





Structural Mass Spectrometry: from theory to practice

XXV IUCr Satellite Workshop: Methods of Structural Biology Workshop CMS - Vestec – 13/08/2021



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Top-down MS



Native MS

Ion Mobility MS





Concept Timeline Output / Bench Perspectives Conclusion

Cross-linking MS



HDX-MS



Native MS and Ion Mobility: Concept





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Concept: non covalent interactions are maintained in the gas-phase

- Use of MS compatible buffer
- Transfer of high-MW species
- Balance between transmission and dissociation









Sharon and Robinson, 2007 Annu Rev Biochem





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Marcoux and Robinson, 2013 Structure



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Marcoux and Robinson, 2013 Structure

Zhou et al, 2011 Science Schmidt et al, 2013 Nature Communications

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Marcoux and Robinson, 2013 Structure

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Rose et al, 2013 Nature Methods

Marcoux et al, 2015 Protein Science





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Ultra High Mass Range Q-Exactive Orbitrap





Output:

• Oligomeric state & subcomplexes





Output:

Oligomeric state & subcomplexes







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Output:

- Oligomeric state & subcomplexes
- Ion mobility and modeling





Hall et al, Structure 2012





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Output:

- Oligomeric state & subcomplexes
- Ion mobility and modeling

• Dynamics







Output:

- Oligomeric state & subcomplexes
- Ion mobility and modeling
- Dynamics
- Ligand binding

Small molecules, lipids, sugars, peptides, DNA, RNA...

 β -lactoglobulin interacting with different fatty acids









1.0

0.8

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Output:

- **Oligomeric state & subcomplexes**
- Ion mobility and modeling
- **Dynamics**





Ion mobility Gas-phase separation of conformers

Spectral cleaning Identification of conformers Collisional cross-sections Colision Induced Unfolding

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Equilibrium titration: 0.25 to 1.6 equivalents of ligand

Timecourse 0 to 15 min



Hyung et al, 2009 Chem Biol



Output:

- Oligomeric state & subcomplexes
- Ion mobility and modeling
- Dynamics
- Ligand binding
- Colision Induced Unfolding





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Native MS and Ion Mobility: Bench

Challenges and Modifications / sample preparation

Need of a volatile buffer, most of the time ammonium acetate 20 mM to 1 M / pH 5 to 9 equilibrated with formic acid or ammonium hydroxide Any kind of buffer exchange: membrane concentrators, SEC (benchtop or FPLC), dyalisis...



Native MS and Ion Mobility: Perspectives^{27/49}

Native MS of Recombinant Proteins from Crude Cell Lysates



Native MS and Ion Mobility: Bench

Nano ESI: Lower sample consumption (2 μl at ~1-100 μM/needle)
 Better sensitivity (ionization efficiency)
 Better salt tolerance/desolvation (less adducts)
 Room temperature, no solvent necessary

Borosilicate micro-pipettes or "needles" pulled manually and coated with gold





Native MS and Ion Mobility: Perspectives^{29/49}

Alternatives to CID: Surface Induced Dissociation

Parent ions are targeted at a surface and daughter ions are analysed

- Transfer of high average internal energy (significant portion of the incident kinetic energy) / fast (ps) single step activation process
- Especially useful for big ions, hard to fragment in CID



SID mode



Zhou et al, 2013 Anal Chem

Native MS and Ion Mobility: Perspectives ^{30/49}

Protein assemblies ejected directly from native membranes



Chorev et al, 2018 Science

Top-Down MS: Concept



Concept: MSMS analysis of entire proteins

- High resolution Orbitraps
- CID, ETD, ECD, EThcD, SID, IRMPD...



Carel et al, 2017 PNAS



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Top-down MS: Timeline







Proteoform: different molecular forms of a gene, including genetic variations, alternative

splicing and PTMs

MPC: Multi-Proteoform Complex



Smith and Kelleher 2013 Nature Methods

Skinner et al, 2016 Nature Methods







Tran et al, 2011 Nature

Ntai et al, 2014 Anal Chem Durbin et al, 2016 JPR

Top-down MS: Output / Bench



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Gersh et al, 2015 Chemistry & Biology

Top-down MS: Output / Bench

Proteome Discoverer (Prosight PD Top Down nodes) Different search modes: Three-Tier Search Absolute Mass Search (100 Da error window) **Biomarker Search**

Absolute Mass Search (1000 Da error window)

Sequence coverage/Scoring in Prosight Lite

Ν		Α	E	R	G	Y	S	F	S	L	Т	Т	F	S	Ρ	S	G	K	L	V	Q	1	E	Y	Α	L	25	Precursor Mass Type: Average Observed: 25,809.79 Theoretical: 25,809.43
2	6	A	Α	V	A	G	G	Α	Ρ	S	V	G	I	Κ	Α	Α	Ν	G	V	V	L	A	Т	Ε	Κ	Κ	50	Mass Diff. (Da): 0.357 Mass Diff. (ppm): 13.85 Scores
5	1	Q	K	S	ſ	L	Y	D	E	R	S	V	Н	Κ	V	E	Ρ	Γ	т	K	Н	I	G	L	V	Y	75	P-Score: 9.8e-76 % Fragments Explained: 43 % % Residue Cleavages: 21 %
7	6	S	G	Μ	G	Ρ	D	Y	R	V	L	V	Н	R	A	R	K	L	Α	Q	Q	Y	Y	L	V	Y	100	Modification (A1) No Modification Custom
10	1	Q	E	Ρ	I	Ρ	Т	A	Q	L	V	Q	R	V	A	S	V	Μ	Q	Ε	Y	Т	Q	S	G	G	125	Uncommon Monomethylation
12	6	V	R	Ρ	F	G	V	S	L	L	I	С	G	W	Ν	Ε	G	R	Ρ	Y	L	F	Q	S	D	Ρ	150	
15	1	S	G	Α	Y	LF	A	W	K	A	Т	A	Μ	G	K	Ņ	Y	V	Ν	G	K	Т	F	L	Ε	K	175	
17	6	R	Y	Ν	Ε	D	L	LΕ	L	E	D	A	I	н	Т	A	I	L	Т	L	K	Ε	S	F	E	G	200	
20	1	Q	Μ	т	Ε	D	Ν	L	E	V	G	I	C	Ν	E	A	G	F	R	R	L	Т	Ρ	т	Ε	V	225	
22	6	К	D	Y	L	Α	A	I	Α	С																		

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Native Top-down MS: Bridging the gap!



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Native Top-down MS: Bridging the gap!



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HDX-MS: Concept









The origins of HDX-MS (before MS)



Hvidt and Linderstrom-Lang, 1956 Biochimica et Biophysica Acta

Rosa and Richards, 1979 J Mol Biol





Katta and Chait, 1991 RCMS

Zhang and Smith, 1993 Protein Science Zhang et al, 1996 Biochemistry

Resing and Ahn, 1998 Biochemistry

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Mehmood et al, 2012 PAS

Sowole et al, 2013 J Mol Biol











HDX-MS: Output / Bench



DynamX software Waters









HDExaminer software (Sierra Analytics)



HDX-MS: Perspectives MDa assemblies



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Conclusions



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Integrated Structural MS and Proteomics or Structural Proteomics

Inserm Workshop 262 Mass Spectrometry for Structural Biology



ORGANIZERS: Cherine BECHARA (IGF, Montpellier), Sarah CIANFERANI (IPHC, Strasbourg), Julien MARCOUX (IPBS, Toulouse)

AIMS: This workshop will present the fundamental and applied aspects of Mass Spectrometry-based structural techniques, as well as data analysis procedures. State-of-the-art lectures will be given by experts in the field, destined to both non-MS and MS specialists.



PHASE I – CRITICAL ASSESSMENT

January 11-13, 2022 - Bordeaux

TOP-DOWN MASS SPECTROMETRY

Frank SOBOTT (University of Leeds, GBR), Michal SHARON (Weizmann Institute, ISR), Julien MARCOUX (IPBS, FRA), Alain BECK (Pierre Fabre, FRA)

NATIVE MASS SPECTROMETRY AND ION MOBILITY

Justin BENESCH (University of Oxford, GBR), Valérie GABELICA (IECB, FRA), Kostas THALASSINOS (UCL, GBR), Cherine BECHARA (IGF, FRA), Charlotte UETRECHT (Heinrich Pette Institute, DEU)

HYDROGEN-DEUTERIUM EXCHANGE COUPLED TO MASS SPECTROMETRY (HDX-MS)

Sébastien BRIER (Institut Pasteur, FRA), Argyris POLITIS (King's College, GBR), Sarah CIANFERANI (IPHC, FRA)

CROSS-LINKING AND INTEGRATIVE MODELING

Carla SCHMIDT (Martin Luther University, DEU), Petr MAN (BioCev, CZE), Matteo DEGIACOMI (Durham University, GBR)

PHASE II – TECHNICAL WORKSHOP

Top-Down - IPBS, Toulouse - Week of 07/02 Native MS & Ion-Mobility - IGF, Montpellier - Week of 14/02 HDX-MS - LSMBO, Strasbourg - Week of 21/02

Hands-on experiments to apply main fundamental aspects developed during Phase I of the workshop. Three different training are proposed together with dedicated bio-informatics tools for data analysis: Native and Ion Mobility MS to analyse multiproteic and ligand binding complexes (IGF, Montpellier), Top-Down MS to identify proteoforms from an immunoprecipitation (IPBS, Toulouse) as well as HDX-MS to study the deuteration of standard proteins (IPHC, Strasbourg).

SELECTION: 8 trainees will be selected for each city among Phase I participants.



Institut national de la santé et de la recherche médicale Information and registration ateliers@inserm.fr https://tinyurl.com/3kt5axhx

Many Thanks

IPBS, Toulouse Odile Schiltz Angelique Dafun Dusan Zivkovic Carine Froment Marie-Pierre Bousquet

Past: Jean Lesne Julien Parra Frédéric Rivière Thomas Menneteau Efthymia Koufogeorgou











