

Biophysical Chemistry

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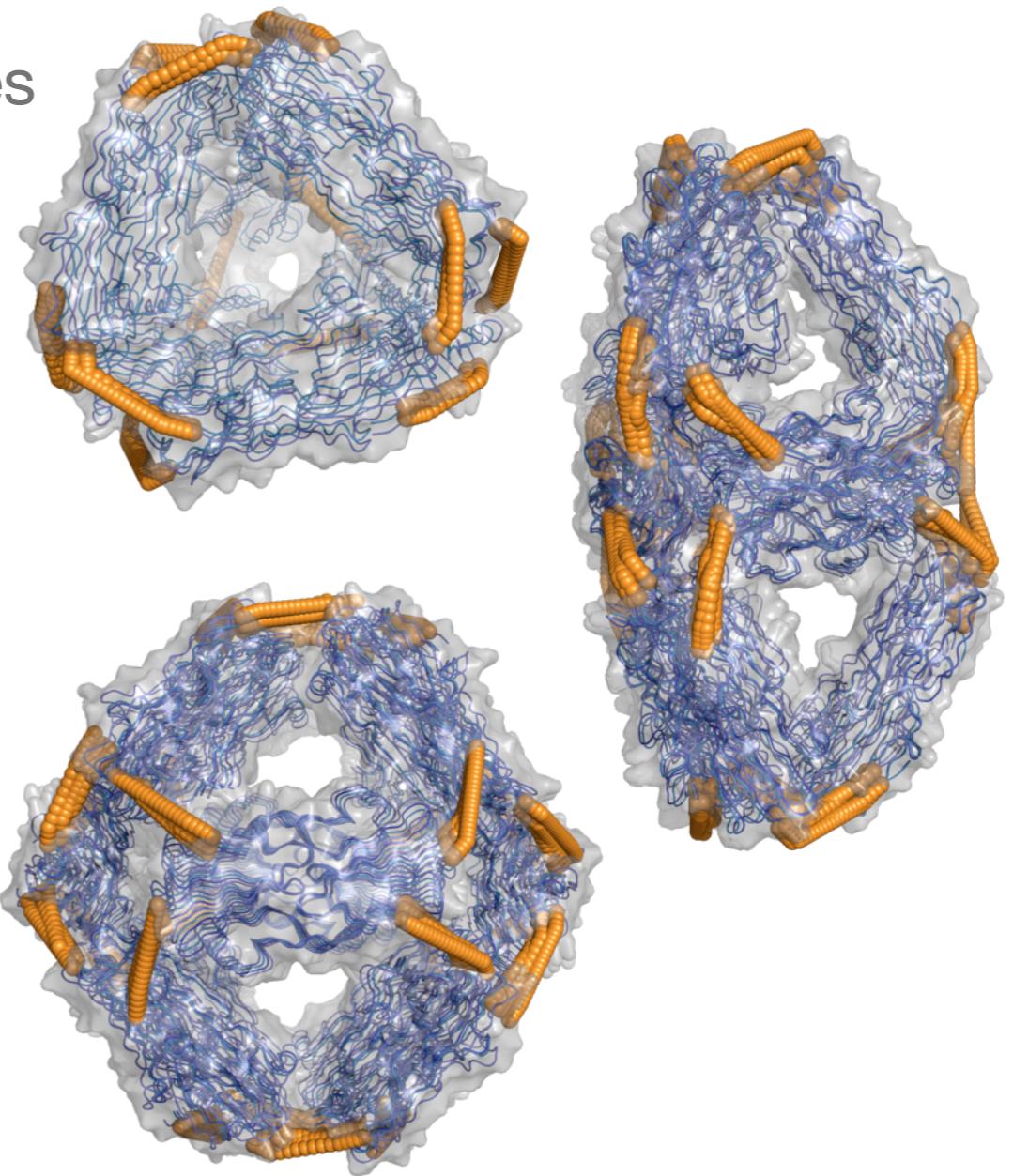
Lecture 8 - Gas-Phase Structural Biology

- **Mass spectrometry of macromolecular assemblies**

- Mass measurement of very large molecules
- Ion mobility spectrometry
- Preservation of structure in the gas phase

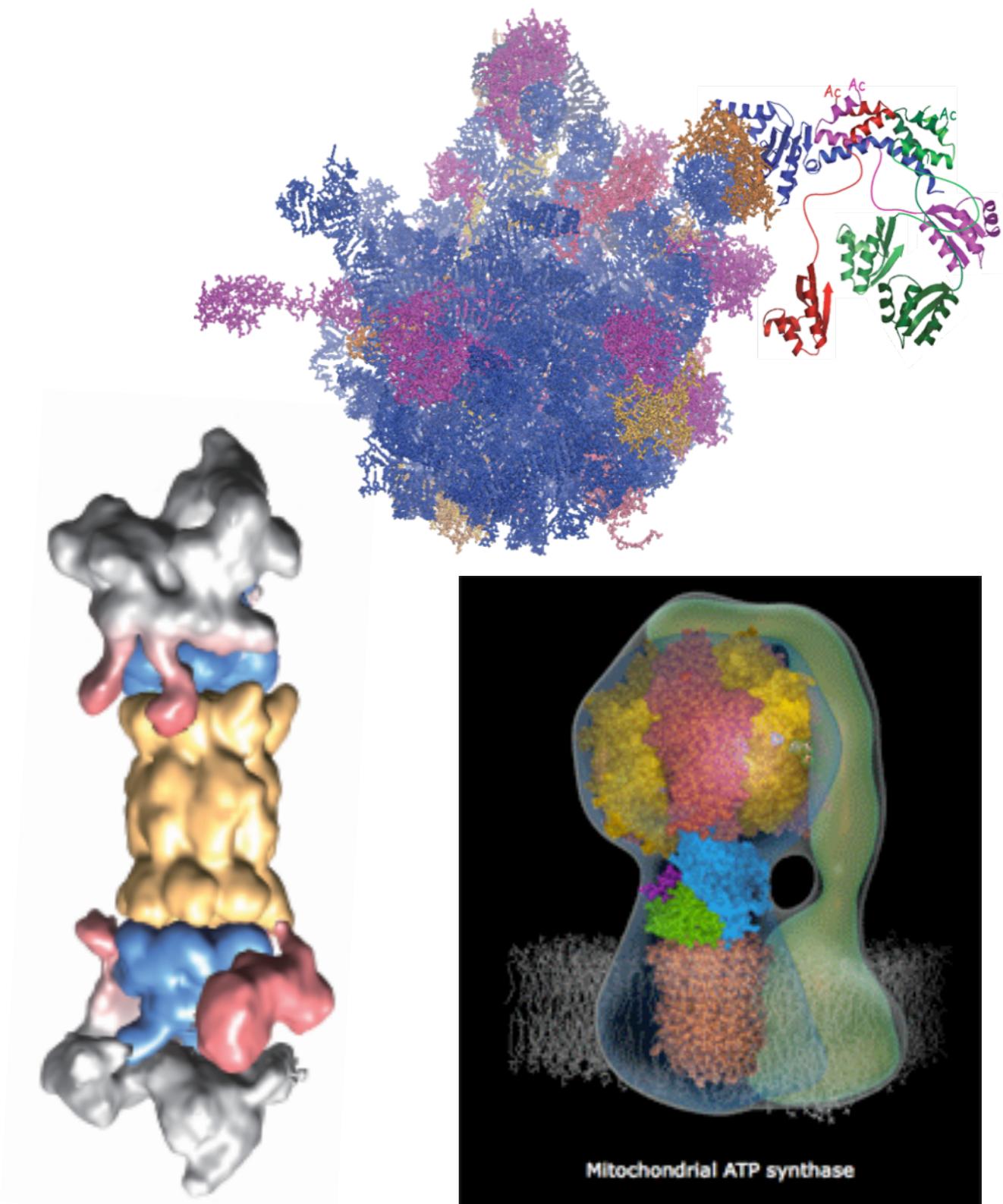
- **Determination of structure and dynamics**

- Protein polydispersity and heterogeneity
- Quaternary dynamics
- Hybrid approaches for structural biology

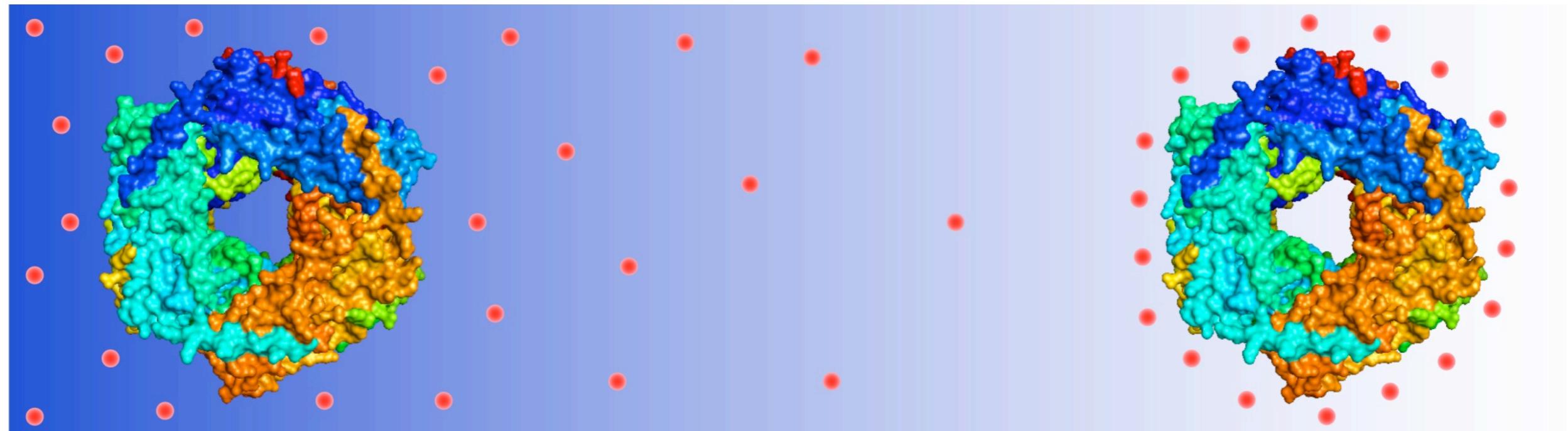


Proteins carry out their functions as assemblies

- Majority of proteins exist as multi-subunit complexes
- Complexes can be homomeric or heteromeric
- Average complex size is 4.9 subunits per oligomer (in yeast)
- This quaternary structure is mediated by noncovalent interactions

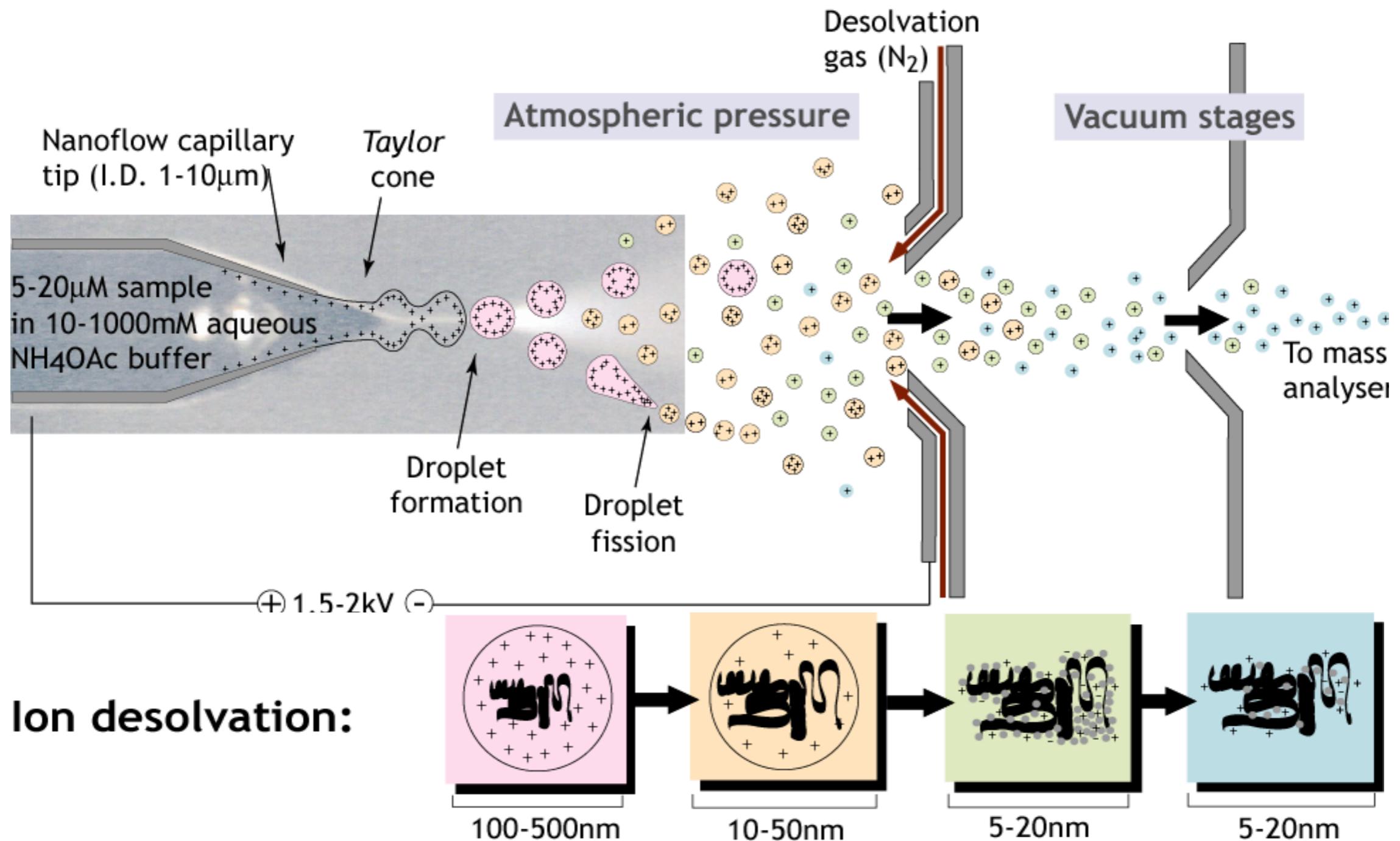


Maintaining noncovalent interactions

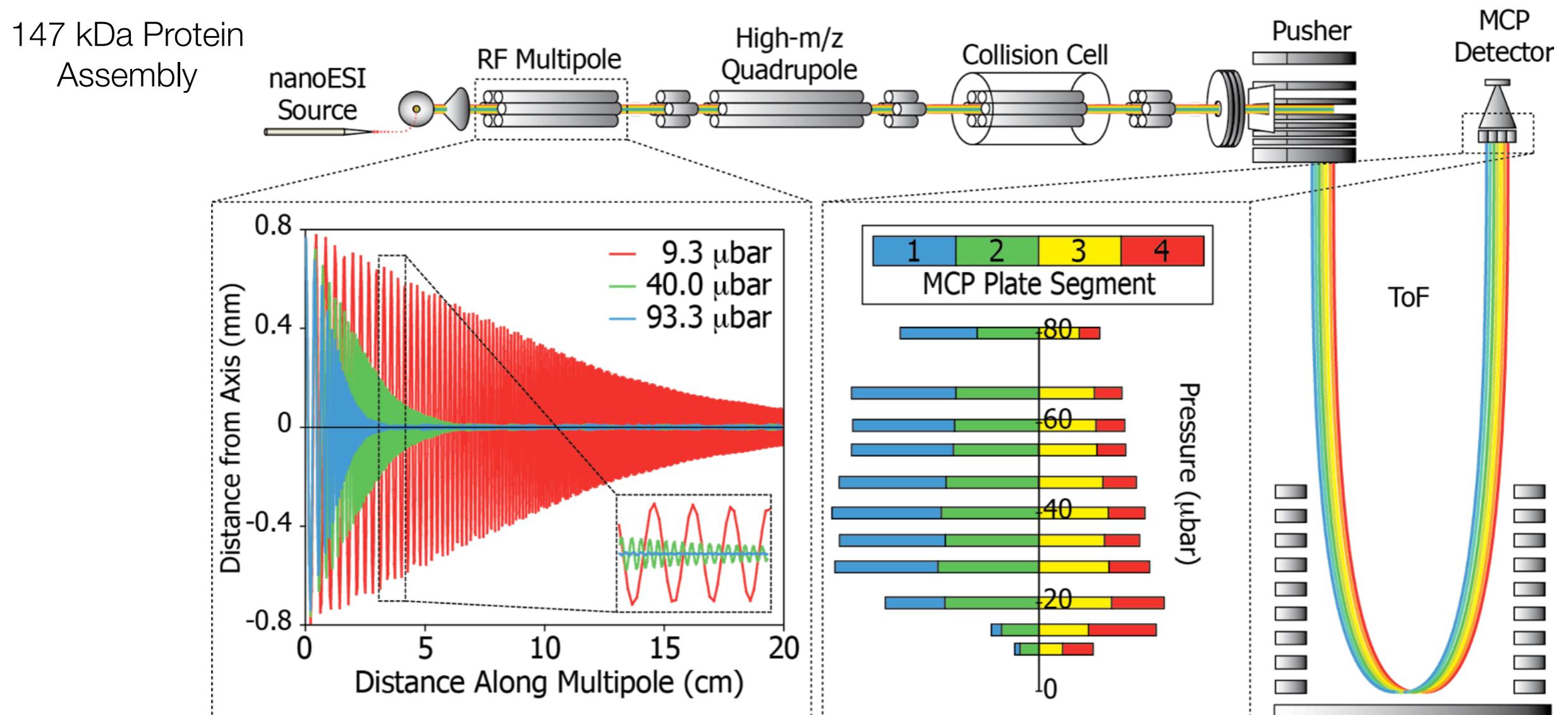


- Transfer multi-subunit protein assembly from solution into gas phase
- Requires control of ionisation conditions, and ion transmission

Nano-electrospray ionisation (nESI)

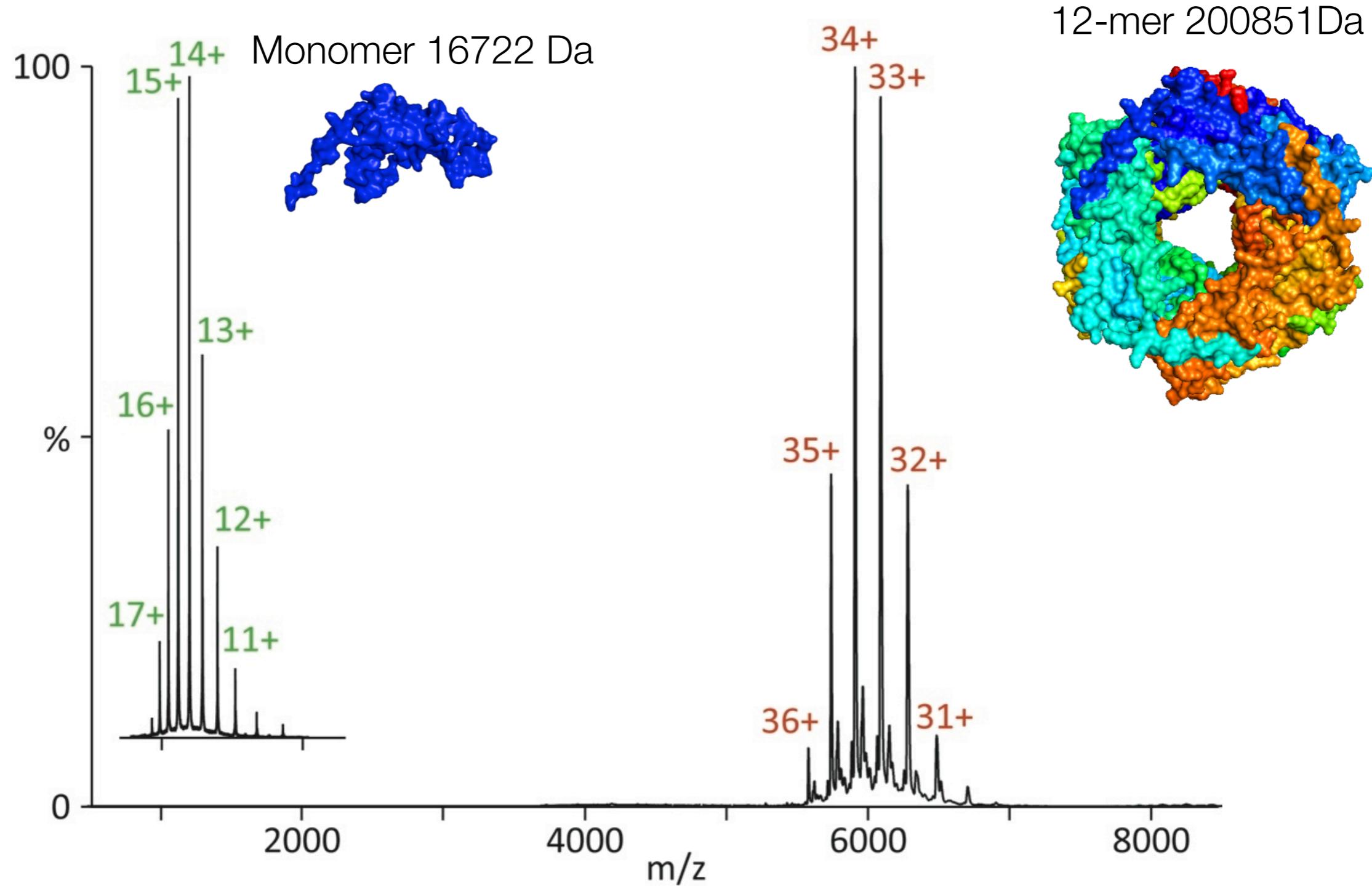


Collisional focussing

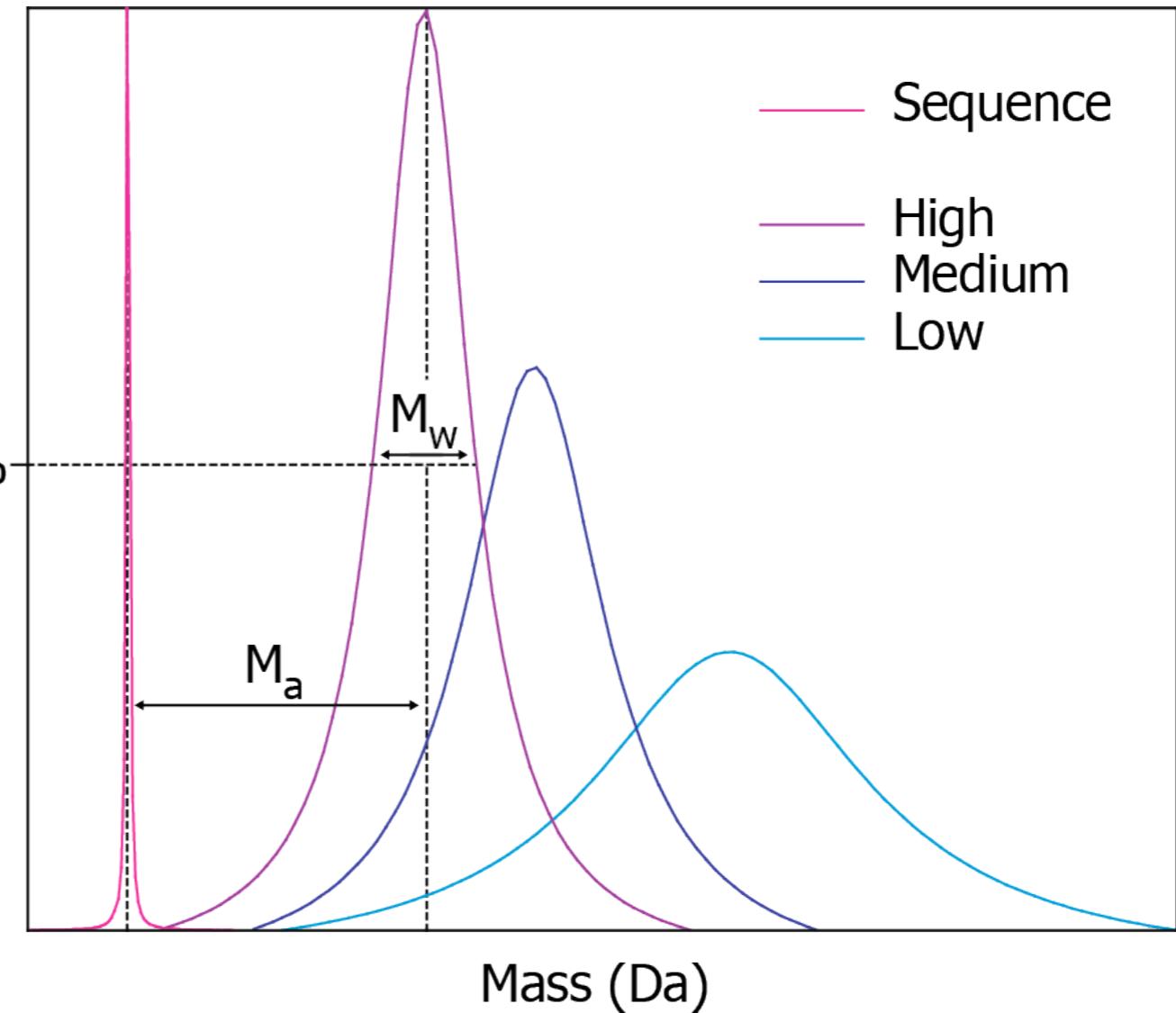
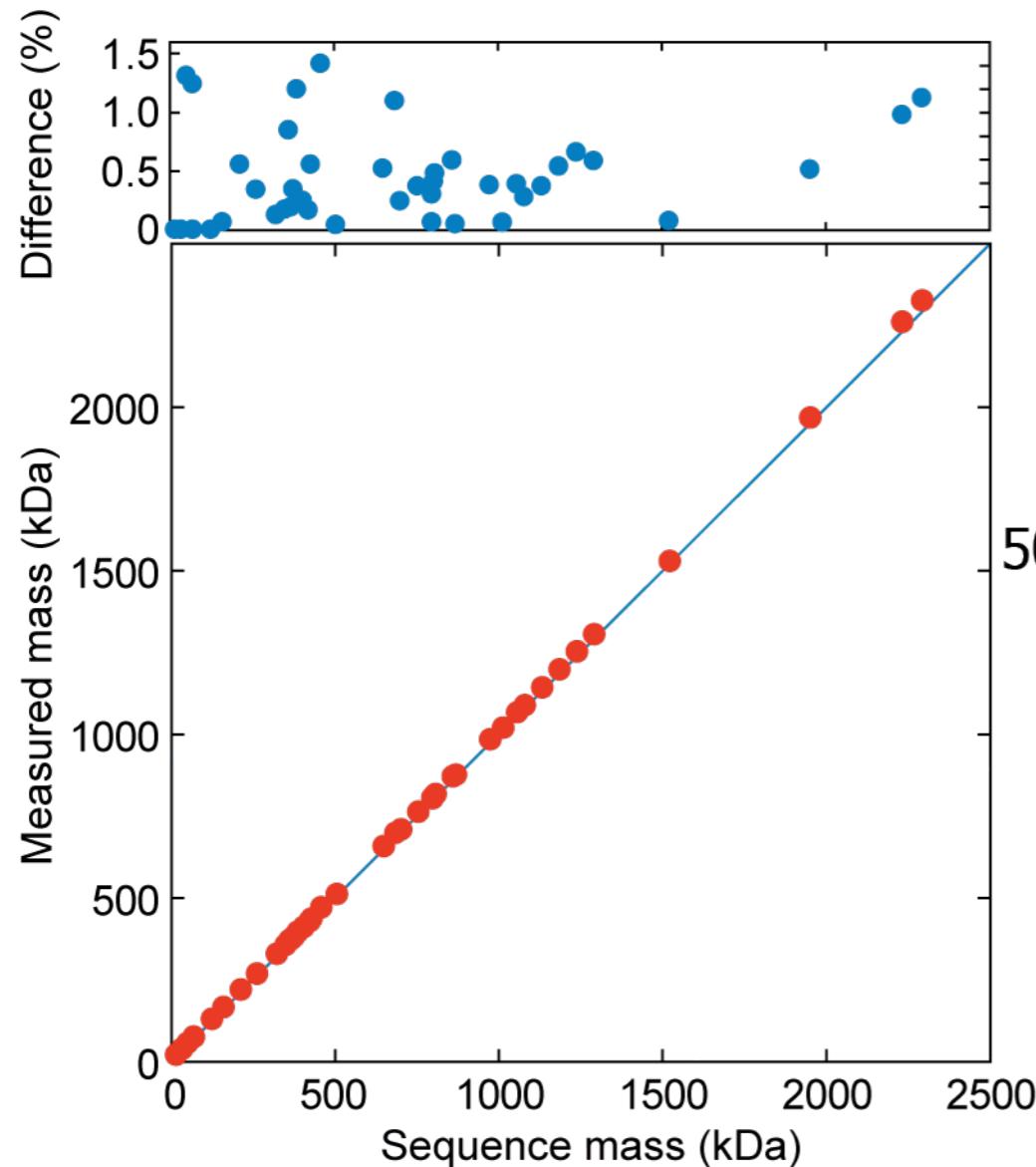


- Both axial and radial components of the ions' velocity can be dampedened by collisions with background gas

nESI mass spectrum



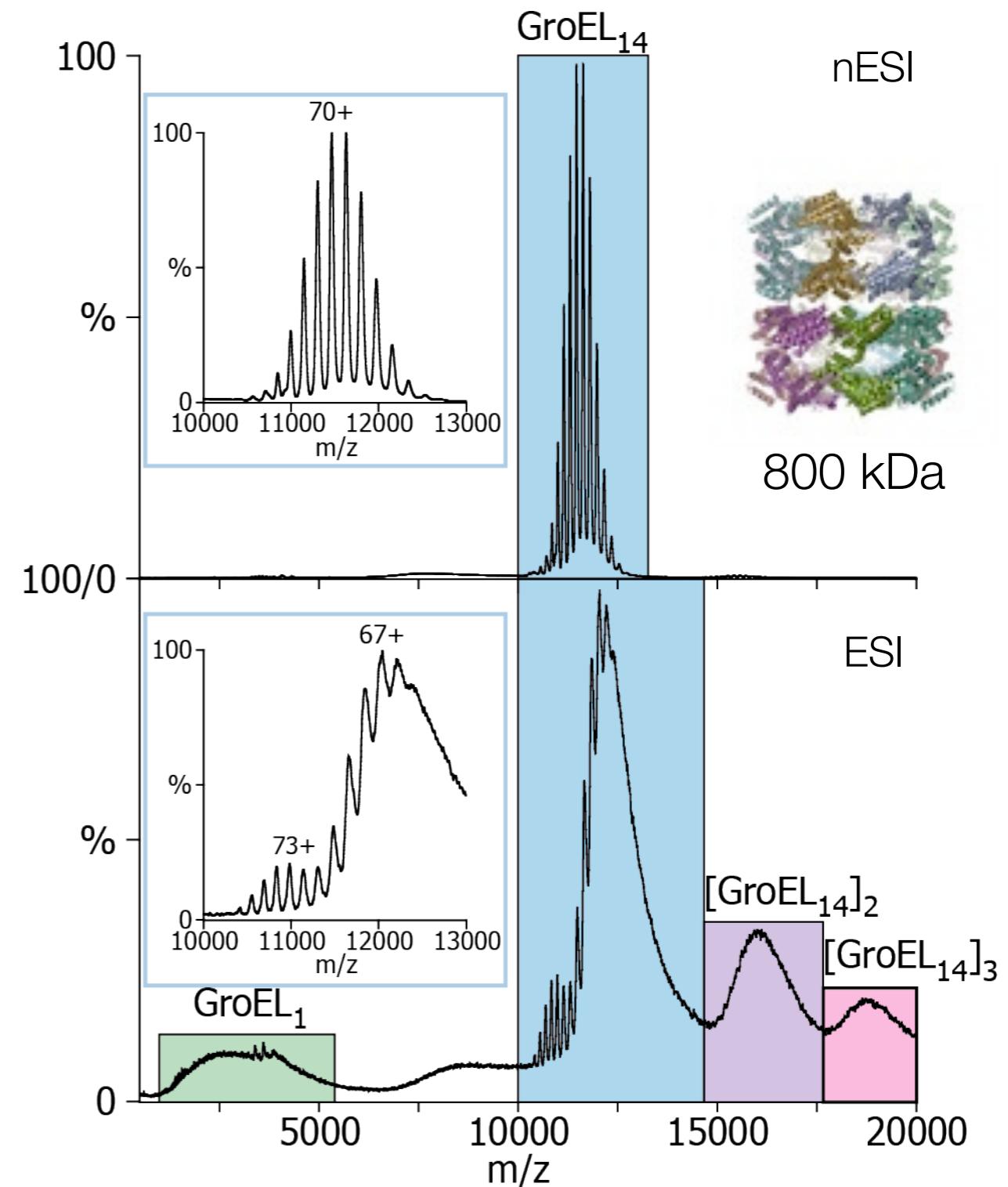
Mass accuracy



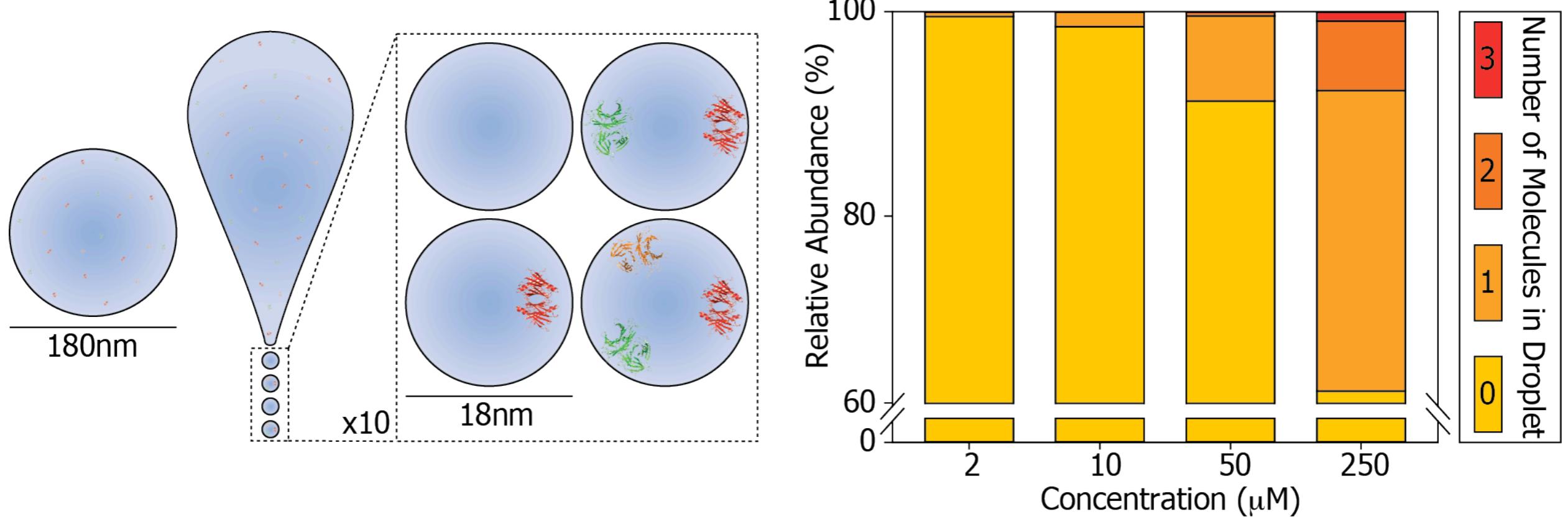
- Additional mass due to adducted solvent molecules and buffer ions
- Number of adducts inversely related to activation

Benefits of nESI

- Lower sample amounts (flow rate approx 10nL/min, vs 5 μ L/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

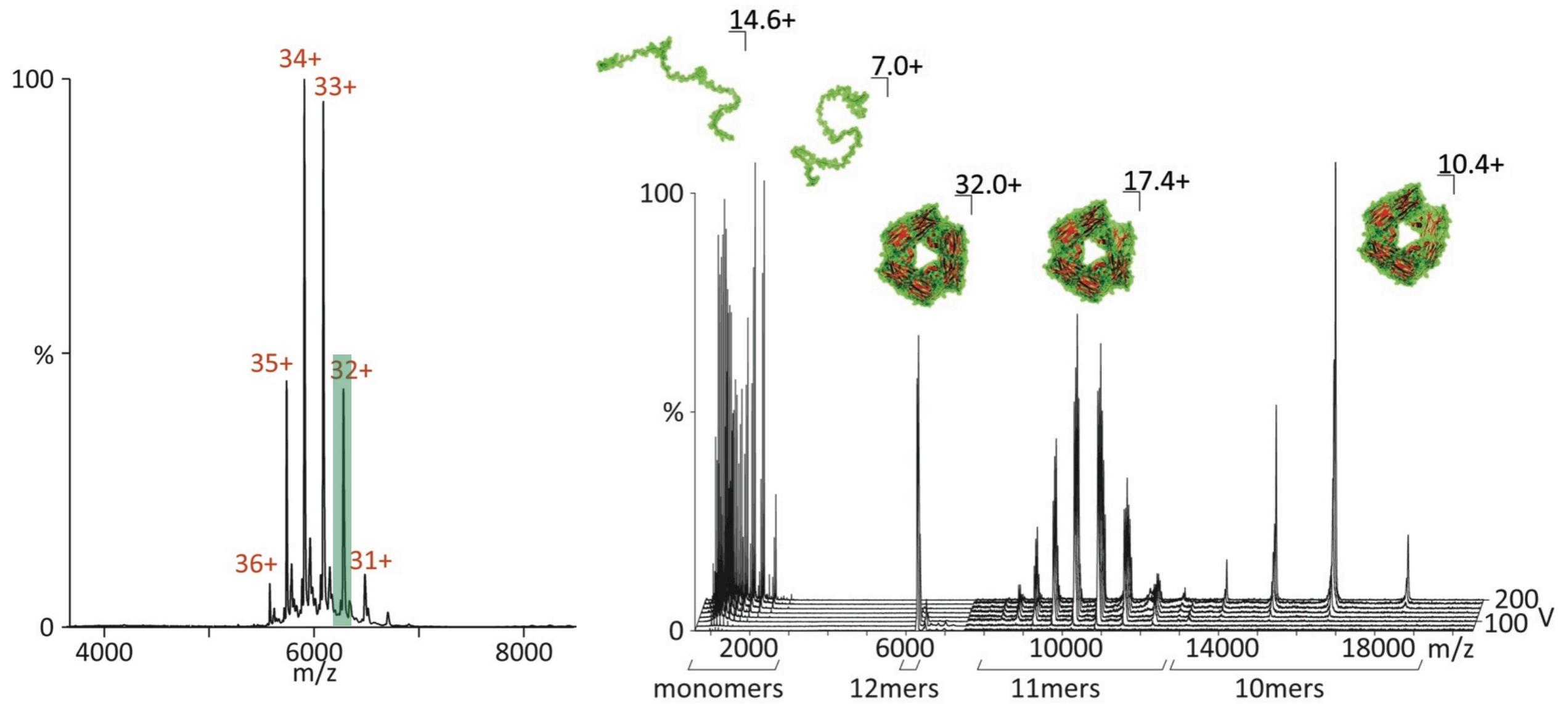


Non-specific associations during ESI



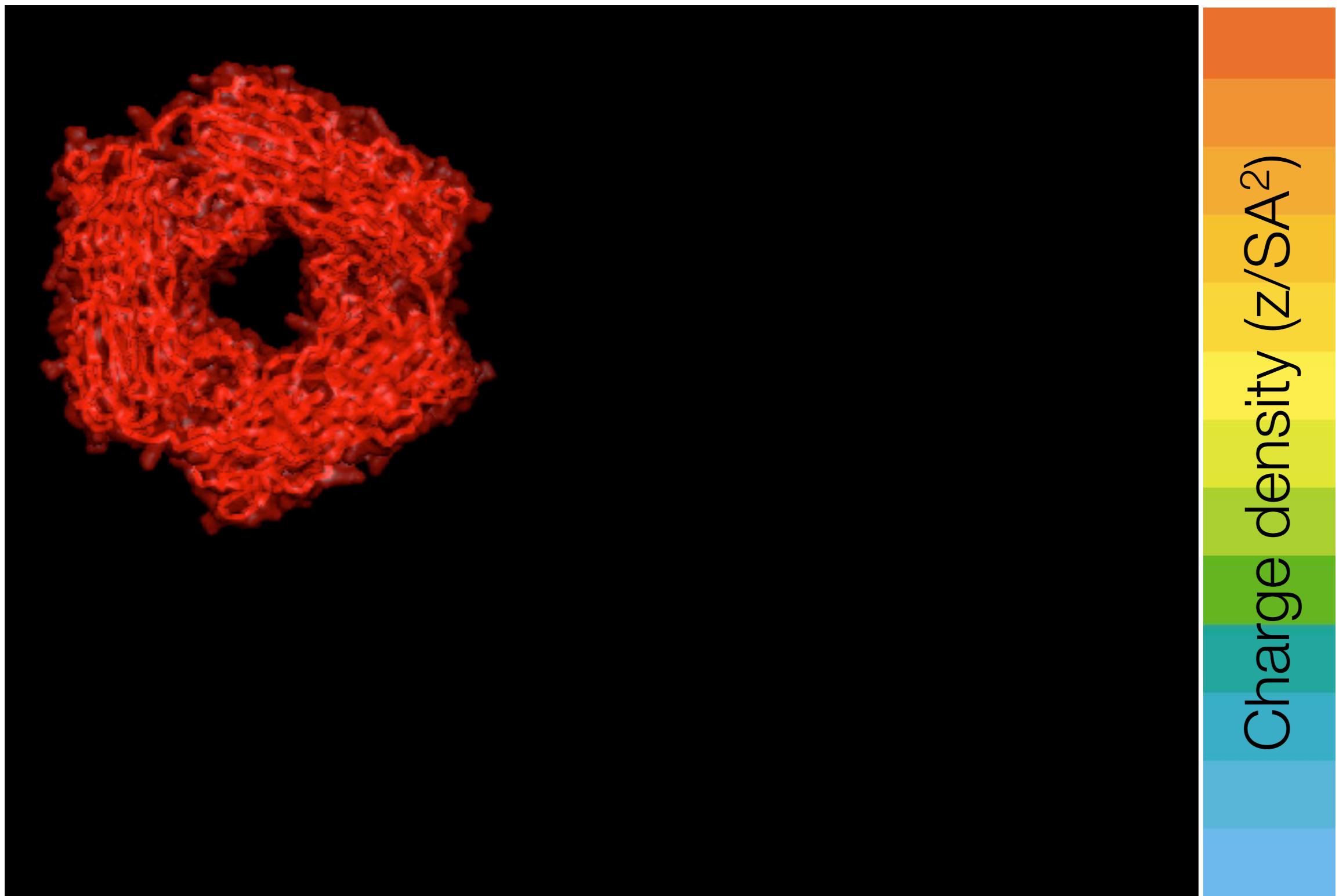
- Probability of there being >1 analyte molecules in ‘final’ ESI droplet
- Most droplets are empty, occupancy increases with concentration
- Decreased initial droplet size in nESI reduces prevalence of non-specific aggregates

Collision induced dissociation of protein assemblies

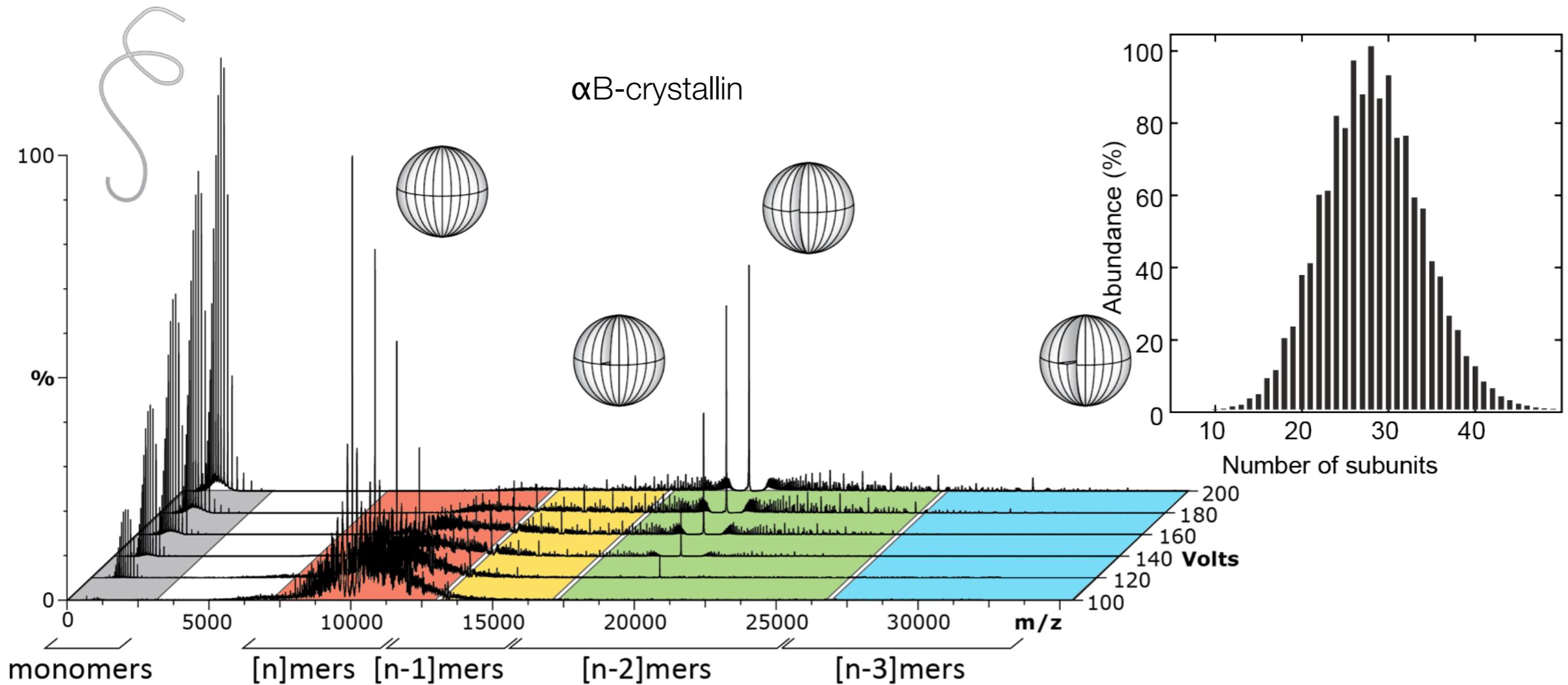


- Dissociation is asymmetric with respect to mass
- Unfolded, highly charged monomers are removed sequentially

Dissociation causes decrease in charge density

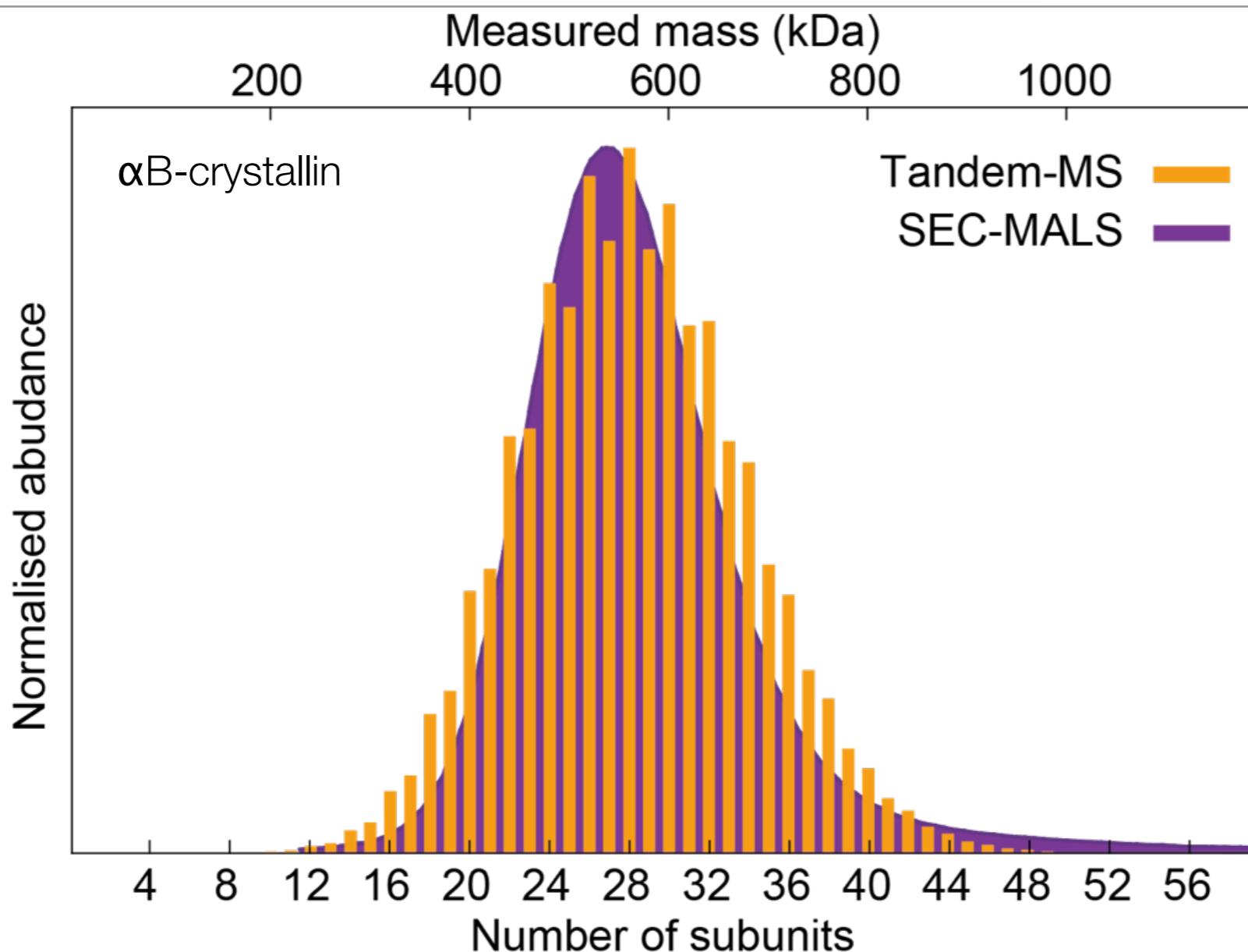


Deconvoluting heterogeneity with CID



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

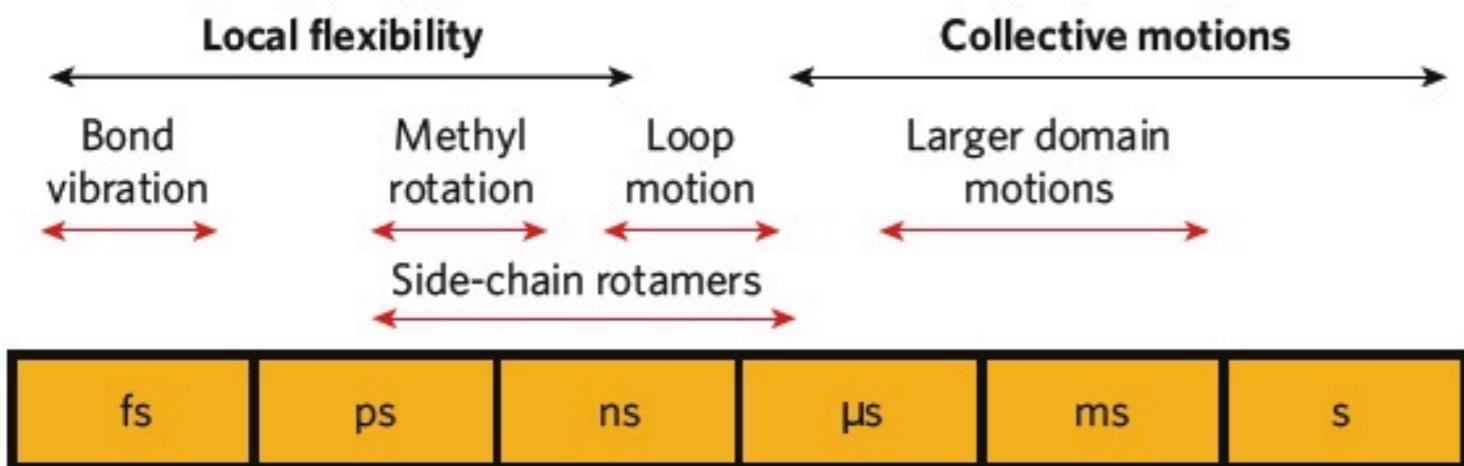
Quantifying stoichiometries



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

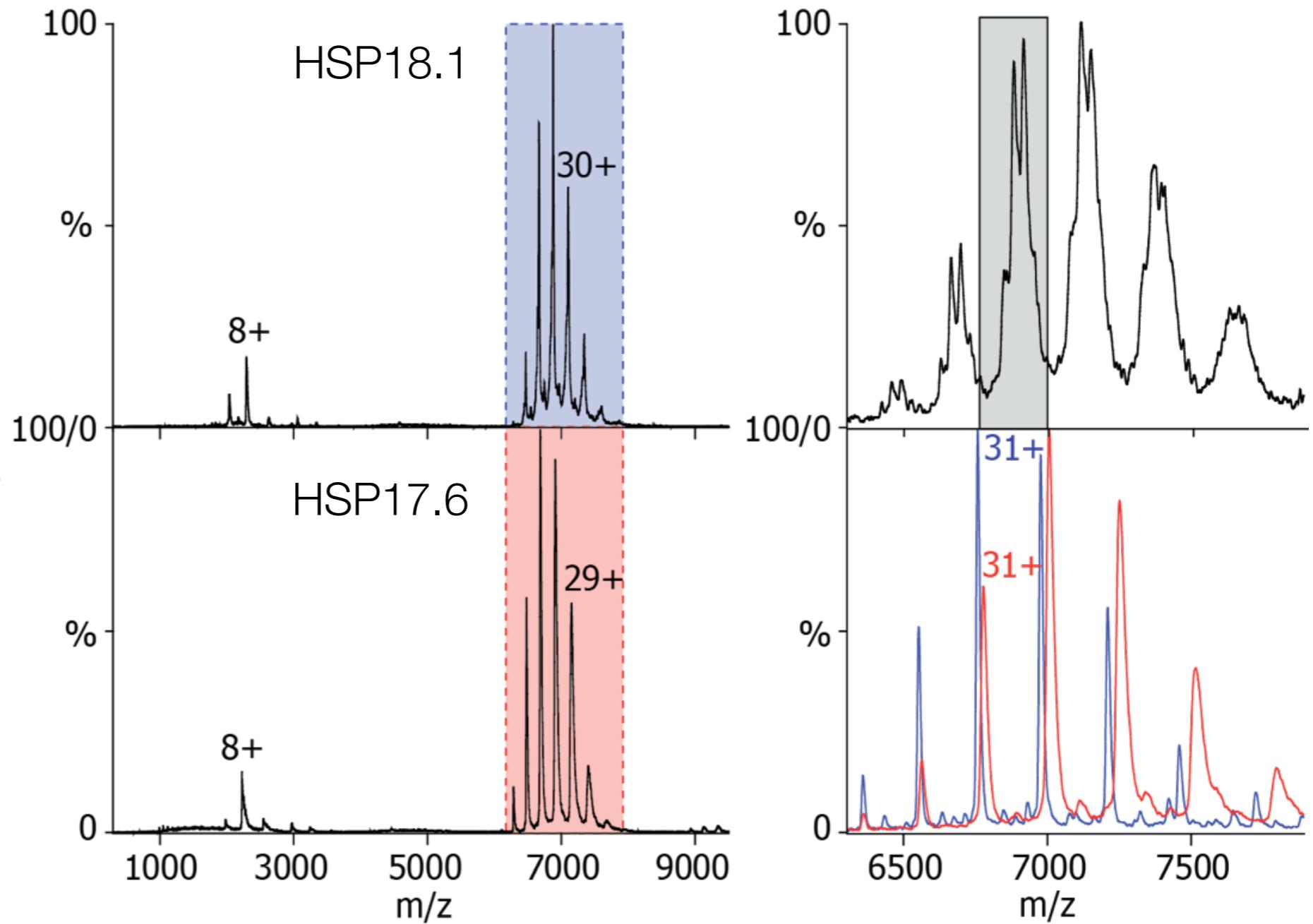
Protein dynamics

- 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



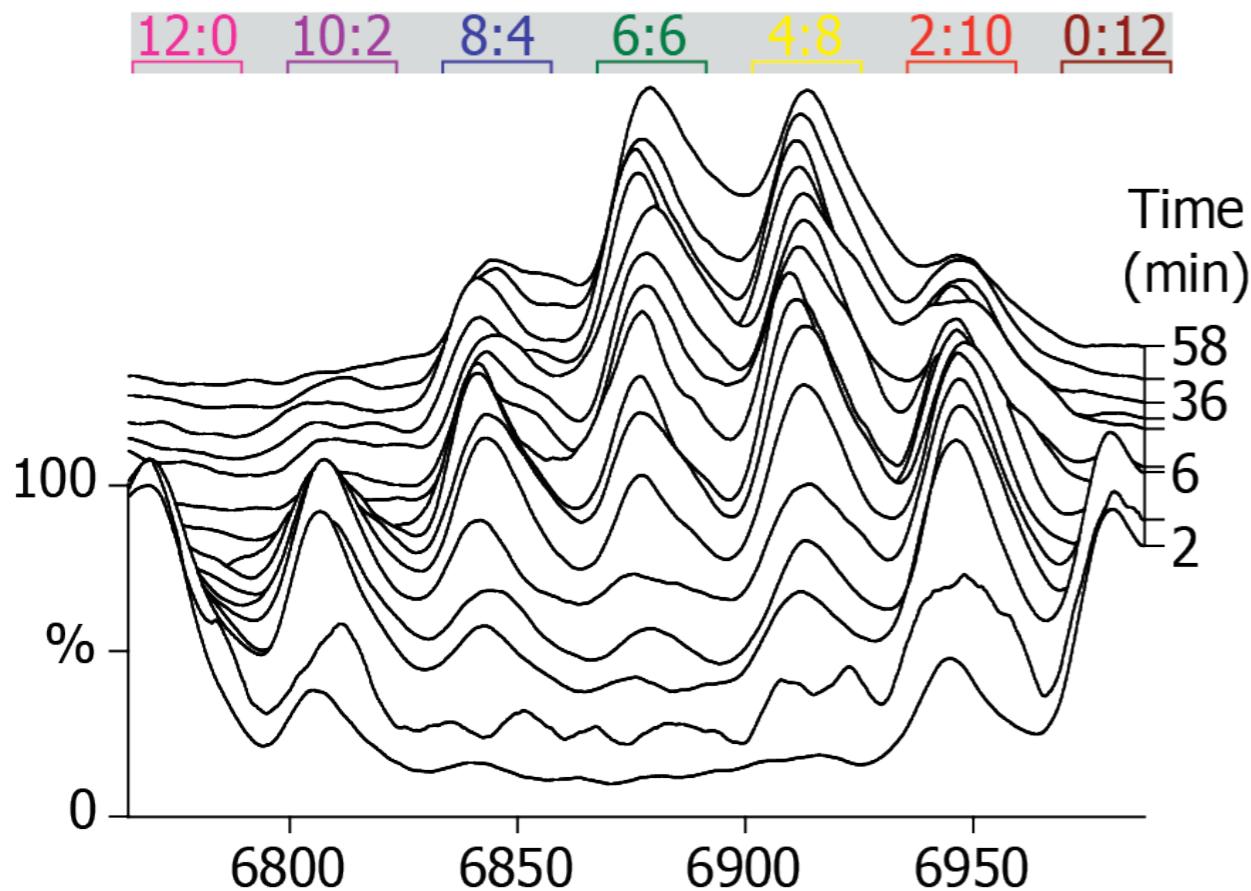
Quaternary dynamics - Example

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

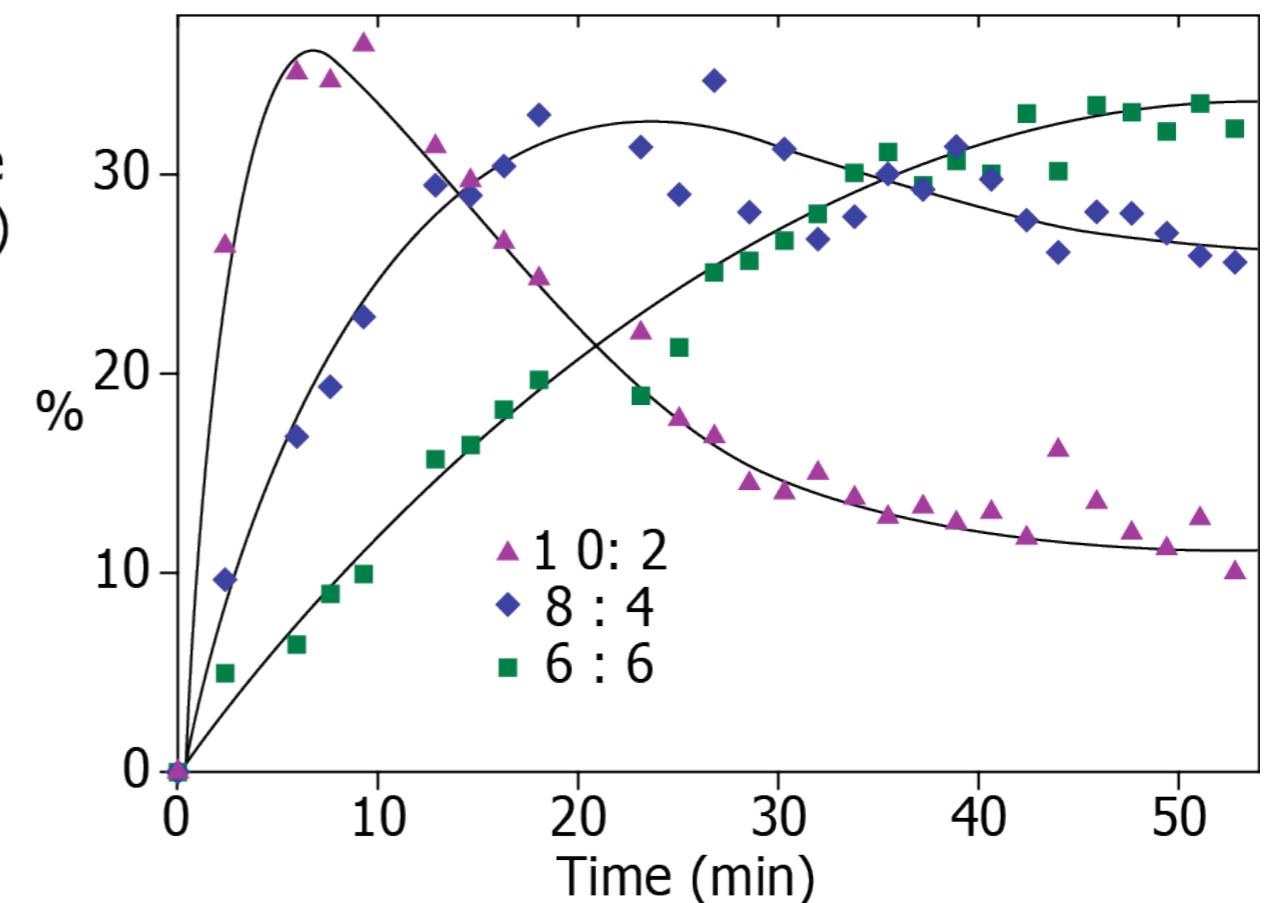


Quaternary dynamics - Example

HSP17.6

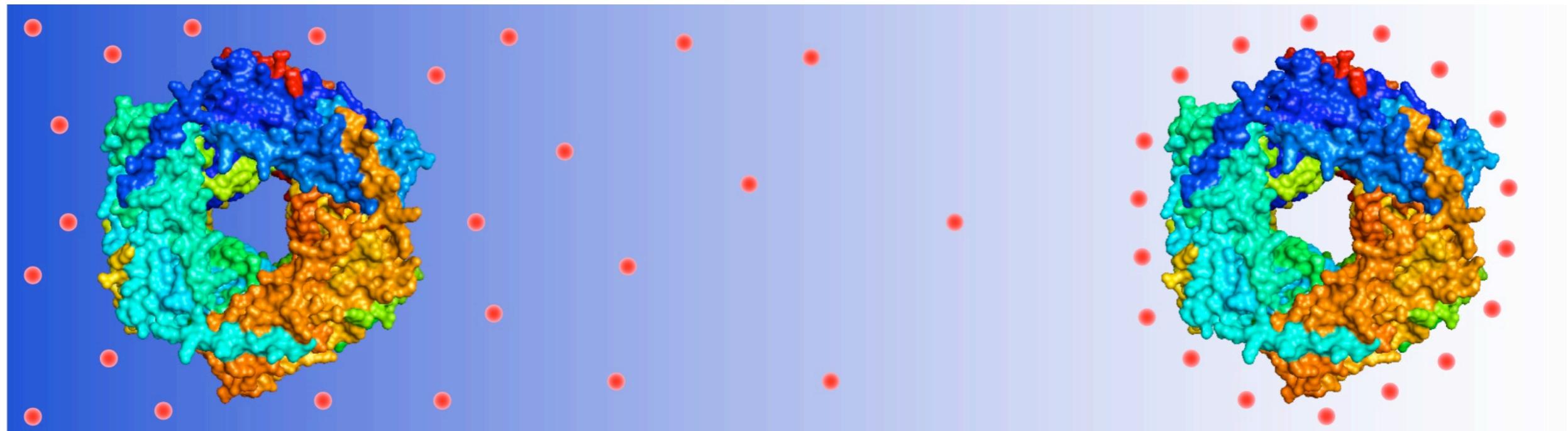


HSP18.1



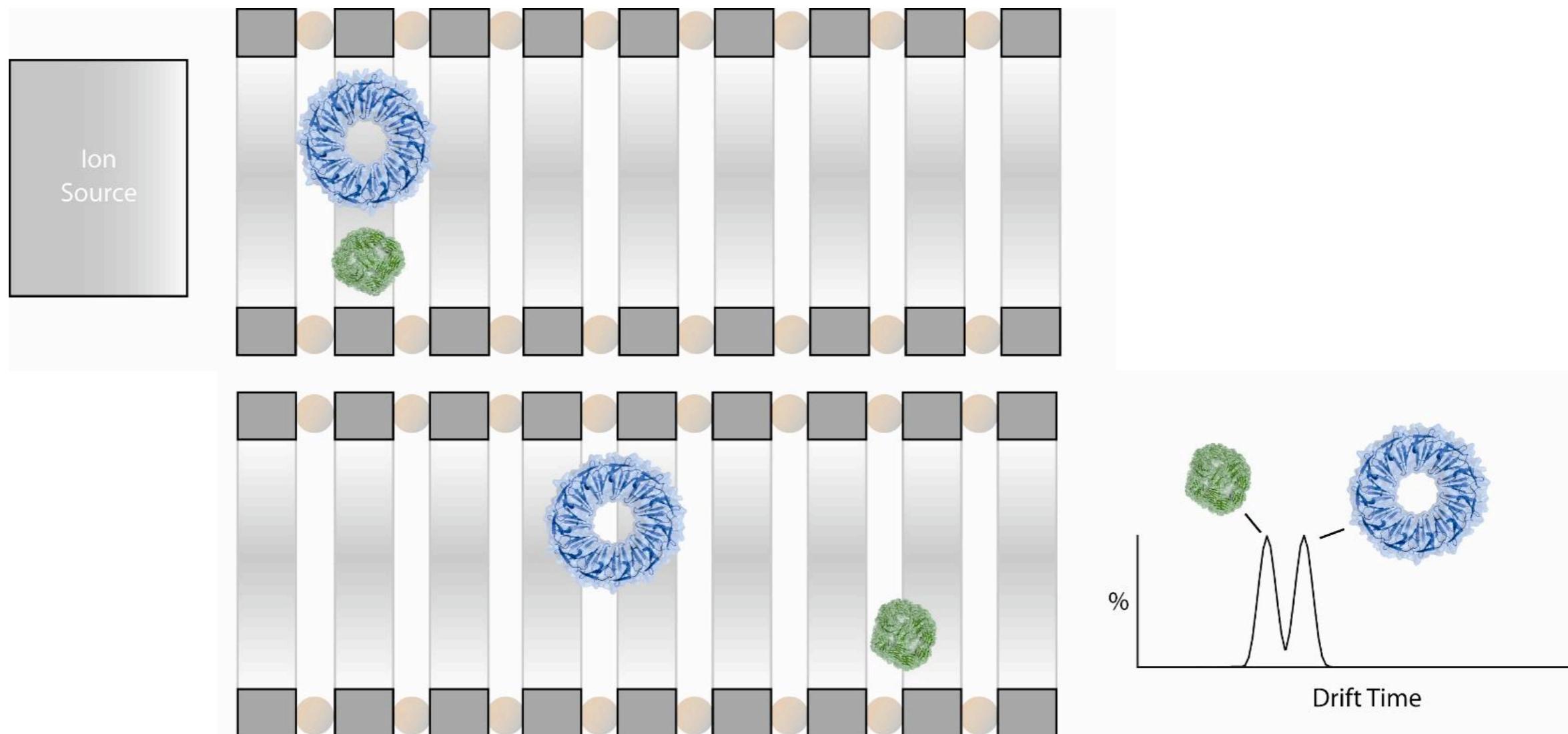
- Exchange proceeds via the movement of dimeric units
- Incorporation is via sequential incorporation of dimers into oligomers
- Hetero-assembly leads to a wide variety of possible oligomers

Preservation of structure



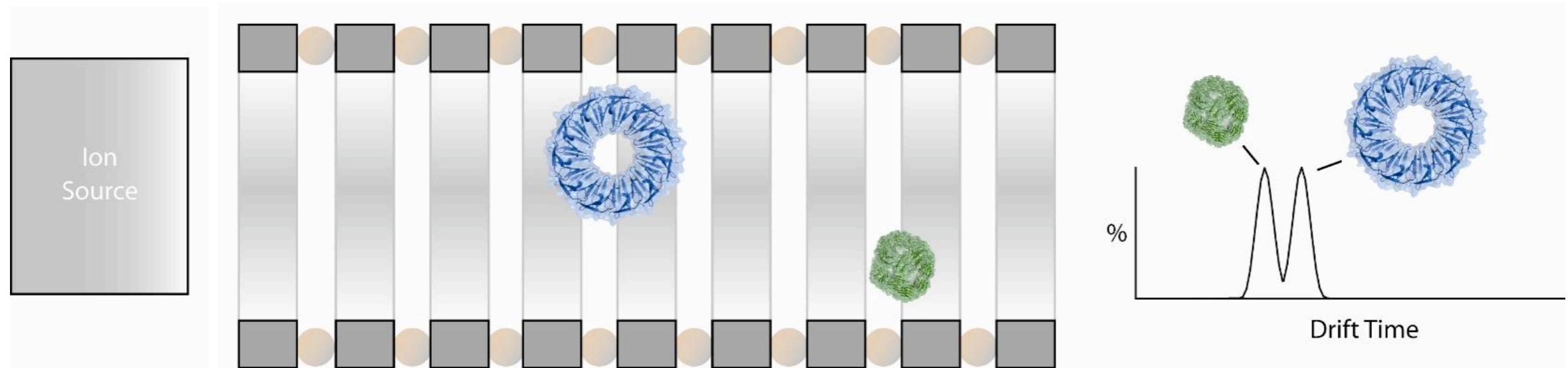
- It is clear stoichiometry is preserved in the mass spectrometer, but can we probe native structure?

Ion mobility spectrometry (IMS)



- Separation of ions according to their ability to traverse a region of gas under the influence of a weak electric field
- Separation is based on ion ‘mobility’, unlike time-of-flight separation (mass)

Factors contributing to IM separation



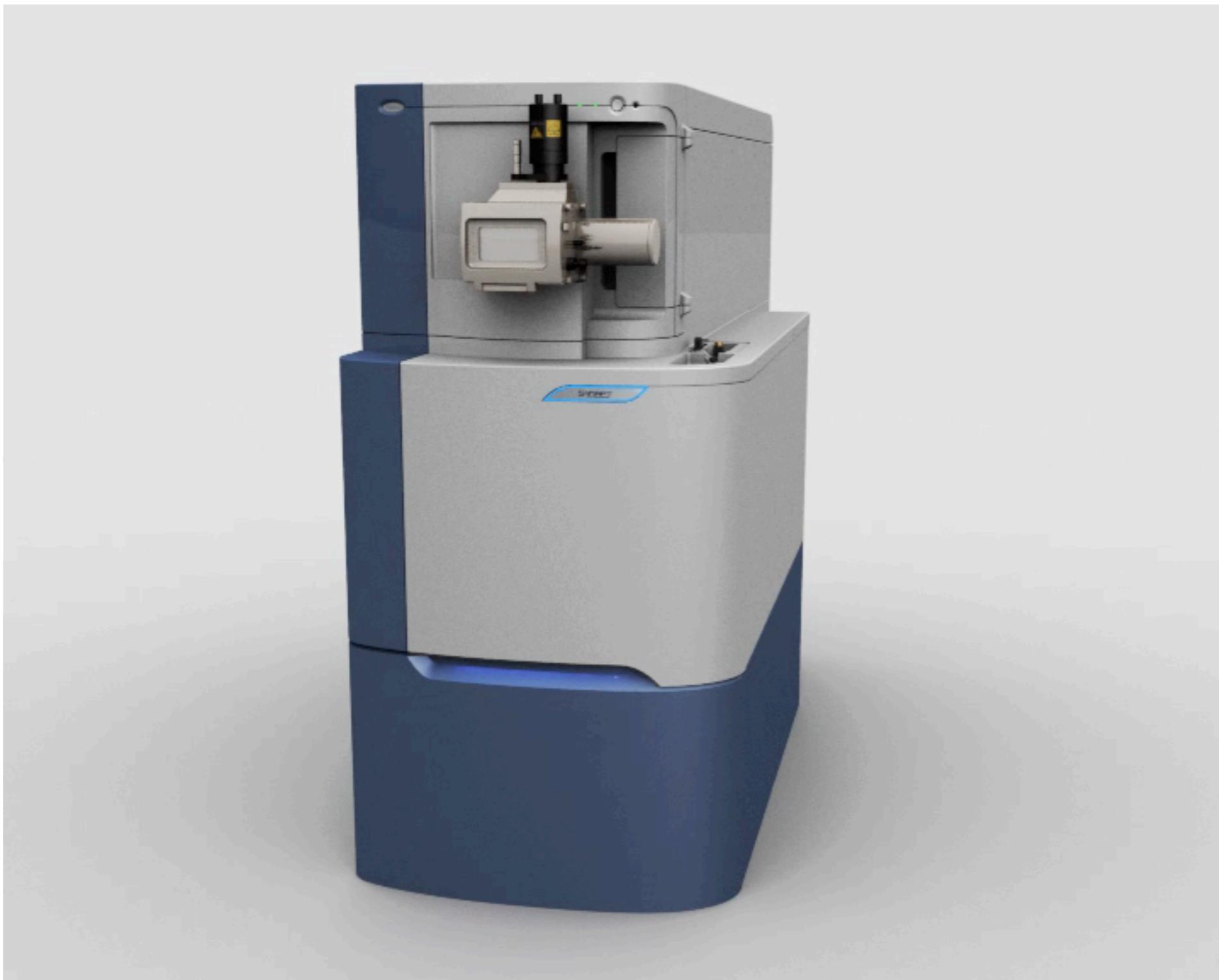
$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_b T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_N} \right]^{1/2} \frac{t_D E}{L} \frac{760}{P} \frac{T}{273.2} \frac{1}{N}$$

- Drift time is inversely proportional to charge
- Drift time is proportional to collision cross section (CCS, Ω)

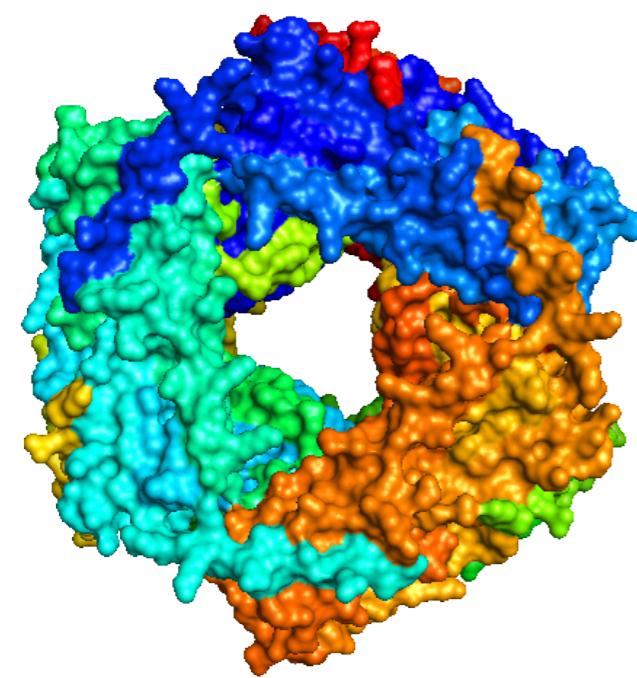
Collision cross section (CCS)

- CCS includes contribution from the gas atom
- CCS results from orientational average of analyte molecules

IM-MS Implementation

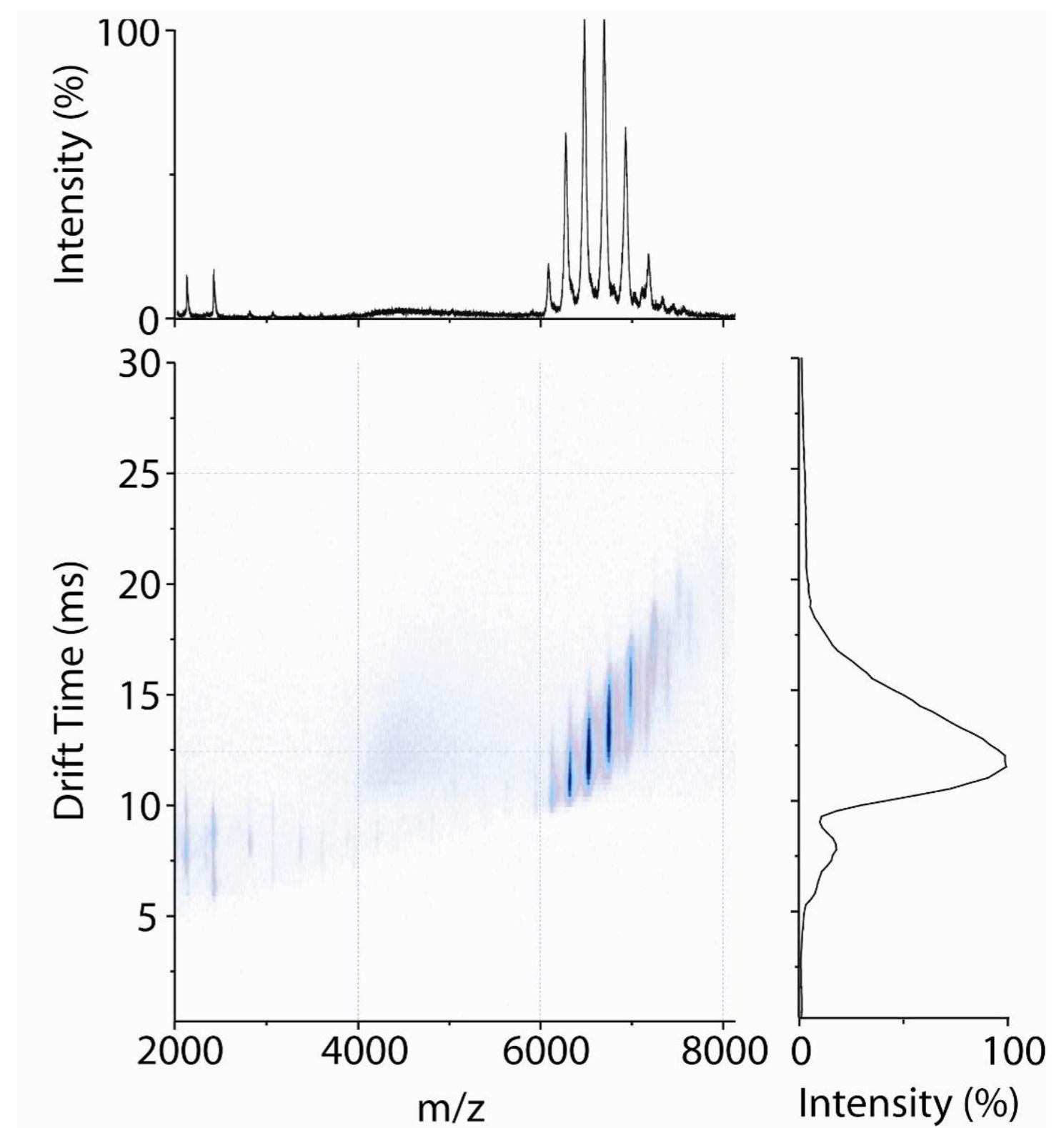


IM-MS spectrum

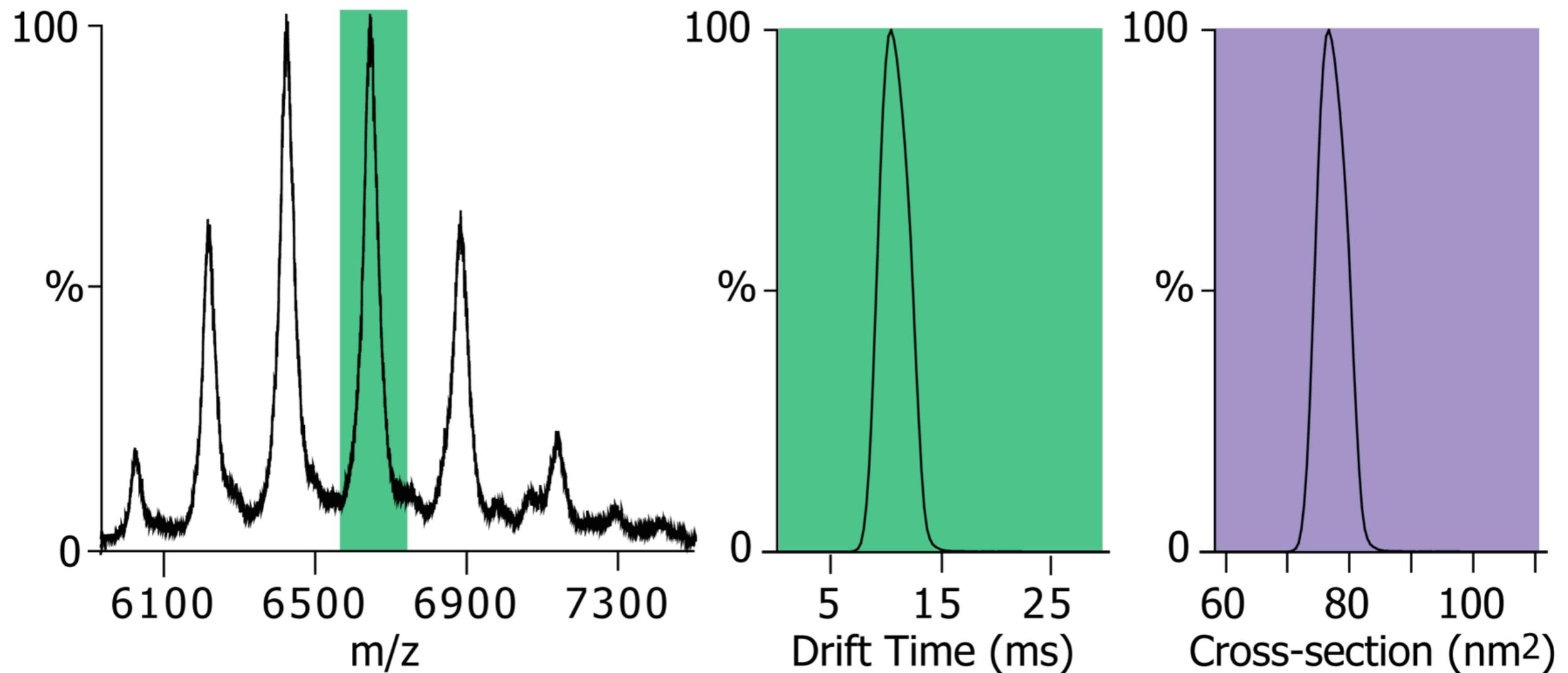


HSP16.9

- Plot of m/z versus drift time

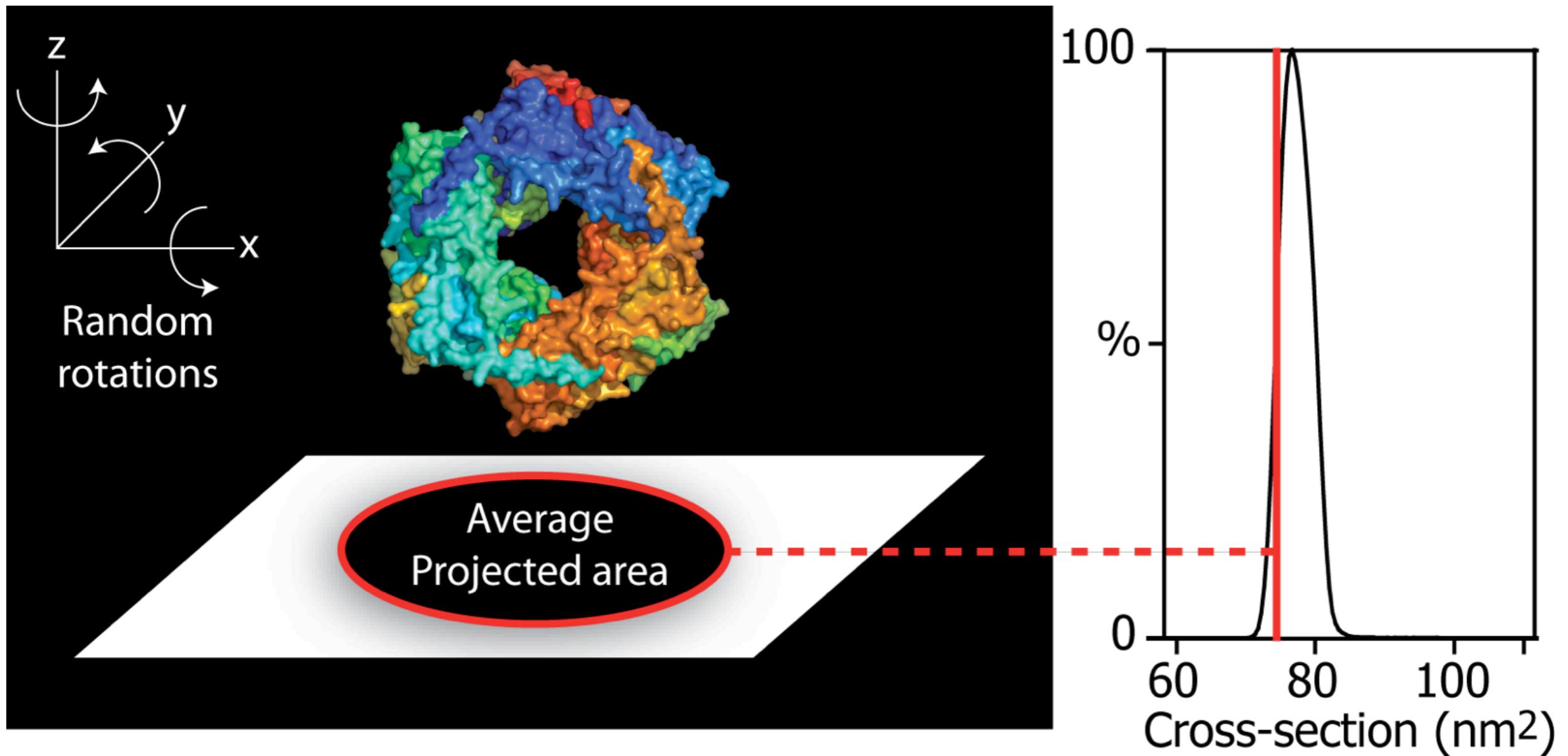


Obtaining an experimental CCS



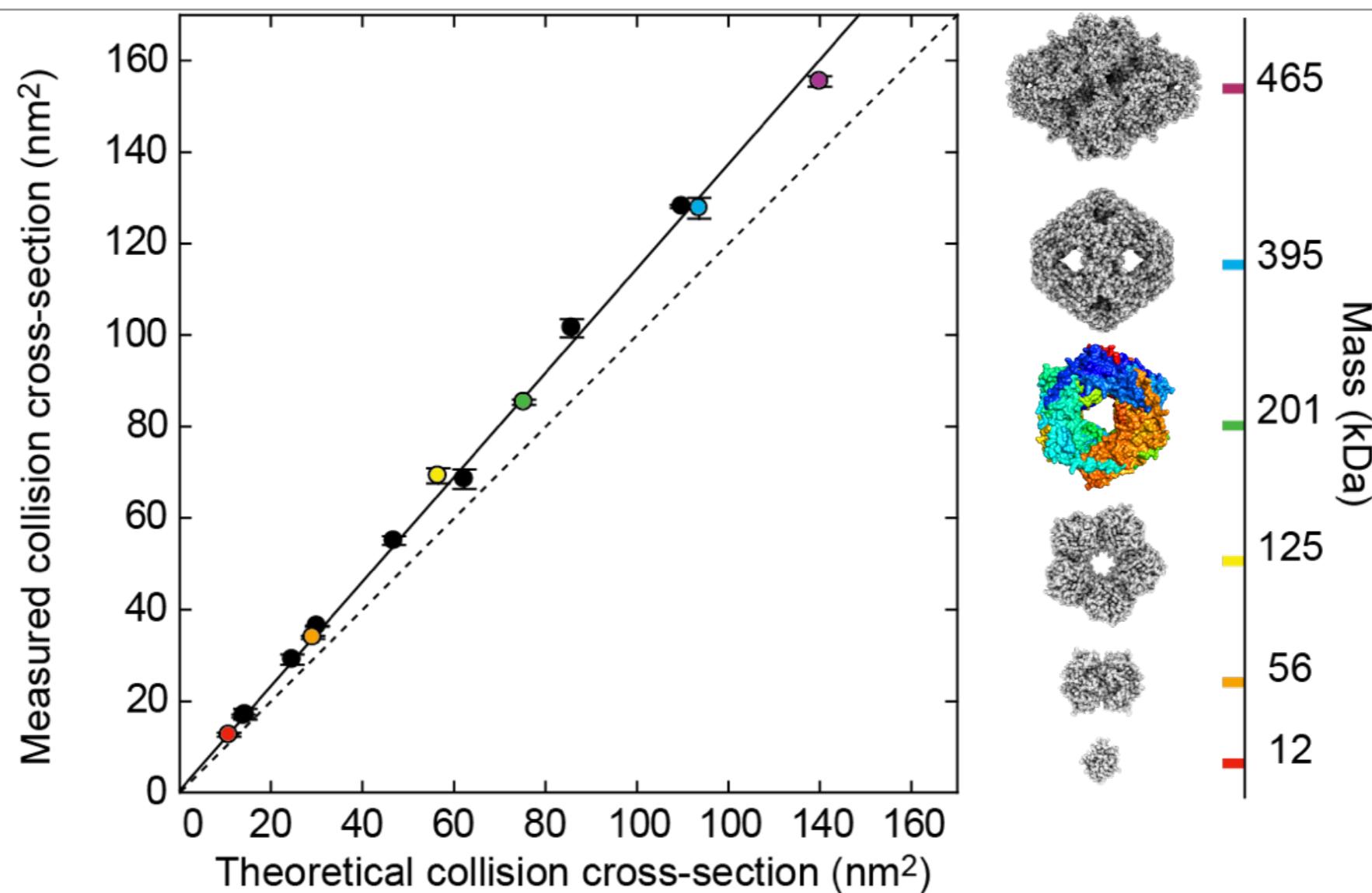
- Every feature resolved in m/z has an associated drift time distribution
- Drift time is converted into CCS either directly or via calibration

CCS values from protein structures



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

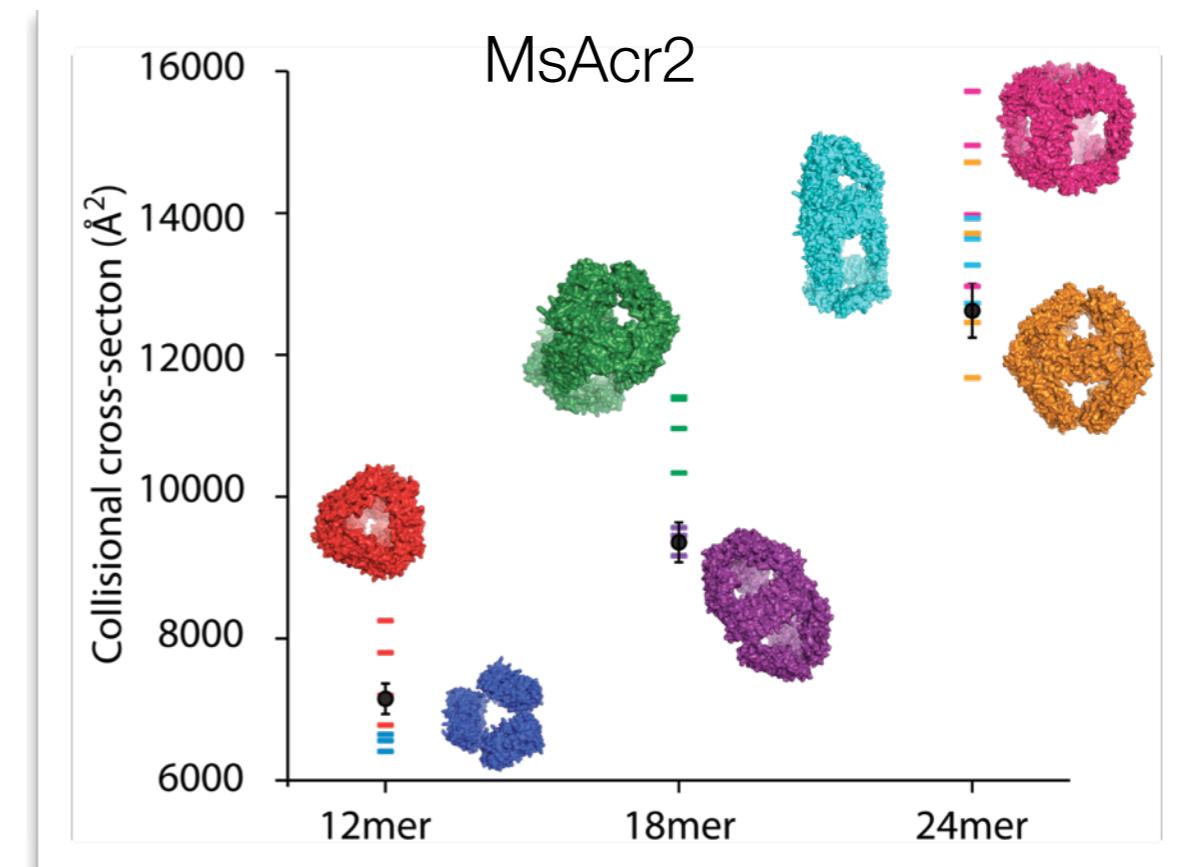
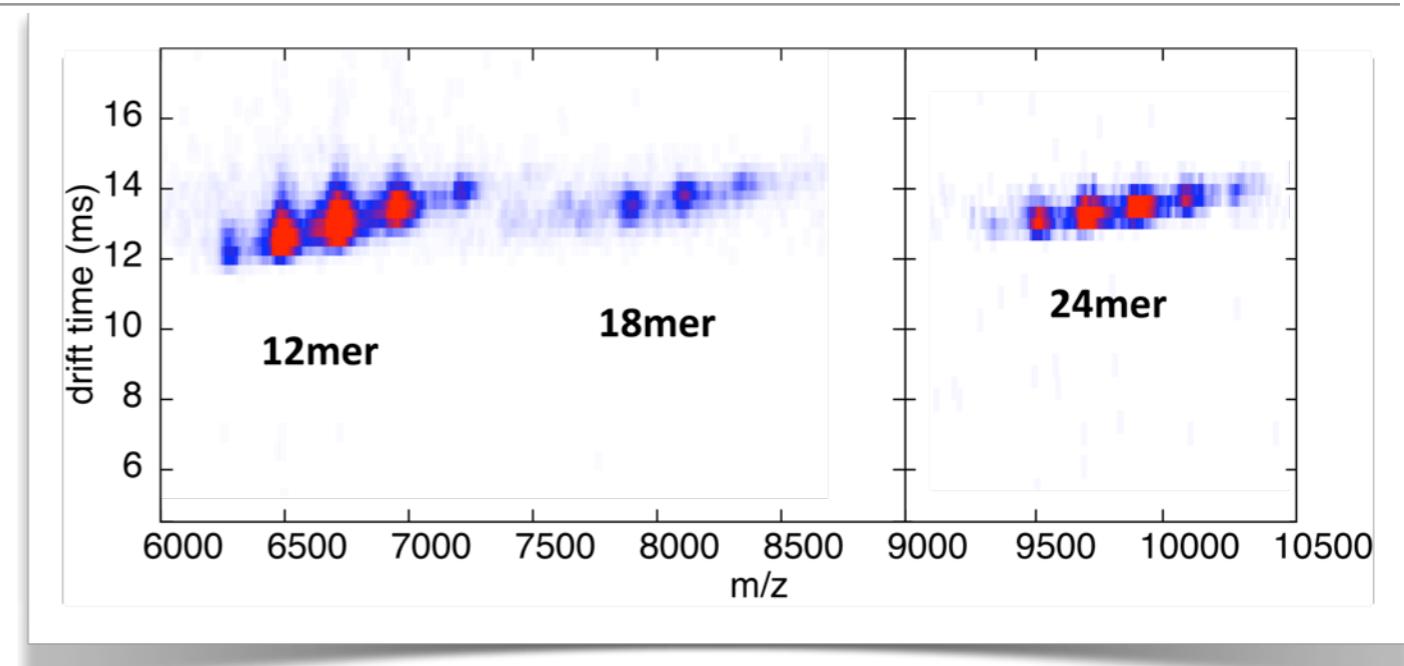
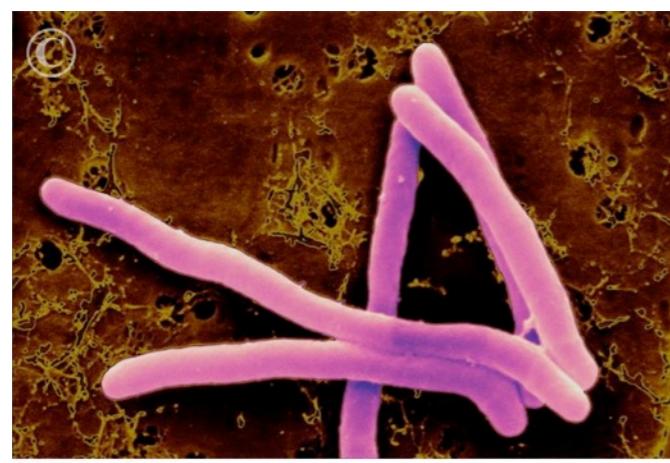
CCS comparison



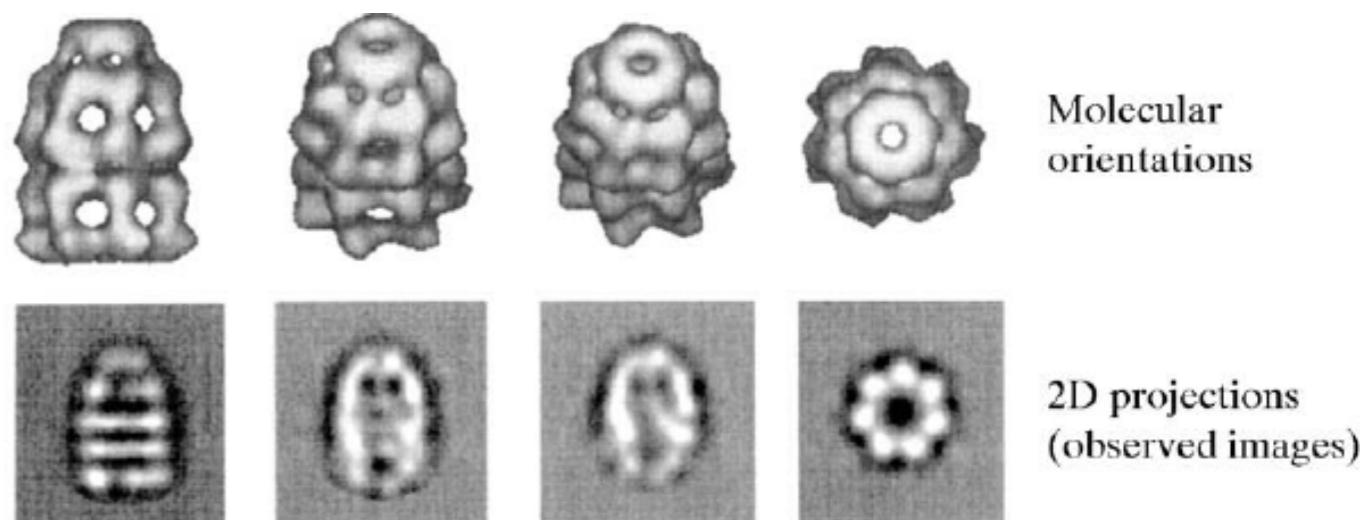
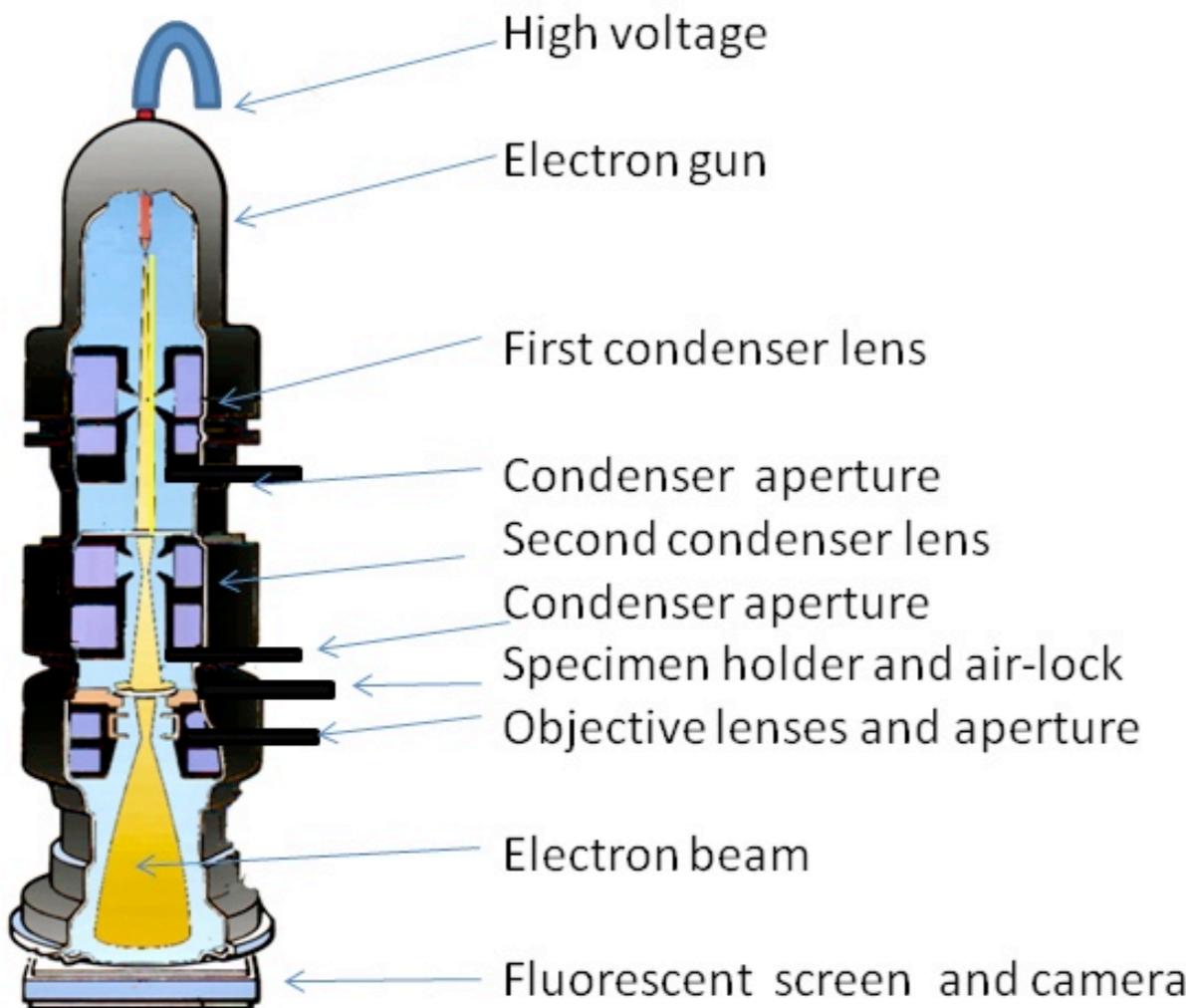
- Excellent correlation between theoretical and measured values
- Discrepancy is due to simplicity of ‘projection approximation’
- Correlation motivates use of IM measurements in assessing model structures

Using IM-MS to filter structures - Example

- Mycobacterial small heat shock protein
- 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

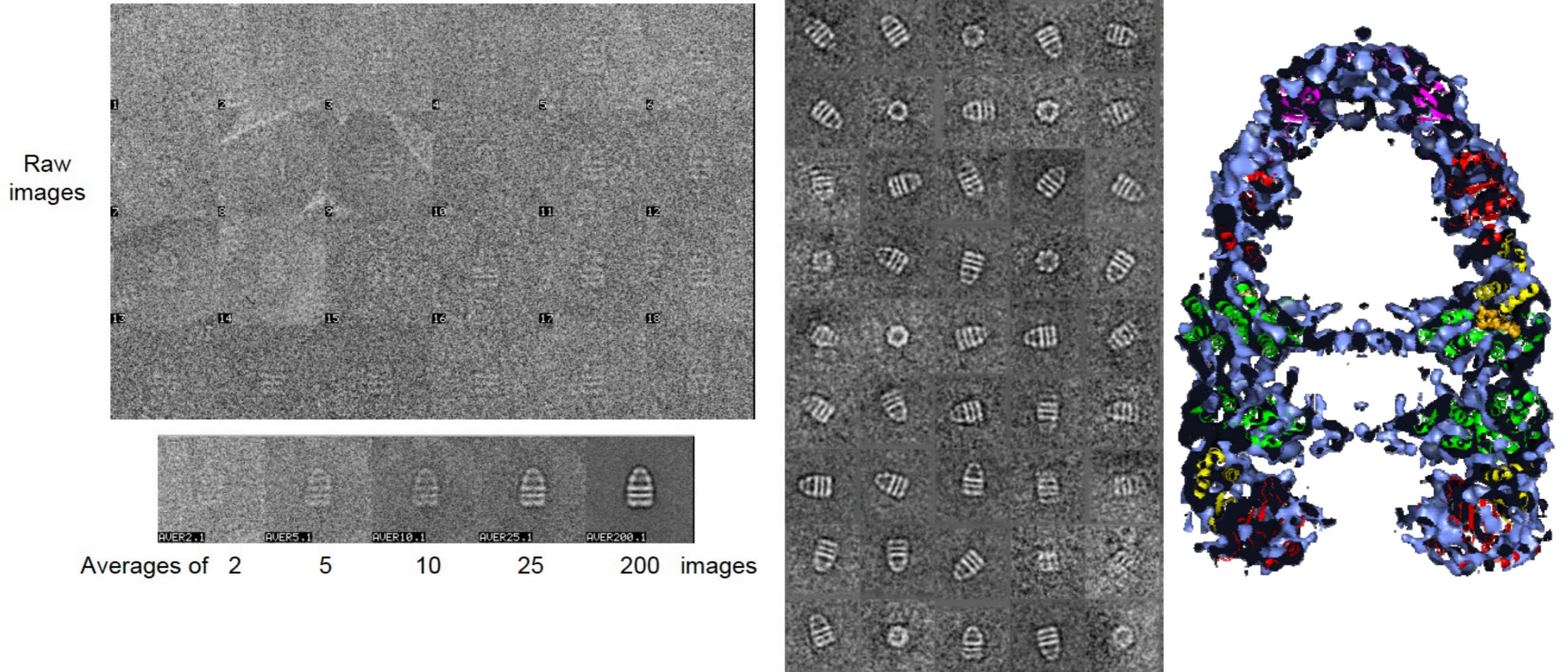


Transmission electron microscopy (TEM)



- TEM gives two-dimensional projections of the molecular electron density

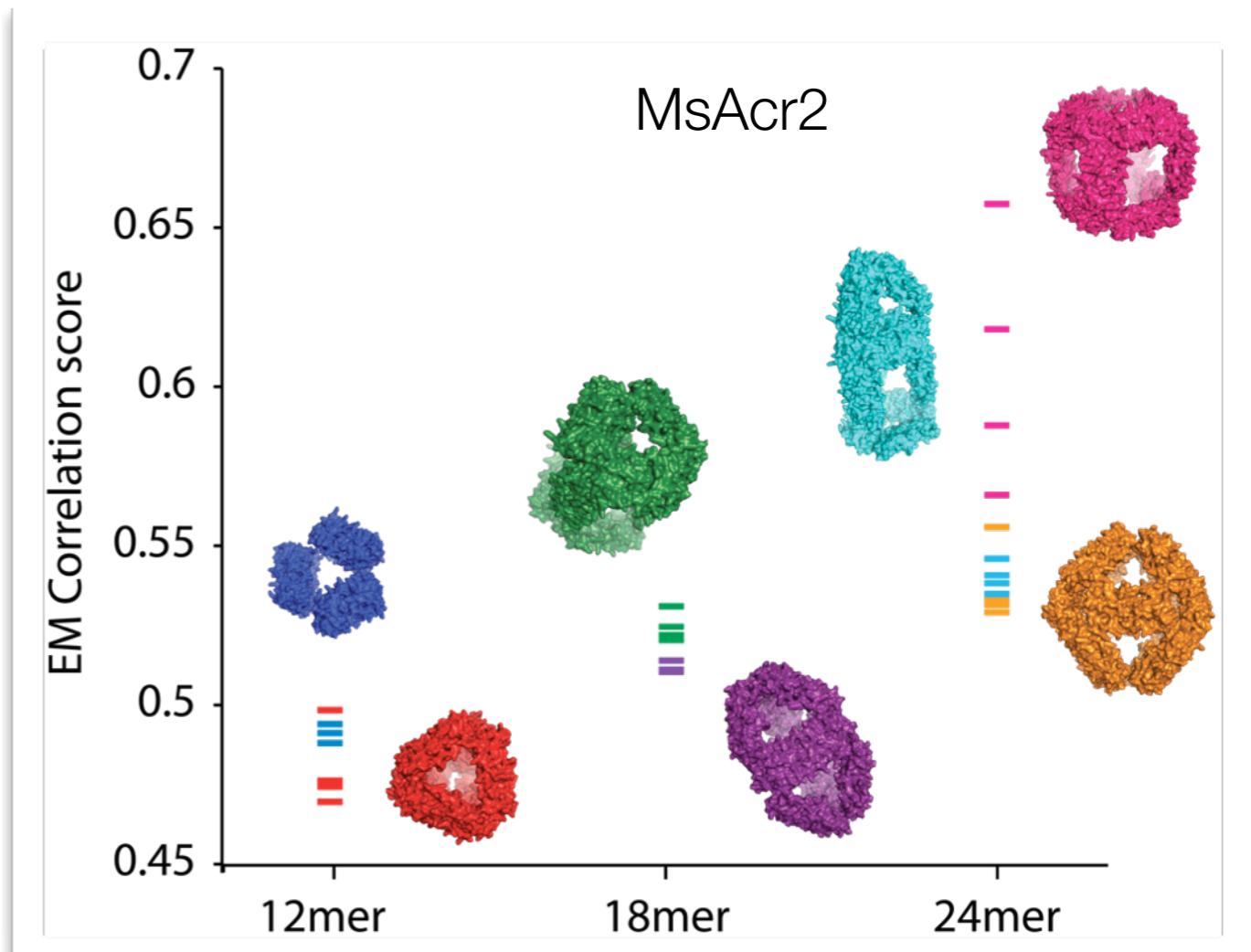
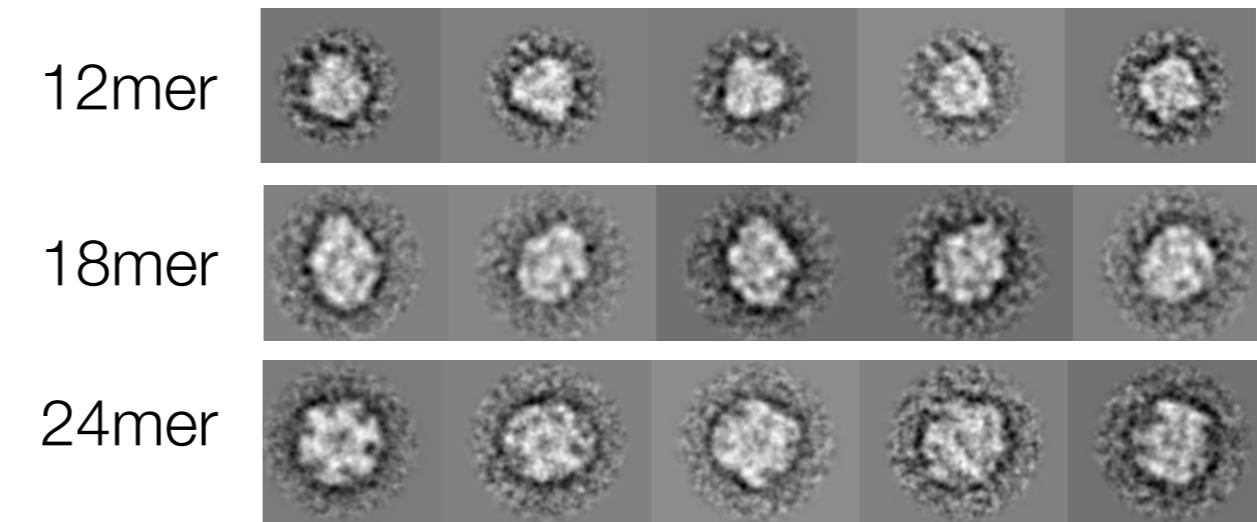
Single particle analysis



- Align particles and average them to improve signal to noise
- Classify particles into ‘class averages’ representing particular orientations
- Combine class averages to generate 3D reconstruction

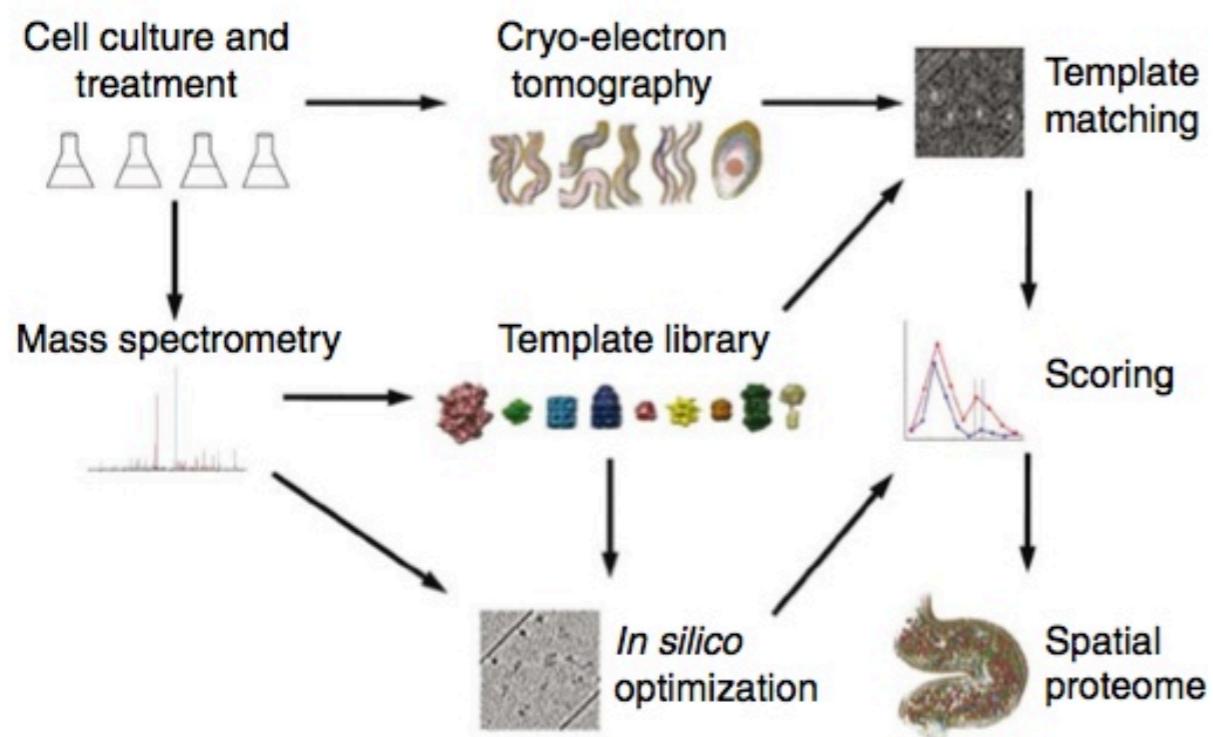
Combining MS with TEM - Example 1

- Compare random rotations of models to TEM class averages
- Lower score is better fit
- Best fit structures are well correlated with those selected using IM-MS (see slide 27)
- Projected area from TEM is analogous to CCS area from IM

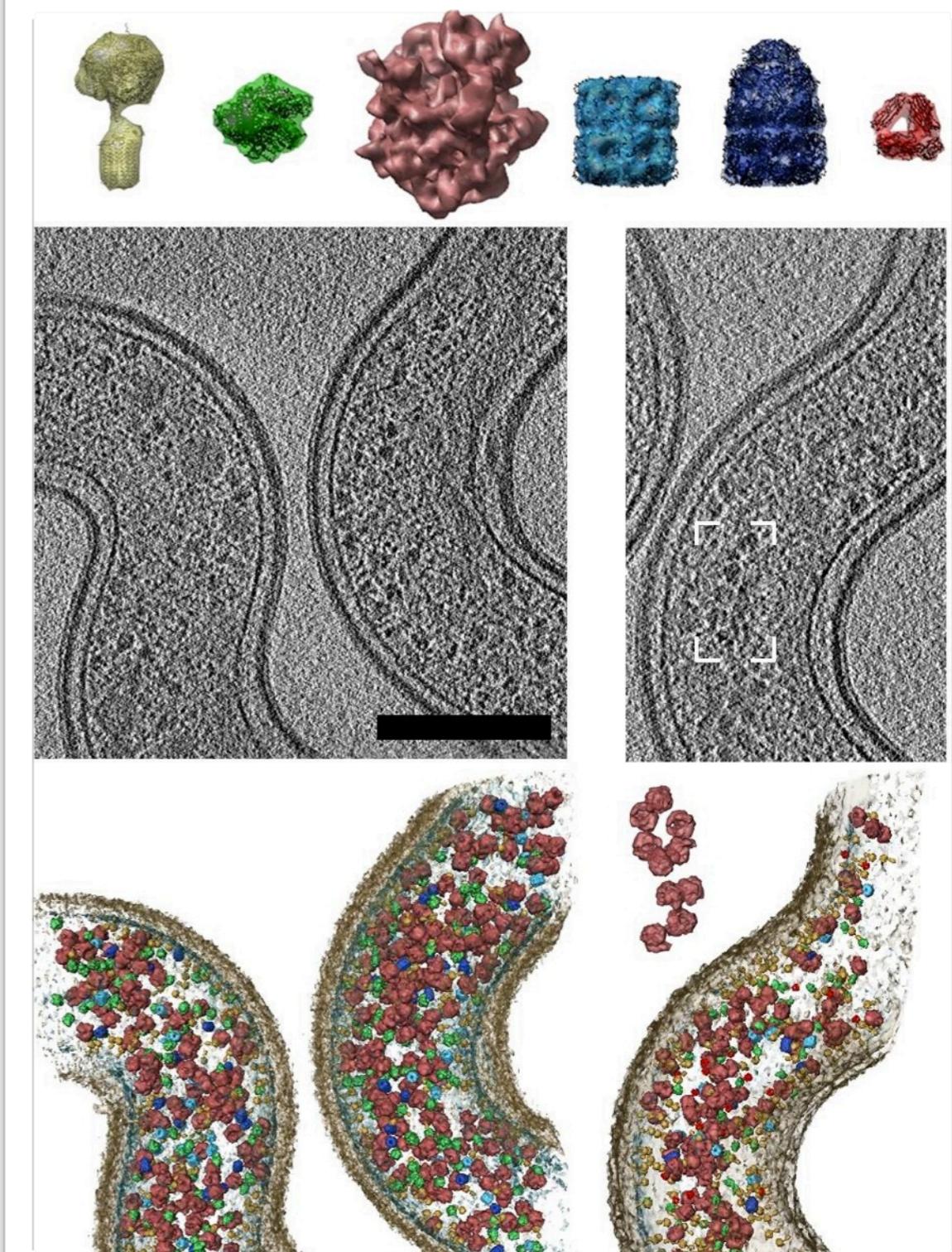


Combining MS with EM - Example 2

- Determine abundances of proteins from MS
- Use these proteins' known structures to match to electron density in 3D reconstruction of cells
- Localise individual proteins and assess their spatial distribution



Beck ... Aebersold, Nature Methods (2009), 6, 817-23



Revision and Problem Class - Th.10, Wk1, PTCL

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