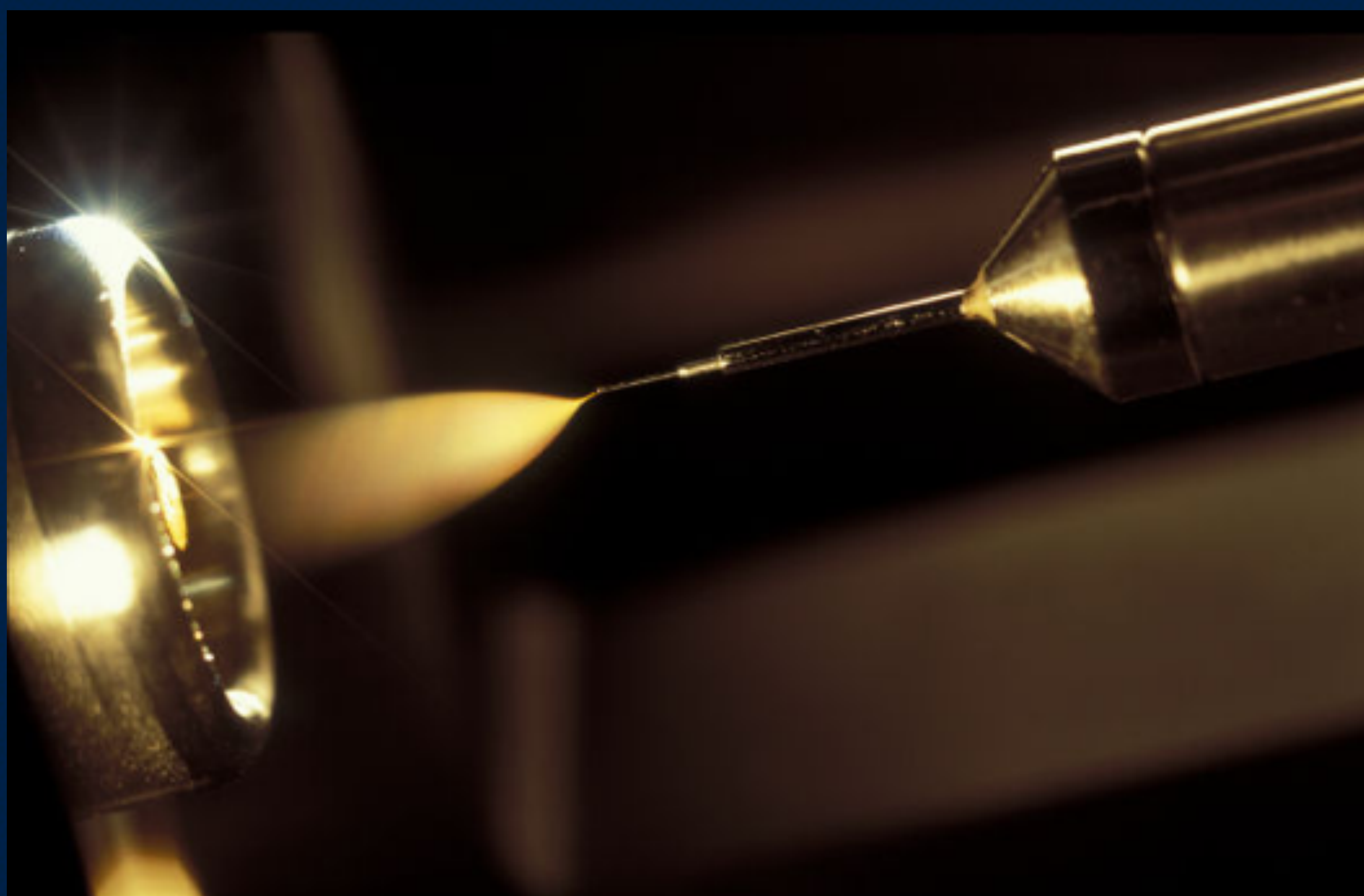
DEPARTMENT OF CHEMISTRY

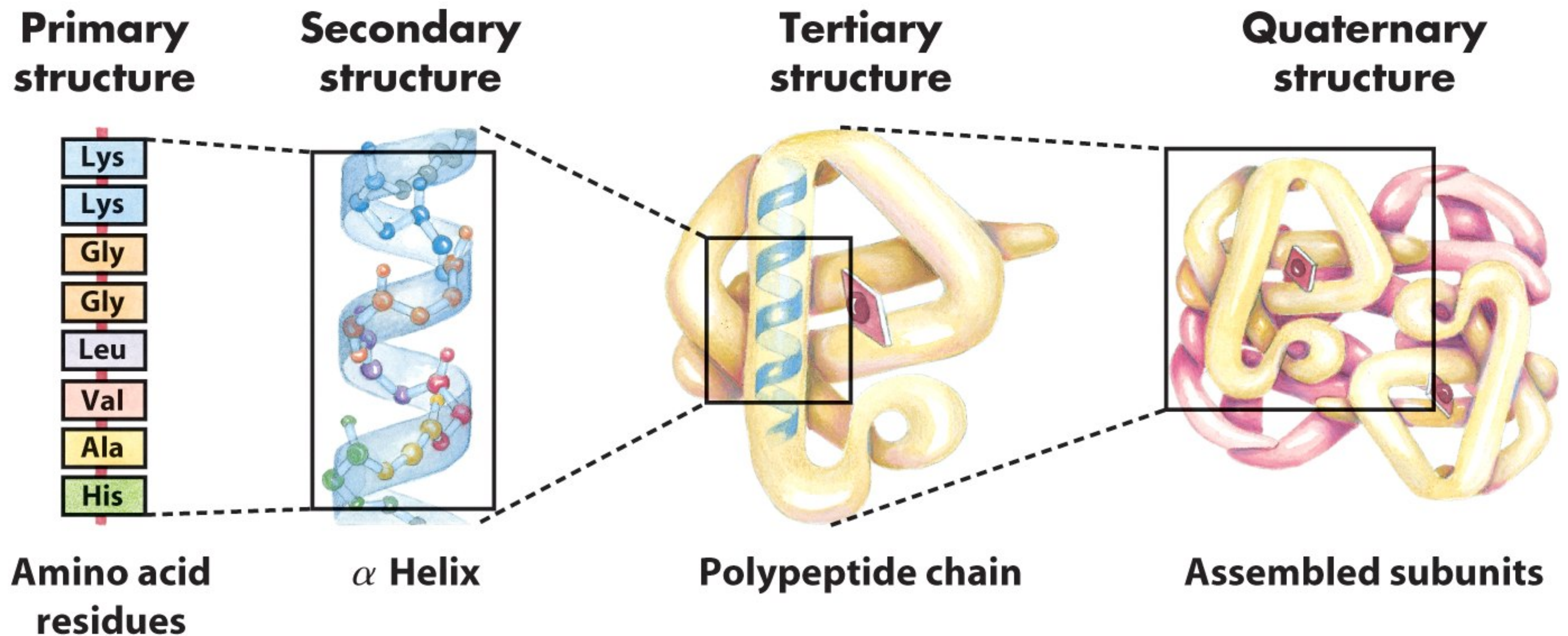
# GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 8

## Mass spectrometry for biophysics and structural biology



Professor Justin Benesch, 23rd November 2016

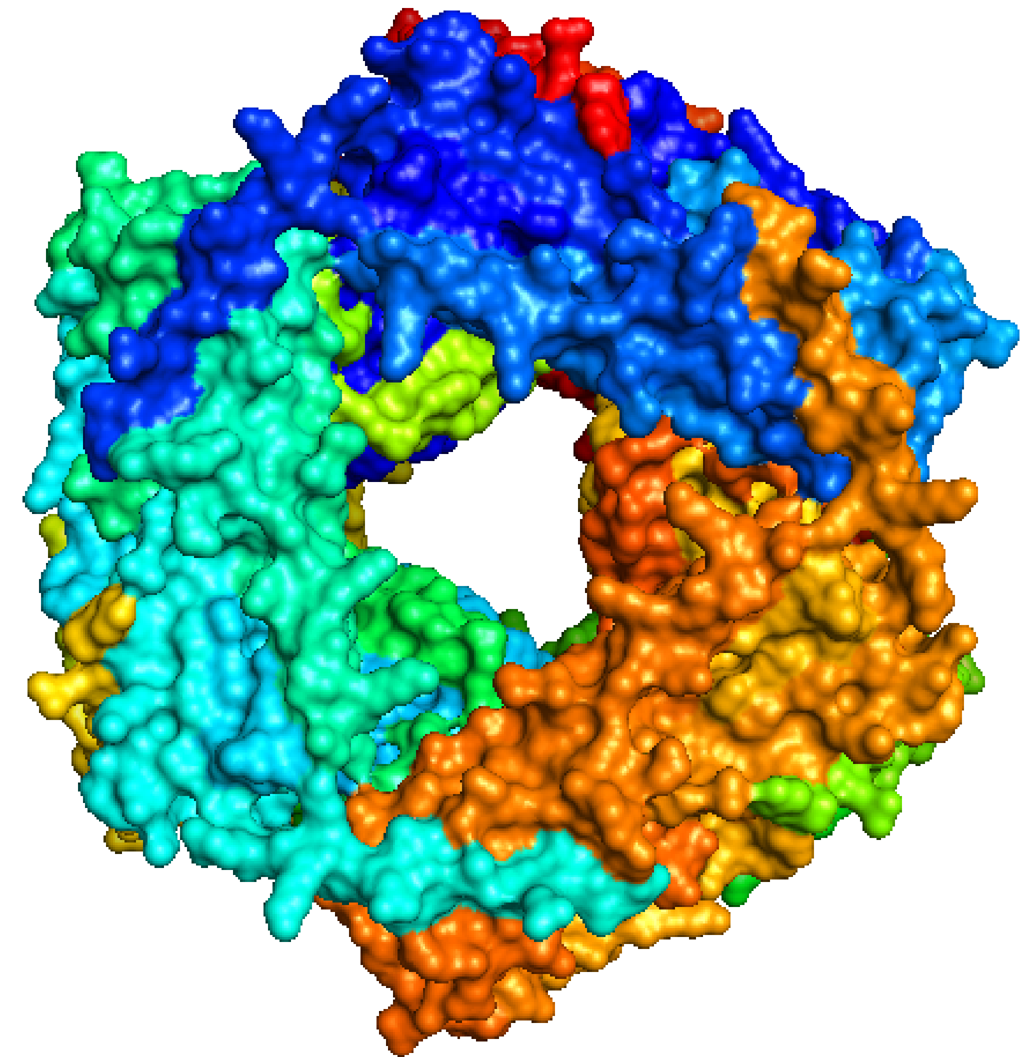
# Levels of protein structure



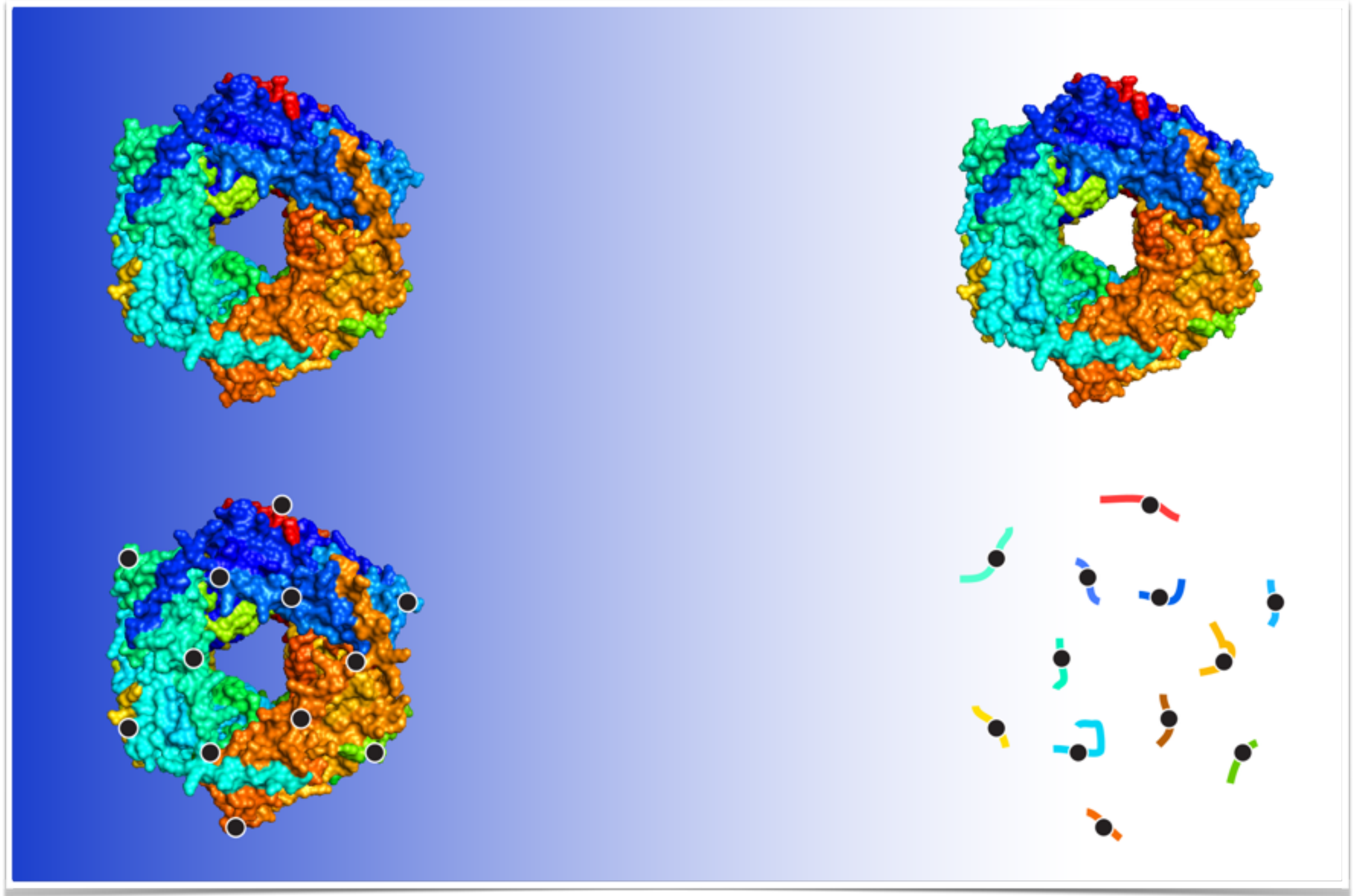

- Mass spectrometry can inform on all levels of protein structure

# What do we want to know?

- Structure
  - Stoichiometry, connectivity, 3D topology, conformations, atomic coordinates
- Thermodynamics
  - Interface and interaction strengths, stability
- Kinetics
  - Rate constants, activation energies

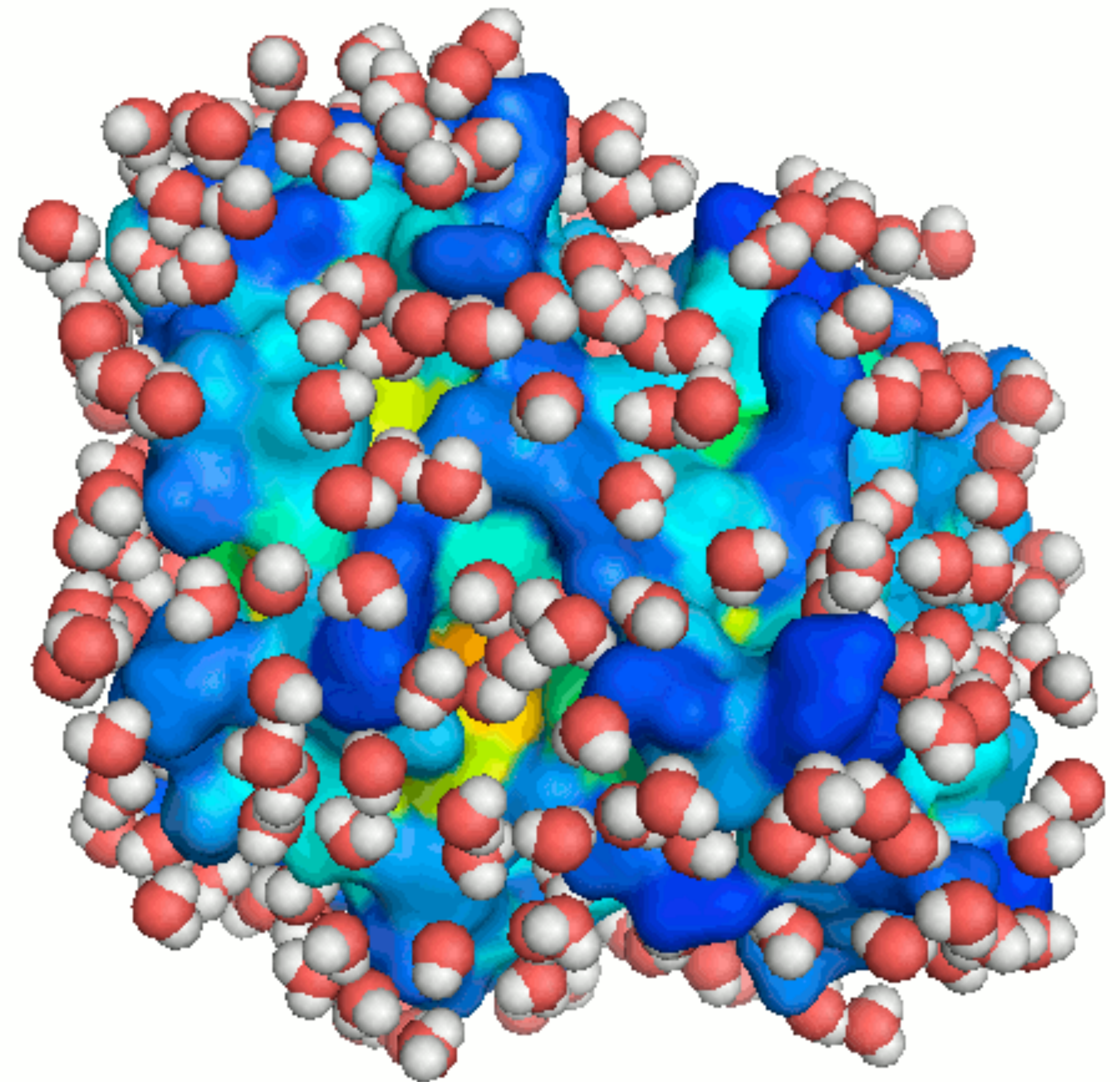


# Two strategies - native or labelling

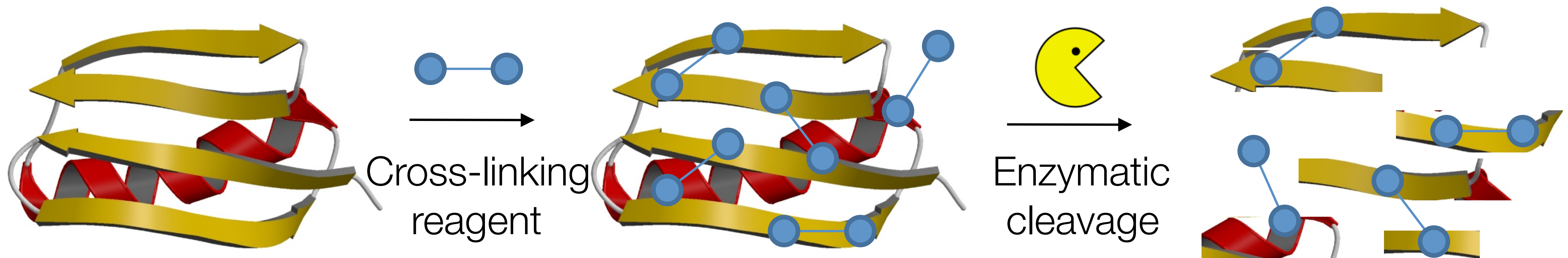


# Labelling technologies with MS

- Label protein, and use MS to localise the individual labels
- Can interrogate the ‘global’ and ‘local’ levels: i.e. protein or peptide level
- Peptide level accessed by enzymolysis of protein, and further interrogation with tandem MS
- Labels reveal solvent accessibility and connectivity
- Provide means of probing protein structure and dynamic fluctuations

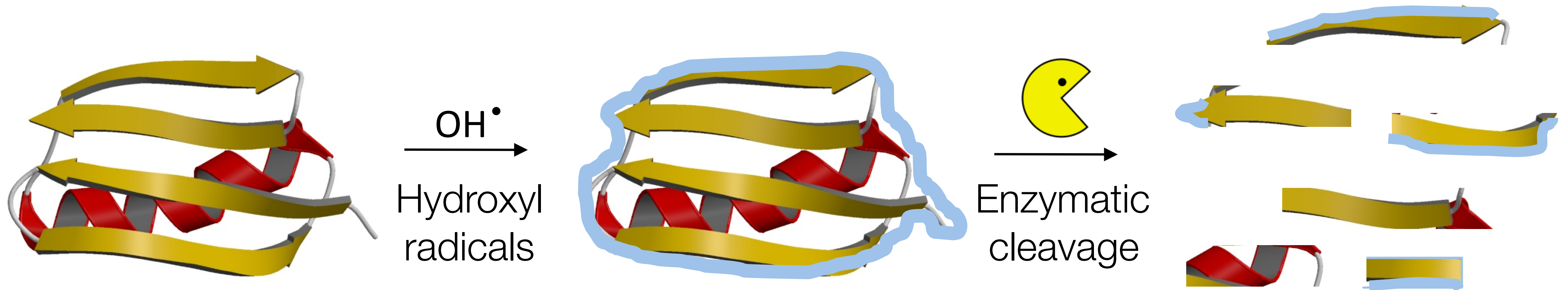


# Cross-linking mass spectrometry




- Cross-linker forms covalent bonds between amino acids with appropriate functionality
- Peptide masses (and fragments) are interrogated to localise cross-linker
- Intra- and inter-protein cross-links can be formed
- Cross-links can be used to determine connectivity, and as a spatial restraint for modelling protein structures
- Problems centre on decreased detection efficiency of cross-linked peptides, kinetic effects, and interpretation of observed links

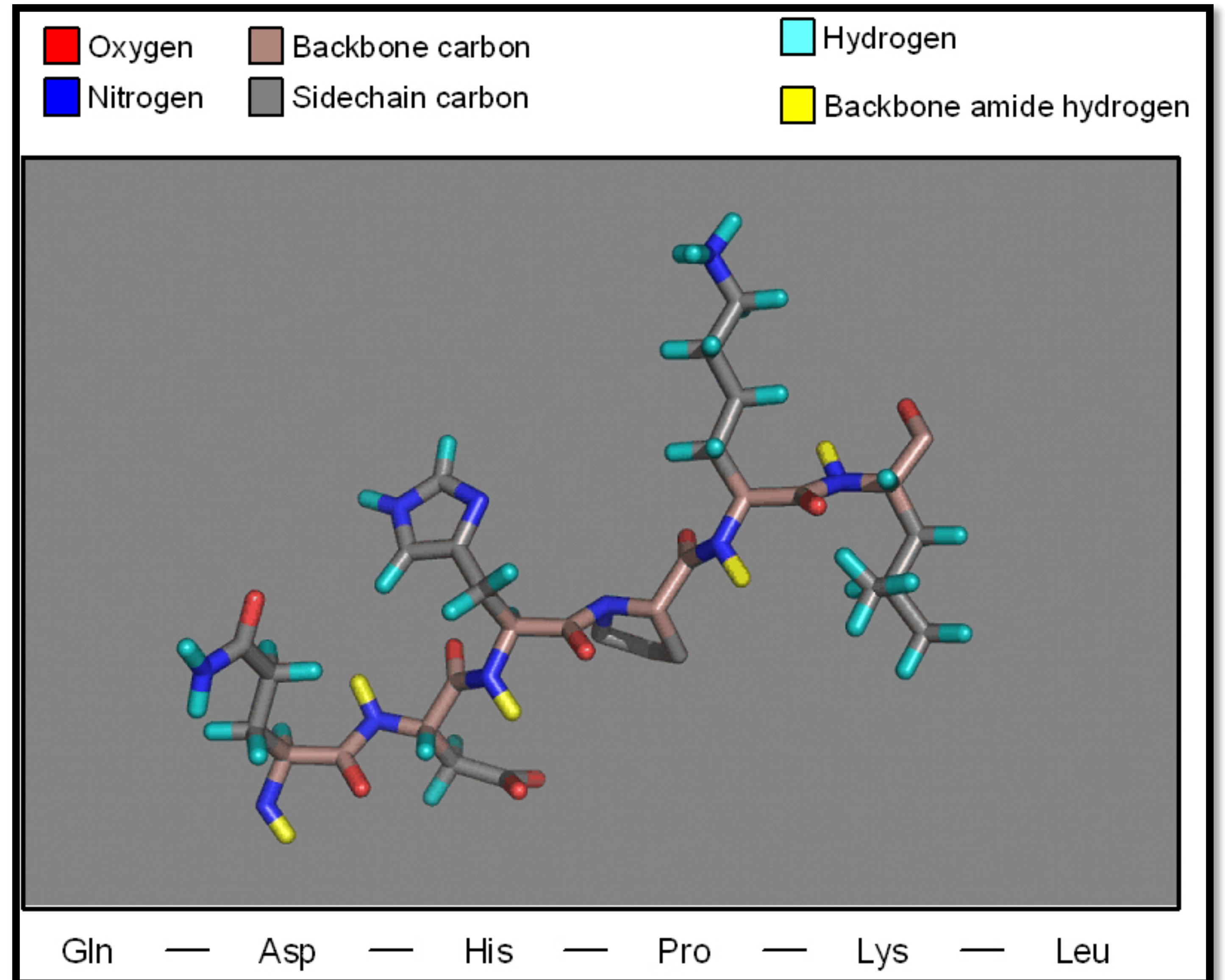
# Oxidative foot-printing




- Solvent accessible amino acid side chains of the protein are oxidised
- Peptide masses (and fragments) are interrogated to localise oxidation sites
- Comparing data from proteins in complex and in isolation allows the determination of interface sites
- Problems centre on data analysis and differential intrinsic reactivity

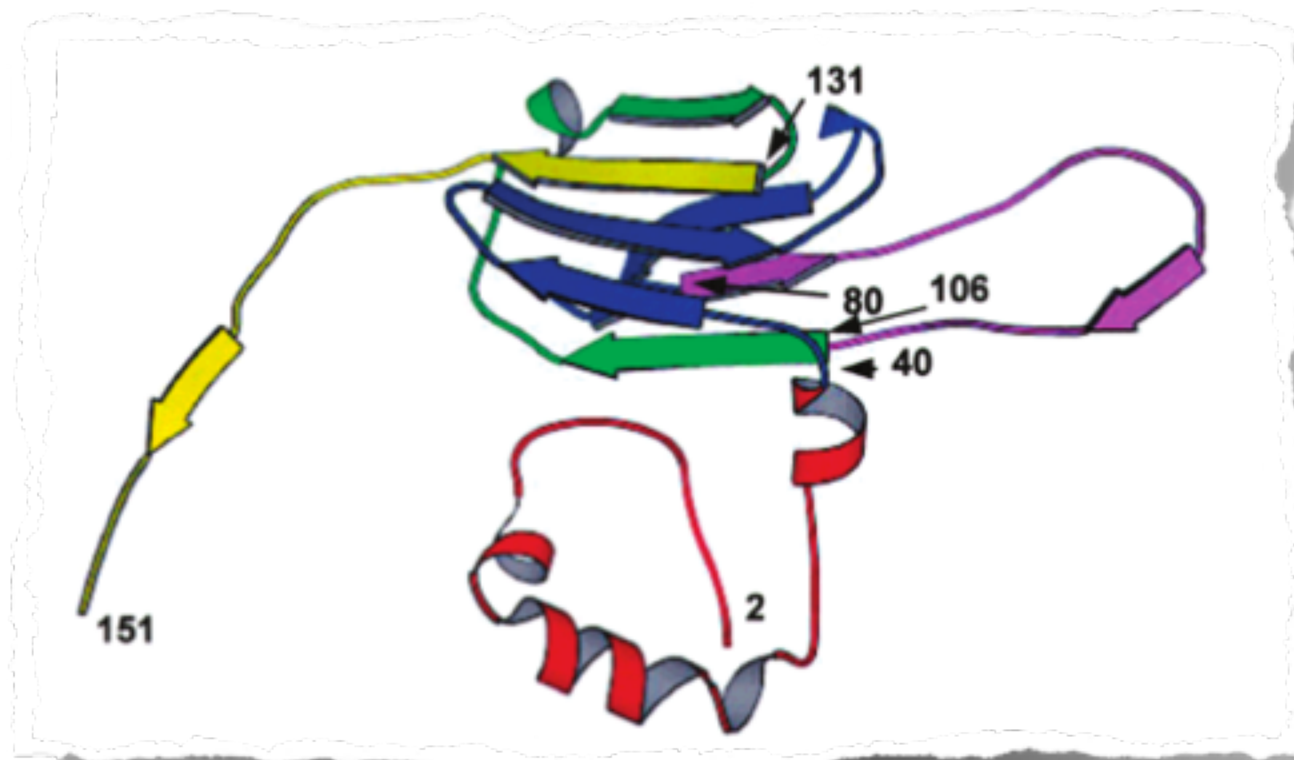
# Hydrogen/deuterium exchange

- Monitor the rate at which protein hydrogens are replaced by deuteriums (or vice versa)
- Three types of hydrogen in proteins, only backbone amide hydrogens exchange at measurable rate
- Exchange can be (effectively) quenched by dropping pH to ~2.5, and temperature to 0ºC
- Exchange rates reveal solvent accessibility

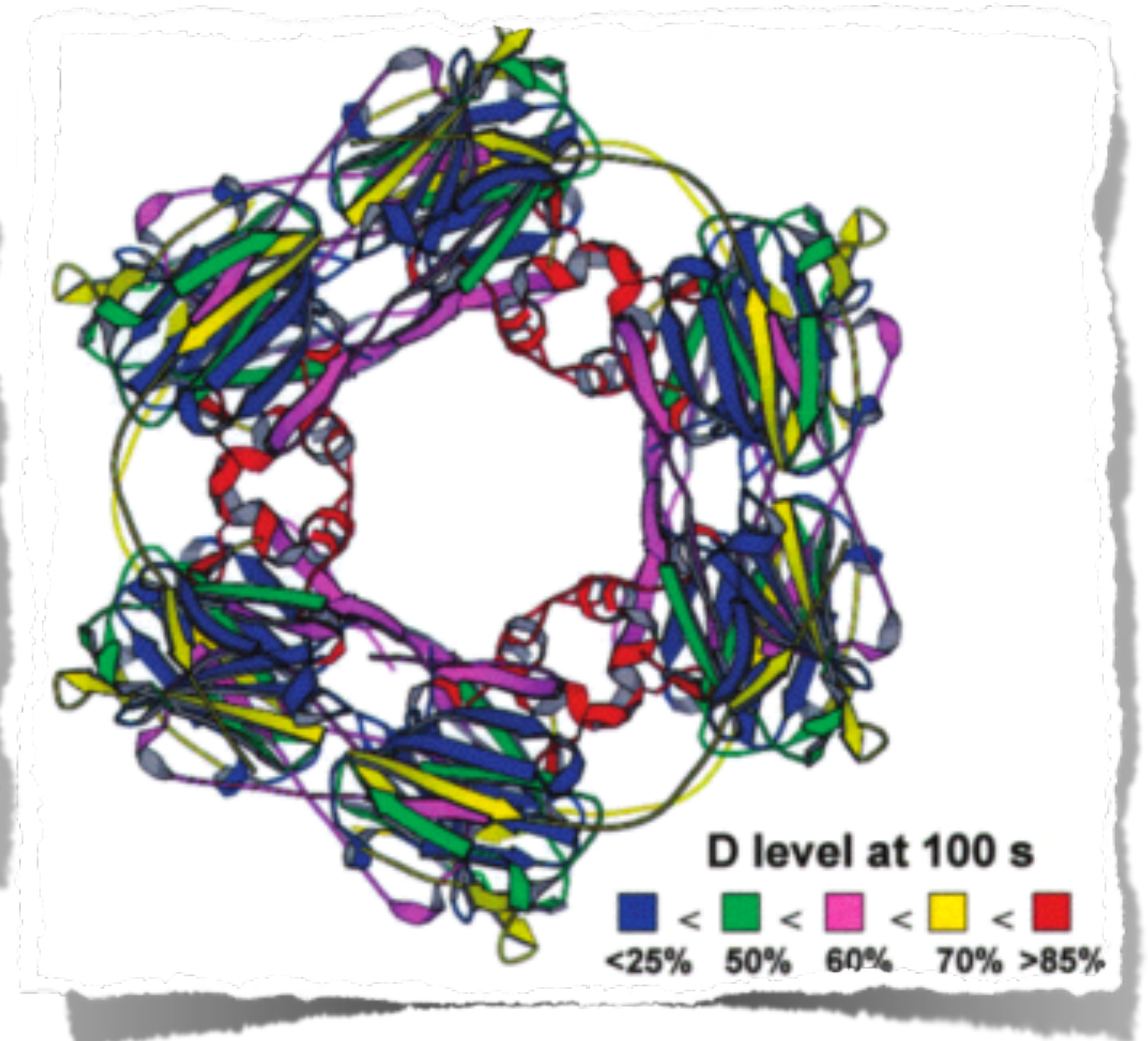



- Problems centre on localisation, and interpretation of solvent accessibility

# Labelling technology - Example




Wintrode ... Smith, Biochemistry (2003) 42, 10667-73




- Hydrogen/deuterium exchange of oligomeric 'molecular chaperone' protein
- Side-chains at interfaces exchange relatively rapidly, suggesting a labile oligomer

# ‘Soft’ Ionisation

- From molecules in solution to ions in vacuum
- Established ionisation techniques resulted in covalent fragmentation of molecules
- Soft ionisation techniques allow ionisation of large ions without their fragmentation
- Current ‘mass record’ is >100 MDa!

Nobel Prize in Chemistry 2002

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

**John B. Fenn**

1/4 of the prize

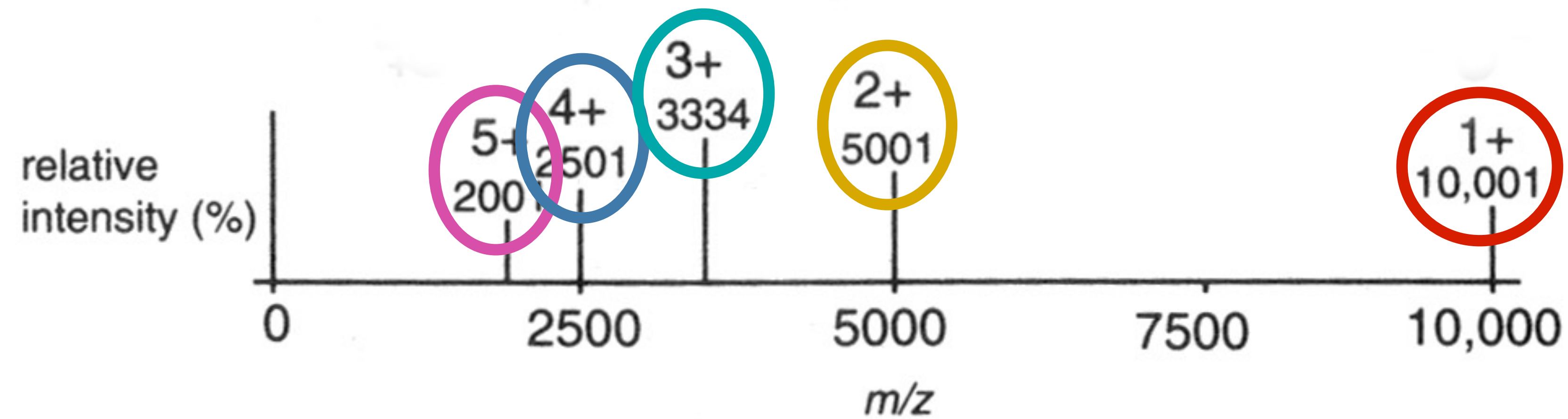
USA

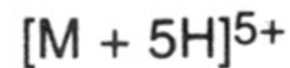
**Koichi Tanaka**

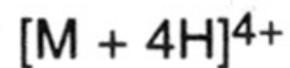
1/4 of the prize

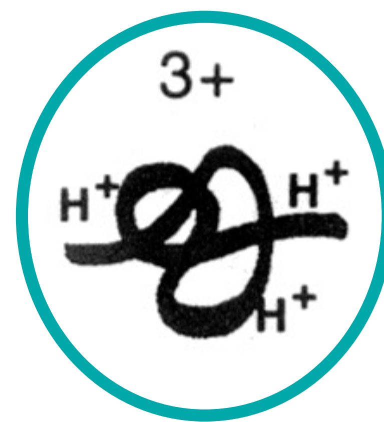
Japan

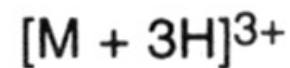
# ESI mass spectrum of single protein

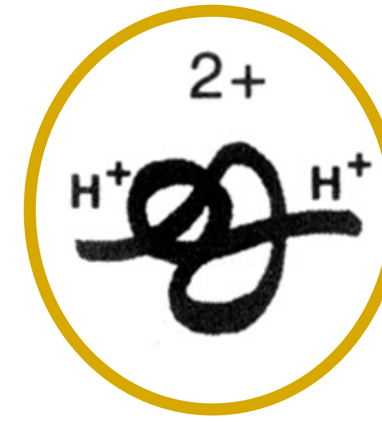


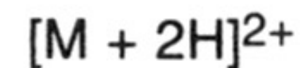


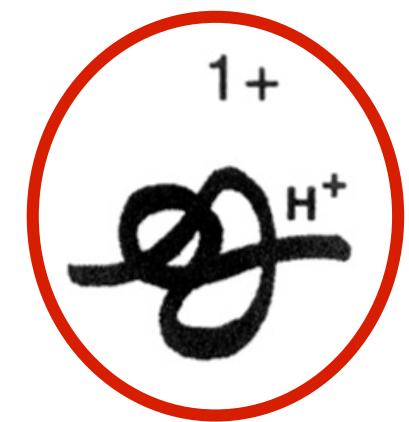




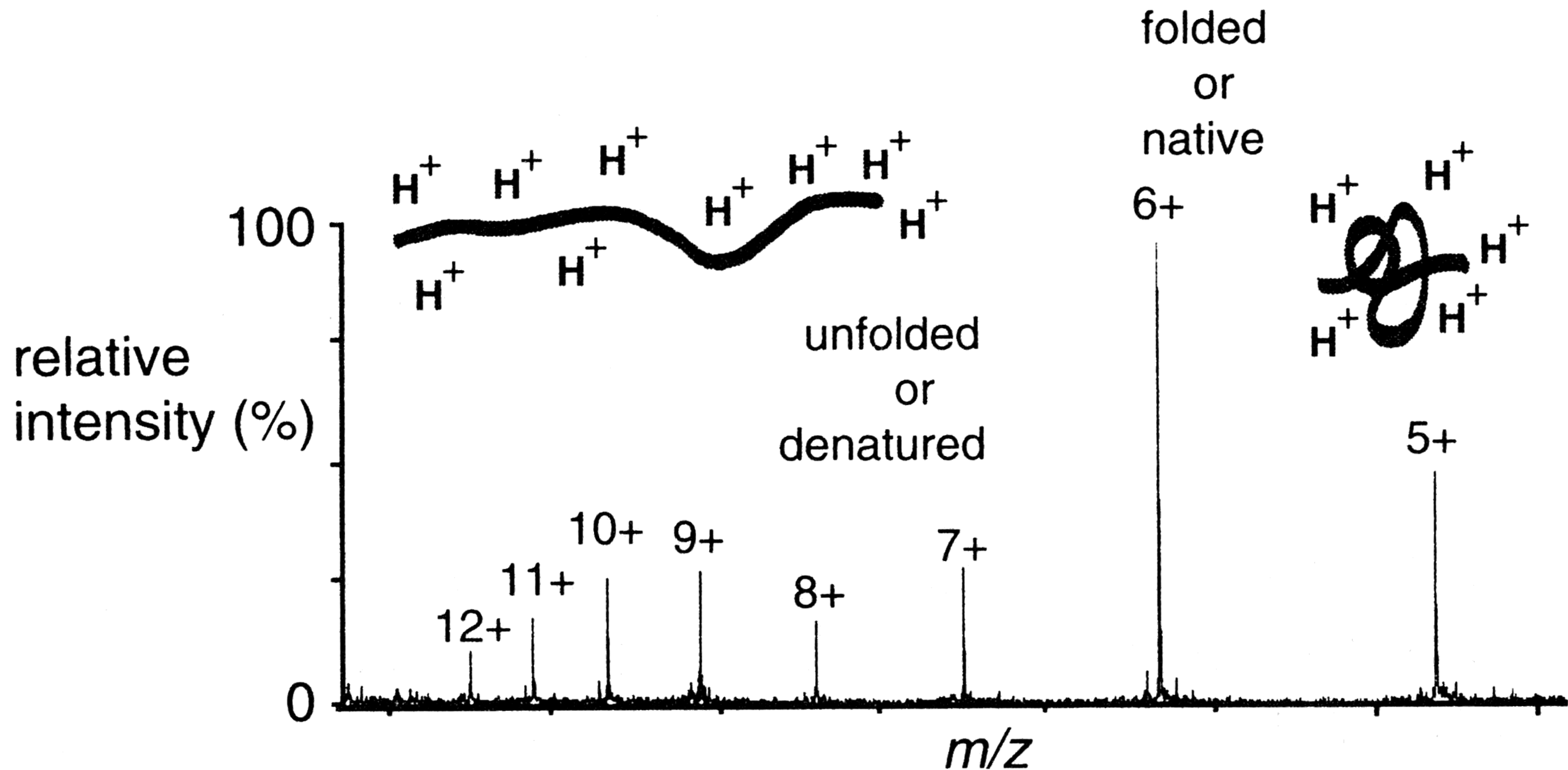


| | | | | |
|---|---|---|---|---|
| $m/z$ = 10,005÷5 | $m/z$ = 10,004÷4 | $m/z$ = 10,003÷3 | $m/z$ = 10,002÷2 | $m/z$ = 10,001÷1 |
| $m/z$ = 2001 | $m/z$ = 2501 | $m/z$ = 3334 | $m/z$ = 5,001 | $m/z$ = 10,001 |

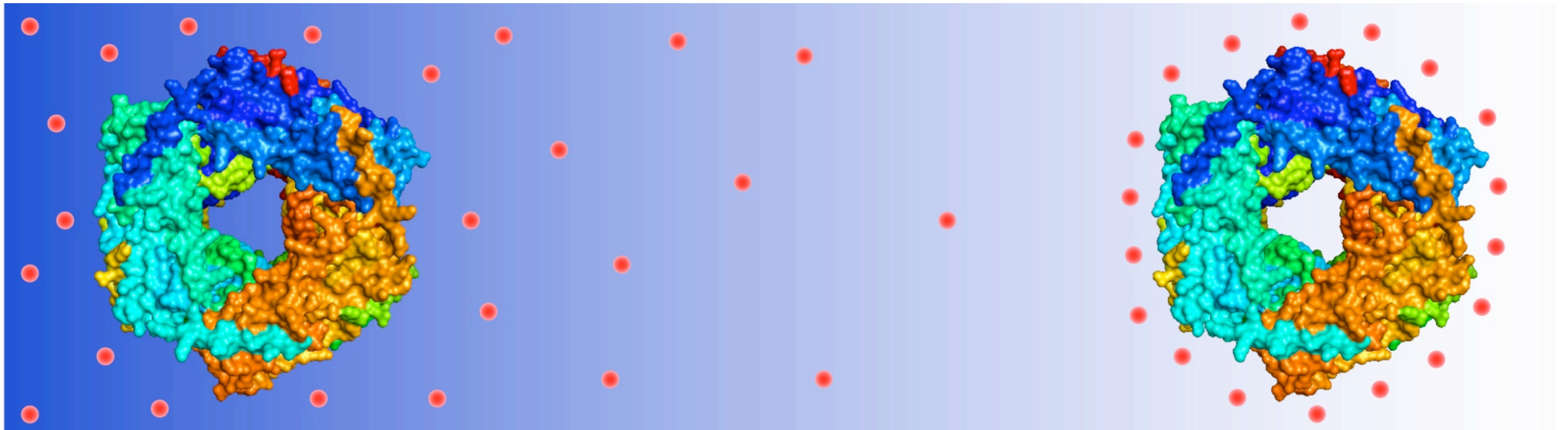
- Electrospray mass spectra show multiple ‘charge states’ for a 10 kDa protein

# Conformational effects on ESI spectra



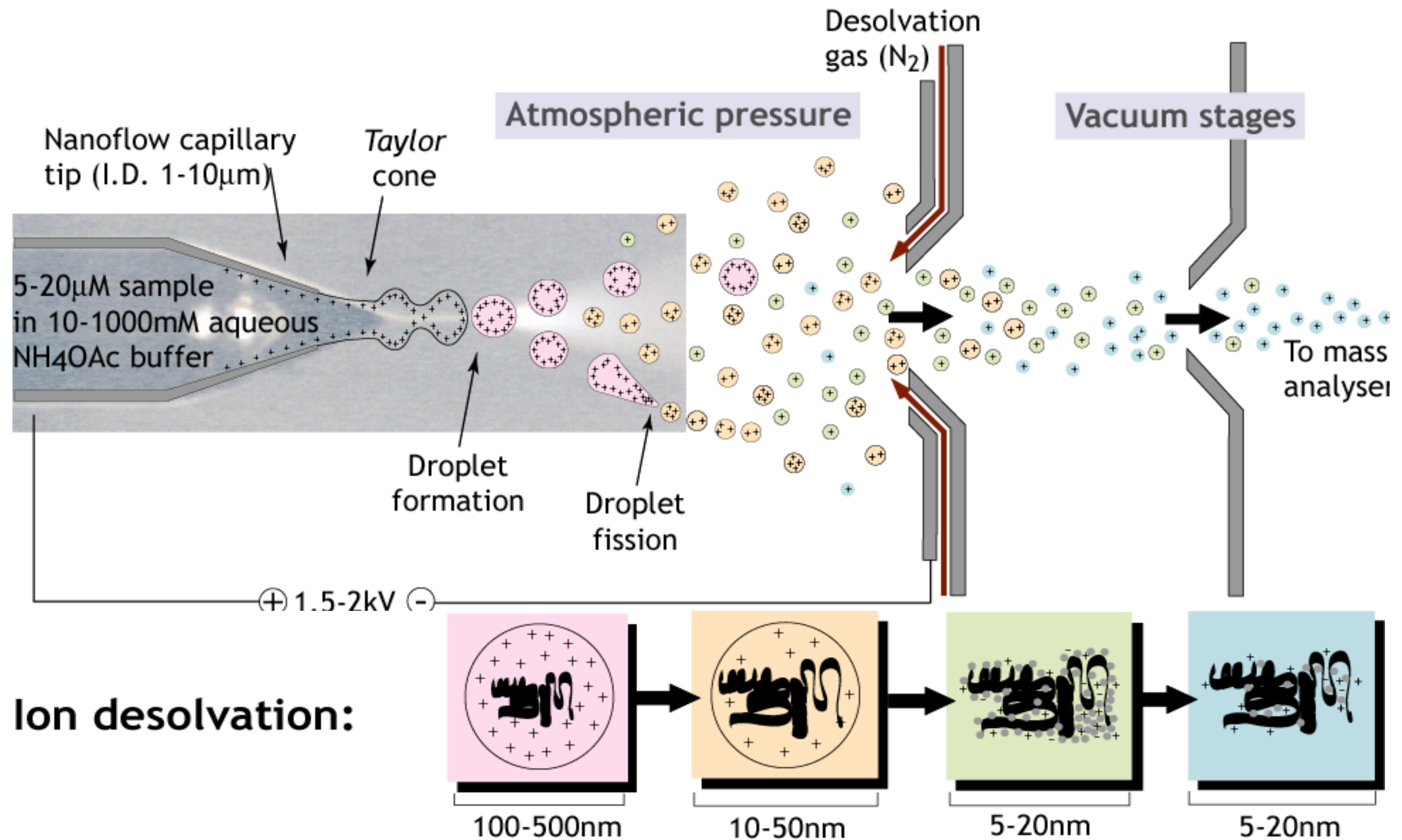

- Folded state of protein governs its surface area, and number of sites available for protonation

# Maintaining noncovalent interactions

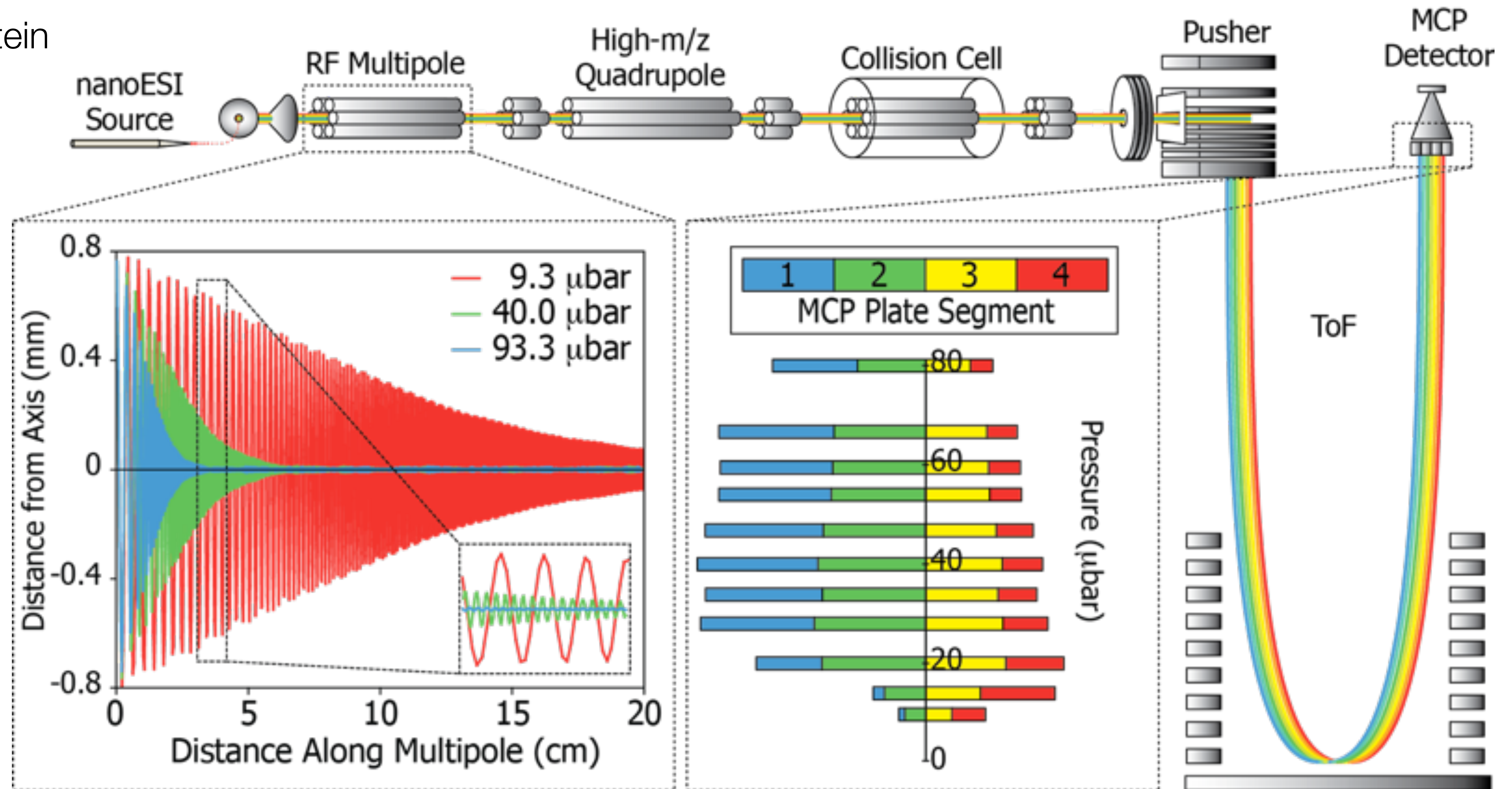


- Transfer multi-subunit protein assembly from solution into gas phase
- Requires control of ionisation conditions, and ion transmission

# Nano-electrospray ionisation (nESI)

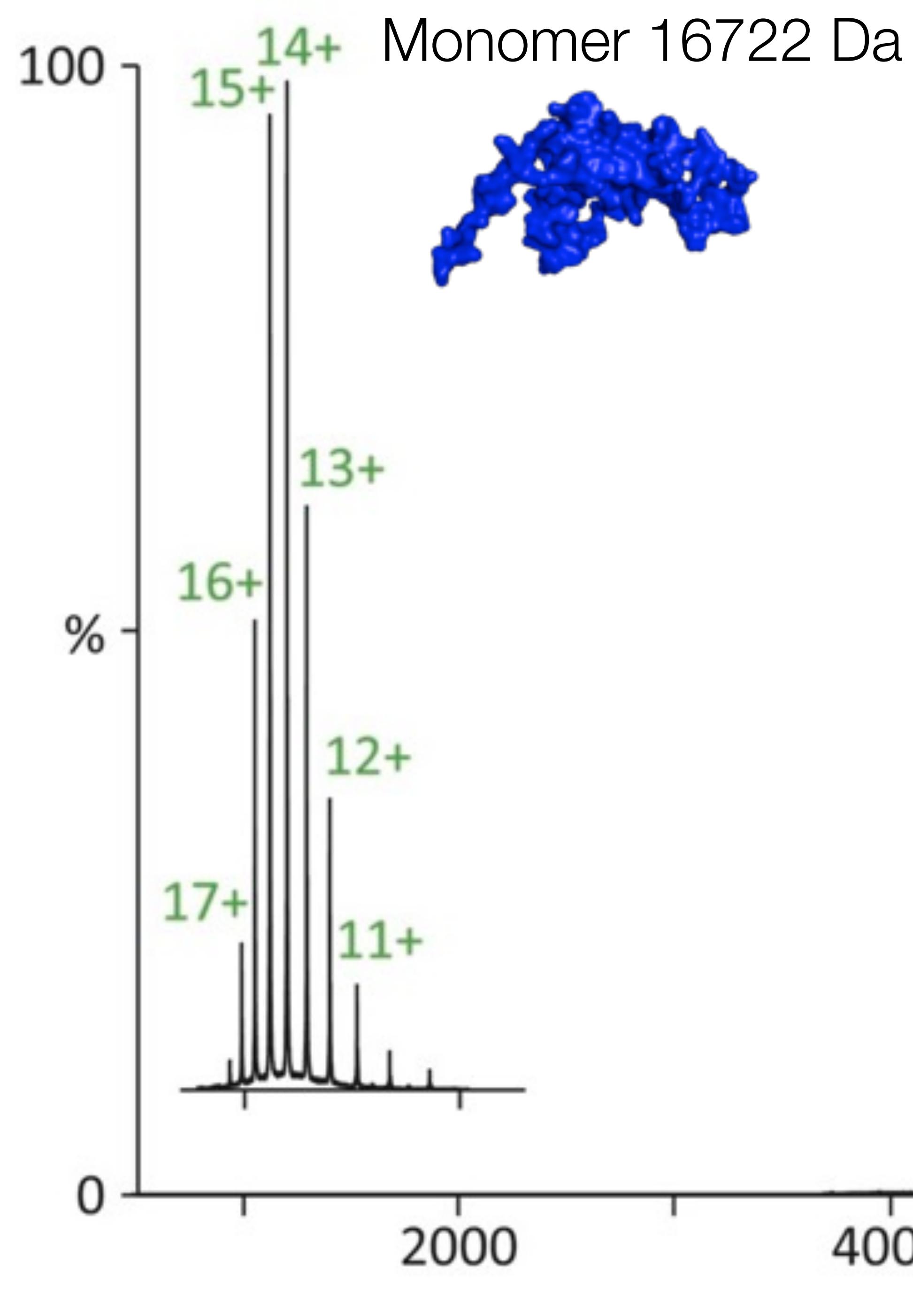


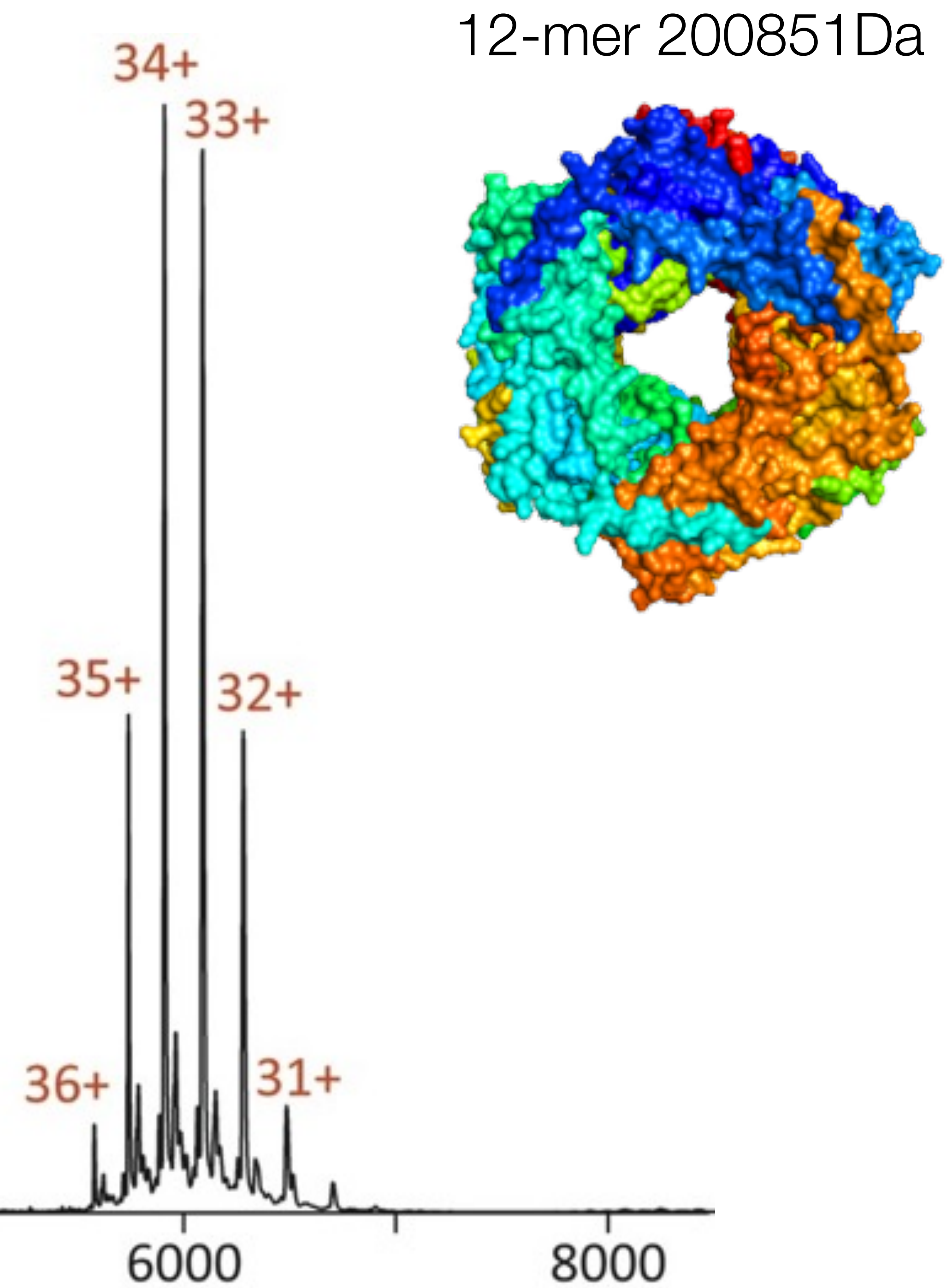
# Collisional focussing



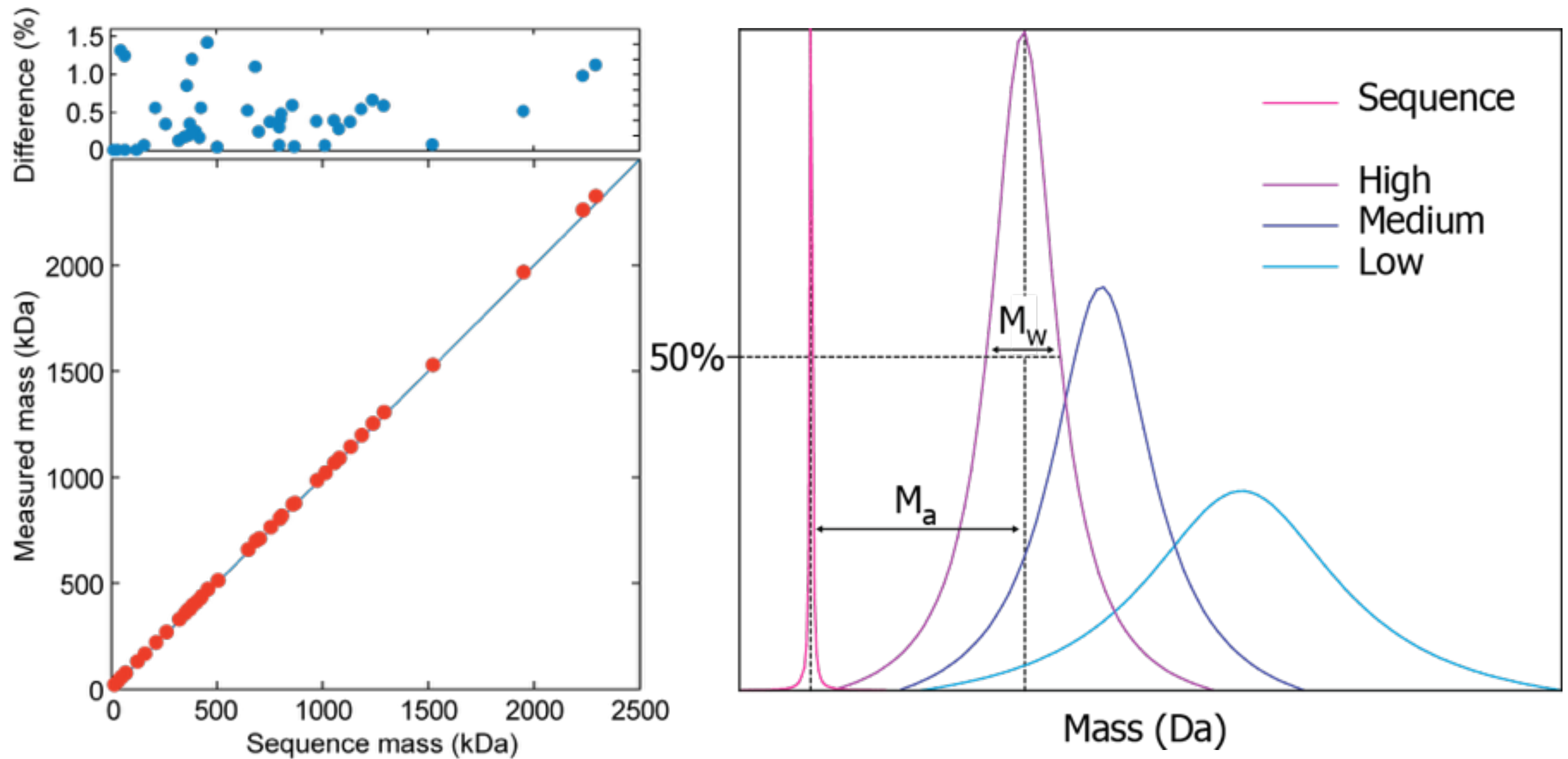

- Both axial and radial components of the ions' velocity can be dampened by collisions with background gas

# nESI mass spectrum



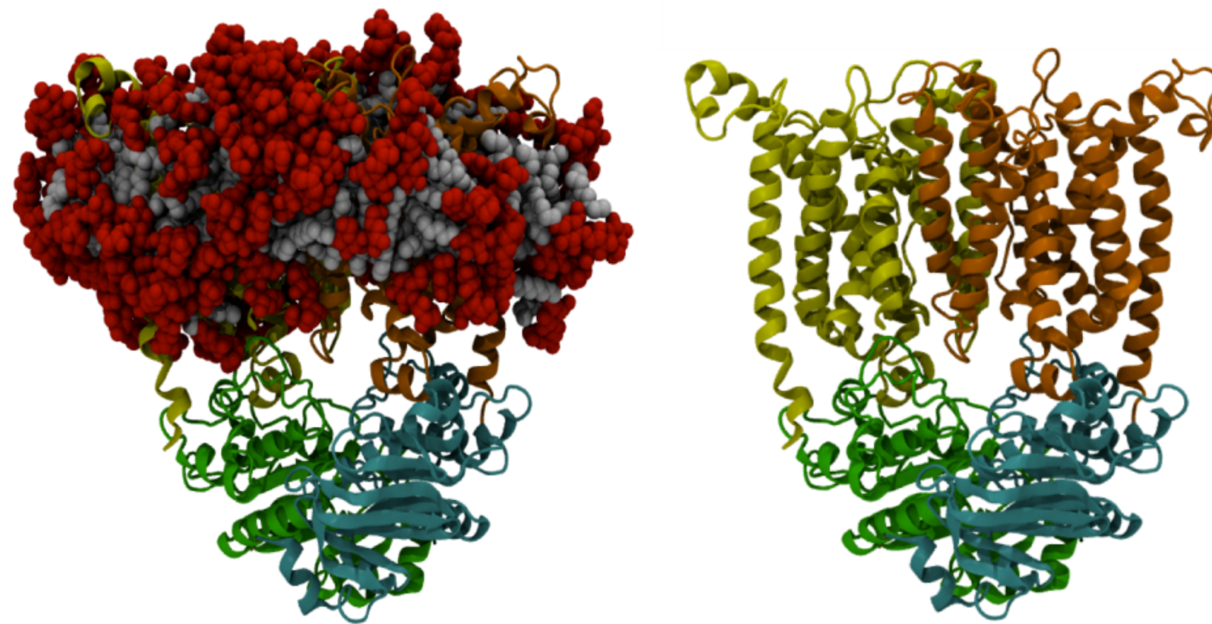



# Mass accuracy

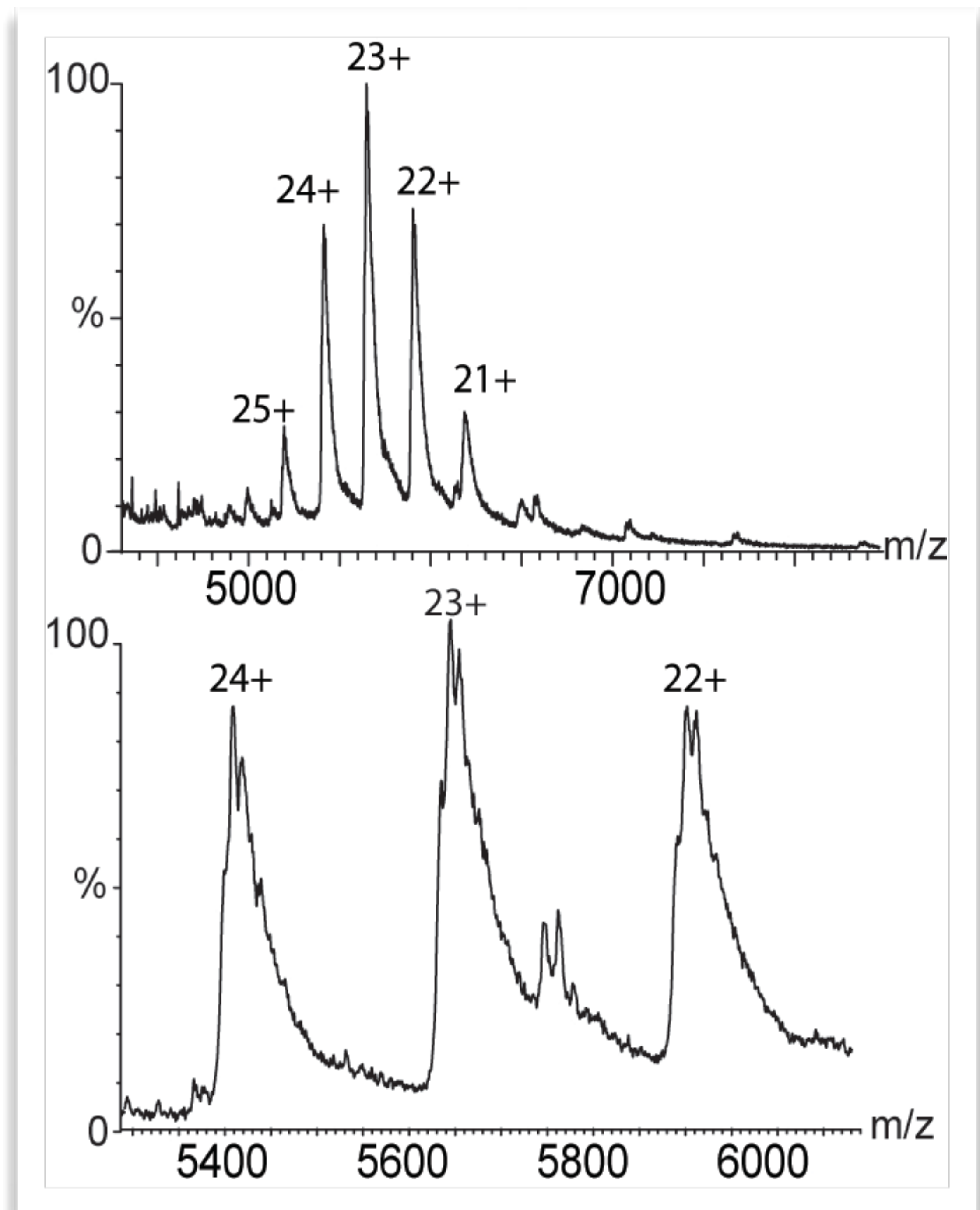



- Additional mass due to adducted solvent molecules and buffer ions
- Number of adducts inversely related to activation

# Membrane protein assemblies are also tractable



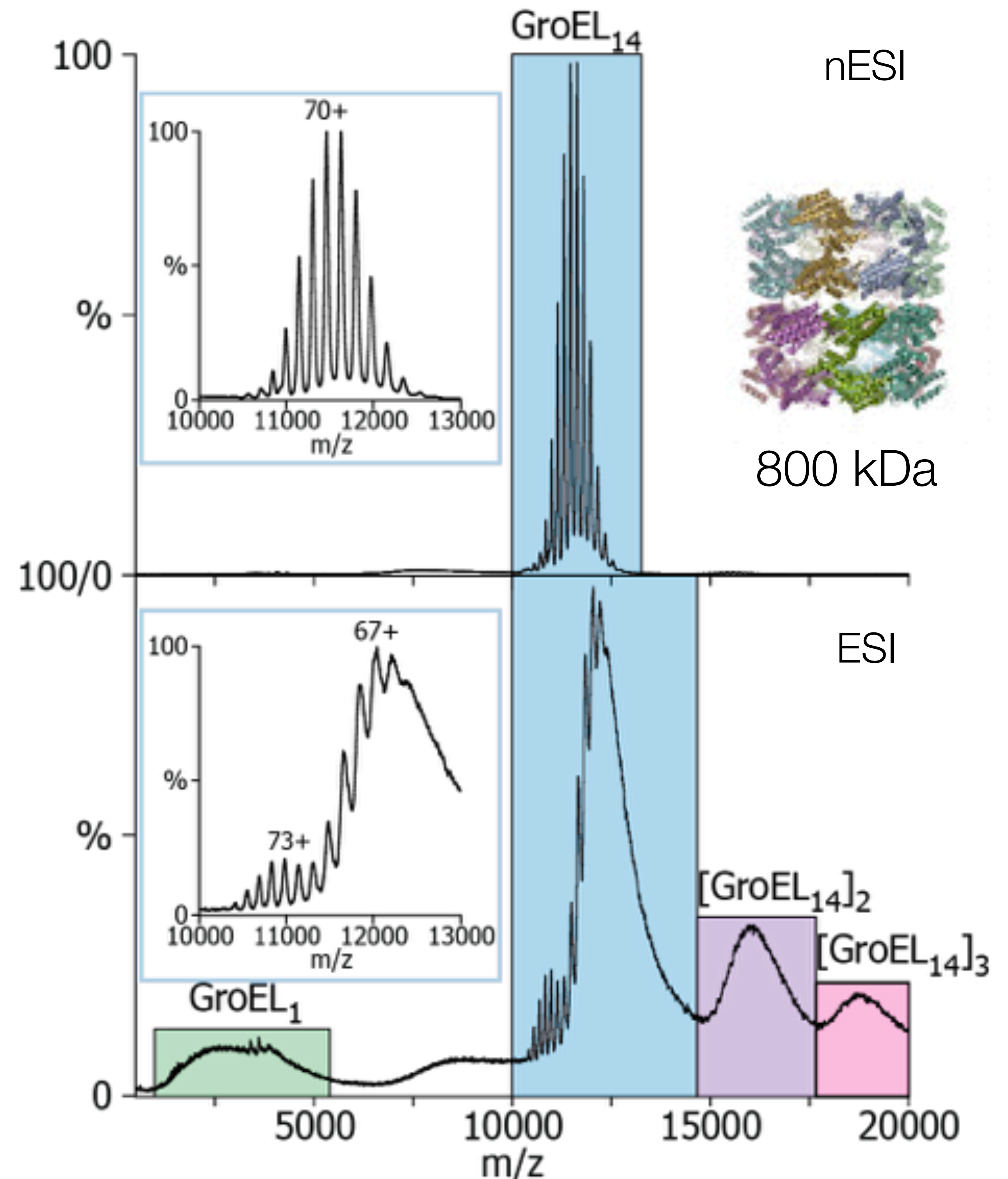
- Sample transferred into vacuum within detergent micelle
- Activation within the mass spectrometer removes detergent to leave “naked” protein assembly



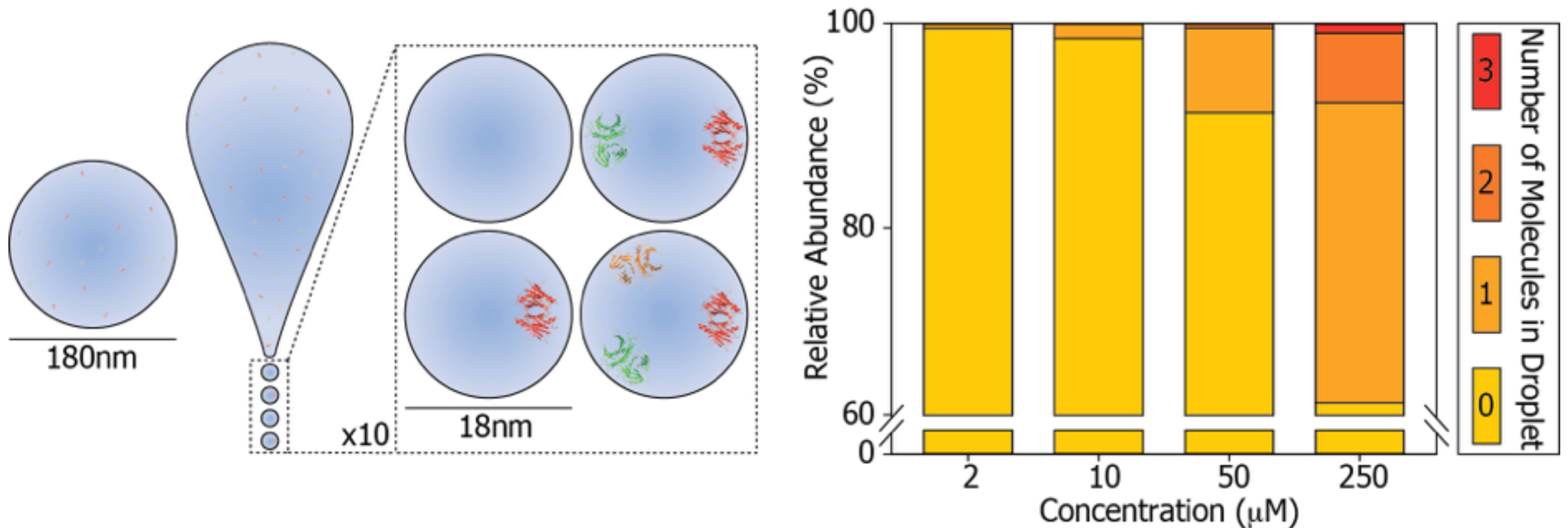

Barrera et al, Science (2008), 321, 243-6

# Benefits of nESI

- Lower sample amounts (flow rate approx 10nL/min, vs 5 μL/min in ESI)
- Can use aqueous buffers and ambient temperatures
- Narrower charge states due to fewer adduction
- Less dissociation of oligomer
- Symmetrical charge state distribution indicative of a single conformation
- Fewer non-specific aggregates

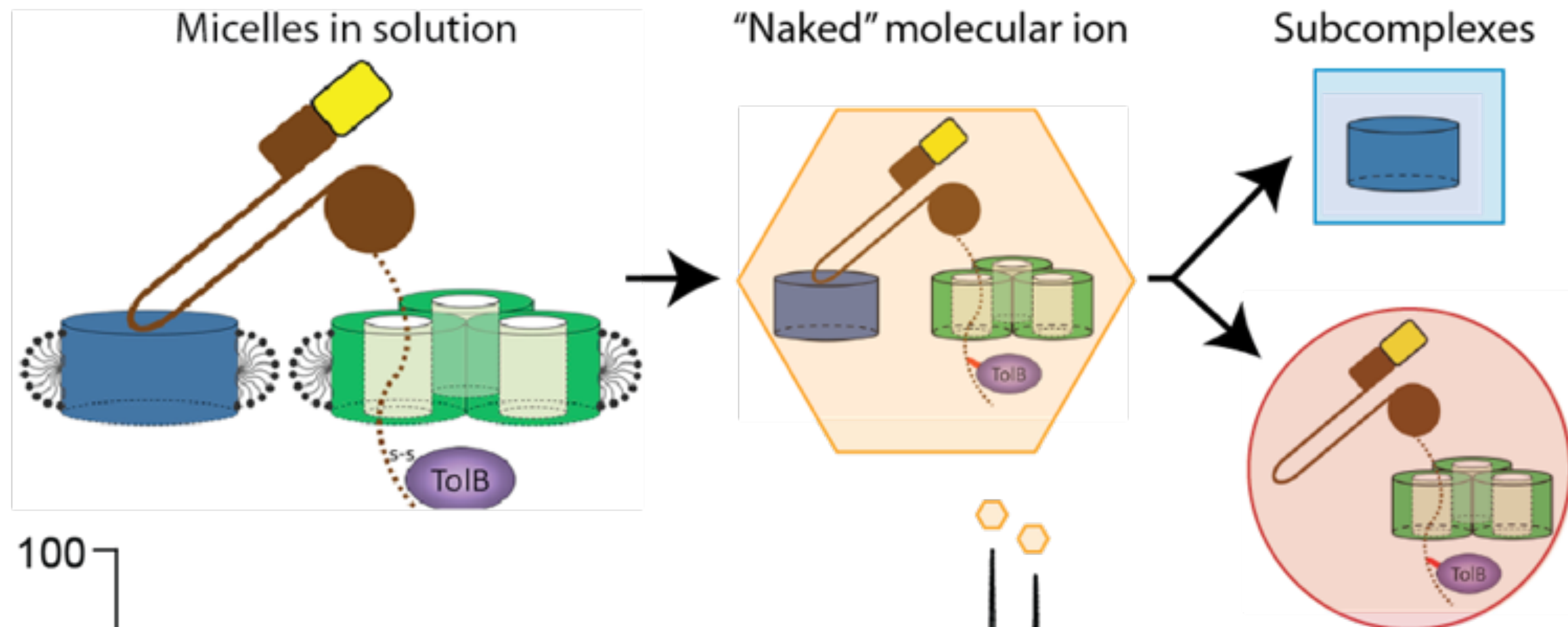


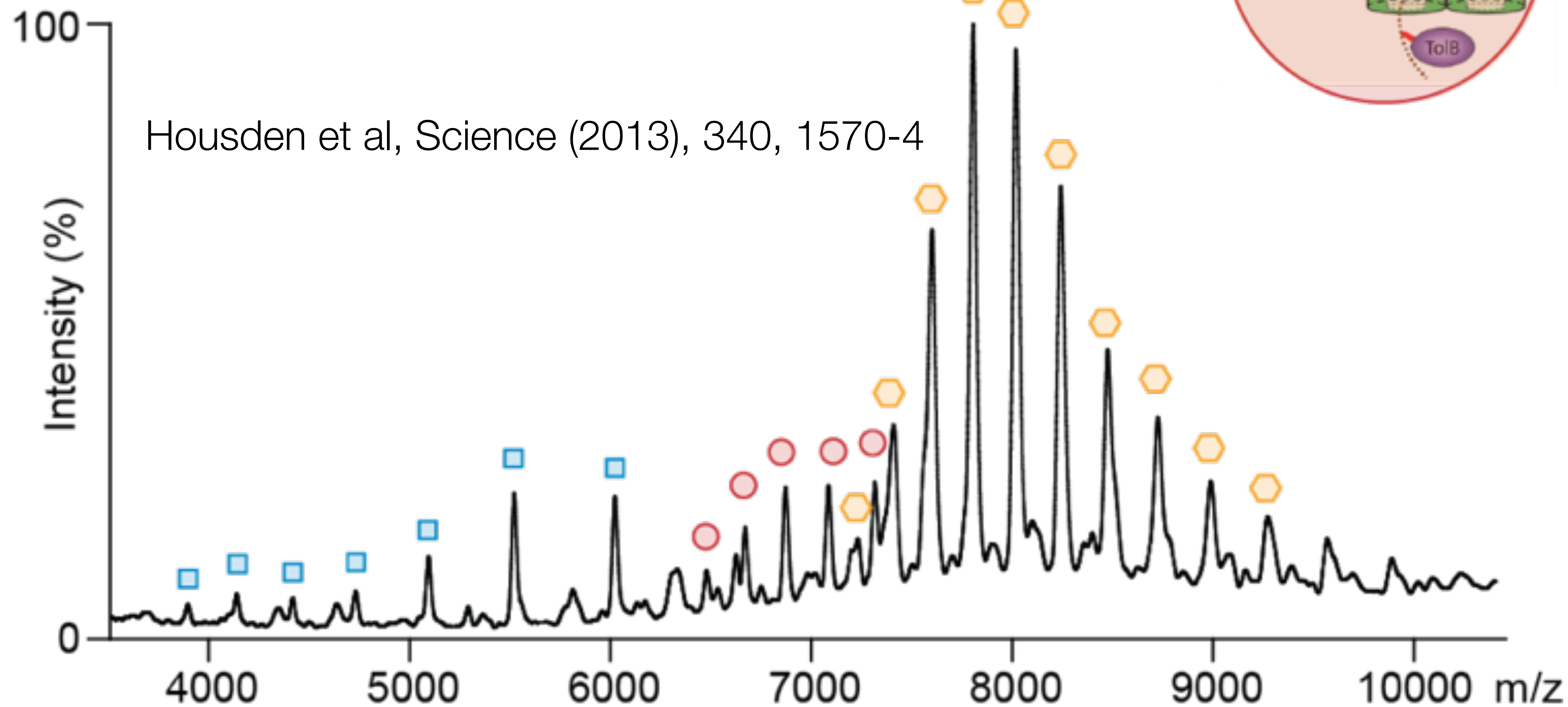
# Non-specific associations during ESI




- Probability of there being >1 analyte molecules in 'final' ESI droplet
- Most droplets are empty, occupancy increases with concentration
- Decreased initial droplet size in nESI reduces prevalence of non-specific aggregates

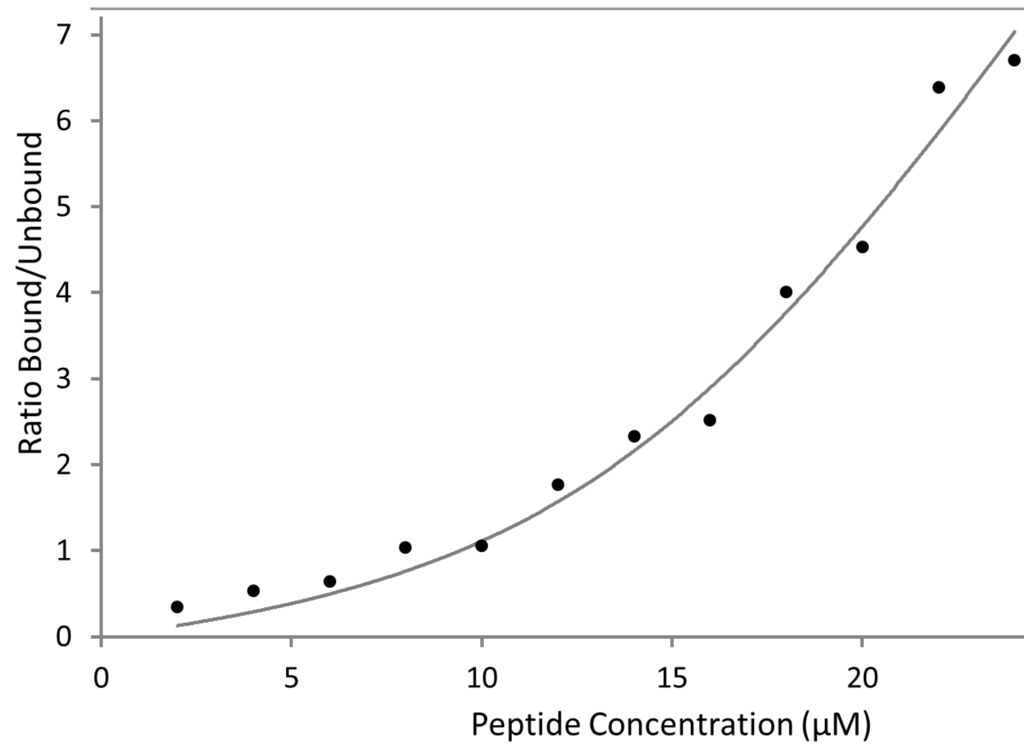
# Intact membrane protein machines - Example



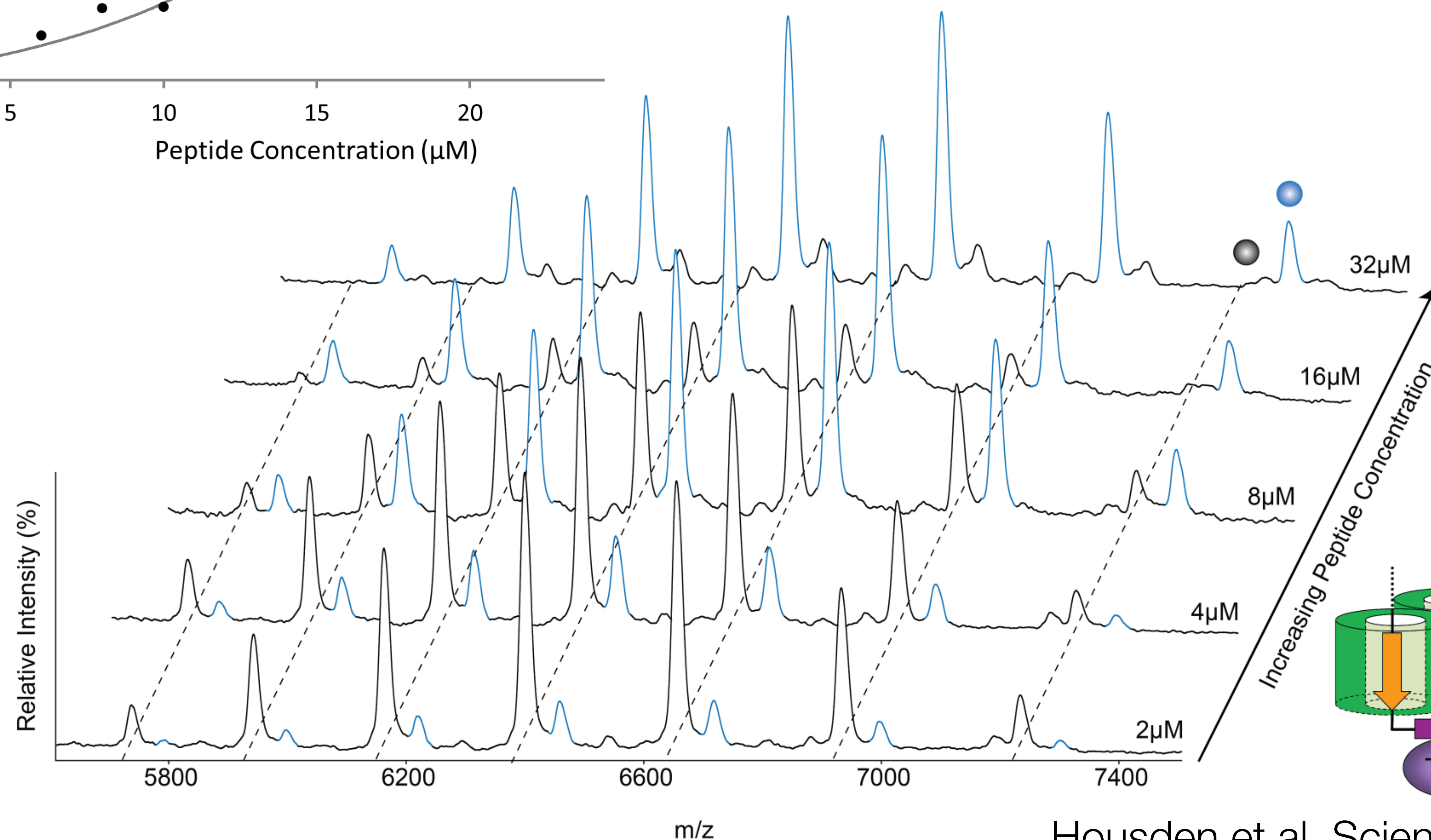



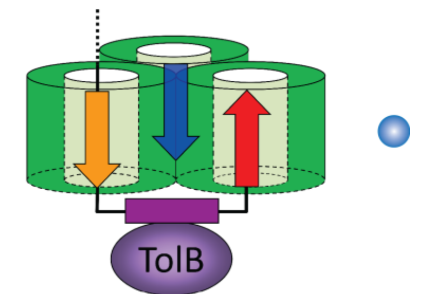

Housden et al, Science (2013), 340, 1570-4

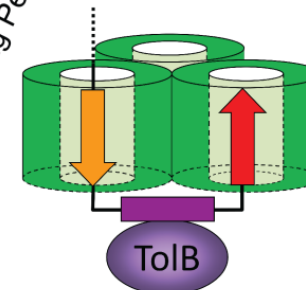
# Determining binding affinities - Example




- Titration experiments allow the determination of binding affinity
- Practicable in the 100 nm - 1 mM range



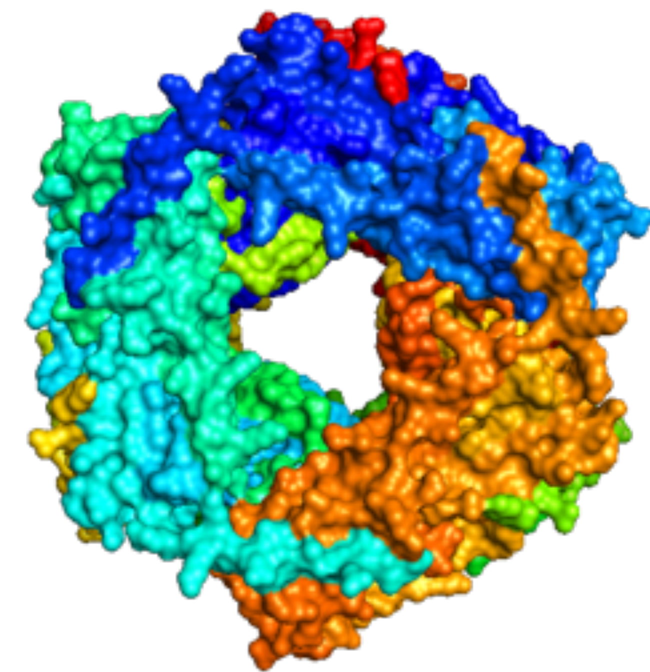




Housden et al, Science (2013), 340, 1570-4

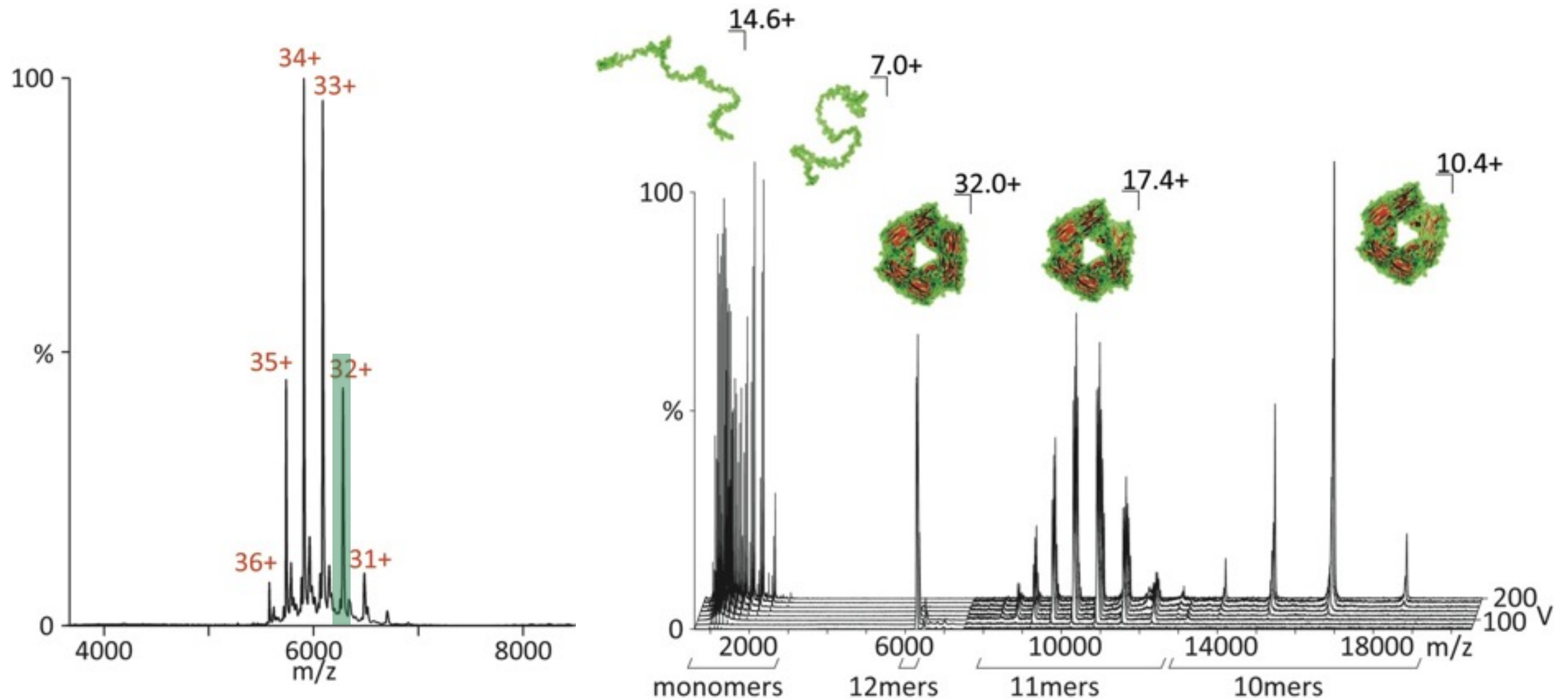
# Effects of activation in the gas phase

- Activating conditions lead to high quality mass spectra
- Same activation can lead to
  - collapse (i.e. smaller CCS)
  - then unfolding (i.e. larger CCS)



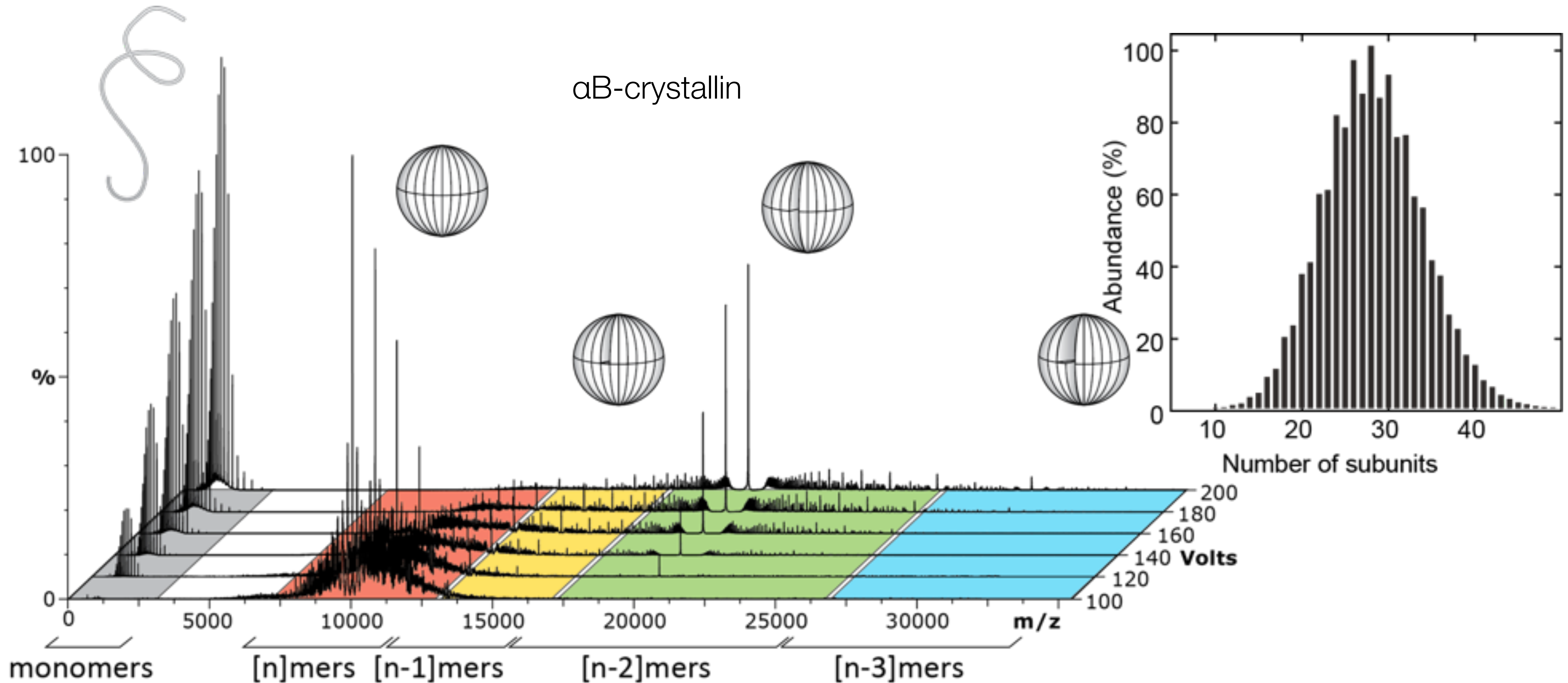
Benesch, J Am Soc Mass Spectrom (2009), 20, 341-8

# Collision induced dissociation of protein assemblies




- Dissociation is asymmetric with respect to mass
- Unfolded, highly charged monomers are removed sequentially

# Deconvoluting heterogeneity with CID




- Peak separation is aided by the charge reduction afforded by CID
- Predictable nature of CID allows back calculation of oligomeric distribution

# Quantifying stoichiometries

Baldwin et al, J Mol Biol (2011), 297-309

Measured mass (kDa)

200 400 600 800 1000

αB-crystallin

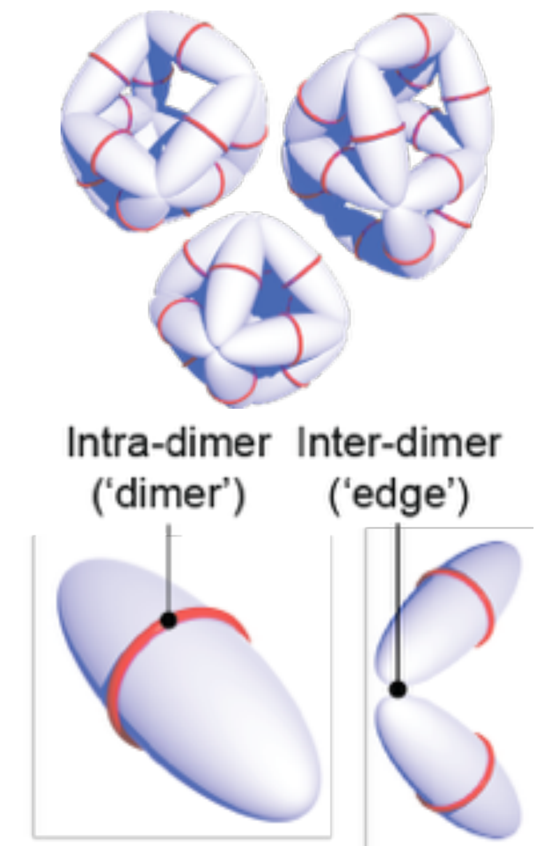
Tandem-MS

SEC-MALS

Normalised abudance

4 8 12 16 20 24 28 32 36 40 44 48 52 56

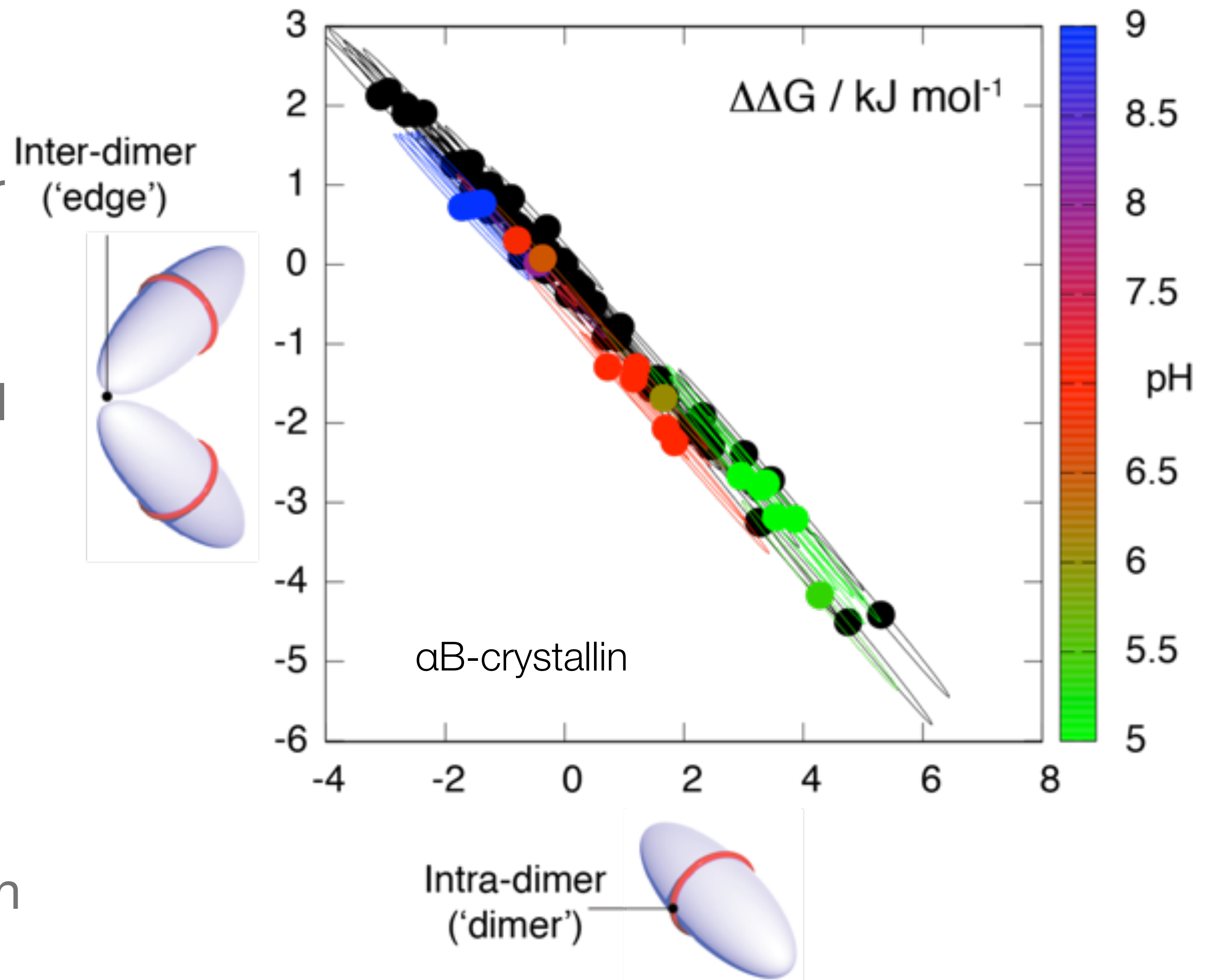
Number of subunits




- MS versus size-exclusion chromatography with multi-angle light scattering
- For proteins of similar composition, abundances match solution values

# Free energies from MS measurements - Example

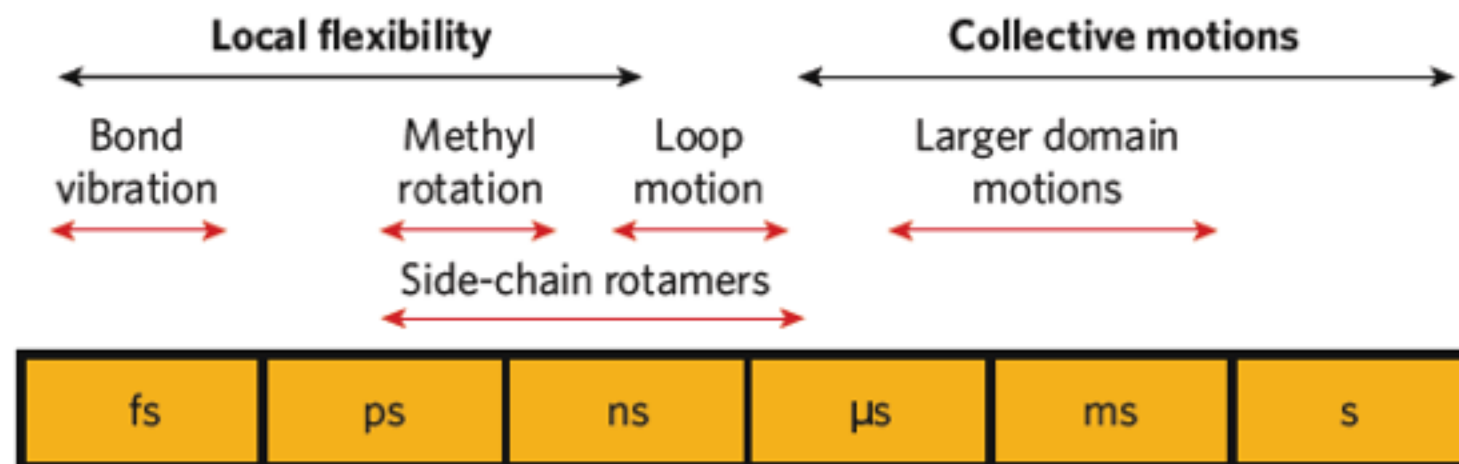
- αB-crystallin forms polydisperse oligomers via inter- and intra-dimer interfaces
- Abundances of stoichiometries are fitted to an appropriate biophysical model for oligmerisation
- Effect of mutation/ environment on free energies extracted
- Allosteric communication between interfaces




Hilton et al, Proc Roy Soc B (2013), 368, 20110405

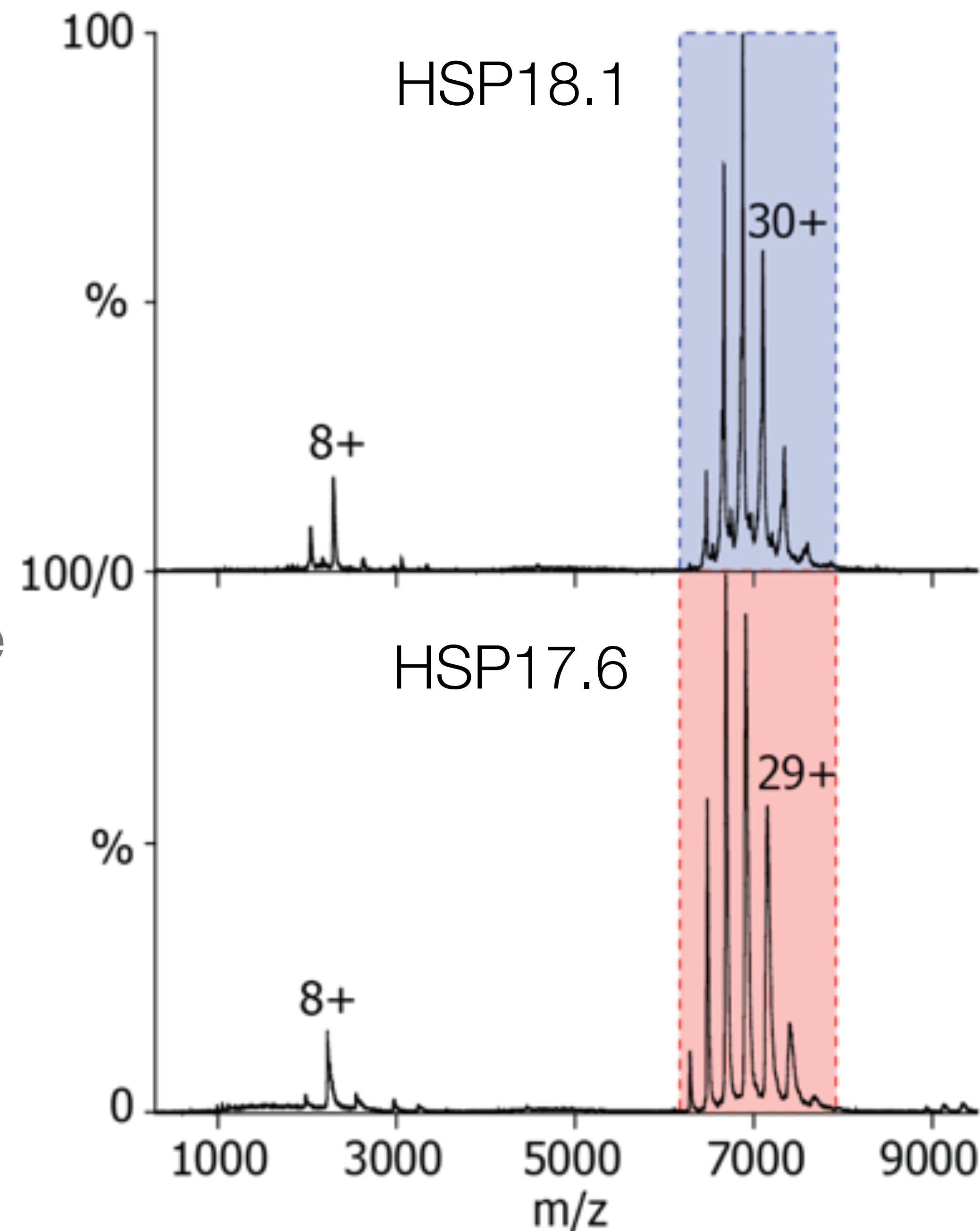
# Protein dynamics

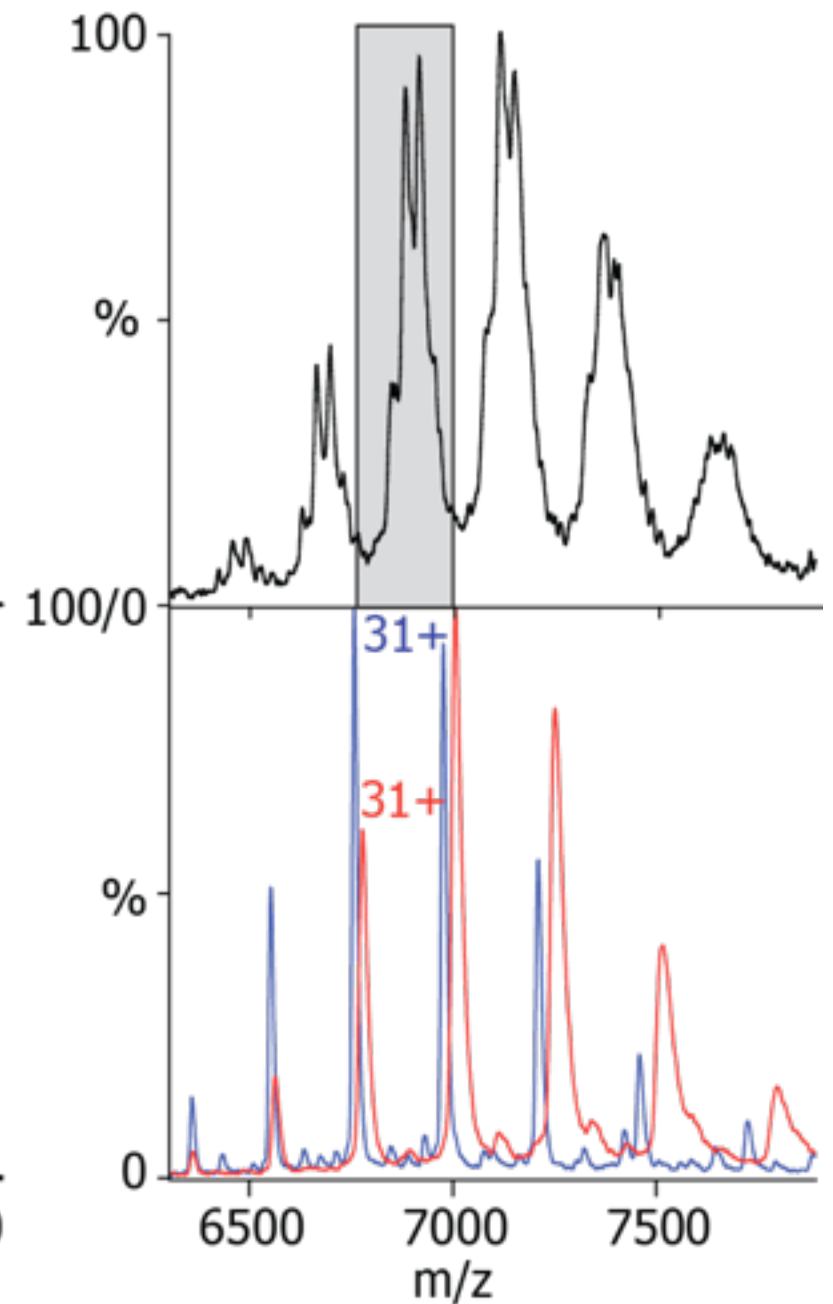
- Proteins are not static structures, but rather undergo fluctuations both at and before equilibrium
- Such ‘protein dynamics’ are crucial to their function in the cell
- These dynamics can span a wide range of amplitudes and timescales



# Quaternary dynamics - Example

- Two homologous proteins from the same cellular compartment incubated
- Subunit exchange results in the appearance of hetero-oligomers

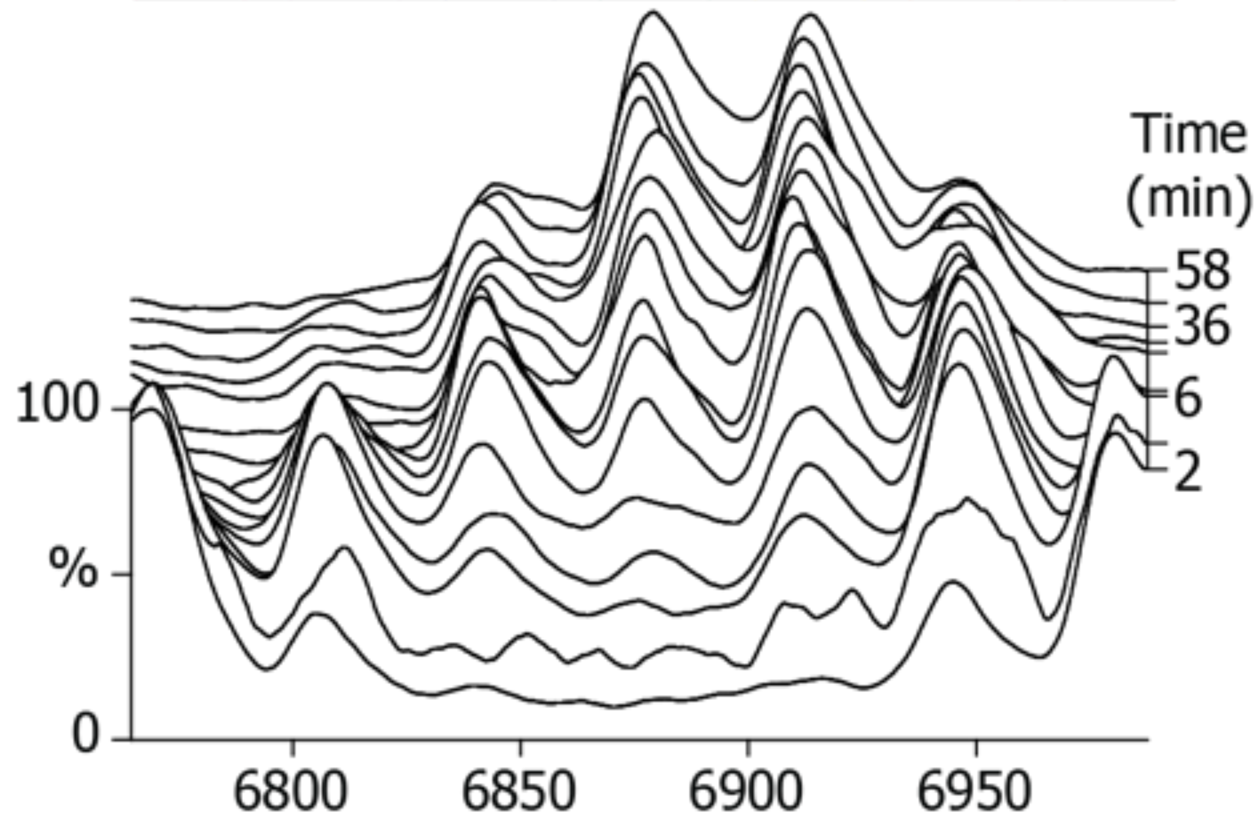


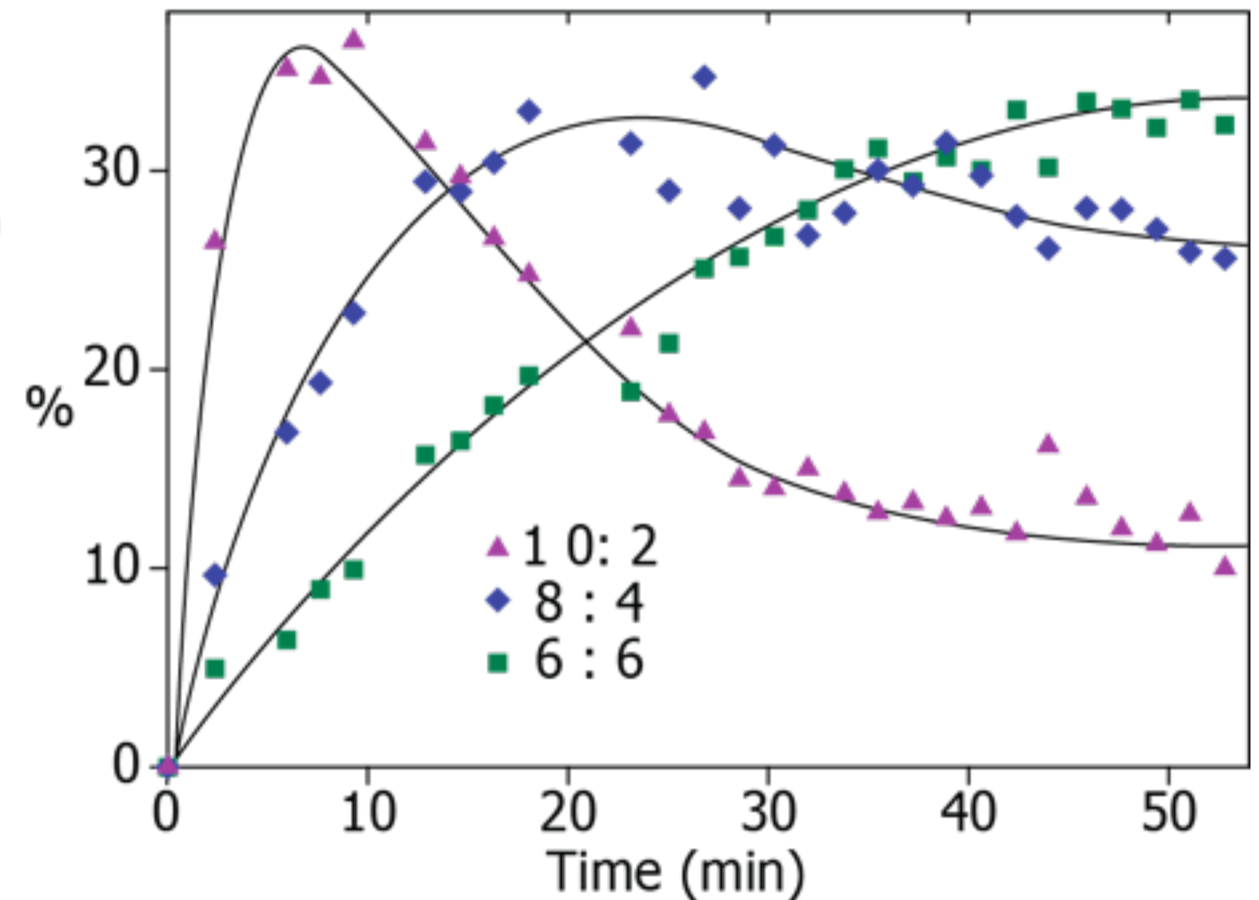



# Quaternary dynamics - Example

HSP17.6 HSP18.1

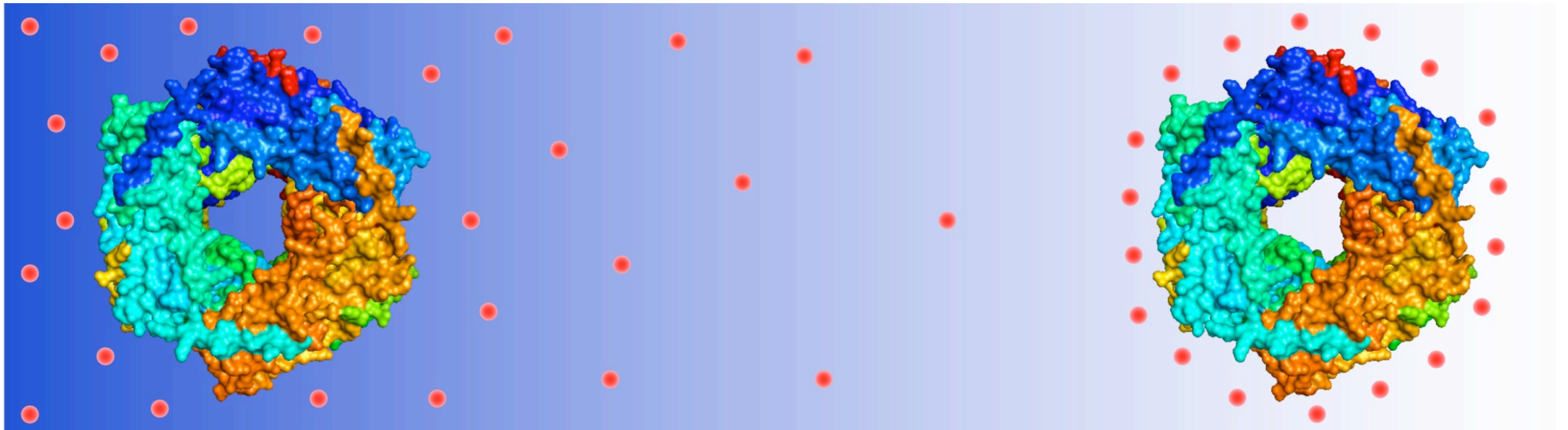
12:0 10:2 8:4 6:6 4:8 2:10 0:12



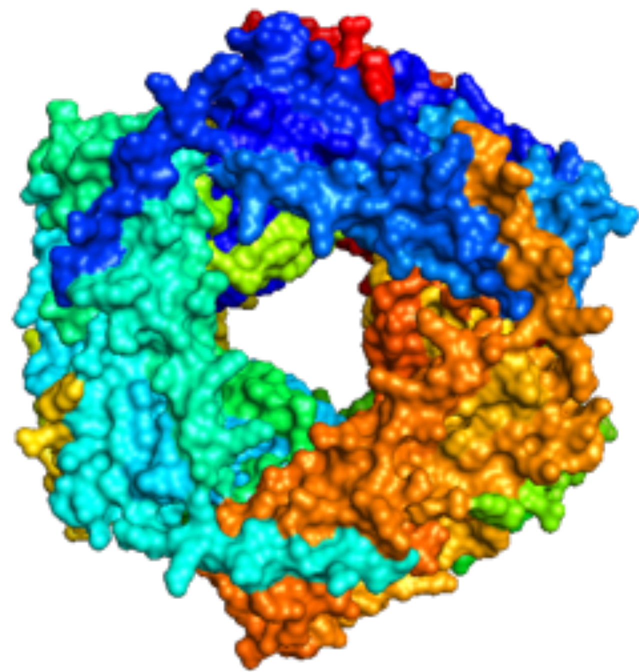

- Exchange proceeds via the movement of dimeric units
- Incorporation is via sequential incorporation of dimers into oligomers
- Hetero-assembly leads to a wide variety of possible oligomers

# Preservation of structure



- It is clear stoichiometry is preserved in the mass spectrometer, but can we probe native structure?

# IM-MS spectrum

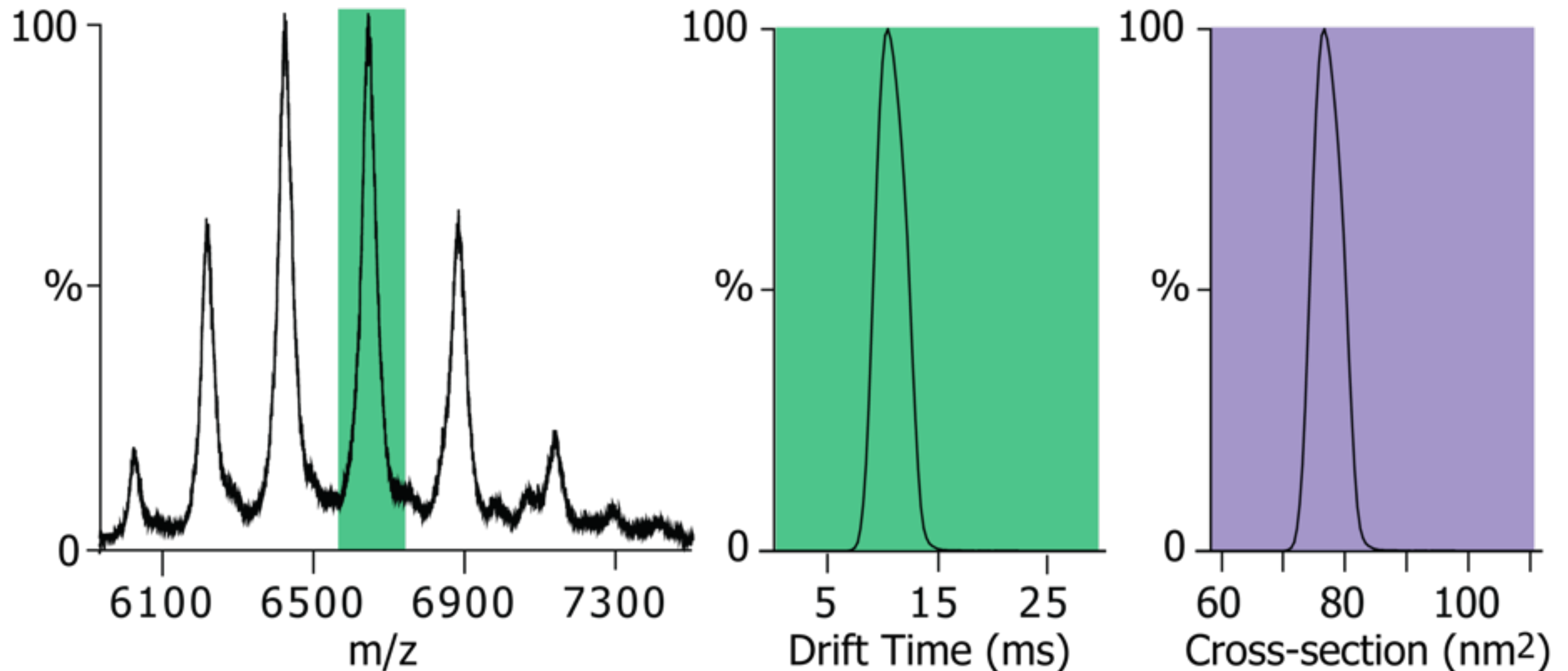


HSP16.9

- Plot of m/z versus drift time

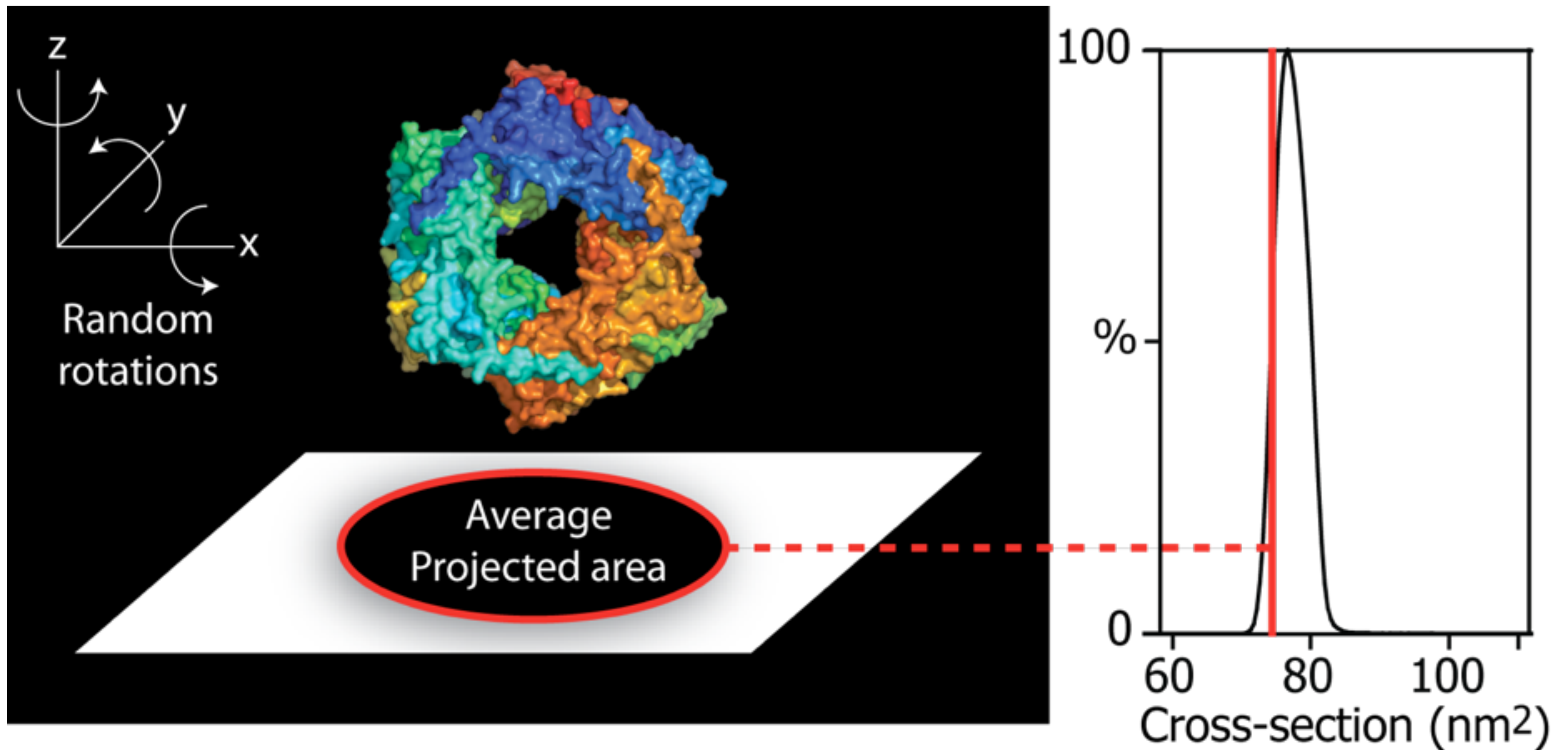
Intensity (%)
Drift Time (ms)
m/z
Intensity (%)

# Obtaining an experimental CCS



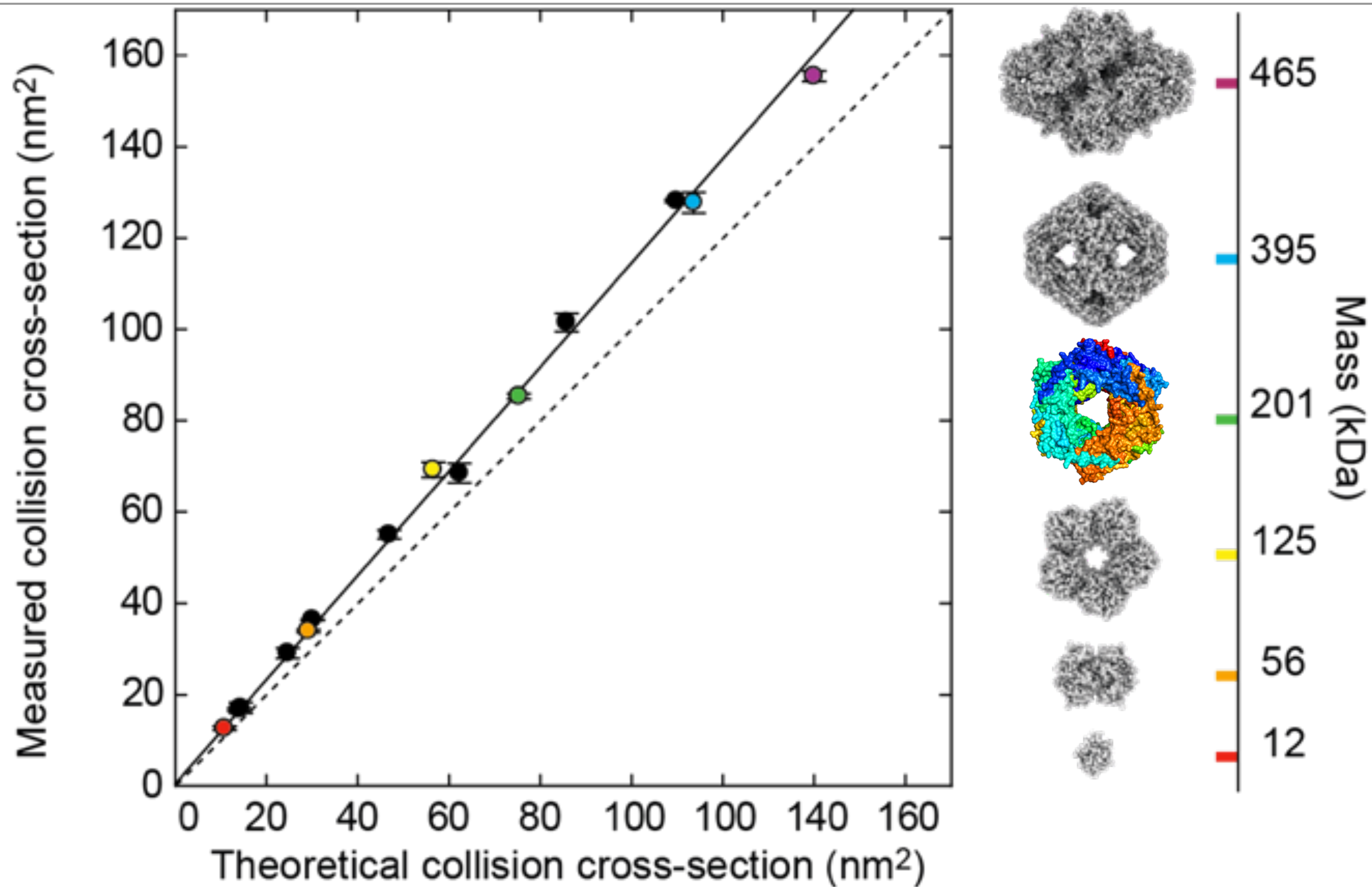

- Every feature resolved in m/z has an associated drift time distribution
- Drift time is converted into CCS either directly or via calibration

# CCS values from protein structures




- Can approximate CCS as rotationally averaged projected area
- Determine 'theoretical' CCSs from solved protein structures

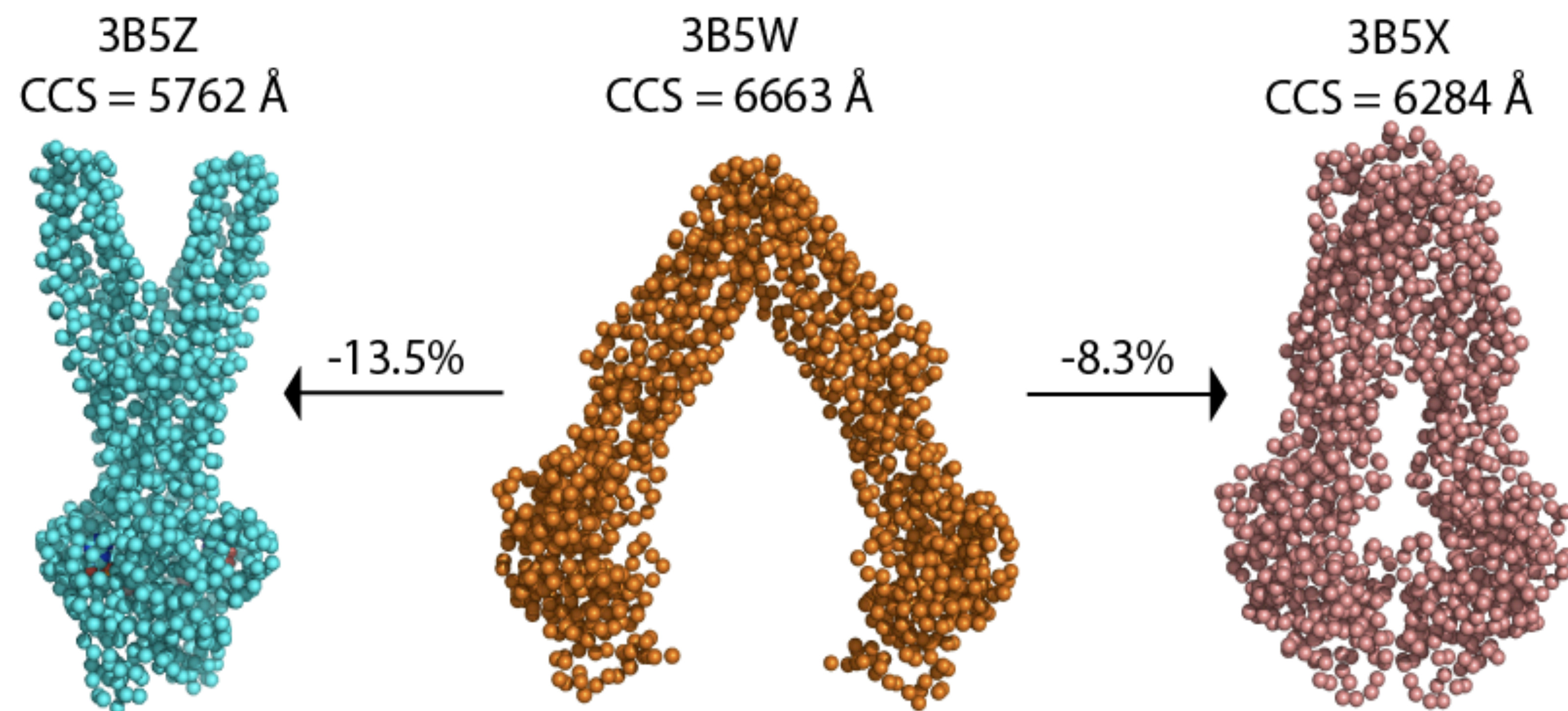
# CCS comparison

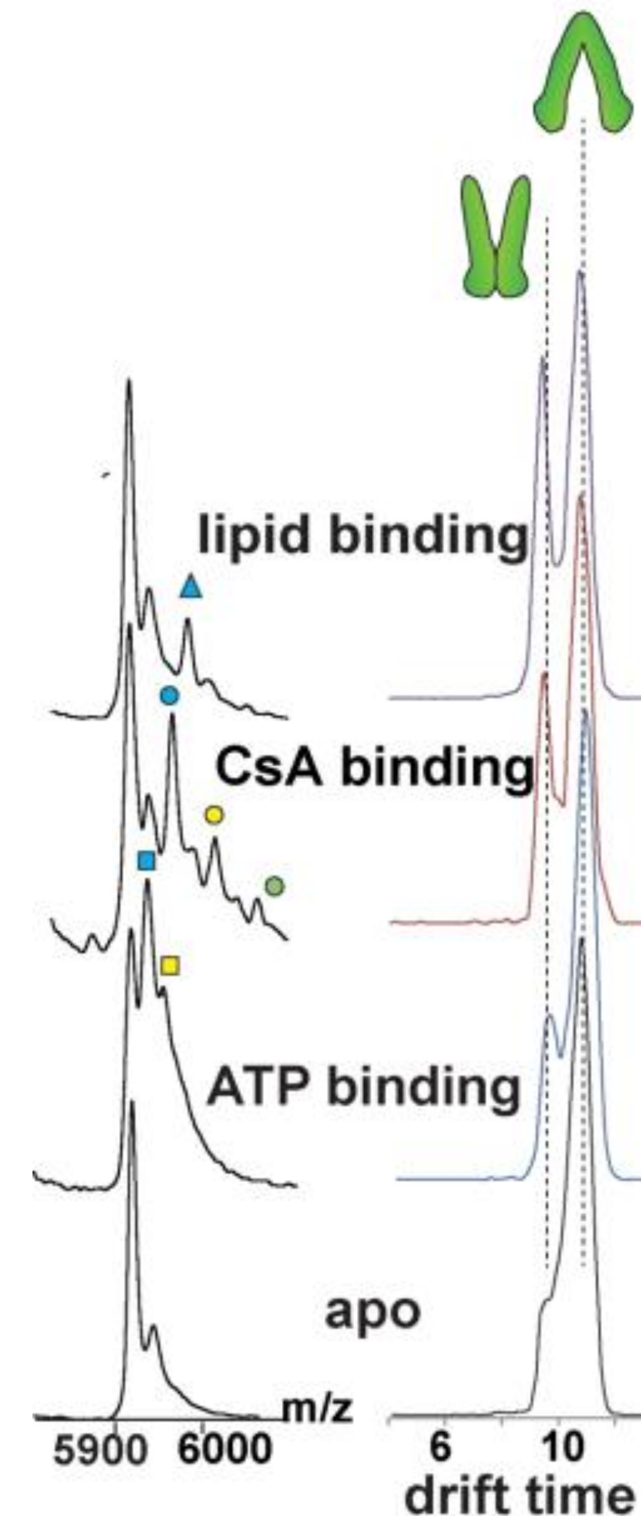



- Excellent correlation between theoretical and measured values for globular proteins
- Discrepancy is due to simplicity of ‘projection approximation’
- Correlation motivates use of IM measurements in assessing model structures

# Using IM-MS to measure conformers - Example

- P-glycoprotein is an low specificity efflux pump which impairs drug delivery
- IM-MS allows the detection of different conformations - outward, inward open, inward closed (left to right)
- Small molecules affect conformational equilibrium

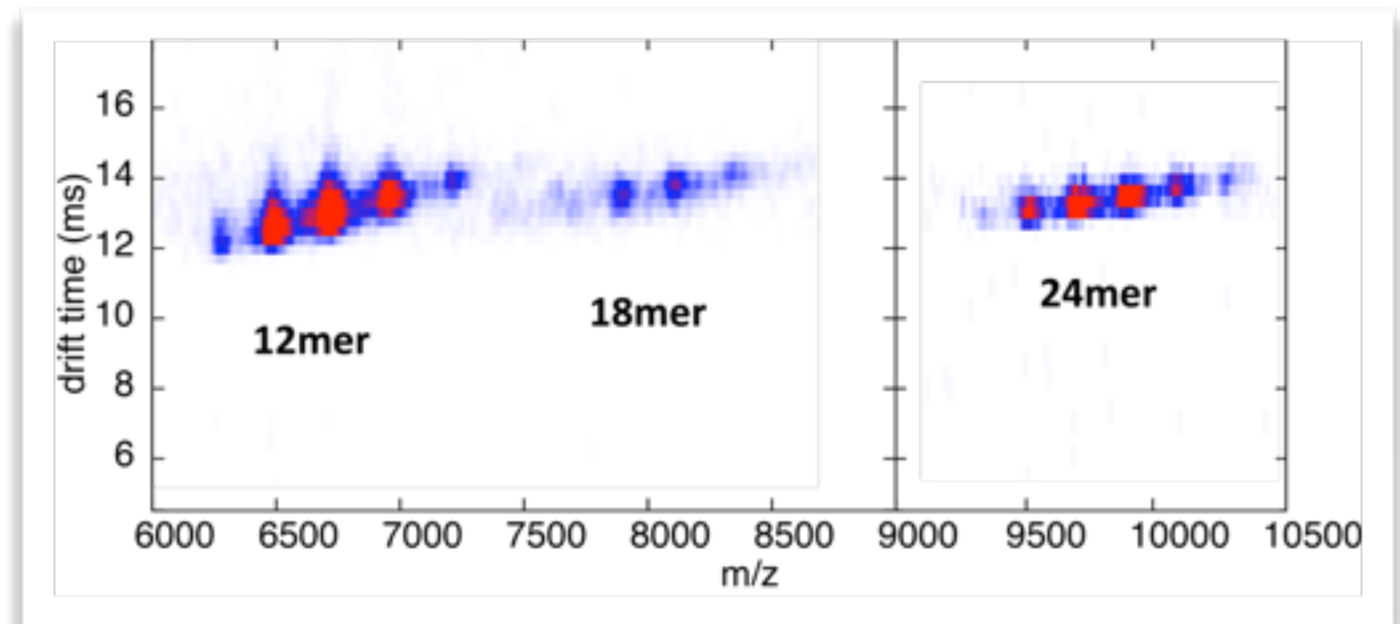


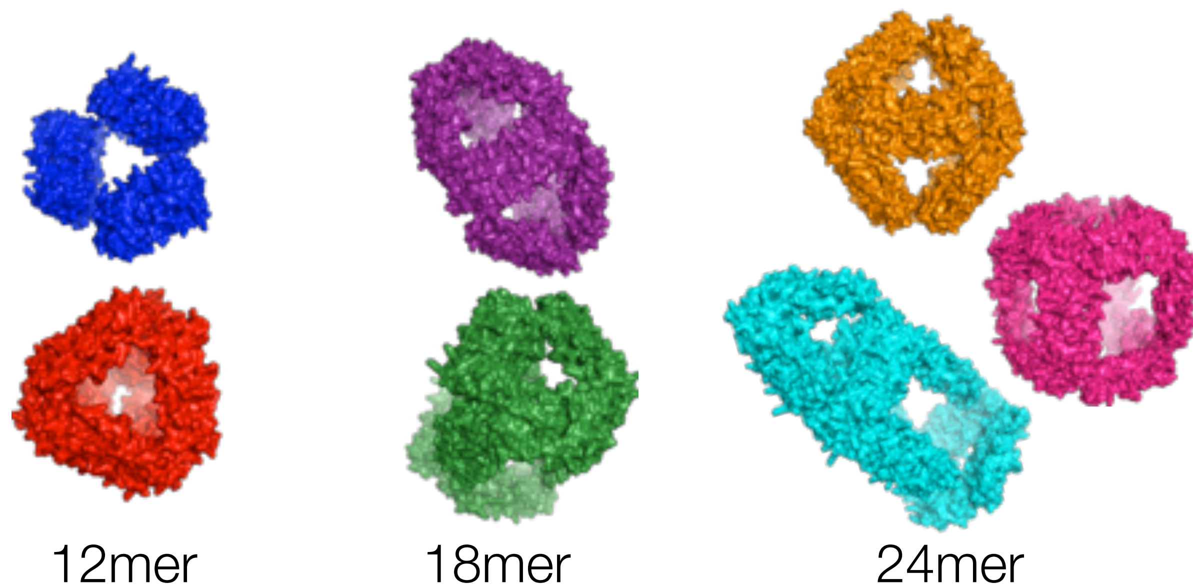



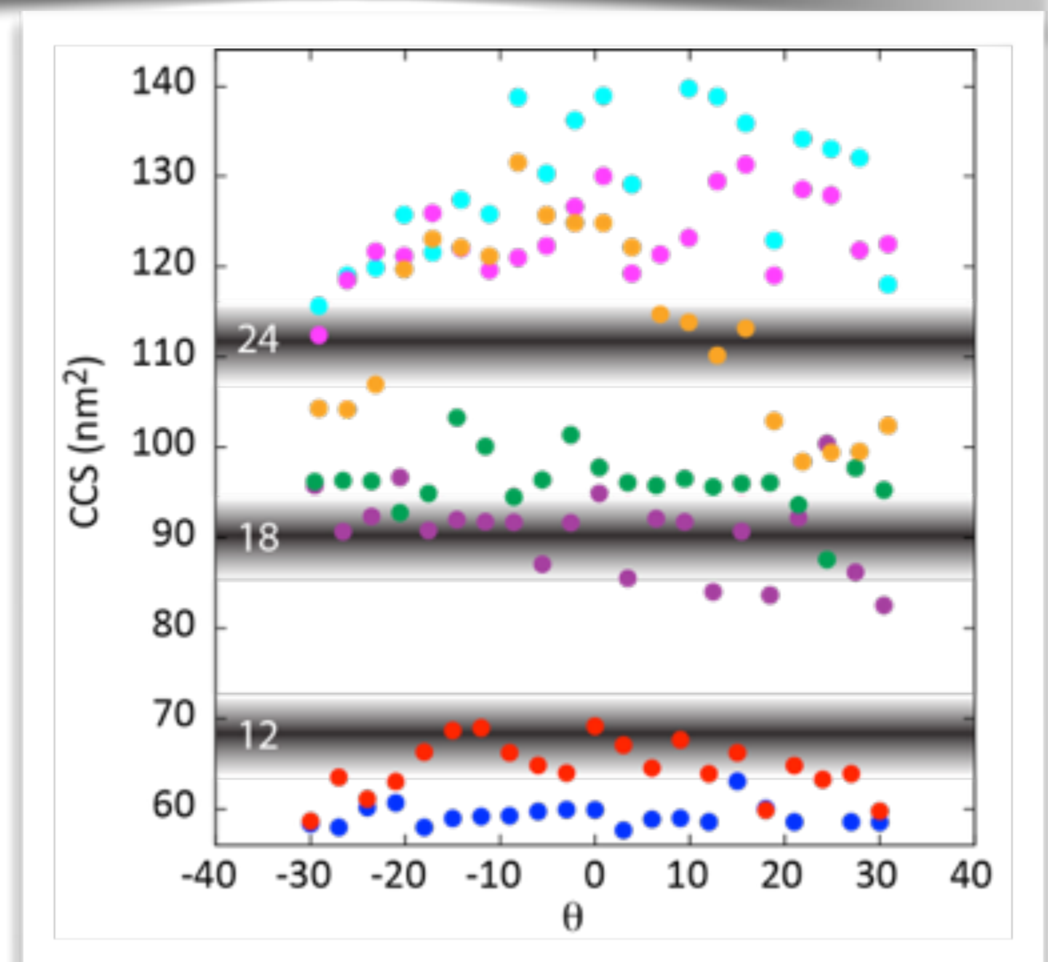

Marcoux et al, Proc Natl Acad Sci USA (2013), 110, 9704-9

# Using IM-MS to filter structures - Example

- Polydisperse oligomeric protein exists in three different stoichiometries
- Based on comparison with homologous proteins likely structures are polyhedral
- Different polyhedral models can be compared to the IMS measurements



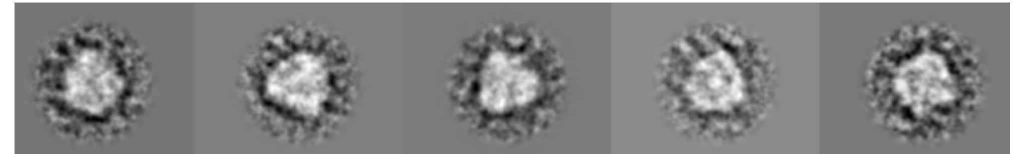



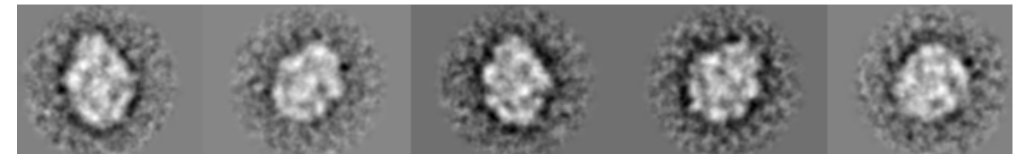
# Using IM-MS to filter structures - Example

- Compare random rotations of models to TEM class averages
- Lower score is better fit
- Projected area from TEM is conceptually similar to CCS area from IM
- Combination of techniques reveals ensemble structures that

12mer



18mer



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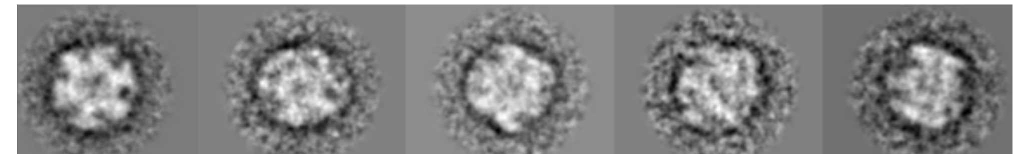

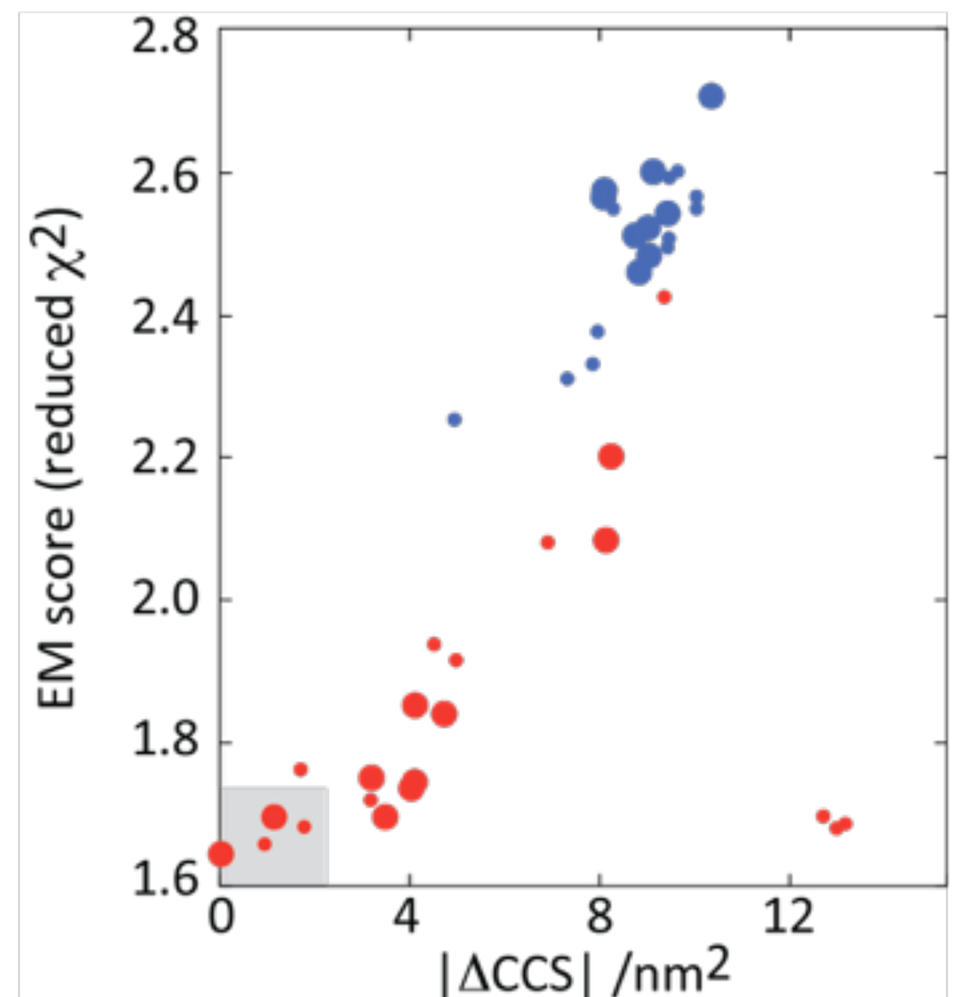


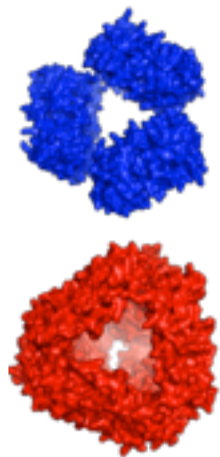

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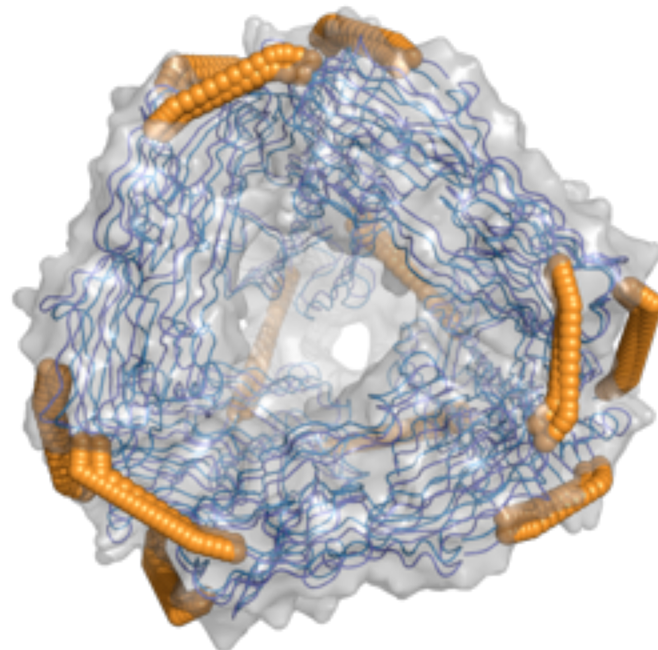
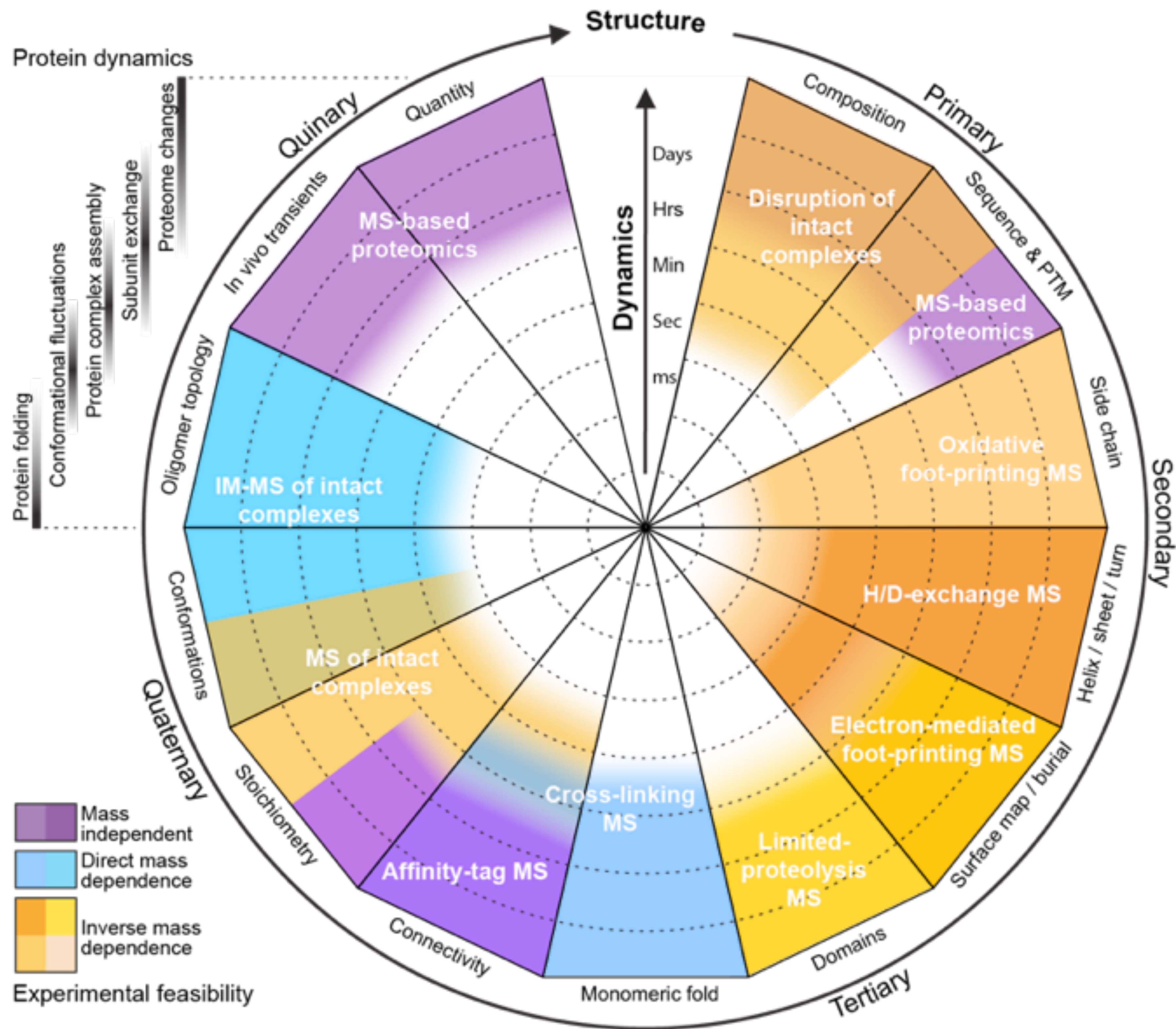
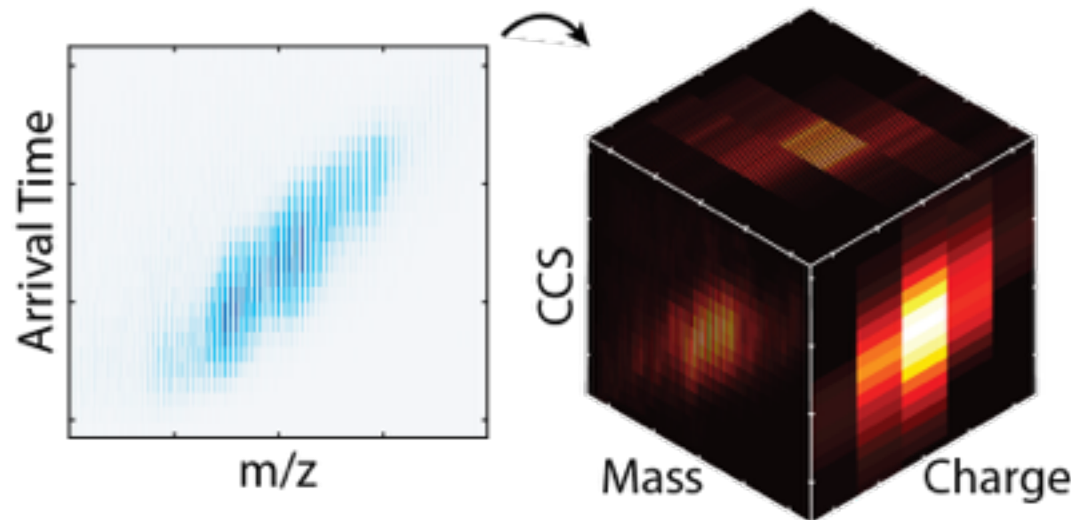


# MS across wide range of time and length scales



# In-house software to enable quantification

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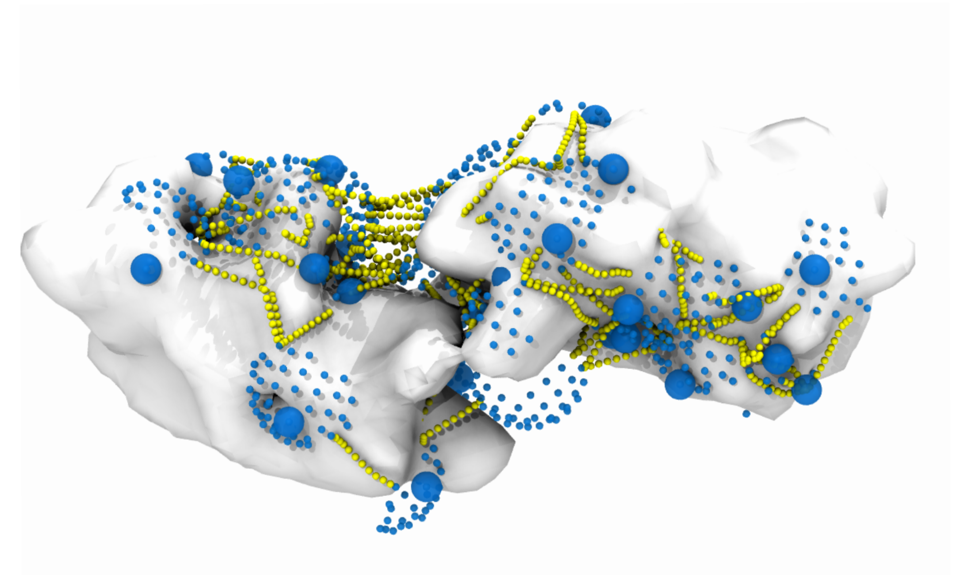
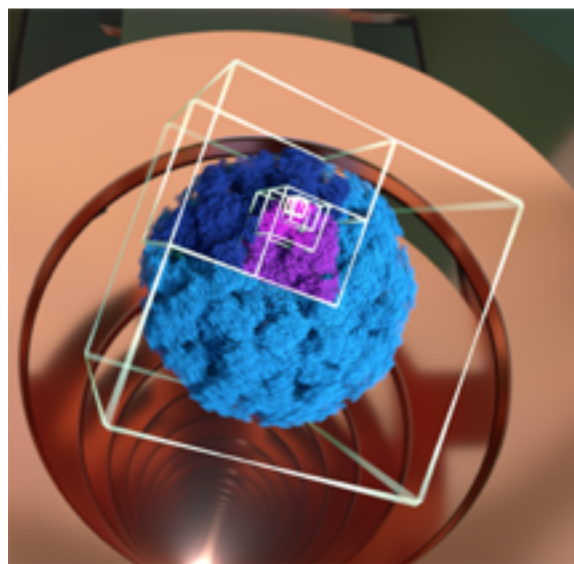


DynamiXL



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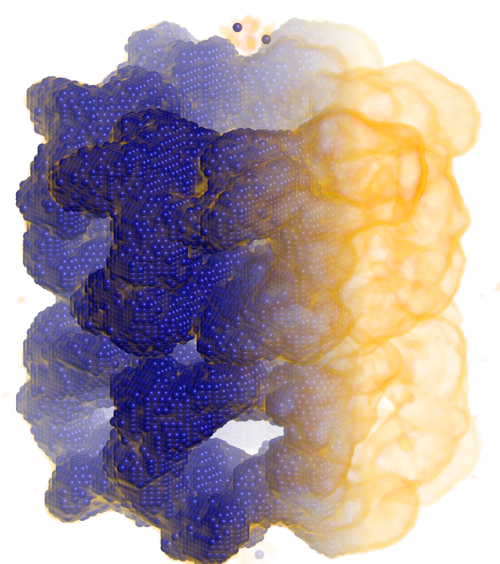
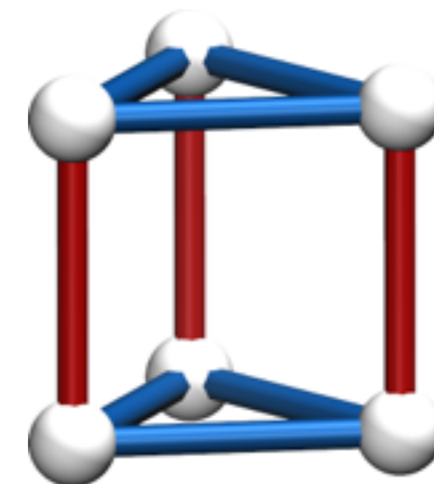


EMnIM.chem.ox.ac.uk

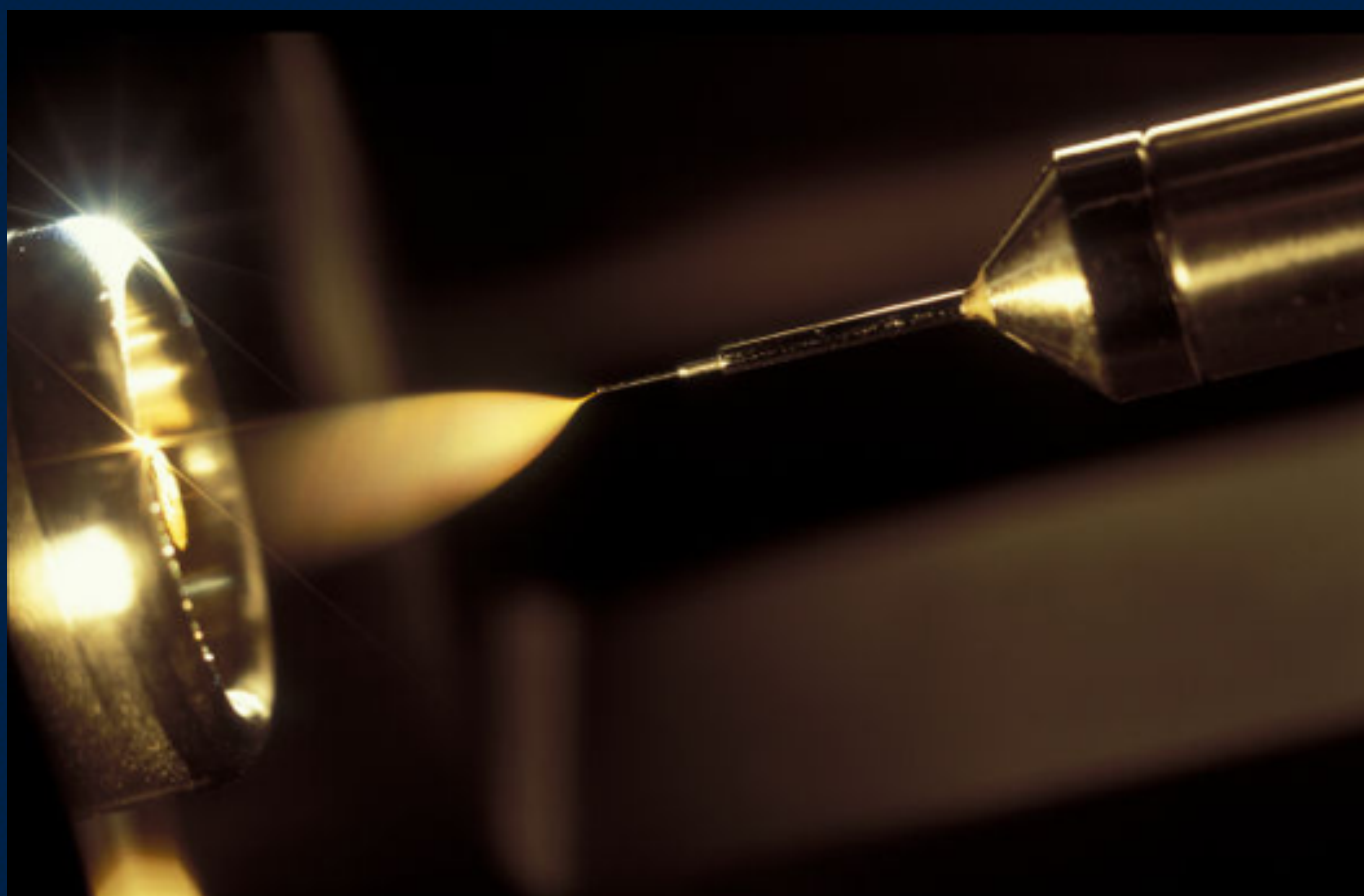


BioBOx

DEPARTMENT OF CHEMISTRY

# GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 8

## Mass spectrometry for biophysics and structural biology



Professor Justin Benesch, 23rd November 2016