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**Towards integrated structural biology:
studying protein complexes by
native mass spectrometry**

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Grenoble

CC22 OIT'S UNIVERSITE Grenoble Alpes

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Outline

- Many types of biological MS
- Intact proteins
- Protein complex challenges
- **Native MS**: preserving interactions
- Dissociation in gas phase
- Dissociation in solution
- **Ion mobility-MS**
- Outlook

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Accuracy and Precision

Accurate= French "juste", "exact"?

Accurate & Precise

Precise, Not Accurate

Accurate, Not Precise

Not Accurate, Not Precise

https://en.wikipedia.org/wiki/Accuracy_and_precision

NC STATE UNIVERSITY

Nomenclature iupac

Mass unit = Da = 1/12 of mass of the a carbon-12 atom = $1.6 \cdot 10^{-27}$ Kg

Accurate mass and exact mass are not synonymous.
Accurate mass refers to a experimentally measured mass.
Exact mass refers to a calculated mass.

Monoisotopic mass (Exact mass): is calculated using the exact mass of the most abundant isotopes of each constituent element (e.g., C12=12.000000, C13=13.003355)

Average or chemical mass: mass of an ion or molecule weighted for its isotopic composition monoisotopic mass. (e.g., C=12.011, H=1.00794, O=15.9994)

Nominal mass: mass of a molecular ion or molecule calculated using the isotope mass of the most abundant constituent element isotope of each element rounded to the nearest integer value and multiplied by the number of atoms of each element.(e.g. CH3OH= 12+4X1+16=32 Da

Murray, 2013 ⁴

Monoisotopic, average masses

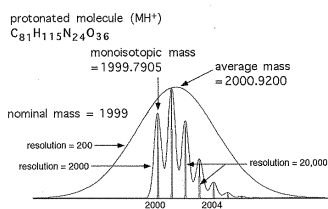


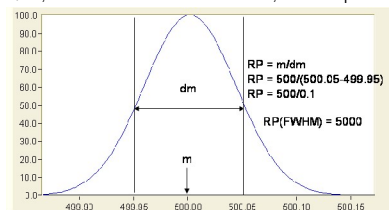
Figure 3.3. The mass spectrum of a protonated molecule obtained at resolving powers of 200, 2000, and 20,000 (using the FWHM definition of resolution). This is an example of how the resolving power can have a dramatic effect on resolving isotopes.

Figure from The Expanding Role of MS in Bio-technology – G. Stuzdak

An Overview of Peptide and Protein Analysis by Mass Spectrometry, S. Carr and R. Annan, in Current Protocols in Protein Science, J. Wiley and Sons (1996)

Nomenclature

Resolution = m/dm , m is the mass of the ion of interest, dm is the peak width



Often dm is taken to be 50% and m is designated as full width at half maximum (FWHM).

Mass error = (exact mass) – (accurate mass)

Mass error in parts per million (ppm) = $(\text{mass error}) / (\text{exact mass}) \times 10^6$

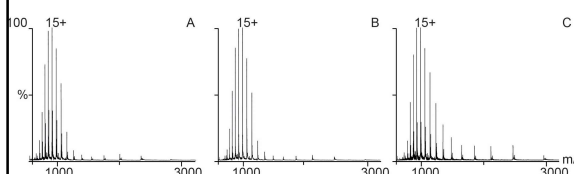
Intact proteins

- MS analyses of intact proteins by:
 - ❖ LC-ESI-TOF: <100 kDa
 - ❖ MALDI-TOF: >100 kDa



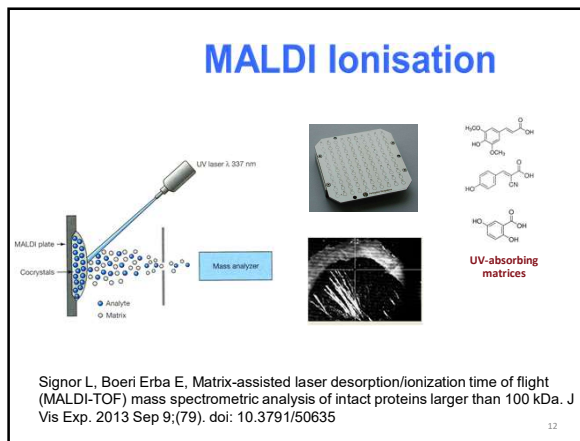
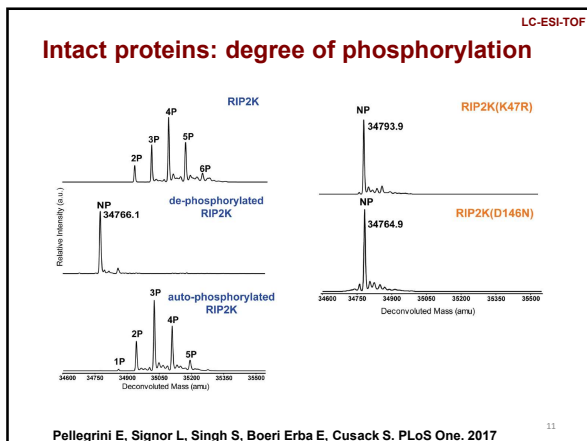
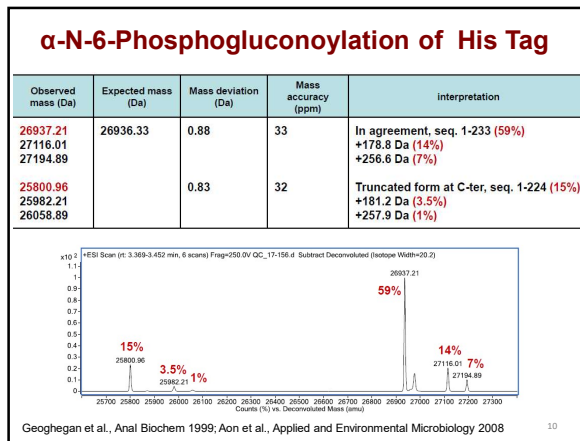
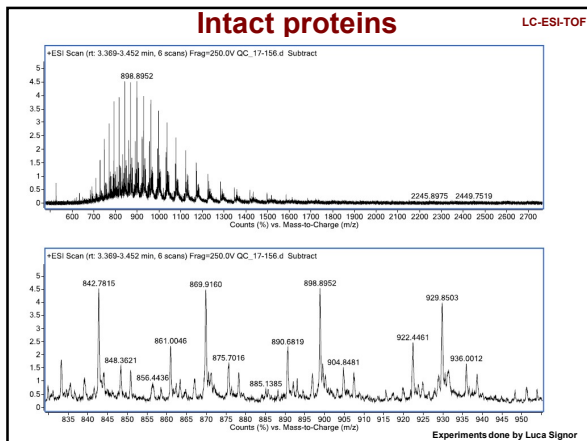
Boeri Erba E*, Signor L., Oliva M.F., Hans F., Petosa C. Methods in Molecular Biology, 2018

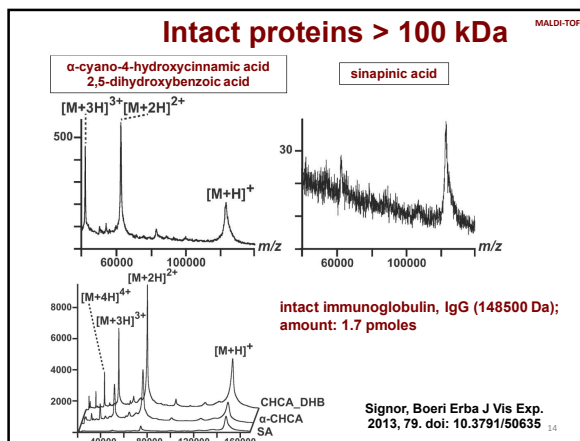
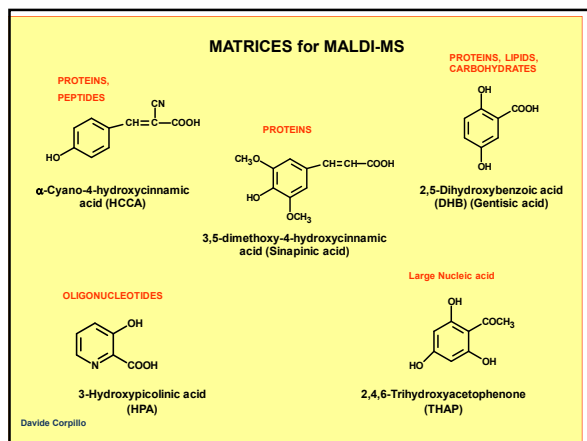
D-, ¹³C¹⁵N- labeling of TTR



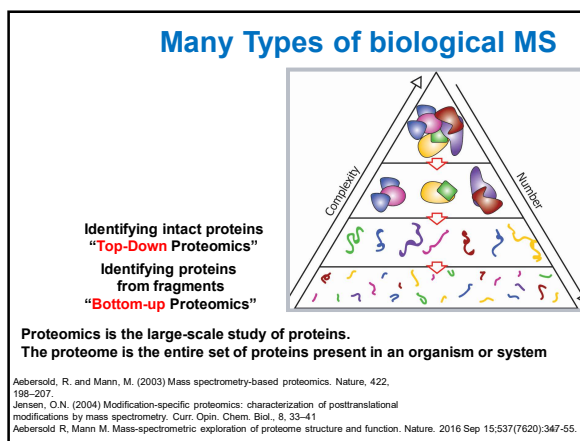
	Calculated mass (Da)	Measured mass (Da)	% of labeling
HTTR	14019.15 ^(a)	14021.03	-
DTTR	14772.15 ^(a)	14771.04	99.85 ^(a)
CNTTR	14809.15 ^(a)	14794.15	98.10 ^(a)

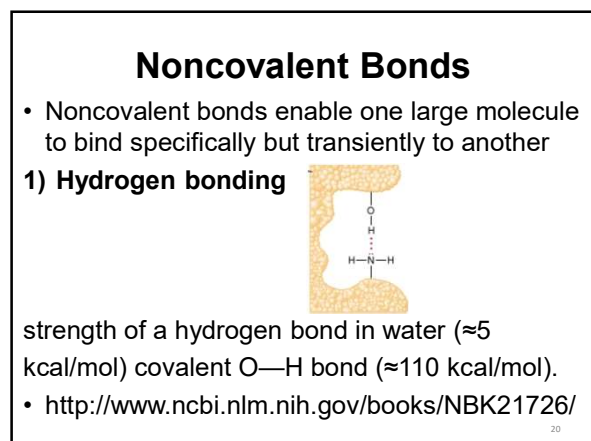
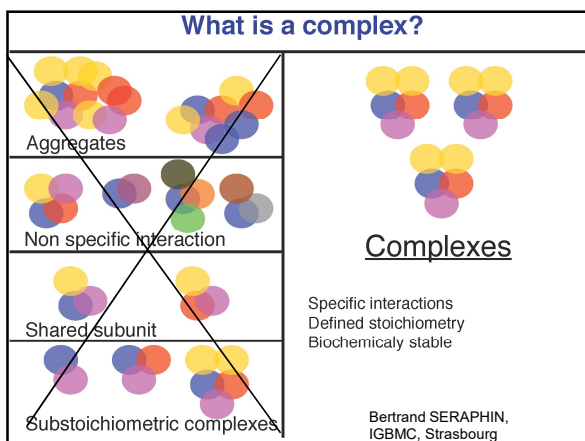
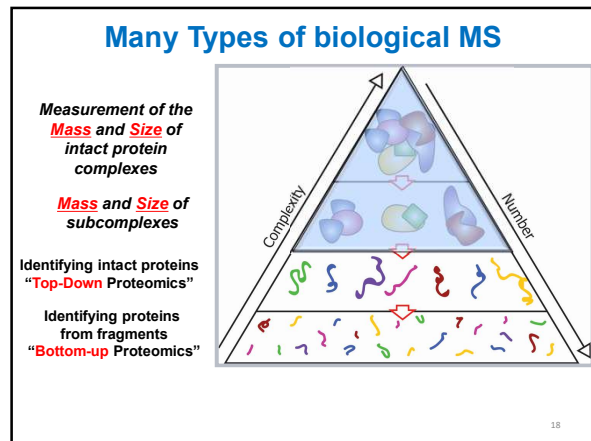
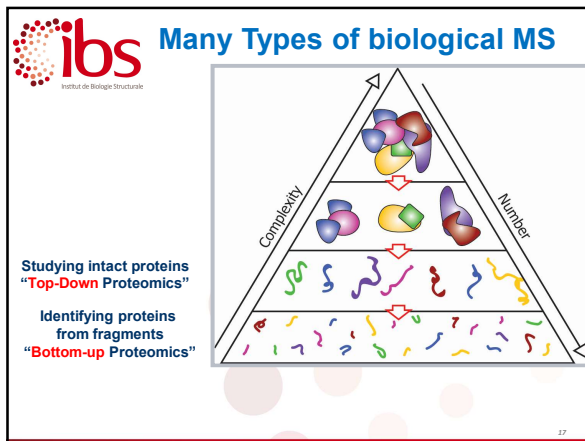
Yee AW..., Boeri Erba E, Forsyth VT. Angew Chem Int Ed Engl. 2016, 55(32):9292-6 Experiments done by Luca Signor





- Outline**
- Many types of biological MS
 - Intact proteins
 - Protein complex challenges
 - **Native MS**: preserving interactions
 - Dissociation in gas phase
 - Dissociation in solution
 - **Ion mobility-MS**
 - **Outlook**
- 15





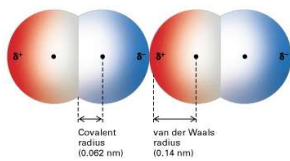
Noncovalent Bonds

2) Ionic Interactions

An attraction of a positively charged ion — a cation — for a negatively charged ion — an anion e.g. Na^+Cl^-

3) Van der Waals Interactions

Caused by electric dipoles; 1 kcal/mol; O_2



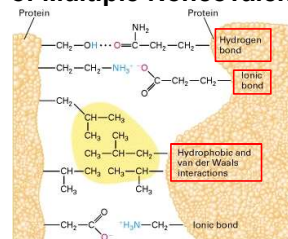
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Noncovalent Bonds

4) Hydrophobic Bonds

Due to force that causes hydrophobic molecules or nonpolar molecule portions to aggregate together rather than to dissolve in water.

Specificity of Multiple Noncovalent Bonds

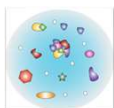


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Analysis challenges



- **Sample features:**
High mass protein complexes
Crucial interactions may be weak



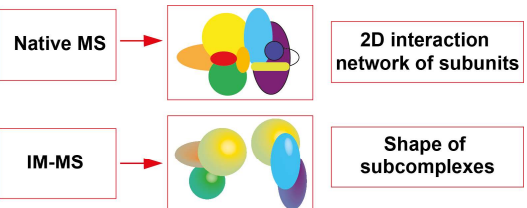
- **Sample Polydispersity:**
Multiple oligomeric states
Mammalian α -crystallin: 24–33 subunits

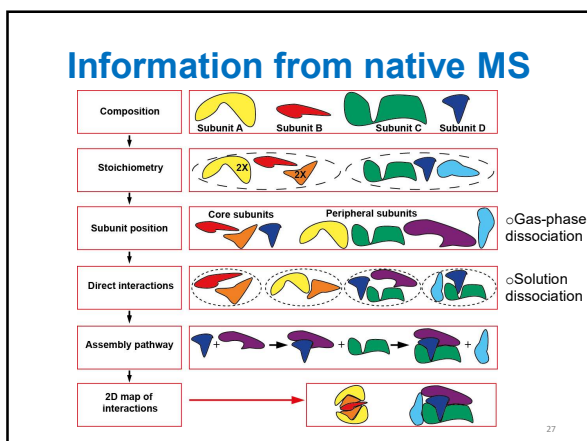
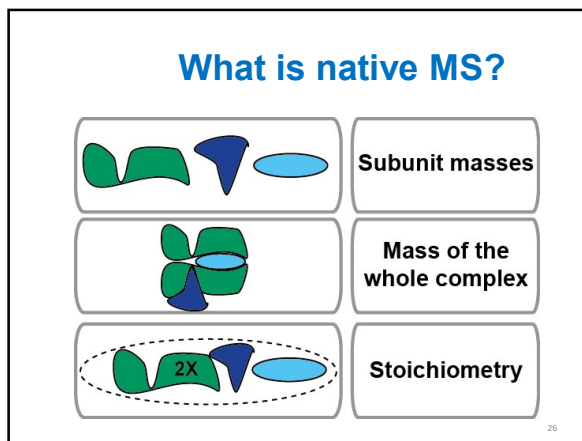
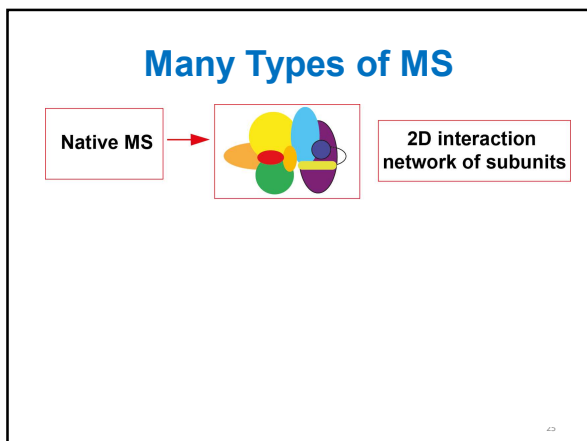


- **Low Sample Concentration**

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Many Types of MS

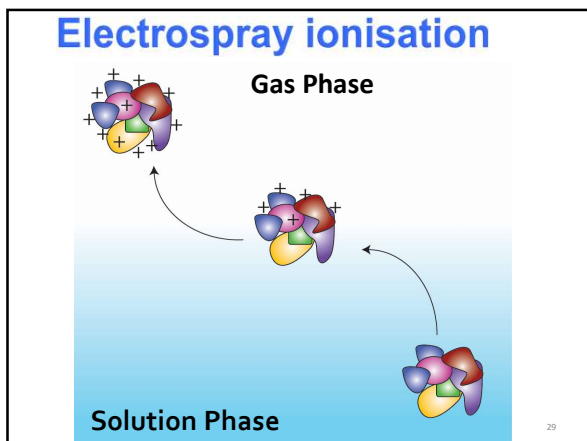




How to **maintain** non covalent interactions in the gas phase?

- **Soft ionisation (electrospray)**

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How to maintain non covalent interactions in the gas phase?

- **Soft ionisation** (electrospray):
- Nano electro-spray source

Helena Hernández & Carol V Robinson. Nature Protocols 2, 715 - 726 (2007)

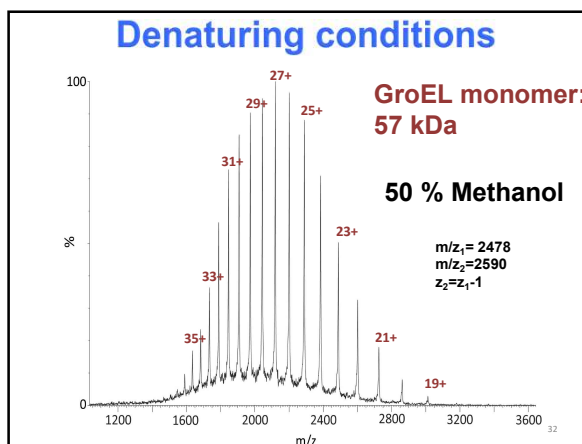
30

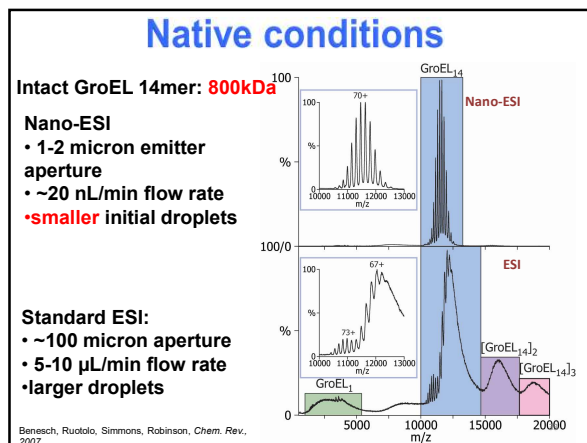
How to maintain non covalent interactions in the gas phase?

- **Soft ionisation:** nano electro-spray
- **Aqueous buffer:**
 - ammonium acetate
 - detergent above critical micelle concentration

Barrera NP et al., Mass spectrometry of membrane transporters reveals subunit stoichiometry and interactions. Nat Methods. 2009 Aug;6(8):585-7. doi: 10.1038/nmeth.1347.

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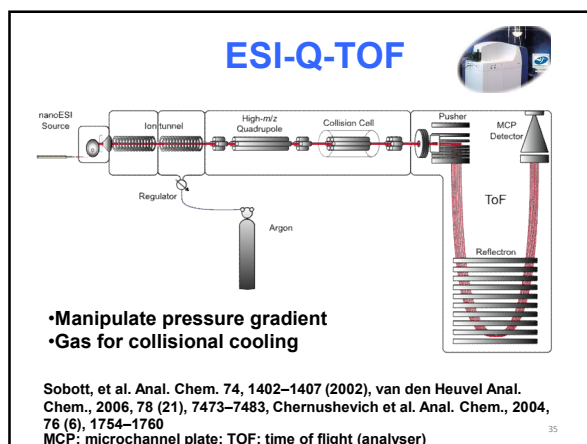
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How to maintain non-covalent interactions in the gas phase?

- **Soft ionisation** (electrospray)
- **Aqueous** buffer (e.g. ammonium acetate)
- **Modified** instruments (Q-TOF, Q-IM-TOF)

Q=quadropole
TOF=time of flight analyser

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What are the sample requirements?

- **Moderate concentrations (2–20 microM)**
- **buffer exchanged to ESI-compatible solutions (Ammonium Acetate)**
- **Buffer exchange: microcentrifuge gel filtration (e.g. Biospin), ultrafiltration devices (e.g. Vivaspins)**

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Examples

- Goals:
 - Stoichiometry
 - Direct interactions between subunits
 - 2D interaction network of proteins

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I. Inactive and active RIP2 kinase

LC-ESI-TOF

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Pellegrini E, Signor L, Singh S, Boeri Erba E, Cusack S. PLoS One. 2017

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I. RIP2 kinase: phosphorylation sites

LC-ESI-TOF

MALDI-TOF

DFG-LS₁₀₀KWRMMS₁₇₄LS₁₇₃QS₁₇₂RS₁₆₉S₁₅₁KS₁₄₉APEGGT₁₄₈IY₁₄₅M-PPPE

- phosphopeptide [173-179] MMS₁₇₄LS₁₇₃QS₁₇₂R
- phosphopeptide [172-182] MMS₁₇₄LS₁₇₃QS₁₇₂RS₁₆₉S₁₅₁K

39

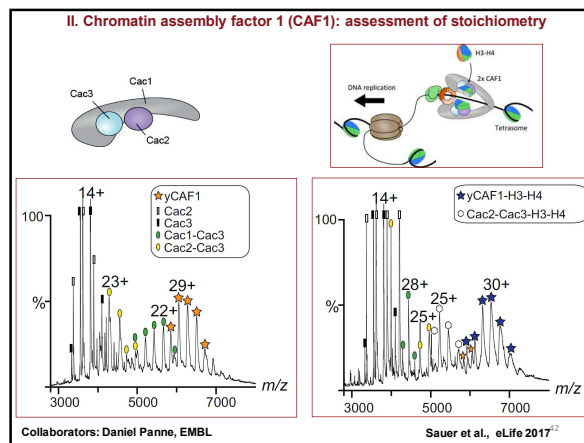
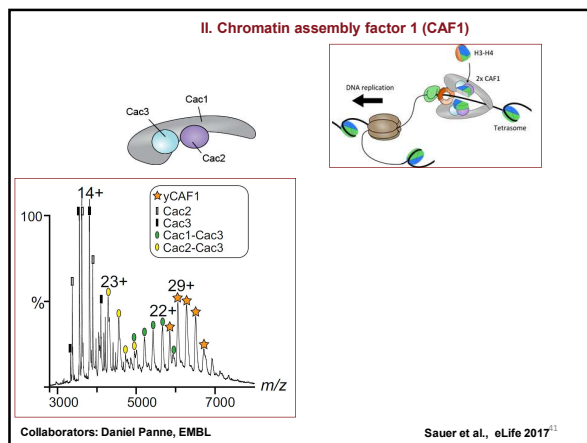
Pellegrini E, Signor L, Singh S, Boeri Erba E, Cusack S. PLoS One. 2017

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I. RIP2 kinase: stoichiometry

40

Pellegrini E, Signor L, Singh S, Boeri Erba E, Cusack S. PLoS One. 2017



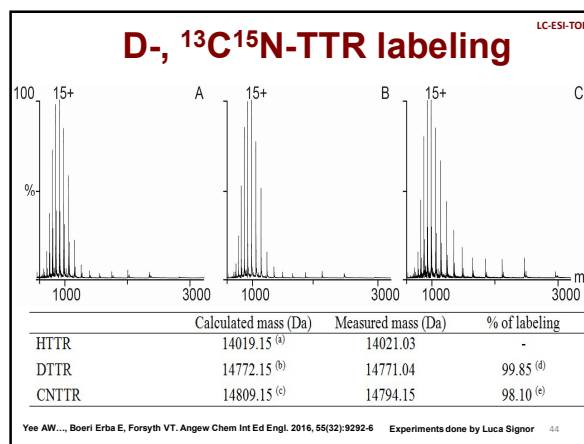
Dynamics of complexes TTR (transthyretin)

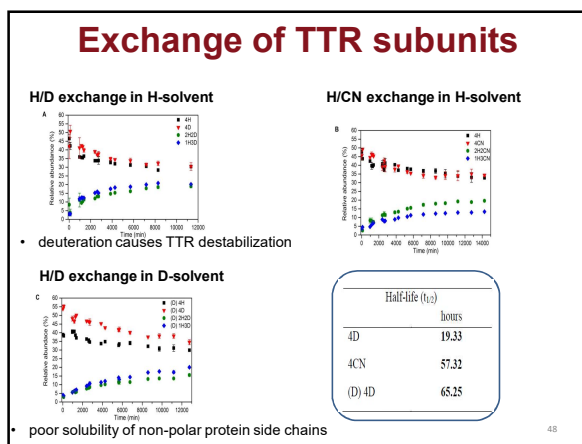
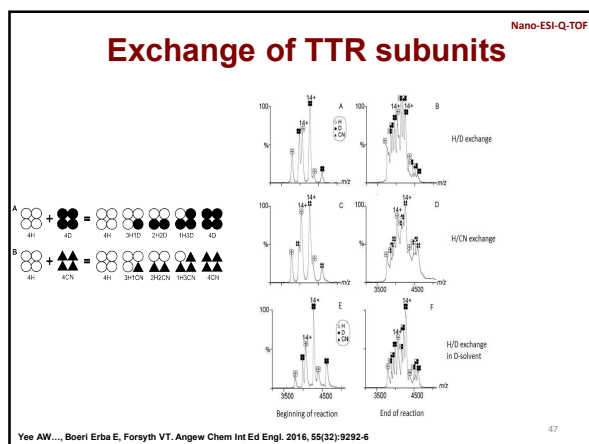
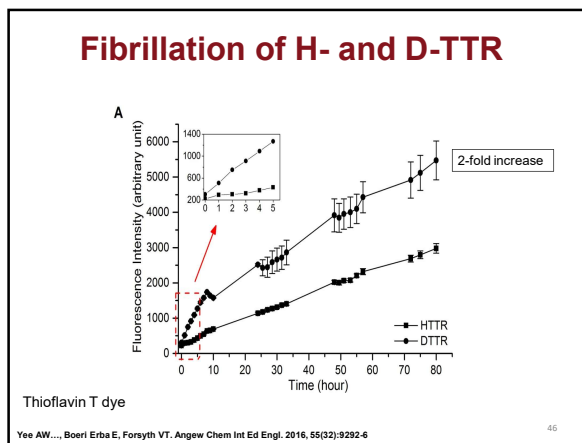
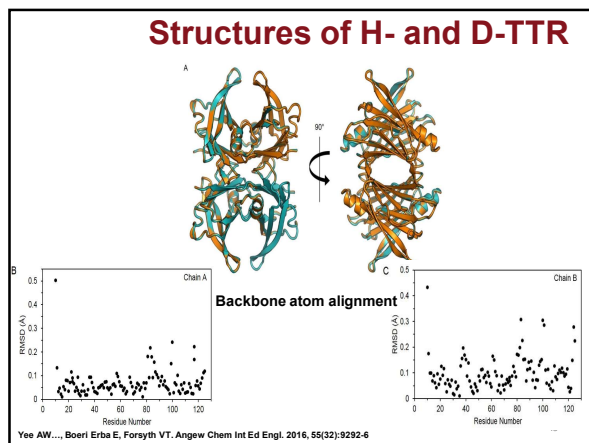
- 4mer in blood plasma and cerebrospinal fluid
- Transport of the thyroxine and vitamin A
- Aggregation into amyloid fibrils

- Influence of amino acidic deuteration
- Influence of a deuterated buffer

Trevor Forsyth, ILL, Grenoble

Yee AW..., Boeri Erba E, Forsyth VT. Angew Chem Int Ed Engl. 2016, 55(32):9292-6





TTR conclusions

- X-ray structures of H and D-TTR are identical
- Amyloid formation accelerated for D-TTR
- Subunit exchange kinetics accelerated for D-TTR
- Slower subunit exchange in D-solvent

Yee AW..., Boeri Erba E, Forsyth VT. Angew Chem Int Ed Engl. 2016, 55(32):9292-6

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• Dissociation in gas phase

• Dissociation in solution

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• Dissociation in gas phase

Stoichiometry



Core and peripheral subunits

Core subunits

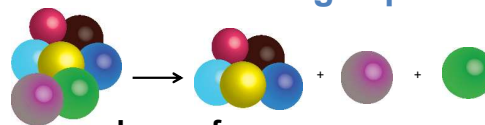


Peripheral subunits



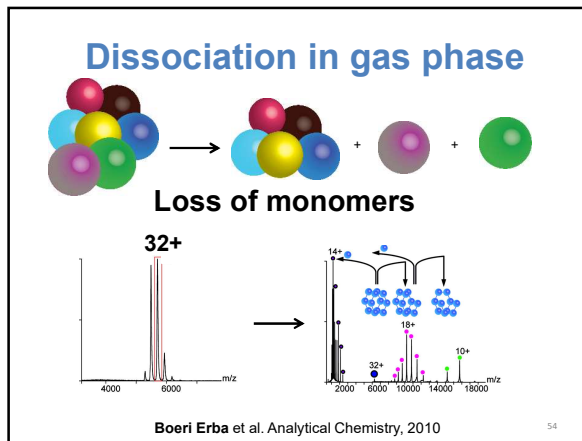
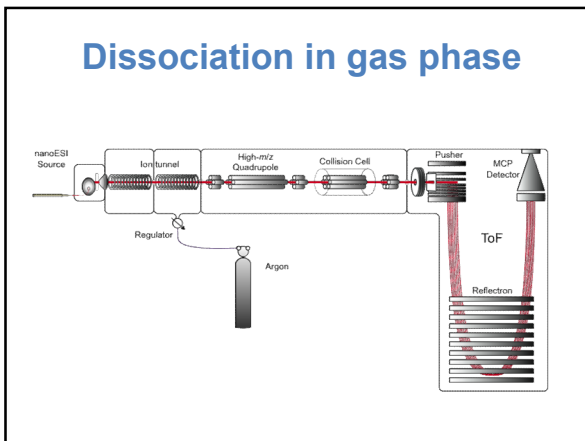
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Dissociation in gas phase



Loss of monomers

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Examples

- Protein complex,
Barbara Callisto, ESRF
- RNA protein assembly,
Carlo Petosa, IBS

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- Dissociation in gas phase
- Dissociation in solution

Subunit interactions

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Dissociation in solution

Levy, Boeri Erba, et al., Nature. 2008, 453(7199):1262-5.
 Assembly reflects evolution of protein complexes.

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Many Types of MS

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Information from IM-MS

Same mass, different shape: ring type and collapsed type

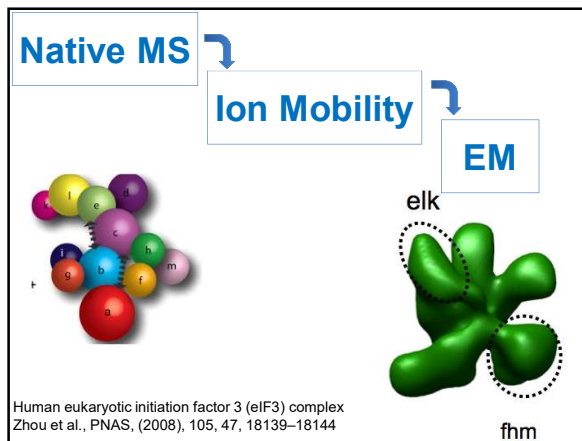
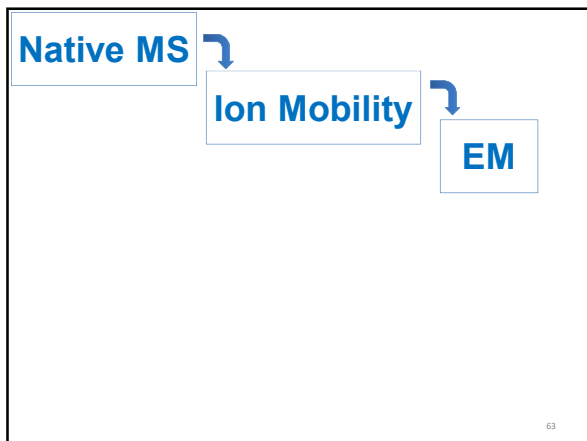
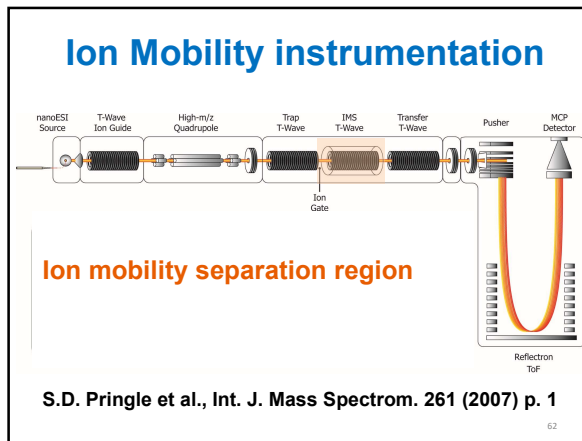
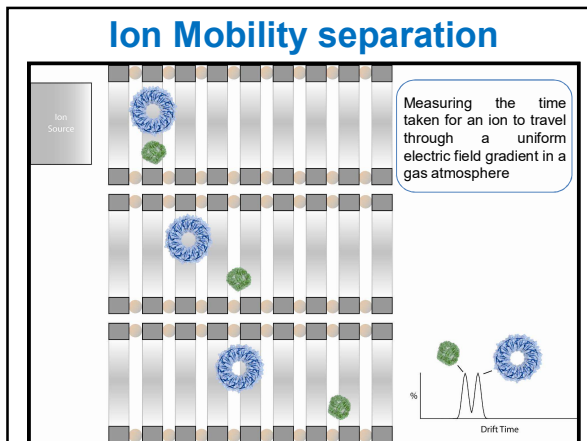
59

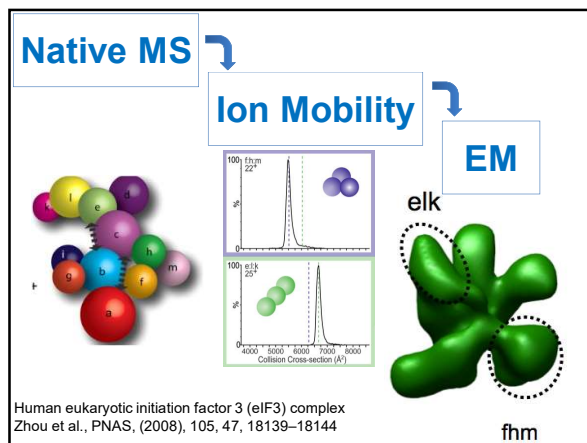
Information from IM-MS

Same mass, different shape: ring type and collapsed type

Collision cross section: the effective surface area of a macromolecular ion when it collides with a neutral gas molecule

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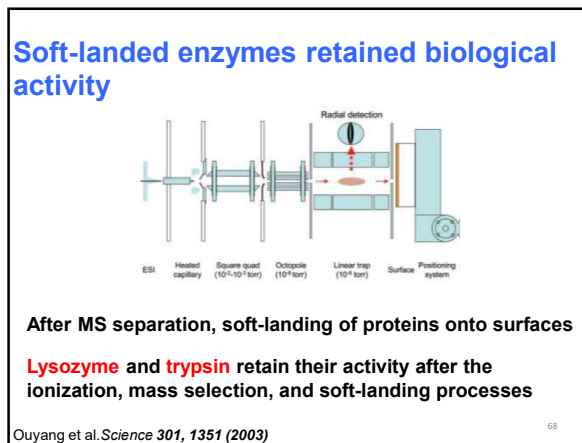
Is the **gas-phase structure** similar to the structure in **solution**?

66

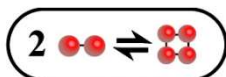
Is the **gas-phase structure** similar to the structure in **solution**?

- Removal of water for milliseconds
- No immediate unfolding of proteins and protein complexes

Meyer et al. 2009 An atomistic view to the gas phase proteome. Structure 17, 88–95.



Solution VS gas phase structure



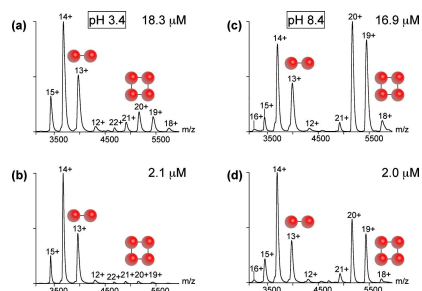
Influence on oligomeric state:

- Solution pH
- Solution concentration

Boeri Erba et al. Anal Chem. 2011.

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Solution VS gas phase abundance



- Changing solution conditions determines different peak intensities

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ibs Why use native MS?

- Moderate quantities of protein complexes (20 μ M, 20 μ L)
- Tolerate composition heterogeneity, flexibility
- Do not require crystals or labeling or crosslinking
- Symmetry in the structure is not an advantage

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ibs Future of native MS and IM-MS

1) Spatial restraints from MS and IM-MS to allocate subunits in EM structure

Jore al. Nat Struct Mol Biol 2011, 18:529-536.
 Lanucara et al. Nat Chem 2014, 6: 281-292

2) High resolution instruments to get both quaternary and primary structure.

Skinner, et al. Nat Methods 2016, 13(3):237-40

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Future of native MS

High sensitivity of MS: analysis of protein complexes affinity-purified directly from cells

1000 versus 10^8 molecules

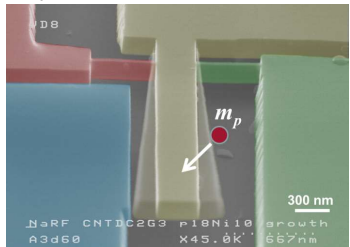
Hanay et al. Single-protein nanomechanical mass spectrometry in real time. *Nat Nanotechnol.* 2012;7(9):602-8
 Sage E, et al. Neutral particle mass spectrometry with nanomechanical systems. *Nat Commun.* 2015. doi: 10.1038/ncomms748

<http://www.edyp.fr/index.php/analytical-methods>
<http://www.youtube.com/watch?v=OM5MW5NrdFA>

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Future of native MS and IM-MS

3) High sensitivity of MS



- Ultra-small mechanical structures (e.g. $1.8 \mu\text{m} \times 120 \text{nm} \times 100 \text{nm}$)
- High mechanical vibrational frequencies.
- Change in the resonator total mass, change in the oscillation resonance frequency

<http://nano.caltech.edu/research/nems-ms.html>

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Reviews

- Boeri Erba E, Petosa C. The emerging role of native mass spectrometry in characterising the structure and dynamics of macromolecular complexes. *Protein Sci.* 2015
- Boeri Erba E. Investing macromolecular complexes using top-down MS. *Proteomics.* 2014
- Sharon M. Biochemistry. Structural MS pulls its weight. *Science.* 2013;340(6136):1059-60
- Barreira NP, Robinson CV. Advances in the mass spectrometry of membrane proteins: from individual proteins to intact complexes. *Annu Rev Biochem.* 2011;80:247-71
- Benesch JL, Ruotolo BT. Mass spectrometry: come of age for structural and dynamical biology. *Curr Opin Struct Biol.* 2011;21(5):641-9
- Hilton GR, Benesch JL. Two decades of studying non-covalent biomolecular assemblies by means of electrospray ionization mass spectrometry. *J R Soc Interface.* 2012;9(70):801-16
- Zhong Y, Hyung SJ, Ruotolo BT. Ion mobility-mass spectrometry for structural proteomics. *Expert Rev Proteomics.* 2012;9(1):47-58
- Uetrecht C, Rose RJ, van Duijn E, Lorenzen K, Heck AJ. Ion mobility mass spectrometry of proteins and protein assemblies. *Chem Soc Rev.* 2010;39(5):1633-55
- Madler S, Boeri Erba E, Zenobi R. MALDI-ToF Mass Spectrometry for Studying Noncovalent Complexes of Biomolecules. 2012; 1-36

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Research laboratories

- Prof. Robinson <http://research.chem.ox.ac.uk/carol-robinson.aspx>
- Prof. Heck <http://www.hecklab.com>
- Prof. Zenobi <http://www.zenobi.ethz.ch/>
- Prof. Ashcroft <http://www.astbury.leeds.ac.uk/facil/mass.htm>
- Prof. Cianferano-Sanglier <http://www.iphc.cnrs.fr/Spectrometrie-de-masse.html>
- Dr. Boeri Erba <http://www.ibs.fr/>


Groups > [Viral Infection and Cancer Group](#) > [Boeri Erba Team](#)

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- Prof. Ruotolo
<http://www.umich.edu/~ruotolo/>
- Prof. Loo
<http://loolab.chem.ucla.edu/index.html>
- Prof. Sharon
http://www.weizmann.ac.il/Biological_Chemistry/scientist/MichalSharon/
- Prof. Frank Sobott
<https://www.uantwerpen.be/en/staff/frank-sobott/prof--dr/research/>

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Integrative Structural Biology

Innovative tool for interaction maps

Crystallography and NMR	EM and SAXS	Native MS
Atomic structures of subunits	3D structure of a whole complex	2D interaction network
↓ ↓ ↓		
 3D architecture of a complex		

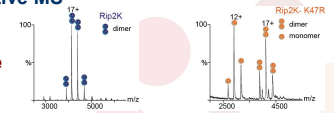
78



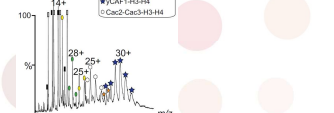
Conclusions

MS analyses of intact proteins
Native MS

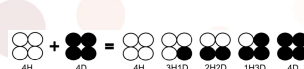
I. Inactive and active RIP2 kinase



II. Chromatin assembly factor 1



III. Dynamics of complexes



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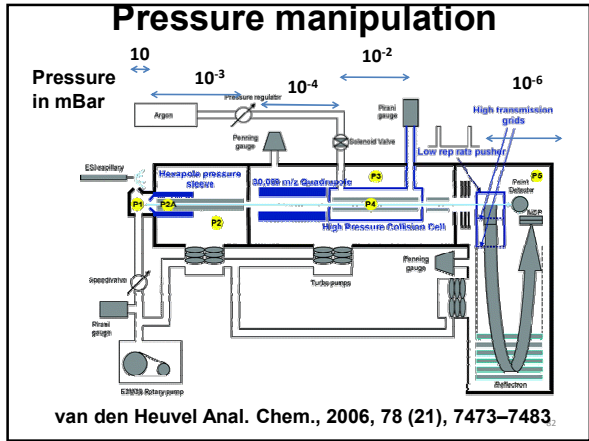
Acknowledgments

- Luca Signor
- Imre Berger
- Simon Trowitzsch
- Sarah Teichmann
- Carol Robinson
- Renato Zenobi
- Barbara Callisto
- Carlo Petosa




Additional information

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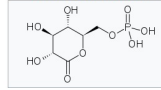


6-Phosphogluconolactone

From Wikipedia, the free encyclopedia

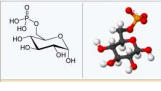
6-Phosphogluconolactone is an intermediate in the pentose phosphate pathway. It is produced from glucose-6-phosphate by glucose-6-phosphate dehydrogenase.

6-Phosphogluconolactone



Names	
Other names	6-phospho-D-glucono-1,5-lactone 6-phospho-D-glucono-5-lactone D-6-phosphoglucono-1,5-lactone D-6-phosphoglucono-5-lactone D-glucono-1,5-lactone 6-phosphate D-glucono-5-lactone 6-phosphate
Properties	
Chemical formula	C ₆ H ₁₁ O ₆ P
Molar mass	258.12 g/mol

Glucose 6-phosphate



Lactones are cyclic esters of hydroxycarboxylic acids, containing a 1-oxacycloalkan-2-one structure, or analogues having unsaturation or heteroatoms replacing one or more carbon atoms of the ring

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