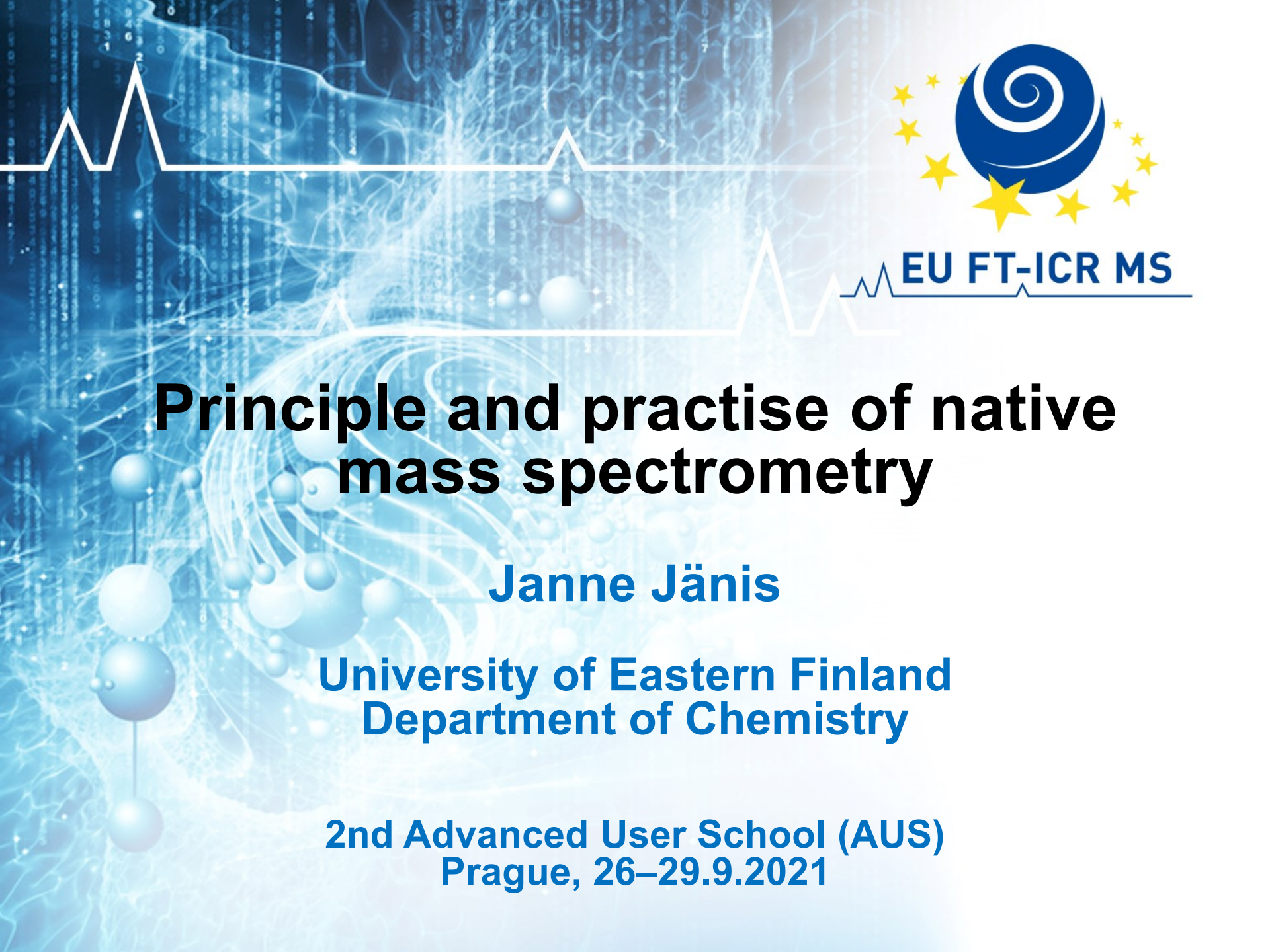


Principle and practise of native mass spectrometry

Janne Jänis

University of Eastern Finland
Department of Chemistry

2nd Advanced User School (AUS)
Prague, 26–29.9.2021

EU FT-ICR MS

"Electrospray Wings for Molecular Elephants"
John B. Fenn (1917-2010), Nobel lecture ***2002***

J. Am. Soc. Mass Spectrom. (2016)
DOI: 10.1007/s13361-016-1545-3

FOCUS: 31st ASILOMAR CONFERENCE,
NATIVE MS-BASED STRUCTURAL BIOLOGY: ACCOUNT & PERSPECTIVE

Native Mass Spectrometry: What is in the Name?

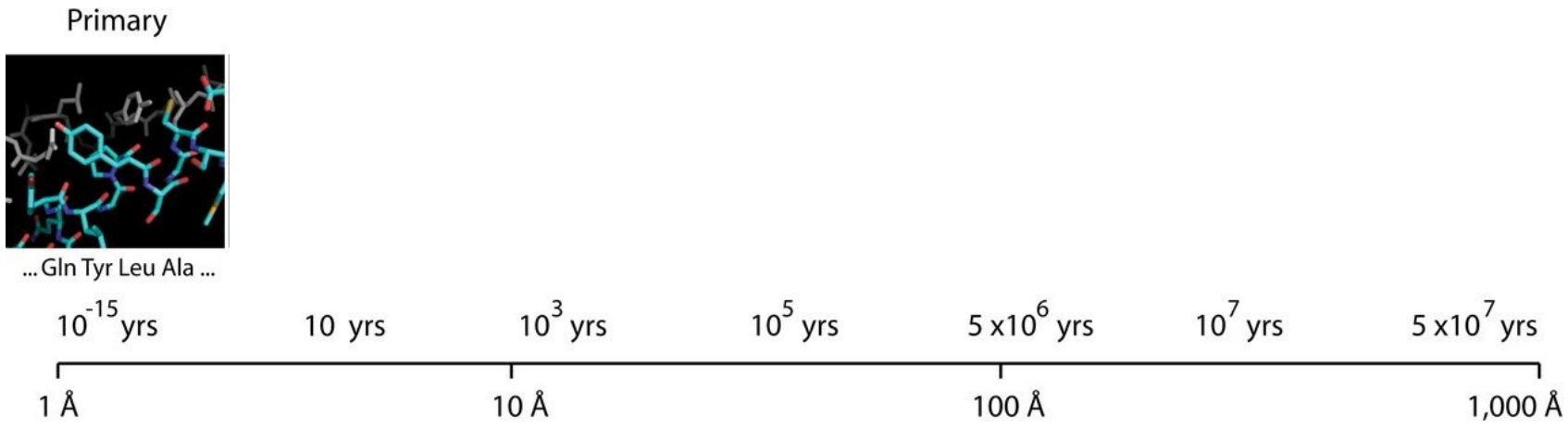
Aneika C. Leney,[1,2] Albert J. R. Heck[1,2]

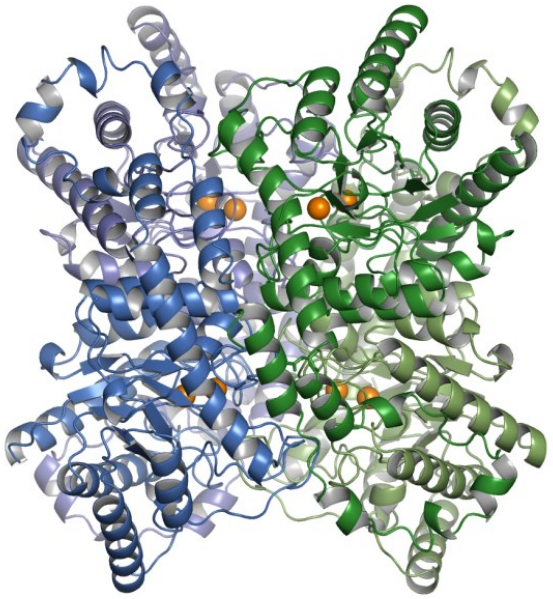
[1]Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584CH, Utrecht, The Netherlands
[2]Netherlands Proteomics Center, Padualaan 8, 3584CH, Utrecht, The Netherlands

"Native MS is a particular approach based on electrospray ionization, whereby the biological analytes are sprayed from a nondenaturing solvent"

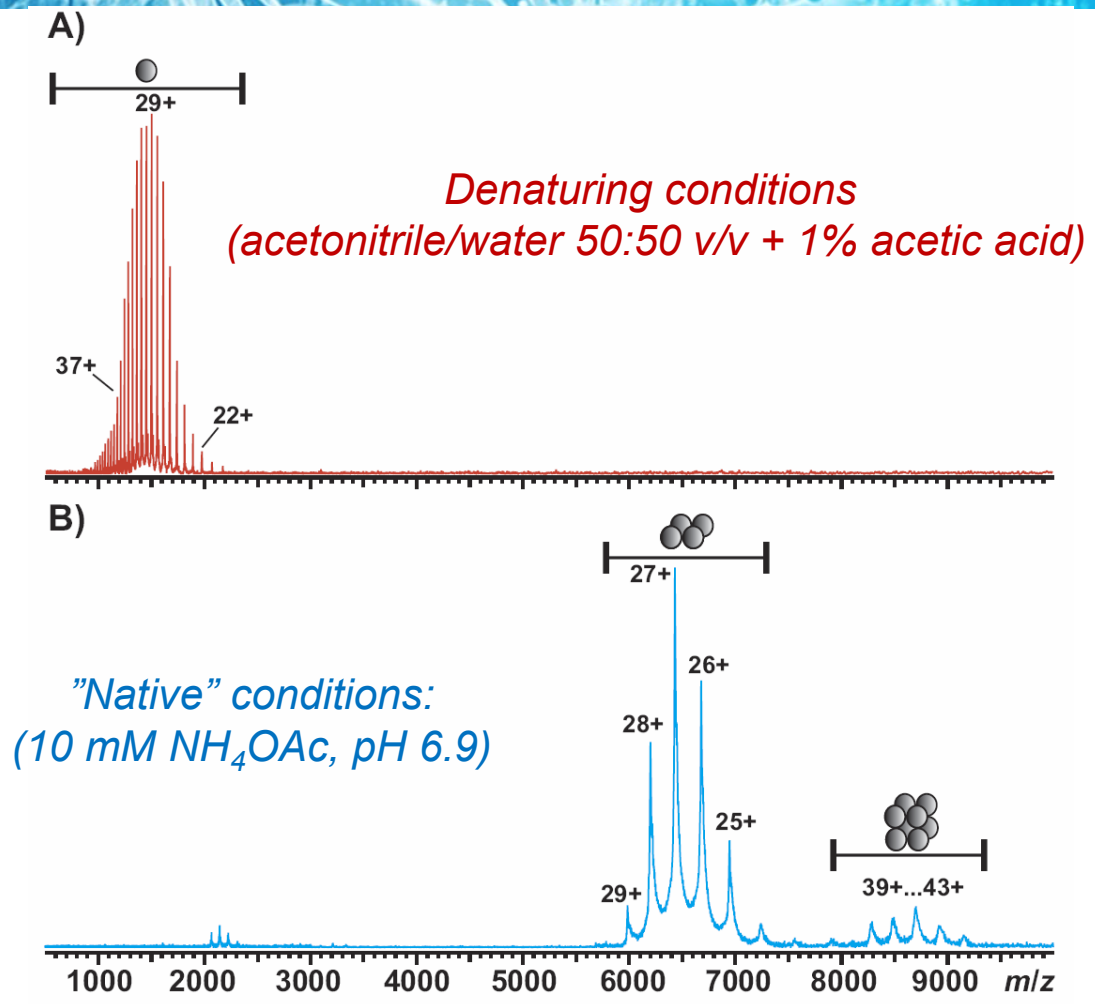
The term describes the biological status of the analytes in solution, prior to the ionization event analytes in solution."

- Levels of protein structure:
  - Primary, secondary, tertiary, quaternary, (quinary)
- Conventional ”denaturing” mass spectrometry
- Native mass spectrometry





Xylose isomerase tetramer:
Exp. 173 129 ± 12 Da
Calc. 173 104 Da for
4 × monomer + 8 × Mg^{2+}



Jänis, J et al. *J. Mass Spectrom.* 2008, 43: 1376-1380.

PERSPECTIVE

Native mass spectrometry: a bridge between interactomics and structural biology

Albert J R Heck

Native mass spectrometry is an emerging technology that allows the topological investigation of intact protein complexes with high sensitivity and a theoretically unrestricted mass range. This unique tool provides complementary information to established technologies in structural biology, and also provides a link to high-throughput interactomics studies, which do not generate information on exact protein complex-composition, structure or dynamics. Here I review the current state of native mass spectrometry technology and discuss several important biological applications. I also describe current experimental challenges in native mass spectrometry, encouraging readers to contribute to solutions.

The analysis of protein complexes and protein interaction networks is an important endeavor as nearly all biological processes involve regulated cooperation between multiple protein subunits in both time and space[1]. Equally important interactions with other biomolecules, such as DNA, cofactors and messenger molecules, also contribute to the complexity of regulation. Identification and structural and functional characterization of the components involved in these machineries and how they interact to carry out their biological functions is the key to understanding biological processes at the molecular level. There are several experimental approaches for investigating protein-complex topology and protein interactions. At one end of the spectrum there are technologies for high-throughput identification of proteins involved in complexes, or 'interactomes', for instance by large-scale yeast two-hybrid screens[2] or by affinity purification mass spectrometry (MS)[3–6]. These studies have generated a plethora of new data on protein complexes, providing an important framework (or catalog) of protein interaction networks. However, further detailed structural and functional characterization of these complexes is required not only to validate these datasets but also to better understand their role in biological processes. At the other end of the spectrum, high-resolution structural data on proteins and protein complexes can be obtained by methods such as nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography and electron microscopy[7]. Such data provide valuable insights into detailed mechanisms at the molecular level, but, with a few notable exceptions[8], obtaining structures of large, heterogeneous complexes, particularly in multiple regulatory states, is still challenging and laborious.

Additional technologies are essential to bridge the gap between 'interactomics' and structural biology. Whereas interactomics technologies do not yield detailed information about structure, dynamics and function, structural biology technologies are often limited by the size of the system that can be studied and by the sensitivity of the methods, and are often dependent on overexpression of recombinant proteins, which are not always representative of the endogenous entities. Native MS, which provides some unique and complementary features, is an emerging technology that could aid in bridging this gap between structural biology and interactomics technologies.

Soon after the introduction of electrospray ionization (ESI), researchers realized that it could be used to monitor intact proteins and even protein complexes by MS[9], but it took more than a decade for the technology to become a robust tool. The term native MS has been coined for this particular area of research[10] to reflect its ability to investigate native-like quaternary structures. Native MS does not yield detailed molecular (and atomic) structure information, but it has some major advantages over traditional structural biology methods, such as its sensitivity (which enables the analysis of endogenously expressed protein complexes in picomole amounts), speed, selectivity and

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PUBLISHED ONLINE 30 OCTOBER 2008; DOI:10.1038/NMETH.1265

Heck AJR, Nature Methods 2008, 5:927

Angewandte Reviews

C. V. Robinson and J. T. S. Hopper

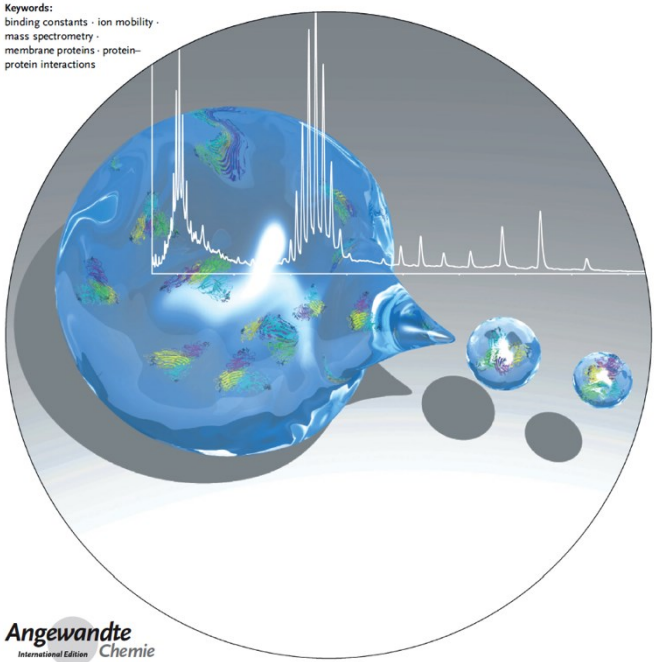
DOI: 10.1002/anie.201403741

Protein Interactions

Mass Spectrometry Quantifies Protein Interactions—From Molecular Chaperones to Membrane Porins

*Jonathan T. S. Hopper and Carol V. Robinson**

Keywords:
binding constants · ion mobility · mass spectrometry · membrane proteins · protein–protein interactions

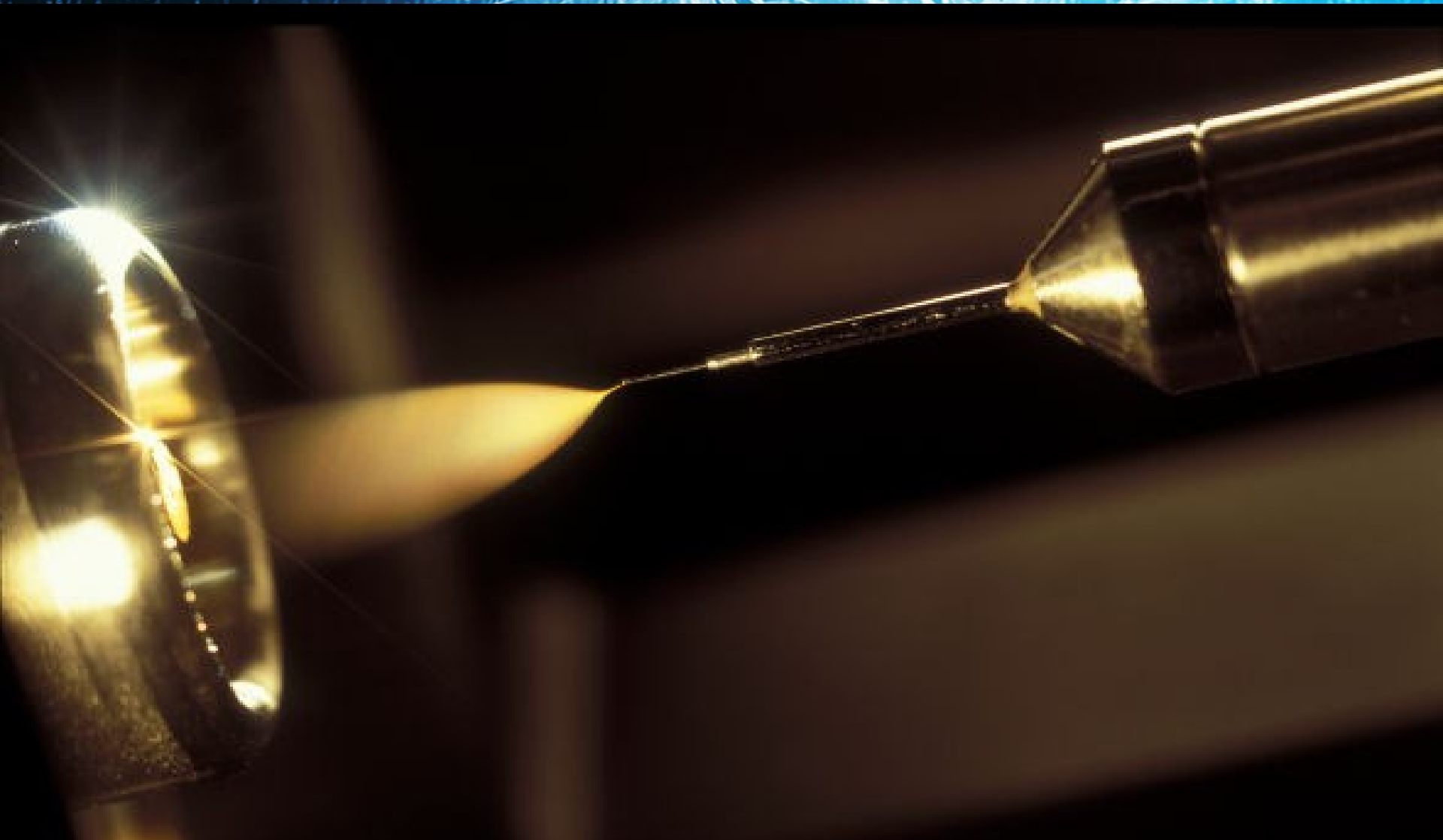


Angewandte International Edition Chemie

Hopper JTS & Robinson CV, ACIE 2014, 53:14002

EU FT-ICR MS

Electrospray ionization (ESI)



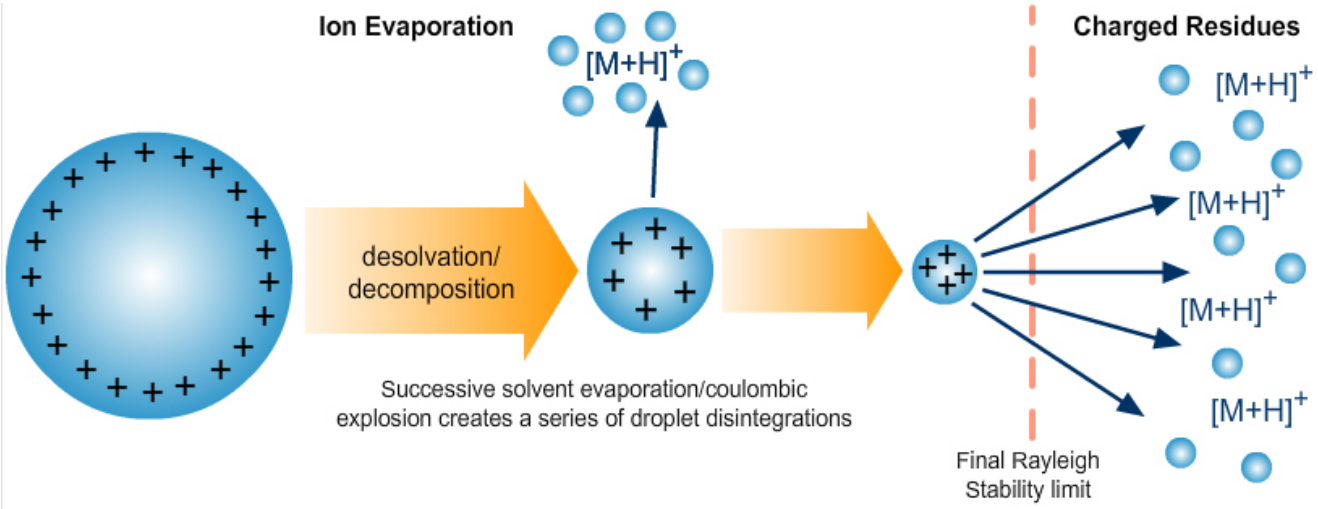
Charge residue model for large protein ions in ESI

- Complicated, multi-phased process
  - A droplet srinkage by evaporation, until the electric field exceeds surface tension (a Rayleigh limit);

$$q^2 \leq 8\ \pi^2 \varepsilon_0 \gamma R^3,$$

q = droplet charge, ε_0 = solvent permittivity, γ = surface tension

R = droplet radius



Kebarle P & Verkerk UH, *Mass Spectrom. Rev.* **2009**, 28: 898–917.

EU FT-ICR MS

Electrospray ionization (ESI)

Electrospray Ionization for Mass Spectrometry of Large Biomolecules

JOHN B. FENN, MATTHIAS MANN, CHIN KAI MENG, SHEK FU WONG, CRAIG M. WHITEHOUSE

Electrospray ionization has recently emerged as a powerful technique for producing intact ions in vacuo from large and complex species in solution. To an extent greater than has previously been possible with the more familiar "soft" ionization methods, this technique makes the power and elegance of mass spectrometric analysis applicable to the large and fragile polar molecules that play such vital roles in biological systems. The distinguishing features of electrospray spectra for large molecules are coherent sequences of peaks whose component ions are multiply charged, the ions of each peak differing by one charge from those of adjacent neighbors in the sequence. Spectra have been obtained for biopolymers including oligonucleotides and proteins, the latter having molecular weights up to 130,000, with as yet no evidence of an upper limit.

MASS SPECTROMETRY CONSISTS IN "WEIGHING" INDIVIDUAL molecules by transforming them into ions in vacuo and then measuring the response of their trajectories to electric and magnetic fields or both. Attempts to extend the sensitivity and accuracy of mass spectrometric methods to the analysis of large polar organic molecules of interest in biology and medicine have long been frustrated by the difficulties of transforming such molecules into gas-phase ions. They cannot be vaporized without extensive, even catastrophic, decomposition. Consequently, one cannot apply the classical methods of ionization that are based on gas-phase encounters of the molecule to be ionized with electrons as in electron ionization, photons as in photo ionization, other ions as in chemical ionization, or electronically excited atoms or molecules as in Penning ionization. Such encounters can remove a negatively or positively charged entity from a neutral molecule, or sometimes attach one, thus transforming it into an ion.

In the last 20 years intrepid experimentalists have developed a number of so-called "soft" ionization methods that have been used with varying degrees of success to produce intact ions from molecular species of ever-increasing size and decreasing vaporizability. One class of such methods is based on very rapid deposition of energy on a surface over which the species to be analyzed (analyte) has been dispersed. The underlying idea, first proposed by Beuhler *et al.*, is that sufficiently rapid energy input may bring about vaporization before decomposition has a chance to take place (*1*). The several methods differ in the way that rapid energy deposition is brought about. In plasma desorption (PD) it results from the impact of a fission product of a radioactive isotope, usually californium-252. So-called secondary ionization mass spectrometry (SIMS) makes use of an incident beam of high-energy ions, such as 40-keV Cs^+ and will therefore be referred to here as fast ion bombardment (FIB). If the ions are neutralized by charge exchange before they strike the surface, FIB becomes FAB (for fast atom bombardment). In laser desorption (LD), photons are the vehicle for energy deposition. These "energy-sudden" techniques have been able to produce intact ions from remarkably large analyte species, even though in an overall sense the processes involved are highly irreversible. Striking improvements have resulted from dispersing the analyte not on a bare surface but in a layer of suitable matrix, for example, thioglycerol for FAB or FIB, nitrocellulose for PD, and nicotinic acid for LD. At this writing the highest molecular weights of ions that have been produced are with LD 210,000 (*2*), with FAB (or FIB) 24,000 (*3*), and with PD 45,000 (*4*). However, product ion currents are usually very small and, except in the case of LD, decrease rapidly with increasing molecular weight of the analyte. When the ions are very large, their detection with multipliers requires postacceleration voltages that are sometimes awkwardly high. Furthermore, the ions often have high levels of internal excitation that can cause substantial peak broadening as a result of predissociation.

Quite different in practice and principle from these "violent" ionization methods are techniques that use strong electrostatic fields to extract ions from a substrate. In so-called field desorption (FD) ionization, the analyte molecules are applied to a fine wire on whose surface is disposed an array of sharp pointed needles or "whiskers." When the wire is placed in a vacuum system and a high voltage is applied while it is carefully heated, the analyte molecules desorb as ions from the tips of the needles where the field strength is very high (*5*). Even though it can transform highly nonvolatile analytes into ions in vacuo, FD has not been widely used because sample preparation is tedious. Finding and maintaining the combination of temperature and voltage that is right for a particular species requires both luck and the right touch. Finally, the desorbed ions have such high energies that relatively expensive magnetic sector analyzers must be used for their analysis. In electrohydrodynamic (EH) ionization, the analyte is dissolved in a nonvolatile liquid (for example, glycerol) and injected into an evacuated chamber through a small capillary tube that is maintained at high voltage (*6*). The

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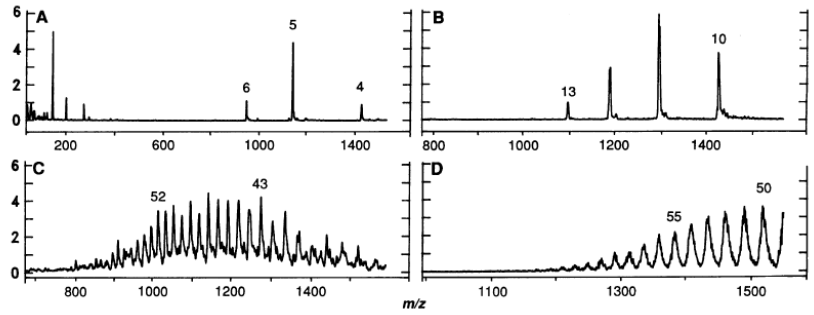

Fig. 6. Representative electrospray mass spectra for protein samples in acidified mixtures of water and methanol. The adduct ions were protons, and the solution flow rate was 1 or 2 μl/min. The spectra are the results of single, 30-s scans. Approximate molecular weight and amount of samples used in the spectrum are as follows: (**A**) insulin, 5,730, 1.7 pmol; (**B**) lysozyme, 14,300, 175 fmol; (**C**) α-anylase, 54,700, 1.7 pmol; and (**D**) conalbumin, 76,000, 200 fmol. See Table 1 for more data.

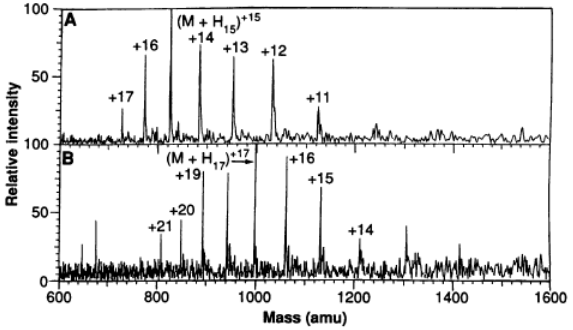

Fig. 9. Electrospray mass spectra obtained by coupling an ES source to a Fourier transform mass spectrometer (*24*): (**A**) cytochrome c (equine); calculated average molecular weight, 12,360.1; measured molecular weight, 12,356.9 ± 0.9; (**B**) myoglobin (equine skeletal muscle); calculated average molecular weight, 16,950.7; measured molecular weight, 16,947.5 ± 1.4.

EU FT-ICR MS

The advent of native MS

8534 *J. Am. Chem. Soc.* **1991**, *113*, 8534–8535

Observation of the Heme–Globin Complex in Native Myoglobin by Electrospray-Ionization Mass Spectrometry

Viswanatham Katta and Brian T. Chait*

The Rockefeller University, New York, New York 10021

Received May 8, 1991

In their native state, globular proteins are tightly folded, compact structures, certain of which may be characterized by the association of the globin with small, noncovalently bound cofactors and prosthetic groups. These proteins can be denatured and caused to unfold by subjecting them to high temperatures, extremes of pH, detergents, and solutions containing high concentrations of compounds like urea, guanidinium chloride, and organic solvents.[1] As the severity of the denaturing conditions is increased, the interaction between the globin and the cofactor can be weakened, with possible separation of the cofactor from the globin. For example, the oxygen-carrying protein myoglobin contains a noncovalently bound heme group in the hydrophobic pocket of the native globin chain that can be induced to unfold under acidic conditions, thus weakening the heme–globin interaction. Under these conditions, the heme moiety can be readily extracted into an organic phase, and this phenomenon forms the basis of widely used procedures for preparing apomyoglobin from the native proteins.[2,3]

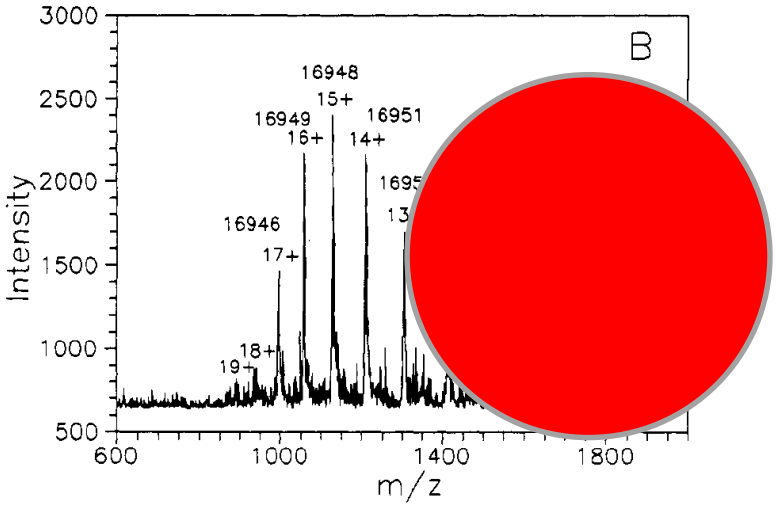

Figure 1. Electrospray-ionization mass spectra of equine skeletal muscle myoglobin obtained from aqueous solutions at different pH values: (A) 3.35; (B) 3.90. The protein concentration is 20–40 μM. The peaks are labeled with the protonation state, n+, and the number of protons, n, attached to the protein molecule. The circled protonation states designate the peaks corresponding to the intact heme–globin complex in myoglobin. The most intense peaks are also labeled with the molecular masses determinated from the measured m/z values.

EU FT-ICR MS

The advent of native MS

Detection of Noncovalent Receptor–Ligand Complexes by Mass Spectrometry

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Cornell University, 925 Warren Drive
Ithaca, New York 14850
Received April 24, 1991

Few methods are known for detecting and identifying enzyme–substrate, receptor–ligand, and antibody–antigen complexes, whose weak noncovalent interactions constitute the essential basis of molecular recognition in the biological world. With newer ionization techniques, mass spectrometry (MS) can be applied to problems of biological interest;[1] however, efforts to date have focused on sequencing carbohydrates,[2] oligonucleotides,[3] peptides, and proteins[4] and detecting other macromolecules.[5]

The newly developed technique of ion-spray (pneumatically assisted electrospray) MS forms gas-phase macromolecular ions directly from solution at atmospheric pressure via protonation and ion evaporation.[6,7] In contrast to electrospray, ion-spray MS can be performed in water without cosolvent, which is ideal for most biological systems. Multiple charging produces a family of molecular ions and dramatically reduces the mass-to-charge ratio, so that even quadrupole mass spectrometers having a typical mass range of 1000–2000 daltons (Da) can determine high MW species with unit mass resolution.

Since ionization of the targeted molecular species occurs under very mild conditions, fragmentation is usually not observed in ion-spray MS experiments, thus suggesting that noncovalent molecular association complexes might be detectable under conditions of real-time[8] reaction monitoring. Here we describe the first successful application of such a technique to a problem of considerable contemporary interest. The method should prove useful in probing a wide variety of macromolecular host–guest interactions.

The macrolides FK506 **1** (MW 804 Da, Figure 1) and rapamycin **2** (RM, MW 913 Da) are promising new immunosuppressive agents with approximately 100-fold better activity than the widely used immunosuppressant cyclosporin A.[9] Both **1** and **2** inhibit T-cell activation in a complex series of events triggered by binding of the drugs to their naturally occurring cytoplasmic receptor FKBP, a member of the immunophilin family of im-

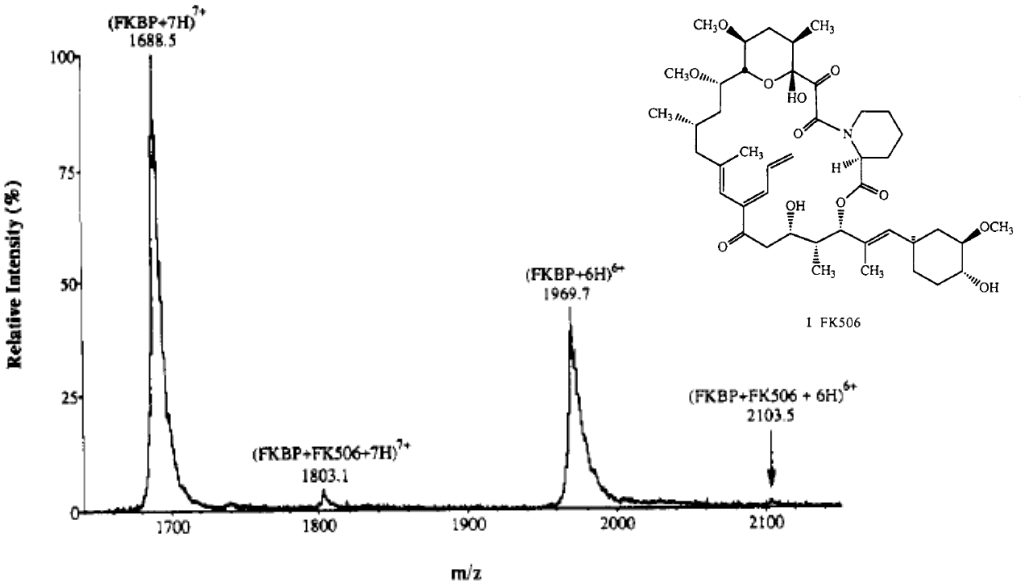


Figure 3. Ion-spray mass spectrum of FKBP + FK506 at pH 7.5.

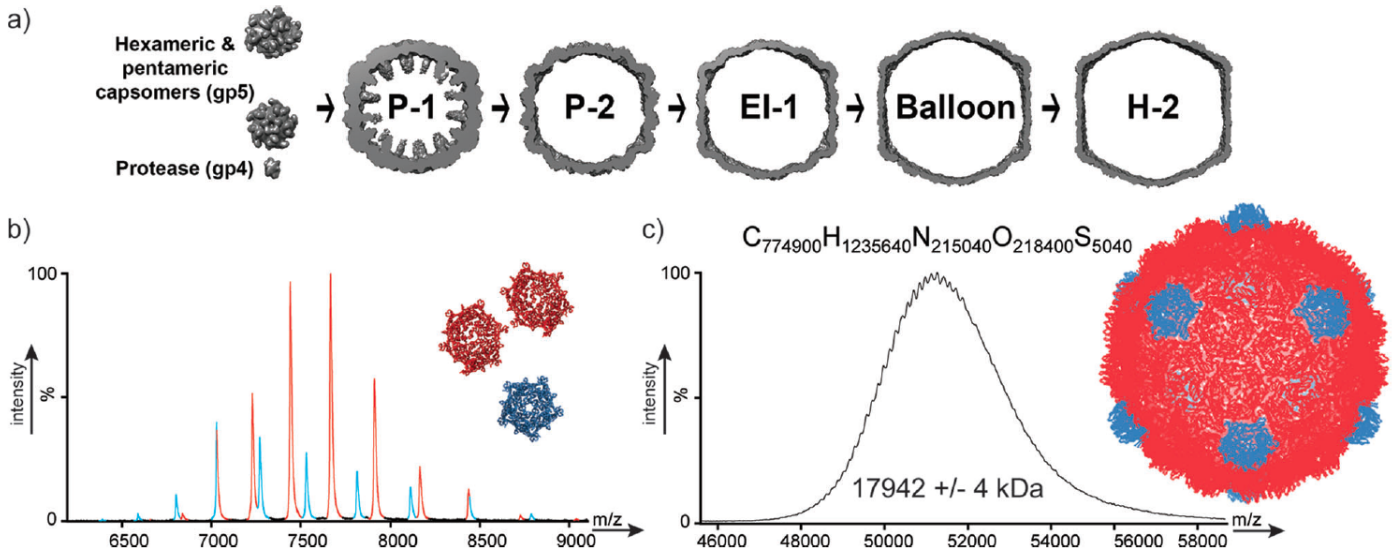
"The newly developed technique of ion-spray (pneumatically assisted electrospray) MS forms gas-phase macromolecular ions directly from solution…
… suggesting that noncovalent molecular association complexes might be detectable.

Ganem B, *JACS* **1991**, 113, 6294-6296

EU FT-ICR MS

Largest native MS complex todate

The largest biomolecular assembly recorded to date with native MS: An 18-MDa viral capsid of the bacteriophage HK97



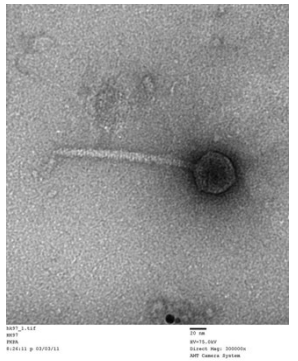


Figure 1. Monitoring the assembly of HK97 capsids with native ESI-MS. a) Assembly and maturation pathway of HK97. b) Free capsomers with penton signal in blue and hexon signal in red. c) Intact Prohead-1 particle. A well-resolved series of charge states is observed allowing the accurate mass calculation.

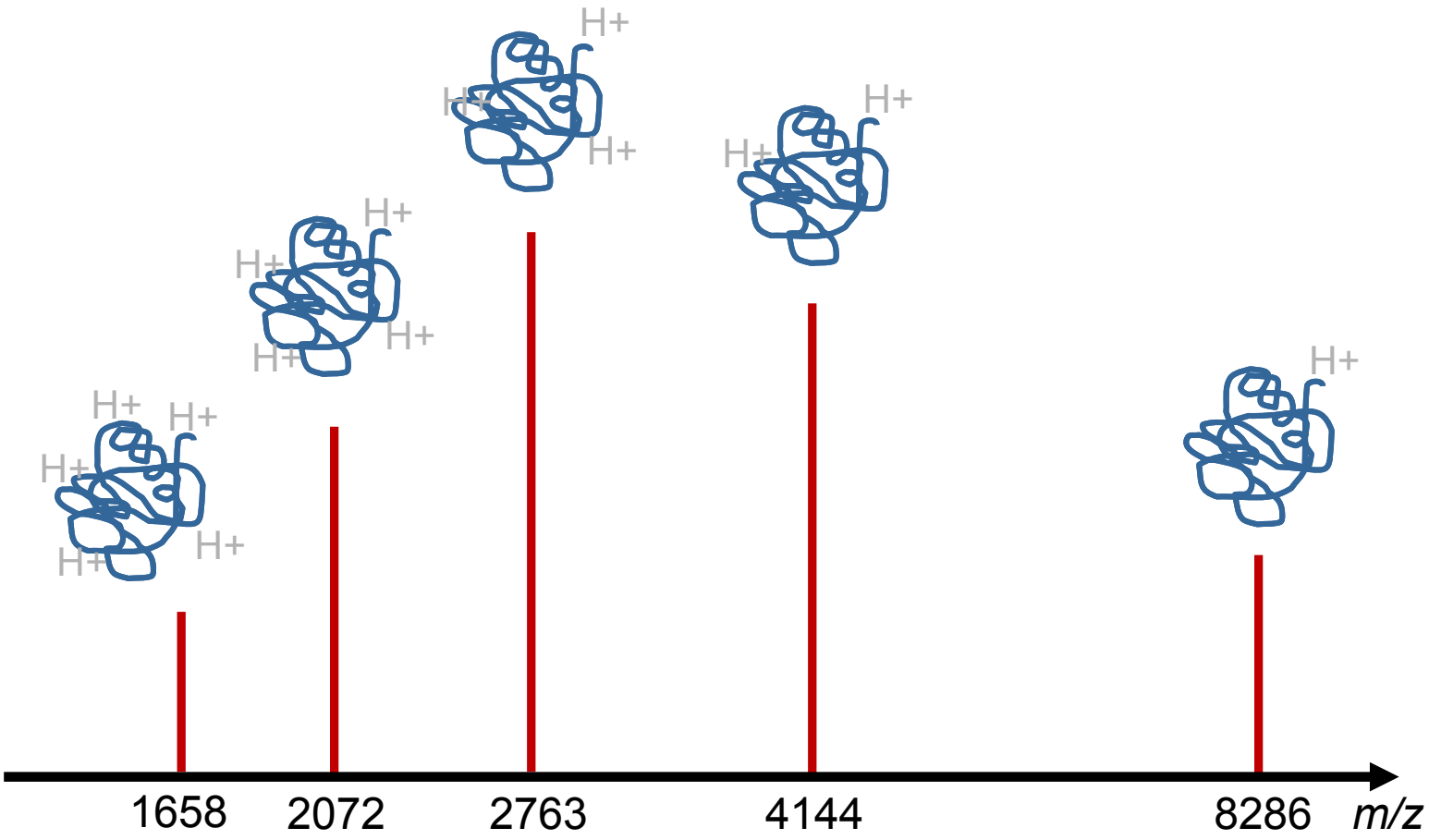
Intact HK97 capsids measured in 12.5 mM ammonium acetate (pH 7) having a collision voltage of 500 V in the desolvation region of the ESI source with Xe as the collision gas.

Snijder et al, *Angewandte Chem. Int. Ed.* **2013**, 52: 1–5

EU FT-ICR MS

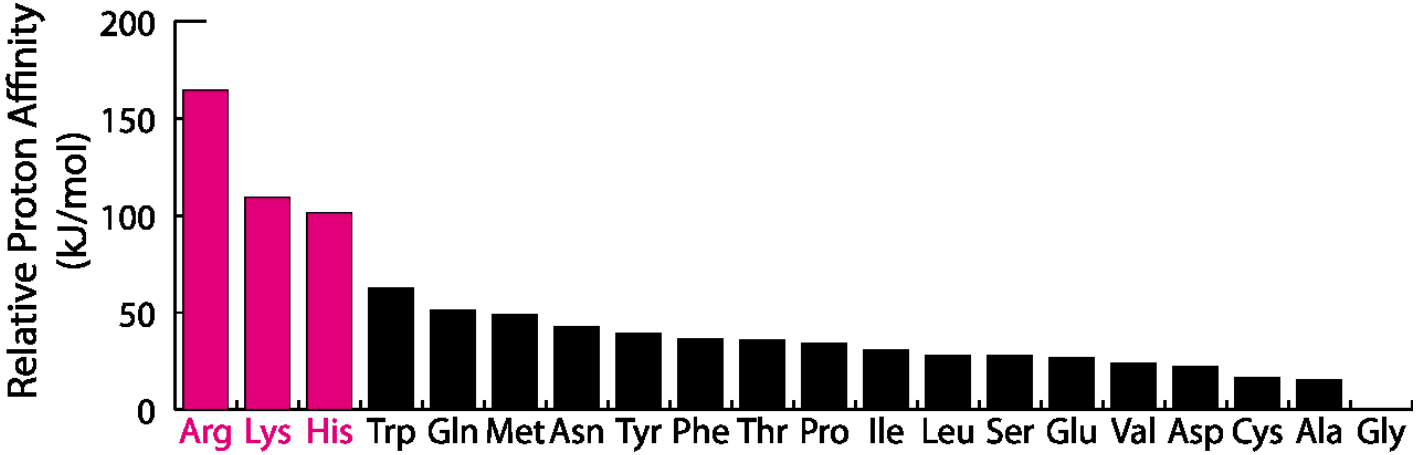
Charge state distribution (CSD)

Small protein, average mass 8285 Da



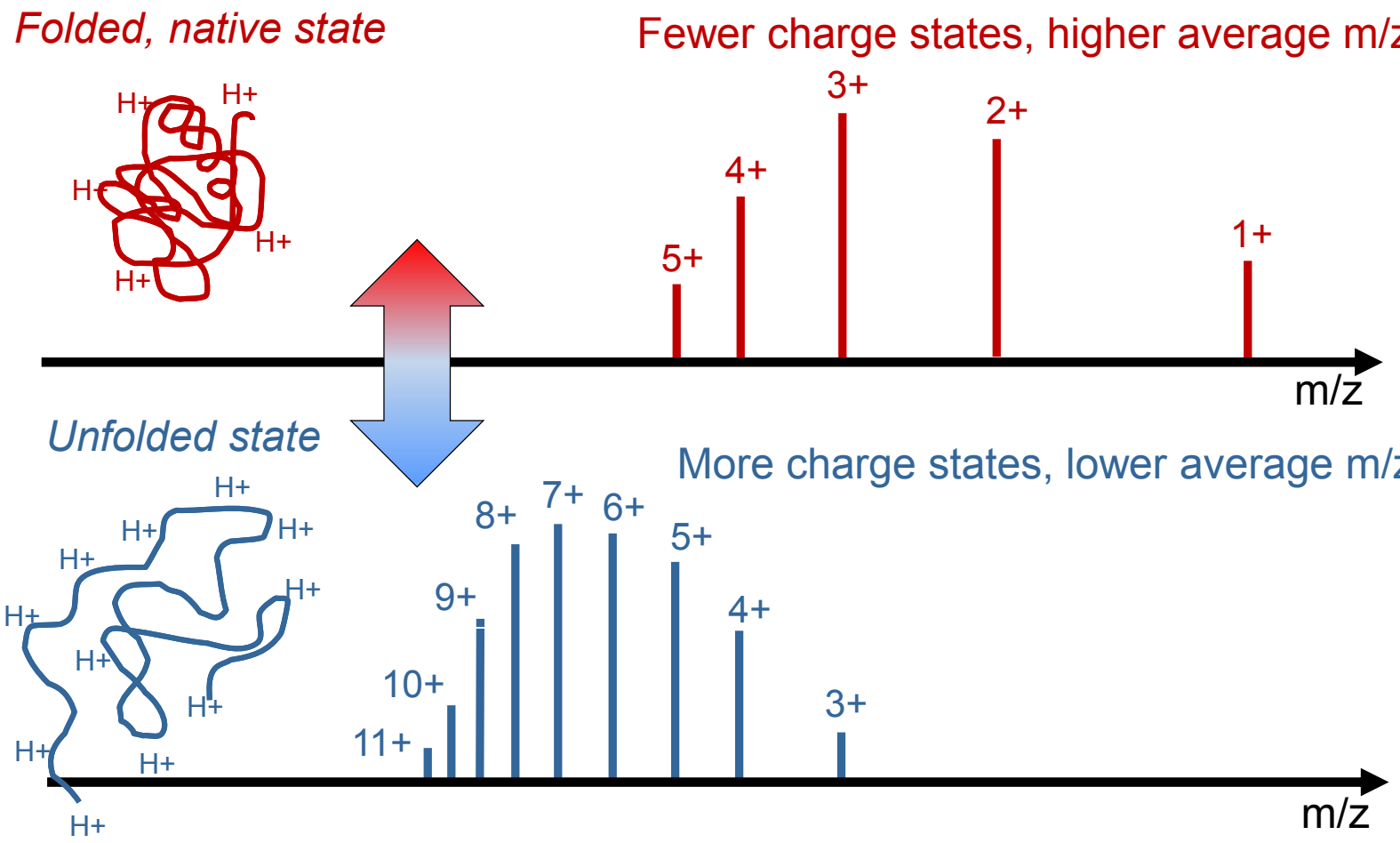
Charge state distribution (CSD)

- ESI of polypeptides gives rise to multiply-charged ions $[M + nH]^+$, where n = 1,2,3…
  - Average <*n*> depends on polypeptide sequence & conformation
  - CSD is dependent on *relative proton affinities* of amino acid residues; thus, it is not easy to predict max. and av. charge state



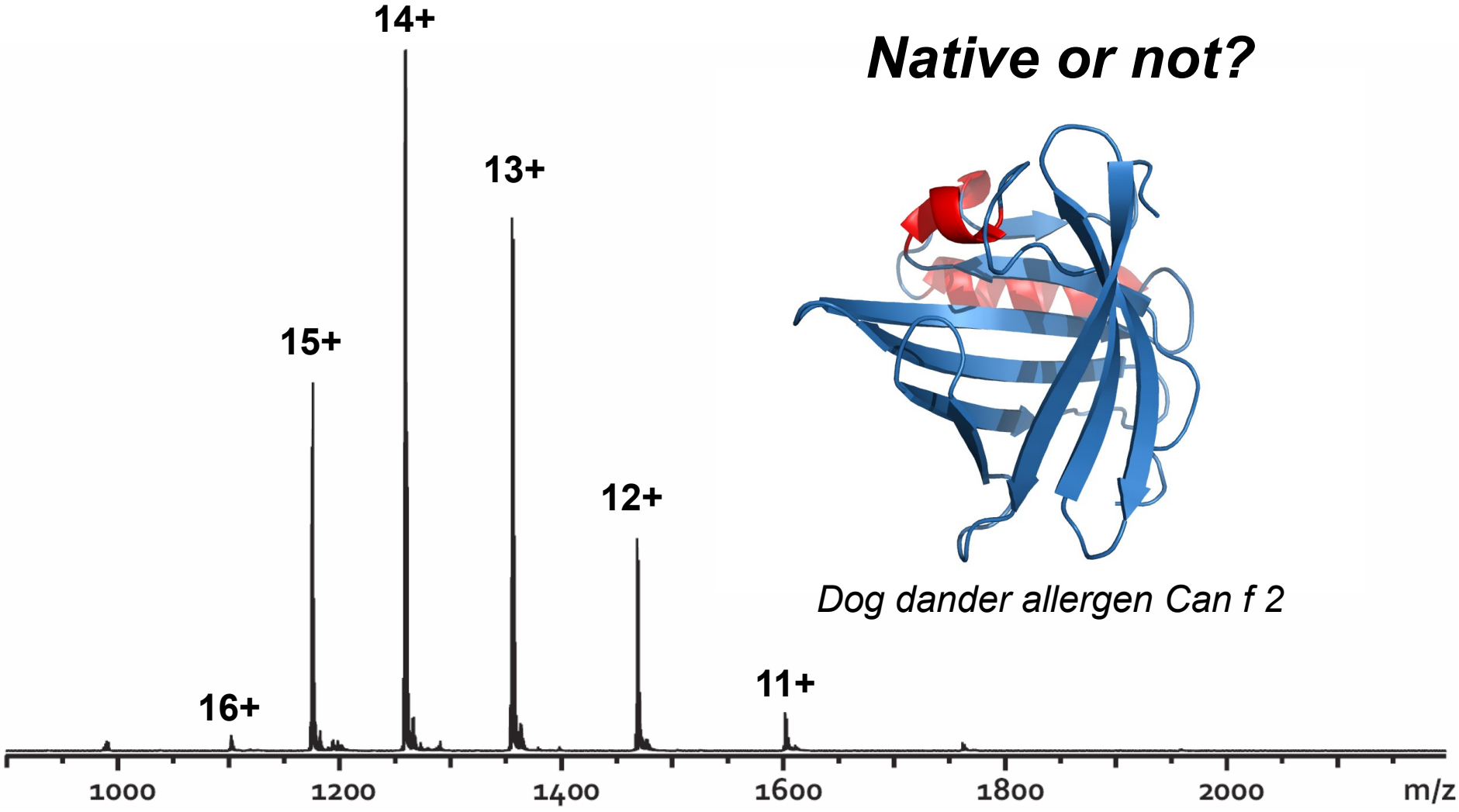
EU FT-ICR MS

Protein ion CSD: a probe for polypeptide conformation



EU FT-ICR MS

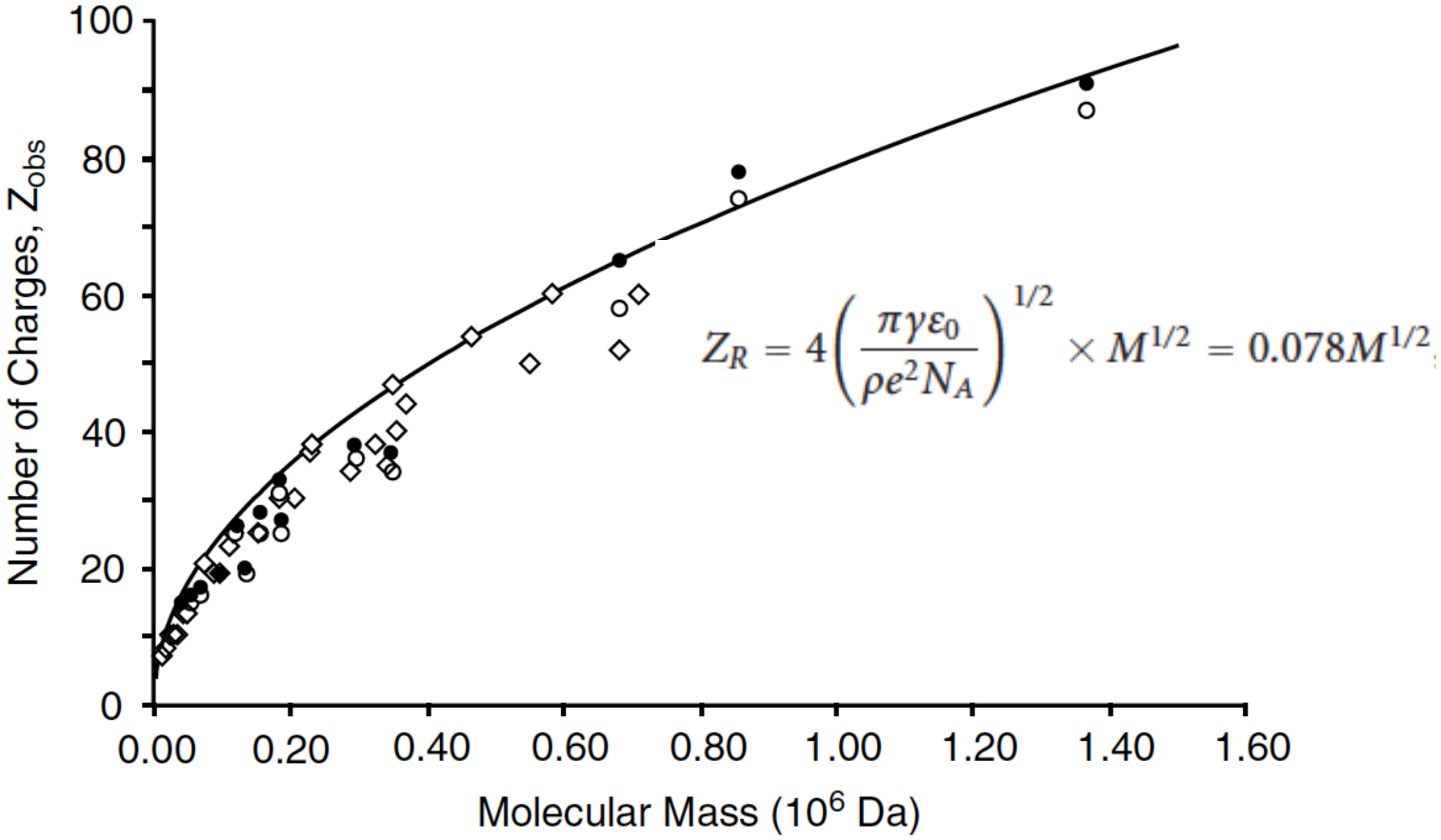
Protein ion CSD: a probe for polypeptide conformation



Native or not?

Dog dander allergen Can f 2

Charge state distribution (CSD)



De La Mora, F. *Anal. Chim. Acta*, **2000**, 406: 93-104.

EU FT-ICR MS

Charge state distribution (CSD)

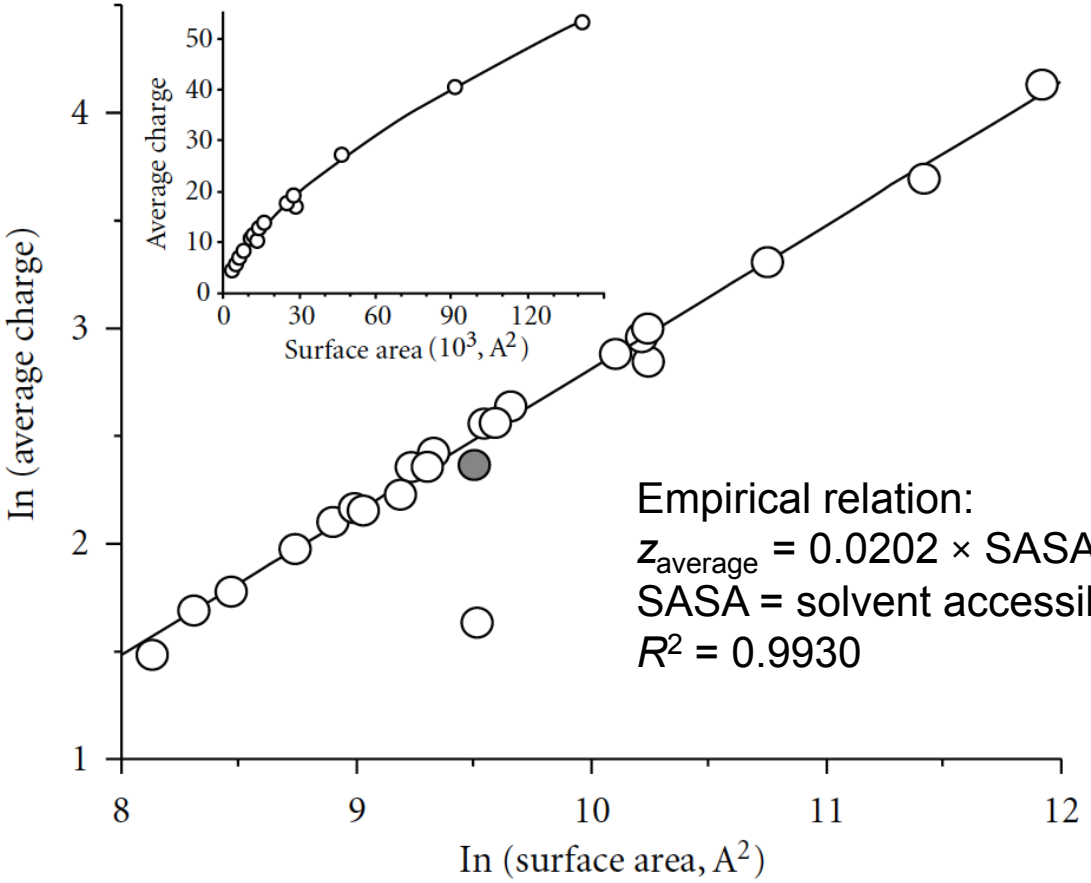
Table 1. Proteins Used To Obtain a Charge–Surface Correlation Shown in Figure 2

protein	PDB id	no. of basic residues (R, K, H)	no. of acidic residues (D, E)	av charge of protein ions	surface area (Å²) (crystal structure)
insulin (bovine)	1APH	1, 1, 2	0, 4	4.4	3396
chymotrypsin inhibitor II	1CIQ	4, 6, 0	4, 6	5.42	4052
ubiquitin (human)	1UBQ	4, 7, 1	5, 6	5.9	4758
cytochrome C (equine)	1HRC	2, 19, 3	3, 9	7.2	6232
cellular retinoic acid binding protein I (mice)	1CBI	9, 9, 2	8, 14	8.13	7341
β-lactoglobulin A (bovine)	1BSY	3, 15, 2	11, 16	8.65	8321
myoglobin (equine)	1WLA	2, 19, 11	8, 13	8.7	8017
H-chain of ferritin (human)	2FHA	7, 12, 10	15, 16	9.33	9816
α1 chymotrypsinogen (bovine)	1EX3	4, 14, 2	9, 5	10.56	10317
ligand binding domain of retinoic acid receptor (human)	3LBD	16, 13, 10	15, 20	11.17	11230
carbonic anhydrase I (human)	2CAB	7, 18, 11	14, 13	10.54	10997
pepsin (porcine)	5PEP	2, 1, 1	28, 13	10.74	13351
β-lactoglobulin dimer (bovine)	1BEB	6, 30, 4	22, 32	12.9	14698
apotransferrin, N-lobe (human)	1BTJ	12, 27, 9	25, 17	13.1	14970
holotransferrin, N-lobe (human)	1A8E	12, 27, 9	25, 17	12.93	13973
ovalbumin (hen egg)	1OVA	15, 20, 7	14, 33	13.89	15586
serum albumin (human)	1AO6	24, 59, 16	36, 62	17.2	28103
hemoglobin (human)	1A3N	12, 44, 38	30, 24	17.8	24548
transferrin (rabbit)	1JNF	26, 57, 18	43, 46	19.8	27395
soluble transferrin receptor (human)	1CX8	48, 88, 24	74, 72	27.2	46530
transferrin–transferrin receptor complex (human)	1SUV[a]	100, 202, 62	166, 154	40.5	91258
H-chain ferritin 24-mer (human)	2FHA	168, 288, 240	360, 384	62.16	141541

[a] Cryo-EM structure of the assembly is refined using X-ray structures of individual subunits.

Kaltashov I. A. and Mohimen, A. *Anal. Chem.* **2005**, 77: 5370-5379

Charge state distribution (CSD)



Empirical relation:

$z_{average} = 0.0202 \times SASA\ (Å^2)^{0.6715}$

SASA = solvent accessible surface area

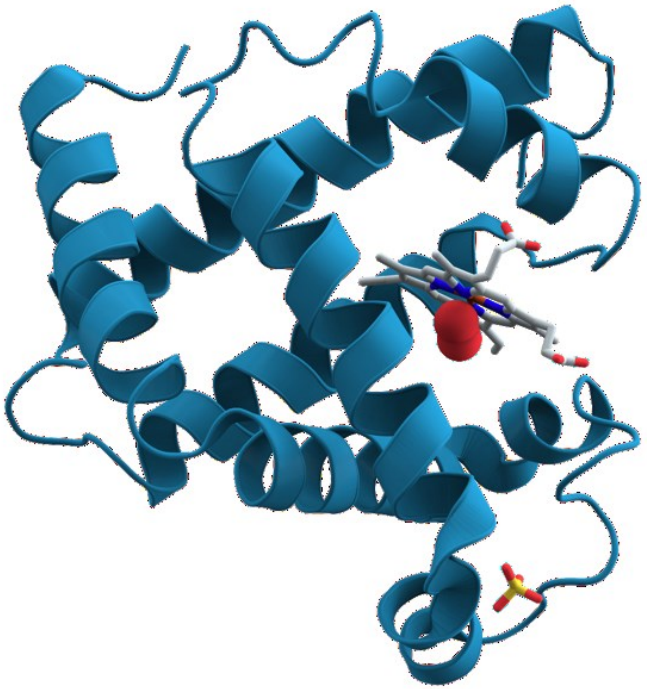
$R^2 = 0.9930$

Kaltashov I. A. and Mohimen, A. *Anal. Chem.* **2005**, 77: 5370-5379

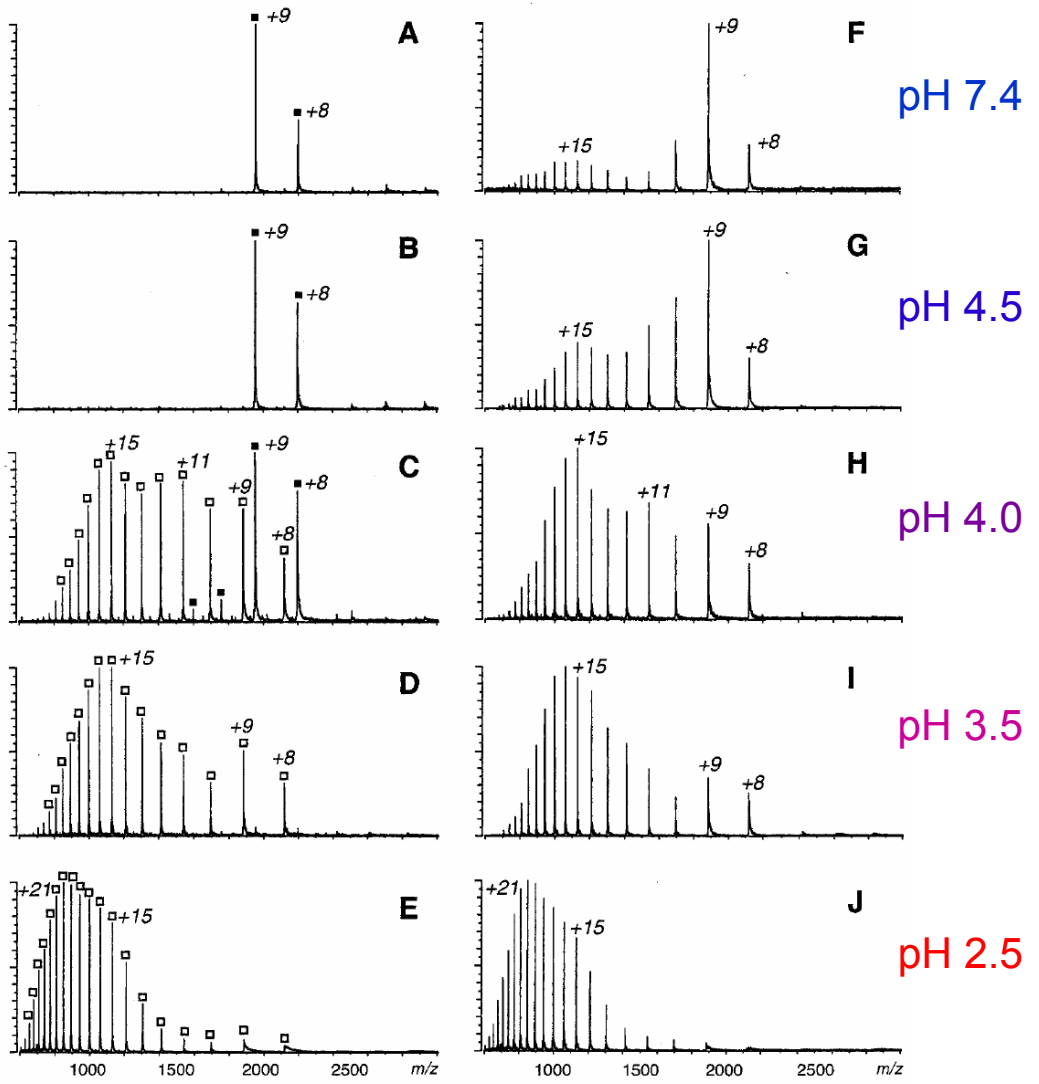
EU FT-ICR MS

Monitoring conformational changes by CSD analysis

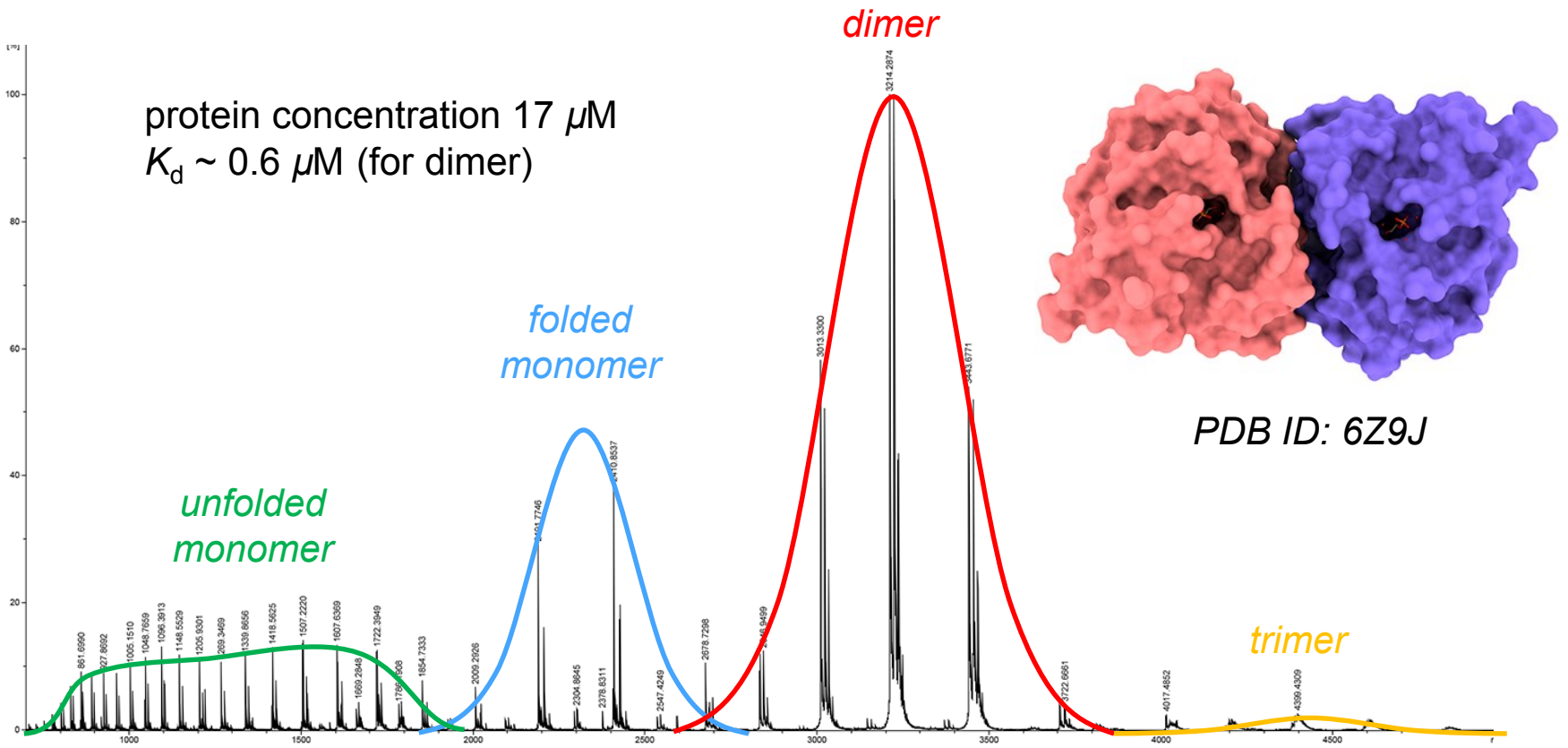
Acid-induced unfolding of myoglobin:
A–E, holo-Mb; F–J: apo-Mb



Konermann *et al.* JASMS 1995



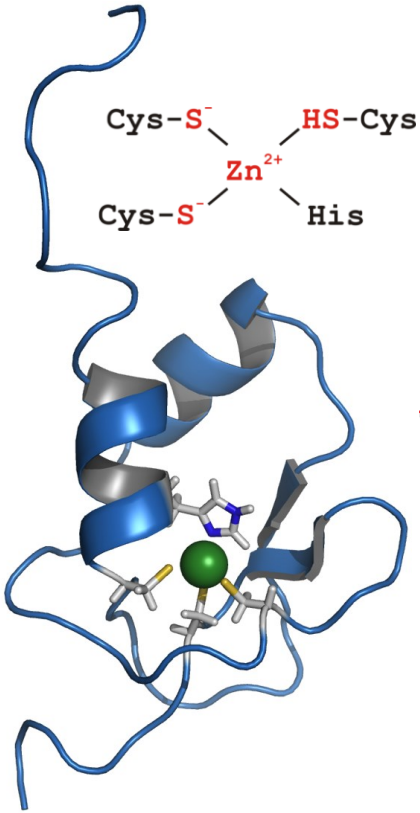
Geobacillus sp. deoxyribose-phosphate aldolase (DERA)
Different structural states of DERA are resolvable



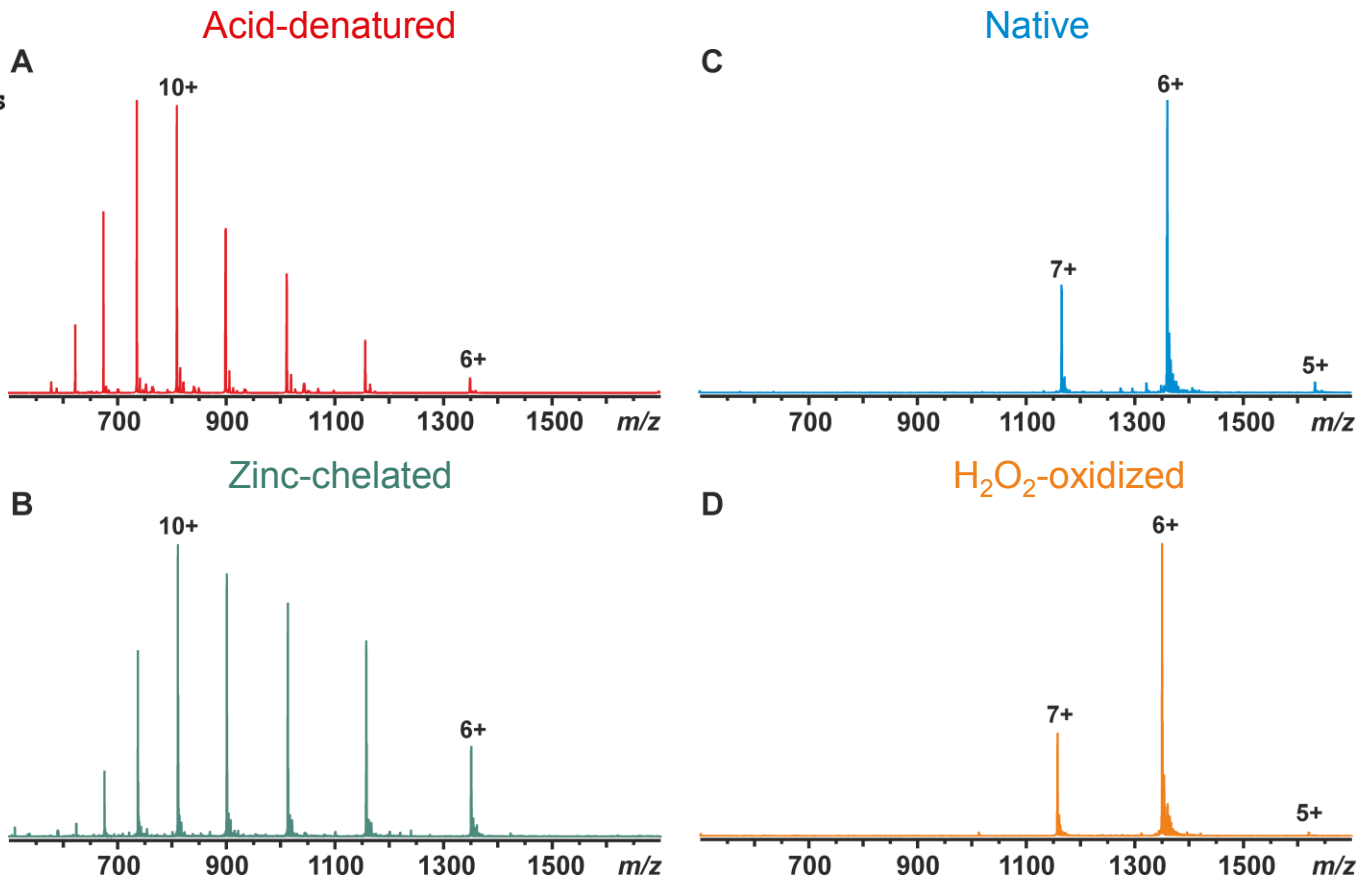
Courtesy of Johan Pääkkönen

EU FT-ICR MS

Metalloproteins: SAP30L zinc finger domain



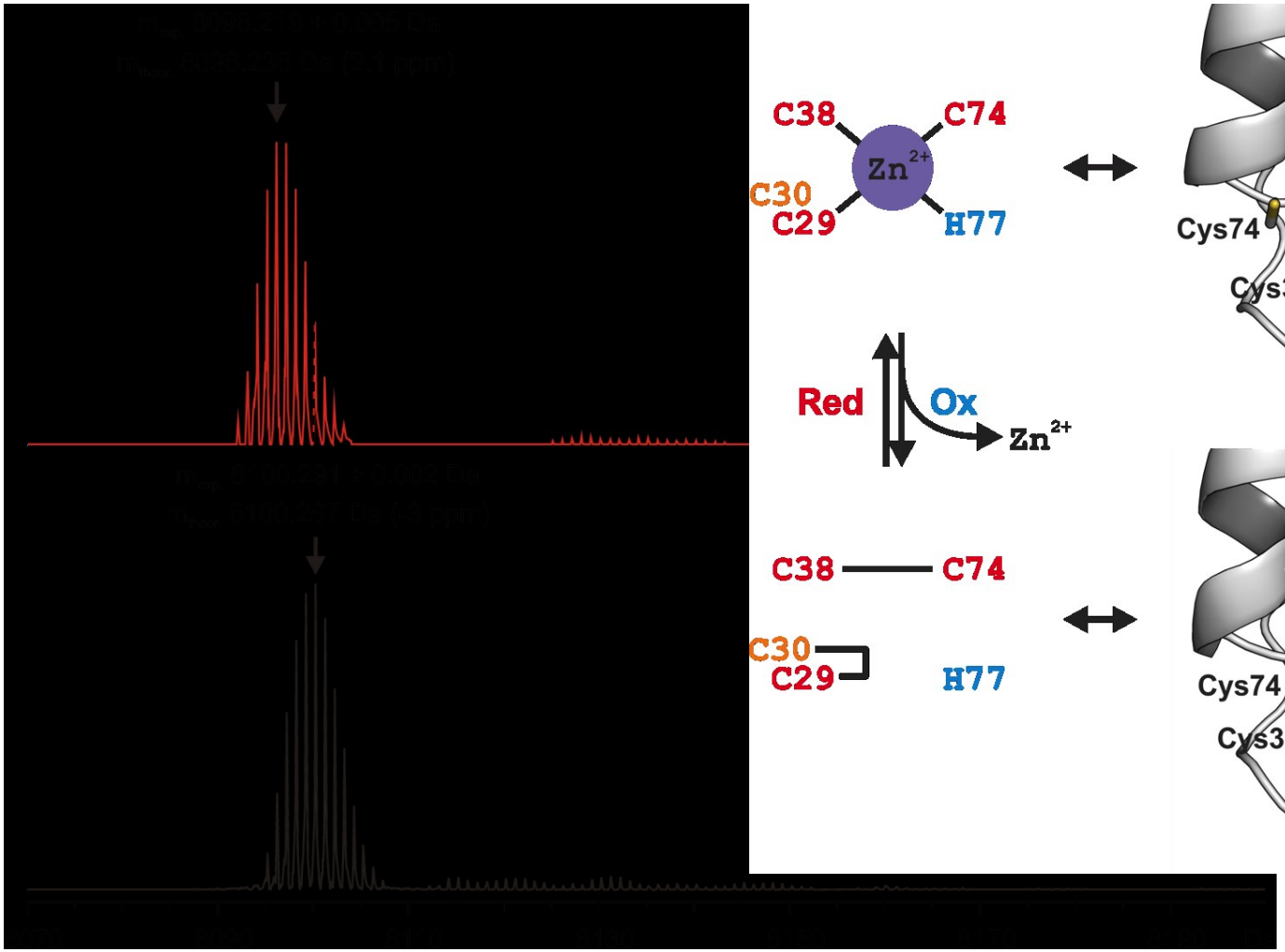
Solution NMR structure, PDB: 2N1U



Laitaoja M, et al. *Protein Sci.* **2016**, 25: 572-586.

EU FT-ICR MS

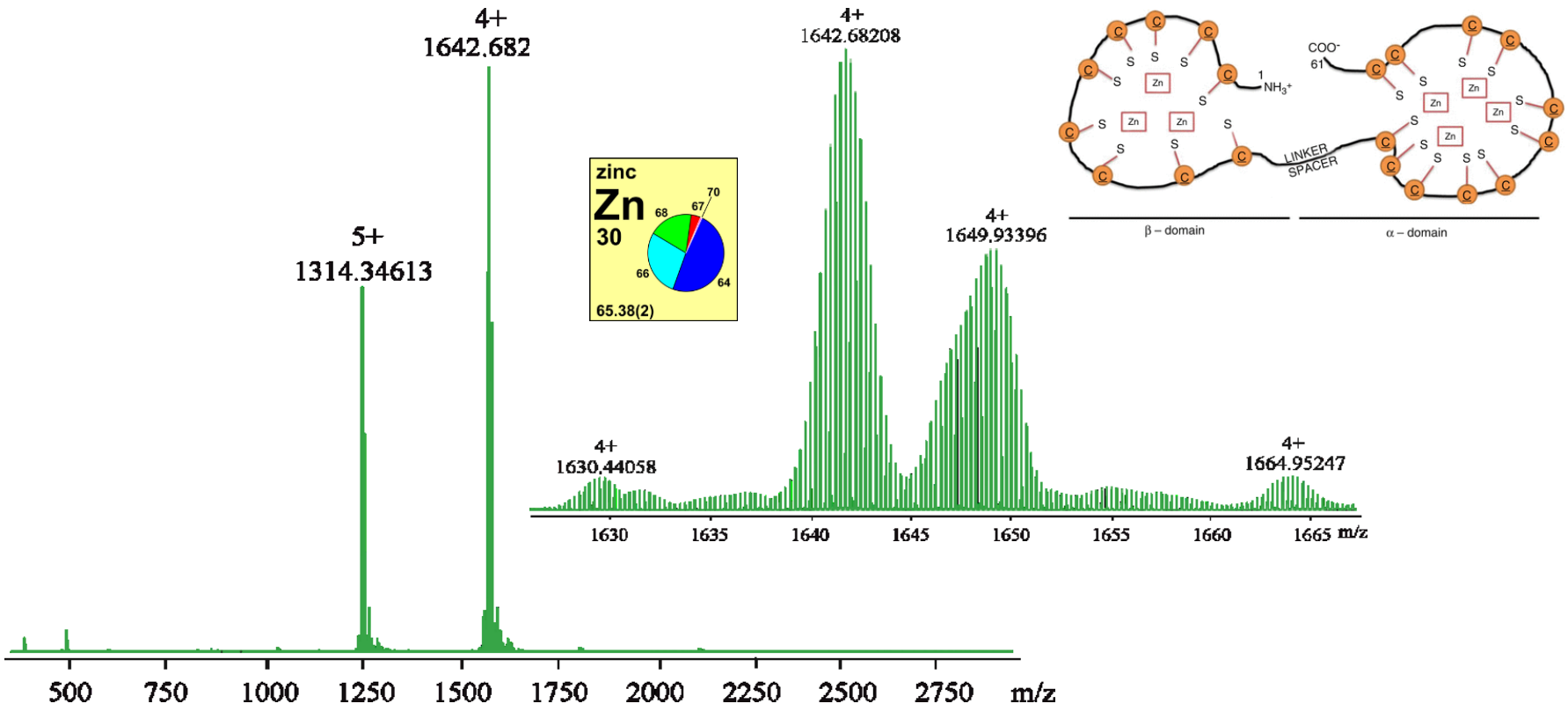
Metalloproteins: ESI FT-ICR MS of SAP30L



Laitaoja M, et al. *Protein Sci.* **2016**, 25: 572-586.

Metalloproteins: rabbit liver metallothionein

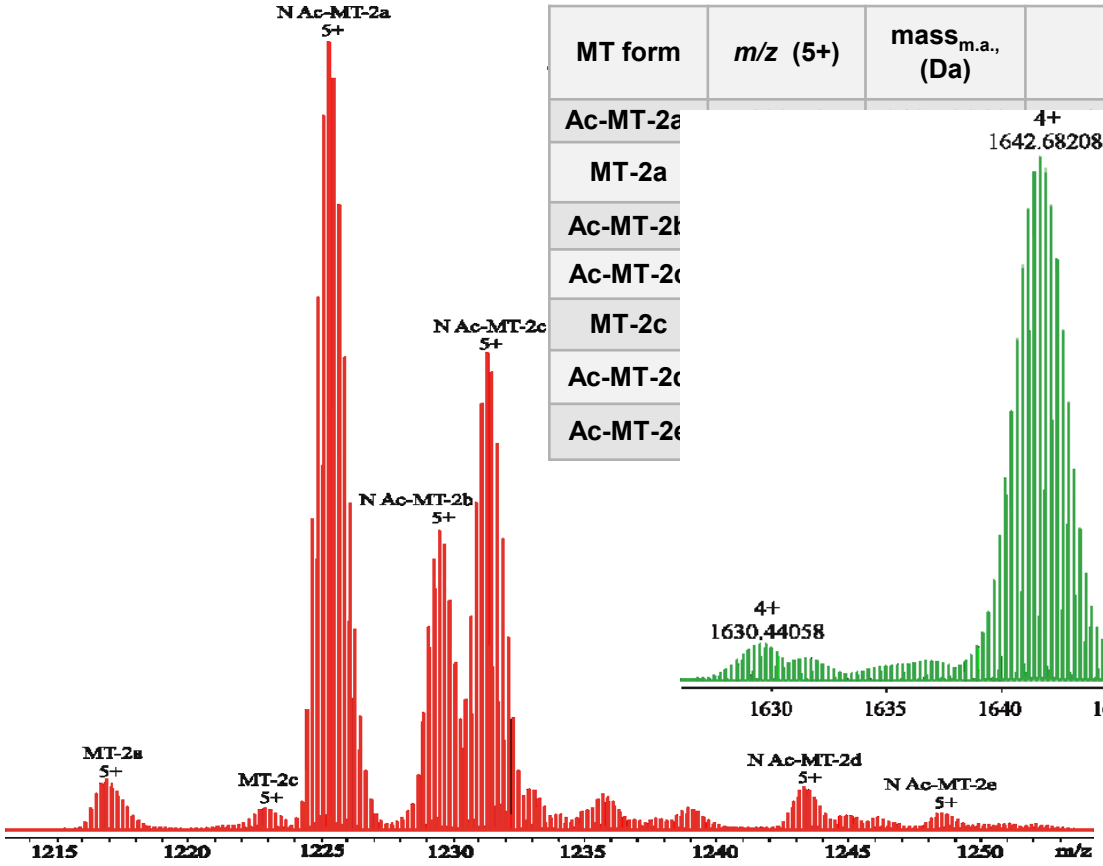
Desalted rabbit liver extract MT-Zn_7 measured in 20 mM NH_4AOc (pH 7) + 20 mM DTT shows broad peak distribution of several overlapping species

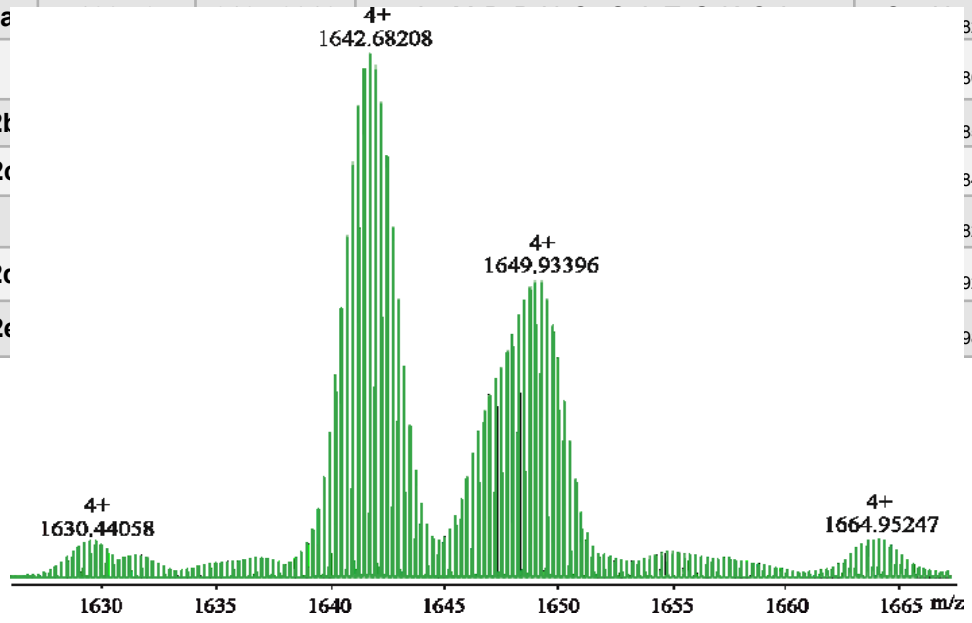


Metalloproteins: rabbit liver metallothionein

Fully demetallated (1 mM 1,10-FEN) rabbit MT shows presence of 9 different proteoforms!

MT form	*m/z* (5+)	$mass_{m.a.}$, (Da)	Amino acid Composition	Molecular Formula
Ac-MT-2a				$_{32}N_{72}O_{84}S_{21}$
MT-2a				$_{30}N_{72}O_{83}S_{21}$
Ac-MT-2b				$_{35}N_{71}O_{86}S_{21}$
Ac-MT-2c				$_{34}N_{72}O_{85}S_{21}$
MT-2c				$_{32}N_{72}O_{84}S_{21}$
Ac-MT-2d				$_{32}N_{74}O_{85}S_{21}$
Ac-MT-2e				$_{98}N_{74}O_{84}S_{21}$



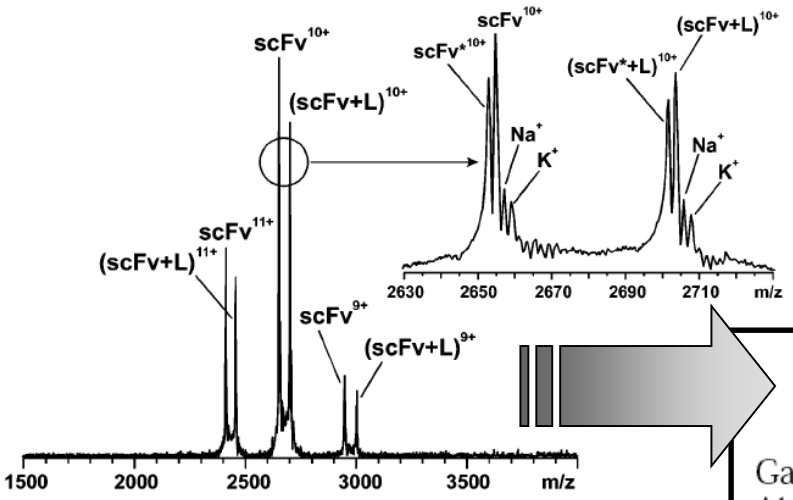


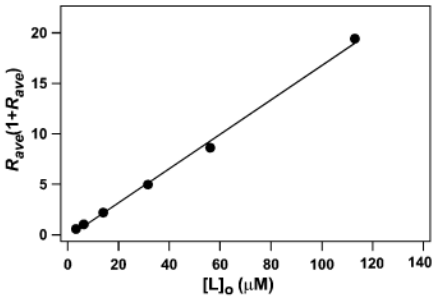
Manuscript in preparation

EU FT-ICR MS

Protein–ligand interactions: Bidning affinity by ESI-MS

- Example: Determination of binding constants for antibody fragment–trisaccharide complexes by direct ESI-MS analysis





$$K_{assoc} = \frac{[PL]_{equil}}{[P]_{equil}[L]_{equil}} = \frac{R}{[L]_{equil}} = \frac{R(1+R)}{[P]_0}$$

$$R = \frac{[PL]_{equil}}{[P]_{equil}} = \frac{\sum_n (I_{(P\cdot L)^{n+}}/n)}{\sum_n (I_{(P)^{n+}}/n)}$$

ligand	$K_{assoc} \times 10^{-5}$ M^{-1} (MS)	$K_{assoc} \times 10^{-5}$ M^{-1} (ITC)[a]
Galα[Abe]Man	1.70 ± 0.05	1.6 ± 0.2
Abe(2-O−CH_3−Man)	1.50 ± 0.20	1.43 ± 0.05
Glcβ[Abe]Man	0.15 ± 0.04	0.30 ± 0.14
GlcGlcGalα[Abe]Man	5.30 ± 0.50	3.81 ± 0.13

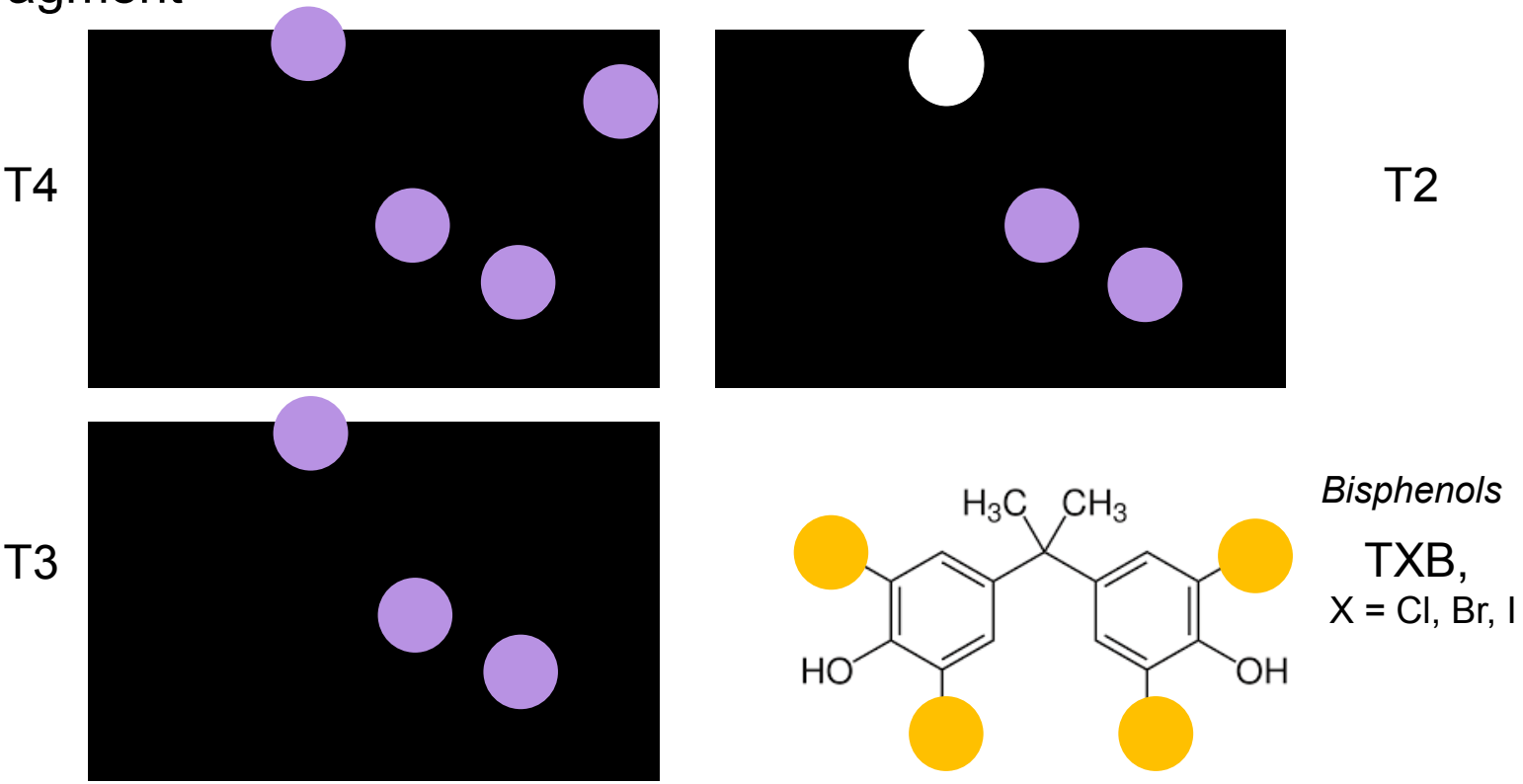
Good agreement between ESI-MS (gas-phase) and ITC (solution)!

W. Wang, E.N. Kitova and J.S. Klassen. *Methods Enzymol.* **2003**, 362

EU FT-ICR MS

Protein–ligand interactions: Bidning affinity by ESI-MS

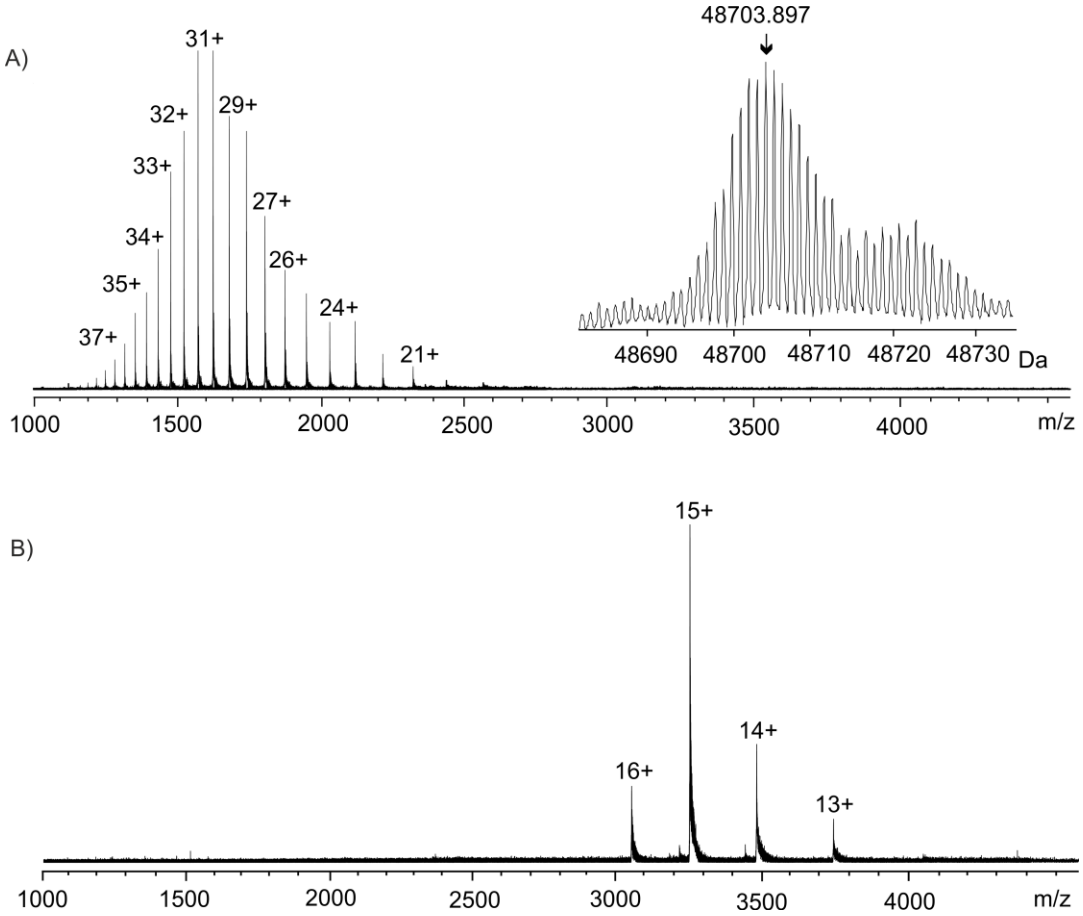
Ligand binding preference and cross-reactivity of anti-thyroxine antibody Fab fragment



Thangaraj S *et al* Quantitation of thyroid hormone binding to anti-thyroxine antibody Fab fragment by native mass spectrometry. *ACS Omega* 4 (2019) 18718-18724.

EU FT-ICR MS

Protein–ligand interactions: Bidning affinity by ESI-MS

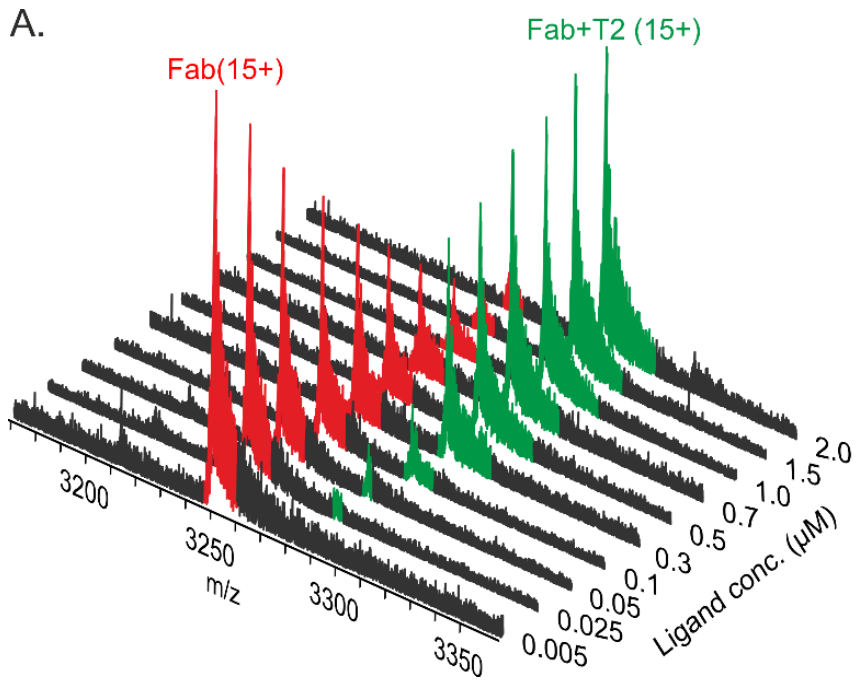


Thangaraj S *et al* Quantitation of thyroid hormone binding to anti-thyroxine antibody Fab fragment by native mass spectrometry. *ACS Omega* 4 (2019) 18718-18724.

EU FT-ICR MS

Protein–ligand interactions: Anti-T4 Fab fragment

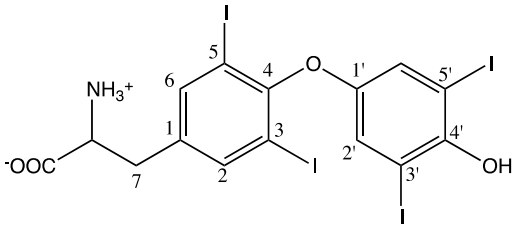
Direct ligand titration of anti-thyroxine antibody Fab fragment with 3,3'-diodo-L-thyronine (T2) – *a reference ligand*



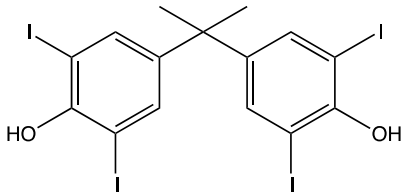
Thangaraj S *et al* Quantitation of thyroid hormone binding to anti-thyroxine antibody Fab fragment by native mass spectrometry. *ACS Omega* 4 (2019) 18718-18724.

EU FT-ICR MS

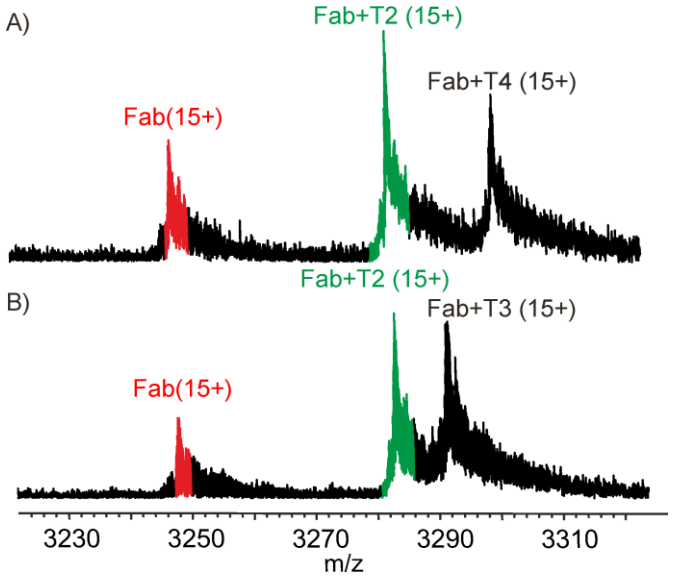
Protein–ligand interactions: Anti-T4 Fab fragment



Thyroxine (T4)



Tetraiodobisphenol A

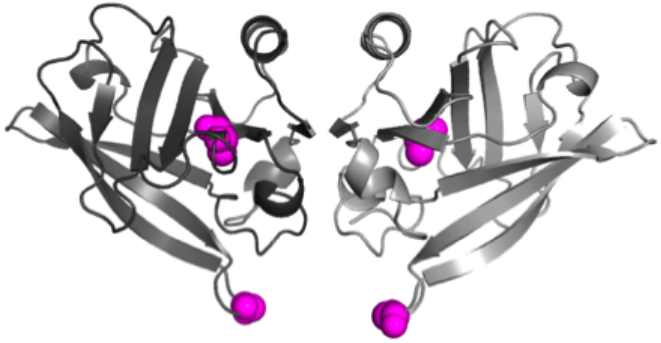


Ligand competition experiments

Ligand	Ligand conc. (μM)	Ref. ligand (T_2) conc. (μM)	K_d (nM)
T_4	0.07	0.5	29 ± 4
T_3	0.05	0.5	3.4 ± 0.7
T_2	0.005 to 2.0	–	260 ± 20
T_0	12	0.07	130000 ± 10000
TIB	0.1	0.7	11 ± 1
TBB	0.5	1.2	87 ± 5
TCB	10	0.4	7800 ± 500

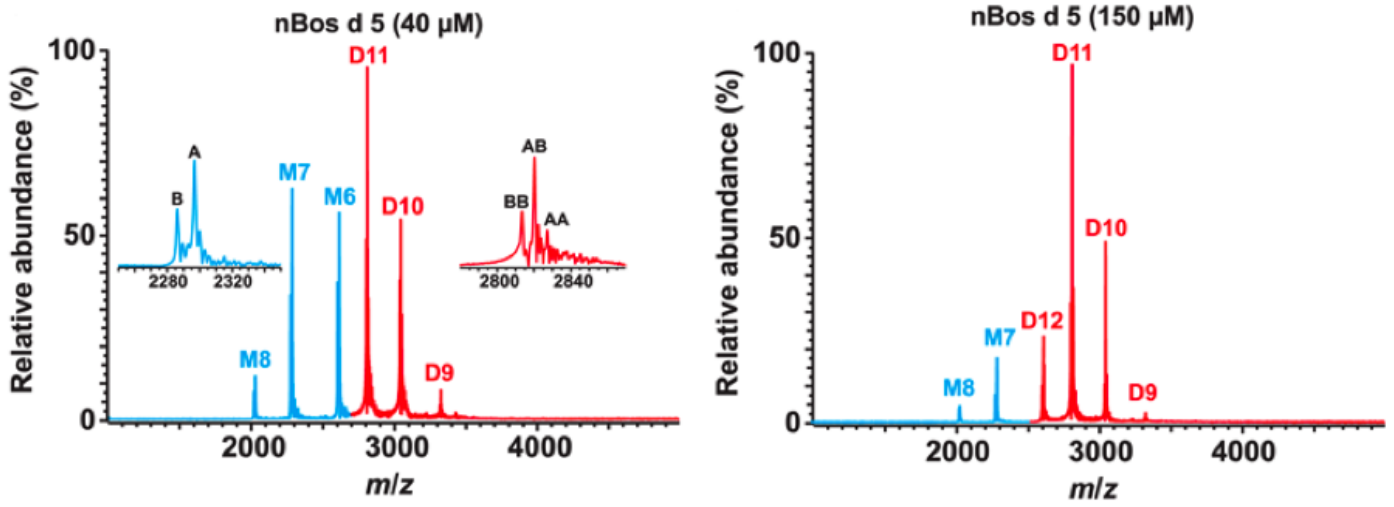
Thangaraj S *et al* Quantitation of thyroid hormone binding to anti-thyroxine antibody Fab fragment by native mass spectrometry. *ACS Omega* 4 (2019) 18718-18724.

Transient homodimerization – β-lactoglobulin (Bos d 5)



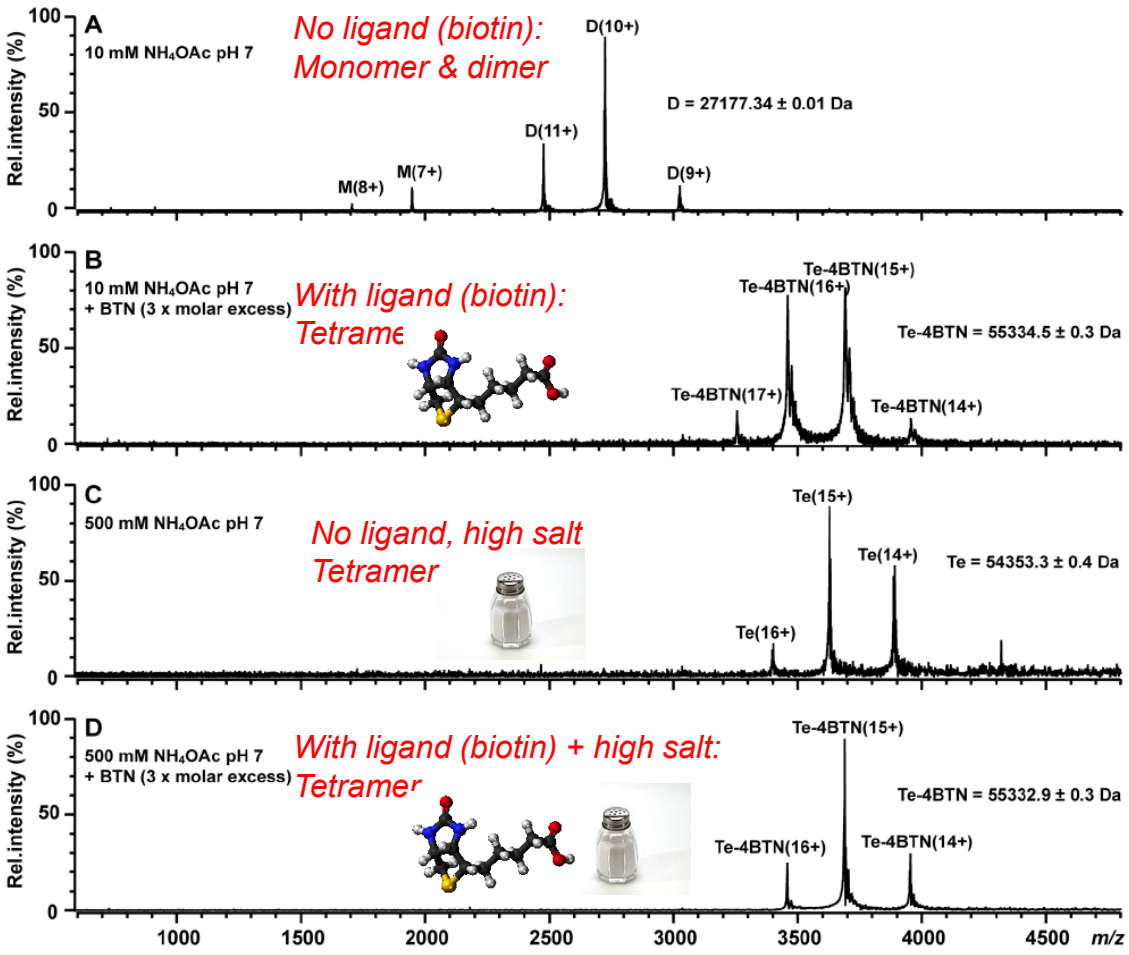
BLG dimer (X-ray structure)

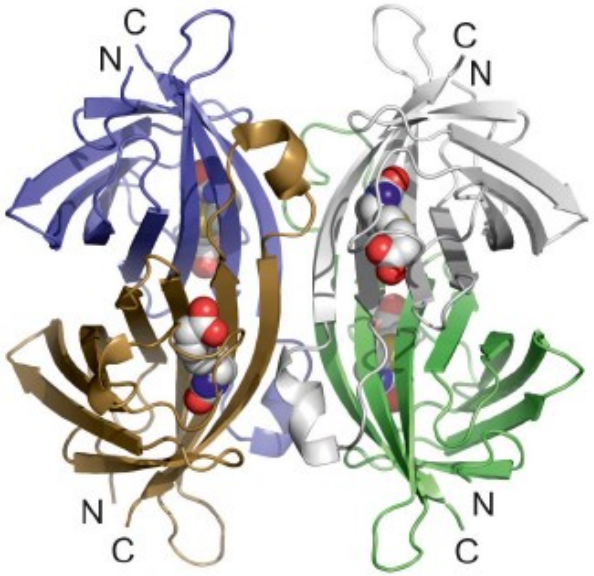
Native-MS of bovine beta-lactoglobulin at two concentrations: blue = monomer, red = dimer



Rouvinen, J., Jänis J., et al. *PLoS ONE* 5 (**2010**) e9037.

Transient oligomerization – zebrafish avidin

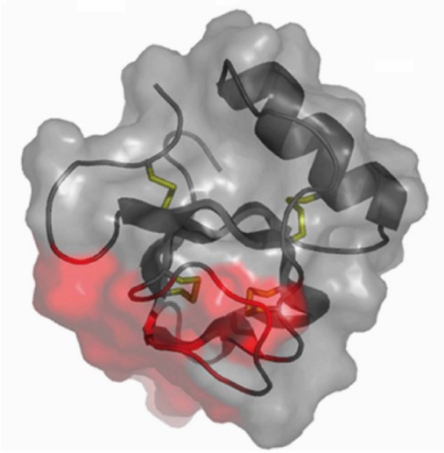


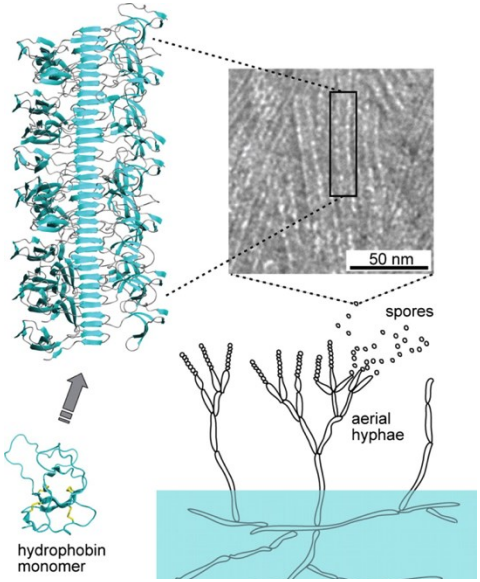


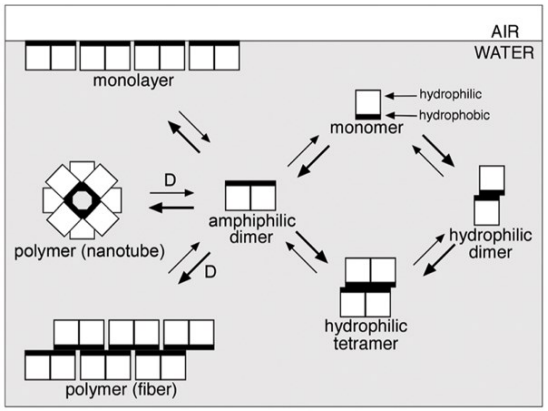
X-ray structure of zebavidin tetramer (PDB entry 2UYW)

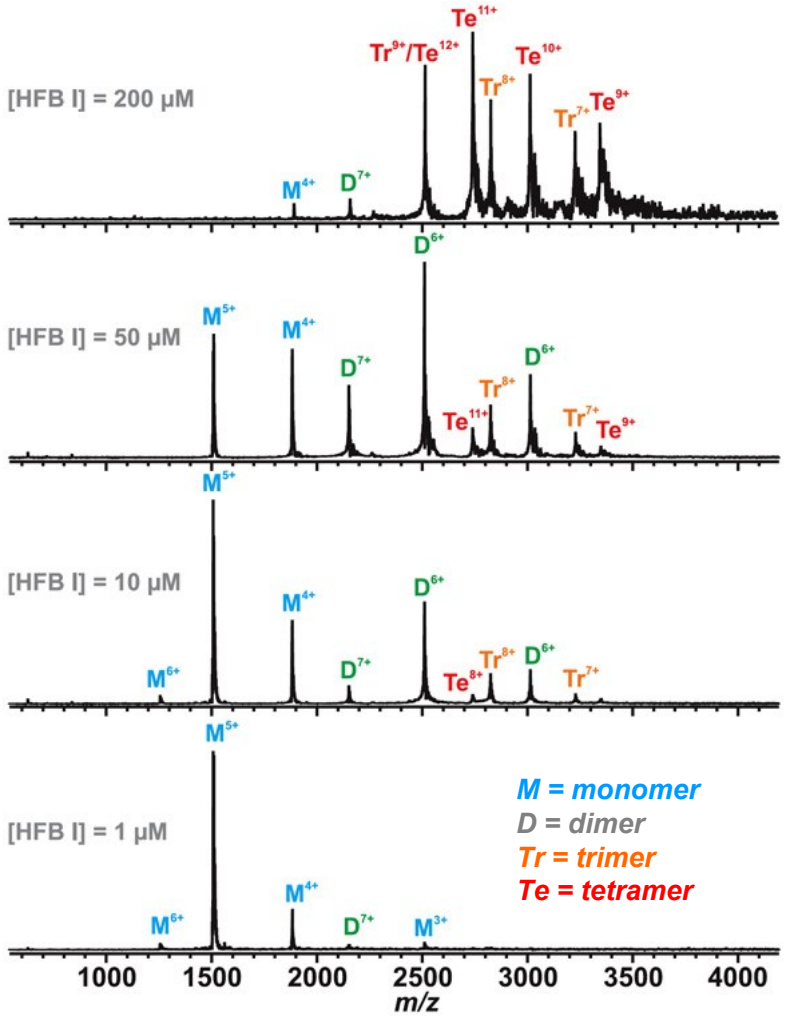
Taskinen B., et al. *PLoS ONE* 8 (**2013**) e77207.

Transient oligomerization – hydrophobin





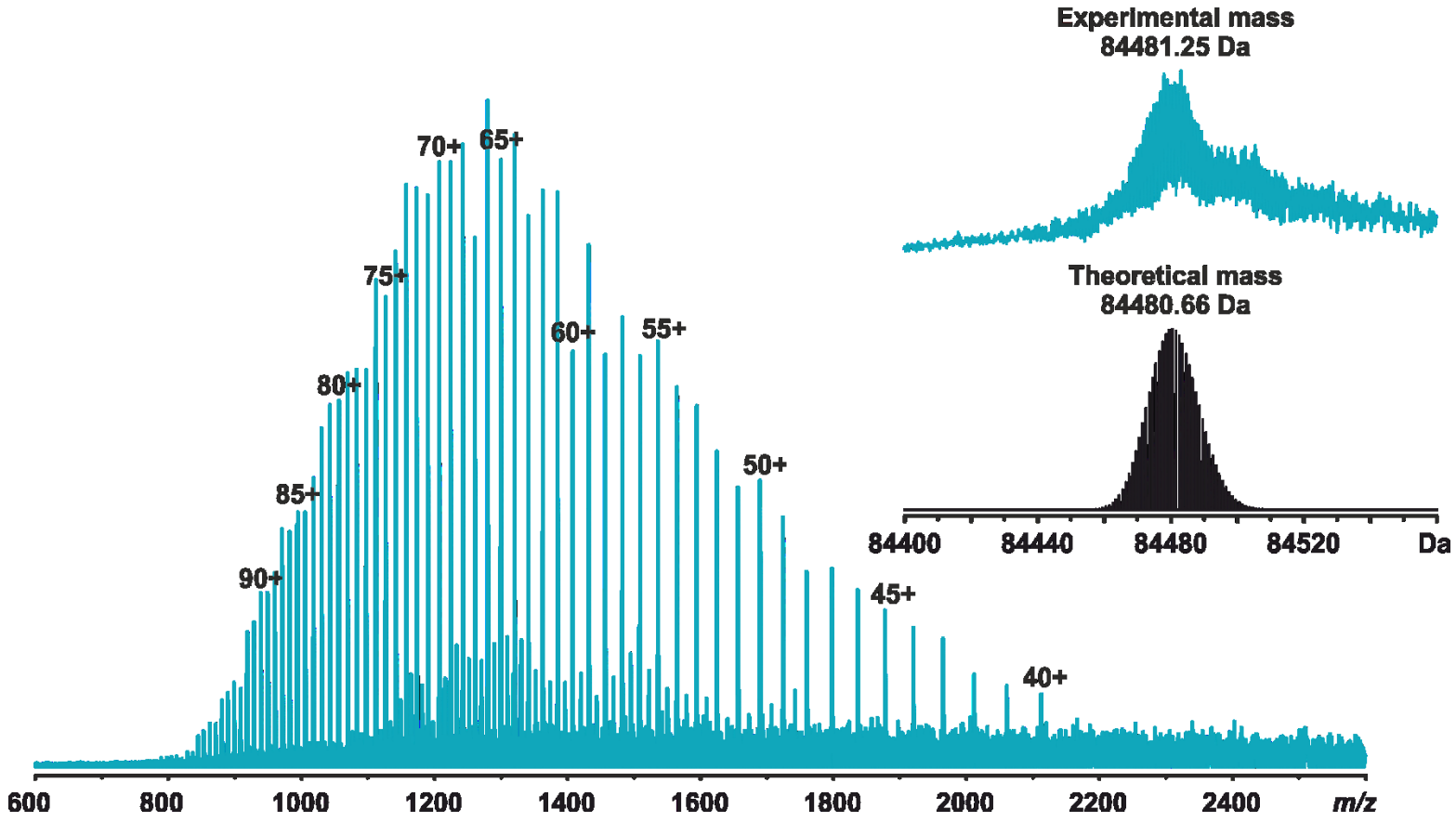




Kallio JM *et al.*, *Chem. Commun.* **2011**, 47, 9843-9845

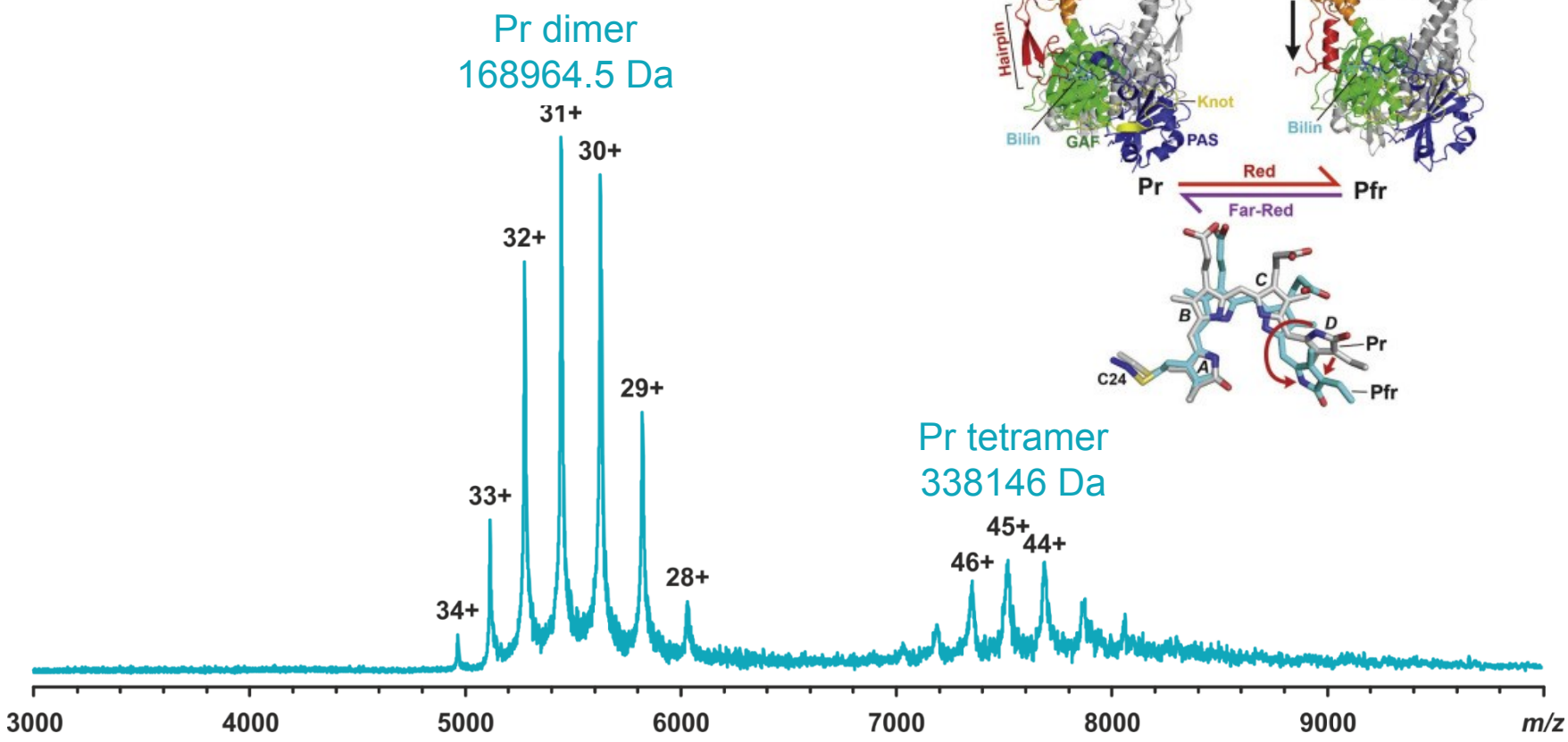
Larger protein complexes with native ESI FT-ICR MS

Native MS of light-sensitive plant phytochrome



Larger protein complexes with native ESI FT-ICR MS

Native MS of light-sensitive plant phytochrome



Largest macromolecular complexes with native ESI FT-ICR MS

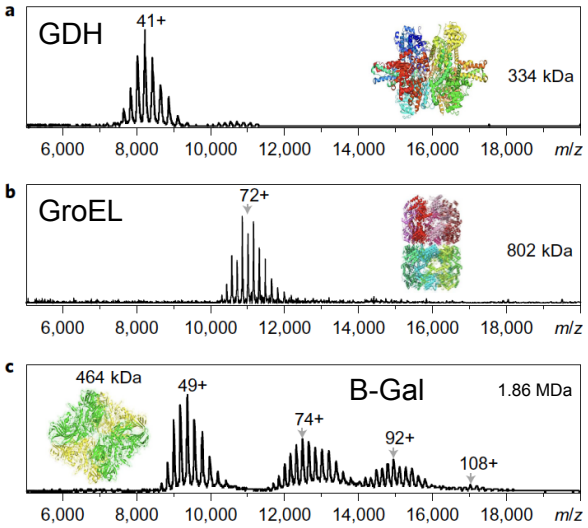
nature chemistry

ARTICLES

PUBLISHED ONLINE: 1 JANUARY 2018 | DOI: 10.1038/NCHEM.2908

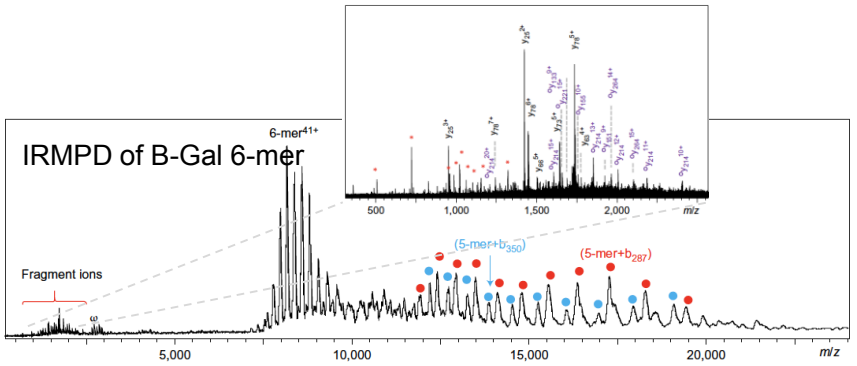
An integrated native mass spectrometry and top-down proteomics method that connects sequence to structure and function of macromolecular complexes

Huilin Li[1]*, Hong Hanh Nguyen[1], Rachel R. Ogorzalek Loo[2], Iain D. G. Campuzano[3] and Joseph A. Loo[1,2]*



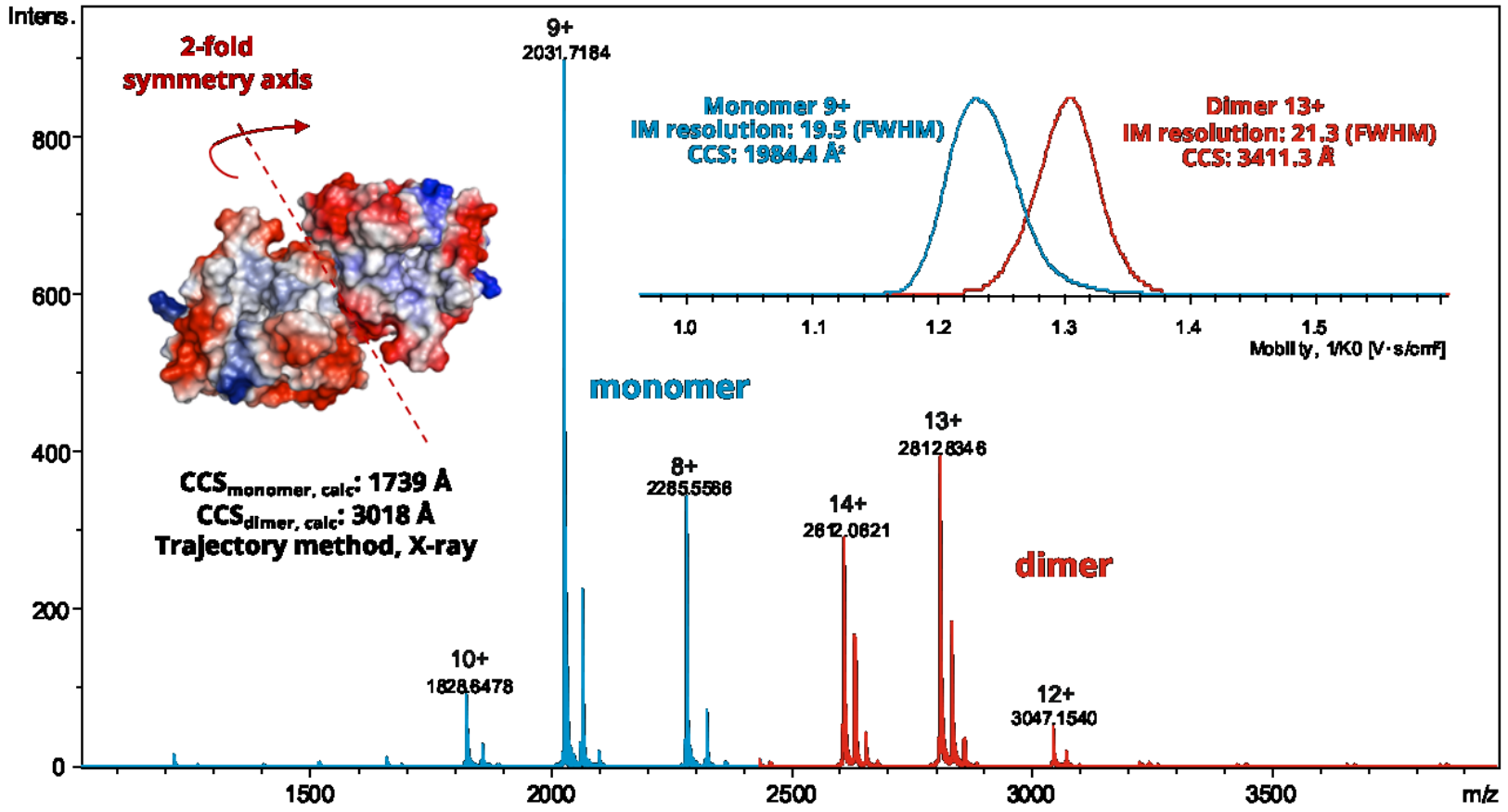
$m_{critical} = 1.20607 \times 10^7 zB^2d^2/(V_{trap}\alpha)$

Since B = 15 T, a = 0.06 m, α = 2.8404, for V_{trap} = 1.0 V, $m_{critical}$ ~3.43 MDa (If z_{CRM} = 144, ion is detected at m/z 24,000)



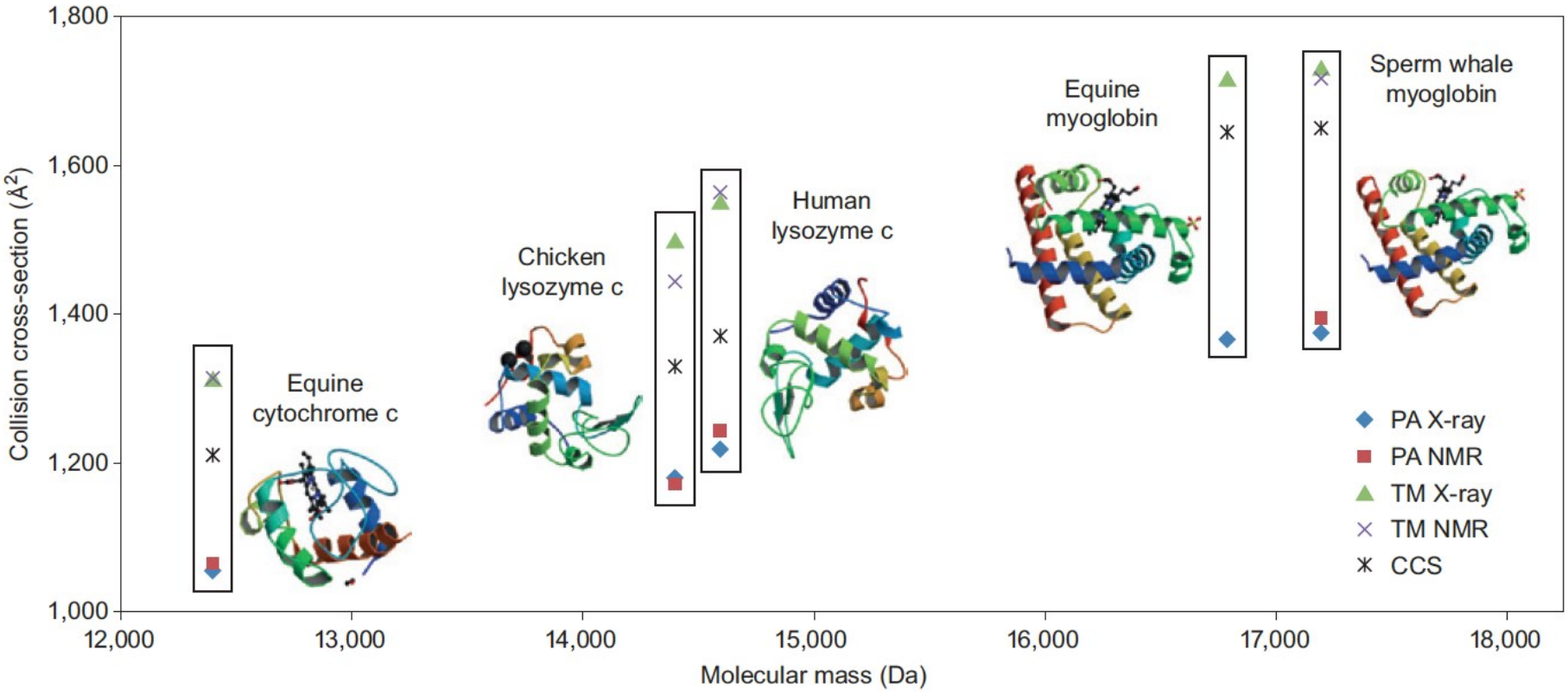
Combining ion mobility with native MS

Native TIMS-TOF bovine b-lactoglobulin



Unpublished results

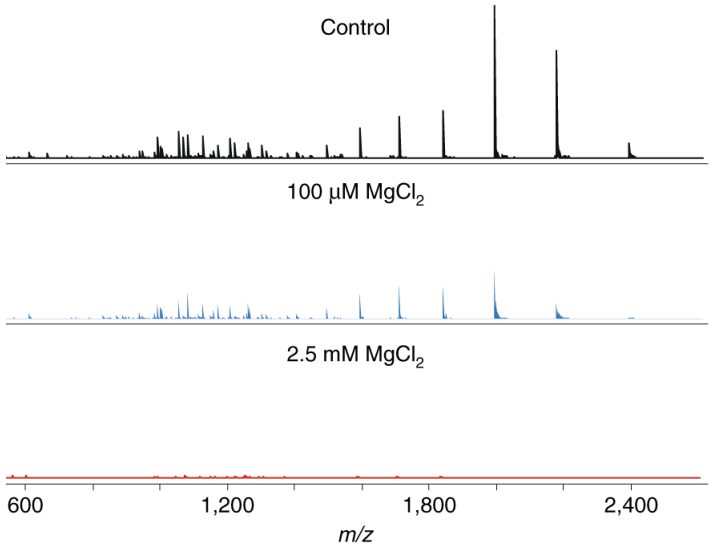
Combining ion mobility with native MS



Lanucara F *et al.* The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics, *Nature Chem* **2014**, 6: 281–294

How to perform native MS?

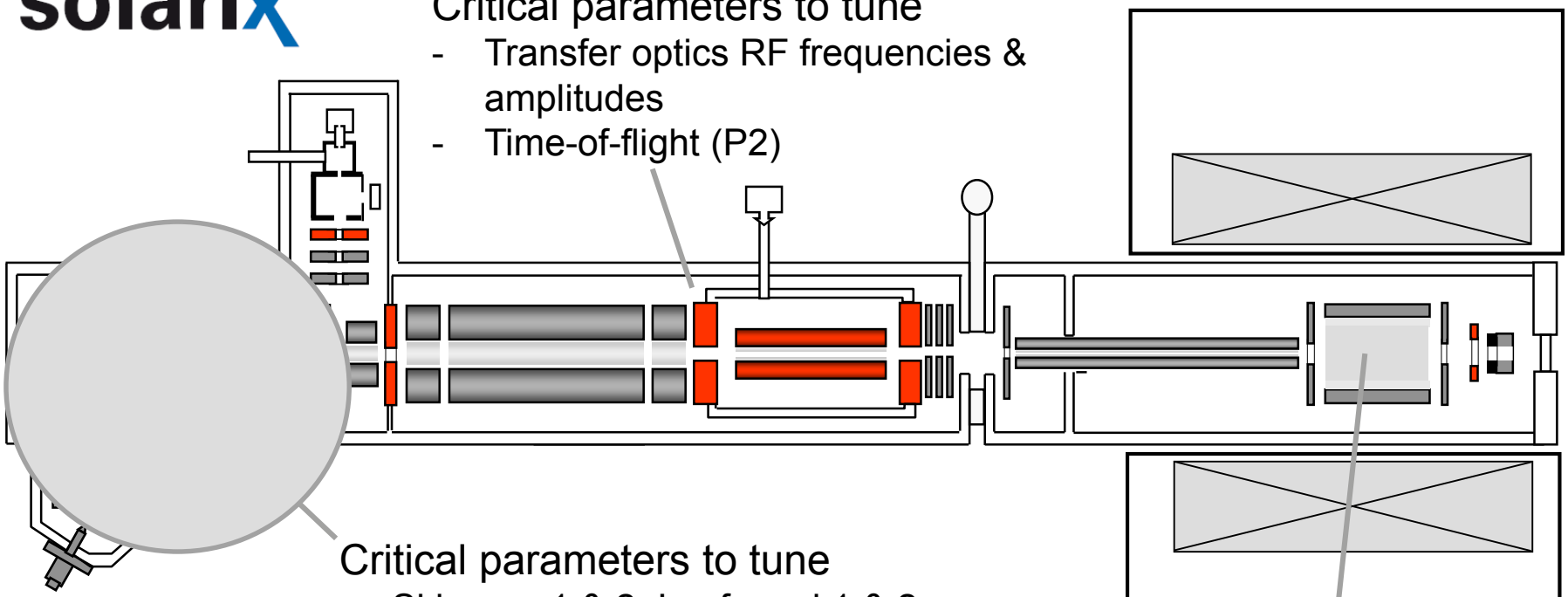
- Important considerations
  - Volatile buffers only (1-1000 mM ammonium acetate or ammonium bicarbonate, pH by formic/acetic acid or ammonia)
  - Extensive desalting and detergent removal needed
  - Fine tuning of instrument parameters to avoid unfolding/complex dissociation and to maintain efficient desolvation, on the other hand
  - Designing experiments: appropriate protein/ligand concentrations



EU FT-ICR MS

Native MS on Bruker Solarix FT-ICR instrument

solarix



Critical parameters to tune
- Transfer optics RF frequencies & amplitudes
- Time-of-flight (P2)

ESI source
- Conventional
- NanoESI
- Microchip

Critical parameters to tune
- Skimmer 1 & 2, Ion funnel 1 & 2
- Collision cell DC offset and RF frequency, ion accumulation time (D10), collision gas
- Drying gas temperature & pressures
- Capillary exit

Critical parameters to tune
- Trapping voltage
- Excitation power

EU FT-ICR MS

Agreement between solution and gas-phase?

THE JOURNAL OF PHYSICAL CHEMISTRY B

ARTICLE

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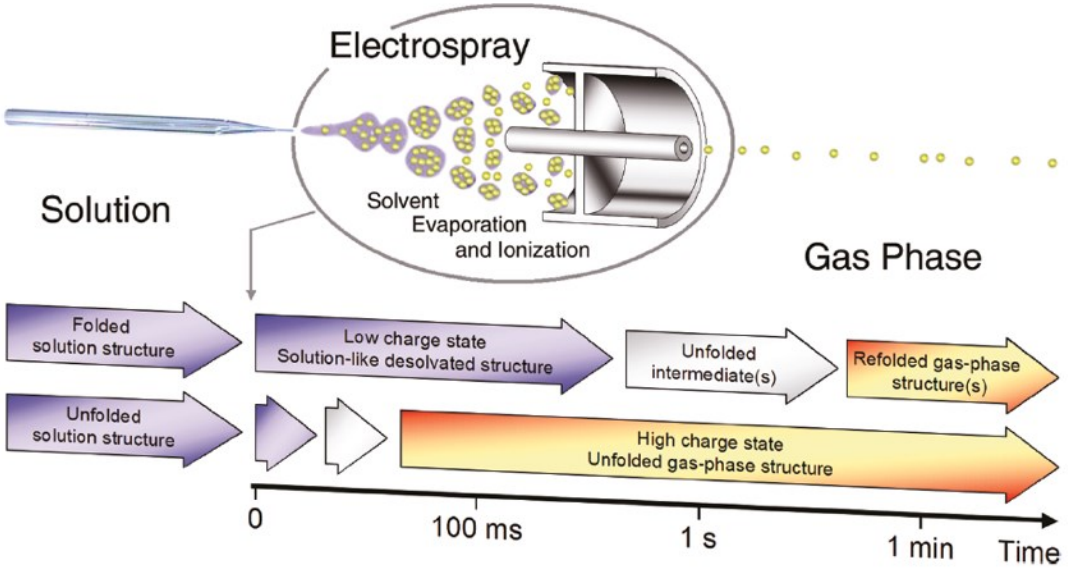
Structural Stability from Solution to the Gas Phase: Native Solution Structure of Ubiquitin Survives Analysis in a Solvent-Free Ion Mobility–Mass Spectrometry Environment

Thomas Wyttenbach and Michael T. Bowers*

Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, California 93106, United States



Wyttenbach & Bowers, *J. Phys. Chem. B* **2011**, 115, 12266–12275

Thank you!

European Union
European Regional Development Fund

Biocenter Finland

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EU FT-ICR MS

European Commission
HORIZON 2020

The info in this presentation reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the info contained therein.

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