n*-3 PUFAin muscle of micro algae fed pigs

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Until now, lipidomic studies in tissues from farm animals fed different diets have so far only been conducted very rarely. The experimental design of the micro algae pig study involved 31 Landrace sows, two dietary groups, one control group (n=15) and micro algae group, (n=16). The study conducted at the experimental pig unit of Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. The pigs were fed indoor (group keeping), and the diet of the piglets of the micro algae group was supplemented with 7% micro algae (*Schizochytrium sp*., DHAgold, Fa. DSM). The pigs of both groups were fed from 33rd to 146th day of age and slaughtered at 100 kg live weight. For lipidomic investigation, samples of the *longissimus* muscle (13-14th rib) were taken. The investigation were performed by Shotgun lipidomics (Q Exactive Plus using the TriVersa NanoMate as nano electrospray source, Thermo, Bremen, Germany) and identification of lipid species by the use of LipidXplorer 1.2.7. For lipidomic data, the R project for statistical computing in combination was used with mixOmix R package using multivariate methods.

The shotgun lipidomics analysis resulted in identification and quantification of roughly 340 lipid species from 17 lipid/phospholipid classes. The main lipid species in pig muscle affected by micro algae intervention were predominantly etherlipids, PS and PA, not so PE, PC and TAGs, however the overall PL class composition was not diet affected. Up to 70% of the lipid species in pig muscle were significantly affected by micro algae supplementation. The results of multivariate lipidomics data evaluation (most variable important coefficients, VIP scores) revealed more than 100 lipid species with VIP scores higher than one, with highest VIP scores for TAG 58:8, PE-O 20:1/16:0, PE 16:0/19:1, PC-O 16:0/22:4 and TAG 56:7. In case of PLs, the Alkyl/Alkenyl PC/PE-Os were most diet affected ones. It's remarkable that the lipid species containing linoleic acid (18:2*n*-6) in micro algae muscle were significantly decreased (shown by GC/FID analysis of fatty acids). DHA, the main fatty in the micro algae (*Schizochytrium sp*.) lipids was incorporated in phospholipids (PC, PE-O, PE and PI) and triglycerides (TAGs) with high VIP scores (TAG 58:8, TAG 56:7, PC 16:0/22:6, PI 18:0/22:6, PE-O 16:1/22:6).

Some lipid species (with high VIP scores) have the potential to function as diet-induced biomarker in pig muscle. In conclusion, lipidomic analysis can be a useful tool for further clarification of muscle lipid metabolism and understanding of skeletal muscle physiology of farm animals.

LIP-19: LipidXplorer 2.0 Web: Online tool for simplified and streamlined lipid identification, visualization and quantification by shotgun lipidomics

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LipidXplorer 2.0 the new version of the popular LipidXplorer shotgun lipidomics software for desktop or online, has the core functionality of identification and quantification of small molecules and improves on the visualization of the results and makes the process more automated and customizable. For the web version there is no need to install and setup the software and the most up-to-date version is always available online. The lipid identification routine for LipidXplorer-Web is vastly simplified and streamlined, and the results are easier to interpret. Identifications are based on the most common forms of molecular ions and fragments of lipids that can be customized and filtered with validation rules that are not fixed on a database of expected lipid species.

Mass Spectrometry in Physics

PHY-01: Gas Phase Analyses of ^{13}C-Labeled Lithium Ion Battery Electrolytes by Means of GC-MS

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The worldwide interest in lithium ion batteries (LIBs) is still growing as an aspect of stationary energy storage and especially electromobility. Furthermore, LIBs are nowadays the state-of-the-art energy storage technology for portable electronic devices.[1] In general the battery consist of a negative graphitic anode, a positive lithium transition metal oxide cathode and an electrolyte. The electrolyte consists of different carbonate-based organic solvents, in which the conducting salt e.g. $LiPF_6$ is dissolved.

During the first charge and discharge cycle of the LIB, electrolyte decomposition takes place, due to reductive potentials at the anode. In this step (formation), a protective layer – so called solid electrolyte interphase (SEI) – is formed. The SEI protects the electrolyte from further decomposition on the anode surface. Besides solid SEI products, different permanent gases and light hydrocarbons evolve during formation.[2] However, the building mechanism and chemical composition of the SEI is not fully understood so far. Analytical methods like gas chromatography (GC) coupled with various detector systems are required to elucidate the important and complex process of SEI building. A stabile SEI is a key factor for long term stability, optimal performance and safety of a battery cell.[3] Therefore, the investigation of gaseous products evolving during formation can help to understand SEI building reactions and to improve the SEI quality.

In this study, a new *in situ* gas sampling port (GSP) integrated in LIB pouch cells was used to investigate the gas phase directly after formation. The used electrolyte contained ^{13}C-labeled ethylene carbonate and unlabeled dimethyl carbonate. GC-MS was used to monitor the ^{13}C-carbons of the gaseous decomposition products, which allows an assignment between solvent and gaseous decomposition products as well as the prediction of possible reaction pathways in LIBs.

References:

[1] R. W. Schmitz et al., *Progress in Solid State Chemistry*, **2014**, *42*, 65–84.

[2] S. J. An et al., *Carbon*, **2016**, *105*, 52–76.

[3] C. Huang et al., *J. Solid State Electrochem*, **2011**, *15*, 1987–1995.

PHY-03: Sandwich-structured substrates embedded in a diode laser-plasma interface for analytical chemistry

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Laser desorption is a common technique used in analytical chemistry for mass spectrometric detection. A key concept is the surface-laser-coupling, that mainly impacts the desorption of an analyte.

Sandwich-structured copper-glass substrates with cavities were constructed to be implemented in a laser-plasma-interface for standardized quantitative analysis by laser desorption. When the beam of a continuous diode laser is focused on the surface, molten substrate material diffuses into the glass, sealing it inside. Therefore, this method can be separated from laser ablation, achieving high ion signals without ablating material from the surface. Flexible Microtube Plasma (FμTP) was selected as ionization source. This laser-plasma-interface was applied to the detection of cholesterol, which showed a limit of detection of 0.4 ng and linear dynamic range of three orders of magnitude in positive ion mode. The dehydrated molecule $[M\text{-}H_2O+H]^+$ was the base peak of the spectrum and no further dissociation or fragmentation was observed. Physical effects of surface modification were investigated, including calculation of laser beam waist to simplify comparison and reproducibility of results.

Metabolomics: Techniques and Applications

MET-01: DeltaMS: a tool to track isotopologues in GC- and LC-MS data

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Stable isotopic labeling experiments are powerful tools to study metabolic pathways, to follow tracers and fluxes in biotic and abiotic transformations and to elucidate molecules involved in metal complexing. In order to introduce a software tool for the

identification of isotopologues from mass spectrometry data we developed DeltaMS. DeltaMS is a R-based software tool which relies on XCMS peak detection and X13CMS isotopologue grouping. Then we implemented the analyses of the data for specific isotope ratios and their relative error of these ratios. It provides pipelines for recognition of isotope patterns in three experiment types commonly used in isotopic labeling studies: (1) search for isotope signatures with a specific mass shift and intensity ratio in one sample set, (2) analyze two sample sets for a specific mass shift and, optionally, the isotope ratio, whereby one sample set is isotope-labeled, and one is not, (3) analyze isotope-guided perturbation experiments with a setup described in X13CMS. To illustrate the versatility of DeltaMS, we analyze data sets from case-studies that commonly pose challenges in evaluation of natural isotopes or isotopic signatures in labeling experiment. In these examples, the untargeted detection of sulfur, bromine and artificial metal isotopic patterns is enabled by the automated search for specific isotopes or isotope signatures. Thus, DeltaMS provides a platform for the identification of (pre-defined) isotopologues in MS data from single samples or comparative metabolomics data sets. All implemented in a graphic and user-friendly layout which allows also non-programmers to use this software tool.

MET-03: Uniting metabolomics data processing and highly confident annotation across six MS instrumental platforms by MetaboScape 5.0

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Metabolomics approaches may need to push different criteria: speed (throughput), separation, and/or accuracy. Tailored to these prioritized criteria different instrumental set ups will fall into favor. Combining different platforms, complementing their respective strengths, ultimately closes the gap between high-throughput and in-depth analysis methods. MetaboScape 5.0 integrates the processing-, dereplicationand unknown annotation-workflows for FIA-MRMS, LCMRMS, LC-ESI-TOF, and LC-ESI-TIMS-TOF and SpatialOMx in a single software. Due to its speed, especially FIA-MRMS is suited to create experiments containing several hundreds of measurements. The T-ReX algorithms perform feature picking, deisotoping, and deadducting across all these measurements. Where LC is involved, RT alignments are computed to ensure solid feature tables. The deadducting is designed to create unanimous ion interpretation across all analyses. This is even true for feature tables combined from positive and negative polarity.

Intelligent filter parameters provide an option to account for ultifactorial designs during feature assessment. MetaboScape provides consistent processing and annotation workflows for multiple instrument types within a single software solution. Process and explore more than a thousand measurements in a single experiment. While using high-throughput and automated tools, deep-dives into details are supported, also featuring machine learning based CCS prediction for 4D Lipidomics.

MET-05: Application of metabolomics methods on LC/GQ-QTOF data to discriminate extra virgin olive oils from different Protected Designations of Origin

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The popularity of extra virgin olive oil (EVOO) and the increasing problem of food fraud have provided the need for quality and authenticity control. The protected designation of origin (PDO) and the protected geographical indication (PGIs) are differentiation strategies used in the olive oil market. Typical problems are mislabeling of PDO or edible oil adulteration. Minor compounds of extra virgin olive oil, such as phenolic and triterpenic compounds, sterols and tocopherols, are highly influenced by agro-technological practices and can be used for olive oil authentication. In this study 126 oil samples from 6 Mediterranean PDOs were analyzed by LC-MS and GC-MS combined to statistical methods. The extracts were eluted with a 15min gradient on an UHPLC using a C18 (2.1x100mm, 1.8µm) column, with acidified water and ACN. The derivatized extracts were injected in GC, using a BR-5 column with a 50min gradient from 150 to 320°C (4°C/min rate). Both systems were coupled to a Compact™ QTOFMS (Bruker) by an ESI and an APCI interface. The power of different sophisticated methods (covering VOO minor fractions) together with statistics to classify oils from diverse origins had been checked. Different 2-class models have been built with the aim of pointing out PDO-markers. The different polarities and platforms logically drove to diverse makers, taking advantage of their complementarity and, consequently, enriching the outcomes of the project. In order to model the seasonal variability too, it would be necessary to enrich the created models by using oils coming from different seasons.

MET-07: Metabolic profiling based on HILIC-MS to characterize mammalian cell cultures

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To reduce the number of animal experiments in toxicological research, the application of cell culture models is an increasingly important alternative to evaluate the toxicity of potentially harmful compounds. However, the outcome of such experiments is limited as the established assays used to determine the cytotoxicity do not yield information concerning the mode of action. The metabolomics approach allows the simultaneous monitoring of cellular metabolic alterations caused by the applied substances and may yield more insight into the mode of action of an unknown compound.

An untargeted HILIC quadrupole time-of-flight mass spectrometry and a targeted HILIC triple quadrupole mass spectrometry metabolomics method were developed to characterize the metabolome in mammalian cell cultures. The sample preparation as well as the instrument parameters were optimized to establish a sensitive and reproducible method. Most optimization of compound-specific triple quadrupole mass spectrometer parameters was achieved using the freeware Skyline, which did not require purified standards.

Since most analytes of interest are very polar small molecules, HILIC was chosen for their separation and analysis. The tailing of anionic molecules was reduced by avoiding metal components in the system and peak shape was further improved using a zwitterionic HILIC phase with PEEK inlining and an alkaline mobile phase. The optimization of the sample preparation showed that the addition of cold acetonitrile/water (4+1, v/v) directly to the cell culture dishes effectively quenched the metabolism and extracted the highest amounts of most analytes.

The incubation of human hepatocarcinoma cells (HepG2 cell line) with substances with known effects on the cellular metabolism allowed the evaluation of the developed methods. As an example, the treatment with 6-aminonicotinamide led to an increased amount of 6-phosphogluconate due to its inhibition of the 6-phosphogluconate dehydrogenase and confirmed that alterations in the pentose phosphate pathway can be detected by the developed method. In addition, the possibility to analyze the influence on glutathione production was confirmed by the incubation of buthionine sulfoximine, which resulted in a significant reduction of glutathione disulfide in the prepared samples. These observations proved the suitability of the developed HILIC-MS methods to characterize the effects of xenobiotics on the cellular metabolism.

MET-09: Visualizing sequestered cardiac glycosides in *Danaus plexippus* and *Euploea core* using high-resolution AP-SMALDI MSI

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During 350 million years of coevolution, plants evolved a multitude of defensive traits against herbivorous insects, including a tremendous diversity of low-molecular-weight chemicals known as secondary metabolites. Strikingly, many insects are not only able to cope with plant toxins but also accumulate the toxic metabolites in their body (sequestration) to defend themselves against predators. The monarch butterfly (*Danaus plexippus*) that sequesters cardiac glycosides from its host plant milkweed (*Asclepias* spp.), represents an emerging model system in chemical ecology and evolutionary biology. Nevertheless, the physiological and molecular mechanisms underlying sequestration are still unknown. To understand the pharmacokinetics of cardiac glycosides in *Danaus plexippus*, we applied atmospheric-pressure matrix-assisted laser/desorption ionization mass spectrometry imaging to visualize the selective uptake, transport, modification and storage of cardiac glycosides in transversal and longitudinal sections of caterpillars. For comparison, we also studied the closely related *Euploea core*, a species known to not sequester cardiac glycosides. All experiments were carried out using an autofocusing AP-SMALDI5-AF high-resolution MALDI imaging ion source (TransMIT GmbH, Giessen, Germany) coupled to a Q Exactive HF Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). First, an optimized sample preparation protocol for transversal and longitudinal sections of 5th instar larvae was established, applicable also to any kind of caterpillars. In *D. plexippus* tissue sections, only five out of 10 detected cardiac glycosides were taken up into the body tissues and finally stored in the larval integument, showing that sequestration is an active and selective process. The concentration of cardiac glycosides were found to decrease along the gut passage, suggesting that the uptake occurs primarily in the anterior region. In contrast, caterpillars of *E. core* did not sequester cardiac glycosides and only had low concentrations in the gut lumen, indicating efficient degradation. To confirm MSI results, gut contents were sampled along the gut by dissecting freeze-dried caterpillars, extracted with methanol and analyzed via HPLC-ESI-MS. Our analysis revealed that the cardenolide concentration was six times higher at the beginning compared to the end of the gut. Furthermore, our results demonstrate how two closely related milkweed butterfly species have evolved diverging strategies to cope with defensive plant compounds and give first insights into the sequestration mechanism of cardiac glycosides in milkweed butterflies. Our approach to visualize low-molecular-weight chemicals in insects suggests that high-resolution AP-SMALDI MSI is a promising technology not only for basic research but also for e.g. studying the pharmacokinetics of insecticides across insect tissues.

MET-11: Integrated workflow with quality control for large cohort and clinical metabolomics research using robust hardware and signal correction

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Metabolomics research relies on precision measurement of statistically powered sets of hundreds or thousands of samples. First, this requires robust analytical hardware with long term stability, capable of generating high precision data. Second, processing of large datasets may require additional mathematical correction to compensate for systematic changes in observed signals. We investigated the long-term stability of an LC-HR-QTOF system by measuring a batch of more than 1000 urine samples and monitoring the effect of data acquisition on MS ion source contamination and detector aging. Six different human urine samples were diluted 1:3 with water and centrifuged. The supernatant was aliquoted for LC separation using a linear RP gradient with a 15 min cycle time. MS data (ESI positive mode) were acquired on an Impact II QTOF-MS (Bruker). Every 7th injection corresponded to a quality control sample which was an equal-parts mixture of each sample. The ion source region was cleaned before and after the sample batch, and detector tuning was performed directly after cleaning. Data processing for untargeted profiling was conducted in MetaboScape and for targeted data evaluation in TASQ (both Bruker). A new workflow solution for population and clinical metabolomics research with outstanding robustness was shown: acquisition of > 1100 injections with an impact II QTOF system without detector tuning, no decrease in peak area, stable mass accuracies <1.5 ppm and stable isotopic patterns. Automated correction of analytical variability helps to reveal subtle but significant metabolic differences: 90% of features had an RSD below 20%.

MET-13: MetaboQuan-R: Rapid plasma profiling of a bladder and lung cancer human cohort

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Cancer is one of the most complex, life threatening diseases, existing in many forms which have unknown pathogenesis. Here, we present a study comparing plasma samples from a cohort consisting of bladder and lung cancer patients, with those of healthy controls using a high-throughput OMICS workflow.

This unique MetaboQuan-R LC/MS workflow is a targeted OMICS solution allowing the rapid screening and semi-quantification of various compound classes and peptides using a single LC-MS platform informatics platform. All LC and MS methods were generated using the Waters Targeted OMICS method library, and did not require any optimization. A known level of at least one labeled analogue from each compound class was added to each sample in order to make the analysis semi-quantitative. The level of each analyte was estimated from its ratio to the appropriate labeled analogue.

Data was collected for 18 plasma samples (6 controls, 6 bladder cancer and 6 lung cancers), each sample was run in duplicate (proteins) or triplicate (acylcarnitines and amino acids). Quality controls (consisting of a pool constructed from all samples) were acquired every ninth injection. In total, 206 injections were performed, equating to a total run time of 22 hours. LC-MS data were processed with Skyline (Washington University, Seattle, USA). Additional data visualization and statistical analysis was performed using Metaboanalyst.

128 compounds were detected and their levels measured using Skyline, generating a coefficient of variation (CV) less than 20% for the QC samples: 80 proteins, 20 acylcarnitines and 28 amino acids. High precision was demonstrated with low observed variance on the QC samples. Valine-d8 (spiked in all samples for the amino acid screening) is used as an example to illustrate the consistency of peak area across the whole study (<5% CV)

Pair-wise comparisons (bladder vs. control and lung vs. control) using a t-test were performed on each compound class. 12 compounds were highlighted as differentially expressed between bladder / control and 11 between lung/control. Although requiring more statistics and validation, some of those compounds were found to have previously been highlighted in cancer studies

Overall the data demonstrated the ability to highlight potential and relevant markers of interest in a simple and rapid manner. There was no need for method development and results were obtained in less than 24 hours. The next step in the study would be to concentrate on the highlighted markers and validate them by performing a more statistically rigorous study.

ME-15: Integrated GC- and LC-MS-based approach for analysis of metabolite patterns associated with desiccation tolerance in moss (*Dicranum scoparium*)

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Drought is one of the main causes for crop losses in the modern agriculture. Therefore, drought tolerance of crop plants needs to be improved. Among other approaches, it can be achieved by means of the genetic material obtained from the organisms tolerant to desiccation. One of the most striking examples of those is represented by mosses, which can survive and restore viability after significant decrease in the tissue water contents. This feature is primary underlied by their ability for a deep and rapid re-organization of their metabolism, accompanied by accumulation of low molecular weight osmoprotectors. However, to implement this feature in practical agriculture, the molecular mechanisms underlying desiccation tolerance of mossesneed to be characterized in more detail. Therefore, here we address the metabolic changes in *Dicranum scoparium* thalli in response to drying (24 hours at room temperature) and rehydration (24 hours at 5°C). To address the analytes of various chemical nature, a comprehensive analysis of primary and secondary metabolites was accomplished with an array of analytical techniques. First, the *D. scoparium* thalli were sequentially extracted in several solvent systems of different polarity, and the resulted extracts were analyzed by gas chromatography and liquid reversed phase/ion-pair chromatography-mass spectrometry (GC- and LC-MS, respectively). The subsequent data processing, i.e. spectrum deconvolution, peaks extraction, alignment of chromatograms by analyte retention time, identification and integration of peak areas, relied on several softwares: Automated Mass spectral Deconvolution and Identification System (AMDIS), MSDial, Xcalibur and LCQuan. Statistical analysis of the obtained data relied on Metaboanalyst and Metfamily on-line tools. The results showed that the moss metabolic response to desiccation and subsequent rehydration was accompanied with a strong accumulation of sugars and alterations in metabolism of lipids (primarily choline-containing phospholipids) and terpenes (biosynthesis of pentacyclic triterpenes). Remarkably, the relative abundance of proline, i.e. the main metabolic marker of drought in flowering plants, did not show any desiccation- or rehydration-related alterations.

Natural Product Mass Spectrometry

NAT-01: MALDI-TOF analysis of fossilized wood

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We are interested in a silicified specimen of fossil wood from the Late Jurassic (150 million years), which has differently colored domains (dark, medium, light). The specimen is part of the tree trunk of a conifer (Araucariaceae) and was collected from silty sandstones of the Six Mile Draw deposit (Brushy Basin Member, Morrison Formation, Utah, USA). We used MALDI-TOF mass spectrometry to analyze and identify the organic compounds in different domains of the varicolored fossil wood. We measured powdered samples from the light, medium, and dark domains and additionally powder produced by desilicifying the dark domain with HF extraction. The specimen is neither a soft tissue nor soluble in an organic solvent. Therefore, we suspended powdered samples in a dimethylformamide (DMF) solution with different matrices. The suspension was then transferred to the MALDI target and dried. The matrices tested were THAP (2,4,6-Trihydroxyacetophenon), DHB (2,5-dihydroxybenzoic acid) and HCCA (α-Cyano-4-hydroxycinnamic acid). At high laser intensity, we succeeded in recording MALDI-MS spectra.

In the light and medium domains, we were able to detect basic building blocks of wood: lignin dimers[1], coniferin[2] and cellobiose[3]. Abundant plain carbon exists in the form of carbon clusters in the dark zone, providing an explanation for the different coloring of the domains.

[1] a) R. Whetten, R. Sederoff, *The Plant cell* **1995**, *7*, 1001; b) P. Araújo, M. S. Ferreira, D. N. de Oliveira, L. Pereira, A. C. H. F. Sawaya, R. R. Catharino, P. Mazzafera, *Analytical chemistry* **2014**, *86*, 3415.

[2] a) A. Yoshinaga, H. Kamitakahara, K. Takabe, *Tree physiology* **2016**, *36*, 643; b) M. Kaneda, K. H. Rensing, J. C. T. Wong, B. Banno, S. D. Mansfield, A. L. Samuels, *Plant physiology* **2008**, *147*, 1750.

[3] S.-f. Ren, L. Zhang, Z.-h. Cheng, Y.-l. Guo, *Journal of the American Society for Mass Spectrometry* **2005**, *16*, 333.

NAT-03: Species identification of marine food with protein-based LC-MS-analysis

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The unambiguous analytical determination of marine species has become important to comply with current conditions concerning labelling and traceability. For the food control authorities and seafood industry it is a major issue to avoid commercial food fraud and guarantee consumer safety [1]. The objective of this work is to develop rapid and reliable protein-based methods for the verification of the correct labelling of crustacean species. Additional food sources (grasshoppers, mussel, mealworm, salmon, tuna) were investigated to verify possible species-specific biomarkers [2,3,4].

In the first step a sample preparation protocol with an appropriate protein extraction for shrimp and lobster was established, which was also usable for other species. After extraction, the protein content was determined by photometric measurement and digested enzymatically with trypsin. The resulting peptides were separated and detected by LC-MS. Due to the fact that a precolumn is used as a trap, these settings were optimized extensively. The developed LC-MS-method was in-house validated according to recovery, linearity, stability of prepared peptides and matrix effects. The peptide profiles of 4 different crustacean species (whiteleg shrimp, giant tiger prawn, American lobster, Norway lobster) were used to establish different statistical methods for the identification of species-specific biomarkers. Including a random forest machine learning algorithm, which was applied for sample classification.

[1] Ortea, I., Cañas, B., Calo-Mata, P., Barros-Velázquez, J., Gallardo, J.M., 2009: Arginine kinase peptide mass fingerprinting as a proteomic approach für species identification and taxonomic analysis of commercially relevant shrimp species. In Journal of Agriculture and Food Chemistry, Heft 57, S. 5665-5672

[2] Yi, L., Lakemond, C.M.M., Sagis, L.M.C., Eisner-Schadler, V., van Huis, A., van Boekel, M.A.J.S., 2013: Extraction and characterisation of protein fractions from five insect species. In Food Chemistry, Heft 141, S. 3341-3348

[3] Zielińska, E., Baraniak, B., Karaś, M., Rybczyńska, K., Jakubczyk, A., 2015: Selected species of edible insects as a source of nutrient composition. In Food Research International, Heft 77, S. 460-466

[4] Buchtova, H., Dordevic, D., Duda, I., Honzlova, A., Kulawik, P., 2019: Modeling some possible handling ways with fish raw material in home-made sushi meal preparation. In Foods, Heft 8, S.459

NAT-05: Unravelling the complexity of mammalian mucin *O*-glycosylation by mass spectrometry

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Mucus glycoproteins, i.e. mucins constitute as major components the mucosal protective barrier of the gastrointestinal tract. Mucins are densely decorated with *O*-glycans exhibiting a vast structural diversity. Specific glycan motifs play key roles in mucosal protection and also in extrinsic interaction with commensal and pathogenic bacteria. Investigations on the bacteria-glycan interplay require the in depth analysis of the *O*-glycan structures. Here, we performed a combination of β-reductive elimination, specific enrichment of oligosaccharides, and mass spectrometry (MS) to get hands on the *O*-glycans of mucins from porcine stomach and from bovine maxillary gland.

MS analysis of *O*-glycans released from the protein backbone of porcine mucin by β-elimination reactions followed by purification making use of solid phase extraction on i-HILIC revealed heterogeneous mixtures of more than 50 distinct neutral

glycoforms harboring core type 1 to 4 structures. Besides simple di- and trisaccharides fucosylated oligosaccharides up to tetradecamers were found and their structures were elucidated by low-energy CID experiments. Chemical liberation of *O*-glycans from bovine mucin yielded a high number of differently fucosylated and sialylated glycoforms based mainly on core 2 and core 3 structures. Taking advantage of the various collisional cross-sections of isomeric oxonium-type fragment ions allowed discriminating different linkage types of terminal sialic acids. The present results demonstrate the potential of MS analysis of *O*-glycans released from mammalian mucins for their in-depth structural characterization. Knowledge on the exact glycan structure is prerequisite for understanding the functional role of *O*-glycans in the interaction of resident and harmful microorganisms with the mucosal barrier.

NAT-07: Investigation of Tetrahydrocannabinol (THC) and Cannabidiol (CBD) in Smoke by Application of an On-Line Photo Ionization Mass Spectrometry

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Photo ionization time of flight mass spectrometry (PI-TOFMS) is well suited for on-line characterization of tobacco smoke. Depending on the photoionization method (single photon ionization, SPI or resonance-enhanced multiphoton ionization, REMPI) smoke constituents such as butadiene, acetaldehyde, naphthalene, phenol or polycyclic aromatic hydrocarbons (PAH, by REMPI) can be detected with high time resolution (puff-resolved). With the increased (legal) availability of marihuana/cannabis and THC containing smoking products not only for medical purposes, the interest in understanding the release processes of the active smoke constituents is increasing as well. Puff by puff emissions analysis of different products ('joints') filled with tobacco mixtures containing dried marihuana flowers, leafs or hashish were performed to investigate the release profile of THC and related smoke constituents in comparison to nicotine as the main active compound of the added tobacco. Within this study, a Laser/Lamp PI-TOF system (Photonion GmbH, Schwerin/Germany) in SPI and REMPI mode was used coupled to a LM1 smoking machine (Borgwaldt KC, Hamburg/Germany). The REMPI methodology enables focusing on aromatic structures primarily relevant for this investigation. Smaller molecules being present in higher concentrations in smoke (e.g. aldehydes), which could lead to a suppression of the target smoke constituents during the measurement, are suppressed effectively compared to SPI (single photon ionization). Environmental gases such as oxygen or nitrogen are suppressed by photoionization anyway. Using a combination of SPI and REMPI the isobaric compounds Tetrahydrocannabinol (THC) and Cannabidiol (CBD) as the most interesting once in cannabis can be separated on-line.

Furthermore, the present study evaluates the influence of activated carbon filters, which become more and more popular to reduce certain smoke constituents, such as PAHs (polycyclic aromatic hydrocarbons), in mainstream smoke of any combustible product.

Proteome Analysis - Basic Research

PRB-01: New chemical cross-linkers for Protein Structure Elucidation with switchable properties: CID stable and cleavable by In-Source Paternò-Büchi Reactions

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This contribution is part of our ongoing efforts to develop innovative cross-linking (XL) reagents and protocols for facilitated mixture analysis and efficient assignment of cross-linked peptide products. In this report we combine in-source Paternò-Büchi (PB) photo-chemistry with a tandem mass spectrometry approach to selectively address the fragmentation of a tailor-made cross linking reagent. The Paternò-Büchi (PB) photo-chemistry, so far exclusively used for the identification of unsaturation sites in lipids and in lipidomics, is now introduced to the field of chemical cross-linking. Based on trans-3-hexenedioic acid an olefinic homo bifunctional amine reactive cross-linking reagent was designed and synthesized for this proof-of-principle study. Condensation products of the olefinic reagent with a set of exemplary peptides are used to test the feasibility of the concept. Benzophenone is photo-chemically reacted in the nano-electrospray ion source to the respective oxetane PB reaction products. Subsequent CID-MS triggered *retro*-PB reaction cleavage of the respective isobaric oxetane molecular ions delivers reliably and predictably two sets of characteristic fragment ions of the XL-linker. Based on these signature ion sets a straightforward identification of XL-derivatized peptides in complex digests is proposed. Furthermore, CID-MS^n experiments of the *retro*-PB reaction products delivers peptide back-bone characteristic fragment ions. Additionally, the olefinic XL-reagents exhibit a pronounced robustness upon CID-activation, without previous UV-excitation. These experiments document that a complete back-bone fragmentation is possible, while the linker-moiety remains intact. This feature renders the new olefinic linkers switchable between a stable, non-cleavable cross-linking mode (CID) and an in-source PB cleavable mode.

Patrick Esch, Moritz Fischer, **Sven Heiles**, **Mathias Schäfer**, *J. Mass Spectrom.* **54** (2019) 976-986. DOI: 10.1002/jms.4474

PRB-03: In-depth characterization of the glycated soy milk proteome

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Thermal processing of soy milk kills pathogens and denatures anti-nutrition factors, such as trypsin inhibitors, therefore ensuring microbiological safety, better digestibility, and longer storage. However, it also induces Maillard reactions of soy proteins yielding glycation (Amadori/Heyns) products and the heterogeneous group of advanced glycation endproducts (AGEs). These products may alter the nutritional value, antigenicity and digestibility of the modified proteins, but are also linked to potentially toxic effects. Very often soy milk products are additionally sweetened, not only by adding additional sugar, but also using fructose, apple juice or agave syrup. Thus, the hexoses (mainly fructose) present in these sweeteners might further increase the glycation degree of the final product.

Here, glycation sites and AGE-modifications were qualitatively and quantitatively analyzed in the soy proteome of unsweetened (natural) soy milk (USM, UHT-treated), soy milk sweetened with hexose(fructose)-containing sweeteners and sucrose (SSM and SSMSu, UHT-treated), and soy-based infant formulas (SIFs) from different companies. Thus, nRPC-ESI-MS/MS identified over 350 peptides including 229 Amadori/Heyns products and 128 AGEs in 53 proteins using a combination of DIA (MS^E) and targeted DDA (CID and ETD modes). Amadori/Heyns products were mainly derived from hexoses, whereas N^{δ}-carboxyethyl-arginine (CEA) and methylglyoxal-derived hydroimidazolone (MGH) were the main AGEs in soy milk accounting for about 70% of all identified AGEs. Early glycation increased similarly with the harsher processing (SIFs) and addition of fructose-containing sweeteners (SSMs). On the other hand, AGE modifications increased significantly after adding fructose-containing sweeteners. Although the total number of AGE modifications did not change in SIFs when compared to natural soy milk, SIFs contained higher content of MGH and CEA similarly to SSMs.

PRB-05: Application of Sample Displacement Batch Chromatography and Intact Protein Mass Spectrometry Analysis for Enrichment and Identification of Proteoforms

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Analysis of protein species (proteoforms) is one of the most challenging problems in analytical chemistry because they are coded by the same gene that makes them physically and chemically very similar. Moreover, they can be present in a very low abundance. In therapeutic proteins, some of these proteoforms may reduce efficacy. There is a risk that some proteoforms may even induce harmful side effects. Enrichment, purification, identification and quantification of these proteoform are needed. Using sample displacement chromatography (SDC), the binding capacity of the stationary phase is used efficiently and offers simple enrichment of proteoforms with higher yield compared to other chromatography modes. For identification purpose, top-down mass spectrometry is preferred to analyse the intact protein so that each proteoforms can be detected. In this work, we apply sample displacement chromatography in a batch (SDBC) using strong cation exchange resin to enrich proteoforms subpopulation for reducing the complexity of proteoform fractions. Top-down mass spectrometry was then used to analyse all SDBC fractions. Conditions for SDBC was obtained by protein purification parameter screening. Commercial ovalbumin was used as a model protein due to the complexity of it is representing therapeutic proteins complexity. With SDBC, more than the double number of proteoforms were identified, compared to the sample prior to SDBC. Low abundance basic proteoforms were well enriched in the early fractions as they have higher affinity toward the cation exchange resin. In the later fractions, most of the basic proteoforms were already removed. Additionally, in the flow-through, other contaminating proteins from in the ovalbumin sample were identified. These results show the utility of sample displacement batch chromatography as a viable and easy to handle approach for enrichment of proteoform subpopulations.

PRB-07: Towards automated proteomics sample preparation - up to 384 complex biological samples per week

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With the advent of novel and fast LC-MS acquisition methods in proteomics (PASEF, DIA, BoxCar), sample preparation has become a major bottle-neck of throughput. To overcome this limitation, we developed a novel semi-automated sample preparation platform for throughput of 384 samples per week [1]. This corresponds to a ~5-fold reduction of lab-time, which is predominantly attributed to the parallel sample handling in 96-well format during the whole workflow (protein purification to LC-MS injection). Our platform can be perfectly exploited (I) for robust and reproducible proteomics and phosphoproteomics (in conjunction with 96-well phosphopeptide enrichment [2]) of hundreds of clinical and biomedical samples and (II) for technical experiments addressing sample preparation conditions (e.g. choice of enzyme or digestion buffer composition).

For semi-automated sample preparation, we employ molecular weight cut-off filter plates for filter aided-sample preparation (FASP) [3], a liquid handling platform for sample loading and addition of digestion buffer and a positive pressure solid-phase extraction unit for filter washing and recovery of peptides after digestion. Samples are directly injected from 96-well plates for both quality control via monolithic column-HPLC (~48 samples/day) and nanoLC-MS analysis. Using this set up we have currently processed more than 1.500 samples.

In first tests, we used HeLa lysate for systematic characterization. Technical variation was excellent with R^2 > 0.95 and highest analyte recovery was achieved with protein loads of 36-100 µg. Notably, also lower amounts can be reproducibly processed at the expense of sample loss (~40 % with 24 µg and 60 % with 12 µg – which is in good accordance with the literature [4]. Further exploitation of our platform for processing biomedical and clinical samples (serum, plasma, cardiac muscle tissue, platelets and megakaryocyte – more than thousand samples in total) revealed error rates of 2-4 % (poor peptide recovery, clogging, or filter rupture) which is approximately three time lower than with manual FASP using centrifuge filter units.

In summary, our novel semi-automated processing platform exhibits high potential to become a key-technology in LC-MS-based proteomics and phosphoproteomics. It paves the way towards higher sample throughput in biomedical and clinical research.

References:

[1] Loroch et al. submitted. 2020

[2] Post et al. J Proteome Res. 2017

[3] Manza et al. Proteomics. 2005 and Wisniewski et al., Nat Methods. 2009

[4] Sielaff et al. J Proteome Res. 2017

PRB-09: Time-resolved proteomic profiling of a mammalian cell cultivation process using the positive pressure workstation Resolvex A200

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The majority of recombinant protein therapeutics are produced in Chinese Hamster Ovary (CHO) cells. Productivity depends on the initial cell line engineering in terms of integration site or choice of an appropriate promotor for the recombinant gene expression, as well as media and process parameter optimization. Here, proteomic profiling with implementation of the Resolvex A200 for a quick and reliable sample preparation is used to evaluate optimization targets for a pharmaceutical relevant cell line system.

Triplicates of CHO cell 2 L bioreactor fedbatch cultivations were performed and daily sampled for proteomic analysis, including extraction, reduction and alkylation of cysteines and tryptic digest. Pre-purification of peptides was performed semi-automatically using the positive pressure workstation Resolvex A200 (TECAN) and the reversed phase polymeric sorbent WWP2 (Cerex). Within a 60 minutes nanoLC gradient peptides were seperated and measured in DDA top10 mode on Orbitrap MS/MS (Q Exactive Plus, Thermo Fisher Scientific) equipped with a dual MALDI/ESI source for increased sensitivity. Data was evaluated using MaxQuant and Perseus software.

Supported by increased automated sample preparation with the Resolvex system, collected data from day 3 (exponential growth, before feeding) up to day 11 (end of stationary phase) showed high pearson correlation of 93.7 ± 4 % with ca. 2500 proteins quantified. Different growth phases were separated by hierarchical clustering and subsequent statistical analysis revealed distinct protein profiles. A specific chaperone with functions in quality control and protein folding showed highest upregulation over time which stands in positive correlation to transcriptomics/RNA-seq data and was validated by Western blotting.

Highly reliable proteomic data of CHO fedbatch cultivation were successfully mapped to growth and metabolic changes during fedbatch cell cultivation and revealed promising new targets, like strong endogenous promotors, for cellular engineering and process optimization of a biopharmaceutical relevant cell line.

PRB-11: Comprehensive proteome analysis of *Xanthomonas campestris* pv. campestris with a focus on proteins involved in the biosynthesis of xanthan

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Xanthomonas campestris pv. campestris (*Xcc*) are Gram negative phytopathogens listed under the top five important phytopathogenic bacteria. They infect *Brassicaceae* and cause increasingly crop yield losses in agriculture. Additionally, they are of great industrial interest due to its capability to produce the secondary metabolite xanthan. The unique rheological properties of xanthan lead to its usage as a thickener and emulsifier in a variety of food and non-food industry applications. Its biosynthesis includes the building and decoration of pentasaccharide subunits, their polymerization and secretion. In our study we worked with the wild type strain *Xcc* B100, whose genome sequence was revealed in 2008. Until now, proteome analysis of *Xcc* B100 were missing a comprehensive view on the proteome, especially those proteins which are needed for the biosynthesis of xanthan.

In this study different protein isolation methods were used to verify the localisation of these proteins. Beside methods for the isolation of fractions, like the periplasmatic, outer and inner membrane or cytosolic proteome, a method for the analysis of the whole proteome of the organism was tested. All samples were prepared in five biological replicates and measured on a nanoLC system coupled to a QExactive Plus. Additionally, different six-frame databases were constructed to identify unknown and small proteins with a maximal number of 70 amino acids.

More than 150 nanoLC MS/MS measurements were performed, leading to a total number of over four million PSMs, which can be associated to more than three thousand proteins representing 73 % of the whole putative proteome repertoire of *Xcc* B100.

In silico prediction revealed that most proteins involved in xanthan biosynthesis are either associated with the membrane or predicted within the cytosolic fraction. Interestingly, those proteins could not be found within membrane- or cytosolic fractions isolated with established protocols. A method based on digesting the whole cell using the organic solvent trifluoroethanol leads to the identification of ten out of twelve proteins which are involved in xanthan biosynthesis. For the purpose to analysis the profile of proteins within the *gum* operon, the new protocol was used for a label-free quantitative analysis of different growth phases of *Xcc* B100. Interestingly, differential regulation of the proteins involved in xanthan biosynthesis depending on the analysed growth phase was indicative for switching between biomass production and xanthan biosynthesis. Conclusively, this work represents the most comprehensive proteome analysis in this strain to date.

PRB-13: Capillary flow micro Pillar Array Columns (µPAC™ capLC): Combining nano flow sensitivity with analytical robustness and throughput for proteomics LCMS

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Mass-spectrometry based proteomics has become an essential tool in biological, biomedical and biopharmaceutical research. Due to the increased sensitivity that can be achieved compared to analytical flow LC-MS, the majority of LC-MS based proteomics research is performed within the nanoflow LC regime, with flow rates typically below 1 µl/min and total run times exceeding 60 min. However, capillary and microflow LC-MS solutions are recently gaining interest: the demand for large quantitative proteomics studies requiring increased throughput and robustness is growing and the sensitivity of MS instrumentation has increased significantly. By working at higher flow rates, the impact of gradient delay and sample loading volumes on the total analysis time can be reduced and low flow rate or column dimension related technical errors (such as electrospray instability, column clogging or the presence of void volumes in the analytical flow path) are minimized.

A micro pillar array column-based solution for capillary flow LC-MS is presented. In contrast to conventional LC columns that contain randomly packed beads as their stationary phase, micro-chip-based pillar array chromatography columns have a separation bed of perfectly ordered and freestanding pillars obtained by lithographic etching of a silicon wafer. The regular mobile phase flow pattern through these micro-chip pillar array columns adds very little dispersion to the overall separation, resulting in better peak resolution, sharper elution and increased sensitivity. The freestanding nature of the pillars also leads to much lower back pressure buildup, and makes it possible to operate longer columns.

This presentation will explain the principles of the micro-chip based pillar array columns. Their contribution to LC-MS workflow robustness will be illustrated with high resolution DIA data of 500 ng human proteome analyzed using either packed bed or micro-chip based pillar array columns operated at different flow rates and gradients, as an opening to an alternative future in proteomics: highly reproducible label-free quantification of 1000's of peptides over large sample batches using capillary LC-DIA.

In addition to proteomics experiments, the µPAC™ capLC column is perfectly suited for many LCMS-based separations where high reproducibility and robustness is desired.

PRB-15: Selective maleylation directed isobaric peptide termini labelling for accurate proteome quantification

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The isobaric peptide termini labeling (IPTL) has been a focus ever since it was proposed, because of providing more accurate and reliable quantification information by the entire fragment ion series. However, its multiplex capacity used to be limited to three. Herein, we present a selective maleylation directed isobaric peptide termini labelling (SMD-IPTL) to extend the multiplexing capacity of conventional IPTL approaches to 7-plex with commercially available 13C/15N-labelled amino acids. With SMD-IPTL, under the background of 4-plex labelled Lys-C peptides of yeast proteins (mixed at ratio of 1:5:1:5), the 1:2:5:10 mixed BSA was quantified as 0.94:2.46:4.70:9.92, which confirms the suitability of SMD-IPTL for the multiplex analysis of complex proteomes.

Proteome Research - Applications in Biology and Biochemistry

PRA-01: Dietary spermidine protects mitochondrial function and delays brain aging via hypusination

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Mitochondrial function declines during brain aging and is suspected of a key role in age-induced cognitive decline and neurodegeneration. Supplementing levels of spermidine (Spd), an endogenous metabolite normally decreasing with age, was suggested to delay aspects of brain aging. We here show that dietary Spd protects from the aging-associated decline of mitochondrial respiratory capacity, oxidative burden in *Drosophila* brains as well as locomotion and memory. Mechanistically, Spd mediated neuroprotection was dependent on hypusination i.e. the catalytic conjugation of the aminobutyl moiety of Spd to eukaryotic translation initiation factor 5A. Moreover, genetic attenuation of hypusination largely occluded the anti-aging protective effects of Spd supplementation on brain mitochondrial respiratory capacity, as well as longevity, locomotion and memory formation. How proteomic alterations might underlie brain and generic aging promoted by compromised hypusination levels are under active investigation. For this, we employed the isobaric tags for relative and absolute quantitation (iTRAQ) technology combined with LC-MS/MS analysis on extracts of mechanically; hand-dissected *Drosophila* brains (about 200 brains per condition). Using iTRAQ, we contrasted brain extracts of isogenic Drosophilae with or without dietary Spd in the food. We chose animals of 15 days old as this is the time window where Spd levels have been shown to drop steeply in aging fly heads and where phenotypic differences consolidate between the Spd-protected and non-protected cohorts. Interestingly, mitochondrial proteins were clearly upregulated under Spd feeding and represented the majority of the 500 most upregulated proteins following unbiased Gene Ontology analysis.

PRA-03: RAPID MALDI-TOF-MS-BASED PROTEOMICS APPROACH FOR RELIABLE DETECTION OF PDO FETA CHEESE ADULTERATION

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"Feta" is a Protected Designation of Origin (PDO) Greek cheese, ripened in brine, produced exclusively from pasteurized sheep's milk or sheep's and goat's milk (up to 30%). However, cow's milk is fraudulently mixed with sheep's milk during the manufacture. Due to its high economic impact, fraud control is therefore vital. A range of analytical methods to detect frauds have been developed, modified, and continually reassessed to be one step ahead of manufacturers who pursue these illegal activities. To assess the authenticity of dairy products and defend consumers' health, a European Reference Methodology (ERM) was established to detect cow proteins in dairy products, based on gel isoelectric focusing (IEF) technique. In the present work, a fast and sensitive MALDI-TOF-MS-based method has been developed for the detection of feta cheese adulteration. Exploiting the total intact protein profile (caseins and whey proteins), an integrated proteomics-based workflow has been elaborated for the detection of the potential feta cheese adulteration with cow's milk. Statistical treatment using advanced chemometric tools, such as unsupervised principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) recognition techniques were utilized for the discrimination/classification of the cheese samples.

PRA-05: POTENTIAL OF TIMS COMBINED WITH LC-HRMS IN FOOD AUTHENTICITY STUDIES: IDENTIFICATION AND CHARACTERIZATION OF SECOIRIDOIDS ISOMERS IN GREEK OLIVE OIL

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Over the last decade, great effort has been put in the field of food authenticity in order to achieve both quality assessment and further characterization of the products. Olive oil, in particular, has recently been found on the frontline of scientific research due to its particular health-promoting effects. The European Food Safety Authority (EFSA) have set the standards for thorough investigation of bioactive compounds Ssecoiridoids is one of the most important class of bioactive compounds in olive oil. They are part of the olive oil phenolic fraction that is also comprised of phenolic acids, phenyl ethyl alcohols, hydroxy-isochromans, flavonoids and lignans. The challenging part in secoiridoids study remains the identification and separation of isomeric compounds already reported but yet to be characterized. A novel method based on Trapped Ion Mobility Spectrometry (TIMS) was applied. The use of ion mobility enhanced the identification providing detailed examination of selected compounds utilizing precise collision cross section (CCS) data. EVOOs of different variety and geographical origin were analysed in order to study differentiations among the samples.

PRA-07: Quantitative Proteomic Profiles generated by non-targeted Liquid Chromatography Tandem Mass Spectrometry distinguishes different Truffle Species

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Truffles of the *Tuber* species as expensive foods are predestined for food fraud. They can be intentionally mislabeled or high quality products can be mixed with inferior product. To distinguish different truffle species, the proteomes as phenotype determining basis were investigated. Here, protein extraction and sample preparation of truffle was tested for non-targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) and then applied to generate quantitative proteomic profiles of different species by which they could be distinguished.

Homogenates from different truffle species were obtained by lyophilization and grinding with a bead mill. Different established in solution bottom-up proteomic sample preparation approaches were applied to one truffle sample. The homogenates were incubated with different detergents/chaotropes for protein extraction. Protein extracts were enzymatically digested by trypsin in solution or with the filter aided sample preparation (FASP) approach. Peptides were analyzed by LC-MS/MS in data-dependent acquisition (DDA) mode on a quadrupole-orbitrap instrument. Identification rates and quantitative reproducibility were compared. The most convenient protein extraction and tryptic digestion from ground truffle was achieved with the sodium deoxycholate (SDC) protocol. This protocol was applied to samples of *Tuber magnatum*, *Tuber melanosporum* and *Tuber aestivum*/*Tuber ucinatum* and by DDA-LC-MS/MS measurements quantitative protein abundance profiles were generated.

By hierarchical clustering and principal component analysis utilizing the generated protein abundance profiles the different truffle species could be well distinguished from each other. The co-specificity of *T. aestivum* and *T. ucinatum*, proven by genomic approaches, could be confirmed on protein basis.

Our work indicates that quantitative proteomic approaches are a powerful tool for distinguishing different truffle species and therefore can be further implemented as a helpful tool in the prevention of food fraud.

PRA-09: Exploring post-translational modification dynamics during dark-light transition in *Arabidopsis thaliana* leaves

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Diurnal dark-light transitions cause fundamental physiological changes in plant metabolism, which are unique to them. In dark-adapted plants mitochondrial respiration is the main energy source. However, during dawn photosynthesis is taking over as primary energy source in autotrophic tissue. Then, starch and sucrose is produced during the day. At the same time, nitrate assimilation and photorespiration starts and the tricarboxylic acid cycle alters its flux supporting nitrogen assimilation and redox balance. These changes affect all green tissues.

As there are already several key metabolic enzymes known to be regulated by post-translational modifications, such as pyruvate dehydrogenase and nitrate reductase, we hypothesize that an important part of this metabolic transition is controlled by post-translational modifications, such as phosphorylation, lysine acetylation, and changes in cysteine redox states.

In this study, we aim to understand these changes from a systems view by monitoring phosphorylation events at several time points around dawn.

PRA-11: COMPLIANT-READY WORKFLOW FOR MASS CONFIRMATION OF OLIGONUCLEOTIDES AND RELATED IMPURITIES

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Advances in the biology, stability and delivery of oligonucleotides have led to a resurgence in the development of oligonucleotide-based therapies. With these advances comes an increasing desire to process oligonucleotide mass spectrometry data and a need for informatics development to enable compliance-ready data capture, processing and reporting, in support of regulatory filings for current and future drug candidates.

Recent improvements to our UNIFI software allow for the mass confirmation of oligonucleotides and their related impurities in a compliance-ready manner, thus supporting GMP development, manufacturing, QC release testing and associated regulatory filings.

The results of these improvements allow the user to specify ‘Mass Only’ target masses and to define and specify custom modifications. It also allows selecting regions of interest using retention time windows. A choice of deconvolution algorithms have been made available so oligos can be processed more effectively based on the mass range.

PRA-13: Mass spectrometry towards more native samples and cells

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Native mass spectrometry is used worldwide for the analysis of proteins and protein complexes. Really native conditions for membrane proteins require a lipid environment, which mimics the cell membrane as closely as possible. However, proteoliposomes or cells are still very difficult to handle in mass spectrometry. It is difficult here to maintain the sample properties, during the transfer from solution to gas phase. The Laser-Induced-Liquid-Bead-Ion-Desorption (LILBID) ionization is a suitable method which we want to employ for this challenge. LILBID is based on the release of protein complexes from the solution phase by infrared laser desorption of a droplet. The MS analysis of membrane proteins is established with LILBID for

detergents and nanodiscs. In this work we want to show that we are on the way to analyze proteins in liposomes and in cells, which corresponds to the natural environment.

For LILBID a piezo-driven droplet generator produces 30 µm droplets with a frequency of 10 Hz. These droplets are transferred into vacuum and irradiated by an IR laser pulse tuned to the absorption wavelength of water (2.94 µm). This leads to the explosive expansion of the droplets, releasing the solvated ions, which can then be mass-analyzed with a reflectron-time-of-flight (TOF) mass spectrometer.

With LILBID-MS we are able to measure biological samples under native conditions. Here we show first results of membrane proteins measured from liposomes or native membranes (ghosts), as well as soluble proteins directly from cells. We could show that it is possible to measure hemoglobin from intact erythrocytes, WT-SRSF3-GFP from Hela cells and DgK in liposomes. Excitingly we can release proteins from these environments without dissociating non-covalent bonds. For example, we observed a high abundance of the expected heterotetramer of hemoglobin in erythrocytes as well as the expected DgK trimer in Liposomes. This will be an important step in the mass spectrometric investigation of proteins in their native environment.

PRA-15: High Sensitivity Phosphoproteomics using PASEF on a TIMS-QTOF mass spectrometer

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High resolution MS-based proteomics is powerful to study signal transduction pathways. Sensitivity, sequencing speed and peak capacity are prerequisites for deep identification and quantification into the phosphoproteome. Here, we present trapped ion mobility QTOF spectrometry (TIMS) using the parallel accumulation –serial fragmentation (PASEF) acquisition mode for deep phosphoproteomics analysis at record acquisition speeds. Using this approach in the analysis of primary hippocampal neurons has enabled us to obtain comprehensive data from his dynamic and complex phosphoproteome. Whole cell lysate of primary cultured hippocampal neurons (DIV 21, 5 million cells in total) was proteolytically digested in a modified FASP protocol. Phosphopeptides were enriched from the desalted peptides using TiO2-and Fe-NTA-based affinity purification strategies. Phosphopeptides enriched were nano-HPLC separated (nanoElute, Bruker) on a 250 mm pulled column (IonOpticks, Australia) and analyzed on a timsTOF Pro (Bruker) using the PASEF acquisition method. . Data were analyzed using PEAKS 8.5 (Bioinformatics solution Inc.) and MaxQuant (Jürgen Cox, MPI Martinsried). PASEF on a timsTOFPro instruments offers the possibility to investigate samples to an unprecedented depth. Sensitive PTM analysis to investigate signal transduction pathways. High-throughput PASEF enables deep proteomics analysis on short gradients.

PRA-17: Elucidation of pathomechanisms of renal Fanconi-syndromes

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Renal Fanconi-syndromes are caused by a dysfunction of the proximal tubulus of the kidney, thus leading to aminoaciduria, glucosuria, phosphaturia, small molecular weight proteinuria, and metabolic acidosis. In the presented studies new, autosomal dominantly inherited forms of renal Fanconi-syndromes were investigated.

Through the combination of proteomic, metabolomic and biochemical approaches, the underlying molecular mechanism of these diseases were elucidated. In the first studied Fanconi-syndrome the mutation of the *EHHADH* gene coding for a peroxisomal protein leads to mislocalization of the protein to mitochondria, thereby impairing mitochondrial fatty acid oxidation and energy production. The diminished energy supply consequently reduces transport efficiency of the proximal tubular cells resulting in the renal Fanconi-syndrome (Klootwijk et al., 2014, N Engl J Med; Assmann et al., 2016, Cell Rep). The second Fanconi-syndrome is caused by mutations of *GATM* inducing oligomerization of the protein within the mitochondria. These oligomers are protected against proteolytic degradation, thus forming fibril-like structures gives rise to impaired mitochondrial fission and mitochondrial dysfunction. This I turn leads to increased cell death resulting in the Fanconi-syndrome and subsequently to kidney failure (Reichold et al., 2018, J Am Soc Nephrol).

Only the combination of various different analytical approaches facilitated the clarification of the molecular mechanisms underlying these pathogeneses.

PRA-19: Quantitative, Tandem-Mass-Tag (TMT)-based LC-MS/MS proteome analysis of FFPE Medulloblastoma tissue reveals new, orthogonally confirmable, molecular signatures for different cancer subtypes

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Medulloblastomas (MB) are predominantly pediatric WHO grade 4 tumors, that are genetically divided into the 4 subgroups: WNT, SHH, Group 3 and Group 4, associated with different molecular pathway aberrations, clinical outcome and oncogenic drivers. MB lack effective target-specific therapies, while surgery, chemo- and radiotherapy often lead to damage in the developing brain. To further understand different MB types and to identify subtype-specific biomarkers and therapeutic targets, proteomic analysis is indispensable as the proteome reflects the addressable phenotype of a disease. Large proteomic studies

on MB are limited by the low availability of fresh frozen tissue. FFPE tissue sections of rare tumor subtypes can be provided in a quantity, that enables reliable statistical analysis but possesses challenges to Mass spectrometry.

Here, we analyzed 43 micro dissected FFPE MB tissue sections, by Tandem-Mass-Tag (TMT) based, quantitative LC-MS^3 analysis using an Orbitrap Fusion ™ Tribrid ™ mass spectrometer subsequent to high-pH-uHPLC-fractionation. A new data analysis and processing pipeline was developed to identify aberrant, quantitative molecular profiles in different MB subtypes. The generated workflow was additionally applied to TMT LC-MS/MS^3 raw data on 45 fresh frozen MB tissue samples obtained from the PRIDE Database. Proteomics data from fresh frozen tissue as well as transcriptomic data on 763 MBs, obtained from the R2 database, were used to corroborate the biological findings of this study.

In total, we quantified 14331 peptides, assigned to 2644 protein groups from FFPE tissue. A distinct separation of all 4 MB subtypes at the protein level was revealed by principle component analysis, based on FFPE and FF tissue. These findings prove the suitability of the method for the differential characterization of MB. Furthermore, the aptitude of both tissue types was demonstrated. In total, 436 proteins, significantly differential abundant among the groups were identified from FFPE samples by ANOVA testing at FDR<0-05. Form these group-specific molecular signatures, orthogonally confirmable biomarker panels were generated. In addition, so far unknown molecular pathway aberrations for MB subgroups, that can potentially improve subtype-specific MB therapy, were disclosed.

PRA-21: Analysis of temporal changes in the plasma proteome of adult and neonatal mice in response to cytomegalovirus infection

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The cytomegalovirus (CMV) belongs to the Herpesviridae DNA virus family. In healthy individuals, a CMV infection only cases mild flu-like symptoms is commonly unnoticed. In Immune-deprived individuals, however, suffer from severe infections which can affect vision, digestive system (colitis, esophagitis, or hepatitis), nervous system or the lungs (pneumonia). Infections in new-born or infection during pregnancy, lead to severe developmental impairments including premature birth, poor liver function, seizures, pneumonia, and abnormally small heads. Therefore, we investigated in a mouse model, the effect of murine CMV (MCMV) infection on the plasma proteome of adult with and without immune impairments, and in neonates in a time dependent manor.

Plasma samples from adult and neonatal mice (164) were collected and analyzed using the Tandem Mass Tag (TMT) strategy of trypsin digested proteins. A master pool consisting of all samples (TMT batch normalization) was generated. Samples were randomized to have an even representation of neonatal and adult plasma samples per batch. After labelling, samples per batch were combined, fractionated by basic reverse phase chromatography and analyzed by a synchronous precursor selection MS3 method on a Fusion mass spectrometer. MaxQuant was used for database searches and quantification, statistical analysis was performed with Perseus.

Data processing revealed about 580 proteins identified and quantified in at least one sample. Principal component analysis (PCA) of proteins present in all samples (~200) demonstrated in the first component a clear difference of the plasma proteome purely based on age of the mice and only in the second component based on infection. Time-dependent sampling of mouse plasma from day of birth to adulthood were analyzed. Several proteins showed time-dependent correlation in abundance either increasing from birth to adulthood or decreasing. Some proteins only changed after entering puberty/adolescence. Others were low at birth increased during growth period and decreased again towards puberty/adolescence.
Effects of MCMV infection were seen in the adult and neonatal groups with some protein abundace changes shared among the group but also specific changes unique to each group. Further, the adult mouse group with impaired immune systems had unique changes in the plasma proteome which were not observed in the otherwise healthy adult group nor the neonatal group.

In conclusion, TMT based protein quantification revealed substantial differences in the proteome of adult and neonatal plasma samples independent of MCMV infection but also demonstrated shared and unique features for the MCMV infection response, indicative of triggered processes.

PRA-23: Modified hippocampal lipid signaling pathways triggered by lifestyle conditions: a multi-omics perspective

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Environmental enrichment (EE) elicits various positive effects on the brain at the molecular, anatomical and behavioral levels including the induction of neural plasticity and enhanced learning and memory. Nevertheless, little is known about the molecular processes involving lipids and proteins of the hippocampal region of the EE animals. The synaptic cleft, as central part of the pre- and post-synaptic junction, is the subcellular location where cell-to-cell communication and signal transduction is mediated. Simultaneous multi-omics extraction and analysis are highly informative technics to probe the interplay of proteins and lipids in neuronal signal transduction. Therefore, we employed the SIMPLEX sample preparation workflow, consisting of a simultaneous MTBE-based extraction of lipid, proteins and metabolites, as a sensitive explorative approach to screen for molecular components of the synaptic cleft. Structural lipids were determined by shotgun lipidomics (HR-MS/MS) while endocannabinoids and proteins were analyzed by nanoLC/ESI HR-MS/MS.

In this study, the molecular inventory of the cleft is accessed and a novel mechanism of reduced endocannabinoid signaling by environmental stimuli was identified. Endocannabinoid signaling was linked to increased surface expression of AMPA receptors, which most likely regulates alterations in synaptic plasticity of mice exposed to an enriched environment.

PRA-25: Targeted Absolute Quantification of Lysosomal Proteins by Multiple Reaction Monitoring and QConCAT Protein Standards

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The absolute quantification of proteins requires externally quantified, stable isotope-labeled peptides, which are spiked into the biological sample. Beside the chemical synthesis of peptides, full length standard proteins can be expressed in bacteria, mammalian cells, or cell free expression systems. With the Quantification Concatamers (QConCAT) strategy, a larger number of internal standard peptides can be generated as an equimolar mixture from one artificial proteolytically cleavable fusion protein. We applied the QConCAT approach for the targeted absolute quantification of the majority of known lysosomal proteins from mouse cells/tissues by Multiple Reaction Monitoring (MRM).

After defining a list comprising a total of 153 verified lysosomal proteins, we selected possible surrogate peptides for these proteins from in-house and public large-scale DDA datasets. This resulted in ~6300 peptides which were evaluated based on their suitability to act as internal standards. Out of these candidates, we manually chose 422 unique tryptic peptides representing 143 lysosomal proteins (if possible 3 peptides/protein). The selected peptides were grouped in 12 fusion proteins (QConCATs) based on their abundance and tryptic cleavage properties, and to each QConCAT a peptide for external re-quantification was added. After optimizing the conditions for bacterial expression and His-tag purification, we evaluated different approaches for tryptic digestion including: SP3, in-solution digestion using 1M GCl or 0,1% RapiGest, Filter Aided Sample Preparation, and in-gel digestion. We analyzed the digests using either Orbitrap Velos/Fusion Lumos or QTrap5500/6500+ mass spectrometers. In-gel digestion and in-solution digestion using RapiGest resulted in lowest missed cleavage rates and highest protein coverage.

Subsequently, we generated spectral libraries and determined peptide-specific retention times using Skyline. We defined six transitions per peptide (three for each, the heavy and the light peptide) and optimized the MRM assay as well as the mixing ratio between the heavy amino acid-labeled internal standard peptides and the endogenous sample peptides. The final assay covers 398 peptides within four runs of 60 minutes gradient time, each in the scheduled MRM mode representing 143 lysosomal proteins. We applied this MRM assay to tryptic digests of whole cell lysates from mouse embryonic fibroblasts as well as nine different organs from C57BL6 mice. For both datasets, we determined absolute protein quantities for lysosomal proteins observing a large dynamic range in copy numbers per cell/µg tissue of up to 2000-fold. These datasets represent the first absolute quantitative study of the quantifiable proteome of a whole organelle, the lysosome.

PRA-27: Proteomics analysis of symbiosis efficiency of pea plant (*Pisum sativum L.)* genotypes.

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Legumes play an essential role in nitrogen cycle and constitute the major source of nutrition for humans. Therefore, improving the efficiency of the symbiotic relationships of such crops with soil microorganisms _ arbuscular mycorrhiza fungi (AMF) and rhizobia (Rh) is of high importance. Based on a previous field study, contrasting genotypes of pea (*Pisum sativum*) plants in terms of efficiency of interaction with soil microorganism (EIBSM) were identified - low EIBSM (K-3358) and high EIBSM (K-8274). Obviously, the information on the changes in the levels of root proteins can provide valuable formation for a better understanding the molecular basis of the legume-rhizobial symbiosis. In this study, the symbiosis-related alterations in root proteome were assessed by nanoHPLC-ESI-LIT-Orbitrap-MS-based bottom-up proteomics after the two genotypes were inoculated individually with Rh, with combination of AMF with Rh, provided with full mineral nutrition (positive control) and untreated soil as a negative control. In total 489 differentially expressed proteins were identified in both genotypes. The proteins were involved in different cellular functions like primary metabolism, protein synthesis and stress response. high EIBSM (K-8274) genotype responded strongly to combined inoculation by up-regulation of proteins associated with primary metabolism and protein synthesis and down-regulation of proteins involved in stress response. However, the low-EIBSM (K-3358) genotype showed more sensitivity to inoculation with Rh in comparison to the high-EIBSM genotype with same treatment, in which, the low-EIBSM genotype showed up-regulation of secondary metabolism and stress related proteins. In the high-EIBSM genotype only one differentially expressed protein was identified. Moreover, both genotypes showed similar response to application to full mineral nutrition by up-regulating of the proteins involved in detoxification and response to abiotic stimuli. Here, we propose that the high-EIBSM line has already established mechanisms for fast recognition and adopting symbiosis relationship with soil microorganisms in contrast to low-EIBSM which respond to soil microorganism by up-regulation of stress and immunity related proteins.

PRA-29: Proteolytic processing of urotensin-II and urotensin-II related peptide and identification of corresponding circulating enzymes

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Urotensin-II (U-II) and urotensin-II related peptide (URP) are cyclic peptide hormones, that are mainly present in the cardiovascular and neurosecretory system. Human U-II and URP consist of 11 and 8 amino acids, respectively, both containing a highly conserved cyclic hexapeptide on their C-terminus, responsible for activation of the Urotensin-II receptor and biological activity. U-II and URP cause strong vasoactive actions and are correlated with various pathological disorders, such as hypertension, atherosclerosis and heart failure. U-II and URP lead to strong vasoconstriction and therefore – in analogy to the peptide hormone Angiotensin-II – the corresponding U-II/URP-generating enzymes have high potential as drug targets for the treatment of cardiovascular diseases like hypertension. However, the proteolytic processing of U-II and URP has not been fully studied. Both peptides are formed through proteolytic cleavage at di- or tribasic proteolysis sites from larger precursors with differing length and sequence resulting in the active forms comprising the completely conserved cyclic hexapeptide. Little is known about the proteases responsible for the release of the active U-II and URP. The plasma fraction Cohn-fraction IV-4 was identified before as suitable biological source exhibiting strong U-II-generating activity using a mass spectrometry based enzyme screening assay (MES assay). Using the MES assay, the conversion of N-terminally elongated pro-forms of U-II and URP to their active sequences was monitored qualitatively and quantitatively by MALDI-TOF- and LC-ESI-TOF-MS. By means of a combination of the MES assay and chromatographic fractionation, an enrichment of the U-II-generating activity within the Cohn-Fraction IV-4 was performed. Protein identification of the most active fractions via LC-MS/MS led to the identification of protease candidates, whose U-II/URP-generating activity was investigated and validated. Further inhibition experiments were used to determine the physiological relevance of the most active protease.

PRA-31: Validation of a filter aided sample preparation (FASP)-based label-free quantification approach for proteomics analysis ofplant tissues

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Plant proteomics engages two main approaches to address the challenge of protein isolation. On the one hand, phenol extraction represents a powerful technique, applicable to diverse biological materials. On the other, protein isolation can rely on treatments with detergent solutions. Quantitative proteolysis of such isolates, containing even hardly soluble plant proteins, can be accomplished by filter-aided sample preparation (FASP). This method represents a common digestion approach of peptide generation for LC-MS analysis allowing sequential concentration of samples, detergent removal, buffer exchange, reduction, alkylation and enzymatic digestion of proteins in one filter device. However, integration of FASP with protein extraction techniques still requires optimization for plant samples. Therefore, here, we address a potential of these two protein isolation protocols in combination with FASP for quantification of *Arabidopsis thaliana* leaf and pea (*Pisum sativum* L.) seed proteins by LC-MS-based bottom-up proteomics.

The proteins were extracted from plant tissues by phenol extraction, and dry protein pellets were reconstituted in 10% (*w/v*) SDS solution. Then, the aliquots of pea seed protein were spiked with bovine serum albumin (BSA) in the following concentrations at 3.125, 6.25, 12.5, 25, 50, 100 % (w/w). The resulting protein mixtures were digested by the FASP approach and desalted by solid phase extraction (SPE). The analysis relied on nanoHPLC-ESI-LIT-Orbitrap MS. Identification of peptides relied on Proteome Discoverer software. The linearity of the sample preparation methods was assessed for six proteotypic BSA peptides by Xcalibur software. The method delivered acceptable linear correlation (>0.9) for relative abundance of individual peptide signals and contents of spiked BSA in samples.

Further, we compared the linearity of two protein isolation methods – phenol extraction and treatment with lysis solution followed by incubation at 95°C. For this, pea seed powder was mixed with Arabidopsis leaf material in the following ratios: 9:1, 3:1, 1:1, 1:3, 1:9, 0:1. Then samples were subjected to phenol extraction or lysis approach. Digestion, pre-cleaning and analysis were performed as described above. The linearity of protein quantification was assessed for three proteins with two proteotypic peptides each by Xcalibur software. Our results showed, that phenolic extraction gave access to better linearity for quantification of Arabidopsis leaf proteins in comparison to the lysis approach which in turn is less time-consuming. Therefore, both protein isolation methods can be considered as applicable for FASP and subsequent MS-analysis.

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Proteome Research - Clinical Applications

PRC-01: Comparison of Targeted Proteomics Approaches on a TIMS-Q-TOF

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Targeted quantitative acquisition methods aim at accurately quantify protein abundances in large set of samples without missing values. It is the method of choice to verify and validate protein biomarker candidates in large sample cohorts. We evaluated the potential of the Trapped Ion Mobility Separation (TIMS) – QTOF platform. and compared the PASEF (Parallel Accumulation Serial Fragmentation) acquisition method, which allows the DDA MS/MSat very high speed (> 100 Hz) with a novel targeted acquisition TIMS-PRM mode. All samples and controls were separated by nano- HPLC (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) with a 60 min gradient and analyzed on a timsTOF Pro (Bruker). The timsTOF was operated in PASEF and TIMS PRM modes. Post-processing analysis was performed with Data Analysis™, PeakX and Skylinedaily™. TIMS-Q-TOF have a strong potential for target proteomics due to an exclusive combination of selectivity, sensitivity and multiplexing. The complementary acquisition modes provide solutions to a variety of analytical challenges. The excellent selectivity and sensitivity obtained by tims-PRM can further be improved by optimizing the acquisition parameters (trapping, collision). Enabling PASEF-PRM will allow targeting 10x more peptides in the same time frame as before.

PRC-03: Molecular signatures of Neuroblastoma by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)

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Objective: Neuroblastoma (NB) is the most common malignant solid tumor in infants and childhood, it derives from neural crest cells of sympaticoadrenal lineage and can develop anywhere in the sympathetic nervous system. The mortality of all pediatric cancers is around 15% on average. The clinical behavior and treatment regimen depend significantly on the pretreatment classification of the tumor subtypes. This is why patients with an high-risk NB subtype (HR) following the international neuroblastoma risk group classification System (INRGCS) have an estimated 5-year survival of less than 50%, whereas low-risk (LR) group has a >85% survival. Usually used histopathological assessment of high risk NB are limited to predict diseases progress and treatment response. Consequently, the untagged technology is useful to explore spatial molecular alterations of high-risk NB, to improve the understanding internal tumor heterogeneity and to develop patient stratification marker. In this study, we used untargeted imaging mass spectrometry (IMS) to investigate the spatial proteomic alterations in 12 human neuroblastoma whole tumor section (6 initial HR, 4 initial LR and 2 primary HR tumors after chemotherapy). This MALDI Imaging analysis results in 205 significant discriminative peptide values (AUC>0.7 or <0.3, p<0.001) and peptide signatures (Components) between HR and LR tumor regions. Using complementary bottom up mass spectrometry 13 proteins were identified which distinguished HR and LR tumor regions. These proteins show also alterations in consequences of chemotherapy.

Conclusion: Our proof of concept study demonstrates that MALDI Imaging shows a high potential to determine peptide alteration in NB, which discriminates between HR and LR tumors. Moreover, we could demonstrate that this technology enables the monitoring of peptide alternation regarding the therapeutic intervention and plays an important role in the search for new proteomic markers of NB for diagnostic and therapeutic target.

PRC-05: Robust and efficient workflow for characterization of clinical biopsy samples by MALDI imaging and MS-proteomics

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Introduction: Deep diagnostics of clinical samples requires state-of-the-art methods and smart analytical strategy. They are expected to provide comprehensive and versatile information, be fast, unexpansive and available on-site. MS-Facilities in cooperation with clinical and research units within the Dresdner Campus established robust and efficient workflow for characterization of clinical tissue samples from FFPE/ FROZEN collections as well as for ongoing cancer projects. It comprises MALDI imaging and MS-proteomics, and is complemented by clinical data.

Experimental details: 20µm biopsy sections of the same sample were used for MALDI Imaging (MSI) and proteomics analysis. MSI was performed on a Rapiflex MALDI Tissuetyper (Brucker Daltonics) using standard protocol. Sample preparation for proteomics analysis included one-step MTBE paraffin removal, beads-assisted tissue desintegration, protein solubilization with SDS, Isopropanol protein precipitation and on-pellet enzymatic digestion. LC-MS/MS analysis was performed on a Q Exactive HF Orbitrap mass specrometer (Thermo Fischer Scientific). Optionally, peptide mixtures were fractionated prior ms-analysis (Stage Tips, Thermo Fischer Scientific). To control the efficiency of sample preparation, fixed amount of BSA was spiked into each sample. For comparison, sample preparation was performed using FFPE FASP protein Digestion Kit (Expedion). Spectra were processed by standard proteomics software packages (MaxQuant, Scaffold); comparative analysis was performed by in-house bioinformatics pipeline.

Results and Discussion: The workflow was tested for FFPE and also H&E-stained biopsys (lymph nods). Applied first, MSI allowed quick screening of clinical biopsy collections and selection of samples for in-depth proteomics characterization. Proteomics analysis identified more than 3000 proteins in unfractionated samples using single tissue sections. The obtained protein dataset represented all celluar compartents, also including membrane-associated proteins. Only 20% of the digests were used for proteomics, the remaining material can be used for technical repeats, targeted and quantitative analysis. The number of detected proteins can be further increased by combining protein material from several sections and peptide fractionation. Our proteomics sample preparation is fast, easy and cost-efficient. In comparison with standard FASP sample preparation method, our results showed less human protein background. Another agvantage is that our sample prpration does not require special skills, kits or equipment and can be performed dirctly in an average clinical lab. Digested samples can be then storied frozen and submitted for proteomics analysis any time later if needed.

The method can be extended to fresh and frozen clinical tissue samples and inclose also lipidomics analysis.

Structural and Functional Proteomics

PRS-01: Studying the Interactions between the C-Terminal Domain of the Tumor Suppressor p53 with S100ß and Human Sirtuins

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The tumor suppressor p53, known as the “guardian of the genome,” plays a significant role in DNA repair, cell cycle control, and apoptosis in human cells. Gaining insights to the full-length, wild-type three-dimensional structure of p53 upon binding to different proteins proves to be challenging due to p53’s flexibility of its intrinsically disordered regions at the N- and C-termini. With the lack of a stable structure in solution, the conformation of an intrinsically disordered protein (IDP) is difficult to characterize. A promising approach is through chemical cross-linking combined with mass spectrometry (XL-MS) as no crystallization is required and low protein concentration is sufficient to obtain structural information.

The regulatory domain located on the C-terminus of p53 plays an important role in transcriptional activity. We are interested in studying the binding of S100ß and human sirtuins to p53. They are known to bind specifically to the regulatory domain and inhibit the transcriptional activity of p53. IDPs often undergo a disorder-to-order transition upon binding. By applying geometric constraints between p53 and its interactors, S100ß and human sirtuins, via XL-MS, these protein-protein interactions can be further investigated and more details on conformational changes in p53 will be gained.

PRS-03: Streamlining mAb Characterization with a PASEF Based DisulfideAnalysis Workflow

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Covalent disulfidebonds (DSBs) define the structure, function and stability of biotherapeutic proteins, such as monoclonal antibodies (mAbs). A common approach to elucidate them are tryptic digests under native and reduced conditions and to awkwardly compare those with significant manual validation. In this work we introduce a combination of improvements for the near-automatic mapping of DSBs. Adalimumab was incubated for 3 h at 37°C in the presence of 5.8 M Guaand 7 mMNEM, diluted 1-50 in digestion buffer and trypsin/LysC(Promega) was then added for an overnight digest. Scrambling of Adalimumab DSBs was initiated by 10 or 30 min incubation at 70 °C before denaturation and digestion. Peptides were separated on a 150 x 2.1 mm 1.7 µm C18 CSH column (Waters) using an Elute UHPLC coupled to a timsTOF Pro (Bruker). Datasets were analysed using Byossoftware (Protein Metrics) built-in disulfidebond workflow. The described approach yielded 98 % mAb sequence coverage from a single digest with PASEF analysis. Byos software confirmed the native DSBs in both mAbs and permitted the detection and identification of scrambled DSB-peptides down to < 0.01 % levels. Data analysis required 5 min operator time and ~2h computer time for searches, quantification and report generation. A single digest of the native protein was sufficient to provide a comprehensive analysis of disulfide structure of antibodies including the scrambled disulfide peptides.

PRS-05: Structural Characterization of the Interaction between the Full-Length Human Tumor Suppressor p53 and DNA by Native MS and Cross-linking/Mass Spectrometry

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The tumor suppressor p53 is a tetrameric, multidomain transcription factor that plays a central role in the cell cycle and maintaining genomic integrity. It binds to specific DNA response elements, is integrated in various signalling networks by a multitude of protein–protein interactions, and is controlled by extensive posttranslational modifications. p53 is a homotetramer, with each monomer consisting of two folded domains, namely the DNA-binding domain (DBD, aa 94–294) and the tetramerization domain (aa 323–360). In addition p53 contains four intrinsically disordered regions (IDRs), the N-terminal transactivation domain (aa 1–67), the proline-rich region (aa 67–94), the nuclear localization signal (NLS)-containing region (aa 303–323), and the C-terminal basic regulatory domain (aa 360–393).

The DBD binds to sequence-specific response elements associated with p53 target gene promoters. It specifically recognizes genes, which contain half-site regions and regulate their function and expression levels. The majority of mutations in p53 are located in the DBD and are responsible for cancer triggering. Even though different models describing the molecular recognition between tetrameric p53 and DNA have been proposed so far, the actual mode of p53/DNA interaction remains elusive. Therefore, we aim to exploit an integrated structural approach combining the complementary use of different MS techniques,

such as native MS and cross-linking/mass spectrometry (XL-MS) to gain structural insights into the p53/DNA interaction. For our experiments, we will use full length, human wild-type p53 as well as the ultrastable tetra-mutant of p53. XL-MS will initially be performed with the MS-cleavable cross-linker disuccinimidyl dibutyric urea (DSBU) that gives a characteristic signature in CID-MS/MS experiments. We will also incorporate photo-reactive, diazirine-containing amino acids to obtain a more complete coverage of cross-links in the N-terminal region of p53. XL-MS will be exploited to derive structural restraints to get insights into the recognition process between human p53 and specific DNA oligonucleotides sequences. Native MS will be employed to check the presence of monomeric and oligomeric forms of p53 and to verify p53's functionality via addition of specific DNA response elements.

PRS-07: Quantification of histone modifications in NaBu and RA treated CHO production cells by Orbitrap-MS after optimized derivatization and isotope labeling

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Histones play an important role as epigenetic regulators in gene expression. The complexity of posttranslational modifications (PTMs) predominantly of histone H3 and H4 can either lead to a condensed heterochromatin structure or to a more accessible euchromatin form. Acetylation of lysine or triple methylation at lysine of histone H3 in position 4 (H3K4me3) relate to high gene transcription whereas modifications like H3K27me3 or H3K9me3 are linked to gene silencing. In recent years approaches have been established and optimized for analysis of histone PTMs by means of high-resolution mass spectrometers. However, there are specific challenges in histone analysis because of the high density of lysine and arginine and a strong dynamics based on the high complexity of interactions. In a previous work a derivatization protocol with propionic anhydride was developed which leads to facilitated histone analysis via MS. Based on this protocol, we generated an optimized strategy for quantification of histone PTMs. After derivatization all unmodified and monomethylated lysines are propionylated. The resulting peptides can easily be separated during chromatography and monitored under MS. In a first approach we investigated the effect of two small inducers (NaBu and RA) on histone modifications of a CHO antibody producing cell line. CHO cells in suspension culture were treated with NaBu or RA. Cells were harvested between 30 min and 24 h after treatment and histones were extracted under acidic conditions. After propionic anhydride derivatization and digest, a labeling reaction with heavy or light MeOH was performed. Through measurement with nLC-ESI Orbitrap MS, also in PRM mode, we could generate ratios of up and down regulation of specific modifications related to effects on gene expression. In order to validate the MS detections, we also performed western blots. Our results illustrate the dynamics of the modifications with different effects of both inducers. As expected, under NaBu treatment there was a high increase of acetylation in context with high product gene expression but also crosstalk with H3K27me3 which showed an upregulation. In contrast, RA led to a decrease of heterochromatin associated modifications, e.g. H3K9me3 and H3K27me3, in relation with higher cell density. The treatment with both molecules resulted in a combination of higher cell density and productivity with a different distribution of histone modification. Proved as well suited in our specific research question, the adapted lab workflow and software evaluation is generally applicable to elucidate complex changes in histone modifications in other contexts.

PRS-09: Analysis of SNARE complex intermediates by structural mass spectrometry

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The SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex mediates signal transmission in neurons and is responsible for the fusion of synaptic vesicles with the presynaptic membrane, resulting in the release of neurotransmitters into the synaptic cleft. The ternary complex is formed by the vesicular Synaptobrevin-2 and presynaptic membrane-bound SNAP25 and Syntaxin-1A. SNAP25 contributes two alpha-helices, while Syntaxin-1A and Synaptobrevin-2 both contribute one alpha-helix forming a stable four-helix bundle in the fully assembled complex. Complex formation proceeds through interactions of a highly conserved sequence, the SNARE motif, present in each of the four helices. While SNARE proteins are well-structured in this four-helix bundle they are natively unstructured in the absence of interaction partners like other SNARE proteins or lipids.

We study the stepwise assembly of the SNARE complex by combining cross-linking and native mass spectrometry (MS). For this, we set out studying the individual, purified SNARE proteins. Cross-linking was performed using bis(sulfosuccinimidyl)suberate (BS3), which preferentially links lysine residues as well as serine, threonine and tyrosine residues to a minor extent. Gel electrophoresis and western blotting of isolated cross-linked proteins indicated the presence of oligomers. Following tryptic digestion, we analyzed the cross-linked peptides by liquid chromatography-coupled tandem MS (LC-MS/MS) to identify specific interactions. Verified cross-links are located across the entire protein sequences confirming their intrinsic disorder. Using native MS, we approved the presence of oligomers.

Following the same procedure, we further analyzed SNARE complex intermediates by incubating the individual proteins to form binary complexes. In contrast to the isolated proteins we could show that, in the presence of their physiological interaction partners, the proteins preferentially form SNARE complex intermediates. In particular, SNAP25 and Syntaxin-1A formed a complex with a 1:2 stoichiometry, imitating the four-helix bundle of the SNARE-complex by incorporating a second Syntaxin-1A molecule.

In conclusion, the combination of cross-linking and native MS is well-suited to study oligomerisation as well as complex formation and will help investigating the assembly of the full SNARE complex in future studies.

PRS-11: Protein Interaction Studies of IGF2BP1 by Chemical Crosslinking/Mass Spectrometry

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Crosslinking/mass spectrometry (XL-MS) in combination with affinity purification of proteins is a valuable tool to identify even weak or transient protein-protein interactions [1]. In this study we employed this approach to identify interaction partners of human wild-type IGF2BP1 (insulin-like growth factor 2 mRNA-binding protein 1) in comparison to its mutated version, possessing a lower RNA affinity due to mutations in all four KH (HNRNK homology) domains.

IGF2BPs present an oncogenic family of RNA-binding proteins that control mRNA transport, translation and turnover during development and in cancer cells [2]. IGF2BP1 has the most conserved 'oncogenic potential' of all three IGF2BPs. The protein enhances an 'aggressive' tumor cell phenotype largely by impairing the miRNA-directed downregulation of mRNAs [2].

Wild type IGF2BP1 and its mutant were expressed as SBP-Flag tagged proteins in the HEK-293T cancer cell line. After cell lysis and affinity pulldown, protein-protein interactions were fixed by chemical cross-linking using 1 mM DSBU [3] (disuccinimidyl dibutyric urea). Afterwards, proteins were eluted and proteolyzed with trypsin following the FASP (filter aided sample preparation) protocol. Samples were analyzed by LC/MS/MS using 180-min gradients on an U3000 nano-HPLC system coupled to a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific). Label-free quantification of proteins was done using Proteome Discoverer 2.4 and crosslinks were identified using MeroX 2.0 [4].

The combined affinity enrichment / XL-MS approach led to the identification of about 2000 proteins. The comparative quantification between the wild type IGF2BP1 and the KH mutant pull-downs allowed us to significantly assign a number of these proteins to be RNA-mediated interaction partners of IGF2BP1, thus potentially playing a role in oncogenesis. Moreover, from the obtained MS/MS data more than 300 unique cross-linking sites could be identified that allow to directly map protein interfaces.

[1] B. Häupl, C. H. Ihling, A. Sinz, *Journal of proteome research* **2016**, *15*, 3686-3699.

[2] S. Muller, N. Bley, M. Glass, B. Busch, V. Rousseau, D. Misiak, T. Fuchs, M. Lederer, S. Huttelmaier, *Nucleic acids research* **2018**, *46*, 6285-6303.

[3] M. Q. Mueller, F. Dreiocker, C. H. Ihling, M. Schaefer, A. Sinz, *Analytical Chemistry* **2010**, *82*, 6958-6968.

[4] M. Goetze, C. Iacobucci, C. H. Ihling, A. Sinz, *Anal Chem* **2019**.

Session 13: Proteomics - Structural and Functional Proteomics; Keynote: Richter

Time: Tuesday, 03/Mar/2020: 3:00pm - 3:30pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Carla Schmidt

RNP-ID generates a picture of the molecular interactions during shuttling and mRNP export

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SR proteins belong to a family of RNA-binding proteins (RBPs) with essential functions as regulators of splicing. Additionally, some family members shuttle continuously between nucleus and cytoplasm and act as export adapters for the main mRNA export receptor NXF1 (Müller-McNicoll, 2016; Botti, 2017). Shuttling of SR proteins is influenced by the extent of NXF1 interaction, methylation of the NXF1 interaction region, binding to different RNA classes and the phosphorylation state of their RS domain (Botti, 2017). Recruited NXF1 dimerizes with its co-factor NXT1 to form a binding platform for nuclear export via the nuclear pore complex.

To understand the regulation of selective mRNA export by SR proteins and NXF1 we developed an MS approach, based on the endogenous expression of GFP-tagged SR proteins, UV-crosslinking, differential RNase digest and stringent pulldowns. We used differential RNase digests followed by mass spectrometry to identify interaction partners of SR proteins that are bound in close proximity on the same short piece of RNA (150 nt). This method is termed RNP-ID. P19 or HeLa cells expressing GFP-tagged proteins were crosslinked with UV_{254nm} at 400 mJ/cm^2. Cell lysates were treated 5min with RNase I to partially digest RNAs and the RNP complexes were purified via magnetic beads. The beads were then subjected to a 30 min RNase T1/A treatment to elute all proteins bound to the bait via a short piece of RNA. Eluted proteins were further FASP digested with a Trypsin/LysC. Proteins remaining on the magnetic beads were eluted with a mixture from Trypsin/LysC. Peptides were purified according to the Stagetips protocol (Rappsilber et al.) or directly injected to the precolumn to capture more hydrophilic peptides. Mass spectrometry data undergoes extensive data mining on the different fractions using four approaches: 1) label free quantification to identify the interaction partners (MaxQuant, Cox et al.), 2) identification of modified peptides using de-novo sequencing and database searches for PTMs (Peaks7.0, Ma et al.), 3) identification of UV derived RNA crosslinks with Peaks7.0 (Panhale et al.) and 4) identification of disulfide peptides with a special Peaks7.0 based search strategy (Singh et al). Our workflow enabled us to devise the specific protein-protein and RNA-mediated interaction partners and identify associated proteins that might explain differences in PTMs and RNA binding. Also it allowed the quantitative comparison of RNPs between cell types or in different cellular conditions and the extraction of diverse parameters describing the composition, modification and dynamics of RNPs.

Session 14: Metabolomics; Keynote: Schmid

Time: Tuesday, 03/Mar/2020: 3:00pm - 3:30pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Anne Schnell
Session Chair: Julica Folberth

New Developments for Small Molecule Identification using Open Source Software

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The separation of complex samples with liquid chromatography (LC) and the detection with high-resolution mass spectrometry (HRMS) rapidly generates gigabytes of information in a single study. Multiple open source tools and workflows have been developed to provide a flexible selection of algorithms for data preprocessing, feature identification, and statistics. One popular software to process small molecule MS data is MZmine, which comes with a user-friendly graphical interface and links to other tools. Since its introduction and release on the GNPS web-platform, molecular networking (MN) has been used for the analysis of non-targeted MS data in various fields, including natural product discovery, microbiome, forensics, biomedical, and environmental research. Based on the principle that ions of two structurally related molecules often result in similar fragmentation patterns, molecular networking aligns the fragmentation spectra (MS^2) and creates a network of MS^2 similarity. In a second step, all experimental spectra are matched against the GNPS spectral reference libraries to yield structure annotations. However, during ionization, an analyte can form multiple ion species with different fragmentation patterns, resulting in separate redundant clusters in a MN. To address this challenge, we developed ion identity networking (IIN) in MZmine and applied the new workflow to 24 publicly available datasets of metabolomics studies.

The core idea is to bundle and reduce signals that originate from the same analyte into one descriptor. This is achieved by grouping of features (chromatographic peaks with a specific mass-to-charge ratio) based on peak shape correlation and subsequent annotation of possible adducts, multimers, and in-source fragments. The high correct annotation rate was successfully validated on a natural products mix by LC-MS with a post-column infusion of different salt solutions to control adduct formation. This verified that sodiated ions (e.g., [M+Na]+ and [2M+Na]+) usually fall into an MN cluster which is separated from ammonium adducts and protonated ion species due to dissimilar fragmentation. With additional connections between ions of the same analyte, the resulting molecular networks have higher identification densities and less unrelated single MS^2 spectra. For instance, ion identity molecular networking successfully connected different adducts as well as the free and conjugated forms of a variety of bile acids in a single networking cluster, which significantly reduced the data redundancy and effort needed to infer structure annotations from the network.

In conclusion, we present the collaborative effort and new developments of the MZmine project surrounding the identification of compounds.

Session 15: ICP-MS: Environment II; Keynote: Meermann

Time: Tuesday, 03/Mar/2020: 3:00pm - 3:30pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: David Clases

Metal based pollutant assessment via diatoms - new possibilities via automated single cell-ICP-ToF-MS

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Diatoms are located at the bottom of the food chain. Toxicological relevant metals can possibly accumulate within the food web and cause harmful effects. Diatoms are a test system in ecotoxicology. Toxicological effects weaken the growth of algae which is by default investigated by means of fluorescence detection. On basis of the expose concentration as well as obtained fluorescence data potential threshold exceedance in e.g. surface waters is assessed.

However, this approach does not allow for the determination of “real” accumulated metal concentration in diatoms. Common approaches are based on bulk analysis via e.g. ICP-MS, ICP-OES or AAS. But, biological variability is completely disregarded.

To tackle this problem, alternative approaches are highly needed. Within the last years, sp-ICP-MS for nanoparticle as well as single cell analysis turned out as a powerful technique to analyze metal contents as well as size distributions on broad size range. But, common ICP-MS systems do not allow for multi-element detection within single particle/cell events [1, 2]. Thus, simultaneous MS detection devices are needed - just recently, ICP-ToF-MS experienced a revival [3].

Within our previous work, we developed an automated sample introduction system based on a HPLC system on-line with single particle-ICP-MS, which allowed for ionic background separation and single algae analysis [4]. However, for unambiguous tracing several fingerprint elements and multielement analysis in single algae (diatoms) is needed. Thus, we coupled our previous setup on-line to ICP-ToF-MS. Test diatom species were exposed to test substances (Zn) as well as nanoparticles (FeNPs) [5].

The developed setup allowed for a fast, automated and multielement analysis in single diatoms. Furthermore, we combined our approach with multivariate data assessment - multielement detection of characteristic fingerprint elements allowed for an unambiguous diatom tracing. Clustering of diatoms according to metal exposure concentration levels was enabled. Our approach is a new potential tool in ecotoxicological testing.

References:

[1] W.-Y. Lau, K.-H. Chun, W.-T. Chan, J. Anal. At. Spectrom. 2017, 32, 807-815.

[2] K.-S. Ho, W.-T. Chan, J. Anal. At. Spectrom. 2010, 25, 1114-1122.

[3] O. Borovinskaya and M. Tanner, Multi-Element Detection of Single Nanoparticles with icp-TOF: Application Note, 2016, https://www.tofwerk.com/application/nanoparticles/).

[4] M. Von der Au, M. Schwinn, K. Kuhlmeier, C. Büchel, B. Meermann, Anal. Chim. Acta 2019, 1077, 87-94.

[5] M. Von der Au, O. Borovinskaya, L. Flamigni, K. Kuhlmeier, C. Büchel, B. Meermann, *submitted for publication.*

Session 16: Ions at Next Generation Lightsources; Keynote: Bari

Time: Tuesday, 03/Mar/2020: 3:00pm - 3:30pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Charlotte Uetrecht

Gas-phase biomolecules at advanced light sources

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The coupling of electrospray ionization sources with synchrotrons and free-electron lasers opens the way to the investigation of the electronic structure of biomolecular systems and of a fine description of their relaxation mechanisms in the vacuum ultraviolet and soft X-ray energy range. The wide-ranging photon energy available at the synchrotrons enables systematic studies of ionization and dissociation as a function of the photon energy. Inner-shell excitations provide a localized site of energy deposition. The extremely high photon flux and fs pulse duration offered by free-electron lasers allow studying the molecular properties in intense fields. Furthermore, using the assets of free-electron lasers in a pump-probe scheme enables the study of the dynamics of charge migration and charge transfer within gas-phase biomolecules.

In this talk, results of mass-spectrometric experiments at advanced light sources will be presented and thereby, for example, secondary damage processes, site-selective dissociation, the influence of photon intensity and molecule size will be discussed.

Session 13: Proteomics - Structural and Functional Proteomics; Oral 1: Piersimoni

Time: Tuesday, 03/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Carla Schmidt

Structural analysis of interaction between lecithin:cholesterol acyltransferase bound to the apolipoprotein A-I belt of high density lipoprotein particles

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Chemical crosslinking of proteins coupled with mass spectrometry (XL-MS) has become a major tool in integrative structural biology for elucidation conformations of protein complexes in solution. XL-MS provides distance constraints, relative subunit orientations, and can validate structures from computational or other structural biology methods.

We used XL-MS to refine the orientation of Lecithin:cholesterol acyltransferase (LCAT) bound to the apolipoprotein A-I (ApoA-I) belt of recombinant high density lipoprotein particles (rHDL). The LCAT-rHDL complex was covalently stabilized with the CID-cleavable crosslinker DC4 and analyzed on an Orbitrap Lumos Tribrid Mass Spectrometer. LCAT catalyzes cholesterol esterification on HDL and is activated by ApoA-I. Structural analysis of LCAT-rHDL interactions will provide a snapshot of a phospholipase engaged with its physiological target, clarifying how LCAT mutations influence the HDL interface in genetic diseases.

We observed 15 unique crosslinks between LCAT and ApoA-I, with most LCAT crosslinks occurring on a common hydrophobic surface proposed as the HDL interaction site. We also observed a crosslink between identical LCAT lysyl residues, suggesting that some LCAT-rHDL complexes have the corresponding LCAT surfaces facing each other. Hyperconnected lysyl residues, diagnostic for flexible domains, were also observed, suggesting some LCAT structural flexibility. However, most crosslinks are found in discrete regions of the ApoA-I belt on either side of the helix 4/6 double-belt segment, indicating the preferred LCAT binding site on HDL.

Crosslinking data reflects the dynamic nature of the LCAT–HDL complex which hampered high resolution crystallographic analysis, but the integration of XL-MS, with negative stain electron microscopy and hydrogen-deuterium exchange MS, allowed refinement of the LCAT–rHDL complex structure. They revealed that LCAT preferentially interacts at the edge of HDL in a manner consistent with direct interactions with ApoA-I helix 6 and with LCAT gaining access to lipids at the edge of the protein delimited lipid bilayer.

Session 14: Metabolomics; Oral 1: Fangmeyer

Time: Tuesday, 03/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Anne Schnell
Session Chair: Julica Folberth

Fast Online Separation and Identification of Electrochemically Generated Isomeric Phase-I Metabolites by means of Trapped Ion Mobility-Mass Spectrometry

Jens Fangmeyer, Simon Gereon Scheeren, Robin Schmid, Uwe Karst
University of Münster, Analaytical Chemistry

The hyphenation of electrochemistry and mass spectrometry (EC/MS) has emerged as a powerful tool in mimicking the phase-I metabolism of xenobiotics catalyzed by cytochrome P450 enzymes. Combined with high resolution mass spectrometry, EC enables identification of unknown metabolites and short-lived intermediates via accurate mass detection. Moreover, fragmentation experiments allow structural elucidation. The distinction between electrochemically generated isomers requires conventional LC separation techniques coming along with increased method complexity and prolonged analysis times. Trapped ion mobility spectrometry (TIMS) offers a time-efficient separation enabling fast analysis of short-lived metabolites after their generation.

Oxidative metabolites were generated in an electrochemical thin-layer cell equipped with a boron-doped diamond working electrode. In order to obtain three-dimensional mass voltammograms, the EC cell was coupled online to an ESI-TIMS-ToF-MS applying a potential ramp by an external potentiostat. Oxidation products were identified via accurate mass detection. In the same analysis run, mobilo voltammograms for each *m*/*z* were recorded to distinguish between isomeric metabolites. Additionally, MS/MS experiments were carried out for each mobilogram signal.

Identification of the different metabolites of the model compound metoprolol was achieved via accurate mass detection provided by mass voltammograms. TIMS separation revealed different isomeric hydroxylations taking place depending on the applied potential. Additionally, subsequent reactions of formed metabolites were detected occurring at higher potentials by overlaying different mobilo voltammograms revealing possible metabolic reaction pathways. MS/MS results after TIMS separation were in good accordance to previous HPLC based experiments. Therefore, the analysis time can be reduced by at least a factor of five.

In this talk the performance of this approach was investigated for global metabolomics studies. An automated processing for semi-quantitation and direct structural identification utilizing the MS/MS^{ALL} data has been is applied to human urine samples. Extending the FIA approach to the second stage of MS made reliable and accurate identification and reproducible quantitation of metabolites possible based on high-resolving MS and MS/MS data.

Session 15: ICP-MS: Environment II; Oral 1: Kautenburger

Time: Tuesday, 03/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: David Clases

Low level analysis for high level nuclear waste disposal: CE- and LC-ICP-MS as tools for analysing metal mobility in clay

Ralf Kautenburger[1], Kristina Brix[1], Sandra Baur[1], Christina Hein[2]
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Nowadays, there is a broad scientific consensus on the technical merits of the disposal of high-level nuclear waste (HLW) in deep and stable geological clay formations. Particularly, clay formations with a high sorption capacity for metal ions are considered as one part of the natural barrier of a future high-level nuclear waste disposal protecting mankind and nature from the possible impact of radionuclide contamination. A wide set of geochemical parameters can influence the mobility of radionuclides originated from a leakage in a waste disposal for example competing ions released from the clay by infiltration of percolating water, natural organic matter (NOM) as complex forming ligands, changes in temperature or pH-milieu of the aquifer. In this study trivalent europium(III) (homologue of americium(III)) uranium(VI), caesium(I) and iodide were used and their retention in Opalinus Clay in the presence or absence of NOM were studied. As methods, capillary electrophoresis hyphenated with inductively coupled plasma mass spectrometry (CE-ICP-MS) was used to study the complexation behaviour of Eu(III) and U(VI) with HA. The influence of metal concentration, the presence of competing cations from clay dissolution as well as cations from clay porewater on the complexation behaviour was analysed. For the sorption/desorption behaviour common batch experiments with clay mineral suspensions are performed, and in comparison a new developed setup of miniaturised clay column experiments (MCCE) with compacted clay was used to study the influence of NOM on the metal mobility in clay. Online coupling of the MCCE with ICP-MS leads to quantitative information on the elemental composition of the eluent directly after determination of the UV/Vis-active compounds in the diode array detector of the LC.

We would like to thank the Federal Ministry for Economic Affairs and Energy (BMWi), represented by the Project Management Agency Karlsruhe (PTKA) for funding (projects: 02E10196, 02E10991 and 02E11415D) and our project partners for the kind collaboration.

Session 16: Ions at Next Generation Lightsources; Oral 1: Marklund

Time: Tuesday, 03/Mar/2020: 3:30pm - 3:50pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Charlotte Uetrecht

Orienting electrosprayed protein complexes for single particle imaging using X-ray free electron lasers

Erik Gustav Marklund, Carl Caleman
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The emergence of free electron lasers (FEL) can transform many areas of the physical and life sciences: the ultrashort and -intense pulses of coherent light that they deliver can probe the nature of matter beyond the limits of previous generations of light sources. The purpose of the MS-SPIDOC project is to enable single particle imaging of protein structures, using X-ray FELs, by devising a sample-delivery system based on native mass spectrometry. Capitalising on the separating abilities of ion mobility mass spectrometry, we strive to determine the structures of challenging systems such as virus capsid assembly intermediates, which are nigh impossible to image using current techniques. Using theory and computations we have discovered that electrosprayed proteins can be oriented in-flight using strong electric fields, and demonstrated the utility of such field-orientation for single particle imaging. Our results show the existence of an “orientation window”, where field strengths are sufficiently high to achieve orientation while being moderate enough to not unfold the proteins. The interaction with a strong electric field can potentially be exploited for other applications too, such as the deliberate unfolding of proteins to probe their stability in mass spectrometry under different conditions analogous to collision-induced unfolding.

Invitation received from Charlotte Uetrecht for the "ions at next generation lightsources" session.

Session 13: Proteomics - Structural and Functional Proteomics; Oral 2: Singh

Time: Tuesday, 03/Mar/2020: 3:50pm - 4:10pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Carla Schmidt

First Draft of the Human Lysosomal Interactome by Cross-Linking MS Reveals Novel Interactions and Structures

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The lysosome, the main cellular organelle for the degradation of macromolecules and recycling of their building blocks, is essential for the correct function of the cell. This is demonstrated by the pathogenic relevance of lysosomal proteins: In humans, genetic diseases for almost all lysosomal hydrolases are known. These disorders are characterized by the accumulation of the enzymes' intermediate degradation products in the lysosome, and are therefore referred to as lysosomal storage diseases (LSDs). Furthermore, altered lysosomal function has been shown to play a role in other conditions such as Parkinson's, Alzheimer's, and cancer.

The lysosomal membrane acts as an interface between the organelle and the cytosol, and proteins located at this membrane are part of a cellular regulatory communication network, which responds to the metabolic state of the cell. Therefore, identification of these proteins is of high interest for a better understanding of metabolic signaling. To date, a number of studies have investigated the lysosomal proteome by mass spectrometry, asserting hundreds of proteins to have a lysosomal localization. However, no studies have been conducted so far, in which interacting partners of lysosomal proteins were investigated in an unbiased manner on a large scale.

In order to identify hitherto unknown lysosomal protein-protein interactions, we applied a cross-linking mass spectrometry approach. We utilized the amine reactive MS-cleavable cross-linker DSSO to fractions, which were enriched for lysosomes using superparamagnetic iron oxide nanoparticles. In order to achieve optimal performance, we compared different strategies for the enrichment of lysosomes followed by the establishment of lysosome-compatible cross-linking conditions, and applied it to lysosomal fractions in an intact and disrupted state. For the enrichment of low abundant cross-linked peptides, we performed strong cation-exchange chromatography (SCX) for 3 biological replicates and collected 40 fractions each. The 20 fractions containing higher charged precursor ions originating from cross-linked peptides were measured on an Orbritrap Lumos mass spectrometer with a MS2/MS3 acquisition strategy. Data were processed with the XlinkX algorithm integrated in Proteome Discoverer resulting to the identification of ~44,700 cross-link specific reporter peaks and ~6300 cross-link spectral matches at 5 % FDR. We were able to identify ~4,300 cross-links from which 882 were assigned to lysosomal proteins. This includes novel interactions as well as such from members of known complexes at the lysosomal membrane. Our data further provide verification of known, and evidence for previously unknown, protein structures. Selected findings were further investigated with biochemical and bioinformatics follow up experiments.

Session 14: Metabolomics; Oral 2: Cakic

Time: Tuesday, 03/Mar/2020: 3:50pm - 4:10pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Anne Schnell
Session Chair: Julica Folberth

Orbitrap Fusion method development for the targeted and non-targeted screening of coenzyme A thioesters

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Identification of coenzyme A thioesters (acyl-CoAs) represents an important tool in the understanding of many biochemical processes. Targeted analysis of acyl-CoAs can lead to the confirmation of specific biochemical hypothesis, while non-targeted screening offers a possibility for discovering and understanding of unknown processes. In this work a method for the screening of acyl-CoAs in bacterial cultures using a high-resolution Orbitrap Fusion tribrid mass spectrometer has been developed. In order to selectively detect acyl-CoAs in complex biological matrices, five different filters are being applied in the method design. Two of them are very specific to all acyl-CoAs and rely on the well-defined fragmentation pattern of the analytes. These are a *targeted mass trigger* for the most characteristic fragment ion (*m*/*z* 428.0365) and a *targeted mass difference* (506.9952), which represents the characteristic neutral loss of the adenosine 3'-phosphate-5'-diphosphate moiety. The utility of the method is demonstrated by analysing culture extracts of the denitrifying betaproteobacterium "*Aromatoleum*" sp. strain HxN1 anaerobically grown with five different substrates. In total, 104 acyl-CoAs have been detected, from which 43 have so far been unambiguously identified by comparison with reference standards. The sensitivity of the method allows detecting acyl-CoAs in biological matrices in highly variable abundances, up to 5 orders of magnitude.

Session 15: ICP-MS: Environment II; Oral 2: Zimmermann

Time: Tuesday, 03/Mar/2020: 3:50pm - 4:10pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: David Clases

Boron and strontium isotope ratio analysis of the Rhine river – tracer for anthropogenic boron emissions?

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Environmental monitoring of complex ecosystems requires reliable and sensitive techniques in order to identify the source, fate and sink of anthropogenic contaminants in e.g. aquatic ecosystems. Within this context, the analysis of stable isotope ratios has been proved a valuable tool. Amongst other applications, the analysis of B and Sr isotope ratios has evolved as promising tracer to differentiate water bodies of different origin and their corresponding mixing processes of e.g. freshwater and seawater.

Due to its versatile use in industries e.g. as sodium perborate, or more recently as dopant for semiconductors, analysis if boron isotope ratios may be of value as a new tracer for anthropogenic B emissions into the aquatic environment.

This contribution aims to investigate, whether it is possible to distinguish between the different discharges of a river on the basis of their B and Sr isotopic signatures, in order to distinguish between natural and human input sources of B into the aquatic environment. Therefore, 76 freshwater samples from one of the most anthropogenically influenced rivers in Germany, the Rhine, were analyzed for their Sr and B isotopic composition. The B isotope composition assessed in the Rhine River shows a large variability of $\delta^{11}B/^{10}B_{NIST951a}$ of ca. 30‰, and a B concentration ranging from 11.6 µg L^{-1} ± 1.3 µg L^{-1} to 65 µg L^{-1} ± 6 µg L^{-1}. In contrast to that. the Rhine tributaries are characterized by significantly higher B loads. Additionally, tributaries are characterized by Sr concentrations and Sr isotope ratios significantly different from those of the Rhine.

The combination of Sr and B isotopic compositions can be used to distinguish different inputs into a complex river system, and can therefore provide a better insight into possible sources and distribution of anthropogenic B inputs.

Session 16: Ions at Next Generation Lightsources; Oral 2: Loru

Time: Tuesday, 03/Mar/2020: 3:50pm - 4:10pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Charlotte Uetrecht

Revealing the make-up of substituted polycyclic aromatic hydrocarbons by discharge sources

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Session: Ions at next generation lightsources

Dr. Charlotte Uetrecht

Detected through the aromatic infrared bands (3-20 mm), polycyclic aromatic hydrocarbons (PAHs) are considered to lock up a large fraction of the carbon in the interstellar medium (ISM) and to significantly impact its physics and chemistry. Despite their importance, much is unknown about their formation and reactivity in the harsh conditions of the ISM.

We are investigating the reactivity of these molecules in the laboratory by means of an electrical discharge nozzle coupled with mass selective IR-UV ion dip spectroscopy at the free electron laser FELIX. In plasma conditions, these molecules are expected to dissociate and recombine to form new species, which are then probed and characterised both via their mass and their unique IR signature.

Herein, we present the products formed upon discharge of PAHs with simple organic molecules that are prevalent in the ISM. We investigated the PAHs naphthalene ($C_{10}H_8$), fluorene ($C_{13}H_{10}$), phenanthrene ($C_{14}H_{10}$), and pyrene ($C_{16}H_{10}$) discharged with acetonitrile (CH_3CN), a nitrogen containing interstellar molecule. A rich chemistry has been observed for all the investigated PAHs, thus providing us with relevant insight into the underlying processes of their chemistry.

Session 13: Proteomics - Structural and Functional Proteomics; Oral 3: Kopicki

Time: Tuesday, 03/Mar/2020: 4:10pm - 4:30pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Carla Schmidt

MHC class I peptide binding monitored by native MS

Janine-Denise Kopicki[1], Raghavendra Anjanappa[2], Maria Garcia-Alai[3], Julia Lockhauserbäumer[1], Rob Meijers[3], Sebastian Springer[2], Charlotte Uetrecht[1,4]

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Major Histocompatibility Complex (MHC) class I molecules selectively bind peptides for presentation to cytotoxic T cells. There are many peptide-bound structures of MHC class I molecules, but the peptide-free state is not explored well due to its instability. We characterized a disulfide-engineered version of the human class I molecule HLA-A*02:01 (dsA2) that is stable without peptide by means of native mass spectrometry. Successful refolding of the heterogenic protein complex was verified, whereas dipeptide used for refolding was detected neither free nor bound to dsA2, which confirms that the protein complex is indeed empty and stable in absence of any peptide. In striking contrast, only a minimal amount of folded wild type A2 (wtA2) was detected in the absence of dipeptide, but wtA2 did remain stable when 0.5 mM dipeptide was present. We conclude that while wtA2 is dependent on the dipeptide at high concentration to maintain its folded conformation, dsA2 is conformationally and thermally stable in the absence of dipeptide. Furthermore, dsA2 was observed to bind a variety of peptides resulting in distinct signals with respective ratios based on their different affinities even when measured from a peptide pool. Thus, we introduce native MS analysis of disulfide-stabilized MHC class I molecules as a versatile tool for peptide screening approaches.

Session 14: Metabolomics; Oral 3: Schöttler

Time: Tuesday, 03/Mar/2020: 4:10pm - 4:30pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Anne Schnell
Session Chair: Julica Folberth

Investigation of genetically determined metabolome changes in *Arabidopsis thaliana* by capillary ion chromatography-MS

Hannah Schöttler, Heiko Hayen
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Genetic engineering is gaining interest in multiple research fields and is progressively integrated in everyday life, i.e. in forms of fuel or food products. To retrace impacts of mutations on the biochemical network of the cells, precise analytical methods need to be developed. Herein, metabolomic studies comprise the identification and quantification of small metabolites present inside organisms. Because of their relevance for its functioning, their investigation is of great interest for comprehensive research and provide information to understand processes and dynamic interactions.

Since metabolites show great similarities in their chemical structures, while undertaking widely different tasks, an adequate and reliable separation and detection is inevitable. Especially the analysis of small ionic metabolites with common chromatography methods is a challenging task due to the lack of a selective and sensitive separation technique.

By coupling capillary ion chromatography (cap-IC) to high-resolution mass spectrometry (HR-MS) these chromatographic problems could be solved and an analysis method with enhanced sensitivity and selectivity for small anionic metabolites was generated. With this method, a set of multiple metabolites from the central carbon metabolism, containing structural isomers and structural related compounds, was separated and identified as their deprotonated molecular ions in negative electrospray ionization (ESI) mode. Additionally, data-dependent fragmentation experiments (MS/MS) with customized collision energies for the labile analytes were performed to differentiate and quantify coeluting isomers by characteristic fragment ions.

Subsequently, the optimized cap-IC-HR-MS/MS method was applied to extracts of wild type and genetically modified *Arabidopsis thaliana* samples. The mutants showed a variation in phenotypical appearance and growth behavior so that it was assumed that the metabolite concentrations could be affected. In order to compare their metabolic composition, an improved extraction method was established, and quantification with an external calibration approach was performed.

Session 15: ICP-MS: Environment II; Oral 3: Horstmann

Time: Tuesday, 03/Mar/2020: 4:10pm - 4:30pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: David Clases

Method development for speciation analysis of MRI contrast agents in river and seawater of Sydney (Australia) by µSPE and HILIC-ICP-MS

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Gd-based contrast agents (GdCAs) are often used to enhance contrasts in magnetic resonance imaging (MRI). Following MRI examination, they are excreted by patients and enter surface and seawater via effluents of local wastewater treatment plants and the frequent application led to an anthropogenic anomaly in the environment, which is particularly pronounced in areas with developed healthcare. The balancing of total Gd levels against those of other lanthanide analogues is the most common way to investigate this anomaly. However, data on Gd species are relatively scarce and so far, not available for any location in Australia. Greater Sydney is the largest metropolitan region (12,400 km^2) of Australia producing more than 1.3 billion litres of wastewater every day and discharging more than 90% into the South Pacific Ocean via deep ocean outfalls. This complicates sampling and confronts speciation analysis with very low concentrations and complex matrices.

In this work hydrophilic interaction liquid chromatography (HILIC) and inductively coupled plasma mass spectrometry (ICP-MS) are used to target individual Gd species in surface and seawaters obtained from the Greater Sydney region. To address low levels of Gd, figures of merit were improved by increasing ion transmission. This was accomplished by employing hard extraction conditions and operating the quadrupole in band pass mode. Furthermore, complex saltwater matrices require efficient matrix elimination and species preconcentration. This was achieved with a novel automated micro-solid phase extraction (µSPE) method.

The increase in ion transmission and the operation of a quadrupole in band pass mode translated in improved figures of merit for the analysis of commonly administered GdCAs (Gd-DTPA, Gd-DOTA, Gd-DO3A-butrol, Gd-DTPA-BMA). The automated µSPE method allowed matrix elimination and analyte preconcentration with quantitative recoveries from river and seawater. Collectively, this decreased limits of detection below 1 ng/L, which was crucial for targeting individual species in the Greater Sydney region.

[1]N. Lloyd, G. Craig, C. Bouman, and J. Schwieters, Technical Note 30464, Thermo Fisher Scientific, Bremen, Germany.

[2]O. Rienitz, A. Pramann, and D. Schiel, *Int. J. Mass Spectrom*. 289 (2010) 47.

[3]A. Pramann, T. Narukawa, O. Rienitz, *Metrologia* 54 (2017) 738.

[4]A. Pramann, O. Rienitz, *Eur. Phys. J. Appl. Phys*. (2019) accepted (DOI 10.1051/epjap/2019190284).

Session 16: Ions at Next Generation Lightsources; Oral 3: Poully

Time: Tuesday, 03/Mar/2020: 4:10pm - 4:30pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Charlotte Uetrecht

Direct effects of ionizing radiation on biological molecular systems

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Charged particles (notably ions) with MeV kinetic energies penetrate into matter and deposit energy along a mostly linear path. Along this path, the linear energy transfer (LET) is first almost constant, and then show the so-called Bragg peak right before the particles stop. Hadrontherapy for cancer treatment (mainly with protons but also carbon ions) takes advantage of this ion-specific interaction with matter to maximize energy deposition in tumors and spare healthy tissues located along the ion path, especially deeper than the Bragg peak location. This precise ballistics adds to a higher relative biological efficiency to kill cancer cells than conventional treatments using X-rays. However, fundamental molecular processes underlying these biological effects are still poorly understood. To bridge this gap, it is crucial to control the experimental conditions. Irradiation of a solution at room temperature mainly leads to the formation of free radicals from the solvent, which chemically react with biomolecules, leading to secondary processes such as bond cleavage, cross-linking and generally quenching of biological activity. All these indirect effects require diffusion of free radicals from the solvent to the biomolecule, which occurs at rates that decrease by several orders of magnitude from room to cryogenic temperatures. To study direct effects, frozen, lyophilized, crystallized, dried but also isolated molecules can be used. These last years, a collaboration between the CIMAP lab (Caen, France) and the Zernike Institute for Advanced Materials (Groningen, Netherlands) has been investigating the irradiation of isolated biological molecular systems of controlled mass and stoichiometry such as collagen mimetic peptides, antibiotic/receptor non-covalent complexes and DNA G-quadruplexes, by means of a home-made experimental set-up coupled to synchrotron or ion beamlines. We have found that interaction with one carbon ion at the Bragg-peak energy or X-ray photon mainly leads to ionization, vibrational energy deposition and intermolecular followed by intramolecular fragmentation. Radical-mediated mechanisms, such as loss of neutral molecules from amino acid side chains or cleavage of the glycosidic bond of the antibiotic vancomycin, have been proposed to account for the very low activation energies observed. Interestingly, our most recent studies on non-covalently bound systems (DNA G-quadruplexes and antibiotic/receptor complexes) reveal a strong influence of geometrical structure on the processes triggered by ionizing photoabsorption. In a near future, we aim at probing the radiation-induced denaturation of biomolecular systems by tandem ion-mobility spectrometry.

Session 14: Metabolomics; Oral 4: Krüger

Time: Tuesday, 03/Mar/2020: 4:30pm - 4:50pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Anne Schnell
Session Chair: Julica Folberth

Targeted HILIC-MS/MS of Foodborn Methylamines: Fermented Dairy and Indirect TMAO

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Methylated amines are highly relevant in the nutrition field. Examples include micronutrients (e.g. choline), food consumption markers (e.g. trigonellline, proline-betaine (stachydrine), N-methylhistidines), and clinical markers (e.g. asymmetric N,N-dimethylarginine, ADMA). In particular, trimethylamine-N-oxide (TMAO) has become a main target due to its strong association with cardiovascular diseases. However, TMAO was also suggested as a putative marker of food consumption.

In recent years, we established a versatile targeted HILIC-MRM method for quantification of up to 30 methylated amines and selected amino acids in urine and blood plasma [1]. This method has been applied in a variety of nutrition studies so far, complemented by ^{1}H-NMR analyses, and we will summarize those activities.

It is known that TMAO can either be taken up directly from fish, or can be produced indirectly by intestinal microbial degradation of precursors such as carnitine or choline, and subsequent oxidation in the liver. In the observational human study KarMeN (Karlsruhe Metabolomics and Nutrition), we confirmed associations with several food groups and covariates [1,2].

However, there are still inconsistencies concerning the origin and relevance of TMAO & Co. A particularly striking example is consumption of dairy foods, for which both increase and decrease of TMAO have been reported. We have analysed samples from two intervention studies with different dairy products: F3 (Function of Fermented Foods) and FoodBAll (Food Biomarker Alliance). Distinct differences between fermented and non-fermented dairy foods/products were found, pointing to the importance of intestinal microbial modulation and variety [3].

[1] Roggensack et al. Rapid Commun. Mass Spectrom. 2019, doi: 10.1002/rcm.8646

[2] Krüger et al. Mol. Nutr. Food Res. 2017, 61(11), e1700363

[3] Burton et al. Nutrients 2020, 12, 234

Session 15: ICP-MS: Environment II; Oral 4: Brix

Time: Tuesday, 03/Mar/2020: 4:30pm - 4:50pm · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: David Clases

On the Trace (Level) of a high-level nuclear waste Disposal – Retention of Cs^+, Eu^{3+} and UO_2^{2+} on Ca-Bentonite

Kristina Brix[1], Christina Hein[2], Sandra Baur[1], Traudel Allgayer[1], Ralf Kautenburger[1]
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Worldwide, nuclear power plants are in operation to grant an energy supply with low CO_2 emission. However, no permanent solution has been found for the accumulating high-level nuclear waste (HLW). The containing substances are radiotoxic for the next hundred thousands of years. It is international consensus to store the HLW in deep geological formations such as salt rock, clay formations or granite bedrock. Until now, worldwide no disposal site is operating and only one is under construction. Nevertheless, bentonite is scheduled as buffer and backfill material in different storage concepts. It should protect the HLW containing canisters from water ingress e.g. the pore water of the surrounding host rock. In addition, bentonite is able to immobilise cations and can consequently bind leaked out cationic radionuclides.

In this study, the retention of different elements relevant for a HLW disposal (Cs^+, Eu^{3+}, UO_2^{2+} and I^-) on raw Ca-bentonite was investigated by using mass spectrometry with inductively coupled plasma (ICP-MS). To simulate realistic conditions, especially for a theoretical disposal in claystone in northern Germany, the experiments were carried out in high saline solutions (up to 5 M NaCl) and also in the hyperalkaline pH range (12.5-13). The latter results from the leaching of cementitious construction materials. Additionally, the elements were investigated in separate and as mixture (Waste Cocktail). These experimental conditions (high salinity, Waste Cocktail) also require the development of new analytical methods.

Even in 5 M NaCl solution, the monocharged Cs^+ can be immobilised by Ca-bentonite. In the Waste Cocktail, competition effects are particularly visible at high concentrations. The retention of Eu^{3+} and UO_2^{2+} is influenced positively by each other probably due to co-precipitation in the hyperalkaline milieu.

We would like to thank the German Federal Ministry of Economics and Energy (BMWi), represented by the Project Management Agency Karlsruhe (PTKA-WTE) for funding (projects: 02E10991 and 02E11415D) and our project partners for the kind collaboration.

Session 16: Ions at Next Generation Lightsources; Oral 4: Kirschbaum

Time: Tuesday, 03/Mar/2020: 4:30pm - 4:50pm · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Charlotte Uetrecht

Resolving Structural Details of Isomeric Glycolipids by Cryogenic Gas-Phase Infrared Spectroscopy

Carla Kirschbaum[1,2], Kevin Pagel[1,2]
[1]Freie Universität Berlin, Germany; [2]Fritz-Haber-Institut der Max-Planck-Gesellschaft, Germany

Glycolipids occur in all organisms ranging from bacteria to men. They are composed of a glycan and a lipid moiety, which can both exhibit different kinds of isomerism. Minute structural alterations are often crucial for the biological functionality of the glycolipid; however, no standalone analytical technique can provide a comprehensive structure elucidation of complex glycolipids to date. Moreover, insufficient tools to distinguish isomers have led to the falsification of research results in the past.

Cryogenic gas-phase infrared (IR) spectroscopy in superfluid helium droplets was recently demonstrated to be a powerful complement to existing analytical workflows for glycolipid analysis. In this technique, mass-to-charge selected glycolipid ions are captured in helium nanodroplets and interrogated by a tunable IR free electron laser. Upon sequential absorption of multiple IR photons, the ion is released from the droplet and detected by mass spectrometry. The resulting IR spectra are highly resolved and can be compared with theoretical calculations.

Cryogenic gas-phase IR spectroscopy was employed for a systematic investigation of isomeric glycosphingo- and glycoglycerolipids. The comprehensive sample sets allowed probing the influence of the glycan, the lipid and the configuration of the interconnecting glycosidic bond on the vibrational spectra. All kinds of isomerism could be unambiguously resolved by diagnostic IR fingerprints. Furthermore, sphingolipids with different double bond positions and configurations were investigated. Due to an interaction between the protonated amine and the double bond, the isomers could be distinguished by characteristic N-H vibrations. Overall, the results underpin the exceptional power of IR spectroscopy to distinguish (glyco-)lipid isomers.

Plenary Lecture 5: R. Boiteau

Time: Tuesday, 03/Mar/2020: 5:20pm - 6:00pm · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Maria Montes-Bayón

Ironing out environmental metal cycles with FTMS

Rene Boiteau
Oregon State University, United States of America

Micronutrient metals are essential for all living organisms, but bioavailable metals are often scarce in alkaline environments such as seawater and calcareous soils due to low solubilities. As a result, primary productivity in over a third of the surface ocean is limited by the availability of iron. Nearly all dissolved iron that reaches these regions is bound to organic ligands that keep it in solution and also affect which organisms can take it up. Assessing the impact of iron on global productivity and carbon cycling requires knowledge of the source and chemistry of the organic ligands. However, identifying metallophores from environmental samples remains a formidable analytical challenge due to the complexity of natural dissolved organic matter (DOM).

To address this challenge, our work employs state-of-the art liquid chromatography mass spectrometry-based methods for identifying and quantifying metal-organic complexes from complicated DOM mixtures. Our approach combines liquid chromatography with inductively coupled plasma mass spectrometry for compound quantitation along with high mass resolution and accuracy Fourier transform mass spectrometry (FTMS), which enables the detection of minor isotopes of intact metal-bound molecules within complex mixtures. Combined with isotope pattern matching data processing algorithms and quality metrics, these methods enable us to survey the composition of metallophores directly from ocean samples. This talk will discuss how this molecular-level insight into the chemistry of organic ligands in the environment sheds light on the mechanistic linkages between metals and marine ecosystems.

Plenary Lecture 6: Maria Montes Bayon

Time: Wednesday, 04/Mar/2020: 9:00am - 9:40am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Carsten Engelhard

Labelled Antibodies and ICP-MS Linked Immunoassays for Quantitative Analysis of Cell Biomarkers: Remaining Challenges

Maria Montes-Bayón, Mario Corte, Daniel Turiel, Alejandro Fernández, Elisa Blanco, Jörg Bettmer
University of Oviedo, Spain

ICP-MS linked immunoassays have been now used in combination with a variety of tagging structures with the final aim to provide signal amplification. The main advantage of using ICP-MS for detection of the labels is that they are not required to possess radioactive, enzymatic, optical, electric or any other special properties. Therefore, a variety of metal-containing structures have been tested as elemental labels. Among them metal chelates, metal containing polymers and inorganic nanoparticles (NPs) are the most widely used antibody tags. In these cases, the amplification of the signal is directly obtained at the moment of the immunochemical reaction thanks to the numbers of labels attached to the detection antibody. However, additional possibilities can be explored by conducting signal amplification after the molecular recognition takes place using, for instance, DNA probes.

In this work, the use of the different labels will be illustrated for the quantitative determination of specific cell biomarkers by ICP-MS. In this first case, the use for Ru-chelates containing labels to evaluate different ICP-MS linked immunoassays to permit the quantification of ferritin and Fe:ferritin ratios will be addressed. The application of such strategies to study the effect of nanoparticulate iron therapy in enterocyte-like cell cultures or the malignancy of different breast cancer cell lines will be illustrated. Secondly, use of DNA probes followed by PCR-amplification (ICP-MS linked immune-PCR) is proposed for the determination of HER2, a breast cancer biomarker whose overexpression is associated with a more aggressive phenotype. Lastly, the use of metal polymers (e.g. DTPA-containing polymers) are also explored for the quantification of TfR1 in different malignancy breast cancer phenotypes in bulk and at individual cell levels. The possibilities and limitations of all the explored strategies will be discussed and critically compared in the presentation.

Plenary Lecture 7: Jana Roìthová

Time: Wednesday, 04/Mar/2020: 9:40am - 10:20am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Mathias Schäfer

Ion Spectroscopy in the Service of Metal Organic Chemistry

Jana Roithova
Radboud University Nijmegen, Institute for Molecules and Materials, Netherlands

Knowing reaction intermediates is the key to understanding of reaction mechanisms. Reactive intermediates are difficult to detect and characterize. We investigate the reactive species by different methods with a particular focus on mass spectrometry.[1] Coupling of ambient ionization techniques and mass spectrometry brings an advantage of a large dynamic range and thus enables detection of low abundant species from a solution as ions in the gas phase. We characterize the mass-selected ions with their IR and UV-vis spectra which allows us to assign the structures to the ionized reaction intermediates.[2]

In the lecture, I will focus on using mass spectrometry and ion spectroscopy for detection and characterization of reactive complexes in the field of biomimetic metal complexes.[3] I will show how classical gas-phase experiments can contribute to understanding of chemistry of these reactive species.[4] Finally, I will show how reactive species generated in the gas phase can provide understanding of chemistry observed in solution.

References

[1] J. Zelenka, R. Cibulka, J. Roithová, *Angew. Chem. Int. Ed.* **2019**, *58*, 15412– 15420.

[2] J. Roithová, A. Gray, E. Andris, J. Jašík, D. Gerlich, *Acc. Chem. Res.* **2016**, *49*, 223–230.

[3] E. Andris, R. Navrátil, J. Jašík, T. Terencio, M. Srnec, M. Costas, J. Roithová, *J. Am. Chem. Soc.* **2017**, *139*, 2757–2765.

[4] E. Andris, R. Navratil, J. Jasik, M. Srnec, M. Rodriguez, M. Costas, J. Roithová, *Angew. Chem. Int. Ed.* **2019**, *58*, 9619-9624.

Session 17: Metabolomics / Glycomics / Proteomics; Keynote: Frolov

Time: Wednesday, 04/Mar/2020: 10:40am - 11:10am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Michael Mormann

A combination of proteomics and metabolomics techniques in characterization of age-related changes in legume root nodule

Andrej Frolov, Tatiana Bilova, Christian Ihling
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Proteomics and metabolomics represent powerful tools of functional genomics, giving direct access to the molecular mechanisms underlying plant response on developmental signals, metabolic pathways, and regulatory networks. The combination of these approaches might give deeper insights into the molecular mechanisms contributing on mutualistic plant-microbial interactions, in particular, legume-rhizobial symbiosis. Here we address age-related changes in determinate and indeterminate root nodules of common bean (*Phaseolus vulgaris*) and pea (*Pisum sativum)*. Juvenile, flowering, and senescent plants were harvested, the proteins were isolated by phenol extraction and digested with trypsin, isolating metabolites by a multi-step extraction with solvents of different polarity. Protein hydrolyzates were analyzed by nanoLC-ESI-Q- and LIT-Orbitrap-MS in data-dependent acquisition (DDA) mode. Database search and annotation of PTMs were based on the SEQUEST algorithm; quantification was performed using a label-free strategy. Functions and localization of the proteins were annotated by an in-house developed workflow, combined with MapMan, Mercator, LocTree3 and String tools.Analysis of primary metabolome relied on the combination of GC-MS and IP-RP-UHPLC-MS, with a secondary metabolite profiling by RP-UHPLC-MS. The analysis revealed 656 and 92 differentially regulated proteins in common bean and pea nodules, respectively. Plant proteins were mostly down-regulated and represented by the molecules involved in signaling and protein metabolism, while most of the bacterial polypeptides were up-regulated and found to be involved in energy metabolism and nitrogen fixation. Age-dependent changes in metabolite profiles were characterized based on the accumulation of carbohydrates and amino acids. Interestingly, mutation in the gene *sym27* resulted in the suppression of signaling and protein biosynthesis in both symbiosis partners. Remarkably, nodule ageing was accompanied with enhanced glycation and carbonylation. In total, 36 age-related glycation hotspots were identified in the *P. vulgaris* nodule proteome.

Session 18: Ion Physics and Chemistry; Keynote: Schäfer

Time: Wednesday, 04/Mar/2020: 10:40am - 11:10am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Uwe Karst

Hydrogen Tunneling in the gas phase investigated by MS, IR Ion Spectroscopy, and theory: first and latest results

Mathias Schäfer[1], Anthony Meijer[2], Thomas Thomulka[1], Katrin Peckelsen[1], Mathias Paul[1], Albrecht Berkessel[1], Jos Oomens[3], Giel Berden[3], Jonathan Martens[3]

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Introduction

Recently, we reported the generation of metal free hydroxycarbenes in a quadrupol ion trap (QIT) at temperatures around 320–350 K and the investigation of their reactivity by IR ion spectroscopy. Charge tagged alpha-ketocarboxylic acid derivatives serve as tailor-made precursors, which deliver hydroxycarbene species upon collision induced CO_2 loss.[1-3] Additionally, quantum mechanical Htunneling was identified as the mode of action for the gas-phase isomerization reaction of a phenylhydroxycarbene into the corresponding benzaldehyde derivative.[1] For the current gas-phase study we synthesized an aliphatic alpha-keto-carboxylic acid precursor ion decorated with a trimethylammonium charge tag. The corresponding hydroxycarbene has alpha methylene hydrogens, which is carefully selected to allow investigating the formation pathway competition to either the enol *or* the aldehyde tautomer.

Methods

The (4-Carboxy-4-oxo-butyl)-trimethyl-ammonium analyte as well as the charge-tagged quinuclidine model compound were synthesized according to a literature protocol as discussed elsewhere.[5] (+)ESI-MS/MS of analyte **1** generates an abundant molecular ion at m/z 174 and similarly to the model compound **3**. Effective CO_2 loss is triggered by CID in a modified spherical quadrupol ion trap (QIT) Bruker Amazon instrument delivering the product ions of interest at m/z 130 (**2** and **4**), which are analyzed by IR ion spectroscopy and theory.[1,3] The IR ion spectra were recorded using the wavelength-tuneable radiation (here, between 5-20 µm) from the FELIX infrared free electron laser (IR-FEL) at the Radboud University in Nijmegen, NL. The IR ion spectra are interpreted in comparison to computed linear IR spectra of candidate structures identified by density functional theory (DFT).[5]

Preliminary data

An aliphatic hydroxycarbene formed by collision induced CO_2 loss from a (4-Carboxy-4-oxo-butyl)-trimethyl-ammonium precursor, was investigated by IR ion spectroscopy.[5] The recorded spectra match computed ones of ring-structured hydroxycarbene candidates held together by a peculiar C---HC hydrogen bond.[4,6] Additional to the predominantly formed hydroxycarbenes the spectra suggest the presence of the enol tautomer in low amounts. Time dependent investigations revealed an outstanding stability of the hydroxycarbene ions. The recorded IR ion spectra show no evidence for the formation of the aldehyde isomer, although this is clearly the most stable tautomer of the three possible alternatives. This finding contradicts the behavior of methyl-hydroxycarbene, which isomerizes cleanly to the acetaldehyde tautomer even at cryogenic temperatures.[2] We assume that the aliphatic model hydroxycarbene is substantially stabilized by the unique C---H-C hydrogen bond interaction, which also explains the absence of the typical hydrogen tunneling reactivity.[4,6] This set of fundamental results suggests a potent strategy for a conformation dependent reactivity switch of carbenes, based on the presence or absence of C---H-C interactions. This may also have far-reaching implications on the understanding of enzyme reactivity and the design of new organo-catalysts.[5]

[1] M. Schäfer, J. Oomens, A. Berkessel, A. J. H. M. Meijer, et al. J. Am. Chem. Soc. **139** (2017) 5779−5786.

[2] P.R. Schreiner, et al. Science **322** (2011) 1300-1303.

[3] A. J. H. M. Meijer, M. Schäfer, et al. Phys. Chem. Chem. Phys. **21** (2019) 16591-16600.

[4] A. J. Arduengo, et al. J. Am. Chem. Soc. **117** (1995) 572–573.

[5] J. Oomens, A. J. H. M. Meijer, A. Berkessel, M. Schäfer, et al., J. Am. Chem. Soc. (2020) in preparation.

[6] M. Paul, M. Breugst, A. Berkessel, M. Schäfer, et al. Chem. Eur. J. **25** (2019) 2511 – 2518.

Novel aspect

A model hydroxycarbene exhibits exceptional stability and shows no hydrogen tunneling reactivity due to intramolecular C···H-C bond interactions

Session 19: Forensic; Keynote: Putz

Time: Wednesday, 04/Mar/2020: 10:40am - 11:10am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Sven Heiles

Metabolite identification studies of trenbolone by hydrogen isotope ratio mass spectrometry and LC-HRMS for doping control analysis

Marlen Putz, Thomas Piper, Thevis Mario
German Sport University Cologne, Germany

Trenbolone is a synthetic anabolic steroid, which has been misused for performance enhancement in sports. The detection of trenbolone doping in routine sports drug testing programs is complex as methods utilizing gas chromatography/ mass spectrometry are limited due to unspecific derivatization products and by-products while liquid chromatography/ mass spectrometry-based assays occasionally suffer from high limits-of-detection. The number of previously reported metabolites in human urine is small, and most analytical methods rely on targeting epitrenbolone, trenbolone glucuronide and epitrenbolone glucuronide.

In order to probe for the presence of additional trenbolone metabolites and to re-investigate the metabolism, an elimination study was conducted. One single dose of 10 mg of 5-fold deuterated trenbolone was administered to a healthy male volunteer and urine samples were collected for 30 days. For sample processing, published protocols were combined considering unconjugated, glucuronic acid, sulfo- and alkaline-labile conjugated steroid metabolites, and the sample preparation strategy consisted of solid phase extractions, liquid-liquid extractions, metabolite de-conjugation, HPLC fractionation, and derivatization. Analytical methods included gas chromatography/ thermal conversion/ hydrogen isotope ratio mass spectrometry combined with single quadrupole mass spectrometry as well as liquid chromatography/ high accuracy/ high resolution mass spectrometry of the hydrolyzed and non-hydrolyzed samples.

Twenty deuterium-labelled metabolites were identified including glucuronic acid, sulfo- and cysteine conjugates and characterized by parallel reaction monitoring experiments yielding corresponding product ion mass spectra. Main metabolites were identified as trenbolone-diol derivatives and potential trenbolone-diketone derivatives excreted in their glucuronic acid and sulfo-conjugated form with detection windows of 5 respectively 6 days. Further characterization was conducted with pseudo MS^3 experiments of the intact conjugates and by comparison of their mass spectra with reference material. For 4,9,11-estratriene-3,17-dione, reference standards are commercially available, and the trenbolone-diol derivative was synthesized in-house.

Session 20: Instrumentation; Keynote: Peretzki

Time: Wednesday, 04/Mar/2020: 10:40am - 11:10am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Tim Esser

Interfacing droplet chips to mass spectrometry

Detlev Belder, Andrea Peretzki
Leipzig University, Germany

Droplet microfluidics is full of promises but chemical selective detection of unlabeled species is one of the main challenges to enable untargeted analysis at picoliter scale. In this context mass spectrometry is a very technology for and sensitive detection of low-abundant analytes. While different approaches to enable the coupling of microfluidic droplet chips to mass spectrometry have been reported, the broad application in practice is still in its infancy. This can also be explained by the lack of systematic work on how to couple different chips to different mass spectrometers.

We demonstrate how the coupling of droplet microfluidic with mass spectrometry is achieved for both in-house fabricated full PDMS as well as full body glass chips. It turned out that especially the inlet potential of mass spectrometer orifice is crucial for an intended MS-coupling of microfluidic glass or PDMS chips.

As electrical fields can disturb upstream droplet processes, approaches for shielding have to be developed to avoid droplet coalescence or fission. The latter aspect is essential if the electrospray emitter is at elevated potential. Recent examples on the application of this technology will be presented such as the analyses of cell metabolism in single droplets as well as optimization of synthesis including the study of catalytic events down to the single particle level.

REFERENCES

[1] K. Wink, L. Mahler, J. R. Beulig, S. K. Piendl, M. Roth, D. Belder, “An Integrated Chip-Mass Spectrometry and Epifluorescence Approach for Online Monitoring of Bioactive Metabolites from Incubated Actinobacteria in Picoliter Droplets,” *Anal. Bioanal. Chem*. **2018**, 410, 7679–7687,.

[2] Detection of antibiotics synthetized in microfluidic picolitre-droplets by various actinobacteria, L. Mahler, K. Wink, R. J. Beulig, K. Scherlach, M. Tovar, E. Zang, K. Martin, C. Hertweck, D. Belder, M. Roth, *Scientific reports* **2018**, 8, 13087

[3] J. R. Beulig, R. Warias, J. J. Heiland, S. Ohla, K. Zeitler, D. Belder, “A Droplet-Chip/Mass Spectrometry Approach to Study Organic Synthesis at Nanoliter Scale,” *Lab. Chip*, **2017,** 17, 1996–2002,.

Session 17: Metabolomics / Glycomics / Proteomics; Oral 1: Vakhrushev

Time: Wednesday, 04/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Michael Mormann

GlycoDIA: Gene Editing and Advanced Mass Spectrometry for O-Glycoproteomics to bridge Next Generation O-glycoproteomics

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Post-translational modifications (PTMs) provide for a nearly limitless expansion of the structural space of the proteome of an organism and add manifold layers of complexity to the interactome. N- and O- are the two major classes of glycosylation. O-linked - mucin-type (GalNAc-type) O-glycosylation is the most diverse and differentially regulated and yet the least understood.

Site-specific O-glycosylation is emerging as an important concept for regulating protein processing and functions. However, full understanding of the nature and functions of this abundant type of protein glycosylation is severely hampered by lack of tools and methods and the scientific community is still awaiting the next breakthrough methodology in high-throughput O-glycoproteomics capable of dealing simultaneously with identifying glycan structures and glycosites.

The "SimpleCell" technology, based on mass spectrometry applied to genetically engineered cells with reduced glycan complexity, has made a significant breakthrough in proteome-wide discovery of localization of O-glycosites (1-2). The major obstacle now is to overcome the difficulty of simultaneous identification of native O-glycan structures at specific sites together with determining the occupancy level.

Data Independent Acquisition (DIA) mode for liquid chromatography–tandem mass spectrometry (LC–MS/MS), may offer the solutions to overcome the challenge of O-glycan stoichiometry level evaluation and site specific determination of native O-glyco landscape.

We developed a sensitive higher energy collisional dissociation (HCD)-MS/MS based O-glycoproteomics strategy employing LC-MS/MS in DIA-mode to break the analytic barriers imposed by current needs for enrichment of glycopeptides and heterogeneity in glycan structures. This approach enables characterization of glycopeptides and structures of O-glycans on a proteome-wide scale with quantification of stoichiometries. We used multiple HCD-based spectral libraries derived from human cell lines and serum to build a high-quality spectral library designated Glyco-DIA, which was further expanded *in silico* to include mono and di-sialylated core1 O-glycans.

We demonstrated that use of the Glyco-DIA method enhances sensitivity of identification and quantification of O-glycopeptides. Applying the Glyco-DIA library to human serum without enrichment for glycopeptides enabled us to identify and quantify 269 distinct glycopeptide sequences bearing up to 5 different core1 O-glycans from 159 glycoproteins in a SingleShot analysis (3).

References:

1. Steentoft C*, Vakhrushev SY*, et al. (**2011**). **Nat Methods**, 8(11): 977-82.

2. Steentoft C*, Vakhrushev SY*, Joshi HJ*, et al. (**2013**). **EMBO J**, 32(10): 1478-88.

3. Ye Z, Mao Y, Clausen H, and Vakhrushev SY (**2019**). **Nature Methods**, 16(9):902-910.

Session 18: Ion Physics and Chemistry; Oral 1: Haack

Time: Wednesday, 04/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Mathias Schäfer

A First Principle Model of Differential Ion Mobility: the Effect of Ion-Solvent Clustering

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Differential Mobility Spectrometry (DMS) is used as a pre-separation tool in mass spectrometry and has increased the interest in DMS over the years. Each ion species exhibits a characteristic difference between the high-field and low-field mobility in an electrical field. Modifying the gas phase with abundant polar small molecules often increases the magnitude of this difference. This leads to enhanced resolving power of the devices. Despite its popularity, the fundamental principles of DMS are still a matter of debate - especially regarding the impact of the gas phase modification. One explanation for the “modifier effect” is dynamic clustering/declustering: At low electrical field strengths (and thus low ion temperatures) the polar modifier molecules cluster around the ion, increasing the collision cross section (CCS). The increase of the ion temperature in the high field strength period through high energy collisions leads to smaller average cluster sizes and a subsequent decrease of the CCS. Thus, ions exhibit a difference in the mobility between high and low electrical field strengths.

This model is in line with experimental results. In this work we aim to provide additional theoretical support for this hypothesis: Differential mobility is calculated from first principles, i.e., by conducting ab-initio calculations to find stable cluster structures, determine the field-dependent cluster population distribution and compute the average CCS from the individual CCS’. Furthermore, dispersion plots are calculated, which are compared to experimental data. It is reported that three effects contribute to differential mobility, i.e., (1) the “hard-sphere” effect, resulting from a change in the momentum transfer between ion and background gas, which leads to a decrease of mobility with field strength, (2) the CCS decreases with effective temperature, leading to an increase in mobility, (3) dynamic clustering occurs with strongly binding modifiers added and leads to a strong increase in mobility. The overall dependence of the ion mobility results from the combination of these three effects and depends on the ion/neutral pair, the ion/modifier pair, the modifier concentration, and the background gas temperature, respectively.

Only when considering all effects, we are able to reproduce semi-quantitatively the experimental results and the observed trends regarding different modifiers, their concentration, and the background gas temperature. Thus, this model strongly supports the dynamic clustering/declustering mechanism.

Session 19: Forensic; Oral 1: Brungs

Time: Wednesday, 04/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Sven Heiles

Identification of Tattoo Pigments in Human Skin Samples by Mass Spectral Library Matching and µXRF

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Popularity of tattoos has grown worldwide. Around 36% of the German population was tattooed in 2018. In the USA the number is even higher with 46%. Despite the grown popularity of tattooing, the regulations on tattoo inks are insufficient. Inks consist of different ingredients, such as inorganic and organic pigments, suspending agents, and preservatives. The applied pigments are often developed for car lacquers or printing inks and are not intended for intradermal use. In some cases, allergic, infectious, or neoplastic reactions occur or autoimmune diseases develop weeks or even years after tattooing. The trigger is often unknown. To follow up on the used pigments and identify the culprits, the analysis of inks and tattooed human skin samples is needed.

In this study, pigments, tattoo inks, and tattooed human skin samples were investigated by micro X-ray fluorescence (µXRF) imaging and laser desorption ionization-mass spectrometry (LDI-MS) imaging. To speed up the data analysis and the identification of most of the commonly used pigments in the samples, the creation of a spectral library and workflow is of great interest. Therefore, a library was created based on LDI-MS^1 and LDI-MS^2 spectra of "pure" pigments. Data processing, library generation, and library matching was performed within the software MZmine.

The µXRF results gave a first hint on which pigments were used. Especially the presence of copper, chlorine, and titanium in pigment regions in human skin thin sections was of great interest. Titanium dioxide is often used as a brightener in tattoo inks and in this study, titanium was found in many skin samples and tattoo inks. In reddish inks or tattooed skin, iron oxide is often used as an inorganic pigment. In some samples, iron can be identified by µXRF in higher concentrations. Copper and chlorine can be part of the pigments or contaminations, which are abundant due to the overall low purity of the pigments. These findings can guide the following LDI-MS analysis if specific elements are detected, such as chlorine. The samples were analyzed as well as the pigments and were matched against the pigment library. First, the matching of MS^1 spectra yields annotations for putative precursor ions of pigments which facilitates the subsequent acquisition of MS^2 spectra. A final structural identification relies on the similarity of the isotope and fragmentation patterns based on combined MS^1 and MS^2 library matching. Some pigments were identified as being restricted in Germany.

Session 20: Instrumentation; Oral 1: Foest

Time: Wednesday, 04/Mar/2020: 11:10am - 11:30am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Tim Esser

Miniaturized combination of Nano-Electrospray and Flexible Microtube Plasma (FμTP) as ionization source for mass spectrometry

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Plasma (LTP, DART, APCI, DBDI) and electrospray (ESI, nESI) based ionization sources are commonly used techniques for mass spectrometry. Both, plasma and electrospray are suitable for different molecular species and are limited to the polarity range of analytes.

On the one hand plasma based techniques are working in the gas-phase at atmospheric pressure and are selective for molecules of low or moderate polarities. On the other hand electrospray type ionization sources are commonly used for compounds of higher polarity in liquids. The combination of both techniques seems to be a further step in development of an ionization source applicable for a wide polarity range of analytes.

The main challenge is the combination of different electrical fields from the nESI and the plasma source. A resulting electrical field or bare electrodes are able to deflect or eliminate produced ions. Therefore, a new Plasma source, the Flexible Microtube Plasma (FμTP), is developed. By adding a FμTP into the vicinity of a nESI, vaporized neutral compounds can be post-ionized. This leads to increasing intensities and additional signals in the mass spectra.

This presentation focuses on the system setup, settings and the application in the field of lipidomics. Therefore, results of reserpine and a complex liver extract is used to compare the combination of both with the standard nano electrospray.

Session 17: Metabolomics / Glycomics / Proteomics; Oral 2: Wudy

Time: Wednesday, 04/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Michael Mormann

High resolution multiple-reaction-monitoring (MRM^{HR}) time-of-flight mass spectrometry to detect cyclolinopeptides in urine and blood after linseed consumption

Susanne Wudy[1,2], Roman Lang[2], Tatjana Lang[2,3], Oliver Frank[2], Gaby Andersen[3], Thomas Skurk[4], Thomas Hofmann[1,2,3,4], Karin Kleigrewe[1]

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Linseed and its oil have recently been suggested as a domestic and cheap alternative to exotic, expensive superfoods. Their various beneficial health effects have mainly been attributed to high amounts of physiologically valuable ingredients such as unsaturated fatty acids, fiber and lignans.[1] However, the role of cyclolinopeptides, which exhibit potent immunosuppressive activity[2], has never been studied with regard to their bioavailability. So far, no data on their metabolism neither in humans nor in animals is available.

Using high resolution multiple-reaction monitoring (MRM^{HR}), we were able to develop a sensitive and reliable UPLC-MRM^{HR}-TOF-MS method to detect cyclolinopeptides in human urine and blood. The enhanced product ion scan application enabled increased sensitivity in addition to the high selectivity obtained by high resolution full MS/MS spectra.

Within an interventional linseed nutrition study, 10 subjects (5 males, 5 females) consumed linseed muffins. Blood and urine samples were collected over 24 hours and were analyzed by UPLC-MRM^{HR}-TOF-MS. Highest concentrations of these cyclic peptides occurred within the first 2 to 4 hours after consumption. This study permits first insights into human metabolism and bioavailability of cyclolinopeptides and states the question about their physiological role.

[1] Shim, Y.Y., Gui, B., Amison, P.G., Wang, Y. & Reaney, M. J. T. (2014). Flaxseed (*Linum usitatissimum* L.) bioactive compounds and peptide nomenclature: A review. Trends in Food Science & Technology, 38(1), p. 5-20.

[2] Wieczorek, Z., Bengtsson, B., Trojnar, J. & Siemion, I. Z. (1991). Immunosuppressive activity of cyclosporine A. Peptide Research, 4(5), p. 275-283.

Session 18: Ion Physics and Chemistry; Oral 2: Überschaar

Time: Wednesday, 04/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Physics: Hall HS1
Session Chair: Mathias Schäfer

Gas-Phase Chemistry in the GC Orbitrap Mass Spectrometer

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Gas-phase reactions of temporally stored ions play a significant role in trapped ion mass spectrometry. Especially highly labile ion species generated through electron ionization (EI) are prone to undergo gas-phase reactions after relaxation to a low vibrational state. Here, we show that in the C-Trap of the Q Exactive GC Orbitrap mass spectrometer, gaseous water reacts with radical cations of various compound classes. High-resolution accurate mass spectrometry of the resulting ions provides a key to the mechanistic understanding of the chemistry of high energetic species generated during EI. We systematically addressed water adduct formation by use of H_2O and $D_2{}^{18}O$ in the C-Trap. Mass spectra of halogen cyanides XCN (X=Cl, Br, I) showed the formation of $HXCN^+$ species, indicating hydrogen atomic transfer reactions. Relative ratios of $HXCN^+/XCN^{+\bullet}$ increased as the electronegativity of the halide increased. The common internal calibrant perfluorotributylamine forms oxygenated products from water reactive fragment ions. These can be explained by the addition of water to an initial cation followed by elimination of two HF molecules. This addition/elimination chemistry can also explain $[M+2]^+$ and $[M+3]^+$ ions that commonly occur in mass spectra of silylated analytes. High-resolution accurate mass spectra of trimethylsilyl (TMS) derivatives revealed these as $[M-CH_3{}^{\bullet}+H_2O]^+$ and $[M-CH_4+H_2O]^{+\bullet}$, respectively. This study explains common fragment ions in ion trap mass spectrometry. It also opens up perspectives for the systematic mechanistic and kinetic investigation of high-energy ion reactivity and allows to explain differences in GC-Orbitrap mass spectra compared to other spectra.

Session 19: Forensic; Oral 2: Paßreiter

Time: Wednesday, 04/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Physics: Hall HS2
Session Chair: Sven Heiles

First steps towards uncovering gene doping with CRISPR/Cas9

Alina Paßreiter, Andreas Thomas, Katja Walpurgis, Mario Thevis
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The discovery of the **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats/**C**RISPR **as**sociated (CRISPR/Cas) system as a programmable, RNA-guided endonuclease, has revolutionised the utilisation of gene technology. Since it enables the precise modification of any desired DNA sequence and surpasses all hitherto existing alternatives for gene editing in precision, robustness and efficiency as well as flexibility and simplicity, it is one of the most frequently utilised tools for editing mammalian genomes these days. But those advantages also facilitate the illicit use of the CRISPR/Cas system in order to achieve a performance-enhancing effect in sporting competitions. Since 2003, this abuse is classified as *gene doping* and is listed in the *Prohibited List* of the *World Anti-Doping Agency* (WADA), which is why there is a pressing need for an adequate analytical method to detect the misuse of the CRISPR/Cas system by athletes. Hence, the initial aim of this study accounts for the identification of the xenobiotic protein Cas9 from the bacteria *Streptococcus pyogenes* (SpCas9), which currently represents the predominantly used endonuclease in genome editing, in plasma samples by means of developing a bottom-up analytical approach via immunoaffinity purification, tryptic digestion, and subsequent detection by HPLC-HRMS/MS. The target analyte was spiked to plasma samples as commercially available recombinant SpCas9 protein. A limit of detection was accomplished at moderate ng/mL-levels using at least two highly specific tryptic peptides. The herewith achieved working range of the method was tested between 25 and 1000 ng/mL. Due to the lack of available post-administration samples, proof of concept data are subject of future studies.

Session 20: Instrumentation II; Oral 2: Rüger

Time: Wednesday, 04/Mar/2020: 11:30am - 11:50am · *Location:* Lecture Hall Building Chemistry: Hall C2
Session Chair: Tim Esser

Cyclic ion mobility spectrometry coupled to high-resolution time-of-flight mass spectrometry — Prospects for complex mixture analysis

Christopher Rüger[1], Johann Le Maître[2], Martin Palmer[3], Eleanor Riches[3], Carlos Afonso[4], Pierre Giusti[2]
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Introduction:

The molecular description of complex organic mixtures, such as Petroleum, remains to be one of the most challenging analytical tasks. Structural and compositional knowledge is a requisite for developing efficient processing strategies and, thus, for optimal ecological/economic utilization of resources. In recent years, various ion mobility spectrometry technologies coupled to powerful mass spectrometric platforms became available. In general, drift-tube and TWIMS devices are limited in resolving power due to the given length of the IMS cell. Very recently, cyclic TWIMS (cIMS) was presented extending the drift length by circular folding of the path and allowing for multiple passes. In this study, the prospects for utilizing cIMS for molecular-level description of complex organic mixtures giving insights into isomeric diversity is presented.

Methods:

Various vacuum gas oils (VGOs), a heavy petroleum fraction in refining, differing in Sulphur- and Nitrogen content were investigated as ultra-complex model matrix. For analysis, a Waters Corporation cyclic ion mobility high-resolution time-of-flight mass spectrometry prototype was utilized. Positive mode electrospray and atmospheric pressure chemical ionization experiments were carried out. IMS resolving power was optimized by stepwise increasing the number of passes before registering a significant "wrapping around" effect, *i.e.*, fast/small ions surpassing slow/large ions in the circular device. Utilizing the Waters application programming interface in combination with self-developed Matlab-based processing schemes allowed to extract the full potential of the recorded multi-dimensional data.

Results:

In agreement with previous studies on VGOs, a continuum of the isomeric complexity, as predicted by the Boduszynski model for Petroleum, was revealed. Nonetheless, with increasing number of passes, individual features could be exposed for selected species. Moreover, the advanced processing of the mobilograms in combination with the mass spectrometric data enabled to overcome the narrow C_3/SH_4 mass split (3.4 mDa). Thus, features, which were previously - with common IMS platforms - not accessible could be analyzed. Aside from the apex CCS and separation of structural groups, the full width at half maximum served as a measure for the isomeric diversity. Higher-order moments, such as kurtosis (tailing) and skewness (symmetry), revealed additional information for the differences in isomeric complexity/diversity between the samples.

Conclusion:

Cyclic ion mobility spectrometry coupled to high-resolution time-of-flight mass spectrometry allowed more-in-depth insights into the isomeric complexity of complex samples from the field of Petroleomics. In particular, separating narrow mass splits by joint processing of the IMS and MS information is expected to have great potential for complex mixture application fields.

Plenary Lecture 8: Susan Richardson

Time: Wednesday, 04/Mar/2020: 12:10pm - 12:50pm · *Location:* Lecture Hall Building Chemistry: Hall C1
Session Chair: Uwe Karst

What's In My Drinking Water? Revealing the Chemicals We Can't See

Susan Richardson
University of South Carolina, United States of America

Drinking water disinfection by-products (DBPs) are an unintended consequence of using chemical disinfectants to kill harmful pathogens in water. DBPs are formed by the reaction of disinfectants with naturally occurring organic matter, bromide, and iodide, as well as from anthropogenic pollutants, such as pharmaceuticals and pesticides. Potential health risks of DBPs from drinking water include bladder cancer, early-term miscarriage, and birth defects. Several DBPs, such as trihalomethanes (THMs), haloacetic acids (HAAs), bromate, and chlorite, are regulated in the U.S. and in other countries, but other "emerging" DBPs, such as iodo-acids, halobenzoquinones, halonitromethanes, haloamides, halofuranones, and nitrosamines are not widely regulated.

This presentation will provide a state-of-the-science overview of the formation of DBPs and how we use gas chromatography (GC) and liquid chromatography (LC) with high resolution-mass spectrometry to comprehensively identify unknown DBPs. In addition, recent work will be presented on the impacts of hydraulic fracturing on DBP formation, as well as new research using granular activated carbon (GAC) to try to remove DBP precursors and make drinking water safer.