10th European Fourier Transform Mass Spectrometry Workshop

April 1-5, 2012
University of Warwick, UK

1991 Bielefeld, Germany	1993 Antwerp, Belgium	1995 Bremen, Germany	1997 Pont à Mousson, France	1999 Warwick, UK	2001 Kerkrade, The Netherlands	2004 Konstanz, Germany	2008 Moscow, Russia	2010 Lausanne, Switzerland	2012 Warwick, UK

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Tuesday, 20 March 2012

Dear EFTMS 2012 Delegates:

Welcome to the 10th European FTMS Conference, at the University of Warwick this year. This conference has been run roughly biennially, since 1991 and originally concerned FTICR mass spectrometers (custom and commercial, with many ionization sources) and ion molecule chemistry. At the time, few people believed that proteins could be routinely sequenced with mass spectrometers, or "unresolved complex mixtures" such as petroleum could now be resolved routinely.

While FTICR and ion chemistry are still relevant and present at this meeting, now 23 years later, the field has greatly expanded and we can now talk about FT-Orbitrap, FT-TOF, other advanced MS techniques which are competitive in performance, and are applied to a much wider range of projects from Top-down Proteomics to Petroleomics. This expansion has been a direct result of continuing improvement in instrumentation and fundamentals, which has naturally led to a tremendous increase in range and depth of the applications.

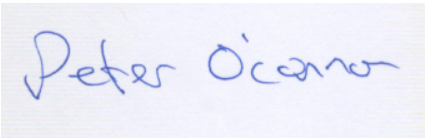
The success of this meeting, over the years, has been to bring together instrumentalists and chemists interested in both fundamentals and applications (both academic and industrial) in a setting which stimulates collaborations and intriguing discussions. We hope to achieve exactly this at the University of Warwick this year.

As chair of this programme, I must personally thank Yury Tsybin and Jon Amster whose recent experience in organizing highly successful meetings has provided both the template and the sounding board for ideas. I must also particularly thank Dr. Mark Barrow and Dr. David Kilgour for much of the organization groundwork and the rest of my research group for assistance throughout. Additionally, I must thank our administrative coordinators, Katie Dingley, Fiona Friel, Samantha Hall, and David Josey for endless help and perpetual patience. I'd also like to thank our Programme committee for assistance in organizing the oral sessions and acting as session chairs.

I would also like to thank our sponsors, Bruker Daltonics, Thermo-Fisher Scientific, Advion, Leco, Waters, and RSC publishing for providing some of the funding which allowed organization of this conference. In particular, I'd like to thank Bruker Daltonics who provided seed money back in 2009 which got the ball rolling.

Finally, thank you for your participation in this meeting, and I hope it is enjoyable by all!

Best wishes,



Prof. Peter B. O'Connor
Programme Chair, EFTMS 2012

BRUKER

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Analyst

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Thermo SCIENTIFIC

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THE SCIENCE OF WHAT'S POSSIBLE.™

10th European Fourier Transform Mass Spectrometry Workshop

Warwick Castle

Included as part of the package, EFTMS Workshop conference dinner will be hosted at Warwick Castle on April 4th. The evening will begin with a drinks reception and delegates will be welcome to visit the Kingmaker exhibit, showing Richard Neville (Earl of Warwick and known as "Warwick the Kingmaker") and his preparations for the Battle of Barnet in 1471. Afterwards, delegates will be guided to the Great Hall and the Undercroft, where there will be a three course meal, with beer, wine, and soft drinks served throughout.

Warwick Castle is situated next to the River Avon in the town of Warwick. The castle's origins began in 1068 when it was built by William the Conqueror, as a fortification in an Anglo-Saxon area, as he advanced further north. Substantial additions have been made since the 11th century and the castle has traditionally belonged to the Early of Warwick. During the 15th century, Richard Neville used Warwick Castle to imprison Edward IV, the king of England. During the 17th century, the defences were improved in preparations for the English Civil War(s) and the castle was controlled by Parliamentarian forces, with Royalist forces later laying siege. The death mask of Oliver Cromwell (who led the overthrow of the monarchy and temporarily created a republican Commonwealth) can still be seen today within Warwick Castle. In the 18th century, the castle grounds and gardens underwent landscaping by "Capability" Brown. Warwick Castle remained in the ownership of the Greville family between 1759 and 1978, at which point it was purchased by the Tussauds Group and later acquired by Merlin Entertainments Group (owners of the Madame Tussauds waxworks display in London, amongst other attractions).

Origins of the logo

The bear and ragged staff is an emblem that has long been associated with Warwick and was first used by the Beauchamp family, who became earls of Warwick in 1268, as a mark of identity in to addition to their own coat of arms. Originally, the two items appear to have been used independently. The bear appears on the tomb of Thomas Beauchamp I, who died in 1369, at St Mary's Church in Warwick.

The earliest known usage of the bear and ragged staff together is associated with Thomas Beauchamp II (Earl from 1369 to 1402), who owned a bed of black material embroidered with a golden bear and silver staff. His great seal of 1397 depicts the Beauchamp coat of arms between two bears, and his privy (or private) seal of the same date a bear on all fours with a ragged staff behind. The great seal of his son, Richard Beauchamp (Earl from 1402 to 1439), has a crest supported by two bears each holding a ragged staff. His tomb can be found at the centre of the Beauchamp Chapel on the south side of St Mary's Church and has an inscription in which the words are separated alternatively by bears and ragged staffs. Richard is known to have used banners embroidered with bears or ragged staffs, although not with the two combined.

Richard Neville, the "Kingmaker" and who married Richard Beauchamp's daughter and heir, was another who used the bear and ragged staff together. From as early as 1454, he used a seal bearing the impression of the bear and ragged staff to authenticate deeds and letters. He also used the emblems as separate badges: in 1458 his retainers are recorded as wearing red coats with silver staffs only embroidered front and rear.

Robert Dudley, Earl of Leicester, favourite of Queen Elizabeth I, and great-great-great-great-grandson of Richard Beauchamp, is known to have used the combined the bear and ragged staff frequently. This can be seen in many places on the walls of the Leicester Hospital in Warwick, which he founded in 1571, and on a chimney piece in his castle in Kenilworth. Inventories of the furnishings of the castle mention cushions, bedcovers, and bookbindings decorated with the design, and his suit of armour, now in the Royal Armoury, is heavily decorated with ragged staffs.

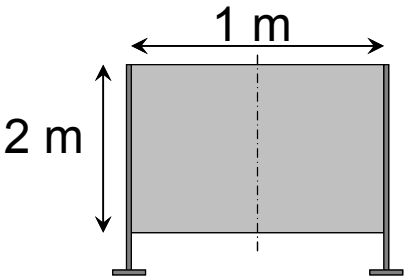
In 1759, Francis Greville, Earl Brooke of Warwick Castle, was created Earl of Warwick. The Greville family were distantly related to the Beauchamp family, and had acquired Warwick Castle in 1604. In the following year, Francis obtained a grant for himself and his heirs of “the crest anciently used by the Earls of Warwick,” that is “a bear erect argent, muzzled gules, supporting a ragged staff of the first.”

The flag below is that used by the county of Warwickshire. The “three cross-crosslets gules” in the county’s arms are taken from the arms of the Beauchamps, who were Earls of Warwick from 1268 to 1449. They are perhaps the most famous of all the families which have held the earldom of Warwick, and this together with the world-wide fame of the Beauchamp Chapel in St Mary’s Church in Warwick, makes the inclusion of their arms in the County’s armorial bearings particularly appropriate.

General Information

The conference will take place at the Ramphall Building on the University of Warwick Campus, with meals and drinks in the Rootes Bar and Restaurant on the 1st floor above Rootes Reception. Check-in for accommodation is at Rootes Reception. Arrows and signs will show the way from venue to venue. For more information, see this link:
http://www2.warwick.ac.uk/conferences/howtofindus/

All mobile phones must be turned off or set to vibrate during all oral sessions. Courtesy is appreciated.



Posters should be set up on Monday morning, April 1st, before the first session at 9 AM and will remain in place until Thursday morning after the last session at 11 AM. Posters must be removed no later than Thursday April 4th, at 12:00, noon. Please see the conference programme for the appropriate poster number. Exhibitor booths and tables will be mingled with the posters.

Presentations can use either the speaker's laptop or a conference PC. Unless otherwise notified, talks should be 20 minutes with an additional 5 minutes scheduled for questions. Student-helpers will be present in the aisles with microphones.

Warwick Conferences Information

Car Parking:
Complimentary car parking is available for conference delegates in the allocated car parks on campus (7, 8a and 15). When you enter the car park, take the token (or ticket in car park 8a) from the machine at the entrance, which you will need to validate at Rootes Building Reception. Disabled parking spaces are available close to the entrance of main buildings. As a University campus, from time to time these car parks become full and when this happens alternative parking will be available, which you will be directed to. We advise that you allow sufficient time for up to a ten minute walk to get to your destination on the Conference Park from the car parks. Some of the car parks are not adjacent to the registration and accommodation areas, it is therefore advisable once you have parked, for you to take your luggage to the Rootes Building where you will be able to leave it with the team in the left luggage facility.

For more information, view the location of the car parks on our campus map:
http://www2.warwick.ac.uk/conferences/howtofindus/colour_map.pdf

Information and luggage point:
Within Rootes Reception there is an information point for all delegates. Here you can:

- Arrange for secure luggage storage
- Validate your car parking ticket or token
- Arrange your log in codes for wifi computer access around campus
- Ask about any lost property
- Enquiries about any parcels which have been delivered in advance of an event
- Access to the Business Centre

Rootes Reception:
The Reception team is available to answer your queries between 7.00am – 11:00pm at the Rootes Building.

Bedroom check in/out
Bedroom keys will be available from 3.00pm to 11.00pm, at Rootes Reception. If you plan to arrive after 11.00pm, please contact your Event Organiser to arrange late key collection. Rooms need to be vacated by 9.30am on your day of departure. Please inform Reception on arrival, of any difficulties you may have in the unlikely event of an evacuation from your accommodation (e.g. hearing or mobility difficulties).

Keys
You will be provided with one key which will access your room and entry door to the residence. Keys can be left at Rootes Reception, Rootes Restaurant or one of the boxes situated in the entrance halls of each residence.

Internet access across campus:
PC's with free internet and email access are available for both day and residential guests in Rootes Reception. These may be accessed 7:00am – 11:00pm Monday – Sunday. If you have your own mobile computer then you can access the wifi network by requesting a log in code at Rootes Reception. The following locations on campus have wifi capacity:

- Warwick Arts Centre – Café Bar
- Science Concourse
- Library Building
- Ramphal – Foyer and Lecture Theatre
- Rootes Building – Panorama, Chancellors, Rootes Reception and the Bar
- Students Union Atrium
- Engineering
- Social Sciences

Internet access in accommodation:
Free access to the internet is available in your bedroom. Ethernet cables are required for this and are available to purchase from reception or alternatively you can bring your own.

Food and Drink:
All meals are provided in Rootes Restaurant located on the first floor of Rootes Building for all delegates (unless your programme indicates otherwise). The restaurant offers an assisted style service of breakfast, lunch and dinner including a range of hot and cold drinks. Your Event Organiser will be able to advise you regarding the specific arrangements for your event. Please have with you your conference badge or room key to gain access to the restaurant. If you have any special dietary requirements then please inform your Event Organiser.

The bar is located on the first floor of Rootes Building and is the ideal place to network and relax after a day's session. The bar serves draught beers, a selection of bottled beers, wines, spirits, soft drinks and a variety of teas and coffees. There are also alternative bars in Warwick Arts Centre and Students Union building's.

Shops, Banks, Café's and Bars on campus:
The campus has many facilities available to all delegates, for all information and opening times please see the website: http://www.warwickretail.com

Sports facilities:
All guests have full use of the comprehensive sports facilities including swimming, squash and tennis.

Details and opening times are available at Rootes Reception or by visiting the website below. Delegates need to present their bedroom key at the reception to gain access. See www2.warwick.ac.uk/services/sport for more information.

For more information:
You can also refer to our Frequently Asked Questions document (FAQ's) which can be found on the website at www2.warwick.ac.uk/conferences/faqconference

History

Year	Organizer	Location
1991	Hanz Friedrich Grutzmacher	Bielefeld, Germany
1993	Luc van Vaeck	Antwerp, Belgium
1995	Karl Peter Wanczek	Bremen, Germany
1997	Jean-Francois Muller	Pont à Mousson, France
1999	Peter Derrick	Warwick, UK
2001	Ron Heeren	Kerkrade, The Netherlands
2004	Michael Przybylski	Konstanz, Germany
2008	Eugene Nikolaev	Moscow, Russia
2010	Yury Tsybin	Lausanne, Switzerland
2012	Peter O'Connor	Warwick, UK


THE UNIVERSITY OF WARWICK
WESTWOOD
SCIENCE PARK
GIBBET HILL
TOCL WOOD
Conference venue
Registration, meals, receptions
Accommodation
FT-ICR Lab
BUILDING KEY
SYMBOLS
For the most up-to-date version of this map go to warwick.ac.uk/go/maps
For further information see the University web site or mobile site www.m.warwick.ac.uk

Sunday, April 1, 2012
15:00 - 19:00 Registration in Rootes Reception
18:30 - 20:30 Dinner in Rootes Restaurant
20:00 - 22:00 Reception in Rootes Bar

Monday, April 2, 2012
08:00 Poster's and Exibitors can set up
07:30 - 09:00 Breakfast in Rootes Restaurant
09:00 Chair's Welcome

09:00 - 10:15 Session 1: The Future of FTICR-MS (chair: Yury Tsybin)
09:00 - 09:50: **Alan G. Marshall**, *FT-ICR MS: Directions for Future Applications*
09:50 - 10:15: **Eugene Nikolaev**, *Progress on Dynamically Harmonized FT-ICR Cell Implementation*
10:15 - 10:20: **Bruker Daltonics***: And now a word from our sponsors!*

10:20 - 11:15 Coffee and Posters

11:15 - 12:55 Session 2: ICR simulation and development (chair: Ron Heeren)
11:15 - 11:40: **Jon Amster**, *Using Particle-In-Cell Simulations for Improving the Design of FTICR Analyzer Cells*
11:40 - 12:05: **Yury Tsybin**, *Filter diagonalization method-based super-resolution mass spectrometry: the first steps*
12:05 - 12:30: **Simon Van Gorp,** *Simbuca, using a graphics card to simulate Coulomb interactions in a penning trap*
12:30 - 12:55*:* **Maria A. van Agthoven***, 2D FT-ICR MS: new instrumental and software developments for protein and polymer analysis*
12:55 - 14:30 Lunch in Rootes Restaurant

14:30 - 15:45 Session 3: Post-translational modifications (chair: Michael Przybylski)
14:30 - 14:55: **Kathrin Breuker,** *Disulfide vs. Backbone Bond Cleavage in Electron Capture Dissociation of Proteins*
14:55 - 15:20: **Joseph Gault,** *Post Translational Modification Key to Pathogenesis in Bacterial Meningitis Designing a One Shot Approach to PTM Analysis*
15:20 - 15:45: **Helen Cooper,** *Direct Surface Sampling Top-down Fourier Transform Mass Spectrometry of Neonatal Dried Blood Spots for the Diagnosis of Unknown Haemoglobin Variants*
15:45 - 15:50: **Thermo-Fisher Scientific***: And now a word from our sponsors!*

15:50 - 16:30 Coffee and Posters

16:30 - 17:45 Session 4: Petroleum analysis (chair: John Headley)
16:30 - 16:55: **Wolfgang Schrader,** *Analysis of asphaltenes from heavy crude oil resources by using 12T LTQ FT-ICR and a research-type high-field Orbitrap*
16:55 - 17:20: **Mark Barrow**, *Application of atmospheric pressure photoionization Fourier transform ion cyclotron mass spectrometry for the comparison*
17:20 - 17:45: **Matthias Witt**, *Analysis of complex mixtures with the new dynamically harmonized ICR cell*

17:45 - 19:00 Reception and Posters
17:45 - 19:00 Simultaneous Bruker Workshop in R0.14
19:00 - 20:30 Dinner in Rootes Restaurant
20:30 - 22:00 Reception in Rootes Bar

Tuesday, April 3, 2012
07:30 - 09:00 Breakfast in Rootes Restaurant

09:00 - 10:15 Session 5: Electrostatic Traps (chair: Pat Langridge-Smith)
09:00 - 09:25: **Ryan Hilger**, *An Electrostatic Linear Ion Trap Mass Spectrometer for Analysis of Small Molecules, Peptides, and Proteins*
09:25 - 09:50: **Jan-Peter Hauschild,** *Pushing the Limits of Orbitrap Benchtop FTMS*
09:50 - 10:15: **Anatoly Verenchikov**, *A Path from Multi-Reflecting TOF to Electrostatic Traps*
10:15 - 10:20: **Leco:** *And now a word from our sponsors!*

10:20 - 11:15 Coffee and Posters

11:15 - 12:55 Session 6: Advances in Fragmentation Methods (chair: Helen Cooper)
11:15 - 11:40: **Jackie Mosely**, *Electron-induced dissociation: an enabling technology for the characterisation of small molecules*
11:40 - 12:05: **Julia Chamot-Rooke**, *What factors influence ECD fragmentation pathways?*
12:05 - 12:30: **Thiago Correra,** *IRPD spectroscopy of X-(H2O)n X = Br-, F-, I- ions*
12:30 - 12:55*:* **Jos Oomens,** *Structure of anionic b- and c-type peptide fragments by IRMPD spectroscopy*

12:55 - 14:30 Lunch in Rootes Restaurant

14:30 - 15:45 Session 7: Protein Structure (chair: Jackie Mosely)
14:30 - 14:55: **Michael Przybylski**, *High Resolution affinity mass spectrometry in the elucidation of oligomerisation-aggregation structures of target proteins for neurodegeneration*
14:55 - 15:20:**Christophe Borchers,** *Deciphering Protein Conformational Transitions by Top-Down ECD/HDX FTMS*
15:20 - 15:45: **Jon Williams**, *ETD and Hemoglobin Variants*
15:45 - 15:50: **Waters**: *And now a word from our sponsors!*

15:50 - 16:30 Coffee and Posters

16:30 - 17:45 Session 8: Analysis of High Resolution Data (chair: Jon Amster)
16:30 - 16:55: **Pat Langrige-Smith**, *Investigating the Molecular Details of p53 Redox Modifications using FT-ICR Mass Spectrometry*
16:55 - 17:20: **Robert Mistrik,** *Novel Type of Spectral Library for the Identification of "Unknown Unknowns"*
17:20 - 17:45: **David Kilgour,** *Deriving confidence metrics for automatic peak assignment through n-dimensional Kendrick defect or mass difference inference networks*
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17:45 - 19:00 Reception and Posters
19:00 - 20:30 Dinner in Rootes Restaurant
20:30 - 22:00 Reception in Rootes Bar

Wednesday, April 4, 2012
07:30 - 09:00 Breakfast in Rootes Restaurant

09:00 - 10:15 Session 9: Improved Instrumentation (chair: David Kilgour)
09:00 - 09:25: **Lutz Schweikhard**, *Penning traps in physics research, developments and applications from highly-charged atomic ions to metal-cluster studies to precision mass measurements of exotic nuclei*
09:25 - 09:50: **Li Ding,** *An Electrostatic Ion Trap with Planar Rotational Field Structure*
09:50 - 10:15: **F. Schinle**, *Improving trapped ion spectroscopy with a conically wired hexapole*
10:15 - 10:20: **Advion:** *And now a word from our sponsors!*

10:20 - 11:15 Coffee and Posters

11:15 - 12:55 Session 10: Imaging (chair: Kathrin Breuker)
11:15 - 11:40: **Delphine Debois,** *Contribution of high mass resolution and accuracy of FTMS to molecular imaging*
11:40 - 12:05: **Don Smith,** *C60 Secondary Ion FT-ICR MS for High Mass Accuracy and High Mass Resolving Power SIMS Imaging*
12:05 - 12:30: **Julia Laskin**, *Chemical Imaging of Biological Systems Using Nanospray Desorption Electrospray Ionization Mass Spectrometry*
12:30 - 12:55: **Ron Heeren**, *Molecular signals on surfaces studied with high resolution imaging mass spectrometry*

12:55 - 14:30 Lunch in Rootes Restaurant

14:30 - 15:45 Session 11: High resolution and Data Analysis (chair: Julia Laskin)
14:30 - 14:55: **Basem Kanawati,** *New coupled frequency to both end caps for enhanced infinity operation of ICR cells. SIMION Study*
14:55 - 15:20: **M. Heck,** *One- and two-pulse quadrupolar excitation schemes of the ion motion in a Penning trap investigated with FT-ICR detection*
15:20 - 15:45: **Huilin Li,** *Protein Flexibility is Key to Cisplatin Cross-linking in Calmodulin*
15:45 - 15:50: *And now a word from our sponsors!*

15:50 - 16:30 Coffee and Posters
16:30 - 18:30 Lab tours and transport to Warwick Castle

18:45 Coaches leave University to Warwick Castle
19:30 - 23:00 Conference dinner at Warwick Castle
23:00 transport from Warwick Castle

Thursday, April 5, 2012
07:30 - 09:00 Breakfast in Rootes Restaurant

09:25 - 10:15 Session 12: Proteins and PTM's (chair: Julia Chamot-Rooke)
09:25 - 09:50: **Pilar Perez,** *Monitoring Deamidation of Collagen Peptides in Ancient Bones Using FT-ICR-MS*
09:50 - 10:15: **Andrea Lopez,** *Binding site identification of glyoxal in Substance P by mass spectrometry.*

10:15 - 11:05 Session 13: 21 Tesla (chair: Mark Barrow)
10:15 - 10:40: **Christopher Hendrickson**, *21 Tesla Hybrid Fourier Transform Ion Cyclotron Resonance Mass Spectrometer: An International Resource.*
10:40 - 11:05: **Jean Futrell,** *Design Considerations for FT-ICR MS at 21 T: A Progress Report*

11:10 Chair's Thanks
11:10 - 12:05 Coffee and Posters
12:05 - 14:30 Packed Lunch and departure

Speakers

FT-ICR MS: Directions for Future Applications

Alan G. Marshall, Huan He, Yuan Mao, Santosh Valeja, Quan Zhang, Christopher L. Hendrickson, Amy M. McKenna, and Ryan P. Rodgers

ICR Program, NHMFL, Florida State U., 1800 E. Paul Dirac Drive, Tallahassee Florida 32310-4005 U.S.A.

As FT-ICR MS heads toward its 40th year, it is appropriate to focus on applications that are uniquely accessible by virtue of its ultrahigh mass resolution and accuracy, in view of continuing improvements in orbitrap and multi-pass TOF instruments [1]. Examples to be discussed range from isotopic fine structure for a single molecule to resolution/identification of components of complex mixtures. Isotopic fine structure provides validation of elemental composition assignment based on accurate mass for a monoisotopic ion, as for sulfated eukaryotic lipids (e.g., human, biofuels [2]). FT-ICR MS continues to contribute uniquely to the field of "petroleomics" (understanding of crude oil properties and behavior at the molecular level): e.g., nature of undistillable "asphaltenes", as well as MS/MS to provide detailed understanding of crude oil distillation. FT-ICR MS enables identification and biological/chemical changes in environmental spills (e.g., the Deepwater Horizon wellhead spill of 2010). Bottom-up proteomics benefits from identification of glycosylation or phosphorylation before MS/MS [3]. Top-down proteomics can access post-translational modifications of proteins up to 150 kDa [4]. Hydrogen/deuterium exchange monitored by FT-ICR MS provides non-perturbative mapping of contact surfaces in large protein complexes: antigen:antibody epitopes [5], tRNA synthetase and protein chaperone complexes, and other protein assemblies inaccessible by NMR or x-ray crystallography. Work supported by NIH (R01 GM78359), NSF Division of Materials Research through DMR-06-54118, NSF CHE-10-49753 (RAPID), and the State of Florida.

1. Xian, F.; Hendrickson, C. L.; Marshall, A. G. "High Resolution Mass Spectrometry," Anal. Chem. 2012, 84, 708-719.
2. He, H.; Rodgers, R. P.; Marshall, A. G.; Hsu, C. S. "Algae Polar Lipids Characterized by On-Line Liquid Chromatography Coupled with Hybrid Linear Quadrupole Ion Trap/Fourier Transform Ion Cyclotron Resonance Mass Spectrometry," Energy & Fuels 2011, 25, 4770-4775.
3. Mao, Y.; Zamdborg, L.; Kelleher, N. L.; Hendrickson, C. L.; Marshall, A. G. "Identification of Phosphorylated Human Peptides by Accurate Mass Measurement Alone," Int. J. Mass Spectrom. (Eyler Issue) 2011, 308, 357-361.
4. Valeja, S. G.; Kaiser, N. K.; Xian, F.; Hendrickson, C. L.; Rouse, J. C.; Marshall, A. G. "Unit Mass Resolution for an Intact 148 kDa Therapeutic Monoclonal Antibody by FT-ICR Mass Spectrometry," Anal. Chem. 2011, 83, 8391-8395.
5. Zhang, Q.; Willison, L. N.; Tripathi, P.; Sathe, S. K.; Roux, K. H.; Emmett, M. R.; Blakney, G. T.; Zhang, H.-M.; Marshall, A. G. "Epitope Mapping of a 95 kDa Antigen in Complex with Antibody by Solution-Phase Amide Backbone H/D Exchange Monitored by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry," Anal. Chem. 2011, 83, 7129-7136.

Progress on Dynamically Harmonized FT-ICR Cell Implementation

Eugene Nikolaev, Roland Jertz, Anton Grigor'ev, Igor Popov, Jens Fuchser, Claudia Kriete, Matthias Witt, Jochen Friedrich, Gokhan Baykut

Leninskij pr. 38 k.2, Moscow 119334 ,Russia

Introduction

Application of FT-ICR MS in the field of top-down proteomics and protein modification analyses via accurate mass measurements is demanding for further increase of the mass resolution. The recently introduced novel FT-ICR cell [1,2] based on a Penning ion trap with specially shaped excitation and detection electrodes prevents distortion of ion cyclotron motion phases (normally caused by non-ideal electric trapping fields) by averaging the trapping DC electric field during the ion motion in the ICR cell. This effect results in mass resolving powers which exceed any values obtained up to now.

Methods

The performance of the novel FT-ICR cell with shaped electrodes has been characterized by isotopically resolved mass spectra of proteins close to 200kDa as well as fine structure of resolved isotopic peaks of peptides and small proteins.

Preliminary Data

The realistic modeling of ion cloud motion using the particle-in-cell approach shows that excited ion clouds in this new ICR cell are very stable and do not lose their integrity for a very long time. Accordingly, detection times of 5 minutes resulting in resolving power close to 40,000,000 have been reached for reserpine at m/z 609 at a magnetic field of only 7 Tesla. Furthermore, fine structures of resolved 13Cn isotopic cluster groups could be measured for molecular masses up to 5.7 kDa (insulin) with resolving power of 4,000,000 at 7 Tesla. Based on resolved fine structure patterns atomic compositions can be directly determined using a new developed algorithm for fine structure processing. Extremely high mass accuracy below 100 ppb (RMS mass error) could be achieved in complex mixtures. As example for high mass species, proteins and multimers of proteins could be measured reaching masses up to 186 kDa (enolase tetramer) with isotopic resolution. For instance, at 7 Tesla resolving power of 800,000 was achieved for enolase dimer (96kDa) and 500,000 for molecular masses above 100 kDa. Experimental data indicate that there is practically no limit for the resolving power of this ICR cell except by collisional damping in the ultrahigh vacuum chamber.

Novel Aspect

Highest FTICR resolving power, determination of atomic compositions by isotopic fine structure evaluation, isotopically resolved highest mass.

Acknowledgements

This work was supported by Russian Foundation for Basic Research through grants Nos. 10-04-13306, 09-04-12130 and 09-04-12225, Russian Ministry of Education and Science contracts 14.740.11.0755, 16.740.11.0369.

References

1.Boldin, I.A.; Nikolaev, E.N. Fourier transform ion cyclotron resonance cell with dynamic harmonization of the electric field in the whole volume by shaping of the excitation and detection electrode assembly. Rapid Commun. Mass Spectrom. 2011, 25, 122-126.

2.Nikolaev, E. N.; Boldin, I. A.; Jertz, R.; Baykut, G. Initial Experimental Characterization of a new ultra-high resolution FT-ICR Cell with Dynamic Harmonization, J. Am. Soc. Mass Spectrom. 2011, 22, 1125-1129"

Using Particle-In-Cell Simulations for Improving the Design of FTICR Analyzer Cells

Josh Driver, Andriy Kharchenko, Ron Heeren, Jon Amster

Department of Chemistry, University of Georgia, Athens, GA 30602-2556 USA

Accurate Image Charge calculations

Particle-In- Cell (PIC) calculations provide invaluable insight into ion motion in an FTICR analyzer cell. This method is able to provide accuracy for large numbers of ions without the large increase in computational time required by most other methods. However, until recently, the level of inference from an ICR cell simulation was limited by the ability to account for the image charge because the latter had been a hard task computationally. The existing capacitance matrix/image charge method (CMM) provided for moderate room for performance optimization due to relying on solving the Poisson equation. On the other hand, the use of another method, such as the charge collocation method (CCM), was not acceptable because it relies on the inversion of large general matrices which, in practice, requires iterative gradient-based approaches, making the process even more computationally expensive than the CMM or even impractical. Fortunately, a new piece of hardware has become widely available, e.g. graphical processing units are ad hoc processors capable of handling matrix-intensive calculations. Following the introduction of unified programming interfaces like CUDA, the use of ad hoc processors in FTICR simulations has allowed for using a significantly higher resolution image charge considerations. Results will be shown comparing the frequency shifts observed for simulations using the charge collocation method via the UGA's GPU cluster.

Spherical Harmonics

A current trend in cell design is static or dynamic harmonization of the trapping potential aimed to minimize cyclotron motion damping and loss of coherence in the motion of like ions as a result of compromised harmonicity of the trapping potential. Examining fields of real cells in the form of spherical or cylindrical harmonic expansion has become a popular framework after works by Marshall, Grosshans, Barlow , Tinkle, and others. Apart from a unified view of a field as an easily interpretable combination of the ideal and a series of detrimental (inharmonic) components, it provides for a way to tweak the inharmonic components independently to improve the cloud destruction process. We will show the results of real ICR cell simulation in the form of spherical harmonic expansions paying special attention to the real field approximation techniques including the approximation with respect to the cloud destruction process.

Filter diagonalization method-based super-resolution mass spectrometry: the first steps

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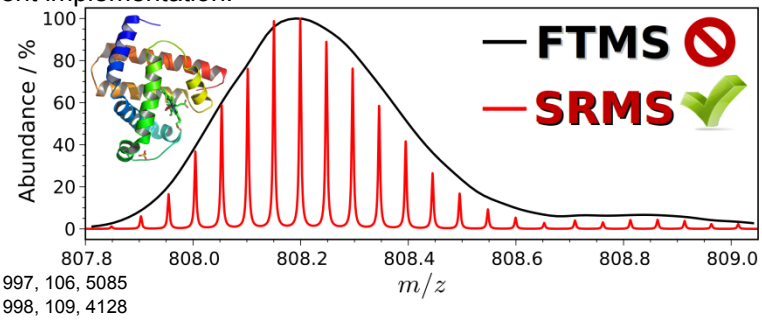
Comprehensive structure analysis of molecules and macromolecules present in the extremely complex samples or performed under time-constrained experimental conditions demands a substantial increase in the acquisition speed of high resolution MS data. Therefore, the advancement of the FTMS-based applications requires achieving required resolution for shorter transient signals or higher resolution for a defined length of the transient signal. Super-resolution signal processing, particularly based on a filter diagonalization method (FDM), should be able, in principle, to provide the required resolution for shorter experimental transient signals in ion cyclotron resonance (ICR) MS compared to the Fourier transform (FT) processing. FDM is a relatively recent method, especially as a signal processing tool. The standard FDM algorithm was published in 1997-1998 [1, 2], further characterized and validated over the years [3, 4, 5]. During the very last years its particular aspects have been further improved and developed [6]. Recently, Aizikov and O'Connor applied generic FDM to trace frequency modulation effects in the experimental transient signals in FT-ICR MS and demonstrated super-resolution mass spectra obtained by FDM for the simulated transients [8]. Here, we will present the preliminary data on the development of a routine FDM-based MS, termed super-resolution MS (SRMS), and demonstrate its initial implementation in ICR MS [9].

MS experiments were performed using 10 T LTQ FT-ICR MS (Thermo Scientific, Bremen, Germany). Transient time-domain signals were recorded in MIDAS data format. FDM-based SRMS implementation on a quad-core workstation using in-house developed Python-based software was extensively tested and optimized against simulated and experimental transient signals. Results indicate that FDM-based SRMS may indeed present a number of advantages compared to other signal processing methods, including FT, Figure 1.

The considered SRMS applications are in bottom-up and top-down proteomics, metabolomics, imaging and petroleomics. We will also discuss the current limitations of the FDM-based SRMS and some technical details of its current implementation.



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Simbuca, using a graphics card to simulate Coulomb interactions in a penning trap

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In almost all cases, N-body simulations are limited by the computation time available. Coulomb interaction calculations scale with O(N^2) with N the number of particles. Approximation methods exist already to reduce the computation time to O(NlogN), although calculating the interaction still dominates the total simulation time. Simbuca [1], a Penning trap simulation package, was developed to speed up the simulation time tremendously by using a graphics card (GPU) to calculate the Coulomb interaction between the ions. This complete and modular Penning trap simulation package was initially developed for the WITCH experiment [2,3] and is currently also being used by around 6 other experiments for analysis as well as investigation of space charge effects. Simbuca uses the output of the Cunbody-1 library [4], which calculates the gravitational interaction between entities on a graphics card, and adapts it for Coulomb calculations. Furthermore the program incorporates three realistic buffer gas models, the possibility of importing realistic electric and magnetic field maps and different order integrators with adaptive step size and error control. The software is released under the GNU General Public License and free for use. This approach is also superior to the octree GPU code [5], which is the first PIC code on a GPU. Finally, the GPU programming field is a novel field that is rapidly finding its way to new applications of which the gains for simulation purposes can be enormous.

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2D FT-ICR MS: new instrumental and software developments for protein and polymer analysis

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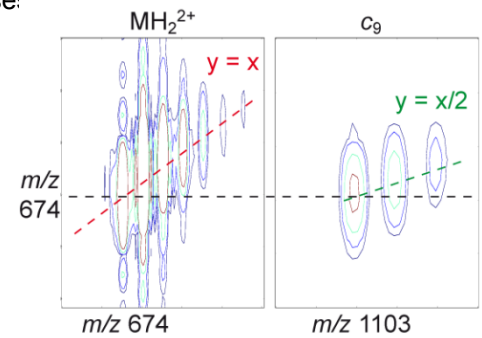
In 2010, we revisited two-dimensional FT-ICR MS developed in 1988 by Gäumann and Bodenhausen, which has no analytical applications yet [1-2]. 2D FT-ICR spectra convey the MS to MS/MS correlations for all compounds in a complex sample in one experiment without precursor ion isolation. We followed three lines: (i) use of gas-free fragmentation modes like IRMPD and ECD instead of in-cell CID to preserve high resolution, (ii) development of new processing algorithms for the large files required for high resolution and for scintillation noise removal [3] and (iii) new pulse sequences to remove self-correlation peaks which are detrimental to sensitivity. We describe the application of these techniques to proteins and PAMAM dendrimers with masses above 10,000 Da.

As proteins, we analyzedubiquitin, myoglobin, and carbonic anhydrase and as polymers PAMAM dendrimers from generations 0 (520 Da) to 3 (6936 Da) which mimick peptides on a Bruker Daltonics 9.4 T hQh-FTICR-MS using nanoESI ionization with a modified experimental script for the Gäumann-Bodenhausen pulse sequence [1] with ECD and IRMPD for fragmentation with mass ranges up to m/z 86-2500 in both dimensions [2]. In our preliminary experiments, time transients were limited to 32k datapoint mass spectra by 2048 scans in the vertical dimension since the 32-bits software could not handle larger data files. The large files generated by 2D FT-ICR MS experiments (>4 Gb) were processed by a dedicated data processing package based on 64-bit Python language using the Hierarchical Data Format (HDF5) and high resolution display. This allows data processing and visualisation of 2D mass spectra expressed in m/z ratios for the first time, with peak-picking and profile-viewing capabilities. To eliminate high-intensity but low-information autocorrelation peaks and harmonics from the 2D mass spectra, we introduced phase cycling in the pulse sequence. This improves the signal-to-noise ratio for fragment ion peaks and eliminates spurious peaks without removing the information about the nature of their precursors. To correct phase errors, we digitally demodulated the data in the vertical dimension to remove secondary autocorrelation lines. Scintillation noise caused by fluctuations in ionization efficiency was filtered out with an optimized implementation of Cadzow's algorithm, as described previously [3]. 2D mass spectra show both fragment ion spectra and precursor ion spectra for complex samples, but also characteristic lines for the capture of one or more electrons in ECD, the evolution of peptide and protein fragmentations with charge, and neutral losses



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Disulfide vs. Backbone Bond Cleavage in Electron Capture Dissociation of Proteins

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Soon after the introduction of electron capture dissociation (ECD) in 1998 [1], it was proposed that cleavage of disulfide bonds is preferred over protein backbone cleavage in ECD [2]. However, in 'activated ion' ECD experiments of ecotin, a 142-residue protein with a disulfide bond between C50 and C87, we have observed nearly complete sequence coverage from c- and z•-type fragments from backbone cleavage in regions of the protein that are not bridged by the disulfide bond, and no fragments from backbone cleavage in the region bridged by the disulfide bond. To further investigate this puzzling observation, we have studied the proteins trypsin inhibitor (180 residues) with two disulfide bonds (C39/C86 and C136/C145) and aprotinin (58 residues), which has three disulfide bonds (C5/C55, C14/C38, and C30/C51), by collisionally activated dissociation (CAD), ECD, and infrared (IR) laser activation.

Consistent with our initial data for ecotin, ECD of trypsin inhibitor and aprotinin did not produce c- and z•-type fragments from backbone cleavage in regions of the proteins that are bridged by disulfide bonds. However, rigorous ion activation by infrared multiphoton dissociation (IRMPD) did produce b- and y-type fragment ions from cleavage in regions bridged by disulfide bonds. Collisional activation before ECD, and infrared activation of reduced molecular ions after ECD, was performed to further investigate the fragmentation of disulfide-bonded proteins. Our data suggest that the competition between disulfide bond and backbone cleavage is more complex than initially anticipated.

Post Translational Modification Key to Pathogenesis in Bacterial Meningitis Designing a One Shot Approach to PTM Analysis

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Post translational modifications (PTM) are increasingly found to be key intermediates in pathogenesis pathways [1-3]. Understanding their role on the molecular level not only greatly increases our comprehension of disease mechanisms but provides an essential basis onto which human intervention strategies can eventually be built.

Mass spectrometry is often implicated somewhere in this process, whether as the primary tool in pinpointing a modified protein or the method of choice for directly demonstrating a role in pathogenesis.

In recent work we identified a post translational modification present on the type IV pili of Neisseria meningitidis. This PTM is induced in vivo after several hours of host cell contact and we hypothesise that the subsequent alteration of the pilus surface ultimately leads to the dissemination of the bacterium; a step that forcibly precedes invasive infection [4].

A combination of top down and bottom up methods were used for characterisation of the protein pilE, the major building block of the pilus, and to localize the modifications of interest. However, an effective top down approach for localisation of all PTM's had at that time not been developed.

Here we present top down results using both Oribitrap and FT-ICR spectrometers and a variety of fragmentation methods, ECD, ETD, CAD, HCD, IRPMD and combinations thereof, for the localisation of the 5 modifications present on this protein. Unusually we also present comprehensive profiling of several of these fragmentation techniques performed on pilE itself rather than the more common, model standards.

Aspects of the techniques, fragmentation mechanisms and applicability to other systems will be discussed along with the major implications of a "one shot" approach to understanding bacterial dissemination.

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Direct surface sampling of dried blood spots coupled with Fourier transform mass spectrometry for quantitative analysis of haemoglobin variants

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Variants of haemoglobin, or haemoglobionopathies, are the most common type of inherited disorders. They are categorized into two groups: the structural variants and the thalassaemias. Structural variants arise via point mutations in the globin chains. The most clinically significant structural variant is the sickle variant HbS. The thalassemias are caused by impaired production of the protein chains that make up haemoglobin. β-thalassemia results from a lack of, or reduced synthesis of, the β-chain. It is characterised by severe anaemia and skeletal abnormalities in infancy. Similarly, α-thalassemia results from absence or reduced synthesis of the α-chain. If undetected, α-thalassemia major will result in stillbirth. Both types of variants are commonly detected in newborn screening programmes using high pressure liquid chromatography and/or isoelectric focusing.

We have previously shown that liquid extraction surface analysis (LESA) of dried blood spots by use of the Advion Triversa Nanomate, coupled with high resolution top-down mass spectrometry, can be applied to the identification of the common structural variants (HbS, HbC and HbD)[1]. Determination of structural Hb variants by this method relies on the identification of mass shifts in either the precursor or MS/MS fragment ions.

Here, we investigate whether the LESA approach may be applied to the identification of thalassemia; i.e., can haemoglobin be quantified by this method? The haemoglobin concentration in blood samples from healthy volunteers were determined by spectrophotometry. Blood samples were spotted onto filter paper and allowed to dry. The dried blood spots were sampled by LESA and either the samples were collected or analysed by mass spectrometry. The haemoglobin concentration in the collected LESA samples were determined by spectrophotometry and compared with (a) the Hb concentration in the whole blood samples and (b) the LESA mass spectra obtained for the dried blood spots.

Analysis of asphaltenes from heavy crude oil resources by using 12T LTQ FT-ICR and a research-type high-field Orbitrap Mass Spectrometer

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Introduction

Due to diminishing conventional resources of crude oil, especially the light crudes, larger parts of the energy have to be obtained from unconventional assets in the intermediate future. These unconventional resources include heavy crude oils, shale oils or the famous Canadian and Venezuelan oil sands. Many of the problems associated with recovery, separation or processing of crude oils are related to the presence of high concentrations of asphaltenes. The asphaltene fraction is defined by its solubility in toluene and insolubility in normal paraffinic solvents (e.g. n heptane). A commonly applied fractionation method uses the different solubilities to separate crude oil into saturates, aromatics, resins, and asphaltene (SARA) fractions, where asphaltenes are initially precipitated by using non-polar solvents. Asphaltenes, as a heterogeneous mixture, are highly disperse in both molecular size and chemical composition, with a high content of heteroatoms (N, O, and S) and metals (i.e. V, Fe) causing them to exhibit polar characteristics and making them difficult to analyze.

Experimental

To obtain the asphaltene fraction, a SARA fractionation procedure was used. The asphaltene was precipitated from a corresponding heavy crude oil using n-heptane. Mass analysis was performed on a 12 T LTQ FT-ICR MS (Thermo Fisher, Bremen, Germany) equipped with commercially available APPI sources. Additional measurements were done by using a research-type high-field Orbitrap MS that allows to obtain data with longer transients of 1.5 and 3 s strongly increasing the resolution capabilities in comparison to standard instruments.

Results

Due to its physical-chemical properties and its complexity, asphaltenes are difficult to analyze. Up til now, FT-ICR MS was the method of choice for the analysis of such a complex mixture. High-field Orbitrap analyzer that are capable of running longer transients of 1.5 or even 3 seconds can achieve resolutions of up to 800000 at m/z 400 corresponding to a resolution of more than 500000 at m/z 600. Here, we present the first data from a research-type Orbitrap based hybrid mass spectrometer that is capable of running these longer transients for the analysis of asphaltene samples and compare the data with results obtained from a 12 T LTQ-FT instrument. It will be shown how well the Orbitrap data correlate to existing ICR data in regards to mass accuracy and whether or not the new Orbitrap is capable of outperforming a standard ICR in the analysis of complex mixtures.

Application of atmospheric pressure photoionization Fourier transform ion cyclotron mass spectrometry for the comparison of water from natural sources and areas of oil sands activity

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Consumption of petroleum continues to increase and, due to pressures on the finite supply, it is necessary to obtain petroleum from less conventional sources, such as oil sands. The Athabasca oil sands are located in Alberta, Canada, and consist of clay, sand, water, and bitumen. Using an alkaline hot water extraction process, it is possible to separate the bitumen, which can then be upgraded to synthetic oil. Approximately three barrels of water are consumed during the production of one barrel of oil. This oil sands process water (OSPW) must be stored in large tailings ponds, as there is a zero discharge policy. It is important to be able to differentiate between those organic components found in the aquatic environment due to natural processes, such as expected seepage of oil sands material, and those which arise due to human activity.

Samples were acquired from a range of natural water and oil sands process water sites in the Athabasca Basin, Alberta, Canada. Concentrates were produced prior to preparation for analysis, which was performed using a 12 T solariX Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Previous research into the characterization of OSPW has typically relied upon the usage of electrospray ionization (ESI), usually due to the targetting of naphthenic acids. Whilst ESI is best suited to the study of polar and ionic species, atmospheric pressure photoionization (APPI) can be applied to the study of less polar species and can produce radical ions in addition to protonated/deprotonated ions. ESI and APPI represent complementary methods, where APPI is an amenable ionization method for the study of a broad range of compounds, such as less polar, sulfur-containing compounds and hydrocarbons which do not incorporate heteroatoms. Mass spectra of complex mixtures that have been acquired using APPI are typically more complex, due to the greater number of components observed and the fact that radical ions are observed in addition. High field FTICR mass spectrometry offers ultra-high resolving power and mass accuracy, which afford high confidence in assignments of species within complex mixtures, which is particularly important for mass spectra generated using APPI.

Following assignments of elemental compositions, it is possible to utilize a variety of data visualization methods, such as categorization according to carbon number, heteroatom content, “hydrogen deficiency” (Z), or double bond equivalents (DBE). Principal component analysis (PCA) has been used to highlight similarities and differences between datasets. Clear differences can be found between water from natural sources and that from areas of oil sands activity, and, furthermore, detailed analyses lead to the ability to distinguish between samples from different companies.

Analysis of complex mixtures with the new dynamically harmonized ICR cell

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Fourier transform ion cyclotron resonance mass spectrometry is known for high mass resolving power and mass accuracy. Therefore, this technique is suitable for different chemical and biochemical applications like proteomics, top-down protein sequence analysis as well as complex mixtures analysis like petroleomics, metabolomics and natural organic matter (NOM) characterization. Especially in complex mixture analysis high resolving power of several hundred thousand is demanding. In FT-ICR mass spectrometry an increase of mass resolving power can be achieved e.g. by higher magnetic fields. However, a new type of FT ICR analyzer cell (dynamically harmonized) based on cylindrical Penning ion trap with shaped excitation and detection electrodes was developed by Nikolaev for better instrument performance even at low magnetic field.

Practically, this new dynamically harmonized cell provides resolving power, which is only limited by pressure damping. This new ICR cell has been tested at Bruker solarix instruments with 7T and 12T magnetic fields using different applications. The solarix systems were equipped with a ESI and APPI source as well as a CaptiveSpray source for NanoLC/MS measurements. Very high resolving power of almost 40 000 000 for isolated compounds like reserpine at m/z 609 could be measured with this new cell at a moderate magnetic field of 7T. Also isolated charge states of proteins could be measured isotopically resolved. For instance, the charge state 51+ of the protein BSA (66 kDa) could be measured at m/z 1303 with a resolving power of 1 500 000. Isolated charge states of even larger proteins and their multimers up to almost 200 kDa could be measured with isotopic resolution in the m/z range between 3000 and 5900. These measurements were done with very low ion populations in the ICR cell by ion selection in quadrupole mass filter in front of the ICR cell.

Therefore, measurements have been performed with complex mixtures (crude oil, NOM, etc.) without quadrupole ion selection to test the dynamic range, resolving power and mass accuracy with high ion population in the ICR cell. High resolving power of more than 1 000 000 and RMS mass errors below 100 ppb could be achieved in broad band mode even for extremely complex samples like crude oil and bitumen. Even better RMS mass errors below 50 ppb could be obtained with quadrupole isolation of complex samples. Results of complex mixture measurements with 7T and 12T instruments have been compared. Additional tests of complex peptide mixtures have been done with an E-coli digest.

An Electrostatic Linear Ion Trap Mass Spectrometer for Analysis of Small Molecules, Peptides, and Proteins

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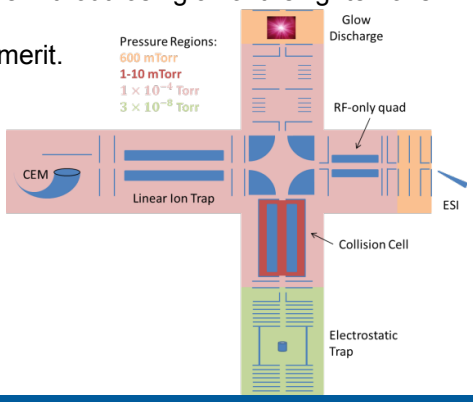
An electrostatic linear ion trap is a mass analyzer that blends multi-reflection time-of-flight with Fourier transform mass spectrometry. Ions are accelerated into the device and trapped between two ion-mirrors. Image current is then recorded while the signal decays due to collisions with the background gas. The resulting transient is Fourier transformed to obtain the mass-to-charge ratios of the ions. While these traps have been used in specialized applications for many years, their utility as a general purpose mass analyzer for bio-ions has never been explored, in part because of the difficulty in interfacing the analyzer with an electrospray (ESI) ion source. We have coupled our trap with a conventional nano-ESI source and demonstrate analysis of small molecules, peptides, and proteins.

Our instrument consists of two ion sources (atmospheric sampling glow discharge (ASGDI) and ESI) and two analyzers (a conventional quadrupole rod array operating as a linear ion trap (QLIT) with mass selective axial ejection, and the electrostatic linear ion trap (ELIT)). A turning quadrupole is used to direct ions from their sources to their destinations. A pre-trap (collision cell) is employed to accumulate ions prior to injection into the electrostatic trap. This setup allows us to directly compare the output of the QLIT with that of the ELIT which provides context for the evaluation of the latter as a general purpose mass analyzer.

In past instruments incorporating electrostatic traps, ions were typically admitted by chopping a continuous beam. This approach was successful when used in conjunction with our ASGDI source that produces a bright beam (10's of nA) of ions, but unsuccessful in conjunction with an ESI source that is much dimmer (pA). Additionally, the performance of the ELIT is highly dependent upon the kinetic energies of the admitted ions, making the kinetic energy distribution of the ions as they leave the source a concern. To solve these problems, we implemented a collision cell which is used to accumulate and thermalize ions prior to injection into the ELIT. We explore the conditions that result in optimal extraction and acceleration of the ions from the accumulation cell into the ELIT.

Use of the accumulation cell posed a new problem: Since the ions will undergo a time-of-flight separation in route to the ELIT, achieving simultaneous transfer of a large range of m/z to the ELIT is tricky. We mitigate the problem by transferring the ions as an intensified beam rather than as a packet. This strategy allows us to wait for the heavy ions to arrive without losing all of the lighter ions.

We also explore questions related to many analytical figures of merit. In many respects, the ELIT is found to be similar to the QLIT. Advantages of the ELIT include a much higher upper m/z limit and no requirement for a sophisticated, high-voltage RF power supply. Disadvantages of the ELIT include the need for ultra-high vacuum and the lack of MS/MS capability.



Pushing the Limits of Orbitrap Bench-Top FTMS

Jan-Peter Hauschild, Eugen Damoc, Olaf Scheibner, Eduard Denisov, Oliver Lange, Frank Czemper, Alexander Kholomeev, Alexander Makarov, Andreas Wieghaus

Thermo Fisher Scientific, Bremen Germany

Recent years have witnessed rapidly growing usage of bench-top Orbitrap mass spectrometers for routine MS applications. They have proved to be versatile high throughput instruments and therefore are increasingly used for the analysis of very complex mixtures as well as proteomic and biopharmaceutical samples. This presentation is devoted to technical improvements that optimize instrument performance for such challenging samples.

As intact proteins create fast decaying beat patterns in Fourier-Transform (FT) image current detection systems, the ability to detect the very first beat is crucial for gaining the most abundant signal. Several changes to the instrument allowed faster voltage settling after the ion injection pulse. The insulators within the Orbitrap assembly were modified and the design was made completely symmetrical with respect to stray capacitances. Additionally, the pre-amplifier circuitry was redesigned to improve recovery time from saturation caused by pulses during injection. In conjunction with advanced signal processing shorter transients can be used to record only the very first beat at high abundance. Though voltage switching during ion injection into the Orbitrap analyzer disturbs image current detection, the implemented hardware changes reduced the duration of this disturbance from about four milliseconds to 250 microseconds. With this improvement, the entire first beat of the transient of intact proteins can be now used for data acquisition.

The large collisional cross sections inherent to intact proteins caused accelerated transient decay, especially in a bench-top instrument with limited vacuum pumping. Reducing gas flow to the HCD cell provides an ultimate vacuum that is similar to hybrid instruments, enabling the resolution of proteins up to ~50 kDa in a targeted mode. For example, baseline isotopic resolution of carbonic anhydrase (29 kDa) and enolase (47 kDa) is demonstrated.

With very complex samples, a dedicated C-Trap charge detection (CTCD) system is shown to improve the accuracy of the prescan-based automated gain control (AGC). The periodic detection of ejected charges from the C-Trap by a charge detector was employed. This allows an internal monitoring and correction of the AGC results when necessary. Higher molecular species coeluting with the analytes of interest may lead to reduced sensitivity and mass accuracy of the instrument due to wrong estimation of the number of charges in the C-trap by the AGC. It could be shown that with the CTCD system in place AGC remains effective even under extreme conditions so that mass accuracy and correct signal intensity were maintained under all conditions.

It could be concluded that bench-top Orbitrap FTMS expands the reach of ultra-high resolution to ever widening range of important analytical applications.

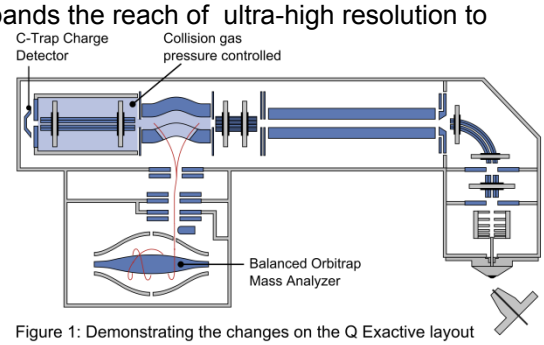


Figure 1: Demonstrating the changes on the Q Exactive layout

A Path from Multi-Reflecting TOF to Electrostatic Traps

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MSC-CG, City of Bar, Montenegro

Electrostatic ion mirrors has been developed to the stage when highly isochronous ion motion can be reached within 10-15% energy spread and for wide and divergent ion packets. Same ion mirrors could be used for variety of mass spectrometric analyzers from Multi-reflecting (MR) TOF to open electrostatic traps, and ultimately, to electrostatic traps with FT detection. Experimental section describes MR-TOF combined with two-dimensional gas chromatography for characterizing O, N and S-content of petroleum samples and their isomeric distributions. To extend MR-TOF dynamic range and space charge limits, the signal has been encoded with a rapid and non uniform start pulse pattern. Signal decoding then resembles FT procedures. Decoding of overlapping TOF-type spectra leads to the idea of open traps, where the ion path is no longer fixed, but rather the number of ion reflections is allowed within a span. Similar signals may be obtained if sampling a small portion of ion packets per every ion oscillation. If using FT signal analysis and charge induced detection, the trap has to be "closed" in all directions. An exemplar hollow cylindrical analyzer automatically provides isochronous boundaries in the drift direction. Particular geometries allow reduction of detector capacity. To accelerate signal acquisition by FDM algorithms, the trap could be filled with continuous ion beam, later pulsed trimmed to a half-trap size. The beauty of multi-reflecting electrostatic traps is in ease of ion injection via a field-free space between the mirrors, and in a large volume available for ion packets, thus improving space charge capacity and charge throughput(estimated as 1E+9 to 1E+10 ions/sec).

Electron-induced dissociation: an enabling technology for the characterisation of small molecules

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Tandem mass spectrometry is a phenomenally important tool for so many applications of mass spectrometry. From the established vibrationally dissociative techniques that occur following activation via collisions (CID) or photons (IRMPD), to the more recent developments of radical driven dissociative techniques that follow interactions with electrons (ECD, EDD and EID etc), the ability to characterise ions is enhanced each time a new and/or different set of fragmentation data is considered.

In an attempt to elicit a greater depth of information for a selection of singly charged molecules, electron-induced dissociation (EID) has been applied to numerous compound classes including pharmaceutical type molecules, synthetic glycopolymers and phospholipids. This has been performed on an FT ICR MS (LTQFT, ThermoFinnigan Corp.) using an indirectly heated dispenser cathode to generate electrons in the energy range 17 eV to 25 eV.

In the case of small organic ions, EID provides data that complements CID very nicely. This advantage has been utilised to great effect for the LC-EID analysis of a pharmaceutical candidate whereby one low-level impurity was characterised, solving an analytical problem that could not be solved by CID and providing a faster and cheaper alternative to preparative isolation and NMR.

During the course of this work sodium, potassium and ammonium adducts were analysed alongside the protonated species. The charge-carrying species has been shown to have a strong effect on the EID spectra, probably due to the location and 'strength' of the charged site(s) within the ion. This can be used to generate yet further complementary information for these pharmaceutical molecules. Metal cation coordination is particularly relevant in the analysis of a synthetic glycopolymer generated by RAFT polymerisation. In this case EID provides sequential cleavage along polymer backbone in one direction only, suggesting the charge is retained by the RAFT agent end-group, and leaves the sugar groups attached. CID cleaves just one sugar group at a time through MSn experiments.

EID has also been successful in the analysis of glycerophosphocholines, systematically cleaving a large portion of only one out of the two acyl chains. By comparing a comprehensive set of oleoyl and palmitoyl acyl chain positions for phosphocholine, the presence of the oleoyl chain would appear to be essential for fragmentation.

Not only do these studies help further understanding of the EID mechanisms, but they demonstrate the wide ranging use of this technique.

What factors influence ECD fragmentation pathways?

Renjie Hui, Gilles Frison, Guillaume van der Rest and Julia Chamot-Rooke

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ECD (Electron Capture Dissociation) is an activation technique available on FT-ICR mass spectrometers that has shown a profound potential for the analysis of peptides and proteins. In ECD, multiply charged even-electron cations are reduced by receiving one electron and are thus converted to intermediate radical-cations which further fragment by backbone cleavage to give mainly *c* and *z*-type ions.

A previous study on the doubly charged AGWLK pentapeptide led to the observation of unusual ECD fragments: only *b/y* and *w* fragments were observed [1]. Stemming from this observation, we pursued a more systematic investigation of different series of peptides (of the AGXLK type, as well as other and larger peptides) to delineate the major factors influencing the type of fragments observed in ECD. An analysis of the SwedECD databasis (11 492 ECD spectra) was also performed to extend our results to a larger data set [2].

Taken together, our results suggest the existence of at least three separate competing pathways in the ECD/ETD fragmentation of peptides: classical N-C_α cleavage leading to *c/z* ions, $H^\bullet$ loss followed by *b/y* ion formation and direct formation of *w* ions from the radical cation. Considering the competition amongst these possible pathways, we hypothesized that the hydrogen bonding network present in the peptide ion could play an important role in the reactivity observed and tested this hypothesis using amino acid substitution to increase hydrogen bonding within the peptides under study.

This work is original as, up to now, most of the experimental and theoretical studies have only focused on the mechanisms leading to the *c/z* fragmentation pathway and much less attention has been brought to the others. Since the precise mechanism leading to the formation of *c/z* fragments in ECD is still a question of debate, the study of factors leading to alternate fragmentation pathways can provide insights into the early steps of the reaction.

[1] R. Antoine et al. *Rapid Comm. Mass Spectrom.* **20**, 1648-1652 (2006)
[2] G. van der Rest et al. *J. Am. Soc. Mass Spectrom.* **22**, 1631-1644 (2011)

IRPD spectroscopy of X-(H2O)$_n$ X = Br$^-$, F$^-$, I$^-$ ions

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Since the first observation of what we now call the Hofmeister effect, in 1888, researchers of many fields are trying to understand the origins of this effect.

The original hypothesis considers that dissolved ions disturb the solvation network, indirectly changing the macromolecule interaction with the solvent molecules in the solution through a long-range patterning effect. Recently this hypothesis was shown to be in agreement with IRPD spectroscopy of sulfate anions in the gas-phase

Another hypothesis considers that the observed change in solution properties arises from the direct binding of dissolved ions to specific macromolecule sites, which would change the protein tertiary structure. This hypothesis was given more strength after a recent neutron diffraction study that proposed the patterning effect of an ion not to range further than the first solvation shell.

To shed some light in this debate and reconcile both experimental approaches, X-(H2O)$_n$, X = F, Br, I e n = 6 - 68 ionic clusters were surveyed by IRPD spectroscopy in the gas phase using a modified 2.7 FT-ICR mass spectrometer interfaced with an OPO-OPA laser system. These clusters were also modeled by DFT calculations, for n = 6 - 12, and molecular dynamics simulations, for n = 25 - 300.

Our results show the existence of a long-range patterning effect exerted by these ions on the surrounding water molecules, extending, at least, up to 68 water molecules. This patterning effect reflects on the number of free OH oscillators on the surface of the cluster. Nevertheless, molecular dynamics simulations suggest that these patterning effects can extend up to several hundreds of solvent molecules, being primarily dependent on the ion formal charge than the nature of the ion.

The ionic OH bond (IOH) vibrational frequency was also analyzed as a function of the solvation number, leading us into the determination of the gas phase coordination number of 8 for F- and 7 Br-, which are also in accordance with the Hofmeister series.

The analyses of the data suggest that both IRPD and neutron diffraction experiments describe different aspects of the Hofmeister effect, with the neutron diffraction probing the water network directly surrounding the ion, being susceptible to the nature of the solute up to the first solvation shell, and the IRPD spectroscopy probing the surface of the ionic cluster, where the long range effect can be witnessed by the number of free OH oscillators.

These results not only show that the patterning effect of anions extends way beyond the first solvation shell, but also suggests that both hypothesis can play an important role in the solvation of biomolecules.

Structure of anionic b- and c-type peptide fragments by IRMPD spectroscopy

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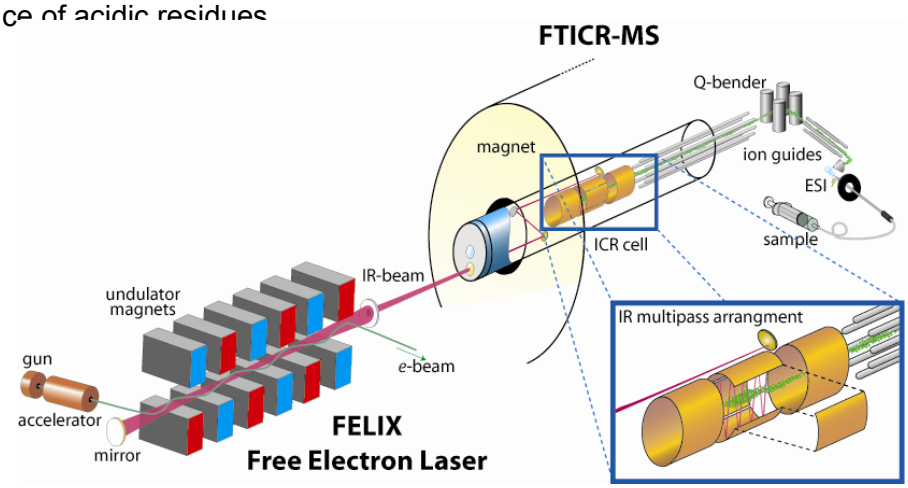
While MS-based peptide sequencing has become a pivotal analytical tool in proteomics research, the chemistry underlying peptide dissociation remains under much debate. Better understanding of the dissociation reaction mechanisms may improve bioinformatics tools used to identify peptides from MS/MS data.

An important parameter in the understanding of peptide dissociation reactions is knowledge of the molecular structures of the (collision-induced) dissociation products formed. In recent years, infrared spectroscopy of such CID product ions has increasingly been applied to obtain accurate information on their structures, where the majority of studies has made use of widely tunable free electron lasers (FELIX, CLIO) to produce IR multiple-photon dissociation (IRMPD) spectra of the fragment ions of interest. In this contribution we present IR spectra and derived molecular structures for b- and c-type fragments of deprotonated (i.e. anionic) peptides.

Whereas CID of protonated peptides yields mainly a-, b-, and y-type sequence ions, anionic peptides are known to yield in addition c- and z-type fragments. Analogous to their positively charged counterparts, anionic b-type fragments have been suggested to possess structures with a 5-membered oxazolone ring at the C-terminus, although lower energy structures are known to exist on the potential energy surface. Anionic c-type fragments are believed to possess linear structures with an amide -C(=O)-NH2 C-terminal moiety. Here we investigate the structures of short anionic b- and c-type peptide fragments and address questions of where these structures are deprotonated and how these structures depend on the presence and absence of acidic residues.



High Resolution Affinity- Mass Spectrometry in the Elucidation of Oligomerisation-Aggregation Structures of Target Proteins For Neurodegeneration

Michael Przybylski, Claudia Cozma, Camelia Vlad, Adrian Moise, Gabriela Paraschiv, Stefan Slamnoiu, Mihaela Stumbaum, Alina Petre, Kathrin Lindner, Marilena Manea, Michael Gross

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FTICR Mass spectrometry in combination with affinity-mass spectrometry approaches have been recently developed in our laboratory as powerful tools for immunoaffinity-proteomics, identification of antibody recognition structures, and key intermediates in oligomerization-aggregation of neurodegenerative proteins. High resolution affinity-mass spectrometry, using selective proteolytic excision in combination with FTICR-MS, has enabled the elucidation of molecular recognition epitopes of neurodegenerative target proteins and their proteolytic fragments [1]. This has been illustrated by the elucidation of oligomer- aggregation pathways in the development of immuno-therapeutic approaches against Alzheimer's disease (AD), using specific antibodies against ß-amyloid (Aß), the key neurotoxic peptide fragment in AD. The differential epitope structure identification by high resolution affinity-MS of (i), therapeutic antibodies that disaggregate Aß plaques, and (ii), physiological Aß-autoantibodies in serum capable of eliciting a protective effect to inhibit the formation of Aß-plaques, provides a breakthrough in the development of AD immuno-therapeutic and –diagnostic approaches [2]. In studies on the aggregation of alpha-synuclein, a target protein in Parkinson's disease (PD), hitherto unknown, highly aggregation-prone proteolytic intermediates of the aggregation pathway have been identified, which provide new insight to "misfolding" –aggregation pathways in neurodegeneration. Furthermore, a new online combination, SAW-biosensor- FTICR- MS will be presented which enables the simultaneous structure identification and quantitative determination of chip-immobilised biopolymer- ligand interactions.

[1] McLaurin, J., et al. (2002) Nature Med. 8: 1263-1269; Macht, M., et al. (2004) Anal. Bioan. Chem. 378, 1102-1111.
[2] Stefanescu, R., et al. (2008) Eur. J. Mass Spectrom. 13, 69-75; Przybylski, M. et al./ Univ. Konstanz & Budapest (2009) Eur. & US Patent Applications ; (2011) US Patent.
[3] Vlad, C. Et al., (2011) ChemBiochem. 12, 2740-2744.
[4] Dragusanu, M., et al. (2010) J. Am. Soc. Mass Spectrom. 21, 1643-1648.

Deciphering Protein Conformational Transitions by Top-Down ECD/HDX FTMS

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Hydrogen/deuterium exchange (HDX) coupled with mass spectrometry (MS) is a powerful tool for characterizing the structure, dynamics, and interactions of proteins. Traditional HPLC-based HDX-MS usually gives peptide-level resolution and incomplete sequence coverage. Recently, we have developed an electron capture dissociation (ECD)-based "top-down" approach that can reach close to amino acid spatial resolution (JACS, 2009, 131, 12081). By fragmenting intact isotopically-labeled proteins in the gas-phase via nonergodic and nonselective backbone bond dissociation, this approach can also significantly reduce back-exchange and reach complete sequence coverage, while eliminating "hydrogen/deuterium scrambling". However, application of this approach has thus far been limited to small proteins (< 17 kDa). Here we demonstrate that drug-induced structural transitions of a larger 29-kDa protein, carbonic anhydrase (CAII), can be probed by top-down HDX. We will also show that the conformational changes during prion protein (PrP) monomer to oligomer conversion can be localized by this technique.

CAII contains 240 amide hydrogens, and global HDX has shown that 163 were protected. After forming a 1:1 complex with furosemide (FSM), CAII displayed 18 additional protected hydrogens compared to its drug-free form. To locate these differentially-exchanged sites by top-down ECD, we used our "ECD-stitching" method to obtain full sequence coverage at a resolution of ca. 2.5 residues. By comparing the amide deuteration status with the crystal structure of CAII-FSM complex, it can be seen that the residues displaying these apparent differences are mostly located in domains that are in close contact with FSM. There are also residues displaying apparent differences that are located in hinge regions distal to the drug-binding site. This finding is in sharp contrast to X-ray crystallographic studies, which showed that the crystal structures of drug-free and FSM-bound CAII were identical.

The second system is PrP, whose oligomerization plays a crucial role in prion disease. Structural characterization of highly dynamic prion oligomers has presented serious challenges to traditional methods such as crystallography and NMR. Here, our HDX experiments showed that approximately 38 amides were protected in the PrP monomer, while only 23 were protected in the oligomer. Top-down ECD data has shown that the structural differences between PrP monomer and oligomer are located in the middle portion of the protein, a region encompassing helix 1 and sheet 2. No significant structural change was observed in helices 2 and 3. These experiments reveal that PrP oligomerization may require unfolding of helix 1 in the monomer.

In summary, our data indicates that top-down HDX-MS is a sensitive tool for structural biology. The ECD-stitching top-down approach should also be applicable to other large protein systems, and may give better insights into solution-phase processes, including conformational changes, than crystallographic methods.

ETD and Hemoglobin Variants

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ETD has been performed on a hybrid quadrupole / ion mobility / oa-ToF mass spectrometer. The instrument incorporates three Travelling-Wave SRIG's prior to the ToF mass analyser. For fragmentation using ETD, a glow discharge source was used to fill the Trap T-Wave cell with quadrupole mass selected ETD reagent anions. During an acquisition, the source polarity and quadrupole set mass are switched to allow multiply charged cations to interact with stored reagent anions in the Trap T-Wave.

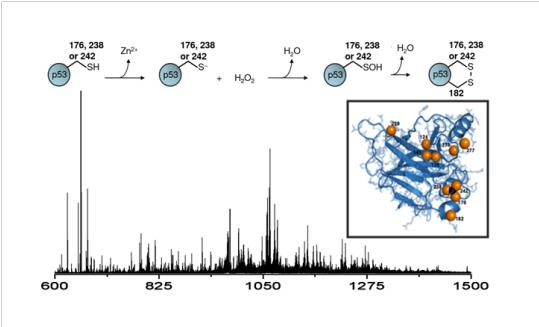
A number of diverse application areas will be described. These will include the use of ETD for the rapid identification of some human hemoglobinopathies, examination of the reaction of $AuPEt_3Cl$, with a number of synthetic peptides to identify the precise site of binding to the peptides and the utility of ETD combined with ion mobility for the analysis of permethylated oligosaccharides.

Investigating the Molecular Details of p53 Redox Modifications using FT-ICR Mass Spectrometry

Jenna Scotcher, David J. Clarke, Stefan K. Weidt, C. Logan Mackay, Ted R. Hupp, Peter J. Sadler and Pat R. R. Langridge-Smith

School of Chemistry,University of Edinburgh, Edinburgh, UK

Many prokaryotic and eukaryotic proteins are now known to have their function controlled via specific oxidation or reduction reactions occurring on critical cysteine residues [1]. The tumor-suppressor protein p53 is a multiple cysteine-containing protein, which is involved in a vast and diverse range of cellular processes including apoptosis, differentiation, senescence, glycolysis, autophagy, oxidative stress and DNA-repair [2]. Mounting evidence suggests that redox-modification of p53 cysteine residues plays an important role in regulating its biological activity [3]. Furthermore, p53 activity has been linked with intracellular levels of the reactive oxygen species (ROS) hydrogen peroxide (H2O2) [4]. However, very few studies have revealed specific details regarding the redox-mechanisms that occur within p53 at the molecular level. We recently characterized the relative reactivity of each of the ten Cys residues in human p53 using the thiol alkylating reagent N-ethylmaleimide (NEM) together with FT-ICR mass spectrometry [5]. Mass spectra of NEM-treated p53 DNA-binding domain (25 kDa) revealed that two cysteine residues in p53, Cys182 and Cys277, display a remarkable preference for alkylation, and alkylation beyond this point initiates rapid modification of the remaining thiol groups, presumably accompanied by protein unfolding. These observations have implications for the re-activation of mutant p53 with cysteine-targeting compounds, which result in the death of tumor cells [3]. Using a combination of top-down and middle-down FT-ICR mass spectrometry, we were able to identify unambiguously the modification state of each thiol group in p53, and thus reveal the two reactive cysteine residues. Our FT-ICR MS/MS results were confirmed by site-directed mutagenesis experiments. We have since used FT-ICR mass spectrometry to investigate oxidation of p53 by the ROS biological signaling molecule H2O2. We have found that the p53 DNA-binding domain forms two disulfide bonds in response to H2O2 treatment. Top-down FT-ICR mass spectrometry was used to unambiguously define the oxidation state of each of the ten cysteine residues, revealing the identity of the four cysteines - Cys176, 182, 238 and 242 - that are involved in disulfide bond formation. Bottom-up FT-ICR mass spectrometry has also been used in order to determine cysteine-connectivity of the disulfide linkages. Based on our previous assignment of Cys182 as a reactive cysteine residue, we propose that H2O2-mediated oxidation of p53 proceeds via initial formation of sulfenic acid (–SOH) on Cys182. [1] C. E. Paulsen and K. S. Carroll, ACS Chem. Biol., 5, 47 (2010) [2] A. J. Levine and M. Oren, Nat. Rev. Cancer., 9, 749 (2009) [3] V. J. N. Bykov, J. M. R. Lambert, et al., Cell Cycle., 8, 2509 (2009) [4] B. Liu, Y. Chen and D. K. St Clair, Free Radic. Biol. Med., 44, 1529 (2008) [5] J. Scotcher, D. J. Clarke, et. al., J. Am. Soc. Mass Spectrom., 22, 888 (2011)



Novel Type of Spectral Library for the Identification of "Unknown Unknowns"

Robert Mistrik; Juraj Lutisan; Mark Sanders; Yingying Huang; Rose Herbold; Tim Stratton; Anthony Taylor

Thermo Fisher Scientific (Schweiz) Reinach, Switzerland

Identification of unknown compounds was traditionally limited to library search techniques and manual spectral interpretation. A few years ago, Precursor Ion Fingerprinting (PIF) was developed. This innovative approach identifies substructural information through the comparison of product ion spectra of structurally related compounds. Structural information is derived by utilizing previously characterized ion structures stored in reference libraries of tandem mass spectral data and matching them with unknown product ion spectra. PIF is a powerful technique that depends upon libraries containing spectra of precursor ions of various chemical classes acquired at various experimental conditions. Here we present a new type of spectral library providing the functionality required for elucidation of unknowns even if compounds are not present in the library.

For the purpose of library creation, precursor ions (isolated, activated, and dissociated ions) can be selected using various criteria; however, the more product ion spectra that are generated, the more comprehensive a library can be built. Spectra and associated experimental data are organized into spectral ion trees, where branches represent precursor ions and nodes the corresponding product ion spectra. The library is implemented in a relational database that will be accessible through a public domain web site of an emerging consortium named "m/z Cloud". Since the consortium is predominantly oriented towards high-resolution, accurate mass spectra, the database design, spectral management, and library search algorithms require a completely new architecture compared to traditional spectral databases.

The project is to create a library of comprehensive spectral ion trees based on structurally characterized product ion spectra to enable the identification of unknown substructures. Each structurally characterized product ion spectrum will contain the precursor ion m/z value, a list of product ion m/z values with mass accuracies, corresponding absolute and relative intensities, ion polarity, charge state, and the structures of the precursor ion and parent molecule. For the assignment of fragment structures to a precursor ion in the process of creating structurally characterized product ion spectra, it is extremely beneficial to have high-resolution spectra since the accurate m/z values of precursor and product ions greatly reduce the number of possible molecular formulas for fragment structures. Also, the determination of the structural arrangement for the elucidated molecule benefits from exact mass measurements by constraining the elemental composition of the elucidated molecule and consistently validating the calculated mass of recognized fragment structures and accurate m/z values of precursor and product ions. The m/z Cloud public domain database aims to provide complete library technology based on spectral ion trees to enable elucidation of unknowns using the precursor ion fingerprinting method.

Deriving confidence metrics for automatic peak assignment through n-dimensional Kendrick defect or mass difference inference networks

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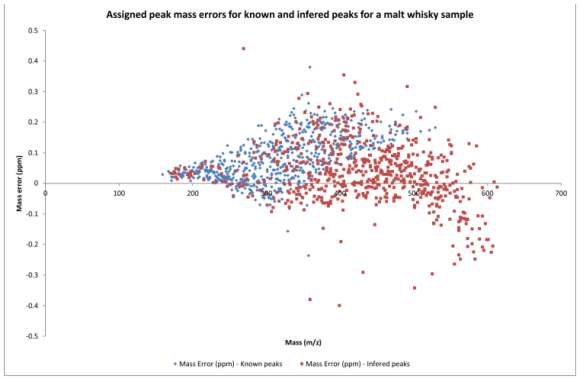
The data volume produced by Fourier Transform Ion Cyclotron Resonance Mass Spectrometers, analysing complex samples (e.g. crude oil, natural organic matter, food products) requires fast and accurate automatic peak assignment algorithms in order to allow efficient interpretation. Kendrick mass defect and peak mass difference formulaic inference techniques have been developed to allow the elemental formulae of unassigned peaks to be inferred from those of assigned peaks – but, how confident can one be in the predictions of these methods? We have developed a novel, n- dimensional Kendrick defect inference network algorithm which uses artificial intelligence methods to produce confidence metrics of the resulting peak assignments. The user can then select the level of confidence they require in the output.

The “n-D KMD” algorithm, written in NI LabVIEW, provides an automatic method for peak assignment. In n-dimensional Kendrick Mass Defect (n-D KMD) space, a mass peak is plotted at co-ordinates corresponding to the Kendrick mass defect of that peak against a set of n Kendrick base masses. Pairs of peaks which have the same difference in Kendrick space have the same relative formula change - this can be used to infer the elemental composition of previously unknown peaks from known peaks. The algorithm provides a metric of confidence in any peak assignment by means of expert and artificial immune system methods. This metric can be used as further selection criteria to identify subsets of the material within required confidence bounds.

Metrics of confidence currently measured in the n-D KMD algorithm are – degree of internal consistency of the n-D Kendrick inference network (to what extent is the assigned formula consistently achieved irrespective of the route taken through the Kendrick network from any known starting peak); level of inter-connectivity within the network; artificial immune system (AIS – a class of artificial intelligence) recognition distance relating the assigned peak mass error, the mass and the original intensity; formulaic sanity checks and stable isotope intensity modelling. The highest confidence setting requires that all assigned peaks pass all confidence tests. Lower setting levels are also available and will be presented.

The “n-D KMD” algorithm has been successfully applied to the study of a variety of samples types: natural organic matter, food samples and biofuel source materials.



Penning traps in physics research, developments and applications from highly-charged atomic ions to metal-cluster studies to precision mass measurements of exotic nuclei

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Penning traps are applied for a large variety of investigations in several areas of physics research. They may serve for

(a) the containment of ions,
(b) the selection of particles with respect to their mass-over-charge ratio,
(c) the analysis of reactions or
(d) the high-accuracy mass determination of exotic nuclear species.

The presentation will give an overview of several activities related to the aspects listed above.

(a) Delicate particles such as atomic ions in very high charge states can only survive if they are in no contact with any other materials. They are produced in electron beam ion sources or traps (EBIS/EBIT) which use some of the properties of “standard” ion-cyclotron-resonance devices.

(b,c) Penning traps are valuable tools in metal-cluster research. They allow a selection of the cluster sizes such that the cluster properties can be determined for one size at a time. Furthermore, simultaneous storage of several species as different as very heavy cluster anions and electrons is possible and their reactions such as the production of multiply negatively charged clusters can be followed as a function of time. Multiple selection, reaction and analysis steps may be applied to cluster ions.

(d) By measuring the cyclotron frequency of ions with even very short-lived atomic nuclei (i.e. in the sub-second range) the nuclear masses and thus their nuclear binding energies can be determined with very high accuracy. This includes nuclides that show exotic decay modes (proton decay or pairs of nuclides between which neutrino-less double-beta decays may occur) and isotopes of very heavy elements (such as nobelium and lawrencium, Z= 102 and 103, respectively).

As much as the application areas vary as much do the techniques that are applied to answer the corresponding research questions. The presentation will give examples of the experiments mentioned above and of recent developments to improve the performance of specific setups. In particular, the current status and performance of ClusterTrap [1], ISOLTRAP [2] and SHIPTRAP [3] will be reviewed.

[1] F. Martinez et al., Eur. Phys. J. D 63, 255 (2011), http://dx.doi.org/10.1140/epjd/e2011-10528-3
[2] M. Mukherjee et al., Eur. Phys. J. A 35, 1 (2008), http://dx.doi.org/10.1140/epja/i2007-10528-9
[3] M. Block et al., Nature 463, 785 (2010), http://dx.doi.org/10.1038/nature08774

An Electrostatic Ion Trap with Planar Rotational Field Structure

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Electrostatic ion trap became popular for high resolution high mass accuracy mass analysis since the success of the Orbitrap. So far, other types of EIT are mainly in form of a linear electrostatic ion trap (LEIT) which was pioneered by Zajfmann and Benner, and it has demonstrated its high resolution for elemental ions and highly charged single protein. However, conventional LEIT easily suffer from the space charge interaction as the ions are trapped within the linear space, so the ion capacity is limited. The design of new EIT is based on construction of 3D rotational trapping field between two planar electrode arrays in shape of concentric rings. For positive ions the outer rings were charged at high positive potential to reflect the ions while some other ring electrodes play role of ion focusing towards the center plane between two planar electrode arrays. Ions oscillate in the trapping field diametrically and occupy the whole circular plane rather than the volume of a beam. With certain initial velocity in tangential direction ion oscillatory motion includes a rotating precession around the centre of the trapping field.

With the planar rotational symmetrical geometry, the spatial focusing in z (rotation axis) direction is only concerned and the time focusing with respect to the initial position and velocity disperses in y, z, and x (tangential direction) are studied. Software AXSIM were used to optimize the voltage settings for time focusing. It has been found that the z focusing voltage can either be positive or negative to achieve comparable performance. One of the optimized setting was shown in the figure and the setting allows to achieve 2nd order time focusing against the initial energy spread in y direction. Ion groups of 609 and 609.12 Th with initial energy spread in y and z directions of 100 eV and 0.1 eV respectively, are baseline separated after only 2 ms flight which indicated a resolving power of ~10 k FWHM. Ion injection from both external toroidal ion trap and a curved electrostatic ion guide are investigated.

Image charge or image current signal can be recorded from any assigned electrode during the simulation. The image charge signals was used in subsequent fast Fourier Transform or used for testing other signal conversion algorithms. With Discrete Fourier Transform, the FFT contains many higher harmonics for each mass, causing a highly scrambled spectrum. With orthogonal projection method, the image charge signal of mixed ions is projected to the space spanned by base signals for all possible masses. Although these base signals are not orthogonal to each other, the resultant spectrum does not contain spurious peaks. However the number of mass points which determines the mass range in one process is limited by the size of matrix that can be handled by current computer. Another method was also proposed which involved a combination of several image charge/ current signals from different pick up electrodes.

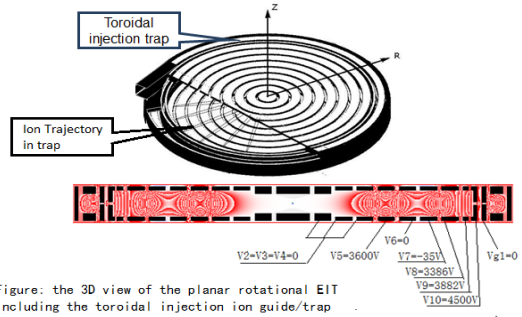

Figure: the 3D view of the planar rotational EIT including the toroidal injection ion guide/trap (top), and the field distribution when voltages are applied to the respective ring electrods.(bottom)

Improving trapped ion spectroscopy with a conically wired hexapole

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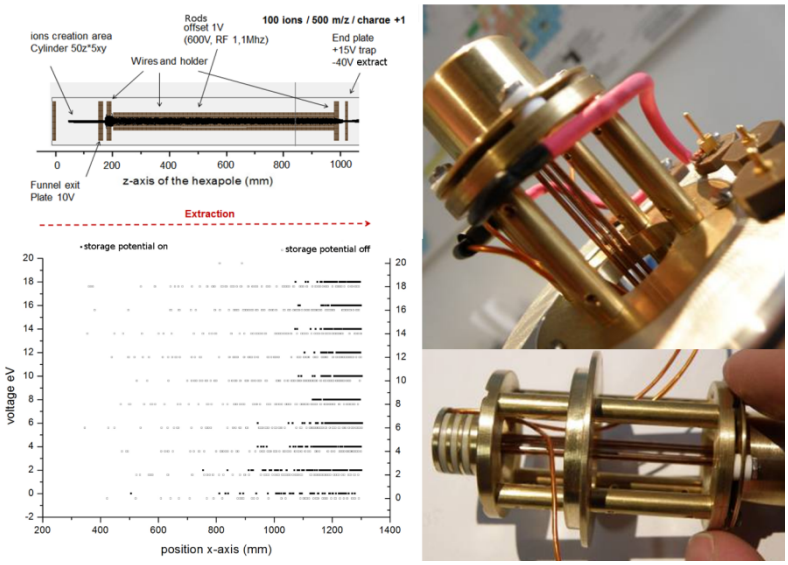
In gas phase spectroscopy, ICR cells can be used for storing and detecting ions under ultrahigh vacuum conditions. In this context Marshall et al. investigated the effect of an accumulation multipole trap used to load ions into a Penning trap. It discretizes the continuous ion flow generated by an electrospray ion source and pulses ion clouds into the Penning trap. [1] The initially proposed modification led to a fivefold improved signal to noise ratio upon detection compared to a Penning trap filled by a standard non pulsed multipole. In 2002 Marshall et al. published calculations for a further modified multipole ion trap with wires conically attached, inducing a voltage gradient while discharging the multipole. This led to a 16 times better signal to noise ratio that can be explained by the reduced extraction time due to the potential gradient. [2]

We present further developments using a conically wired hexapole, but this time with the wires on a constant bias: a potential gradient is present while accumulating the ions. For this purpose we built a conically wired hexapole and interfaced it to our 7T-FT-ICR (Bruker Daltonics). The behavior of this setup was modeled with Simion 8.0.4. The simulations indicate that one can influence the mean kinetic energy of the ions and the mean position during storage in the multipole, without losing the advantages described previously [1,2]. This allows a tuning of the internal energy of the stored ions and/or selective dissociation by modifying the voltage. The reduced velocity distribution of the extracted ion cloud and the localization of the ions at the end of the storage device in simulation, as well as preliminary results suggest that a further improvement of the signal to noise ratio can be expected.

[1] M.W. Senko, C.L. Hendrickson, M.R. Emmett, S.D.H. Shi, A.G. Marshall, JASMS 1997, 8, 970.
[2] B.E. Wilcox, C.L. Hendrickson, A.G. Marshall, JASMS 2002, 13, 1304–1312



Contribution of high mass resolution and accuracy of FTMS to molecular imaging

Delphine Debois

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Liege Belgium

Since its first implementation in 1997, MALDI Mass Spectrometry Imaging (MALDI MSI) has become an important tool in the proteomic arsenal, especially for biomarker hunting. First dedicated to high molecular weight, MALDI MSI is more and more used to map the distribution of small molecules too (lipids, drugs and metabolites,…). Last developments tend to improve the sample treatments to obtain the best spatial resolution as possible. From this perspective, great efforts have been made on the MALDI matrix deposition methods.

Now, one of the remaining challenges for MALDI-MSI users consists of identification of detected molecules. For high molecular weight, methods inspired by classical proteomics techniques, are regularly used. Bottom-Up (PMF obtained after in situ trypsin digestion) and Top-Down (in situ In-Source Decay) approaches have been used directly from a tissue slice, leading to the identification of some of the most abundant proteins present at the surface of the tissue. When small molecules are analyzed, the identification is more straightforward. Indeed, tandem mass spectrometry can easily be used, leading to the fragmentation of the detected compounds which allows their unambiguous identification. This identification is even more reliable when high resolution exact mass measurements can be performed.

In this talk, I will present how in our lab, we profit of the exceptional features of FT-ICR mass spectrometry for imaging and especially for identification purposes. The first example will deal with the benefit of high mass accuracy and high mass resolution for ISD-based protein identification. The mass accuracy and high mass resolution coupled with the use of a “cleaning” software allow unequivocal assignment of ISD fragments of proteins, in the low mass range (m/z between 300 and 900), whether from pure solutions or from tissue slices. The next examples will deal with the imaging of small molecules. The identification of drugs and their metabolites is facilitated with high mass accuracy. In our lab, we work on the localization of methadone and its first metabolite, EDDP (real name) in necrophagous fly larvae. In the mass range of these compounds (278-310 m/z), many matrix ion peaks are detected and the unique features of FT-ICR allows for unambiguous identification thanks to exact mass measurements. We also use MALDI Imaging to map the messenger molecules between plant roots and beneficial bacteria. The comparison of spectra recorded with a TOF/TOF instrument and with a FT-ICR demonstrates that high resolution allows for detecting molecules which could have been missed otherwise. It also allows to distinguish unknown compounds from alkali adducts of known molecules.

C60 Secondary Ion FT-ICR MS for High Mass Accuracy and High Mass Resolving Power SIMS Imaging

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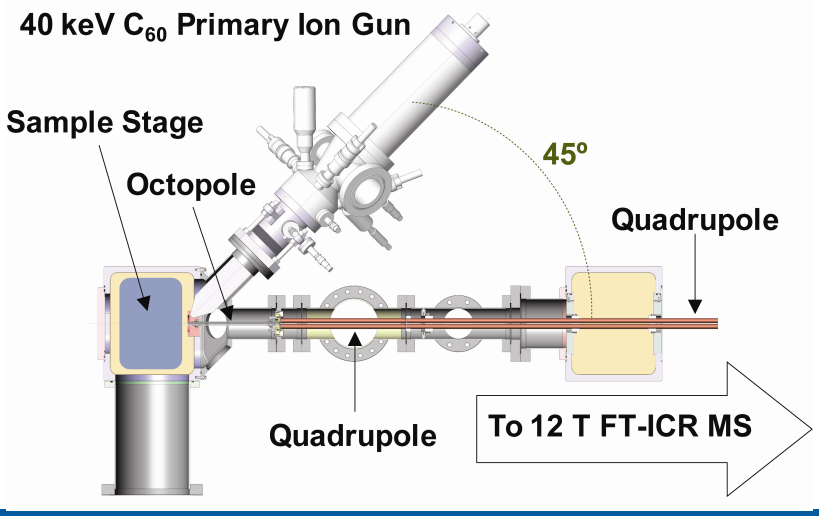
Secondary ion mass spectrometry (SIMS) has long been used for high spatial resolution (< 1 µm) MS imaging. The development and commercial availability of cluster and polyatomic primary ion sources (e.g., Au and Bi cluster and buckminsterfullerene (C60)) have led to exciting SIMS instrumentation development that take advantage of the higher secondary ion yield and decreased fragmentation of surface species that these sources provide. In particular, these SIMS instruments aim for better mass accuracy and mass resolving power than is attainable on more common TOF-SIMS instruments. Further, tandem-MS capabilities for structural identification of secondary ions are highly desirable.

Here we describe the development of a FT-ICR MS SIMS system with ultrahigh mass resolving power and high mass accuracy. Mass resolving power > 100,000 ($m/\Delta m_{50\%}$) and root-mean-square mass measurement accuracies below 1 part-per-million are demonstrated. In addition, tandem MS of secondary ions from standards and biological tissues is shown.

A 40 keV electron-impact based C60 primary ion gun was interfaced to 12 T and 9.4 T Bruker solariX FT-ICR mass spectrometers. Secondary ions are collected by an RF only octopole and transferred into the commercial FT-ICR via RF only quadrupoles. The C60 gun is operated in direct-current mode for fast collection of secondary ion populations, for optimum FT-ICR MS spectral performance. Secondary ions subjected to collision induced dissociation yield fragment ions which can be assigned unique elemental compositions, leading to parent ion identification. MS imaging experiments on tissue yield hundreds of tissue specific ions, many of which cannot be resolved on lower performance mass spectrometers. Instrument design, performance, applications for biological tissue imaging anc



Chemical Imaging of Biological Systems Using Nanospray Desorption Electrospray Ionization Mass Spectrometry

Julia Laskin, Brandi S. Heath, Ingela Lanekoff, Patrick J. Roach

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Ambient ionization imaging mass spectrometry enables detailed chemical characterization of biological samples in their native environment. We have recently developed nanospray desorption ionization (nano-DESI) - a new ambient pressure ionization technique for analysis of complex molecules on substrates. In nano-DESI the analyte is dissolved into a liquid bridge between two capillaries and ionized through self-aspirating nanospray. This enables precise control of desorption and ionization processes. The sampled area is determined by capillary diameter and solvent flow rate. We have recently applied this technique for ambient imaging of biological samples on an LTQ/Orbitrap instrument. Examples include highly sensitive chemical imaging of tissue samples with high spatial resolution and spatial profiling of living bacterial communities directly from their natural growth medium. Our initial results demonstrated the utility of nano-DESI for imaging of tissue samples with spatial resolution better than 12 μm without sample preparation. This presents a significant improvement over the existing ambient ionization techniques. Spatially resolved chemical analysis of living bacterial colonies directly from agar provides a unique insight on the distribution of various metabolites inside and outside the bacterial colony. High-resolution MS and MS/MS analysis of metabolites extracted from biological samples using nano-DESI is essential for unambiguous identification of the observed species. Nano-DESI mass spectrometry imaging combined with high detection efficiency and high mass resolution enables new applications in clinical diagnostics, drug discovery, microbiology, and biochemistry.

Molecular signals on surfaces studied with high resolution imaging mass spectrometry

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The spatial organization of molecules on cellular and tissue surfaces and its dynamics lies at the heart of many problems in biology and biomedicine. Studies search for direct relationships between molecular structure and function in these complex system to elucidate molecular signaling pathways and cellular communication to improve our fundamental understanding of disease diagnosis and prognosis. It is an area of research in which many science disciplines come together. This has led to the concept of integrative systems biology, in which different information resources are merged to resolve and provide insight in the complexity of biological systems.

Imaging mass spectrometry [1,2] as a new and rapidly growing discipline within the analytical sciences is taking full advantage of this development. New technologies that improve spatial and spectral resolution are rapidly emerging. Structural identification capabilities emerge that directly benefit from new approaches in mass spectrometry based proteomics and metabolomics. New developments in high resolution imaging MS are driven by innovative technology and application development. High resolution imaging mass spectrometry developments focus on increasing spatial resolution and increasing mass spectral resolution by the application of high end technologies.

Unraveling the complexity of molecular profiles at biological surfaces with imaging MS is severely hampered by the achievable mass resolution, sensitivity, dynamic range and spatial resolution of conventional ToF based mass spectrometric systems. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry offers unique capabilities is this respect and has already demonstrated to allow the resolution of new spatial features from complex surfaces. [5] Secondary Ion Mass Spectrometry is one technique that could greatly benefit from the mass spectral capabilities of FTICR-MS. We have combined, for the first time, the advantages of a C60 primary ion source with the ultrahigh mass resolving power and high mass measurement accuracy of FT-ICRMS [6]. SIMS-FTICRMS with a mass resolving power in excess of 100 000 ($m/\Delta m50\%$) is demonstrated, with a root-mean-square mass measurement accuracy below 1 part-per-million. Imaging of mouse brain tissue at 40 µm pixel size demonstrated the benefit of a high resolution approach. Tandem mass spectrometry of ions from biological tissue is employed to assign molecular formulas of through the determination of fragment ion elemental compositions.

The innovative imaging MS approaches described above have been employed to elucidate several molecular signaling pathways in a variety of diseases. They have been combined with several optical and other hyperspectral technologies in a true high performance multi-modal imaging approach. It demonstrates how integrative imaging MS has evolved to a problem solving tool that spans several scientific disciplines and provides fundamental insight into complex tumor biology.

New coupled frequency to both end caps for enhanced infinity operation of ICR cells. SIMION Study

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Coupling of the radial ion exciation RF waveform to both end caps of ICR cells, well known as infinity operation mode is important to minimize ion loss caused by increased axial vibrational amplitudes of radially excited ions. We tested this operation mode under hard RF excitation conditions: Off-resonance radial ion excitation by an emitted frequency which equals to ω+ + 3.5kHz. This causes ion Z-ejection if no RF is coupled to both end caps. If RF coupling to end caps is activated and when RF radial excitation amplitude of 200Vp-p at this off-resonance frequency is chosen, all masses in the range (150-800)amu were ejected not through axial Z ejection but by developing a large magnetron radius upon RF radial ion excietation which let ions to collide in the XY plane onto the central ring electrode. A reduced radial RF amplitude of 150Vp-p was capable to maintain all ions in the mass range (150-800)amu in the cell but a critical magnetron radius was developed which can threaten ions to be ejected if this magnetron radius has to increase further as a result of ion-neutral collisions. In an attempt to overcome these drawbacks in the mentioned infinity operation mode especially at high RF amplitudes such as 200Vp-p, another characteristic frequency is specifically triggered on the end caps only during radial ion excitation. We called this new operation mode “Enhanced Infinity” and it was successfully capable to maintain perfect ion Z-axialization without developing any noticeable magnetron motion.

One- and two-pulse quadrupolar excitation schemes of the ion motion in a Penning trap investigated with FT-ICR detection

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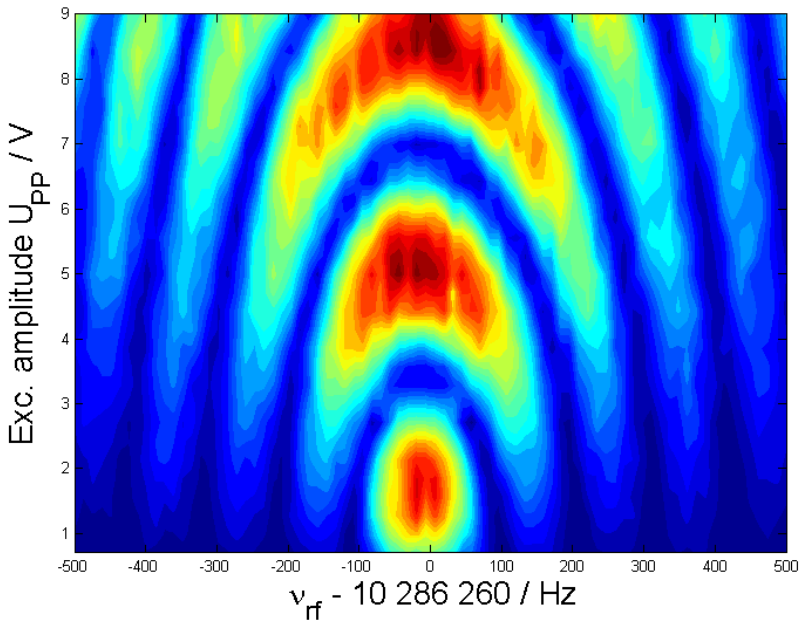
Penning traps are widely used as storage devices for charged particles in the fields of analytical and precision mass spectrometry. The two radial motional modes of the trapped ions are coupled by applying an azimuthal quadrupolar radio frequency (rf) field at a frequency near the true cyclotron frequency. The interaction of the ions with one- and two-pulse (Ramsey) quadrupolar excitation fields causes an interconversion of the radial motional modes. This interconversion has been probed by Fourier transform ion cyclotron resonance (FT-ICR) detection. The dipolar-detection of the FT-ICR signal at the modified cyclotron frequency has been studied as a function of the interaction parameters such as excitation frequency, amplitude and duration. The figure shows the measured FT-ICR signal intensity in a color-coded contour plot as a function of the detuning and rf amplitude for the one-pulse excitation scheme. Systematic amplitude-dependent frequency shifts of the modified cyclotron and the sideband frequency are also measured as a function of the number of stored particles. Results including frequency-ratio studies of stable lithium isotopes will be presented.



Protein Flexibility is Key to Cisplatin Cross-linking in Calmodulin

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Chemical cross-linking in combination with Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) has significant potential for studying protein structures and protein-protein interactions. Previously, cisplatin has been shown to be a protein cross-linking reagent and cross-links multiple methionine (Met) residues in apo-calmodulin (apo-CaM). However, the inter-residue distances obtained from NMR structures are inconsistent with the measured distance constraints by cross-linking: Met residues lie too far apart to be cross-linked by cisplatin. Here, by combining FTICR MS with a novel computational flexibility analysis, the flexible nature of the CaM structure is found to be key to cisplatin cross-linking in CaM. In both calcium-containing and calcium-free forms of CaM, it is found that the side chains of Met residues can be brought together by flexible motions. The possibility of cisplatin cross-linking calcium-containing CaM is then confirmed by mass spectrometry (MS) data. Therefore, flexibility analysis as a fast and low-cost computational method can be a useful tool for predicting cross-linking pairs in protein cross-linking analysis and facilitating further MS data analysis. Finally, flexibility analysis also indicates that the cross-linking of platinum to pairs of Met residues will effectively close the nonpolar groove and thus will likely interfere with the ability of CaM to bind to protein targets, as was proved by comparing assays for cisplatin¨Cmodified/unmodified CaM binding to melittin. Collectively, these results suggest that cisplatin cross-linking of apo-CaM or Ca¨CCaM can inhibit the ability of CaM to recognize its target proteins, which may have important implications for understanding the mechanism of tumor resistance to platinum anti-cancer drugs.

Binding site identification of glyoxal in Substance P by mass spectrometry.

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During hyperglycemia the high concentration of glucose leads to protein function and structure damage extracellularly and intracellularly as well. Glucose during its non-enzymatic reaction with proteins and peptides generates fructosamines (an early glycation adduct), which decomposes to form, among others, glyoxal (G). Glyoxal is also produced by glucose auto-oxidation and lipid peroxidation in the human body. Thus, during diabetes, glucose, glyoxal, fructosamine and other α-dicarbonyl compounds generate the glycation products commonly named Advanced Glycation End-product (AGEs). These AGEs also react with proteins in biological systems1 forming the irreversible toxic AGE adducts;2,3 which are strongly associated with diabetic complications4,1 such as diabetic neuropathy,5 nephropathy, retinopathy, cataract,6 and hearth disease.7 Hence, glycation is a post-translational modification (PTM) that involves a variety of reactions being the products formed or AGEs a heterogeneous group of compounds. Thus, glycation was studied here using Substance P as a model binding to glyoxal. The binding site (under the conditions of the experiment), was determined at the arginine residue using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). Binding site identification has been achieved due to the ability to perform Electron Capture Dissociation (ECD),8 double resonance ECD (DR-ECD),9,10 (Figure 1) and Collisionally Activated Dissociation (CAD),11 with a confident assignment of the modified amino acid with a mass error < 1 ppm.

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Monitoring Deamidation of Collagen Peptides in Ancient Bones Using FT-ICR-MS

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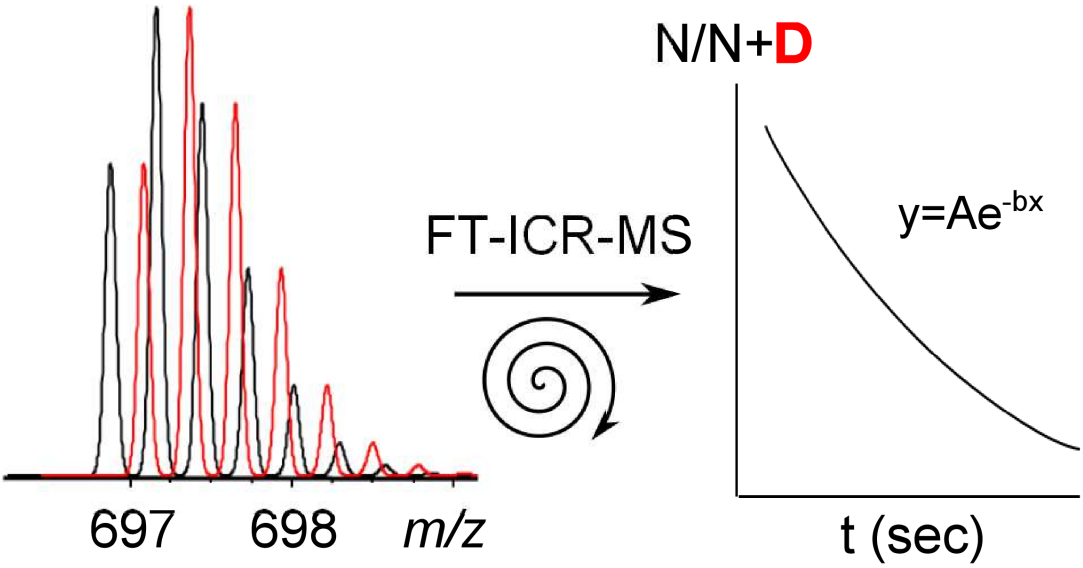
Collagen is the major component of skin, tendons, ligaments, teeth and bones, it provides the framework that holds most multi-cellular animals together, and type I collagen constitutes the major fibrillar collagen of bone. Due to the complexity of collagen's structure the study of post-translational modifications such as deamidation for this protein is challenging. Although there is no evidence of this protein being used for age assessment; it has been shown that deamidation of collagen is remarkably increased in old bones from mammals. Nonspectrometric methodologies have been used for the determination of the extent of deamidation by measuring the amount of amide nitrogen released in ammonia as well as the rate constants for deamidation of asparagine in collagen. In general these methodologies required more sample and separation processes. To understand if collagen plays a significant role in the aging process of fossil materials, we aim to apply the methodology recently developed in our lab to determine the extent of deamidation in collagen, but this time in collagen extracted from ancient bones. The present work shows how to determine the extent of deamidation in collagen from ancient bones (1,000 years old) using Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) along with Collisionally Activated Dissociation (CAD) and Electron Capture Dissociation (ECD). The measured deamidation half-life for the tryptic peptides from collagen (I) was found to range from 4000-5000 seconds under high temperature conditions (~62 °C) and pH 7.5.



21 Tesla Hybrid Fourier Transform Ion Cyclotron Resonance Mass Spectrometer: An International Resource for Ultrahigh Resolution Mass Analysis

Christopher L. Hendrickson, Steven C. Beu, Greg T. Blakney, Tong Chen, Nathan K. Kaiser, Alan G. Marshall, and John P. Quinn

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Fourier Transform Ion Cyclotron Resonance (FT-ICR) provides the highest broadband mass resolving power and measurement accuracy of any mass analyzer. FT-ICR resolving power, acquisition speed, mass accuracy, and dynamic range all improve with increasing magnetic field strength, which extends application and impact into increasingly complex mixtures of proteins, peptides, lipids, metabolites, or natural organic matter (e.g., petroleum or humic substances). We describe design and optimization of a 21 Tesla hybrid FT-ICR mass spectrometer, which will be the highest field superconducting ICR magnet in the world when installed in 2013. The 21 Tesla magnet stores 36 MJ in a compact coil design that is roughly the size of our 14.5 T (6 MJ) magnet, but with equivalently low stray field and temporal drift rate, and better absolute spatial homogeneity. Challenges include precise ion injection through the large magnetic field gradient, and careful minimization of perturbing magnetic and electric field imperfections and ion-ion interactions. Integration of a second mass analyzer for parallel analysis and/or rapid and efficient mass selection and dissociation facilitates MS/MS at <1 Hz while high field maintains ultrahigh resolving power. The 21 T FT-ICR instrument will be available to all qualified users through the National High Magnetic Field Laboratory user program (http://www.magnet.fsu.edu/). Initial applications are expected to include Top-Down protein characterization beyond 150 kDa, and heavy petroleum crude oil analysis that requires >one million resolving power at m/z 1000.

Work supported by NSF Division of Materials Research through DMR-06-54118, NSF Division of Chemistry through CHE-1016942 (21 T Magnet) and NSF CHE-1019193 (21 T Spectrometer), Florida State University, and the National High Magnetic Field Laboratory in Tallahassee, FL.

Design Considerations for FT-ICR MS at 21T: A Progress Report

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Scientist teams at the Environmental Molecular Sciences Laboratory (EMSL) at PNNL and the ICR Group at the National High Magnetic Field Laboratory are developing prototype spectrometer platforms for very high resolution and mass accuracy next generation 21 T FTICR instruments. That all critical performance parameters of FTICR scale linearly or quadratically with magnetic field strength is well known. However, realizing these advantages of higher field in a multiplicative manner depends critically on design features of the entire instrument. All spectrometer components, including ion sources, ion trapping, manipulation and storage elements, multiple stages of differential pumping and ion injection must be configured and combined in ways that do not compromise final system performance. Some components—particularly ICR cells and RF storage elements-require careful attention to new understanding of properties of non-neutral plasmas

A combination of numerical simulation and testing of prototype “next generation” ICR cells has begun at both laboratories as the first step in this design effort. AT PNNL these simulations involve both commercially available SIMION 8.1 and Charged Particle Optics (CPO) and customized electric field and particle-in-cell (PIC) ion trajectory calculation codes. SIMION, CPO and electric field codes utilize standard multi-processor workstations, while the PIC code runs on Chinook, EMSL’s highly parallelized supercomputer. Three candidate cells address limitations in ICR performance introduced by nonlinearity in trapping fields by approaching as closely as possible the ideal quadratic field of a Penning trap. These include a multiple segment trapping and shim segments to approximate the ideal quadratic field, the recently characterized, dynamically harmonized cell and a new concept hybrid cell combining these concepts. Preliminary results for these designs, including results from limited experimental tests of prototype cells utilizing 12 T and 15 T superconducting magnets will be presented. The challenge of injecting ion packets from a relatively modest fringe field into the homogeneous high field “sweet spot” of the magnet is addressed by a combination of simulation and testing. These experiments will utilize a 12 Tesla surrogate test magnet for which precise field maps are available. This information is imported into the SIMION and CPO platforms for modeling combinations of RF and DC ion optical elements for injecting ions into the ICR cell. Based on these simulations, one or more of these candidate systems will be tested in the surrogate magnet now being installed at EMSL.

Posters

Poster 1

Using Electron Induced Dissociation to Characterise Polyketides and Polyketide Synthase Intermediates

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Polyketides are an important class of natural products, particularly in the drug discovery process, as they are one of the main sources of medicinal compounds. The identification of their biosynthetic pathways and the characterisation of their structures is of interest to chemists and biochemists who wish to prepare them on a large scale and investigate their bioactivity.1 Electron Induced Dissociation (EID) is an MS/MS technique used on Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometers and involves the fragmentation of species using electrons with typical energies of between 10 and 20 eV. Its potential for unveiling structural information has previously been demonstrated on different classes of biomolecules including peptides2 and fatty acids.3 Herein, EID has been used in tandem with Collision Induced Dissociation (CID) in order to characterise the structures of known polyketides, namely erythromycin A, lasalocid A and iso-lasalocid A, as well as of putative trapped intermediates in their biosynthesis.4,5 In a comparison with CID, EID caused fragmentation through multiple pathways, providing complementary structural information previously inaccessible through the use of CID alone. The use of EID in tandem with CID has therefore proved to be a valuable tool for determining detailed structural information on polyketides, and can assist in the characterisation of intermediate structures, helping to identify their biosynthetic pathways.

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Poster 2

Direct surface sampling of dried blood spots coupled with Fourier transform mass spectrometry for quantitative analysis of haemoglobin variants.

Rebecca L. Edwards, Paul Griffiths, Josephine Bunch and Helen J. Cooper.

School of Biosciences, University of Birmingham

Variants of haemoglobin, or haemoglobionopathies, are the most common type of inherited disorders. They are categorized into two groups: the structural variants and the thalassaemias. Structural variants arise via point mutations in the globin chains. The most clinically significant structural variant is the sickle variant HbS. The thalassemias are caused by impaired production of the protein chains that make up haemoglobin. β-thalassemia results from a lack of, or reduced synthesis of, the β-chain. It is characterised by severe anaemia and skeletal abnormalities in infancy. Similarly, α-thalassemia results from absence or reduced synthesis of the α-chain. If undetected, α-thalassemia major will result in stillbirth. Both types of variants are commonly detected in newborn screening programmes using high pressure liquid chromatography and/or isoelectric focusing. We have previously shown that liquid extraction surface analysis (LESA) of dried blood spots by use of the Advion Triversa Nanomate, coupled with high resolution top-down mass spectrometry, can be applied to the identification of the common structural variants (HbS, HbC and HbD)[1]. Determination of structural Hb variants by this method relies on the identification of mass shifts in either the precursor or MS/MS fragment ions. Here, we investigate whether the LESA approach may be applied to the identification of thalassemia; i.e., can haemoglobin be quantified by this method? The haemoglobin concentration in blood samples from healthy volunteers were determined by spectrophotometry. Blood samples were spotted onto filter paper and allowed to dry. The dried blood spots were sampled by LESA and either the samples were collected or analysed by mass spectrometry. The haemoglobin concentration in the collected LESA samples were determined by spectrophotometry and compared with (a) the Hb concentration in the whole blood samples and (b) the LESA mass spectra obtained for the dried blood spots.

Poster 3

Investigating changes in protein structure following deamidation using topdown FTICRMS methods and ion mobility

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Department of Chemistry, University of Warwick

The post translational modification of proteins occurs continuously in vivo and can result in changes to protein global structure that alter functionality with potential health implications. Here, ion mobility, FTICRMS topdown HDX-ECD and infra red absorption dependant unfolding-ECD are used to assess the structural changes of native state gas phase Calmodulin and Beta-2-microglobulin following post translational modification. Briefly, proteins were deamidated (conversion of Asparagine to Aspartic Acid or Iso-Asp via amine group removal and water addition) in NaOH at a pH of 9 overnight before being desalted, purified and resuspended in 10mM Ammonium acetate. The previously mentioned mass spectrometry methods were then carried out using nanospray alongside non deamidated controls. Deamidation was selected as a modification primarily because it occurs frequently with protein aging in vivo and also because it involves the conversion of an uncharged residue (Asn) to a negatively charged one (Asp) making it more likely for the modification to have an effect on local and global structure, similarly Calmodulin and B2M both possess multiple rapidly deamidating Asparagine residues. Ion mobility is a robust method for this type of study and results from the topdown FTICRMS methods will be compared with this data to assess the extra structural information they can provide over solely utilising ion mobility.

Poster 4

Improving Intact Protein and Top-Down Analysis by Orbitrap Mass Spectrometry

Mathias Mueller, Eugen Damoc, Martin Zeller, Kai Scheffler, Hartmut Kuipers, Jens Griep-Raming, Eduard Denisov, Alexander Makarov, Dirk Nolting and Thomas Moehring

Thermo-Fisher Scientific, Bremen, Germany

The ultrahigh resolution of the Orbitrap Elite hybrid mass spectrometer is one of the most important prerequisites for intact protein characterization and top-down analysis. However, resolving power of large intact protein mass measurements is limited by the pressure in the Orbitrap mass analyzer due to fast transient decay caused by collisions with residual gas. We have modified a Thermo Fisher Scientific Orbitrap Elite ETD instrument for improved high molecular weight ion characterization. The modifications of the instrument relate to the trapping scheme of the ions in the C-trap, the implementation of a switching valve in the C-trap collision gas supply used to control respectively reduce the pressure in the Orbitrap mass analyzer as well as changes in the instrument control software. Reduction of the gas load in the C-trap/HCD collision cell allows for the reduction of the pressure in the Orbitrap mass analyzer and hence reduces the decay of transients of intact proteins and allows for the detection of more discrete beats in the transient of high molecular weight ions, thus increasing the upper mass limit of proteins isotopically resolved in online analysis up to ~50 kDa. Lowering the pressure in the C-trap/HCD collision cell however results in undesired reduction of trapping efficiency in the C-trap. Thus, in this modified instrument we have implemented the trapping of ions in the HCD cell for all modes of operation with subsequent gentle transfer of cooled ions into the C-trap prior to injection into the Orbitrap analyzer. In addition, lower number of collisions during ejection from the C-trap was found to result in an increased signal even for the first beat. This significantly increases the signal-to-noise in mass spectra of very large proteins such as antibodies and improves the analysis of their glycosylation status. To demonstrate the improved instrument performance resulting from the implemented modifications we have analyzed an intact monoclonal antibody in non-reduced and reduced condition by LC-MS. The intact antibody respectively the separated light and heavy chains were analyzed in Full MS experiments as well as with top-down experiments using in-source CID (SID), CID, HCD and ETD fragmentation techniques. For data evaluation ProSight PC 2.0 and Protein Deconvolution 1.0 software packages were used.

Poster 5

Identification of protein ligands by FT-ICR-MS/MS

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Department of Biology, University of York

The presence of non-covalently bound ligands in proteins can have a variety of significant biological effects; examples are numerous including biological synthesis, cell signalling, and transport. Accurate identification of the ligands involved is of vital importance in these processes. However, conventional mass spectrometry and even X-ray crystallography cannot always provide definitive identification, especially where sample is limited in amount. The high mass accuracy of FT-ICR-MS, in tandem with its ability to couple with a variety of ion dissociation options, offers good scope for the identification of these biological molecules. We have demonstrated that the Bruker solariX equipped with a 9.4-tesla magnet can be used to accurately identify non-covalent protein ligands. Ligand identification by FT-ICR-MS was performed under denaturing conditions to maximise signal quality and mass accuracy. Acquisition parameters were adjusted to aid release of the ligand and optimise sensitivity in the low m/z region where the ligands are found. Important acquisition parameter changes included increasing the source accumulation time and reducing the TOF time. Typically, identification of elemental composition was possible from the intact m/z alone, with indications of structure arising from fragmenting the ligand using different dissociation methods. The solariX affords a variety of dissociation options; so far we have performed fragmentation of ligands using CID in the quadrupole and SORI in the Infinity™ cell. These approaches have yielded differing and complementary product ion spectra that aid the assignment of ligand structure. In the case of L-alanyl-γ-D-glutamyl-mesodiaminopimelate the y1-ion pedominated in the SORI spectrum while the y2-ion was only detected following CID. Example of ligands identified include: L-alanyl-γ-D-glutamyl-mesodiaminopimelate; glutathione; sialyl amide and S-adenosyl, S-carboxymethylhomocysteine. Identifications were consistent with results of structural binding assays or X-ray crystallography data.

Poster 6

Electron Capture Dissociation Tandem Mass Spectrometry of Cyclic Side-Chain Containing β-peptides

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Electron capture dissociation (ECD) tandem mass spectrometry of cyclic side-chain containing β-peptides was performed. Doubly charged precursor ions produced mainly c-, a- and b-type fragment ions. The observed c ions were due to N-Cβ bond cleavages. No N-Cα bond cleavages were observed. The formation of b ions in the absence of basic amino acid residues (BAARs) is similar to what has been observed with α-peptides. Surpisingly, b ions from the β-peptides containing BAARs were radical species (i.e., b* ions). Previously, it has been proposed that the formation of b-ions in ECD is due to the initial protonation at the amide nitrogens, which should lead to complementary b* and y ions. However, in practise only even-electron b ions have been observed which could be explained by hydrogen atom transfer within a long-lived b*/y ion complex. To the best of our knowledge, this is the first time the formation of b* ions in ECD is reported. An extended fragment ion nomenclature for peptides composed of homologated amino acids (e.g., β-peptides) is also proposed.

Poster 7

Tandem mass spectrometry of chlorophyll a

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The breakdown of chlorophyll a is an enigma for a long time. As its irreplaceable role mainly depends on the asymmetric and conjugated structure, electron-based MS/MS can probably fragment it in a well-regulated way, which may help us to understand the degradation mechanism of chlorophylls. Singly charged chlorophyll a is fragmented by electron induced dissociation (EID) using fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Fragments induced by EID are compared with those leaded by collisionally activated dissociation (CAD) and infrared multiphoton dissociation (IRMPD). Accordingly, the breakdown rule of chlorophyll by MS/MS is illustrated, which can also provide some clues of how chlorophyll a degradation works in vivo.

Poster 8

The gas-phase thermochemistry of Hf2+ and Ta2+ oxide ions

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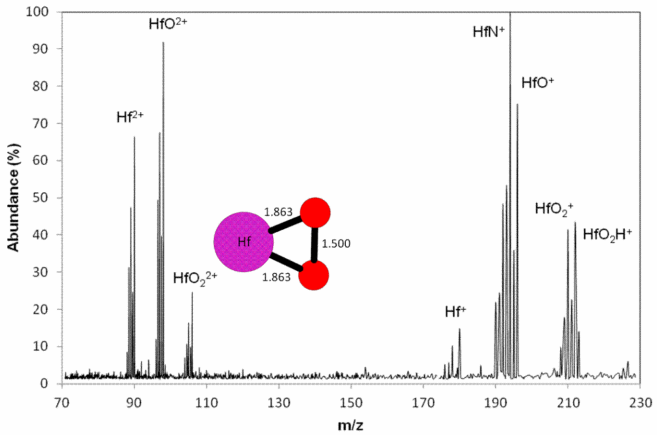
We have been examining the gas-phase chemistry and thermochemistry of transition metal oxide ions by FTICR/MS, to provide fundamental chemical models for complex multi-charged systems, and to obtain new thermodynamic data for ionic and neutral species containing these elements. The experiments have focused on the gas-phase reactions of Hf2+ and Ta2+ ions with several oxidants, ranging from the thermodynamically facile O-atom donor N2O to the ineffective donor CO, as well as intermediates donors O2, CO2, NO and CH2O. All oxidants reacted with Ta2+ by electron transfer yielding Ta+, in accord with the high second ionization energy of Ta (ca. 16 eV). Hf2+ reacted by electron transfer with N2O, O2, NO and CH2O, in agreement with the lower second ionization energy of Hf (ca. 15 eV). HfO2+ and TaO2+ were produced with N2O, O2 and CO2, oxidants with ionization energies above 12 eV. It was also found that HfO2+ and TaO2+ reacted efficiently with N2O yielding HfO22+ and TaO22+, respectively. Density functional theory (DFT) calculations indicated that “hypervalent” HfO22+ is actually a peroxide whereas TaO22+ is a bent dioxide with oxygen-centered radical character. Estimates were made for the second ionization energies of MO and MO2 (M = Hf, Ta), and of the bond dissociation energies of doubly charged Hf and Ta monoxide and dioxide ions. The experimental bond dissociation energies were compared with those predicted by DFT calculations. Figure: Mass spectrum for the Hf2+/N2O reaction showing the formation of HfO2+ and HfO22+ (2.5x10-7 Torr N2O, ca. 3.7x10-6 Torr Ar, reaction time 0.1 s); the insert shows the ground state peroxide structure (C2v-1A1) for HfO22+ (DFT - B3LYP/ SDD (Hf) : TZVP (O)).
Acknowledgments This work was supported by the Fundação para a Ciência e a Tecnologia (projects PTDC/QUI-QUI/108977/2008 and Pest-OE/QUI/UI0100/2011); by the Università della Calabria; and by the Director, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences and Biosciences of the U.S. Department of Energy at LBNL, under Contract No. DE-AC02-05CH11231. This research used resources of the National Energy Research Scientific Computing Center (NERSC), which is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231



Poster 9

A novel ESI-MS rearrangement of 2,5-disubstituted tetrazole metabolites studied by LC-FTICR mass spectrometry

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Chemical Works of Gedeon Richter, Spectroscopic Research

In the early-phase drug discovery high-resolution mass spectroscopy plays a unique role. The detection and structure elucidation of in-vitro metabolites of potential drug candidates have an impact on the drug design, as it is necessary to determine the metabolic soft spots of the candidate molecules for further lead optimization. Up until now very few papers have been published about the use of the FTMS technology in drug metabolism studies. The test compound was synthesized – among several other 2,5-disubstituted tetrazole derivatives – as a potential drug candidate for the prevention and/or treatment of mGluR5 receptor mediated disorders, particularly neurological disorders, psychiatric disorders, acute and chronic pain and neuromuscular dysfunctions of the lower urinary tract. This parent compound was incubated with rat and human liver microsomes, subsequently the incubation samples were analyzed on a Thermo LTQ-FT Ultra instrument coupled with a Thermo Accela HPLC. During the structure elucidation of the main metabolites a novel gas-phase ESI-MS rearrangement – involving the disruption of the tetrazole ring – was observed. At first sight in the MS/MS spectrum of the parent compound only a single analogue rearrangement fragment ion peak was observed, but careful inspection of the parent compound's high resolution MS/MS spectrum revealed all of the analogue rearrangement fragment ions in the close vicinity of the ^{13}C isotopic peaks of the main fragments. The rearrangement process was also observed in the direct MS analysis of analogue 2,5-disubstituted tetrazole derivatives. Altogether four kinds of rearrangement ions were formed and their origin was studied by high-resolution MSn measurements. The parent compound of the in-vitro metabolism study was chosen as a model compound and theoretical calculations (B3LYP/6-31+G*) were carried out for the explanation of the phenomena observed. In our ongoing research project the rearrangement routes have already been partly rationalized by the formation of cyclic transition state intermediates and/or an ion-molecule complex. Beyond its theoretical importance, the exploration of new gas-phase ESI-MS rearrangements enables the determination of more accurate and reliable chemical structures in mainly LC-MSn based structure elucidation tasks, such as in-vitro metabolite identification.

Poster 10

Metabolomic Profiling in Drug Discovery: Understanding the factors that influence a metabolomics study and strategies to reduce biochemical and chemical noise

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Introduction Metabolomics is used within the pharmaceutical industry to investigate biochemical changes resulting from pharmacological responses to potential drug candidates. The ability to identify markers of toxicity/efficacy can significantly accelerate drug discovery and helps define the appropriate clinical plan. Data from LC-MS metabolomic profiling experiments contains large amounts of chemical background which often confounds biomarker discovery. New mass spectrometer technology and data processing software were utilized here to reduce chemical background; animal experiments were designed to investigate the influence of animal age and nutrition in relation to drug-induced changes. Methods Blood samples were taken from groups of male rats (fully satiated, acute and chronic fasting, different ages). LC-MS analyses were performed in positive and negative modes using a hybrid Orbitrap mass spectrometer capable of fast scanning at ultra high resolution (>50K), and a 12-minute UHPLC separation. Study data was analyzed using new component detection algorithms in beta SIEVE 2.0 software to determine metabolic effects of food deprivation and aging rats. Preliminary data In typical LC-MS metabolomics studies much of the data is redundant (multiple ions per component) and irrelevant (chemical noise). External factors that influence metabolic profiles (age, nutrition) increase biological variation. Since many of the chemical entities are unknowns, it is especially important to filter false positives before implementing structure elucidation. Ultra-high resolution instruments combined with UHPLC separations address the issues of chemical noise and redundancy by providing sufficient resolution to distinguish metabolites from chemical background. Accurate mass data allows sophisticated processing needed to recognize related signals leading to significant reduction in data size and providing improved quantitation of targeted metabolites. Biological factors have profound impact on metabolic profiles and even modest metabolic changes can obscure drug-induced metabolic effects. Understanding normal metabolic changes in rats helps to minimize “biological noise” and provides more confidence in assigning drug-related metabolic changes. Novel Aspect New hardware, software and a database of normal rat metabolic responses to external stimuli are applied to reduction of noise in metabolomics studies.

Poster 11

Technological Advances in Metabolomics: The Role of Ulltrahigh-Resolution FT-ICR MS

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Biological metabolites vary in size, polarity, charge, structure, and elemental composition and reside as a highly complex mixture in a biological matrix. Large-scale experiments aimed to identify and quantify metabolites currently suffer from analytical and computational limitations, including detection of all chemical matter, correct assignment of isotopic distribution and speed of analysis. No single ionization technique can effectively deal with all chemical classes of metabolites. Mass spectrometry (MS) detection can also be limited by low resolution and the frequent need to perform MSn analyses. We will present new developments that will supply key improvements to overcome current limitations in metabolomics, focusing on metabolite ionization (fast +/- ion switching), detection, and data reduction/analysis. Sub-ppm mass accuracy alone is often insufficient for elemental assignment due to the large number of possible atomic configurations for a given nominal mass, but coupled with high mass accuracy and ultrahigh-resolution isotopic fine structure information, compounds can be uniquely identified. A series of standard chemical compounds that represent different chemical classes of biologically important metabolites (S-methyl-L-cysteine, alpha-ketoglutarate, L-phosphatidylcholine, dopamine, fructose 1,6 diphosphate) were analyzed on a ultrahigh-resolution Bruker Solarix 12T FT-ICR MS equipped with a dynamically harmonized ICR cell. Six were ionized by (+) ion ESI and 2 by (-) ion ESI (m/z range ~90 -~760, average mass resolving power 350K). All spectra showed detailed isotopic fine structure for each test compound. For example, the isotopic fine structure of the first 13C peak of S-methyl-L-cysteine at m/z 137.04603 revealed four baseline-resolved, heteroatom-containing peaks: 12C41H1015N116O232S1 at m/z 137.03972, 12C41H1014N116O233S1 at m/z 137.04207, 12C313C11H1014N116O232S1 at m/z 137.04603 and 12C41H92H114N116O232S1 at m/z 137.04896. Each heteroatom's peak height correlated to the correct isotopic abundance. The latest revision of a high-resolution based algorithm was applied to these data sets to determine elemental composition of each metabolite. The process involved simulating ultrahigh-resolution isotopic patterns that were folded with the monoisotopic peak shape of the experimental profile to make sure the correct peak shape was created. For each nominal mass of the pattern having at least two peaks above a threshold, these peaks were folded with the peak shape in the corresponding range around the monoisotopic mass, which resulted in a simulated profile. The profiles for each isotope cluster were compared and scored using a statistical goodness of fit. The algorithm successfully assigned the correct elemental composition for all metabolite standards without MSn analysis. Future studies will be to apply this methodology with fast +/- ion switching to metabolomic analysis (including polar lipids) on an LC time scale, in the study of glioblastoma and glioblastoma-derived cancer stem cells.

Poster 12

Combining non-selective and selective fragmentation and high resolution accurate mass for metabolite screening and identification in early in vitro studies

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Metabolic stability screening assays are a common in vitro test used to screen drug discovery candidates for favorable properties. In the past these screening assays were commonly analyzed by LCMS using triple quandrupole mass spectrometers which need to be optimized for each compound. While the quantitative quality was sufficient, qualitative data was limited without significant additional method development. Here we show how data obtained on a high resolution accurate mass benchtop high operating in both all ion fragmentation and selective MS2 modes, combined with screening software, provides both quantitative stability as well as early qualitative metabolite information. The same data, without reinjection, can be further scrutinized for more definitive metabolite structure using selective MS2 scan data. The metabolic stability of nortriptyline (0.5 µM) in liver microsomes (0.5 mg/mL) was determined by timecourse incubation with sample aliquots taken at five time points. Samples were analyzed by UPLCMS on a novel benchtop quadrupole Orbitrap MS with a higher energy collision dissociation (HCD) cell. Data was acquired as a full scan followed by an all-ion-fragmentation (AIF) scan. Precursor isolated HCD MS2 scans were obtained near the peak maxima on components observed above threshold. The data was acquired using a beta version of MetQuest 1.1 metabolic screening software with automatic relative quan/qual data analysis. Initial processing of the timecourse samples was performed automatically using a list of 50 commonly found biotransformations as well as screening for unexpected metabolites. Eleven expected metabolites were detected whose area when compared to the control differed by more than 5X and whose exact mass value was within 5 ppm of the expected value (minimum acceptance criteria for an expected metabolite). Metabolite peaks were integrated using a parameterless peak detection algorithm to provide results with minimal user interaction. Metabolite relative quantitative abundance as a % of nortriptyline at t0 was reported along with collected MS2 fragmentation data. They ranged from 0.5% to 28% of parent. Detected mass accuracies were better than 2 ppm. The high resolution accurate mass data was also used to assign elemental compositions to the proposed metabolites based on an algorithm specifically designed for data from this type of instrument. The same raw data files and metabolite information were used to further scrutinize the results and to assign fragment structures of nortriptyline and determine its metabolite structures. Theoretical fragments for nortriptyline were generated and combined with the list of modifications provided from the Quan/Qual study. Initial fragmentation explanation based on nortriptyline with the allowed modification set provided identification for 3 of the 5 metabolites without any user intervention. Further scrutiny of the predicted fragment structures allowed for close assignment of the 4th metabolite and a regional assignment to the 5th metabolite.

Poster 13

Advanced characterisation of organic matter in complex mixtures by Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS)

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Crude oils as well as coals are highly complex mixtures of organic compounds, which show a huge variety in their elemental compositions and chemical structures. Besides hydrocarbons, oils and coals contain variable but sometimes very high amounts of heteroatom-containing compounds (nitrogen, sulfur and oxygen (NSO) compounds). These heteroatomic compounds can provide geochemical information about geological origin, thermal maturity or microbial degradation, however, from a production perspective they may cause problems e.g. during refining or storage. To understand the influence of heteroatom-containing constituents on the properties and quality of fossil fuels, their isolation from the hydrocarbon fractions and subsequent identification is an important challenge for industry and scientists. Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS) in connection with specific ionization methods is a powerful tool for the detailed characterisation of complex mixtures of heteroatomic and high molecular weight compounds. This presentation will give an overview of the composition of the NSO compounds and the n-hexane-insoluble asphaltenes in two coal samples from Germany and three reservoir core samples from a North Sea oil field. The analytical characterisation is based on the extraction of the soluble organic matter and its subsequent separation into asphaltenes, aliphatic hydrocarbons, aromatic hydrocarbons, neutral NSO compounds and carboxylic acids. The asphaltene, neutral NSO compound and carboxylic acid fractions have been further analysed by direct infusion atmospheric pressure ionization coupled to ultrahigh resolution FT-ICR-MS. Electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI) mass spectra were acquired in the negative and positive ion mode using a solarix FT-ICR mass spectrometer equipped with a 12 Tesla refrigerated superconducting magnet (Bruker Daltonik GmbH) to study the chemical composition of the sample set on the molecular level. Different ionization methods were used to detect compounds of different polarity.

Poster 14

Molecular characterization of bio-oil through APPI FTICR

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Exhausting petroleum resources, combined with political and environmental concerns about fossil fuels, are fostering the research of renewable and environmentally sustainable substitutes. Among various energy forms, liquid fuels are convenient for storage and transportation, and are suitable for existing fuel distribution infrastructures. Solid wastes of organic origin are potential feedstock[1] for the production of liquid biofuels, which in turn could be suitable alternatives to fossil fuels in the transport and heating sectors. In this work FTICR MS direct analysis have been used to characterize a Bio-Oil sample obtained by thermochemical conversion of waste biomass. The wet biomass, subjected to a liquefaction process, is partially transformed into a water-insoluble oil-like organic phase, called bio-oil. The bio-oil has a high percentage of carbon and a high content of etheroatoms, in particular nitrogen and oxygen. The sample has been first analyzed in GC-MS with a single quadrupole mass spectrometer. Although the gas chromatographic separation yields a partial coelution of many different compounds, the main classes of compounds have been identified, considering the electron ionization mass spectra. Direct analysis through APPI-FTICR MS, recently utilized for the molecular characterization of crude oil,[2] has been used for the characterization of bio-oil. The ion source is connected to a LTQ-FT Ultra (Thermo Scientific) instrument with a FT-ICR cell surrounded by a 7 Tesla magnet. Photoionization[3] is very effective for the ionization of low-medium polarity and aromatic molecules. The spectrum has been acquired in positive mode with an average resolving power of 400000. Very high mass accuracy together with high resolution allows the attribution of thousands of molecular formulas per mass spectra. Data have been processed by the software Xcalibur (Thermo Fisher Scientific Company) after the selection of some restrictions of the range of elements: 10-60 12C, 0-2 13C, 10-100 H, 0-6 N, 0-6 32S, 0-1 34S, 0-6 O and the error range was set to 2.5 ppm. These restrictions are required because of the great number of possible different combinations of elements that can be generated from a single accurate mass. In this bio-oil mass spectrum we have assigned a molecular formula to more than 6000 peaks through a custom built software (ISOMASS). The main classes found contains one or two nitrogen atoms and 0-2 oxygen atoms (N2,O1N2,O1N1,O2N1). This approach has been used also for the molecular level characterization of bio-oils during the upgrading steps of bio-oil into diesel-like products.

[1] M. Munster, H. Lund, Waste Management (2010), 30, 1251-1263.
[2] A. G. Marshall et al. Energy and Fuels (2007),21, 2869-2874.
[3] D. B. Robb, T. R. Covey, A. P. Bruins, Anal. Chem.,(2000)72, 3653-3659.

Poster 15

Orbitrap high resolution mass spectrometry characterization of Athabasca oil sands acids in environmental samples

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The Athabasca oils sands region of Alberta, Canada is one of world's largest bitumen reserves with a proven 170 billion barrels of crude oil. Approximately 3 barrels of river water are used during the alkaline/hot water extraction process of the mined oil sand for every barrel of oil produced. During the extraction process, acidic bitumen components (including naphthenic acids) are solubilized in the water. This oil sands process water (OSPW) is stored in tailings ponds in accordance with a zero discharge policy. There is a growing need for development of analytical methods that can distinguish between compounds found within industrially derived OSPW from those derived from natural weathering of oil sands deposits. This is a difficult challenge as possible leakage beyond tailings ponds containments will likely be in the form of a mixture of water soluble organic acids that are similar to those leaching naturally to aquatic environments. An overview is given on the progress of analytical developments and the current state of mass spectrometry analysis of environmental samples. The potential for Orbitrap high resolution mass spectrometry and accurate mass measurements for chemical fingerprinting of oil sands acids from tailing ponds, interceptor wells, groundwater and reference river surface waters is evaluated. Particular emphasis is given to (i) new developments which recognize that the oil sands acid fraction contains more components than the traditional structures of naphthenic acids; and (ii) the influence of extraction procedures on what is being measured. Significant differences in high resolution mass spectrometry results were observed for the same sample set that are attributed to the extraction procedure and/or extraction solvent of choice.

Poster 16

Characterization of oil by ESI, APPI and GC/APCI FTMS

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Crude oil as well as oil fractions represent an extremely complex mixture of organic compounds consisting of various elemental compositions and chemical structures. The composition of many compounds is not exactly known. Oil consists mostly (>95%) of hydrocarbons. However, the remaining part is composed mainly of hetero atomic compound classes containing oxygen, sulfur and nitrogen. These compounds are polar and most of them can be detected by mass spectrometry using atmospheric pressure ionization. The composition of the hetero atomic compounds is a fingerprint. Therefore, the oil industry is highly interested in qualitative and quantitative information of compound classes to optimize refining and catalytic processes by avoiding emulsion formation, coke formation and corrosion. Speciation of heteroatomic compounds like sulfur or nitrogen containing compounds with specific double bond equivalents (DBE) is necessary for oil classification on the molecular level. Polar compounds can be detected by electrospray ionization mass spectrometry. Direct infusion experiments can be carried out using an instrument with an ultra-high resolving power and mass accuracy to achieve mass peak separation and correct annotation of the molecular formula of all peaks in the mass spectrum. Several studies have been made in positive and negative ion mode by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) using electrospray ionization. However, other atmospheric pressure ionization methods like atmospheric pressure chemical ionization (APCI) or atmospheric pressure photo ionization (APPI) can be used to analyze crude oil. Another alternative to detect polar and semi-polar volatile compounds is GC/APCI. Even isomers can be separated by GC and the molecular ions are detected by APCI mostly without any fragmentation in contrast to GC/MS instruments with an EI source. Nearly all peaks in a mass spectrum can be assigned with exactly one molecular formula by FT-ICR mass spectrometry with a resolving power of more than 400,000 (200,000 with GC/APCI) and a mass accuracy better than 0.5 ppm. Two different oil samples were analyzed by ESI, APPI and GC/APCI using a solarix 12 T FT-ICR mass spectrometer (Bruker Daltonics, Billerica, MA). A few thousand elemental compositions have been detected and identified for each sample in positive and negative ion mode. The classification has been done by intensity plots of compound classes and DBE (double bond equivalents) vs. C plots. The oil samples could be distinguished by the relative abundances of their compound classes. However, a detailed analysis by DBE vs. C plots of specific compound classes improves the confidence for the oil characterization. GC/APCI can be used to compare samples by their relative abundances of specific isomers, for instance alkylated benzothiophenes and dibenzothiopenes as well as PAHs. Therefore, FT-ICR MS can be used to distinguish very similar oil samples on the molecular level.

Poster 17

GC/APLI-FTMS – a new sensitive method to detect aromatic compounds in complex mixtures

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A new method, GC/APLI-FTMS, for the detection of aromatic compounds in complex mixtures has been developed which combines GC as a high performance separation method, APLI as a very sensitive ionization method for aromatic compounds and FTMS which is known for accurate mass measurements. APLI is soft ionization method in contrast to common GC/MS systems with EI source. Therefore, this hyphenation method is well suited for the characterization of volatile aromatic compounds of complex mixtures like waste water or oil samples. GC/APLI has been coupled to an FT-ICR instrument to detect polycyclic aromatic hydrocarbons (PAHs) and polycyclic aromatic sulfur heterocycles (PASHs) in fuel oil and gas oil. Waste water samples were analyzed concerning specific PAHs. A GC system was coupled with a GC/APLI source to a solarix 12 T FT-ICR mass spectrometer. Typical GC temperature gradients (5 °C/min and 10 °C/min) have been used. Mass spectra have been acquired with 1Hz resulting in a resolving power of 250.000 at m/z 200. 1 μL of sample dilutions (between 1:103 and 1:106 of oil sample) have been injected on a VF-5ms, 30m, 0.25 mm ID, GC column (0.25 μm film thickness). Transfer temperature of 290°C between GC and ion source was used. The elemental compositions of PAHs and PASHs in gas oil and fuel oil as well as PAHs in waste water have been analyzed by exact mass and retention time in positive ion mode. Samples with different amount of sulfur between 10 ppm to more than 1000 ppm have been tested. PAHs and PASHs can be easily identified just by retention time and accurate mass combined with the isotopic pattern of the molecular ion. Extracted ion chromatograms (EICs) with 1 mDa mass tolerance have been used to identify isomers of PAHs and PASHs. Species with a small mass difference with similar retention times can also be separated due to high resolving power of the FT-ICR MS technique. All results have shown that GC/APLI-FTMS allows the detection and characterization of low abundant PAHs and PASHs in complex mixtures. Therefore, this new analytical technique might be a new method to detect and quantify very low amounts of PAHs in waste water or even drinking water.

Poster 18

Analysis of sulfur-rich crude oil and bitumen by APPI-FTMS

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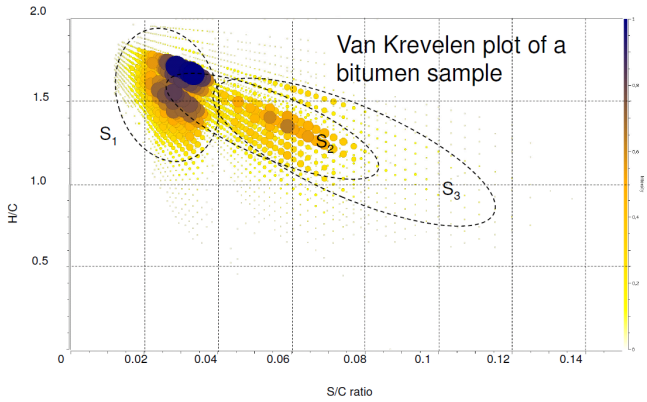
Crude oil consists mostly (>90%) of hydrocarbons. Remaining compounds in oil mainly contain hetero atom classes with oxygen, sulfur and nitrogen. Commercial oils vary in the composition of the type and amount of compounds as well as compound classes. Polar compounds in crude oil can be detected easily by atmospheric pressure ionization (API) mass spectrometry. Direct infusion experiments of crude oil samples can be carried out by FTMS to achieve mass peak separation and correct annotation of the molecular formula of all peaks in the mass spectrum. Compounds containing only sulfur and no other hetero atoms are difficult to detect by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) due to the fact that these compounds are difficult to protonated or deprotonated. APPI is able to generate radical cations using multi-photon ionization to detect these compounds without chemical modification using for instance methylation reagents. Sulfur containing compound classes like benzo thiophenes (DBE 6) or dibenzo thiophene (DBE 9) can be identified based on the number of double bond equivalents (DBE). More than 95% of all mass peaks could be assigned with exactly one molecular formula with a resolving power of 600,000 at m/z 400 and a mass accuracy better than 0.5 ppm using internal calibration. Four crude oils and two Bitumen samples from the company SINOPEC, China, were analyzed. The spray solutions of the crude oils were prepared without any further purification. 10 mg of the crude oil were dissolved in 200 µL dichloromethane. These sample stock solutions were diluted 1:300 with 50% methanol, 50% toluene for APPI measurements in positive ion mode. Mass spectra were acquired with a 12 T solarix FTMS. Formula calculation was done in Composer 1.0.2 (Sierra Analytics) using a maximum formula of CnHhN3O3S3. DBE vs. carbon number plots as well as compound class and Van Krevelen plots were generated with Composer. The samples could be separated in three groups based on the detected compound classes: low amount of sulfur (crude oil), medium amount of sulfur (crude oil) and high amount of sulfur (bitumen). Even compound class S3 with a relative abundance of about 8% is present in the bitumen samples. The average DBE of compound class S1 is mainly between 9 and 15 of the crude oil samples whereas DBE is mainly DBE 6 and 9 of the bitumen samples. DBE is shifted up to higher DBE values for compound class S2 for all samples. Van Krevelen plots have been generated in addition to the compound class plot (see figure) to show the degree of unsaturation of each class directly in the plot.



Poster 19

Analysis of peat bog after solid phase extraction by FTMS

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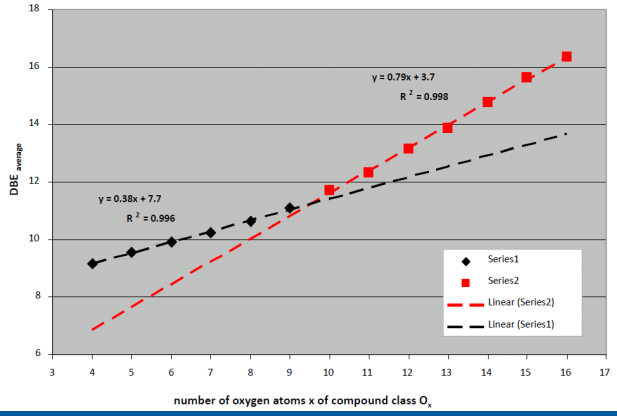
Natural Organic Matter (NOM) is a complex mixture of highly degraded organic compounds present in surface and ground water. Structures and chemistry of these compounds are of high interest. NOM is abundant in terrestrial, limnic and marine environment. Major contributors are humic substances, sedimentary and dissolved organic matter (DOM). Masses range from 150 to 1000 Da measured by FTICR mass spectrometry. Structural information of organic molecules of NOM is difficult to gain from molecular formula. A huge variety of chemical isomers is possible even at small masses with different structures and functional groups. However, relative abundance of functional groups like hydroxyl or carboxyl groups can be tried to determine by plotting DBE vs. number of oxygen atoms. In this study a sample from peat bog treated by solid phase extraction was analyzed. Molecular formulas of organic compounds in DOM were determined by FTMS in electrospray negative ion mode using a resolving power of 480.000 at m/z 400. Measurements have been performed with a solarix 12T system. After molecular formula assignment of all mass peaks Van Krevelen plots were created for compound class Ox, NOx and SOx. Additionally, class distribution plots were created for these three compound classes as well as DBE distribution plots. These plots were generated by Composer (Sierra Analytics). Class distribution, DBE distribution and DBE vs. oxygen atom plots were generated with Windows Excel using the tables generated with Composer. The RMS error was only 159 ppb using more than 7000 mass peaks. Due to the high mass accuracy of FT-ICR results, peaks could be assigned with their molecular formula unambiguously up to masses at m/z 800 using standard restrictions for DBE, H/C and O/C ratios. Van Krevelen plots have been generated for the compound classes Ox, NOx and SOx for a detailed analysis of the data. More than 5000 compounds have been found for compound class Ox compared to nearly 1000 compounds for compound class NOx and about 600 compounds for compound class SOx. The relative distribution of DBE of class Ox ranging from low to high number of oxygens is very interesting. DBE shifts to higher values with increasing number of oxygen atoms. However, two series have been observed, one series with a slope of only 0.38 for compounds containing 4-9 oxygen atoms, the other series with a slope of about 0.8 containing 10-16 oxygen atoms. Good linear regression factors have been calculated for both series. One series seems to be dominated by carboxyl groups, whereas the other series seems to be dominated by carboxyl as well as carbonyl groups. The intercept of the linear regression can be used to get information of the core structure of both series. Core structure of the molecules with low number of oxygen atoms contain about eight double bonds or rings whereas the core structure of the series with high number of oxygen atoms contain only four double bonds or rings.



Poster 20

Advanced Front-End Electronics for Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry

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The preamplifier, the first-stage electronic device in a signal processing chain, of the high mass accuracy/performance Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry plays a critical role for improving the signal-to-noise performance. The Green's and the O'Connor's group recently reported a transimpedance preamplifier, in which its feedback resistor is also used to bias the detection plates of an ion cyclotron resonance (ICR) cell to preserve ion trajectories during detection. Such preamplifier was tested with improved signal sensitivity and noise performance for a 12 T FT-ICR system. The thermal noise from the biasing resistor becomes another voltage noise source apart from the intrinsic noise of the preamplifier. A novel transimpedance preamplifier with new feedback and biasing setup was designed to limit the thermal noise whilst maintaining the gain and bandwidth performance. Data have shown that a transimpedance preamplifier designed for a 12 T FT-ICR system using an 18 kΩ feedback resistor and using the AD8099 provides excellent flat mid-band transimpedance of about 85 dBΩ over a frequency range from around 3 kHz to 10 MHz, while the measured lowest input current spectral density could be as low as 1 pA/√Hz. In such a preamplifier system, the feedback resistor was used not only to generate negative feedback to stabilize the system but also to bias the detection plates of the ICR cell. With a designed bandwidth of 10 MHz, a typically-estimated parasitic capacitance of 80 fF within a 0805 surface mount resistor limits the maximum feedback transimpedance to about 200 kΩ. This proposed novel feedback arrangement and a new lower-noise amplifying stage have a theoretically lower intrinsic noise, and about a 10-fold lower thermal noise voltage generated by the biasing circuitry, with similar gain and bandwidth performance. Preliminary data measured from the circuit on a single-layer printed circuit board showed an excellent agreement with the theoretical calculation. Comparisons between different circuit design strategies and the electronic limitations will be discussed at the conference.

Poster 21

Absorption-mode: The Next Generation of Fourier Transform Mass Spectra

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The Fourier transform ion cyclotron resonance (FT-ICR) mass spectrum is conventionally presented in the magnitude-mode. However, it is well known that the absorption-mode display gives a much narrower peak shape which greatly improves the spectrum. Our recent papers show that the successful solution of the phase equation allowed broadband phase correction which makes it possible to apply the absorption-mode routinely in FT-ICR. The new spectra yield a 50 - 100% improvement in resolution, a 20 - 100% improvement in mass accuracy, and a 40% improvement in signal/noise ratio at no cost in instrumentation! Here, we apply the method to both top-down protein and crude oil spectra, to demonstrate the advantages of the absorption-mode in the ultra-complex spectra. The result is substantially more advantageous than we had previously realized. With all improvements above obtained simultaneously, the effect is similar in improvement ratio to increasing the magnetic field strength of our instrument from 12 tesla to 17-24 tesla.

Poster 22

Isotopically Resolved FTICR Mass Spectrometry at 7 Tesla

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FT-ICR continues to be the leading technique in mass resolution and mass measurement accuracy. Its application in the field of top-down proteomics and protein modification analyses via accurate mass measurements is demanding further increase mass resolution. Higher mass resolution needs higher magnetic fields or principally new measurement methods. The new type of FTICR cell [1] which has been presented recently is based on a cylindrical ICR cell with shaped excitation and detection electrodes for dynamic harmonization of the electrostatic trapping field. In this new type of cell the effect of ion cyclotron motion dephasing during signal detection is almost eliminated [2], it provides a mass resolution that is practically only limited by the pressure in the ICR cell region. Its performance has been experimentally characterized in a moderate magnetic field of 7 Tesla [3] using several samples covering a wide mass range. In the case of smaller molecules, a maximum resolving power of almost 40,000,000 with a detection time of 5 minutes could be reached for reserpine at 609 m/z. Isotopic fine structures within resolved 13Cn isotopic cluster groups were measured for molecular masses up to 5.7 kDa (insulin) with resolving powers of about 6,000,000. Based on these resolved fine structure patterns atomic compositions can be directly determined using a new algorithm for fine structure processing. Also large proteins and their multimers up to a molecular mass of 186 kDa (Enolase Tetramer) could be measured isotopically resolved. At this moderate magnetic field of 7 Tesla a typical resolving power of up to 500,000 for proteins >100 kDa could be achieved.

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Poster 23

High-resolution ion isolation by swept frequency with phase reversal in Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Selective ion isolation without the loss of resolution is required for MS/MS studies ranging from protein-level identification and quantification to ion/molecule reaction kinetics and thermodynamics [1,2]. FT-ICR MS is equipped with an in-built quadrupole and an in-cell ion selection but ion losses and low resolution render these methods inefficient for the isolation of low abundance ions and ions in complex samples. Ion isolation has been achieved by fixed frequency, chirp and Stored Waveform Inverse Fourier Transform (SWIFT) ejection. The unwanted excitation of ions prevents the first two methods from providing high-resolution isolation. Although SWIFT is efficient in providing uniform excitation over a wide frequency range and mass-selective ion ejection, it requires specialized computers, interfaces and software for operation. As FT-ICR MS provides the possibility of manipulating the trapped ions by excitation-detection pulses in the ICR cell, ions of interest can be isolated by ejection of other ions. Exploiting this property, Vulpius and Houriet demonstrated a highly selective ion ejection method by reversing the phase of the sweep frequency to select a desired ion and confine it to the center of the ICR cell, ejecting all other ions [3]. Easy implementation with chirp frequency instruments, providing high resolution and sensitivity with high speed, makes notch-ejection an advantageous technique. We implemented isolation by notch ejection on a commercial Bruker Daltonics 9.4 T ApexQE FT-ICR mass spectrometer with substance P, insulin and ubiquitin. We optimized the sweep rate of the radiofrequency excitation pulses and reversed the phase of the pulse at the cyclotron frequency of the selected ion. For substance P, we isolated the monoisotopic ion of MH22+ and fragmented it by ECD, leading to the identification of MH2•+ as separate from the 13C isotope of MH+. For insulin and ubiquitin, we isolated monoisotopic multicharged ions with both high and low abundances, without any loss of intensity, with a resolution of over 10000. Ejection resolution decreases with mass-to-charge ratio, but by optimizing the power levels of the excitation pulses we could isolate ions of higher m/z ratios without loss of resolution. This high-resolution isolation method can be an exceptional tool for MS/MS for reducing the complexity of the fragment ion spectra, enabling better assignment and quantification of complex samples. We plan to selectively isolate more than one ion species in complex samples and then fragment them through ECD and IRMPD techniques. [1] A.G. Marshall, C.L. Hendrickson and G.S. Jackson, Mass Spec Rev. (1998) 17, 1-35. [2] S. Guan and P.R. Jones , J.Chem.Phys. (1989) 91,5291-5295. [3] T. Vulpius and R. Houriet, Int. J. Mass Spectrom. (1989) 88,283-290.

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