SEVENTH EUROPEAN WORKSHOP ON FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

WELCOME TO THE EFTMS 2004

Dear FTMS friends and colleagues

It is my pleasure to welcome you on behalf of the members of the scientific and organising committee and the members of our laboratory to the 7th European Workshop on Fouriertransform mass spectrometry.

I am certain you are all aware of the growing importance and application areas that FTMS is experiencing as **the** High Performance MS method, with an astounding development in instrumentation, new methodology, and – *nota bene* – bioanalytical applications.This development is duly reflected both by the growing number of academic FTMS laboratories and of instrument manufacturers – compared to the just two FTMS manufacturers at the time of the 6th EFTMS. A number of new FTMS laboratories have been established in Europe, partially being funded by special programmes. According to this development the 7th EFTMS is presenting a scientific programme encompassing fundamental aspects, new instrumental developments and methods, and analytical applications of FTMS, with all efforts of assembly in a balanced and fruitful manner to maximise scientific communication. In addition to the plenary lecturers, an additional special highlight of the 7th EFTMS will be the honorary session to the scientific work, and the awarding of a special issue of *European Journal of Mass Spectrometry* to Jean Futrell and Burnaby Munson.

In order to take full advantage of the capabilities of FTMS and its future development the introduction and research training of young scientists is of the utmost importance (*who is to succeed the present F1 champion in a car of comparable performance to Ferrari?)*. This urgent need is being met by a course immediately preceding the workshop *PROTEOME ANALYSIS USING HIGH RESOLUTION MASS SPECTROMETRY* which has been receiving the highest participation amenable to our laboratory – please see the course programme which has been included in this conference volume.

Last but not least – the organising team has been making a big effort to follow the great example of the 6th EFTMS in Kerkrade headed by Ron Heeren and Albert Heck, to provide all hospitality possible and fun at the University of Konstanz, City, and Lake surroundings. Science and discussion should go best within an appropriate atmosphere and this is already the motto for the opening lecture, first poster session and banquet reception.

We welcome you to the 7th EFTMS and trust you have fun at this meeting.

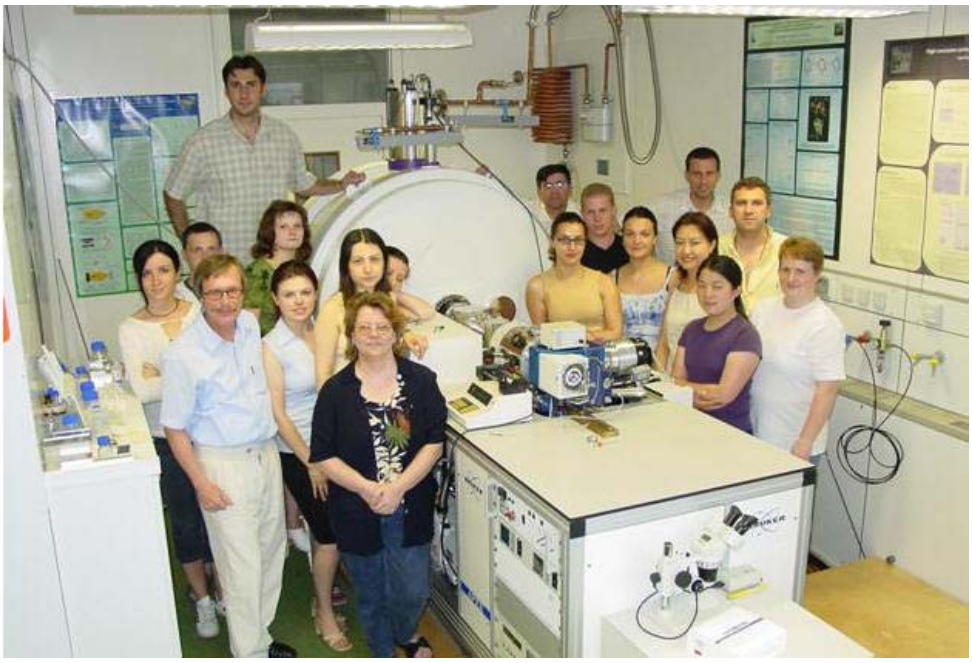
Chairman, Scientific Committee

Scientific Committee

Catherine E. Costello	Boston University
Peter Derrick	University of Warwick
Jochen Franzen	Bruker Daltonik, Bremen
Jürgen Grotemeyer	University of Kiel
Per Håkansson	Uppsala University
Ron Heeren	AMOLF-Institute for Atomic and Molecular Physics, Amsterdam
Michael Linscheid	Humboldt University Berlin
Alan G. Marschall	Florida State University, Tallahassee
Fred W. McLafferty	Cornell University, Ithaca
Nico Nibbering	Vrije Universiteit, Amsterdam
Jasna Peter-Katalinic	University of Münster
Reinhold Pesch	Thermo Electron, Bremen
Michael Przybylski	University of Konstanz
Helmut Schwarz	Technische Universität Berlin
Kenneth Tomer	National Institutes of Environmental Health Sciences, USA
Joachim R. Wesener	Bayer AG, Leverkusen
Roman Zubarev	Uppsala University

ORGANISATION COMMITTEE

- Susanne Becker, University of Konstanz
- Nicolaie-Eugen Damoc, University of Konstanz
- Ron Heeren, AMOLF-Institute for Atomic and Molecular Physics, Amsterdam
- Svetlana Mack, University of Konstanz
- Michael Przybylski, University of Konstanz
- Ekaterina Solovieva, University of Konstanz
- Xiaodan Tian, University of Konstanz
- Reinhold Weber, University of Konstanz
- Joachim Wesener, Bayer AG, Leverkusen
- Nikolay Youhnovski, University of Konstanz



UNIVERSITY OF KONSTANZ

A Brief History of a Modern Campus University

With the University of Konstanz, the prototype of a modern and compact campus university was built.

In 1965 the founding committee of the University developed the concept of a reform university with new forms of study and teaching, a central administration and central facilities for technology, computers, and language training. New forms of self-administration replaced traditional university structures. This new spirit found its expression in the establishment of a central, freely accessible library.

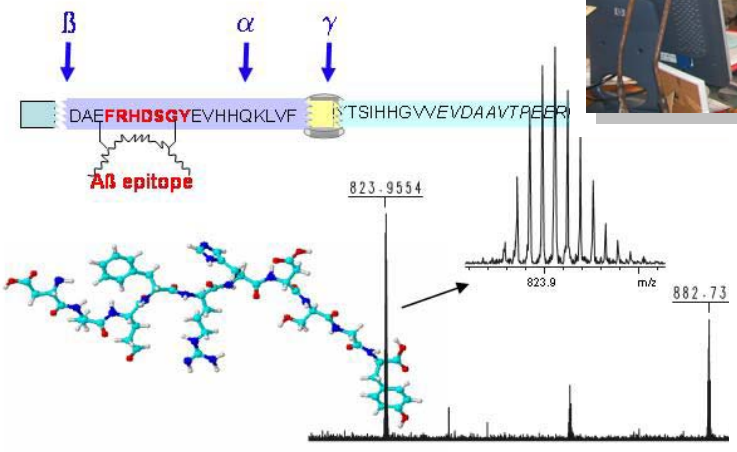
In 1966 the University began its work in a wing of the Inselhotel, formerly a Dominican monastery.

The beginning was makeshift - in the middle of Sonnenbühl on the fringe of the suburb Petershausen on the right side of the Rhine, with a handful of professors and a few dozen students. Starting in 1967, today's campus was developed through individual construction projects on the hill known as the Gießberg. Until today, the university has continued to be structurally altered and expanded.

From its inception, the University was planned as a place of life and learning in which new forms of research and teaching were to be reflected in the architectural style. Compact construction, short paths, the absence of large auditoriums in exchange for a large number of seminar rooms, as well as the variety of work, traffic, and quiet areas, are all part of the architectural concept. In addition, there was the idea of creating lasting "Kunst am Bau" (structural art) accents, so that the building of a desolate concrete landscape could be avoided in an effective manner.

LABORATORY OF ANALYTICAL CHEMISTRY AND BIOPOLYMER STRUCTURE ANALYSIS AT THE DEPARTMENT OF CHEMISTRY

The research profile of the Laboratory of Analytical Chemistry is focussing for a number of years on (i) biopolymer structure analysis and proteomics, (ii) molecular recognition and epitope structures, and (iii) vaccine peptides and proteins and associated molecular affinity systems. In all research areas biopolymer mass spectrometry and its combination with micro-separation and –affinity techniques play a central role among methods of structure analysis. Present biochemical and medical applications include neurodegenerative proteins, lung alveolar proteomics, and cryoglobulin antigens and comprise several international collaborations, e.g. with the Universities of Cardiff, Toronto, Budapest, EPF Lausanne. The Mass Spectrometry Laboratory serves as an interdepartmental facility for both the Departments of Chemistry and Biology. An FTICR-MS facility was awarded by the Deutsche Forschungs-gemeinschaft and is operative since 2000.



http://www.ag-przybylski.chemie.uni-konstanz.de/

SPONSORS

We gratefully acknowledge financial support of the 7th EFTMS by the following companies:

- Advion BioSciences Ltd
- Bioptic Lasersystems AG
- Bruker Daltonik GmbH
- IM Publications
- Intavis AG
- Ionspec Corporation
- Siemens AG
- Thermo Electron GmbH

GENERAL INFORMATION

Venue

The workshop will take place at the campus of the University of Konstanz.

All lectures will be held in the Audimax (Building A). Signs will show the way to all venues of the workshop.

All poster sessions will take place in the area around the Audimax. Posters will be mounted on Sunday afternoon, 28 March, from 2 pm and will be displayed during the entire conference. The opening poster session will be held during the opening reception on Sunday, 7.30 pm for all posters. Poster session A (odd poster numbers) will take place on Monday, 29 March, 1 pm, poster session B (even poster numbers) will take place on Wednesday, 31 March, 1 pm. Authors of posters are kindly requested to be present during these sessions *(which may be rewarding because of prizes for the best poster presentations)*.

Exhibition booths are located in the general entrance area of the main campus building on the way to the Audimax. Exhibitions will be open from Sunday, 28 March for the entire conference.

The conference secretariat will be situated in front of the Audimax and will be open 8.00 am to 6.00 pm

EFTMS 7 Conference Secretariat
Attn Mrs Ekaterina Solovieva
University of Konstanz
FB Chemie
78457 Konstanz, Germany

Phone: +49 7531 88 2497
Fax: +49 7531 88 3097
e-mail: eftms7@uni-konstanz.de
http://www.ag-przybylski.chemie.uni-konstanz.de/

HOW TO REACH THE UNIVERSITY OF KONSTANZ

By car

From Stuttgart (180 km)

Take the A 81 towards Singen. Exit at junction no. 40 in direction of Konstanz and follow the signs to Konstanz. First, follow the directions to the city centre (Zentrum), then directly to the the university.

From Zurich international airport by car (75 km)

The motorway N7 leads to Kreuzlingen / Konstanz. After the border please follow the signs "Mainau". Once you reach the old Rhine Bridge, signs will lead you to the university.

Signs will lead you to one of the university car parks; (1.30 Euro per day, automat does not return money!). Please note that on account of the annual amphibian crossing the university is accessible after 7.00 pm via the route from the city only.

By train

From Zurich by train

A train leaves Zurich airport every hour *:18 h from the underground station and arrives in Konstanz at the Swiss station (adjacent to the main station) 1:15 h later. Continue by local transport

Local transport

Bus

On Sundays you can reach the university using bus line number 4 leaving Central Station every half hour (*.08 and *.38) arriving in Egg – the stop for the university - approximately 14 Minutes later. The bus driver will be glad to assist you. A short walk will take you to the university.

On weekdays lines 9A and 9B take you to the university from the city centre via central station. It runs at 15 minute intervals.

Taxi
Taxis will bring you to the main entrance any day (including Sunday).

We wish you a pleasant trip and look forward to welcome you at the EFTMS 2004.

FTICR MS Course Preceding the Workshop

PROTEOME ANALYSIS USING
HIGH RESOLUTION MASS SPECTROMETRY

24 – 27 March 2004

Laboratory of Analytical Chemistry
Department of Chemistry

PROGRAMME & SCHEDULE

(L) Lecture (seminar room M 630)
(P) Practical (MS laboratory L 846. seminar room L 829)

Wednesday, 24 March 2004

(L)	1.30 pm	Welcome
		M. Przybylski Introduction; fundamentals of biopolymer-MS Introduction of instructors (practicals):
(L)	2.30 pm	**M. Przybylski** Ionisation-desorption methods for biopolymers Introduction /Fundamentals of FTICR-MS
		break
(P)	4.00 pm	**E. Damoc and N. Youhnovski** ESI-FTICR-MS of bioorganic/biopolymer molecules
(P)		**R. Weber and M. Manea** Sample preparation for MALDI-FT-ICR-MS

Thursday, 25 March 2004

(L)	9.00 am	**M. Przybylski** High-resolution biopolymer-MS using FT-ICR: techniques, instrumental, analytical development, applications
(L)	10.30 am	**N. Youhnovski** Combination FTICR-MS with microseparation techniques
		break
(L)	11.15 am	**N. Youhnovski** FTICR combined with nanotechnology nanotechnology/biosensor techniques
		lunch
(P)	1.30 pm	**N. Youhnovski** LC-MS of peptide mixture/ protein digests
(P)		**S. Becker** Combination with Edman micro-sequencing
(P)		**E. Damoc** Biopolymer fragmentation in FT-ICR-MS; determination of peptide sequences; analysis of combinatorial mixtures

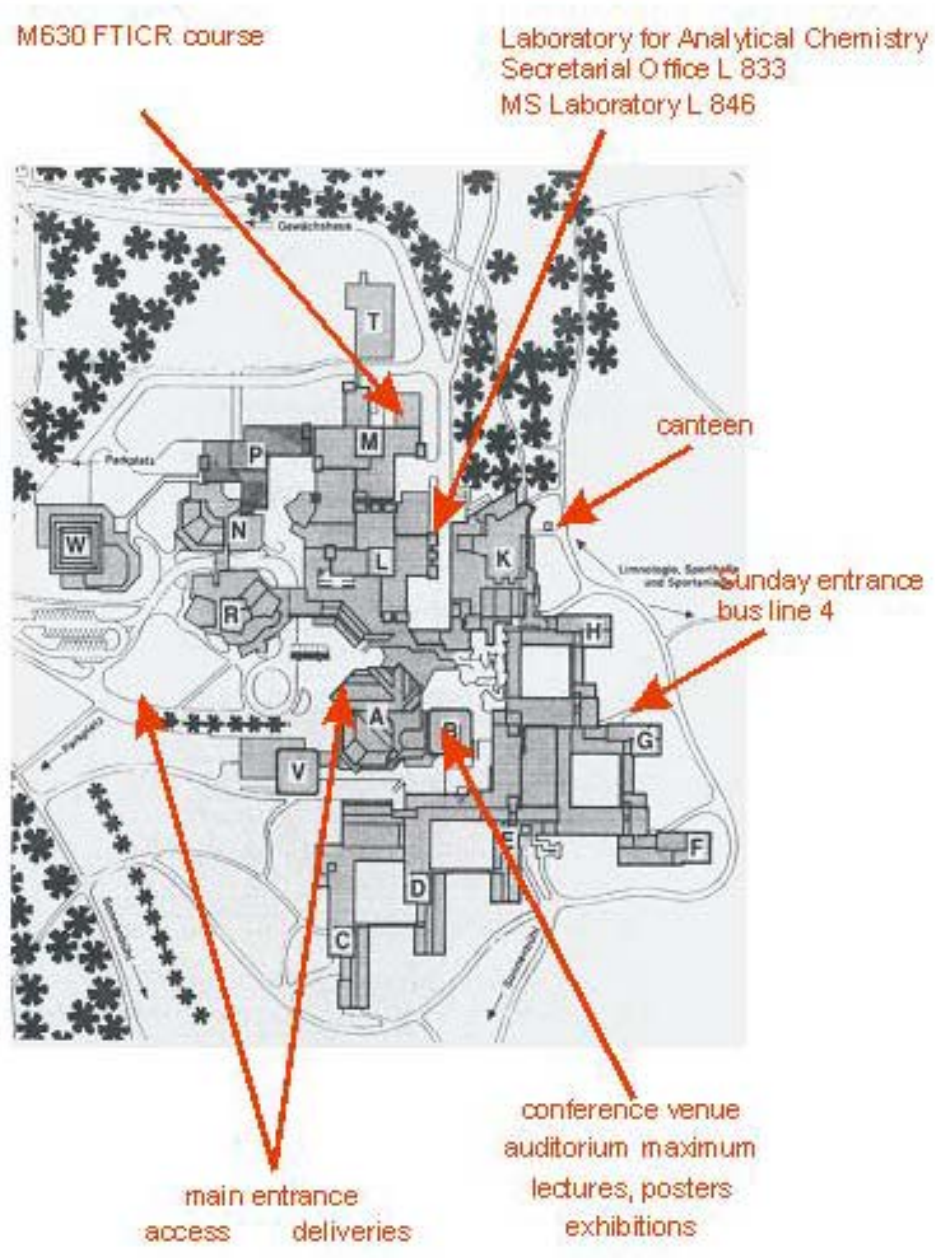
Friday, 26 March 2004 (c. 9 a.m. – 5 p.m.)

(L)	9.00 am	**M. Przybylski** Gas phase ion chemistry determination of protein primary structures, post-translational modifications
		break
(L)	11.15 am	**N. Youhnovski and R. Cecal** Higher-order biopolymer structures; non-covalent interactions (ESI-FTICR-MS), analysis of conformational states; deuterium exchange techniques
		lunch
(P)	1.30 pm	**E. Damoc and N. Youhnovski** Bioanalytical applications to proteome analysis
(P)		**R.Cecal and X. Tian** Conformational probing by H/D exchange

Saturday, 27 March 2004

(L)	8.30 am	**M. Przybylski** Biopolymer-MS in proteomics; principles, analytical strategies, techniques
		GUEST LECTURES
(L)	9.00 am	**Ken Tomer, NIEHS (USA):** Mass spectrometric characterization of neutralizing anti HIV antibodies
(L)	9.45 am	**Joachim Wesener, Bayer AG Leverkusen (Germany):** Automated accurate mass measurement with electrospray FT-ICR-MS
		break
(P)	11.00 am	**S. Becker, E. Damoc, N. Youhnovski** Bioanalytical applications to proteome analysis
		lunch
(P)	1.00 pm	**C. Damoc, R. Weber** Data base evaluation & protein identification
(P)		**E. Amstalden, A. Marquardt** Affinity proteomics
(L)	3.00 pm	**M. Przybylski** High-selectivity/affinity-proteomics: Application to molecular recognition structures, epitope analysis
		Concluding discussion

MAP OF THE UNIVERSITY OF KONSTANZ



Conference Proceedings – Special FTMS Issue of European Journal of Mass Spectrometry

Fouriertransform Mass Spectrometry 2004: State-of-the-Art and Future Perspectives

(Eds: Catherine Costello, Peter Derrick, Michael Przybylski)

A special issue of European Journal of Mass Spectrometry (EJMS) will be published from selected presentations of the EFTMS. Details regarding manuscript submission, instructions for manuscript preparation and deadlines will be announced in the course of the meeting, and further information will be available at the publisher's desk.

SEVENTH EUROPEAN WORKSHOP ON FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

UNIVERSITY OF KONSTANZ, GERMANY

28 MARCH TO 1 APRIL 2004

PROGRAMME

Sunday, 28 March

6.00 **OPENING OF CONFERENCE**

Welcome by Michael Przybylski, University of Konstanz
Jürgen Grotemeyer, Chairman of DGMS

6.30 **Plenary lecture 1:** **Chair:** J. Grotemeyer

Alan Marshall, Florida State University, USA
Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: Thirty Years and Counting

7.20 **OPENING OF POSTER SESSION (ALL NUMBERS)**

OPENING RECEPTION AND BANQUET

Monday, 29 March

I. NEW METHODS IN FTMS

Chair:
J. Wesener

9.00 L1: **R. Heeren**, R. Mihalca, I. Taban, A.J. Kleinnijenhuis, A.J.R. Heck, M.C. Duursma, L.A. McDonnell, and T.H. Mize
FOM- Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands
Hot and cold FTICR-MS Dissociation Studies with New FTMS Instrumentation

9.40 L2: **M. Linscheid**, S. Beck, T. Hagemeister, and V. Livadaris
Humboldt Universität zu Berlin, Germany
Fourier-Transform Mass Spectrometry Using the Finnigan LTQ FTMS - High Resolution - High Accuracy as Analytical Routine

10.10 L3: **J. Pittman**, B. Thomson, and P. O'Connor
Boston University School of Medicine, USA
A New Hybrid ESI-QQQ FTMS

10.40 **Break**

II. BIOANALYTICAL APPLICATIONS A

Chair:
N. Kelleher

11.10 L4: A. Kondakova and **B. Lindner**
Research Center Borstel, Germany
Structural Characterization of Complex Bacterial Glycolipids by FT-MS

11.50 L5: **M. Mormann,** L. Bindila, M. Froesch, A. Zamfir, and J. Peter-Katalinić
University of Münster, Germany
FT-ICR MS of Complex Carbohydrates

12.20 L6: **C. Hendrickson,** G. Blakney, M. Chalmers, L. Mackay, M. McFarland, J. Quinn, and A. Marshall
Florida State University, USA
Instrumental Advances in FT-ICR MS/MS of Biomolecules

12.50 **Lunch break**

1.00 **POSTER SESSION (A)** (odd numbers)

III. FUNDAMENTALS

Chair:
H. Schwarz

2.20 L7: **H. Schnöckel**
University of Karlsruhe, Germany
Bonding and Reactivity of Structurally Known Metalloid Cluster Species in the Gas Phase

3.00 L8: **K. Wanczek** and K. Garbade
University of Bremen, Germany
Understanding Ion-Ion Reactions in the Gas Phase

3.30 L9: **I. Balteanu.** P. Balaj, B. Fox-Beyer, M. Beyer, V. Bondybey
Technische Universität München, Germany
Use of Isotopically Enriched Material in Gas Phase Cluster Studies

4.00 **Break**

IV. SPECTROSCOPY AND ATOMIC CLUSTERS

Chair:
P. Wanczek

4.30 L12: **D. Moore**, J.Oomens, R. Dunbar, D. Ridge, L.v.d.Meer, G.v.Helden, G. Meijer, A. Marshall, J.Valle, and J.R.Eyler
FOM Institute "Rijnhuizen", Nieuwegein, The Netherlands
Structural Characterization of Gas Phase Ions by Infrared Spectroscopy

5.00 L11: **L. MacAleese**, J. Lemaire, J.-M. Ortega, and P. Maître
University of Paris, France
Infrared Spectroscopy Under FT-ICR-MS Conditions : Experimental Setup and Applications to Organometallics

5.30 L10: **L. Schweikhard,** K. Blaum, A. Herlert, G. Marx
Ernst-Moritz-Arndt-Universität, Greifswald, Germany
Atomic Clusters and Ion Cyclotron Resonance Mass Spectrometry - A Fruitful Combination

6.00 **Guided tours:**

Walls tell tales
Popes, heretics, courtesans

Tuesday, 30 March

V. NEW METHODS AND INSTRUMENTATION

Chair:
R. Heeren

9.00 L13: **G. Niedner-Schatteburg**
University of Kaiserslautern, Germany
A New and Versatile Multi Ion Source FT-ICR-Setup: F.R.I.T.Z.

9.30 L14: **W. Metelmann-Strupat**, K. Strupat, J. Griep-Raming, and H. Münster
Thermo Electron, Bremen, Germany
Accurate Mass and High Scan Speed – Using Two Detectors in One Instrument

10.00 L15: D. Davis, K. Gallaher, **W. Rimkus**
Siemens Applied Automation, Bartlesville, USA
Use of a Miniature High Resolution Fourier Transform – Mass Spectrometer for Support of Product and Process Analysis

10.30 **Break**

VI. NEW BIOANALYTICAL METHODS

Chair:
J. Peter-Katalinic

11.00 L16: **B. Spengler;** D. Kirsch, W. Bouschen, V. An Thieu
Justus-Liebig-University, Gießen, Germany
Peptide Composition Analysis and Composition Based Sequencing of Peptides: The Power and Beauty of Accurate Mass Values

11.40 L17: **M. Allen** A. Sterling, and M. Baumert
Advion BioSciences, Norwich, UK
Fully Automated High Throughput Analysis Using Chip-Based Nanoelectrospray FT-ICR Mass Spectrometry

12.10 L18: **C. Hagman,** M. Ramström, P. Håkansson, J. Bergquist
Uppsala University, Sweden
Quantitative Analysis of Tryptic Protein Mixtures Using Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

12.40 L19: **R. Geels,** A. Heck, S. van der Vies, and R. Heeren
FOM- Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands
Dissociation Analysis of GroES via SORI-CID in an FT-ICR-MS

1.10 **Lunch break**

VII. DISSOCIATION TECHNIQUES

Chair:
G. Baykut

2.00 L20: C. Adams, B. Budnik, K. Haselmann, F. Kjeldsen, and **R. Zubarev**
Uppsala University, Sweden
Electron Capture Dissociation Detects the Presence of a Single D-Amino Acid in a Protein and Probes the Neutral Tertiary Structure

2.40 L21: **K. Breuker** and Fred W. McLafferty
University of Innsbruck, Austria
Native Electron Capture Dissociation

3.10 L22: **H. Cooper**
University of Birmingham, England
Electron Capture Dissociation of Non-Standard and Modified Peptides

3.40 L23: **Y. Tsybin**, M. Witt, G. Baykut, G. Weiss, P. Håkansson
University Uppsala, Sweden
Optimization of ECD: Mechanistic Studies, Top-Down and Bottom-Up Applications

4.10 **Break**

VIII. BIOANALYTICAL APPLICATIONS B

Chair:

M. Linscheid

4.40 L24: **M. Freitas** and L. Zhang
Ohio State University, USA
Cracking the Histone Code: A Mass Spectrometry Based Approach for the Determination of Histone Modifications

5.10 L25: **M. Ramström**, I. Ivonin, A. Johansson, H. Askmark, K. E. Markides, R. Zubarev, P. Håkansson, S.-M. Aquilonius, and J. Bergquist
Uppsala University, Sweden
Protein Patterns in Cerebrospinal Fluid Revealed by Liquid Chromatography Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

5.40 L26: **A. Sinz,** D. Schulz, C. Ihling, S. Kalkhof, and M. Clore
University Leipzig, Germany
Mapping Protein Interfaces by Chemical Cross-Linking and FTICR Mass Spectrometry: Application to Calmodulin/Peptide Complexes

6.10 L27 **X. Tian,** R. Cecal, J. McLaurin, R. Stefanescu, M. Manea, S. Grau, M. Ehrmann, P. St George-Hyslop, and M. Przybylski
University of Konstanz, Germany
Application of FTICR-MS in Characterisation of Target Peptides/Proteins of Alzheimer's Disease and Elucidation of an Amyloid Plaque-Specific Epitope

6.40 **Departure:**

Guided tour of Joh. Albrecht Brewery with evening meal
Wine Tasting in the Spitalkellerei Konstanz

Wednesday, 31 March

IX. NEW FTMS METHODS

Chair:
P. Håkansson

9.00 L28: **E. Nikolaev**, C. Masselou, G. Anderson, and R. Smith
The Institute for Energy Problems of Chemical Physics; Moscow, Russia
Some New Approaches in Ion Trapping and Detection in FT ICR

9.40 L29: **P. O'Connor**
Boston University School of Medicine, USA
Current Thoughts Regarding the Cryogenic FTMS

10.10 L30: V. Frankevich, X. Guan, M. Dashtiev, and **R. Zenobi**
Swiss Federal Institute of Technology (ETH), Zurich, Switzerland
Laser-Induced Fluorescence of Trapped (Bio)Molecular Ions

10.40 **Break**

X. ANALYTICAL APPLICATIONS

Chair:
B. Spengler

11.10 L31: **B. Koch,** T. Dittmar, R. Engbrodt, M. Graeve, M. Witt, G. Kattner
Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany
Composition of Marine Dissolved Organic Matter: First Chemical Formulas as Detected by FTMS

11.40 L32: **A. Stopford**, N. Polfer, P. Langridge-Smith
University of Edinburgh, Scotland
A Study of Electron Capture Dissociation Efficiency of Peptide Dications Using a Dispenser Cathode

12.10 L33: **T. Bristow**
LGC, Teddington, England
A Comparison of Calibration Methods for Accurate Mass Measurement of Low Molecular Weight Molecules Using FTMS

12.40 **Lunch break**

1.00 **POSTER SESSION B (EVEN NUMBERS)**

CEREMONIAL ACT IN HONOUR OF THE SCIENTIFIC CAREER OF JEAN FUTRELL AND BURNABY MUNSON

2.00 **Honorary address**:
G. von Graevenitz, Rector, University of Konstanz

Chair:
P. Derrick

P. Derrick: Laudatio on Jean Futrell
F. McLafferty: Laudatio on Burnaby Munson

AWARD OF SPECIAL EJMS ISSUE

2.50 **Honorary Lecture**:
Jean Futrell, Pacific Northwest National Laboratory, Richland, USA
Kinetics of Activation and Dissociation of Large Molecular Ions in the Gas Phase

Chair:
F. McLafferty

3.20 **Honorary Lecture**:
Burnaby Munson, University of Delaware, USA
Chemical Ionization Mass Spectrometry: A Useful Accident

Chair:
M. Przybylski

3.50 **Break**

4.10 **Plenary lecture 2:**

Chair:
A. Marshall

H. Schwarz, Technische Universität Berlin, Germany
Elementary Processes in Catalysis:Looking at and Learning from "Naked" Transition. Metal Ion Chemistry and Physics - Theory and Experiment in Concert

5.00 **Departure to Meersburg Castle and Conference Dinner**

Thursday, 1 April

XI. PERSPECTIVES FOR FTMS

Chair: K. Tomer

9.00 L34: **O. Trapp,** J.R. Kimmel, O. Yoon, I.A. Zuleta, R.N. Zare
Stanford University, USA
Hadamard-Transform Time-of-Flight Mass Spectrometry

9.30 L35: **B. Paizs**, M. Schnölzer, U. Warnken, and S. Suhai
German Cancer Research Center, Heidelberg, Germany
Ion Intensity Relationships for the MS/MS Spectra of Protonated Peptides

10.00 L36: C. Koy, P. Serrano, S. Möller, B. Ringel, S. Mikkat, R. Martin, S. Drynda, J. Kekow, H.-J. Thiesen, and **M. Glocker**
Proteome Center Rostock, Germany
Identification and Structure Characterization of Disease-Associated Marker Proteins Using MALDI QIT ToF MSn in Clinical Proteome Research with Patients Suffering from Rheumatoid Arthritis

10.30 **Break**

XII. BIOANALYSIS HIGHLIGHTS IN FTMS

Chair: R. Heeren

11.00 L37: **N. Kelleher**
University of Illinois at Urbana-Champaign, USA
Of Biomarkers and Modifications: Top Down Mass Spectrometry of Proteins Across the Phylogenetic Tree

11.40 AWARDS

Chair: M. Przybylski

12.20 **Plenary lecture 3:**

F. McLafferty, Cornell University, Ithaca, USA
Structural Characterization of Biomolecules, Unique Advantages of FTMS

13.10 CLOSING REMARKS AND END OF CONFERENCE

ABSTRACTS OF LECTURES

PLENARY LECTURE 1

FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY: THIRTY YEARS AND COUNTING

Alan G. Marshall

ICR Program, NHMFL, Florida State University, Tallahassee, USA

This Workshop marks the 30th anniversary of the first FT-ICR publication [1]. Although that first spectrum was limited to narrowband detection of simple methane cations, mass resolution was already at the milliDa level. The past 30 years have witnessed a host of improvements and extensions, leading to today's routine broadband ultrahigh-resolution detection based on ionizsation methods including EI, CI, electrospray, MALDI, field desorption, and atmospheric pressure photoionization, for analysis of complex mixtures ranging from biological fluids to petroleum crude oil, with applications ranging from proteomics to forensics. Throughout this evolution, advances have come from increasingly deeper understanding of the fundamental nature of ion motions in magnetic and electric fields as well as ion structure, energetics, and reactivity. The peak capacity of FT-ICR MS (200x higher than any single-stage wet chemical separation) can even make it possible to eliminate one or more separations prior to mass analysis. In this talk, I shall try to review and connect some selectied technique developments in FT-ICR MS.

1. Comisarow, M. B.; Marshall, A. G. "Fourier Transform Ion Cyclotron Resonance Spectroscopy", Chem. Phys. Lett. 25, 282-283 (1974)

Plenary Lecture 2

ELEMENTARY PROCESSES IN CATALYSIS: LOOKING AT AND LERNING FROM „NAKED" TRANSITION. METAL ION CHEMISTRY AND PHYSICS – THEORY AND EXPERIMENT IN CONCERT

Helmut Schwarz

Fakultät für Chemie, Technische Universität Berlin, Germany

Concepts for the activation of methane, the most difficult of all hydrocarbons to be selectively functionalized and one of the great challenges of contemporary catalysis, are derived from ion-molecule reactions of mass-selected electronic ground state transition-metal ions M^+ and small clusters M_x^+ (x = 1 – 5). It will be shown that gas-phase experiments are ideally suited to probe at a molecular level and in the absence of obscuring "environmental" effects mechanistic details of elementary steps in bond activation and bond coupling reactions. Particular attention will be paid to the role, the electronic structure of M^+ as well as relativistic effects play in these fundamental processes. Finally, unprecedented examples for the operation of co-operative effects in the gas-phase chemistry of heteronuclear metal clusters will be presented.

For further reading, see:

1) H. Schwarz, D. Schröder, *Pure Appl. Chem.* **2000**, *72*, 2319.

2) D. Schröder, S. Shaik, H. Schwarz. *Acc. Chem. Res.* **2002** *33*, 138.

3) I. Kretzschmar, D. Schröder, H. Schwarz, P. B. Armentrout, *Adv. in Metal and Semiconductor Clusters*, **2001**, *5*, 347.

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Plenary Lecture 3

STRUCTURAL CHARACTERIZATION OF BIOMOLECULES, UNIQUE ADVANTAGES OF FTMS

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The unique resolving power and tandem mass spectrometry advantages of the Fourier-transform ion cyclotron resonance instrument are basic to its choice for "Top Down" proteomics, but it is electron capture dissociation that makes possible its high accuracy in pinpointing posttranslational modifications. This is illustrated in a combinatorial study of structure-function relationships of peptide nucleic acids and with the elucidation of pathways for the biosynthesis of thiamin pyrophosphate, cysteine, and Coenzyme A. The extensive, specific fragment ion data also make detailed kinetic studies efficient, both without and with isotopic labeling. Biosynthesis illustrations include distinguishing between pathways and identifying rate limiting steps. Ribonuclease B occurs naturally as a mixture of isoforms with five different sugar side chains. Their reductive unfolding kinetics involving cleavage of 4 S-S bonds through isomeric intermediates could be followed quantitatively. A new ESI/FTMS technique yields 3050-3800 cm-1 infrared photodissociation spectra of gaseous ions from proteins and smaller biomolecules to provide unique new information on hydrogen bonding and zwitterionic structures.

Honorary Lecture

KINETICS OF ACTIVATION AND DISSOCIATION OF LARGE MOLECULAR IONS IN THE GASE PHASE

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Because of their large number of internal degrees of freedom the kinetics of dissociation of large molecular ions are qualitatively and quantitatively different from the analogous behaviour of small molecule counterparts. This makes the study of complex systems interesting from both a fundamental and applied (analytical mass spectrometry) perspective. We have utilized surface-induced dissociation (SID) as a means for depositing very rapidly (a few hundred femtoseconds) relatively large and controllable amounts of internal energy in protonated model peptides as a technically important class of complex molecules. These experiments have been carried out on a specially designed research FTICR instrument that incorporates mass selection, collisional relaxation, kinetic energy selection and focusing of primary ions on a semiconducting surface inside a very efficient ion trap ICR cell. The principal surface used is a C12 self assembled monolayer (SAM) on gold. We have compared the time dependant dissociation of these ions using an RRKM-based model developed in our laboratory to describe their kinetics. Molecular mechanics modeling has been used to provide insights into complex rearrangements these molecules undergo. Finally different semiconductor surfaces have been investigated to explore the effect of "stiffening" the molecular structure used for energy transfer into gas phase ions.

A rather surprising result is the precision with which we can determine thermodynamic and kinetic parameters for these large molecules. This precision results from two factors—the large kinetic shift that offsets experimental decomposition curves by an order of magnitude more than the thermochemical threshold difference and the efficiency, typically 20% or less, of conversion of kinetic energy into internal energy in SID. Consequently ion survival curves are shifted by a factor of the order of 50 compared to the thermochemical difference in dissociation energy—e.g., offset by 4 eV for a thermochemical difference of 80 meV. A corollary is that energy differences as small as 10 meV can be measured in these measurements.

Comparison of internal energy distributions achieved in SID with thermal decomposition data and multiple-collision activation experiments demonstrated that energy distribution functions are well-represented by high temperature Maxwell-Boltzmann distributions. This is true after a single collision with hydrocarbon-type surfaces or a relatively small number of gas phase collisions. Master equation modeling rationalizes these results by demonstrating efficient energizing (and de-energizing) of complex ions in collisions.

Another important discovery is a dramatic change in kinetics with ion kinetic energy. For octapeptides, for example, a dramatic increase in the number of ions formed occurs above 25 eV. Most have low mass and no detectable time dependence—that is, they are formed instantaneously within our ability to investigate the time evolution of ions. We describe this phenomenon as "shattering" taking place on or very near the surface. A detailed analysis suggests that this is the principal kinetics mode for any class of mass spectrometer that detects ions on the sub-millisecond time scale, including most tandem instruments that have been used to study these phenomena to date.

We have also investigated the effect of changing the surface. A "soft" hydrocarbon SAM introduces energy in small increment and spreads out decomposition curves very efficiently. A harder but still "soft" surface such as a fluorinated SAM transfers energy more efficiently while maintaining a narrow energy distribution function. Both generate high quality data from which accurate thermodynamic and kinetic information can be deduced. Diamond is an example of a very hard surface that both deposits energy very efficiently and has a broad energy distribution function. Diamond is therefore a very promising surface for analytical applications.

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Honorary Lecture

CHEMICAL IONIZATION MASS SPECTROMETRY: A USEFUL ACCIDENT

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Chemical Ionization mass spectrometry, CIMS, was an accidental outgrowth of gas phase ion/molecule reactions at the then ultra-high pressure of ~ 2 torr. Pressure studies with ultra-high purity methane gave irreproducible results for the distribution of major ions at m/z 17, 19, and 29 which resulted from trace levels of water in the vacuum system. The ions at m/z 17 and 29 did not react with methane, as confirmed later in ICR experiments.

Pressure studies on methane with small amounts of other compounds established the reactions of m/z 17 and 29 with most compounds. Studies with isomeric hydrocarbons showed the analytical potential of the technique for molecular weight and structure identification. CIMS is now standard on many commercial instruments and is often used for selective detection and quantification.

L1

HOT AND COLD FTICR-MS DISSOCIATION STUDIES WITH NEW FTMS INSTRUMENTATION

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The use of temperature to get insight into reaction and dissociation pathways is routinely employed to study biopolymer reactions in liquids. Here we have used gas-phase temperature control to manipulate the internal energy and structure of both small and large biomolecular ions. In particular we have performed electron capture dissociation studies using both hot and cold ion populations of various peptides and proteins. These studies have been made possible through the use of a cold cell design [1] and a new modular PXI base data acquisition and control system. Especially ECD at low tempratures reveals some interesting phenomena. certain fragments dissapera completely whereas the intensity of other fragments increases. The studies will be shown to demonstrate the importance of both hydrogen mobility and the non-ergodic nature of the dissociation process.

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L2

FOURIER-TRANSFORM MASS SPECTROMETRY USING THE FINNIGAN LTQ FTMS - HIGH RESOLUTION - HIGH ACCURACY AS ANALYTICAL ROUTINE

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The new Thermo Finnigan LTQ FTMS is a hybrid mass spectrometer expressedly designed for analytical highest-level routine MS work and LC/MS. In our laboratory the mass spectrometer is in use for several months now as a multipurpose Electrospray and AP-MALDI instrument. Regularly, even the day to day analytical measurements such as the identification synthetic of modified PDNA´s, nucleotides or biologically active substances - if high resolution is necessary - are performed very efficiently. In addition, it became evident that many more analytical questions can be answered, which could not even be seen without high resolution and accuracy.

A few examples will be discussed here: Firstly, the detailed analysis of synthetic polymers using Atmospheric Pressure MALDI at high resolution was shown to be possible with an analytical depth unthinkable without high resolution and accuracy, since different copolymers and isotope signals can be separated.

Secondly, cis-Platinum modified nucleotides were analyzed using MS^N techniques at high resolution allowing the differentiation of various fragmentation pathways previously undiscernible.

Lastly, the LC/MS analysis of peptides of membrane proteins from Bdellovibrio Bacterivorus will used to demonstrate the analytical capabilities of the instrument due to its resolving power, accuracy, stability and reliability. For this measurement an Agilent Nano HPLC system was interfaced to the LTQ FTMS.

L3

A NEW HYBRID ESI-QQQ FTMS

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A new electrospray qQq-FTMS has been assembled using an MDS Sciex triple quadrupole ion source mounted onto the Boston University home built FTMS. Ions are selected in Q1, accumulated in a Linac equipped RF only quadrupole, and pulsed out through a series of hexapole ion guides to the capacitively coupled closed cylindrical cell. Ion transmission efficiency is >75% from the accumulation quadrupole to the cell.

This system has been built specifically to study labile or low abundance post-translational modifications in proteins. Data is available now in which minor component phosphopeptide and glycopeptides are accumulated and fragmented by ECD, and it is expected that more such data will be available prior to presentation. Typical performance tests have been done and will also be presented. The sensitivity and selected accumulation ability of this system shows promise for post-translational modification determination.

L4

STRUCTURAL CHARACTERIZATION OF COMPLEX BACTERIAL GLYCOLIPIDS BY FT-MS

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Bacterial glycolipids are amphiphilic molecules which are important for the properties of bacterial membranes and which play a major role in the activation of immune competent host cells. Already small alterations of the chemical structure can influence their biological activity tremendously. Due to their intrinsic heterogeneity and complexity part structures have to be isolated for unequivocal chemical and NMR analyses. Applying various ESI FT-MS methods structural information can be obtained from the intact native glycolipids. CSD as well as SORI-CID- and IRMPD-MS/MS analysis are compared and their advantages to provide structural information of diagnostic importance are discussed. By means of recently analysed lipopolysaccharides (LPS) it is demonstrated how these methods can be used to screen the structural heterogeneity of the lipid portion of LPS (number, type and distribution of fatty acids, substitution with P, P-Etn etc.) as well as that of the oligosaccharide moiety (sugar sequence and substitution pattern). Furthermore, fragmentation can be induced allowing to differentiate between heterogeneity within the inner and the outer core region.

L5

FT-ICR MS OF COMPLEX CARBOHYDRATES

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Complex carbohydrates represent an ubiquitous class of biopolymers. Complex carbohydrate biology and biochemistry gained attention in the past decade due to increased evidence of glycoconjugate functional significance, like in cell trafficking, clearance in blood stream and leucocyte adhesion to endothelium. Glycosylation of proteins represents the most abundant modification of proteins while structural elucidation of protein glycosylation remains an elusive goal due to the high diversity and heterogeneity of glycosylation patterns giving rise to complex mixtures. The comprehensive characterization of a glycoprotein comprises the determination of the amino acid sequence, the glycan sequence and branching pattern, and its attachment site to the peptide backbone.

FT-ICR MS techniques represent a powerful tool to study glycoconjugates of high complexity. The high resolving power, mass accuracy and sensitivity have been explored in analysis of the isotopic fine structure of single carbohydrate structures in complex mixtures, like normal and oversulfated chondroitin/dermatane sulfate glycosaminoglycans released from proteoglycans, mucin-type *O*-GalNAc and *O*-Fuc-glycosylated peptides, highly glycosylated amino acids and peptides from urine and gangliosides from human brain. SORI-CID and ECD provide distinct mechanisms for fragmentation reactions to be applied in different contexts of structural analysis. The potential of ECD fragmentation analysis to address five structural parameters of *O*-GalNAc glycosylated mucins in a single experiment and the mechanisms involved therein will be discussed.

L6

INSTRUMENTAL ADVANCES IN FT-ICR MS/MS OF BIOMOLECULES

Christopher L. Hendrickson, Greg T. Blakney, Michael J. Chalmers, Logan Mackay, Melinda A. McFarland, John P. Quinn, Alan G. Marshall

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Biomolecular structural characterization by FT-ICR MS/MS has made significant advances. Several fragmentation techniques are available and complementary, including CAD, IRMPD, and ECD. We describe here the instrumentation and techniques used in our laboratory for rapid and efficient MS/MS. Each of the dissociation techniques can be used separately, successively, or simultaneously to elucidate biomolecule sequence and modification. Millisecond timescale dissociation and mass selective ion accumulation facilitates MS/MS on a timescale compatible with online separation methods. Finally, advances in data acquisition makes automated, data-dependent MS/MS routine at one scan per second.

L7

BONDING AND REACTIVITY OF STRUCTURALLY KNOWN METALLOID CLUSTER SPECIES IN THE GAS PHASE

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The large number of FT-ICR investigations about "naked", i. e. structurally not exactly known metal atom clusters is in contrast to the fact that similar research on metalloid clusters which have been structurally characterized in the solid state e.g. via X-ray diffraction experiments, is completely missing. Here we present FT-ICR experiments on some examples of metalloid Ga- and Al-clusters exhibiting their so far unknown stabilization of the nearly naked core containing only metal atoms, by means of carbene-analogous AlR- resp. GaR-entities. The fragments obtained via SORI-CAD experiments furthermore allow to get some experimental insight in the bonding of unsaturated MnRm (n > m) species like the one discussed for the GaGa triple bond. Besides the different results on Al and Ga clusters we will present some preliminary data on gas phase dissociation experiments of a Tungsten halide cluster.

In any case, the stepwise collision induced decay in the gas phase gives first ideas for the formation mechanism of these clusters via the concept of retro synthesis.

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L8

UNDERSTANDING ION-ION REACTIONS IN THE GAS PHASE

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Ion-ion reactions in the gas phase in ion traps are diccussed. A few typical results of other research groups are compared with our own findings. There are only very few theoretical papers in the literature. A simple theory based on the works of McLuckey and of Su and their coworkers is developed for a special case of ion-ion reactions, the charge exchange reaction of doubly charged ions with singly charged ions of opposite charge polarity.

L9

USE OF ISOTOPICALLY ENRICHED MATERIAL IN GAS PHASE CLUSTER STUDIES

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Ion molecule reactions of ionic metal clusters with small organic molecules serve as gas phase models for heterogenous catalysis. Size and charge state dependence of the reaction rate constant reveal valuable insight into geometric and electronic effects governing the reactivity. A major drawback of using FT-ICR mass spectrometry for catalytically important materials like platinum or palladium is their unfavorable natural isotope distribution, which dilutes the intensity e.g. of a Pd_20 cluster into 30 different peaks. This also hampers the identification of the reaction products. Isotopically enriched target material circumvents this problem. We present here first results of cationic and anionic platinum clusters with up to 25 atoms, with an isotopic enrichment of 97.2% 195Pt. Quantitative analysis of the reactivity as a function of size and charge state is now feasible with a wide variety of reactants.

L10

STRUCTURAL CHARACTERIZATION OF GAS PHASE IONS BY INFRARED SPECTROSCOPY

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Infrared multiple photon dissociation (IRMPD) spectroscopy has been used to characterize a wide range of gas phase ionic species in an FTICR mass spectrometer. Systems studied include cationic transition metal-organic molecule complexes, proton-bound ether dimers, and anionic transition metal carbonyl clusters. Comparison of the experimental results with calculated spectra allows unambiguous structural determination in many cases. For example, for the Cr+-aniline complex, the experiment resolves a computational ambiguity between different potential binding sites. For the proton-bound species, the spectra show several conserved features, and also have similarity with the IRMPD spectrum of proton-bound water dimer. It is argued that this may be evidence for a spectroscopy signature of the O-H+-O moiety. For the anionic iron-carbonyl cluster, we demonstrate how SORI (Sustained Off-Resonance Irradiation) can be used to direct gas-phase synthesis of selected clusters by ion-molecule reactions. These clusters are then analyzed spectroscopically, initially showing a pronounced blue-shift with increasing cluster size, which converges at ~2000 cm-1 for the larger clusters.

L11

INFRARED SPECTROSCOPY UNDER FT-ICR-MS CONDITIONS : EXPERIMENTAL SETUP AND APPLICATIONS TO ORGANOMETALLICS.

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Infrared spectroscopy of ions isolated under Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) will be presented. We will present our experimental setup featuring a coupling of a Mobile ICR Analyser (MICRA) to an IR Free Electron Laser (FEL). IR spectroscopy offers a significant improvement for the structural characterization of mass-selected ions under FT-ICR-MS conditions and we will particularly focus on the characterization of organometallic reactive intermediates.

The spectroscopic method used in our experiment is IR Multiple Photon Dissociation (IRMPD). Despite its multiple photonic character, this particular spectroscopy gives excellent results in terms of structure identification, as will be shown on various examples. Different aspects regarding the effects of the IR irradiation parameters on the dissociation rates will be presented.

L12

ATOMIC CLUSTERS AND ION CYCLOTRON RESONANCE MASS SPECTROMETRY – A FRUITFUL COMBINATION

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Clusters consisting of a few atoms build the bridge between individual atoms and the condensed phase of matter and they are thus of high general interest. Over the last two decades considerable progress has been made in the study of their properties, but there are still some "missing links". Ion storage techniques, in particular the use of ion cyclotron resonance (Penning) traps, are important tools for advanced investigations. Vice versa, cluster ions can serve to evaluate the properties of the ion traps. Furthermore, they are ideally suited for the calibration of mass spectrometers and for the search for systematic uncertainties in high-accuracy mass determinations. The present report includes examples from the research areas mentioned, i.e. the investigation of cluster properties and the application of cluster ions for Penning trap studies and mass calibration.

L13

A NEW AND VERSATILE MULTI ION SOURCE FT-ICR-SETUP: F.R.I.T.Z

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Conceptually FT-ICR-MS may combine with many - if not any - type(s) of external ion source. Only one at a time, however. This is due to the non-negligible magnetic stray fields which dictate strictly on axis operation so far. Only recently passive and active shielding of superconducting magnets became available. Reduced stray fields allowed, for the first time, an off axis operation of ion sources. This lead us to propose the new concept of simultaneous operation of multiple ion sources at a time. Switching is enabled by an ion wedge as realized through a quadrupole bending field.

Proper operation of the new multi ion source FT-ICR-MS-setup (7 Tesla, actively shielded) was achieved only after an accurate re-alignment of ion transfer with respect to the magnetic field axis while operating the external ion beam and by applying position sensitive ion detection. The thus worked out alignment scheme is universally applicable to any other ICR-setup with ion transfer from an external ion source.

We achieved instant overall operation and proof of principle of the ion wedge. Two of three ion sources are operational by now: electrospray ionization (ESI) and pulsed nozzle laser vaporization (LVAP). First results arose from the investigation of transition metal coordination complexes as well as of transition metal clusters reactions.

Cooperation with Manfred Kappes and Oliver Hampe (Universität Karlsruhe and Forschungszentrum Karlsruhe) is gratefully acknowledged.

L14

ACCURATE MASS AND HIGH SCAN SPEED – USING TWO DETECTORS IN ONE INSTRUMENT

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The interest in detecting unusual modifications or species present only in minor concentration is increasing causing a demand for more powerful mass spectrometers and a clear need for accurate mass determination and MS/MS both compatible with the chromatographic time constraints.

The knowledge of the accurate mass of an MS/MS precursor ion allows for a significantly higher reliability of protein identification resulting in less false positive hits, and increased database search speed. FTICR-MS is the most suitable way to achieve the required mass accuracy. MS/MS spectra can be generated with high sensitivity and high acquisition speed in a linear ion trap mass spectrometer.

In the *Finnigan* LTQ-FT both mass analyzers can operate independently from each other. The linear ion trap is characterized by a high ion storage capacity combined with a high scan rate. The FTICR- MS routinely achieves a mass resolution of R = 100.000 @ m/z 400 with a scan rate of 1 scan/s, while achieving ≤ 2 ppm mass accuracy with external calibration.

The quality of an ICR analysis is directly dependent on the time used to acquire the transient signal. In order to achieve the above performance, the transient needs to be detected for more than 700 ms in the LTQ-FT. During this acquisition time the linear ion trap MS can perform fully independent experiments, i.e. both mass analyzers can be used in parallel. This allows for example the acquisition of several MS/MS scans concurrent to the acquisition of a HR MS spectrum. These MS/MS scans can be run in a *data dependent* mode, referring to a preview into the accurate mass scan. Also detection of several MS^n scans in the linear trap is possible while detecting MS^n scans in the ICR cell.

The presentation will give an overview over the potentials of parallel processing in proteomics with real life samples.

L15

USE OF A MINIATURE HIGH RESOLUTION FOURIER TRANSFORM – MASS SPECTROMETER FOR SUPPORT OF PRODUCT AND PROCESS ANALYSIS

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In 2001 Siemens introduced a compact, cost-efficient, high resolution FTMS based on a 1 Tesla permanent magnet - The Advance Quantra. The system, which costs many hundreds of thousands of dollars less than its bigger relatives still boasts extremely good performance. Priced at under $75,000 the world of High Resolution is now affordable and open to uses in the general analytical and process analysis disciplines. This presentation will describe results and milestones in the growth and development of the Quantra since its introduction.

Catalysts - In cases where catalytic reactions involve heteroatoms (oxygen, nitrogen etc.) use of a high-resolution, high mass accuracy mass spectrometer can often facilitate the rapid analysis of products. This can be either for assisting in catalyst development or for process monitoring and control. With a mass resolution of approximately 20,000 at m/z 131, the Quantra has sufficient resolution to resolve most ion pairs (<m/z 200) with the same nominal mass, but different molecular formula. Process control of a catalytic pilot plant will be presented.

Corrosive/aggressive Sample analysis - Conceived as a rugged process control mass spectrometer, the Quantra has unique design features that allow the analysis of very aggressive and corrosive species. Examples of NF3, Phosgene, semiconductor Fluorocarbons, and chemical weapon surrogates are presented.

Automated operation and process control - Coupled with the Siemens NAU (Network Access Unit) a standard controller for their process GC, the Quantra is capable of fully automated analysis, calibration and process control, including stream switching, various modes of digital and analog I/O, as well as connection to factory and process plant DCS (Distributed Control Systems).

L16

PEPTIDE COMPOSITION ANALYSIS AND COMPOSITION BASED SEQUENCING OF PEPTIDES: THE POWER AND BEAUTY OF ACCURATE MASS VALUES

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A new strategy is described for the determination of amino acid sequences of unknown peptides employing FT-ICR mass spectrometry [1]. Different from de novo sequencing, the new method is based on a two-step process. In the first step the amino acid composition of an unknown peptide is determined on the basis of accurate mass values of the peptide precursor ion and of a small number of fragment ions. Protein database information is not used. In the second step the sequence of the found amino acids of the peptide is determined by scoring the agreement between expected and observed fragment ion signals of the permuted sequences. It was found that the new approach is highly efficient if accurate mass values are available and that it easily outstrips common approaches of de novo sequencing being based on lower accuracies and detailed knowledge of fragmentation behavior.

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L17

FULLY AUTOMATED HIGH THROUGHPUT ANALYSIS USING CHIP-BASED NANOELECTROSPRAY FT-ICR MASS SPECTROMETRY

Mark Allen, Alistair Sterling, Mark Baumert

Advion BioSciences Ltd, Norwich UK

Data will be presented demonstrating the rapid analysis of a variety of molecules using a robotic, chip-based infusion device (NanoMate 100, Advion Biosciences) coupled to FT-ICR mass spectrometers.

Samples from different compound classes including drugs and metabolites, peptides, proteins, polymers, and ligands were analysed to obtain accurate mass information (<1ppm) from small sample volumes (3 to 5µL).

The ability to spray directly from solutions in excess of 20% DMSO will also be presented highlighting the potential for rapid, accurate mass analysis of compound libraries or files with no LC separation required.

The chip-based system uses a conductive pipette tip to introduce samples through the back of a fully integrated ESI chip consisting of a 10x10 array of nozzles etched from the planar surface of a silicon wafer. Each sample is aspirated and infused via a unique flow path thus eliminating sample carry over.

L18

QUANTITATIVE ANALYSIS OF TRYPTIC PROTEIN MIXTURES USING ELECTROSPRAY IONIZATION FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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For the first time quantitative analysis of tryptic protein mixtures, labeled with Quantification-Using-Enhanced-Signal-Tags (QUEST)-markers, were performed with electrospray ionization and a 9.4 T Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer. Labeling tryptic protein mixtures with QUEST-markers resulted in enhanced ionization efficiency and therefore also a higher probability of identifying low abundant components. An algorithm was developed to identify labeled tryptic peptides and determine the intensity ratio of the assigned labeled pairs. The amount of identified labeled peptides increased after coupling a High Pressure Liquid Chromatography (HPLC) separation step prior to the mass analysis. The range for the determined intensity ratios of two peptides in a labeled pair was large, and the obtained median intensity ratio correlated very well with the corresponding concentration ratio. This method can be used for observing protein dynamics in a specific cell type, tissue, or in body fluids.

L19

DISSOCIATION ANALYSIS OF GROES VIA SORI-CID IN AN FT-ICR-MS

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The dissociation pathways of GroES are investigated with FT-ICR-MS SORI-CID analysis. The ion storage capabilities and long time frame of the FTMS are ideal for internal energy manipulation. The biologically active conformer of GroES is a 71kDa heptameric non-covalently bound complex. It forms an essential part of the *E. Coli* GroEL/GroES chaperonin complex. After isolation of the heptameric complex ion in the ICR cell its dissociation dynamics have been investigated. The internal energy of the protein was increased via SORI-CID with argon gas, until it fragmented into mostly hexameric and monomeric reaction products. The fragmentation products had an asymmetric charge distribution, presenting evidence that one monomeric unit was squeezed out of the heptameric ring while unfolding and taking up extra protons.

L20

ELECTRON CAPTURE DISSOCIATION DETECTS THE PRESENCE OF A SINGLE D-AMINO ACID IN A PROTEIN AND PROBES THE NEUTRAL TERTIARY STRUCTURE

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First results are reported on the application of ECD FT ICR MS in analysis of stereoisomers of Trp-cage (NLYIQWLKDGGPSSGRPPPS). This smallest and fastest-folding of all known proteins exhibits in solution a tightly folded tertiary structure. The chiral recognition based on the ratios of the abundances of z_{18} and z_{19} fragments in ECD of 2+ ions was excellent, even for a single amino acid (Tyr) D-substitution (R_{chiral} = 8.6). The chiral effect decreased with an increase of temperature in the electrospray ion source. At the same time, 3+ ions of the stereoisomers gave similar ECD mass spectra (R_{chiral} = 1.5). A general approach is suggested for charge localization in n+ ions by analysis of ECD mass spectra of (n+1)+ ions. Application of this approach to 3+ Trp-cage ions revealed the protonation probability order in 2+ ions: Arg_{16} >> Gln_5 >≈ N-terminus. The ECD results for native form of the 2+ ions favor at least partial preservation of the solution-phase neutral tertiary structure, and the chiral recognition through the interaction between the charges and the neutral hydrogen-bond network. Conversely, ECD of 3+ ions supports the dominance of ionic hydrogen bonding which determines a different gas-phase structure than found in solution. Vibrational activation of 2+ ions indicated greater stability of the native form, but the fragmentation patterns did not provide stereoisomer differentiation, thus underlying the special position of ECD among other MS/MS fragmentation techniques. Further ECD FTICR MS studies should yield more structural information as well as quantitative single-amino acid D/L content measurements in proteins.

L21

NATIVE ELECTRON CAPTURE DISSOCIATION

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As shown recently, Electron Capture Dissociation (ECD) provides detailed information on the higher order structure of stable gas-phase conformers of protein ions (K. Breuker, H.-B. Oh, D.M. Horn, B.A. Cerda, F.W. McLafferty, J. Am. Chem. Soc. 2002,124, 6407-6420; H.-B. Oh, K. Breuker, S.-K. Sze, Y. Ge, B.K. Carpenter, F.W. McLafferty, Proc. Natl. Acad. Sci. USA 2002, 99, 15863-15868). We show here that Native Electron Capture Dissociation (NECD), a newly discovered phenomenon (K. Breuker, Fred W. McLafferty, Angew. Chem. Int. Ed. 2003, 42, 4900-4904), provides instead detailed information on the higher order structure of NATIVE proteins and their unfolding as a result of desolvation during ESI.

L22

ELECTRON CAPTURE DISSOCIATION OF NON-STANDARD AND MODIFIED PEPTIDES

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Structural elucidation of post-translationally modified proteins is of key importance in the understanding of an array of biological processes. The value of ECD, and its complementarity with IRMPD, in determining sites of phosphorylation and glycosylation in proteins is well-documented. We have applied ECD to the analysis of aberrant glycosylation of IgA1, implicated in IgA nephropathy, and to the analysis of ubiquitinated peptides, a modification which regulates many cellular functions. We have also studied the ECD of some non-standard peptide structures, including branched peptides, where branching is achieved through a bivalent lysine residue, as in ubiquitination, in order to gain further insight into the processes that occur following electron capture.

L23

OPTIMIZATION OF ECD: MECHANISTIC STUDIES, TOP-DOWN AND BOTTOM-UP APPLICATIONS

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The performance of electron capture dissociation (ECD) FTICR MS was improved by increasing the number of ICR trap loads with ions using gas assisted dynamic ion trapping (GADT). The increased number of ions allowed the investigation of ECD FTICR MS efficiency dependence on the experimental parameters, including electron energy, irradiation time, electron flux, and ion trapping method in the electron energy range 0-50 eV.

Electron kinetic energy distributions and emission currents were measured for ECD and hot ECD. Two electron injection systems were employed: one with injection of a pencil electron beam (disk dispenser cathode) and another with a hollow electron beam (ring dispenser cathode).

ECD with a hollow or pencil electron beam was obtained for both sidekick and GADT FTICR MS with an electrostatic ion transfer line. The experimental results are completed by a theoretical (SIMION) study of ion motion in the ICR trap following sidekick trapping.

FTICR MS with a more sensitive pre-amplifier was used for improved (LC) ECD FTICR MS analysis of post-translationally modified peptides and proteins. The results are compared with CID and IRMPD data.

L24

CRACKING THE HISTONE CODE: A MASS SPECTROMETRY BASED APPROACH FOR THE DETERMINATION OF HISTONE MODIFICATIONS

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The post-translational modifications of core histones play a critical role in gene activities. Furthermore, there is a growing need to characterize the modulation of histone modification in conjunction with the application of new chemoprevention strategies that employ drugs that target enzymes that modify histones (Histone DeACtylase Inhibitors). We identified 20 novel modifications by FTICR MS on the core histone of bovine thymus. Two novel sites of modification (H4 K59 and K91) have been examined in yeast and have been shown to play important roles in gene silencing and DNA damage repair. LC-MS assays have been developed to screen for modifications in tumor cell lines and patients with acute myeloid or chronic lymphocytic leukemia.

L25

PROTEIN PATTERNS IN CEREBROSPINAL FLUID REVEALED BY LIQUID CHROMATOGRAPHY FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

M. Ramström*, I. Ivonin**, A. Johansson***, H. Askmark***, K. E. Markides*, R. Zubarev**, P. Håkansson****, S.-M. Aquilonius***, J. Bergquist*

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*** Department of Neuroscience, Unit of Neurology
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Neurodegenerative disorders, such as Alzheimer's disease and amyotrophic lateral sclerosis (ALS) cause a heavy burden for patients and relatives. Today, there is no single biomarker that can be used for the diagnosis of these disorders. In this study, we present a novel proteomic approach to be used for investigations of protein patterns in body fluids. The method involves on-line packed capillary FT-ICR MS of tryptic digested cerebrospinal fluid (CSF), followed by pattern recognition. The results demonstrated the possibility to correctly identify in vitro added proteins of low concentrations and to detect alterations in very complex biological matrices. When comparing mass chromatograms from ALS-patients with those from healthy controls, no single biomarker was identified from the classification peaks. However, it was possible to correctly assign unknown samples into the correct classes based on the protein patterns.

L26

MAPPING PROTEIN INTERFACES BY CHEMICAL CROSS-LINKING AND FTICR MASS SPECTROMETRY: APPLICATION TO CALMODULIN/PEPTIDE COMPLEXES

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Chemical cross-linking has gained renewed interest in combination with mass spectrometric analysis of the reaction products as a valuable tool to identify interacting sequences in protein complexes. We present a strategy for mapping protein interfaces using chemical cross-linking and analysis of the created cross-linking products by nano-HPLC/nano-ESI-FTICR mass spectrometry. The method was employed to define interacting regions in the calcium-dependent calmodulin/melittin and calmodulin/myosin light chain kinase peptide (M13) complexes.

We applied several amine-reactive homobifunctional cross-linkers spanning distances between ~6-16 Å as well as a 'zero-length' cross-linker. Following chemical cross-linking, the reaction mixtures were first separated by one-dimensional gel electrophoresis and subjected to enzymatic in-gel digestion yielding intricate mixtures of cross-linked and non-cross-linked peptides. Those highly complex mixtures were separated by nano-HPLC on reversed phase columns applying water-acetonitrile-gradients with flow rates of 200 nl/min. The nano-HPLC system was directly coupled to an FTICR mass spectrometer (Apex II, 7 T, Bruker Daltonics) equipped with a nano-electrospray ionization source (Agilent Technologies). The obtained mass spectra were screened for possible cross-linking products by employing customized software programs.

For the calmodulin/M13 complex our cross-linking data were in good agreement with NMR spectroscopic data of the complex. Using the distance constraints derived from the chemical cross-linking data in combination with computational methods of conjoined rigid body/torsion angle simulated annealing, we were able to generate a low-resolution three-dimensional structure model for the calmodulin/melittin complex, for which no high-resolution structure exists to date. Our data provide evidence for the first time that calmodulin is able to recognize target peptides in two opposing orientations.

L27

APPLICATION OF FTICT-MS IN CHARACTERISATION OF TARGET PEPTIDES/PROTEINS OF ALZHEIMER'S DISEASE AND ELUCIDATION OF AN AMYLOID PLAQUE-SPECIFIC EPITOPE

X. Tian*, R. Cecal*, J. McLaurin**, R. Stefanescu*, M. Manea*, S. Grau***, M. Ehrmann***, P. St George-Hyslop**, M. Przybylski*

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In this work, the detailed chemical structures and post-translational structure modifications of amyloid precursor protein (APP) and APP fragments have been studied using Fourier transform ion cyclotron resonance mass spectromertry (FTICR-MS), in particular, both the chemically synthetic and recombinant cytoplasmic domain of ßAPP has been characterised by ESI FTICR MS, which helps to understand at the molecular level of the formation of the amyloid peptide and of amyloid plaques in AD and other cerebral disorders.

Immunisation of transgenic mouse models of AD with Aß(1-42) has been recently effective to inhibit and disaggregate Aß-fibrils, and to reduce both AD-related neuropathology and memory impairments. The mechanism underlying these therapeutic effects has been as yet unclear. Using selective proteolytic digestion of the antigen:antibody complex (epitope excision) in combination with high resolution FTICR-MS, we have identified the epitope recognised by the therapeutically active antibody as the N-terminal Aß(4-10), , which opens new lead structures for effective AD vaccine development.

L28

SOME NEW APPROACHES IN ION TRAPPING AND DETECTION IN FT ICR

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** Biological Sciences Division and Environmental Molecular Science Laboratory, Richland, USA

Three innovations in ion trapping and detection methodology are discussed:

1. New FT ICR cell design. Ion transfer from an accumulation multipole to the ICR cell causes ion axial excitation in the cell in all trapping methods, such as gas assisted trapping and gated trapping. This excitation distorts mass resolution as well as mass accuracy. It is necessary to cool ions either by gas collisions or by introducing a delay between ion capturing and frequency measurement for Coulomb cooling. Both methods extend the total measurement time by at least 0.5 to 2 seconds. This amount of time is comparable to other events, such as ion accumulation in the multipole and ion detection, and is significant when fast mass spectral measurements are needed. We report on the new FT ICR cell design, which permits externally accumulated ions to be trapped without further cooling and present initial experimental results obtained using this design.

2. Improvements to new FT ICR cell design. We report on the attempts to improve the FT ICR cell design by two approaches: i) simultaneously trapping of both positively and negatively charged ions and ii) suppression of the inhomogeneous electric field created by trapping electrodes to measure the unperturbed ion cyclotron frequency directly.

3. Novel methodology for ICR signal measurements. FTICR mass spectrometry provides the highest mass measurement accuracy (MMA) over a broad m/z range. MMA depends strongly on the total number of ions in the cell ("global" space charge") as well as on the distribution of different m/z ions inside total charge population, which causes local deviation from mass calibration curves. The main source of "local" mass measurement errors is a Coulomb interaction of a particular m/z ion cloud with ion clouds of different m/z ions. We propose a novel methodology for ICR signal measurements in which the most abundant ions are ejected from the total ion ensemble during the signal detection event following excitation of cyclotron motion. On the fly ejection is preceded by FT of the portion of time domain signal just after excitation to choose the most abundant ions for ejection. We have shown that this new methodology provides an improvement in MMA for all the spectra studied.

L29

CURRENT THOUGHTS REGARDING THE CRYOGENIC FTMS

Peter B. O'Connor

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The Cryogenic FTMS offers interesting potential advantages in terms of magnetic field strength, base pressure, and preamplifier noise. Operating the the cell and chamber at 4K allows the FTMS to be built in the 4K vertical bore of a superconducting magnet, eliminating the need for insulation to bring the bore to room temperature. This allows use of narrower bore diameter magnets, which are less expensive in terms of cost/Tesla.

The 4 K vacuum chamber walls are also cryopumping, which will greatly decrease the base pressure in the cell. Estimated base pressure is <1x10-12 mbar, and the estimated pumping speed is >10000 liter/second at the cell. Furthermore, 4K chamber can be used to cool the amplifer to reduce Johnson noise and shot noise in the amplifier. However, it is imperative to build a preamplifier either with GaAs FETs, or with HEMT's which function at 4 K in order to achieve the predicted noise benefits.

Heat flow analysis is critical in the design of the vacuum chamber, ion optics, and wiring to prevent unnecessary thermal stress on the cryogenic system. These and other concerns and solutions will be discussed.

L30

LASER-INDUCED FLUORESCENCE OF TRAPPED (BIO)MOLECULAR IONS

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Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

The combination of laser-induced fluorescence with mass spectrometry opens up new possibilities both for detection purposes and for structural studies of trapped biomolecular ions in gas phase. However, this approach is experimentally very challenging, and only a handful of studies have been reported so far. In this presentation, a novel scheme for Laser-induced fluorescence measurements of ions trapped inside a FTICR mass spectrometer will be introduced. It is based on an open FTICR cell design, continuous wave axial excitation of the fluorescence, orthogonal photon collection by fiber optics, and single photon counting detection. Rhodamine 6G ions generated by an internal MALDI source were used to develop and test the set-up. An innovative ion tomography method was used to align the excitation laser. Due to photodissociation processes, the excitation laser power and the observation time window have to be carefully optimized. Applications for studying the gas-phase structure of green fluorescent protein and for investigating fluorescence resonance energy transfer (FRET) of trapped donor-acceptor pairs will be discussed.

L31

COMPOSITION OF MARINE DISSOLVED ORGANIC MATTER: FIRST CHEMICAL FORMULAS AS DETECTED BY FTMS

Boris Koch, Thorsten Dittmar, Ralph Engbrodt, Martin Graeve; Matthias Witt, Gerhard Kattner

Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

Marine dissolved organic matter (DOM) represents an enormous amount of organic carbon comparable to that of atmospheric CO2. A major part of DOM is refractory and resistant to chemical decomposition and despite manifold analytical approaches the bulk of its chemical structure remains completely unknown. This study addressed the question: What are the sources and formation processes of DOM in the ocean? The Southern Ocean is a key area to study DOM of pure marine origin. However a considerable amount of terrestrial DOM is transported by rivers to the oceans. The modification of terrestrial DOM by photo-degradation was examined in a 10-day degradation experiment. Software was developed to process chemical formulas for several thousand mass peaks in the FTMS spectra. On this basis the elemental contribution of dissolved oxygen and hydrogen to DOM was estimated. Selective losses of compounds with high O/C and low H/C ratios indicate loss of aromatic compounds and carboxylic acids during photo-degradation. The chemical formulas varied significantly between marine and terrestrial DOM sources and between marine DOM samples of differing maturity states.

L32

A STUDY OF ELECTRON CAPTURE DISSOCIATION EFFICIENCY OF PEPTIDE DICATIONS USING A DISPENSER CATHODE

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** FOM Institute for Plasma Physics "Rijnhuizen", Nieuwegein, The Netherlands

Electron capture dissociation (ECD) has been shown to be a useful technique for the characterisation of post-translational modifications (PTMs) in peptides and proteins. The advent of high-surface dispenser cathodes has enabled electron irradiation times (10's ms) that are sufficiently low to be compatible with liquid chromatographic separation prior to fragmentation by ECD. These dispenser cathodes generate much higher electron fluxes than conventional electron filaments (30 mA vs 1 mA) and over a much wider area (20 mm2 vs < 1 mm2), improving ion cloud/electron beam overlap.

The electron capture cross-section in ECD is believed to be dependent on the square of the charge state and inversely dependent on the electron kinetic energy. In this study we have examined the efficiency of ECD as a function of electron kinetic energy for two model peptide dications. The fragmentation of the doubly protonated precursor ions of substance P (SubP) and (d)Trp(6) luteinising hormone releasing hormone (LHRH) was investigated using a 9.4T fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer following electron irradiation with a BaO dispenser cathode (5-200ms). The yield of the remaining precursor ions and the sum of the reduced fragment species were determined for different irradiation times and electron kinetic energies. The depletion rate of [subP]2+ and [LHRH]2+ was 3-5x greater than the rate of singly charged fragment formation suggesting the low abundance of these species or a two electron capture process. ECD efficiency was estimated using rate constants determined for the reduction of the doubly charged precursor and the singly charged fragments at different initial kinetic energies. The results of a retarding field analysis study, used to determine the kinetic energy of electrons entering the ICR cell, is supported by electron trajectory simulations using the program Simion 7.0 ©.

L33

A COMPARISON OF CALIBRATION METHODS FOR ACCURATE MASS MEASUREMENT OF LOW MOLECULAR WEIGHT MOLECULES USING FTMS

Tony Bristow

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The results from a comparison of a number of techniques for accurate mass measurement of small molecules (a single ion at m/z 476) using FTMS are presented. These were external calibration, internal calibration using mutiple calibrant ions and internal calibration using a single calibrant ion (lock mass) at various m/z. The goal of the study was to evaluate the methods to reduce the mass measurement error, improve the precision and therefore reduce the uncertainty associated with the derivation of elemental formulae from such measurements. For each calibration protocol twenty-five mass measurements of the analyte ion (m/z 476) were made (five replicates on five separate days). A single point internal calibration using m/z 462, produced the most accurate measurement and smallest precision (1 standard deviation) of 0.23 ppm +/- 0.16 ppm. This compares to 0.35 ppm +/- 0.29 ppm for internal calibration using multiple calibrant ions and 0.50 ppm +/- 0.98 ppm for external calibration alone. The m/z difference between the internal calibrant ion and m/z 476 had a significant effect on mass accuracy. The significance of both the mass accuracy and precision for determination of elemental formulae is also discussed. The use of expanded uncertainty is shown to improve the confidence with which an elemental formula is selected.

L34

HADAMARD-TRANSFORM TIME-OF-FLIGHT MASS SPECTROMETRY

O. Trapp, J.R. Kimmel, O. Yoon, I.A. Zuleta, R.N. Zare

Stanford University, Department of Chemistry, USA

Mass spectrometry is one of the most important analytical tools to characterize and identify minute amounts of molecules in complex mixtures and is becoming a very sensitive detector in separation sciences. We are developing a unique form of time-of-flight mass spectrometry (TOFMS), called Hadamard transform TOFMS (HT-TOFMS), which offers faster acquisition rates and higher signal-to-noise ratios than traditional TOFMS methods. In this method a continuous ion beam emerging from an ion source is accelerated and then modulated by a pseudo-random sequence of "on" and "off" pulses. The pseudo-random sequence is derived from the Hadamard matrix. The data is acquired synchronously with the modulation of the ion beam, and the modulation sequence is deconvoluted from the data using a Hadamard transformation to extract the time-of-flight distribution of the ions. This multiplexing scheme increases the ion usage to approximately 50% (normal multi-channel plate detector) or approximately 100% (structured multi-channel plate detector) and improves the signal-to-noise level considerably over that of conventional TOFMS. The fundamentals, development, and application of this technique will be discussed.

L35

ION INTENSITY RELATIONSHIPS FOR THE MS/MS SPECTRA OF PROTONATED PEPTIDES

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** Department of Molecular Biophysics, German Cancer Research Center, Heidelberg, Germany

Bioinformatics-aided tandem mass spectrometry (MS/MS) is a significant component of the exploding field of proteomics. The currently available MS/MS bioinformatics tools compare the theoretical MS/MS spectra predicted for entries of protein and/or translated nucleic acid databases and the measured experimental data exploiting only the first (fragment ion mass to charge ratios) and disregarding the second (fragment ion intensities) dimension of the latter. The corresponding protein identification algorithms could be no doubt further refined by the incorporation of reliable MS/MS ion intensity relationships (IIRs) into the existing schemes.

In this presentation we will show that some IIRs of the MS/MS spectra of peptides can be understood and predicted [1]. To this end, we present both experimental and modeling data on the fragmentation pathways of various peptides including the VX, XF, GGX, and GXF series (X is varied), A5, Leu-enkephaline, and G5R (both singly and doubly charged). IIRs of the experimentally determined MS/MS spectra will be discussed and explained by considering the underlying fragmentation mechanisms derived from modeling and by application of free-energy relationships.

[1] B. Paizs and S. Suhai, Mass Spectrom. Rev., in press.

L36

IDENTIFICATION AND STRUCTURE CHARACTERIZATION OF DISEASE-ASSOCIATED MARKER PROTEINS USING MALDI QIT TOF MSN IN CLINICAL PROTEOME RESEARCH WITH PATIENTS SUFFERING FROM RHEUMATOID ARTHRITIS

C. Koy*, P. Serrano*, S. Möller*, B. Ringel*, S. Mikkat*, R. Martin**, S. Drynda***, J. Kekow***, H.-J. Thiesen*, **M.O. Glocker***

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** Shimadzu Biotech, Manchester, UK
*** Clinic of Rheumatology, University of Magdeburg, Germany

We found conspicuous differences in the abundance of disease-relevant soluble proteins between synovial fluids (SF) and plasmas of patients diagnosed to suffer from either Rheumatoid Arthritis (RA) or Osteoarthritis (OA). Detailed analysis of 2DE-separated spots by MALDI-MS and MALDI QIT ToF MSn revealed that the haptoglobin a2 chain is present in both body fluids in at least four variants. The heaviest form harbours an arginine residue at the carboxy-terminus whereas the others lack this characteristic C-terminal residue. The abundance of the "full-length" variant is significantly lower in SF than in plasma, while the spot volumes of the truncated variant with highest abundance are significantly higher in SF than in plasma. Also, MRP14 was identified as a discriminatory marker protein in SF by global proteome analysis. To examine the applicability as a diagnostic marker, plasma levels of the MRP8/MRP14 complex were validated by ELISA and showed that RA patients can be clearly differentiated from OA patients. Longitudinal studies on samples from RA patients indicate that plasma levels of the MRP8/MRP14 complex also might become a useful marker in monitoring anti TNFa therapy.

L37

OF BIOMARKERS AND MODIFICATIONS: TOP DOWN MASS SPECTROMETRY OF PROTEINS ACROSS THE PHYLOGENETIC TREE

Neil L. Kelleher

Department of Chemistry, University of Illinois at Urbana-Champaign, USA

New tools to drive the emergent "Top Down" approach to proteome analysis will be described. They combine informatics and software (1) with a Quadrupole/Fourier-Transform hybrid mass spectrometer (Q-FTMS) and molecular weight-based separation methods to enable efficient characterization of biological events that change the mass of protein molecules from that predicted by an annotated genome sequence. It is now possible to automatically interrogate hundreds of proteins using new Top Down technology. Further, "hypothesis driven" experiments target specific protein classes (e.g., histones) for measurment of putative PTMs thought to exist by gazing through sequenced genomes. Such targeted detection of proteins from complex proteomes of methanogens, yeast, and humans will be presented. For combinatorially-modified proteins, Electron Capture Dissociation combined with a new database strategy termed "prescriptive annotation" enable automatic detection and localization of up to six post-translational modifications. More generally, Top Down Mass Spectrometry holds promise for direct identification of disease biomarkers and a deeper understanding of the signaling and regulatory "codes" Eukaryotic cells use that are written in the language of post-translational modification.

1) Taylor, G. K.; Kim, Y-B; Forbes, A.J.; Meng, F.; McCarthy, R; Kelleher, N.L. "Web and Database Software for Analysis of Intact Proteins By Top Down Mass Spectrometry," Anal. Chem., 2003, 75, 4081-4086.

Poster Programme

Poster sessions:

Sunday, 28 March, 7.30 pm: all numbers

Monday, 29 March, 1.00 pm Session A (odd numbers)

Wednesday, 31 March, 1.00 pm Session B (even numbers)

- Instrumentation and Methods -

P1 **O. P. Balaj**, I. Balteanu, B. S. Fox-Beyer, M. K. Beyer, V. E. Bondybey
Technische Universität München, Germany
The Development of a Temperature Controlled ICR-Infinity Cell

P2 **M. Dashtiev**, V. Frankevich, R. Zenobi
Swiss Federal Institute of Technology (ETH), Switzerland
Optimization of Laser-Induced Fluorescence Setup for High Mass Biomolecules

P3 **M.A. McFarland***, A.G. Marshall*, P. Fredman**, J.-E. Mansson**, C.L.Nilsson***
* Florida State University, USA, ** Sahlgrenska University Hospital, Goteborg University, Molndal, Sweden, *** Institute of Medical Biochemistry, Goteborg University, Goteborg, Sweden
Structural Characterization of GM1 Gangliosides by Infrared Multiphoton Dissociation, Electron Capture Dissociation, and Electron Detachment Dissociation

P4 **V. Frankevich**, M. Dashtiev, R. Zenobi
Swiss Federal Institute of Technology (ETH), Switzerland
Effect of Buffer Gas in Internal MALDI Source FTICR-MS

P5 **M. Froesch***, L. Bindila*,**, Ž. Vukelić***, A. Zamfir*,**, J. Peter-Katalinić*
* University of Münster, Germany, ** National Institute for Research and Development in Electrochemistry and Condensed Matter, Timisoara, Romania
*** University of Zagreb, Croatia
Ionisation And Fragmentation Of Highly Sialylated Glycosphingolipids by FT-ICR

P6 **D. Galetskiy***, R. Weber*, J. S. Becker*, E. Damoc*, N. Youhnovski*, J. Rossier**, N. Lion***, F. Reymond**, H. Girault***, M. Przybylski*
* University of Konstanz, Germany, ** DiagnoSwiss SA, Monthey, Switzerland,
*** Laboratoire d'Electrochimie, EPFL-DC, Lausanne, Switzerland
HIGH-PERFORMANCE PROTEOMICS USING A THIN-CHIP MICROSPRAY SYSTEM
WITH INTEGRATED MEMBRANE-BASED DESALTING AND FT-ICR MASS SPECTROMETRY

P7 T. Giroldo, M. Sena, L. A. Xavier, J. **M. Riveros**
University of São Paulo, Brazil
A COMPARISON BETWEEN IRMPD AND DISSOCIATION BY A HEATED FILAMENT FOR SIMPLE IONS

P8 **G. Hübner**, C. Crone, and B. Lindner
Research Center Borstel, Leibniz Center for Medicine and Biosciences, Borstel, Germany
IRMPD-MS/MS ANALYSIS OF GLYCOLIPIDS: INFLUENCE OF DIFFERENT TRAPPING METHODS

P9 **C. Ihling**, A. Sinz
University of Leipzig, Germany
ANALYSIS OF COMPLEX PROTEIN MIXTURES BY LIQUID CHROMATOGRAPHY IN COMBINATION WITH FTICR MASS SPECTROMETRY

P10 **J. Lemaire*** **, L. M. Aleese*, J.-M. Ortega**, P. Maître*
* Laboratoire de Chimie Physique, University of Paris, Orsay, France
** CLIO-LURE, University of Paris, Orsay, France
VIBRATIONAL SPECTORSCOPY OF SELECTIVELY PREPARED GAS PHASE IONS BY COUPLING A PERMANENT MAGNET BASED FT-ICR-MS WITH A FREE ELECTORON LASER

P11 **E. Mirgorodskaya***, R. Jertz** G. Baykut**, A. Haase***, J. Gobom*
* Max-Planck-Institute for Molecular Genetics, Berlin, Germany, ** Bruker Daltonik GmbH, Bremen, Germany, *** Bioptics Lasersysteme AG, Berlin, Germany
FORMATION OF DOUBLY CHARGED PEPTIDE IONS BY ELEVATED-PRESSURE IR-MALDI- FTMS WITH A 2.94 MM ER:YAG LASER

P12 **H. Oesten***, D. Davis**, W. Rimkus**, S. Bichlmeier*
* Siemens AG, Karlsruhe, Germany, ** Siemens Applied Automation, Bartlesville, OK, USA
QUANTITATIVE AND QUALITATIVE APPLICATIONS OF FT ICR MS SPECTROMETRY FOR PROCESS CONTROL

P13 **J. Oomens***, D.T Moore*, A.F.G. van der Meer*, G. von Helden**, G. Meijer**, J. Valle***, J.R. Eyler***, A.G. Marshall****
* FOM Institute for Plasma Physics, Nieuwegein, The Netherlands, ** Fritz-Haber-Institut der Max-Planck-Gesellschaft, Berlin, Germany, *** University of Florida, Gainesville (FL), USA, **** National High Magnetic Field Laboratory, Tallahassee (FL), USA
INFRARED SPECTROSCOPY OF GAS-PHASE MOLECULAR IONS IN AN FTICR MS

P14 M. Šamalikova, **R.Grandori**
Johannes Kepler University, Linz, Austria
ROLE OF SOLVENT SURFACE TENSION IN ELECTROSPRAY-IONIZATION OF PROTEINS

P15 **I. A. Tarasova**, M.V. Gorshkov.
Institute of Energy Problems of Chemical Physics, Russian Academy of Sciences, Moscow, Russia
CHARACTERIZATION OF FTICR MASS SPECTROMETRY AT LOW MAGNETIC FIELD

P16 **Y. O. Tsybin***, M. Witt**, G. Baykut*, G. Weiss**, P. Håkansson*
*Division of Ion Physics, The Ångström Laboratory, Uppsala University, Uppsala, Sweden, ** Bruker Daltonik GmbH, Bremen, Germany
OPTIMIZATION OF ECD: MECHANISTIC STUDIES, TOP-DOWN AND BOTTOM-UP APPLICATIONS

P17 **A. Uhl**, B. M. Reinhard, S. E. Joos, A. Lagutschenkov, G. Niedner-Schatteburg
Technische Universität Kaiserslautern, Germany
F.R.I.T.Z. (FOURIERTRANSFORM REACTIVE INVESTIGATION TEST ZOO) AND WHAT TO DO WITH IT

P18 **R. Weber**, E. Damoc, J.S. Becker, N. Youhnovski, M. Przybylski
Laboratory of Analytical Chemistry, Department of Chemistry,
University of Konstanz, Germany
HIGH PERFORMANCE PROTEOME ANALYSIS WITH UV- AND IR- MALDI-FTICR MASS SPECTROMETRY

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** Laboratory of Neuromorphology, Department of Anatomy, Semmelweis University, Budapest, Hungary
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POSTER-ABSTRACTS

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P1

THE DEVELOPMENT OF A TEMPERATURE CONTROLLED ICR-INFINITY CELL

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The variable temperature FT-ICR cell under development has a wide range of potential applications:

- extracting activation energies from BIRD (Black-body infrared radiative dissociation) experiments through master-equation modeling
- studying ion-molecule reactions under variable and well-defined collision-gas temperatures
- using water clusters as model systems for polar stratospheric clouds and study their reactions under realistic temperature conditions
- increase the lifetime of non-covalent complexes and thus enhance resolution for large biomolecules, which otherwise might undergo lifetime broadening
- increase resolution by decreasing the pressure in the ICR-cell, whose surfaces act as a cryo-pump
- extracting binding energies from temperature-resolved radiative association kinetics.

The strength of our design, with an almost complete enclosure of the cell interior with cooled walls lies in the well-defined radiation temperature down to 100 K.

P2

OPTIMIZATION OF LASER-INDUCED FLUORESCENCE SETUP FOR HIGH MASS BIOMOLECULES

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Laser-induced fluorescence (LIF) in combination with Fourier-transform ion cyclotron resonance mass spectrometer is an ideal combination for obtaining structural and conformational information of high-mass biomolecular ions in gas-phase. An improvement in ICR cell design has been shown to increase the fluorescence detection efficiency for trapped biomolecular ions. In LIF experiments trapped ions must be very well axialized. Quadrupolar excitation was used for ion focusing and axialization. The efficiency of axialization was monitored by means of ion tomography with Rhodamine 6G ions. Preliminary results showed stable LIF signals of heavy biological molecules.

P3

STRUCTURAL CHARACTERIZATION OF GM1 GANGLIOSIDES BY INFRARED MULTIPHOTON DISSOCIATION, ELECTRON CAPTURE DISSOCIATION AND ELECTRON DETACHMENT DISSOCIATION

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Gangliosides play important biological roles in the central nervous system, such as cell-cell recognition, contact inhibition, cell adhesion, and tissue differentiation. The carbohydrate portion of the ganlioside mediates molecular recognition, while the ceramide portion acts as a modulator of glycolipid structure and function. Therefor, structural studies of glycolipids should include characterization of both the lipid and the carbohydrate moieties. Here we employ FT-ICR MS-MS dissociation techniques to demonstrate the combination of ECD and IRMPD for positive mode and EDD and IRMPD for negative mode structural characterization of the isomeric ganglosides GM1a and GM1b. Fragments corresponding to cleavage at each sugar of the carbohydrate as well as both the fatty acid and long chain base portions of the lipid are present. Fragmentation of sulfatides will also be presented as a conformation of the ceramide moiety's fragmentation pattern.

P4

EFFECT OF BUFFER GAS IN INTERNAL MALDI SOURCE FTICR-MS

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A pulse of buffer gas in proximity to a MALDI sample significantly enhances the yield of positive ions. We interpret this effect as a result of decreased number of negative ions in the MALDI plume and reduced fragmentation under high-pressure conditions. Photoelectrons are shown to play an important role in MALDI. Most MALDI matrices have maximum negative ion [M-H]- formation rate at an electron energy of about 1 eV. Less efficient electron capture dissociation in the MALDI plume was observed in the presence of the buffer gas. This is due to reduction of the electron energies by collisions. It is proposed that the fragmentation in internal MALDI FTICR sources is mostly a result of collision-induced dissociation (CID) in the plume. The efficiency of CID is lower at high buffer gas pressure and results in decreased fragmentation.

P5

IONISATION AND FRAGMENTATION OF HIGHLY SIALYLATED GLYCOSPHINGOLIPIDS BY FT-ICR

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Sialylated and sulfated glycosphingolipids (GSL) are particularly abundant in the nervous system as plasma membrane and myelin building components involved in cell signaling and cell-to-cell recognition/communication mechanisms. Mass spectrometry has been already demonstrated as a method of choice for the structural analysis of GSL mixtures [1,2]. Our studies are focused on the development of viable strategies based on nano-electrospray ionization Fourier Transform Ion Cyclotron Resonance mass spectrometry (nanoESI FT-ICR MS) for screening of complex saccharide mixtures from biological matrices. nanoESI FT-ICR MS was already implemented for analysis of complex glycopeptide mixtures from urine of patients suffering from Schindler‘s disease [3]. By (-)nanoESI FT-ICR MS single components present in the mixture could be identified and by optimization of sustained off-resonance irradiation collision induced dissociation mass spectrometry (SORI-CID MS) the type and sequence as well as sialylation pattern were determined. FT-ICR MS at 9.4 T is reported here for investigations of a purified fraction of a trisialoganglioside from normal human brain with respect to the optimization of ionization and fragmentation conditions.

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P6

HIGH-PERFORMANCE PROTEOMICS USING A THIN-CHIP MICROSPRAY SYSTEM WITH INTEGRATED MEMBRANE-BASED DESALTING AND FT-ICR MASS SPECTROMETRY

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The development of low cost polymer microchips has recently provided the basis for the development of new high performance micro-analysis systems. The coupling of the microchip system with ESI-FT-ICR mass spectrometry has provided protein analysis with high sensitivity and mass determination accuracies in the low- and sub-ppm range, enabling new application dimensions of high resolution proteomics, the characterisation of protein complexes and post-translational modifications [1]. A new concept for both desalting and sample preconcentration in proteomics applications prior to mass spectrometric analysis is presented. This new device for chip-ESI-FT-ICR-MS is based on the coupling of a nanospray microchip with a membrane-based trapping system. Since captured proteins are eluted in a small volume, this immobilization step leads to preconcentration and desalting of the proteins providing both high sensitivity and ultra-high resolution, suitable for bio-analytical applications [2]. The microchip-based desalting approach has been successfully applied to the direct FT-ICR-MS analysis of digested tryptic peptide mixtures from human microtubule-associated tau protein, which has gained widespread interest as a target protein for formation of neurofibrillary tangles in Alzheimer's disease. The high analytical performance of chip-ESI-FT-ICR-MS is illustrated in structural studies of tau.

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P7

A COMPARISON BETWEEN IRMPD AND DISSOCIATION BY A HEATED FILAMENT FOR SIMPLE IONS

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In the last few years we have developed a simple technique that makes use of a heated filament to promote the dissociation of relatively simple ions by absorption of high temperature near-blackbody radiation in a small cubic cell of a simple Fourier Transform ion cyclotron resonance spectrometer. Under special conditions, dissociation energies can be determined from these experiments. On the other hand, the technique is very sensitive in recognizing different isomeric species of molecular ions. We have studied a number of aromatic systems and cluster ions with this technique and comaprd with the dissociation induced by IRMPD experiments using a CO2 laser. A comparison of the quantitative and qualitative information that can be obtained from these experiments, along with their limitations, will be described for these different systems.

P8

IRMPD-MS/MS ANALYSIS OF GLYCOLIPIDS: INFLUENCE OF DIFFERENT TRAPPING METHODS

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Glycolipids play an important role in immunological processes and signal transduction. Monitoring structural alterations and changes in the overall lipid composition of functional compartments may contribute to understand biological interaction mechanisms. ESI FT-MS combined with IRMPD-MS/MS is a powerful tool for glycolipid identification without time consuming standard chemical analysis. We have investigated the influence of different trapping methods on the quality of the IRMPD-MS/MS spectra with respect to sensitivity, S/N-ratio, mass resolution and degree of fragmentation in the positive and negative ion mode. Since sidekick-trapping may force ions off axis, laser ion interaction can be substantially reduced. Analogous to recent approaches using resonant excitation pulses prior to irradiation of isolated ions we used sustained off-resonance irradiation during laser interaction to enhance fragmentation. Although this procedure leads to an improvement, no significant increase in sensitivity and S/N ratio could be obtained compared to gated trapping without sidekick. Gas assisted dynamic trapping gave best results but needed more elaborate tuning.

P9

ANALYSIS OF COMPLEX PROTEIN MIXTURES BY LIQUID CHROMATOGRAPHY IN COMBINATION WITH FTICR MASS SPECTROMETRY

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The basic problem of complexity poses a significant challenge for proteomic studies. To date, 2D-PAGE (two-dimensional polyacrylamide gel electrophoresis) followed by enzymatic *in-gel* digestion, extraction of the peptides, and subsequent identification by mass spectrometry is the most commonly used method to analyze complex protein mixtures. However, 2D-PAGE is a slow and labor-intensive technique, which is not able to resolve all proteins of a proteome. To overcome these limitations we are developing gel-free approaches on the basis of high-performance liquid chromatography (HPLC) and FTICR (Fourier transform ion cyclotron resonance) mass spectrometry. The extremly high signal capacity and excellent mass measurement accuracy in FTICRMS provides a basis for simultaneous analysis of numerous compounds [1].

In the present study, the small-protein subfraction of an *E. coli* cell lysate was analyzed using C4 reversed phase chromatography for protein pre-separation and nano-HPLC / nano-ESI-FTICRMS for analysis of the peptide mixtures after tryptic digestion of the protein fractions. We demonstrate that the combination of LC separation on the protein level followed by nano-HPLC separation and FTICR mass spectrometry of the enzymatically digested fractions has the potential to be a powerful method for analyzing complex protein mixtures. Strengths and limitations of this approach will be discussed.

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P10

TITLE

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The Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR-MS) used for these experiments is a Mobile ICR Analyser (MICRA). It is based on a structured permanent magnet cylinder producing a nominal field of 1.24 Tesla.
The ICR cell has an open structure so that the output of the Free Electron Laser can easily be focussed on the ion cloud. Primary ions can be generated either by electron ionization, laser ablation or Matrix Assisted Laser Desorption Ionization (MALDI).
The infrared Free Electron Laser (FEL) CLIO based at Orsay (France) provides a high intensity output over a wide wavelength range (3- 120 μm) and gives access to the infrared molecular fingerprint region. The laser relative bandwidth is of ca 0.5%. The temporal structure consists of macropulses fired at 25 Hz and each macropulse contains 500 micropulses of a few picoseconds duration, separated by 16 ns.
The effect of the infrared laser intensity on the dissociation efficiency for different molecular systems will be discussed.

P11

FORMATION OF DOUBLY CHARGED PEPTIDE IONS BY ELEVATED-PRESSURE IR-MALDI FTMS WITH A 2.94 MM ER:YAG LASER

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The success of UV MALDI FTMS of biomolecules was for a long time limited by the short lifetime of the produced molecular ions. The development of elevated-pressure and high-pressure MALDI ion sources for FTMS decreased the metastable decomposition and thereby led to improvement. In parallel to this development, the use of infrared (IR) Er:Yag MALDI lasers was reported to have superior performance over UV lasers for FTMS analysis of labile biomolecules. Here we evaluate the effect of performing IR MALDI FTMS under elevated pressure, with the focus on the generation of doubly charged ions, which was observed with high-pressure MALDI FTMS.

The MALDI source of a Apex III FTMS was modified to accommodate both UV and IR laser. A number of previously reported IR MALDI matrices were tested with a set of peptides to evaluate if elevated-pressure IR MALDI would enhance the formation of doubly charged ions. The initial results showed a 3:1 intensity ratio of doubly to singly charged ions for ACTH (18-39). The production of doubly charged ions appears to be matrix-dependent and decreases with increased laser power. Different sample preparations are currently being evaluated.

P12

TITLE

Authors

Affilitation

A new FT ICR MS instrumentation is presented. The Siemens Quantra comes to the FT ICR MS world with a very compact design, flexible installation capabilities and a new application focus. Provided with 1 Tesla permanent magnets, a maintenance free ion getter pump, patented gas inlet valves and online spectra acquisition tools, the Quantra fulfils all requirements not only for laboratory, but also for continuous process use.

Quantra is able to analyse continuously different industrial or pilot plant process streams, calculate concentration data online and show changes in the composition as time vs. concentration charts. Additional information about unexpected components, combined with high resolution capabilities make the Quantra an interesting tool to optimize continuous industrial or laboratory processes.

As examples, applications like headspace analysis of fermentation off-gases and assisting process catalyst development are given. In the first case, the knowledge of fermenter headspace composition can increase fermenter performance by: a) increasing knowledge about the reactions, b) optimizing yield, c) decreasing reaction time d) and preventing ruined batches.

In the second case, the progress of a catalyst-induced reaction was observed under changing feeds. In this particular case, an unexpected component could be identified due to the high mass accuracy of the instrument. In toxic gas reactions, Quantra is helpful in monitoring the reactor shutdown in order to avoid possible contaminations of ambient air.

P13

INFRARED SPECTROSCOPY OF GAS-PHASE MOLECULAR IONS IN AN FTICR MS

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The infrared spectroscopy of gas-phase molecular ions has long been impeded by the low number densities that can be obtained as a consequence of space charge effects. Over the past years we have pioneered a method based on the infrared multiple photon dissociation (IRMPD) spectroscopy of stored ions using the widely tunable IR radiation from a free electron laser (FEL). Initial experiments were performed in a simple rf quadrupole ion trap, where a variety of polyaromatic ions has been investigated in the 5-20 micron wavelength range.

Recently, an FTICR mass spectrometer has been coupled to the FEL beamline, which offers not only a superior mass resolution but in addition many new possibilities to form, mass-select and store “exotic” gas-phase molecular ions. The instrument is based on a home-built 4.7-T superconducting FTICR-MS and a MIDAS data station. The IR radiation is continuously tunable from 3 to 250 micron and has an intensity of typically 60 mJ per 5 microsec pulse. Infrared spectra have among others been obtained for proton bound dimers, metal cation coordination complexes and iron-carbonyl complexes.

P14

ROLE OF SOLVENT SURFACE TENSION IN ELECTROSPRAY-IONIZATION OF PROTEINS

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Although applications of ESI-MS to protein folding studies have developed rapidly, the conformation dependence of protein charge-state distributions (CSDs) is not well understood yet. According to the most accredited model, the observed charge states are limited by the Rayleigh charge of the droplets that generate the gas-phase ions. This hypothesis is based on the observation that the maximum charge state of folded proteins in ESI-MS is between 65% and 110% of the charge calculated by the Rayleigh equation for water droplets of the same radius as the globular protein structure. A testable prediction of this model is that protein CSDs in ESI-MS should respond to changes in the surface tension of the droplets according to the Rayleigh equation. We show that protein CSDs in the presence of low-surface-tension, low-vapor-pressure additives are either the same as in the control samples or present much smaller changes than calculated by the Rayleigh equation. Thus, they do not seem to be limited by the surface tension of the solvent and, rather, appear to be quite protein-specific.

This work was supported by the Austrian Science Foundation (FWF projects T135, P15311 and P13906).

P15

CHARACTERIZATION OF FTICR MASS SPECTROMETRY AT LOW MAGNETIC FIELD

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The qualitative parameters of the FTICR-MS improve with increasing magnetic field [Marshall, A.G.; Guan, S., Rapid. Com. Mass Spectrom. 10 (1996) 1819]. However, in many instances, the larger size of FTICR trap compensates the disadvantages of lower field [Gorshkov, M.V., et al., Eur. J. Mass Spectrom. 8 (2002) 169]. Also, while field strength plays important role in achieving high performance, it is possible to obtain mass resolution and accuracy exceeding the relative field homogeneity. This work focuses on detailed consideration of FTICR-MS characteristics, such as sensitivity, resolution, and mass accuracy at low magnetic fields, as well as instrument design and effect of field homogeneity on these characteristics. The work was supported by Russian Basic Science Foundation, grant 03-04-48228.

P15

OPTIMIZATION OF ECD: MECHANISTIC STUDIES, TOP-DOWN AND BOTTOM-UP APPLICATIONS

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The performance of electron capture dissociation (ECD) FTICR MS was improved by increasing the number of ICR trap loads with ions using gas assisted dynamic ion trapping (GADT). The increased number of ions allowed the investigation of ECD FTICR MS efficiency dependence on the experimental parameters, including electron energy, irradiation time, electron flux, and ion trapping method in the electron energy range 0-50 eV.

Electron kinetic energy distributions and emission currents were measured for ECD and hot ECD. Two electron injection systems were employed: one with injection of a pencil electron beam (disk dispenser cathode) and another with a hollow electron beam (ring dispenser cathode).

ECD with a hollow or pencil electron beam was obtained for both sidekick and GADT FTICR MS with an electrostatic ion transfer line. The experimental results are completed by a theoretical (SIMION) study of ion motion in the ICR trap following sidekick trapping.

FTICR MS with a more sensitive pre-amplifier was used for improved (LC) ECD FTICR MS analysis of post-translationally modified peptides and proteins. The results are compared with CID and IRMPD data.

P17

F.R.I.T.Z. (FOURIER TRANSFORM REACTIVE INVESTIGATION TEST ZOO) AND WHAT TO DO WITH IT

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We have modified a commercial Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Apex III) for the simultaneous use of up to three different ion sources at a time. With F.R.I.T.Z. It is now possible to switch between such sources as Electrospray-Ionization (ESI), Laser Vaporization (LVAP) and very soon also either an Aggregation source without significant delay. F.R.I.T.Z. Combines the high mass resolving power of a 7 Tesla ICR-MS with a variety of ion-sources. This gives us the opportunity to investigate ion molecule reactions as of relevance to many areas of chemistry.

P18

HIGH PERFORMANCE PROTEOME ANALYSIS WITH UV- AND IR-MALDI-FTICR MASS SPECTROMETRY

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Upon sequencing complete genomes, proteome analysis has recently become a focus area of scientific interest. Since complete protein complements of a genome under defined conditions are highly complex, dynamic systems, new analytical methods of high resolution, and selectivity are required. Due to its unrivalled performance in mass determination accuracy and high resolution, FT-ICR mass spectrometry in combination with efficient "soft" ionisation methods such as MALDI has become a powerful tool for this tasks.

In addition to UV-MALDI, a previously installed IR-laser (Bioptic, Berlin) provides advantages in terms of "softer" ionisation, avoiding in-source fragmentation.

Examples cover the analysis of nitropeptides (as nitration is an important pathogenic post-translational modification [1]) with UV- and IR-MALDI, demonstrating that with the IR-laser intact peptides can be desorbed, whereas under UV-conditions fragmantations occur.

Further examples show the general applicability of IR-MALDI FT-ICR mass spectrometry by protein identification from brain tissue after 2-D gel electrophoretic protein separation and tryptic in-gel digest using peptide mass fingerprint.

EN.REFLIST

P19

HIGH MASS ACCURACY MEASUREMENTS USING EXTERNAL CALIBRATION ON A LINEAR TRAP FOURIER TRANSFORM MASS SPECTROMETER

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The combination of a linear ion trap mass spectrometer and a Fourier transform ion cyclotron resonance analyser enables high resolution, accurate mass determinations and MS^n for routine high-throughput analysis. Robustness, reliability and confidence in the results are the key to success for the daily routine work. This is of utmost importance for accurate mass measurements where the Finnigan LTQ FT shows superior performance. The high mass accuracy at external calibration is based on the automatic gain control (AGC) of the ion trap. It is better than 2 ppm RMS over a broad mass range with external calibration which is especially useful for experiments in which addition of an internal standard is detrimental or not possible.

In this contribution we explain how the external calibration is realized and demonstrate the stability and robustness of the external calibration under changing experimental conditions, i.e. positive or negative ion mode, infusion experiments or on-line LC/MS runs, full scan MS or MS^n experiments, singly or highly charged ions, and varying sample concentrations.

P20

TITLE

Authors

Affilitation

Fourier Transform Mass Spectrometry (FTMS) has been used for many years as a technique for exact mass assignment. With the introduction of hybrid instruments using quadrupole or trap devices for Collision Induced Dissociation (CID) in front of the FTMS analyzer, the long-time lack of MS/MS capabilities could be overcome. In modern FTMS instruments, MS as well as MS/MS data sets can be analyzed highly sensitive with mass accuracies in the range of 1 ppm.
Parallel to the development of those hybrid instruments, Electron Capture Dissociation (ECD) was introduced as an alternative MS/MS technique especially for peptide fragmentation. Due to improper electron guns, irradiation times for ECD were previously in the range of several seconds, resulting, unfortunately, in an insufficient sensitivity and analysis rate for real-life applications. Only with the introduction of indirectly heated dispenser cathodes, ECD can now be performed in the ms-time range and is, therefore, now well suited to be coupled to online HPLC. We present an experimental setup of an indirectly heated hollow dispenser ECD cathode, allowing in addition Infrared Multiphoton Dissociation (IRMPD) to be performed with an on-axis IR-laser beam passing through a hole at the centre of the ECD cathode.
As ECD and CID generate fragments of different types (c, z and b, y ions, respectively) and in a different way, complementary information of the structure for the characterization of peptides and proteins can be obtained. ECD is mostly used when information on post-translational modifications are required. Moreover, ECD in combination with an ion pre-activation by IRMPD transforms a high level of energy onto the ions, reflected by the possibility of fragmenting even intact proteins or generating w- or d-ions which are rather typical for high-energy collisions in TOF/TOF or sector field analyzers. Shown will be data illustrating different applications in CID and ECD in particular with HPLC coupling.

P21

TITLE

Authors

Affilitation, Germany

Since the introduction of time of flight mass spectrometers equipped with an API interface the determination of precise mass has been established as a routine job in LC/MS. More and more the calculation of elemental formulas based on accurate mass measurement is an important tool in structure elucidation. Typically a resolution of 5.000-10.000 is sufficient to determine accurate masses with a precision in the range of 1- 10 ppm. However, specific conditions have be kept in mind to obtain mass accuracies in this low ppm range some. One of the most critical point using this type of technology is the optimum numbers of ions. Mass precision significantly decreases if the ion intensity is too high.

Investigating chemicals one often deals with complex mixtures of compounds with unknown structures and different polarities. Within one sample the response factors of different analytes may change dramatically under ESI/APCI conditions. Furthermore, one major demand in industrial analyses is the detection of the main products and all impurities within the same LC run. By this, the main products often result in ion numbers, which are too high for precise mass measurement. Automatic determination of the accurate mass fails and data dependent MS/MS-experiments may be difficult as well in terms of mass accuracy.

To answer this problem, one possibility is the attempt to increase the dynamic range of the detector. Another way to overcome the problem of too much ions has been realized in the LTQ/FT instrument. The combination of an ion trap and a FTMS results in a strong increase in the dynamic range for precise mass measurement if the compounds are separated by HPLC. The poster represents typical data of mass precision obtained on a TOF-MS and a LTQ/FT-MS. The dynamic range of the precise mass obtained with both type of instruments will be discussed in the case of HPLC/MS and direct inlet measurements as well.

POSTER-ABSTRACTS

APPLICATIONS

P22

ANTIBODY PROTEOMICS: ELUCIDATION OF PARATOPE STRUCTURE RECOGNISING A ß-AMYLOID SPECIFIC EPITOPE USING HIGH RESOLUTION FT-ICR MASS SPECTROMETRY

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The elucidation of the paratope structure of an anti-amyloid antibody recognising a ß-amyloid specific epitope has been obtained with the use of an epitope-affinity column. The monoclonal antibody anti Aß1-17 was chosen for the structure recognition due to its high binding affinity to the ß-amyloid peptide in previous experiments. The antibody was first characterised using two-dimensional gel separation followed by high resolution FT-ICR mass spectrometry and peptide mass fingerprint identification. For the paratope elucidation a small peptide (Aß1-30) containing the antibody-specific epitope N-terminal was bound to NHS-activated Sepharose . The antibody was first degraded in solution, and the peptide fragment mixture applied to the column (antibody extraction). After removal of the unbound peptides the affinity bound fragments were eluted from the column and analysed by MALDI-FT-ICR mass spectrometry. After mass fingerprint identification specific peptides from the variable region of the antibody were found, including both heavy and light chains.

P23

HIGH RESOLUTION PROTEOME ANALYSIS OF SURFACTANT PROTEINS A AND D USING FT-ICR MASS SPECTROMETRY

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Surfactant proteins A (SP-A) and D (SP-D) belong to the collagenous lectin group of proteins. They are important host defence components of the lung. These multimeric collectin mediate the clearance of pathogens and modulate immune cell functions via C-terminal carbohydrate recognition domain (CRD) [1-2]. FT-ICR MS has been recently developed as a most powerful mass spectrometric tool in proteome analysis, depending on its high mass resolution and mass accuracy [3-5]. In the present study, one- and two-dimensional gel electrophoresis combined with high resolution FT-ICR-MS have been applied as a powerful approaches for the proteome analysis of surfactant proteins SP-A and SP-D, including identification of structurally modification (hydroxy-proline). The high resolution mass spectrometric proteome analysis should facilitate the identification of subunits, aggregations and modification of surfactant proteins and hence contribute to understanding the mechanistic basis of lung disease pathogenesis.

P24

DETERMINATION OF ELEMENT CONCENTRATIONS BY LA-ICP-MS OF ALZHEIMER'S DISEASE BRAIN PROTEINS COMBINED WITH PROTEIN IDENTIFICATION BY HIGH RESOLUTION FT-ICR-MS

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The neurofibrillary, microtubule associated protein tau has recently gained increasing interest as a target protein for Alzheimer´s disease. A novel combination of molecular and atomic mass spectrometry using FT-ICR-MS and LA-ICP-MS are proving highly efficient tools for determination of protein structures, proteome analysis, phosphorylation and concentrations of P and metals. Phosphorus, sulfur, silicon and metal concentrations (Al, Cu and Zn) were determined in human brain proteins by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) after separation of protein mixtures by 2-D gel electrophoresis. MALDI-FTICR-MS was applied to proteome analysis, yielding the identification of several brain proteins from gel spots, and efficient methods have been developed for the identification of phosphorylation sites using high resolution MS data. Results of structure analysis of human brain proteins by MALDI-FTICR-MS were combined with those of the direct determination of phosphorous, sulfur, silicon and metal concentrations in protein spots by LA-ICP-MS.

[1] J.S. Becker, M. Zoriy, J. Su. Becker, C. Pickhardt, M. Przybylski, J. Anal. At. Spectrom. 2004, 19, 149.

P25

IN GEL SCREENING OF P, CU, ZN AND FE IN YEAST MITOCHONDRIA BY LA-ICP-MS AND IDENTIFICATION PROTEIN STRUCTURES BY MALDI-FTICR-MS AFTER SEPARATION BY TWO-DIMENSIONAL GEL ELECTROPHORSIS

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A new screening technique using two-dimensional gels was developed in order to rapidly identify various elements in well-separated protein spots. Yeast mitochondrial membrane proteins were separated using two-dimensional gel electrophoresis (blue native/ SDS 2D-PAGE) and marked by silver staining. The 2-D gels were systematically analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) using a double-focusing sector field instrument. From more than 60 protein spots of mitochondria in two-dimensional gels phosphorus, those proteins that contained sulfur and selected metals (Cu, Zn and Fe) were detected in a short analysis time by screening 2-D gel by LA-ICP-MS using a focused laser beam. In selected protein spots a quantitative element determination was performed.

MALDI-FTICR-MS was applied for structure analysis and identification of proteins. Results of the structure analysis of separated proteins in mitochondria by MALDI-FTICR-MS were combined with those of the direct determination of phosphorus, sulfur and metal concentrations with LA-ICP-MS.

P26

REACTION MECHANISMS OF HYDRATED ELECTRONS (H2O)N- IN THE GAS PHASE

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The reactivity of hydrated electrons (H2O)n- with small molecules is studied by Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. The ionic nanodroplets are generated by laser vaporization of a solid zinc target and supersonic expansion of the hot plasma into high vacuum in a helium/water mixture. In bulk solution, hydrated electrons are short lived due to a variety of recombination processes, e.g. with protons to form hydrogen atoms. In the nanodroplet, no species are available for recombination. If the droplet is sufficiently large, i.e. n>30, hydrated electrons are stable on the time scale of seconds. The only decay channel is loss of individual water molecules due to heating by black body radiation, while smaller species may also exhibit electron detachment. In reactions with small molecules, one can classify at least three different reaction mechanisms:

a) Ligand exchange, where e.g. a methanol molecule replaces water

b) core switching, where a radical anion is formed, e.g. O2- or CO2-

c) chemical reactions, where e.g. a hydrogen atom is transferred to acetonitrile, forming CH3CHN and OH(H2O)n-

P27

EPITOPE EXTRACTION TECHNIQUE USING PROTEOLYTIC MICROREACTOR COMBINED WITH FTICR-MS AS TOOL FOR HIGH-THROUGHPUT SCREENING OF POTENTIAL VACCINE LEAD PROTEINS

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The scope of this study was to apply highly reactive and specific enzyme microreactors which enable complete and specific substrate fragmentation. As a model polypeptide the ß-amyloid-peptide Aß(1-40) was utilized. The choice of the matrix (chemical and magnetic properties, particle size and distribution, porosity etc.) was a key factor influencing the quality of the enzyme microreactor. The alginate coated ferrite microparticles with immobilized TPCK-trypsin (0.095mg of Trp/mg of particles) provided the best working efficiency with zero non-specific sorption; the digestion times were significantly reduced (from 3 hours to 5 minutes), and sensitivity and specificity of fragmentation was confirmed by FTICR-MS analysis. For specific isolation and identification of the main immunogenic peptides the sepharose microreactor with anti-amyloid-ß-protein monoclonal antibodies, or immunoaffinity microreactors were utilized. In this mode the epitope excision and epitope extraction technique coupled with FTICR-MS analysis is shown to be an excellent basis for high-throughput screening of potential vaccine lead proteins.

P28

CHARACTERIZATION OF TRANSITION METAL-CARBIDO SPECIES: ASSEMBLY AND DECOMPOSITION OF THE NEW CARBON-CENTERED TUNGSTEN CLUSTER [CW6CL18]N- (N=1,2)

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Transition metal-carbon clusters have been of interest as models for intermediates in heterogeneous catalysis and as fragments of refractory metal carbides. Recently Zheng et al. and Welch et al. reported on the synthesis of some new carbon-centered trigonal prismatic tungsten clusters [CW6Cl18]n- (n=1,2,3). [1], [2], [3] In our studies we performed MS/MS experiments by using Sustained Off Resonance Induced Collision Activated Dissociation (SORI-CAD). Observing the stepwise decomposition, we were able to isolate each fragment and to follow its further decomposition. In addition we obtained some structural informations from DFT-calculations. Finally we proposed a mechanism for the formation of [CW6Cl18]n- by applying retro synthesis.

[1] Zheng, Y.-Q.; von Schnering, H. G.; Chang, J.-H.; Grin, Y.; Engelhardt, G.; Heckmann, G. Z. Anorg. Allg. Chem. 2003, 629, 1256.

[2] Welch, E. J.; Crawford, N. R. M.; Bergman, R. G.; Long, J. R.; J. Am. Chem. Soc. 2003, 125, 11464-11465.

[3] The studies has been initiated by H. G. v. Schnering and the samples has been provided by Prof. H.-J. Meyer, University of Tübingen, 72076 Tübingen.

P29

EPI-TAG: SELECTIVE CARBOXY-TERMINAL LABELLING OF EPITOPE PEPTIDES IN PROTEINS BY PROTEOLYTIC ^{18}O-INCORPORATION AND FT-ICR MASS SPECTROMETRY

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The determination of molecular recognition structures (epitopes) in peptide and protein antigens by selective proteolytic degradation of immune complexes (epitope excision), followed by dissociation and mass spectrometric analysis has been established as a powerful tool for molecular epitope mapping in our laboratory. A specific N-terminal epitope of the neurotoxic ß-amyloid peptide fragment, Aß(1-42), the major plaque-forming product of Alzheimer's (AD) ß-amyloid precursor protein (APP) was recently identified from therapeutic antibodies produced upon Aß-immunisation of a transgenic AD mouse model (J. McLaurin et al., Nature Med. 2002). We report here the selective tagging and proteolytic cleavage analysis of C-terminal groups in epitope peptides by ^{18}O-incorporation and FT-ICR-MS ("EPI-TAG").This isotopic labelling technique involves the incorporation of ^{18}O into the alpha-carboxy groups liberated during enzyme-catalysed hydrolysis of epitope-containing peptides and proteins, followed by mass spectrometry to identify the C-terminal epitope peptides. A series of standard proteins and amyloid peptides were digested in normal and highly ^{18}O-enriched water using serine proteases and other proteolytic peptides employed for protein structure determination, and the extent of ^{18}O incorporation into the resulting peptide fragments characterized by ESI and MALDI-FTICR-MS. Using trypsin as protease, the incorporation of two ^{18}O atoms was found specifically for Arg-peptides, resulting in a mass shift of +4 amu, in contrast to only one ^{18}O incorporated in Lys-peptides. The reason for this difference will be discussed with regard to the proteolytic mechanism of trypsin.

P30

BINDING OF MODEL BASES TO IRON PORPHYRINS IN THE GAS PHASE

Authors

Affilitation

A vast number of metalloproteins performing vital functions is endowed with iron-protoporphyrins-IX (heme) as the prosthetic group. The gas phase chemistry of Fe(III)heme$^+$ and Fe(II)-hemeH$^+$ ions with neutrals selected among model or natural ligands has been investigated to provide an understanding of the intrinsic factors responsible for the binding to the heme group. Electrosprayed heme$^+$ were transferred and reacted with neutrals into the cell of a Bruker BioApex 4.7T FT-ICR mass spectrometer at 300K. The kinetics for the radiative association reaction as well as the ligand exchange equilibria have been studied and reported. In the presence of MeOH, stationary concentrations of heme$^+$ and hemeL$^+$ were established and an association equilibrium was attained. The so derived $\Delta G1°$ value provided an anchor to obtain a list of heme$^+$ cation basicity (HCB) for the various ligands. In turn HCB's correlate with the proton basicity of the ligands. Both heme$^+$ and hemeH$^+$ species are very reactive in binding to NO in spite of its low proton basicity. The efficiencies for the association reaction were found to correlate with the increasing $\Delta G°1$ values.

P31

ELUCIDATION OF PHOSPHORYLATION STRUCTURE IN HUMAN CHROMATIN PROTEIN DEK BY NANO-ELECTROSPRAY MASS SPECTROMETRY

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DEK protein is phosphorylated by CK2 in vitro and in vivo and its role must have consequences of physiological importance. DEK in its dephosphorylated form, is involved in the binding of phosphorylated DEK to chromatin in vivo . The phosphopeptides tend to loose the phosphor moiety upon low-energy collisional activation in a tandem mass spectrometric experiment, which serves as a signature for a phosphopeptide or a phosphate containing peptide. The advanced scan modes of neutral loss, precursor ion scans combined with information-dependent acquisition experiment were used for scanning phosphorylated peptides and identification of phosphorylation sites in DEK protein. The phosphorylation sites identified by nano-ESI-QTrap MS in combination with nano-ESI-FTICR-MS, and are located in the C-terminus of DEK protein, region involved in DEK-DEK interactions (di-or multimerization). We demonstrate how the properties of nano-ESI-QTrap MS such as neutral loss and precursor ion scan can be used to address problems involving the determination of phosphorylation sites in DEK protein. The quantification of phosphorylation level was done by ICP-MS.

P32

HIGH RESOLUTION PROTEOME ANALYSIS OF SPECIFIC HUMAN BRAIN REGIONS USING MALDI-FTICR MASS SPECTROMETRY

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The protein expression levels in specific human brain regions were investigated using a combination of 2-DE and high resolution FT-ICR-MS. Proteins from six different brain regions (somatomotor cortex, medial prefrontal cortex, parahipocampal cortex, cerebellar cortex, putamen and ventral thalamic nuclei) were separated by 2-DE and the 2-D proteome maps were compared using PDQuest software (Bio-Rad). Gel comparison revealed the presence of several minor, probably allelic, differences but also of several major differences. Several proteins found on/off or up/down regulated in specific brain regions were unambiguously identified by FT-ICR mass spectrometry (e.g. glial fibrillary acidic protein, GFAP which is a marker for neuronal decay and brain damage) and quantified using PDQuest software. Of all mass spectrometric techniques currently used in proteomics, FT-ICR-MS is by far the method providing the highest mass determination accuracy. In most cases, the accurate mass determination allows the setting of low tolerance thresholds during database searches which greatly improve the selectivity of protein identification with a minimum number of proteolytic peptides.

P33

CREATINE KINASE NON COVALENT INTERACTIONS WITH VARIOUS LIGANDS STUDIED BY FT-ICR MASS SPECTROMETRY

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Mammalian creatine kinase (CK) is an 86 kDa homodimeric enzyme. It catalyzes the following equilibrium, involved in the energetic balance of muscle cells:

ADP + phosphocreatine <-> ATP + creatine

Electrospray ionization in non-denaturating conditions (10 mM ammonium bicarbonate) yielded the intact dimer. Addition of the ligands in various concentrations led to the formation of complexes, which were detected in the gas phase. Interaction between a high molecular weight protein and a small ligand leads to a small mass shift and therefore requires the mass accuracy afforded by an FT-ICR mass spectrometer. The specificity of the interaction observed after ESI was assessed by these titration experiments. In the case of ADP, a dissociation constant of 0.008 mM was determined in the absence of any metal cation. Biochemichal titration experiments, performed in the presence of various divalent metal cations, measured a value between 0.11 mM and 0.06 mM [1]. The influence of the cation on the binding constant is discussed in this work. The methods set up for this model study will be applied to other multimeric kinases.

[1] D.C. Watts, in The Enzymes, Vol. 8, P. D. Boyer Ed., 383 (1973).

P34

INVESTIGATION OF THE 'ROGUE' ATOM IN OCTAHAEM CYTOCHROME C BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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Developments in soft ionisation techniques have enabled the gas-phase study of increasingly larger biological molecules. Isotopic resolution of proteins in excess of 100 kDa has been achieved by FT-ICR MS, leading to determination of accurate mass. Haem containing proteins have a vital biological role as they are involved in such functions as oxygen transport, reduction of peroxides and nitrates, dehydrogenation. The octahaem cytochrome C was identified from the genome of the bacterium Shewanella oneidensis MR-1. Its primary sequence codes for 461 AA and has a molecular size of ~ 51.4 kDa. Its crystal structure has been solved and it indicates an unidentified atom proximal to a cysteine residue. The mass obtained using FT-ICR MS is ~ 62 ± 1 amu higher than predicted from the sequence. The identity of the 'rogue' atom is probed by several gas-phase dissociation techniques. ESI-FT-ICR mass spectra indicate that each of the eight irons in the haem groups is present in its highest oxidation state, i.e. Fe3+. Furthermore, the lowest observable charge state is +26, which corresponds to a charge contribution from iron of +24.

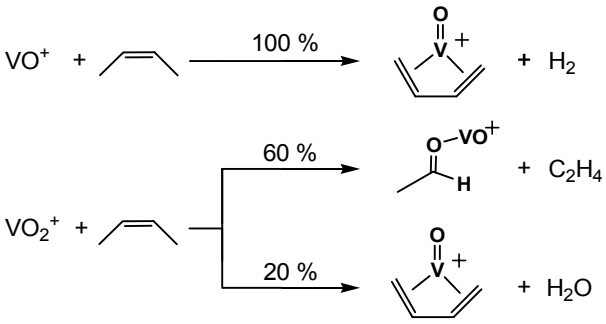
P35

GAS-PHASE OXIDATION OF ALKENES BY SMALL CATIONIC VANADIUM OXIDES

Authors

Affilitation, Germany

Vanadium oxides are among today's most important transition-metal based catalysts for the selective oxidation of propane and butane. Yet, the extremely complex reaction mechanisms are still not understood. Answering the question of what role "isolated" vanadium-oxygen units may play therein can be aided by gas-phase studies. FTICR-MS is used as a particularly powerful tool to investigate the ion/molecule reactions of the two smallest cationic vanadium-oxides VO^+ and VO_2^+ with hydrocarbons under single-collision conditions. Besides information gained about the energetics and kinetics of the observed reactions, the structures of the ionic products are deduced via MS/MS-experiments and selective deuterium-labeling. The study of alkenes presented here is an extension of our earlier work on alkane oxidation.



The oxidation state of the metal greatly influences the ions' reactivity towards hydrocarbons. VO^+ dehydrogenates alkanes and alkenes as does V^+, no oxygen transfer reactions occur. The oxygen atom acts like a spectator ligand which decreases reactivity and enhances selectivity. In contrast, VO_2^+ is a strong oxidizing agent and permits oxidative dehydrogenation, oxygen transfer and the scission of C-C bonds. Interestingly, the total cleavage of the double bond is a strongly favored reaction pathway. In most cases, the reactions of VO_2^+ involve the reduction of the metal to V^{III}.

P36

TITLE

Authors

Affilitationy

In a 5 sections cylindrical ICR-cell the chemistry of Tris(trifluoromethyl)phosphine in negative mode has been investigated. Thermal Electron attachment produces a phosphide $(CF_3)_2P^-$ which reacts with the neutral molecules under $3x10^{-7}$ mbar to produce two phosphoranides: $(CF_3)_2PF_2^-$ and $(CF_3)_3PF^-$. This is accompanied with the appearance of CF_3^-, F^-, and $C_2F_3^-$ ions when the phosphide has been accelerated. Only CF_3^- and F^- have shown to contribute to the production of the two phosphoranides via the phosphide $(CF_3)_2P^-$. Differences in behaviour between the two Phosphoranides regarding their Ion-Molecule reactions with the neutral molecules were also investigated.

P37

REACTION OF C+ (^{12}C+ AND ^{13}C+) IONS WITH POLYCYCLIC AROMATIC HYDROCARBONS

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Reactions of the C+ ions (^{12}C+ and ^{13}C+) with polycyclic aromatic hydrocarbons (PAHs), studied using Fourier Transform ion cyclotron resonance mass spectrometry (FTICR), provide evidence for the formation of large PAHs. Product ions consist of adducts formed by association accompanied by the loss of a hydrogen atom. The relevance of the association reaction channel increases with the size of PAHs. Starting from a given distribution and following the temporal evolution it was possible to obtain useful information on how the distribution changes or by comparing the obtained distribution with our observed one deduce the "original" distribution before distortion in the interstellar medium. Two initial distributions have been used: Gaussian and exponential. This last is the most probable distribution, because it is similar to the interstellar grain size distribution.

P38

REDUCTIVE NITRILE COUPLING IN NIOBIUM-ACETONITRILE COMPLEXES PROBED BY FREE ELECTRON LASER IR MULTIPHOTON DISSOCIATION SPECTROSCOPY AND FT-ICR-MS

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Experimental IR multiphoton dissociation spectra of metal containing ion-molecule complexes with the formal stoichiometry $[Nb,nCH_3CN]^+$, n=4-5, are compared to ab initio IR absorption spectra throughout the spectral "fingerprinting" range 780-2500 cm^{-1}, as provided by the Free Electron Laser at Clio, Orsay, France. The vibrational spectra provide clear evidence for a square planar high spin quintet $[Nb^I(NCCH_3)_4]^+$ complex. For n=5 additional vibrational bands between 1300-1600 cm^{-1} are interpreted in terms of C-C coupling in $[Nb,5CH_3CN]^+$. Screening on the basis of ab initio calculations leads to an assignment of the recorded spectrum in terms of a metallacyclic species $[Nb^{III}(NCCH_3)_3(N=C(CH_3)C(CH_3)=N)]^+$ with an electronic triplet state. The observed processes upon fourfold and fivefold coordination of Nb^I with CH_3CN in the gas-phase are complexation only and reductive nitrile coupling respectively. The minimum energy pathways of the reductive nitrile coupling reaction from $[Nb^I(NCCH_3)_n]^+$, n=4-5, investigated for singlet, triplet and quintet states (S=0,1,2) by density functional theory, account well for the observed (non-) reactivity. In ground state (triplet, S=1) $[Nb^I(NCCH_3)_5]^+$, the reaction is found to be exothermic and the activation barrier amounts to approximately 49 kJ mol^{-1}, whereas for ground state quintet (S=2) $[Nb^I(NCCH_3)_4]^+$ the reaction is endothermic and requires activation of >>116 kJ mol^{-1}. Close correspondence of IR-MPD and IR absorption spectra is discussed in terms of the underlying mechanism.

P39

ENCAPSULATED GUEST MOLECULES IN DIMERS OF OCTAHYDROXYPYRIDINE[4]ARENES

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The crystal structure of the pyridine[4]arenes shows head-head dimers attracted by hydrogen bonding. These dimers form a cavity which may include molecules or ions if the guest fits into the cavity. Those guest incorporations can be observed by ESI FT mass spectrometry: Beside the most abundant pseudomolecular ion [C+H]+, also the gasphase dimer [C•C+H]+ of the calixarene C is observed. The addition of small amounts of a guest results in the incorporation into the cavity of the dimer. There is only room for small variations. We find incorporation of guests like formic acid, acetic acid, d4-acetic acid, trifluoro acetic acid, propionic acid, acetamide and trifluoro acetamide. Guests that are more bulky than propionic acid are not incorporated any more.

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P40

"AFFINITY-PROTEOMICS": DIRECT PROTEIN IDENTIFICATION FROM BIOLOGICAL MATERIAL USING MASS SPECTROMETRIC EPITOPE MAPPING

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We describe here a new approach for the identification of affinity-bound proteins by proteolytic generation and mass spectrometric analysis of its antibody bound epitope peptides (epitope excision). The cardiac muscle protein troponin T was chosen as a protein antigen due to its diagnostic importance in myocardial infarct and its previously characterised epitope structure. Two monoclonal antibodies (IgG1-1B10 and IgG1-11.7) raised against intact human troponin T were found completely cross reactive with bovine heart troponin T. A combination of immuno-affinity isolation, partial proteolytic degradation (epitope excision), mass spectrometric peptide mapping, and database analysis was used for the direct identification of Tn-T from bovine heart cell lysate. The peptide masses identified by mass spectrometry were used to perform an automated database search, combined with a search for a common "epitope motif". This procedure resulted in the unequivocal identification of the protein from biological material with only a minimum number of peptide masses, and requiring only limited mass determination accuracy.

P41

SYNTHESIS, STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF CONJUGATES CONTAINING A ß-AMYLOID PLAQUE SPECIFIC EPITOPE

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One possible approach to treat or prevent Alzheimer's disease (AD) is immunotherapy and the use of nonfibrillar/nontoxic ß-amyloid homologous peptides might be a safer vaccination approach in humans. Based on the identification of Aß4-10 as the predominant B-cell epitope recognised by therapeutically active antisera from transgenic AD mice, we synthesized and characterized conjugates containing the Aß4-10 epitope that could be used as possible vaccines. In order to produce immunogenic constructs, the Aß4-10 epitope elongated by Cys residue was attached to a chloroacetylated tetratuftsin derivative as a carrier via thioether linkage either directly, or by insertion of a spacer moiety and the conjugation reaction was followed by HPLC and MS. Another group of conjugates containing an amide bond between the carrier and the epitope were obtained by stepwise synthesis. Structures and molecular homogeneity of all peptide-conjugates were ascertained by HPLC, MALDI and ESI-FT-ICR MS. The secondary structure of compounds was studied in water and in TFE by CD spectroscopy. The binding properties of the conjugates with a specific monoclonal antibody were determined by a direct ELISA.

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MASS SPECTROMETRIC CHARACTERISATION OF THE LECTIN-LIKE DOMAIN OF HUMAN TNF-ALPHA

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Tumour Necrosis Factor-alpha (TNF-alpha) is a protein secreted by activated macrophages and lymphocytes which induces hemorrhagic necrosis of certain tumours in vivo, initiated by binding to high-affinity receptors. It also has a lectin-like affinity of unknown molecular specificity and interacts with certain parasites, e.g. inhibiting the development of Trypanosomes. The trypanolytic and lectin-like affinity is mediated by the a TNF-alpha domain, called TIP-domain, which is functionally and spatially distinct from the receptor binding sites[1]. In our study, we synthesised different linear and cyclic TIP-peptides and investigated their proteolytic stability in several biological media like serum amd plasma samples, showing that these peptides are extremly stable against proeolytic digestion. Using ESI-FTICR mass spectrometry, we examined the interaction of these peptides with carbohydrates like chitobiose and cellubiose.

[1] Lucas, R., S. Magez, R. De Leys, L. Fransen, J.P. Scheerlinck, M. Rampelberg, E. Sablon, and P. De Baetselier, Mapping the lectin-like activity of tumor necrosis factor. Science, 1994. 263(5148): 814-7.

P43

SPECIFIC NITRATION AT TYROSINE-430 OF BOVINE PROSTACYCLIN SYNTHASE REVEALED BY HIGH RESOLUTION FT-ICR MASS SPECTROMETRY

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Prostacyclin is formed via isomerization of PGH_2 by PGI_2-synthase in vascular endothelial cells and vascular smooth muscle cells. Prostacyciln synthase, with nitric oxide, is an essential factor for a functional endothelium by providing anti-aggregatory, anti-proliferative and vasorelaxing properties to the vessel wall. Peroxynitrite generated from nitric oxide (NO) and superoxide ($.O_2^-$) can react with Tyr or Tyr- containing proteins under formation of 3-nitrotyrosine.

Following nitration of bovine aortic microsomes with 25 μM PN, PGI_2-synthase was isolated by gel elecrophoresis and subjected to proteolytic digestion. Digestion with thermolysin for 24 h provided a single specific peptide which was isolated by HPLC and identified as PGI_2-(427-430), nitrated at the tyrosine-430 residue. The structure of this tetrapeptide, LKNY(3-nitro), was established by precise mass determination using ESI-FT-ICR mass spectrometry. As a control the authentic nitrated tetrapeptide and the fragment 419-432 were synthesized using SPPS-Fmoc synthesis and the correct structures were established by MS. Complete digestion by pronase to 3-nitrotyrosine was obtained after 72 h, suggesting that the nitrated Tyr residue is embedded in a tight fold around the hem binding site.

[1] Schmidt P., Youhnovski N., Daiber A., Balan A., Przybylski M., Ullrich V., *J. Biol. Chem.,* **278** (2003) 15, 12813-12819.

P44

COMPLEXATION OF ZNII IONS BY L-CARNOSINE IN THE PRESENCE OF WATER

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Combined FT-ICR-MS and correlated ab initio studies of the interactions of Zn(II) ions with L-carnosine (ß-alanyl-L-histidine) in the presence of water have revealed two different competing complexation modes:
Zn(II) is either complexed internally within a binding pocket or externally at the surface of the carnosine ligand, the latter structures being stabilized by hydration.
Evidence is provided for the occurrence in bulk solution of both forms.

P45

GAS PHASE ION CHEMISTRY OF POLYPHOSPHATE ANIONS. THE $H_5P_2O_8^-$ IONS

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The hydrolytic cleavage of phosphate esters bonds is an essential and ubiquitous reaction in biological systems. Over the last decades the determination of the dominant mechanism of phosphate hydrolysis in aqueous solution has been the subject of an enormous experimental effort but, despite that, a considerable uncertainty still remains about the actual mechanism. Previous mass spectrometric and theoretical calculations [1] allowed us to characterise the $H_3P_2O_7^-$ ions as having the diphosphate anion connectivity. In this work, we report the preparation and structural characterization of the $H_5P_2O_8^-$ ions obtained from CH_4/CI of gaseous diphosphoric acid and water and from electrospray ionization of a $CH_3CN/H_2O/H_4P_2O_7$ solution. The joint application of Triple Quadrupole (TQ) and Fourier Transform Ion Cyclotron (FTICR) mass spectrometric techniques and theoretical calculations performed at the B3LYP/6-31G* level of theory allowed to get insight into the gas phase ion chemistry of $H_5P_2O_8^-$ ions and the hydrolysis mechanism of diphosphate ion.

[1] Pepi, F.; Ricci*, A.; Rosi, M.; Di Stefano, M.; Chem.Eur.J., 2004, in press.

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P47

SIMULTANEOUS DETERMINATION OF THE DIFFERENT BILE ACIDS IN BILE AND SERUM OF ALPHA-METHYLACYL-COA RACEMASE-DEFICIENT MICE BY ESI-FT-ICR-MS

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The enzyme α-methylacyl-CoA racemase (AMACR) is required for the degradation of branched-chain fatty acids and is also involved in the synthesis of bile acids from cholesterol. The inherited deficiency of the enzyme in humans causes adult-onset sensory and motor neuropathy. To study the physiological role of the enzyme, we have generated an AMACR-deficient mouse strain.
Bile acid composition of bile and serum from knock-out animals and from their normal litter-

mates was quantitatively analyzed by mass spectrometry. The extremely high resolution of FT-ICR-MS allowed the simultaneous determination of all relevant compounds without prior chromatographic separation. In the bile of enzyme-deficient animals, total concentration of mature (C24) bile acids was reduced to approx. half the normal content, while the C27 precursors, which are barely detectable in normal controls, made up for most of the difference. Similar changes were found in serum and in liver tissue.
The presence of significant amounts of normal bile acids, in spite of the block of an essential step indicates the operation of an alternative pathway, with lower capacity.

P48

CLEAVAGE SITE IDENTIFICATION OF A NOVEL HEAT SHOCK PROTEASE ON AMYLOID TARGET PEPTIDES USING HIGH RESOLUTION MASS SPECTROMETRY

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A secreted human protease HtrA1 is likely to be involved in the degradation of extracellular matrix proteins which could be important for arthritis, tumor progression and age-related diseases. There appears to be a preference for valine or isoleucine as residue preceding the clevage site, although this is not the sole cleavage site determinant. For the analysis of the proteolytic cleavage specificity of the HtrA1 protease, a 108 amino acids peptide fragment containing the fragment 671-770 of the amyloid precursor protein and a carboxy-terminal histidine-tag (C99) with the total molecular weight of 12336 Da was expressed in E.coli.

After characterisation of the C99 polypeptide by FT-ICR mass spectrometry the peptide was digested with human Htra1 at 50º C. Aliquots were taken during 72 hours and analysed by MALDI-TOF and MALDI-FT-ICR. The cleavage specificity was also analysed using the fragment 724-770 of the amyloid precursor protein which was synthesised according to Fmoc Solid Phase Peptide Synthesis strategy. The results suggest a possible cleavage pattern of human HtrA1, which might help to understand whether the enzyme plays an important role in age-related diseases.

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FT-ICR mass spectrometry offers the possibility for the investigation of both dissociation and ion-molecule reactions in the gas phase. This unique advantage helps to find an answer to two fundamental questions concerning the metal-metal bonding.

First, binary, bare metal clusters $M_mM'_n$ are subject of great interest since they exhibit interesting electronical and structural properties. The investigation of the metalloid cluster $Ga_{19}R_6$ with FT-ICR mass spectrometry using LDI as ionisation method results in a variety of bare metal clusters containing only gallium and silicon atoms, $Ga_nSi_m^-$ (n = 5...43, m = 1...3). The silicon atoms deriving from the ligand sphere of the metalloid cluster. SORI-CAD experiments on the one hand and quantum chemical calculations on the other hand give insights into the structural and electronical properties of those clusters. The results of which show the similarities and differences to another group of binary clusters containing atoms from both group 3 and group 4, the well known carboranes as e. g. $B_{10}C_2$..

Secondly, multiple bonding between main group metals still is an open question in inorganic chemistry. The investigation of the tetrahedral $Al_4Cp^*_4$ Cluster with FT-ICR mass spectrometry using LDI as ionisation method results in a couple of aluminium clusters, that all can be interpreted as fragments of the metalloid cluster $Al_8Cp^*_4$. Some of those fragments react with Cl_2 in the gas phase. The investigation of one of those reactions $Al_2Cp^{*+} + Cl_2 \rightarrow AlClCp^{*+} + AlCl$ together with quantum chemical calculations gives a new approach to the question whether or not an aluminium-aluminium double bond exists.

P50

HIGH RESOLUTION ESI-FT-ICR-MS STUDY ON GAS-PHASE KINETICS OF SQUARE-PLANAR PLATINUM-AMMONIA COMPLEXES

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Platinum coordination compounds serve as active anticancer drugs, most notably cis-dichlorodiammineplatinum(II), which is also known as cis-platin. The corresponding trans-isomer does not show anticancer activity. Therefore the investigation of differences in the chemical behaviour of cis- and trans-isomers of square-planar platinum-ammonia complexes is of high interest [1].

ESI-FT-ICR-MS of cis- and trans-platin solvolysis products reveals the unexpected coordination complexes cis- and trans-$[Pt(NH_3)_2Cl(N_2)]^+$, which originate from endoergic gas-phase ligand exchange of H_2O against N_2 in the monoaqua complex $[Pt(NH_3)_2Cl(H_2O)]^+$. Gas-phase reactions of $[Pt(NH_3)_2Cl(N_2)]^+$ and $[Pt(NH_3)_2Cl(H_2O)]^+$ and of further cis- and trans-platin derivatives with gaseous ammonia are recorded and the rate constants of the ligand exchange reactions of N_2 and H_2O against NH_3 are determined.

[1] Cisplatin, Chemistry and Biochemistry of a Leading Anticancer Drug, edited by Bernhard Lippert, VHCA Zürich / Wiley-VCH Weinheim, 1999.

AUTORENVERZEICHNIS

Zuleta, I.A. L34