# Non-covalent or Native Mass Spectrometry

We can ionize intact protein complexes using ESI !!

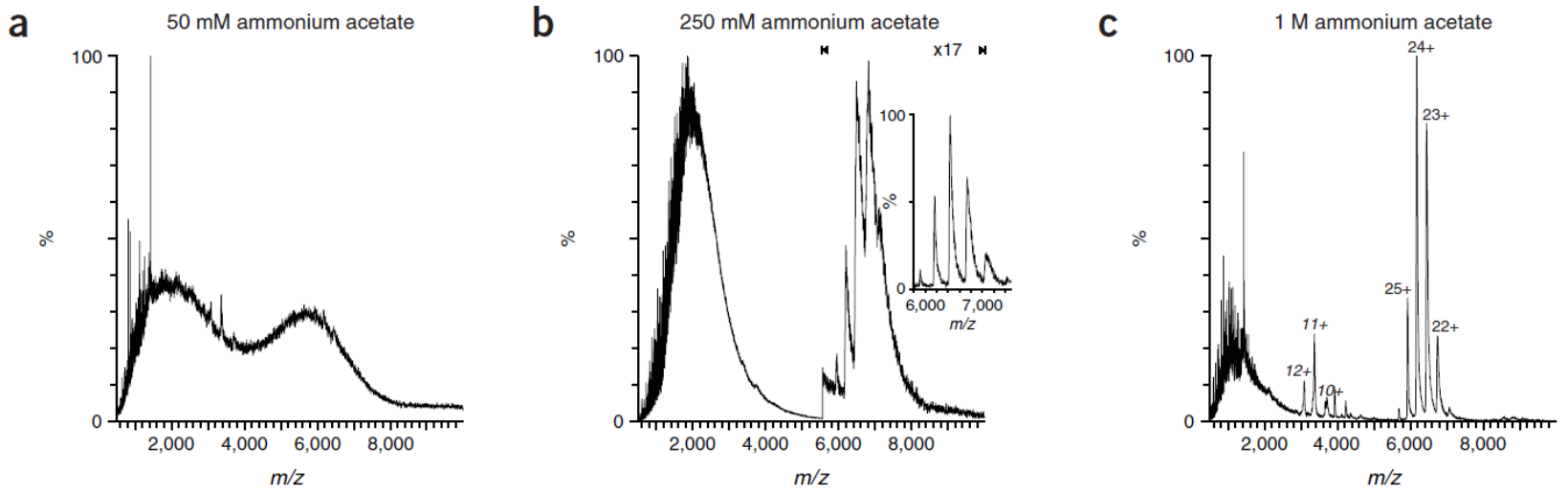
# What Can We Learn?

- Stoichiometry of complex
- Relative affinity and topology
- Rates of subunit exchange
- Assembly pathways

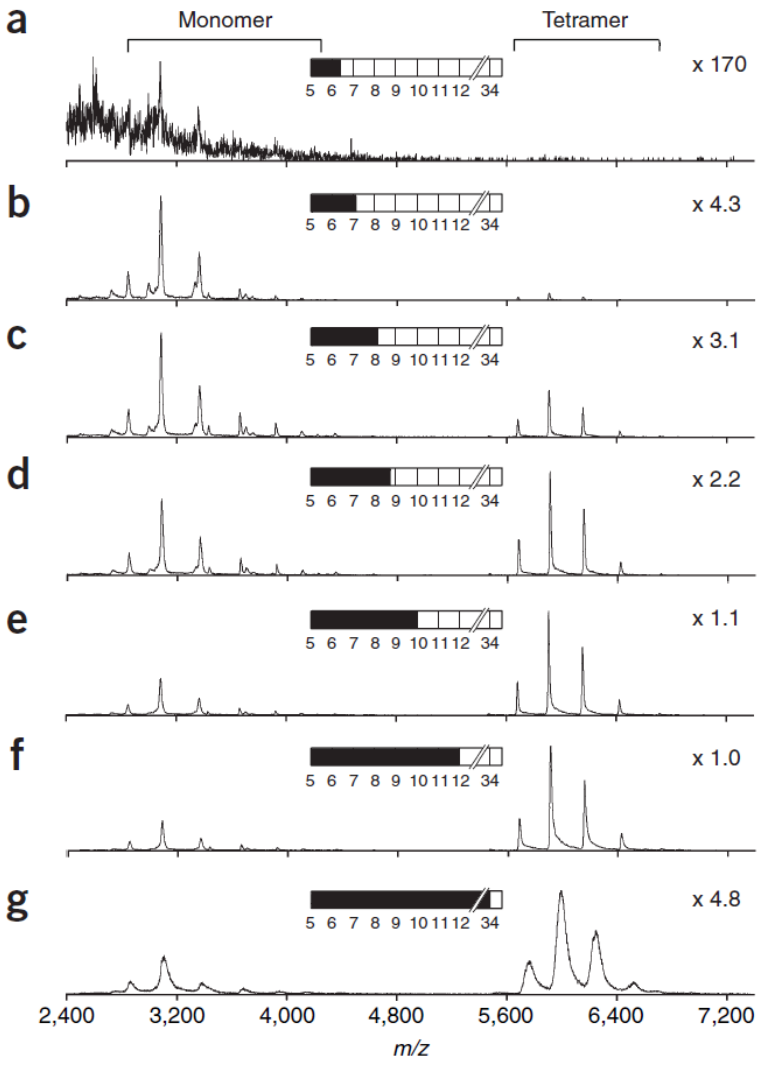
# Parameters for Non-covalent Mass Spectrometry

- Generally done with ToF instruments
- Direct (nanospray) infusion out of volatile buffers (typically ammonium acetate)

# Displacement of “Contaminating” Buffer by Competion

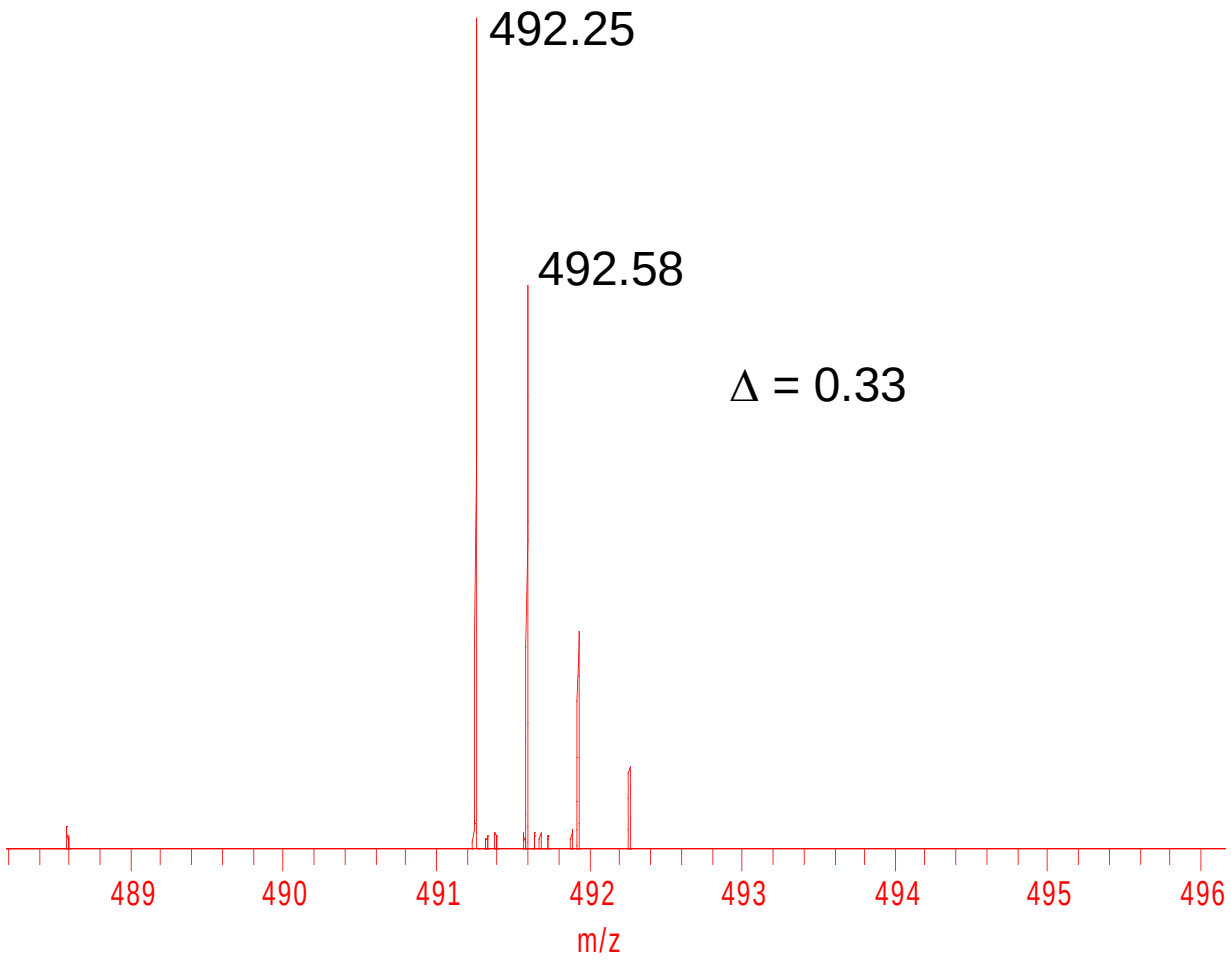


# Effect of Pressure on Spectral Quality

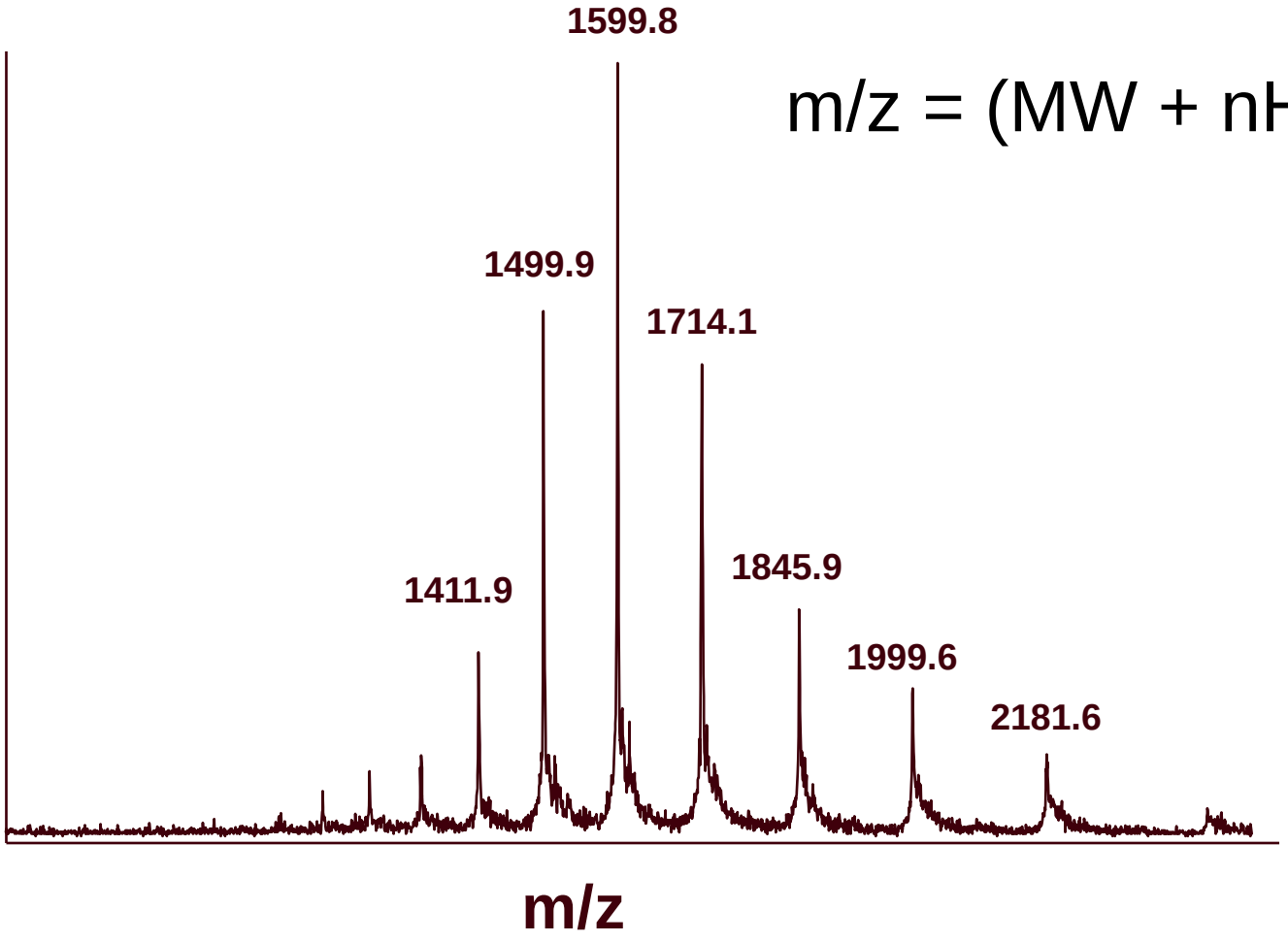



Increasing vacuum

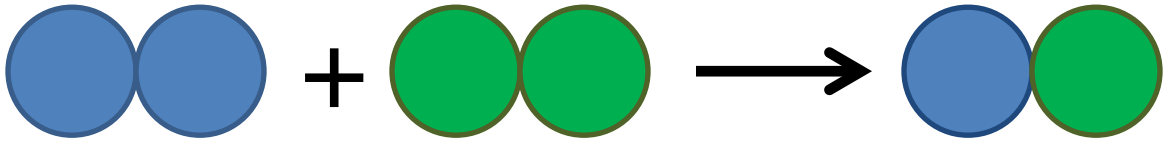
# How Do We Determine Charge State?



# How Do We Determine Charge State of a Protein?

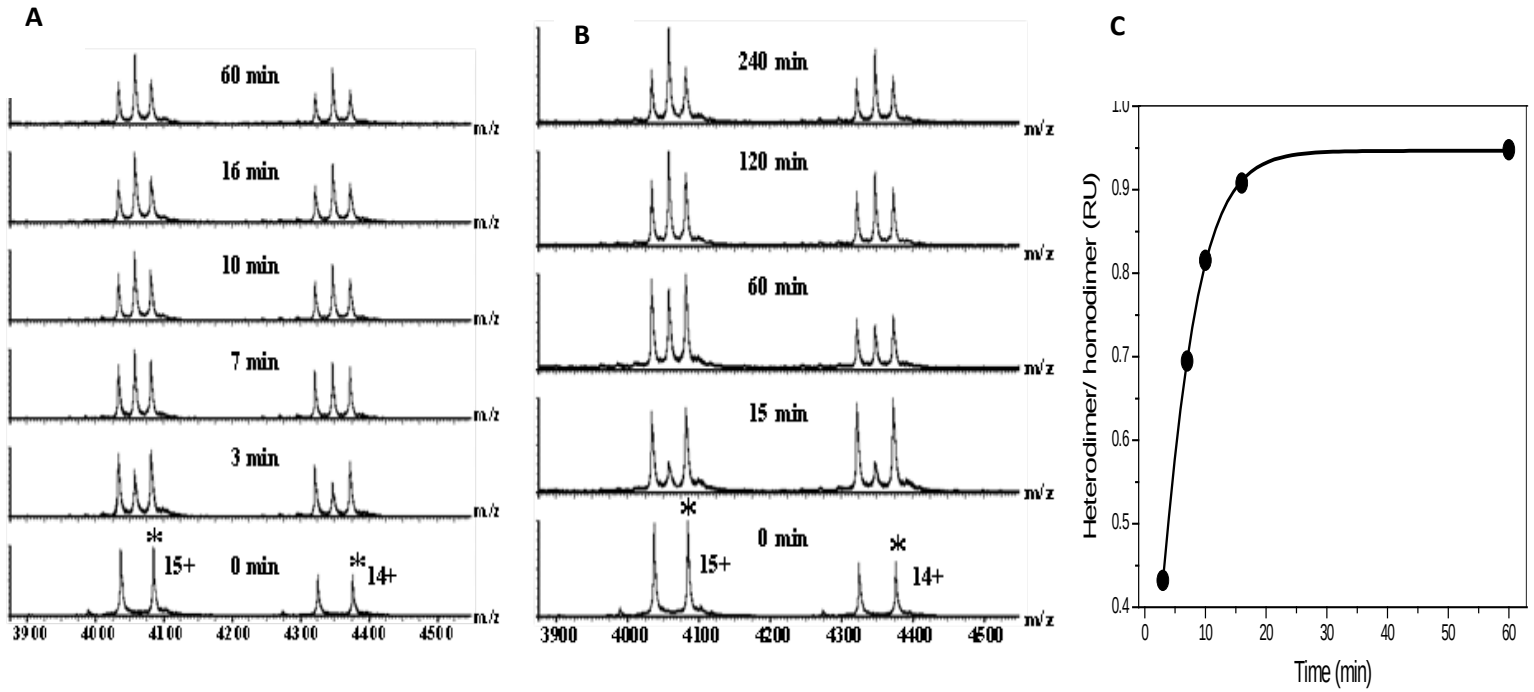



$$m/z = (MW + nH^{+})/n$$

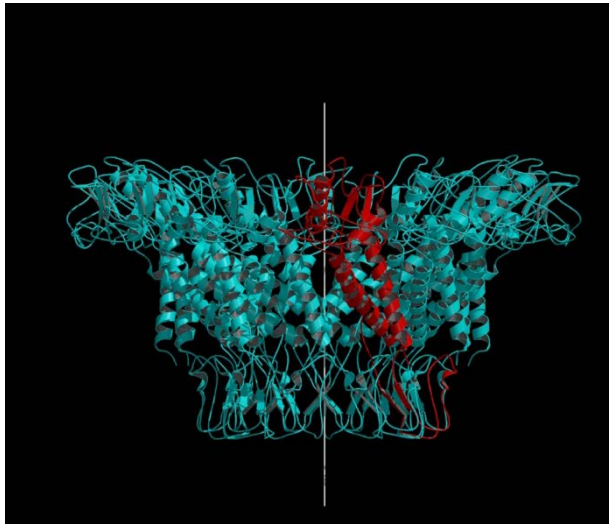


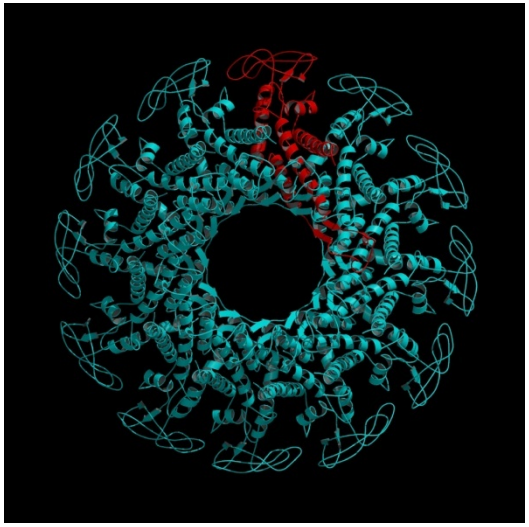
$$K_d = k_{off} / k_{on}$$

# Subunit Exchange in NAD Synthetase



# Portal Motor Packages DNA Into Phage Head





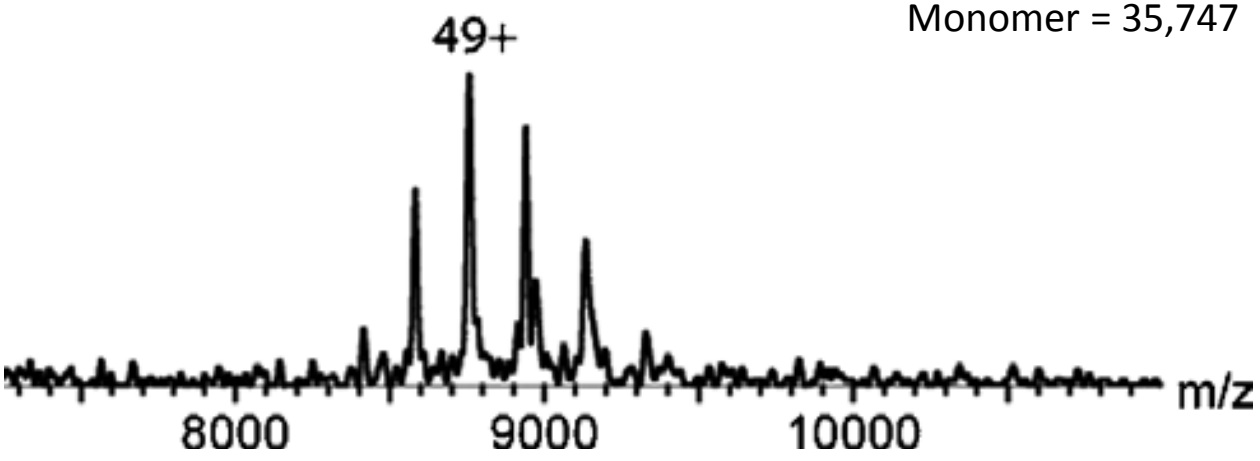
# Native Mass Spectrometry Can Determine the Stoichiometry of Macromolecular Complexes

Phi-29 Portal Complex

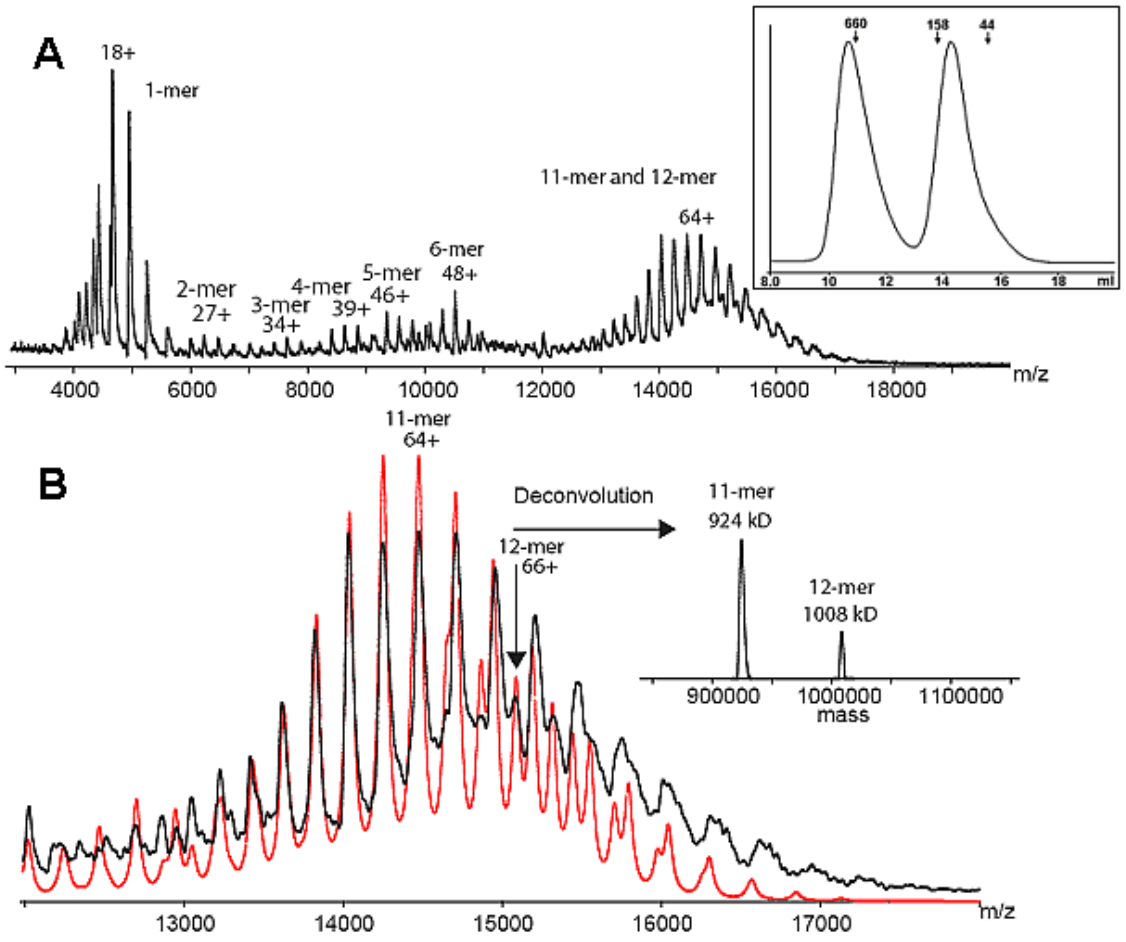
m/z = 8938
m/(z+1) = 8756
m/(z+2) = 8581

m = 429,052

m/12 = 35,754
Monomer = 35,747

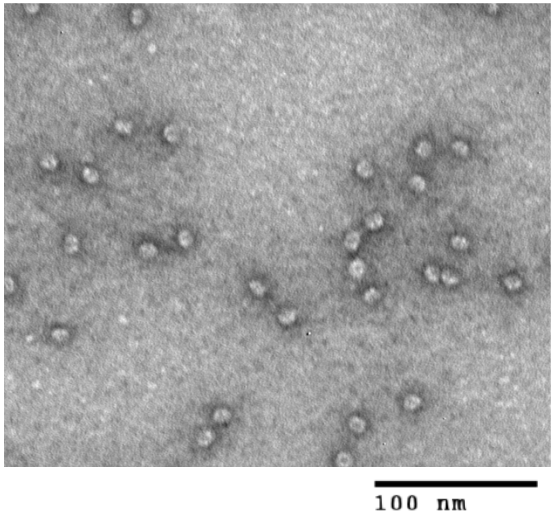


# Detection of Intermediates and Sub-populations

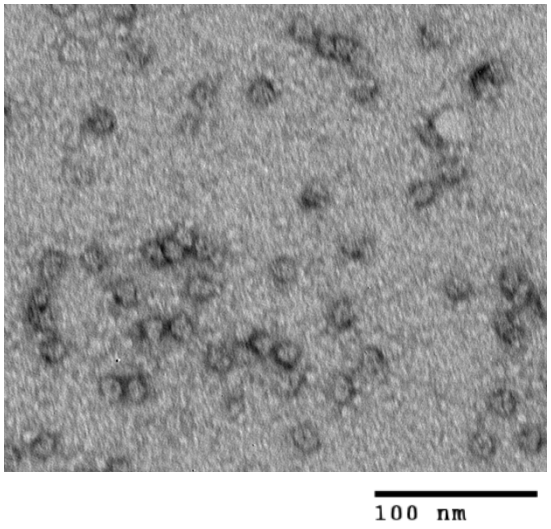


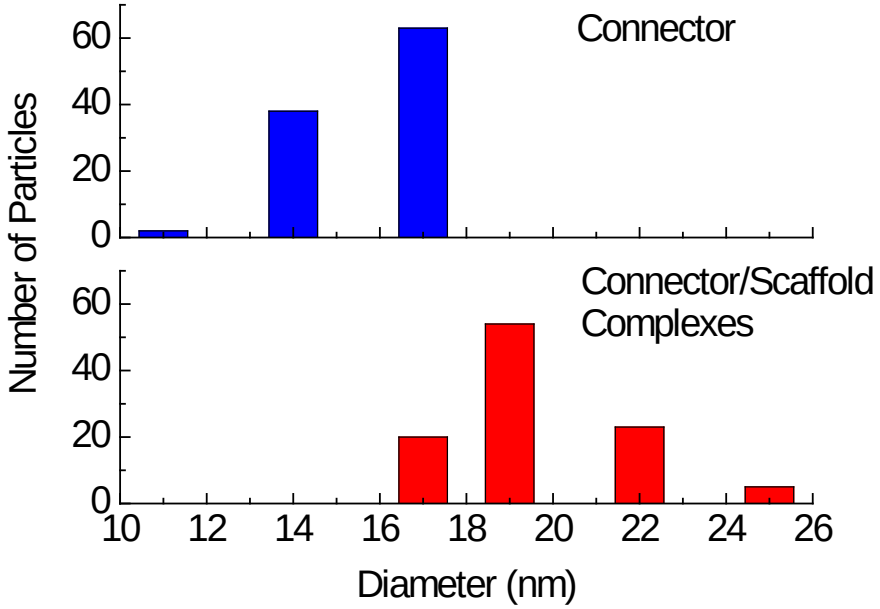
# Complexes Formed *in Vitro* Larger Diameters

Connector Dodecamers




Complexes

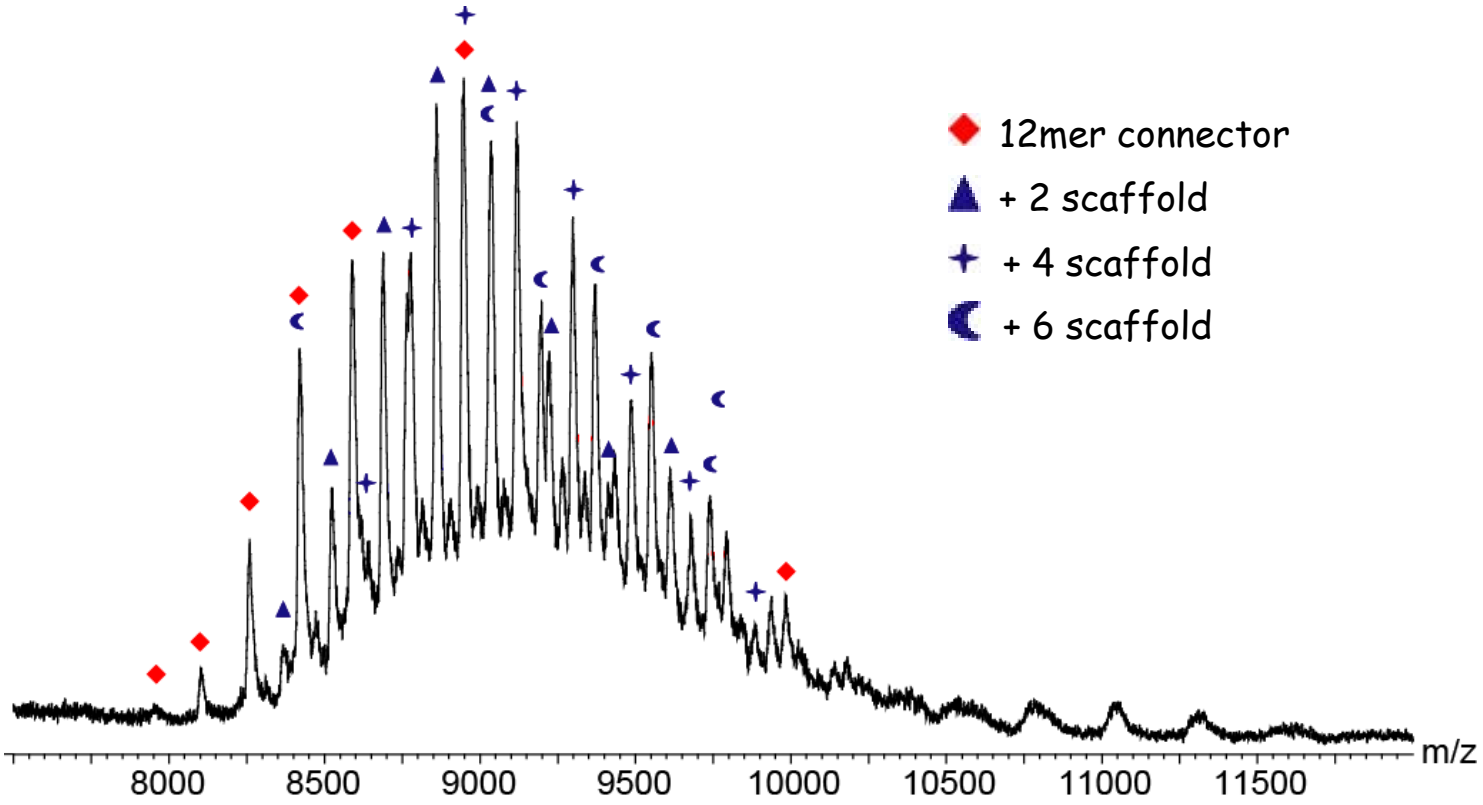


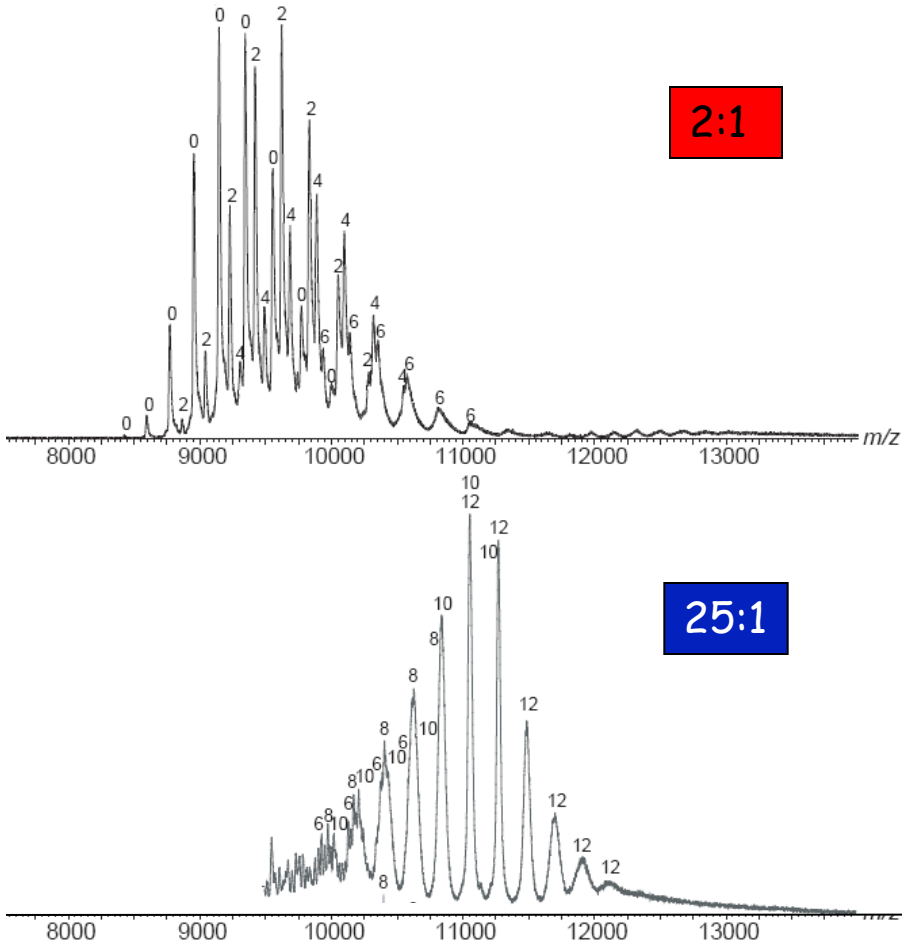

Negative-stained EM

# Native Mass Spectrometry Demonstrates Scaffold Binds as a Dimer

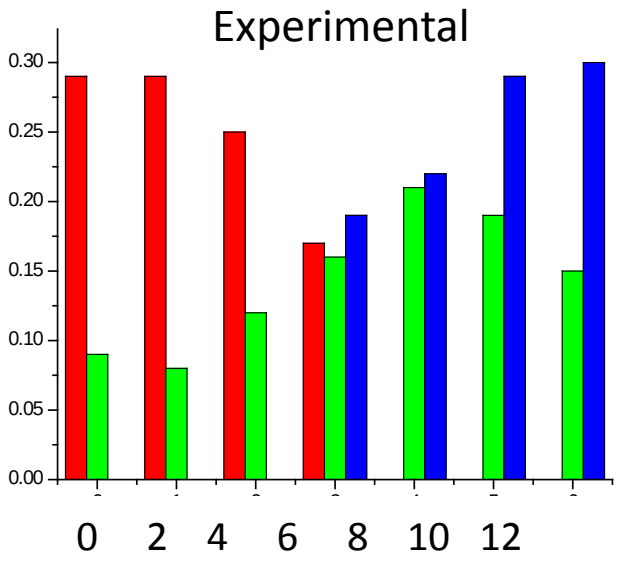
Scaffolding :Connector at 2:1 Input Ratio

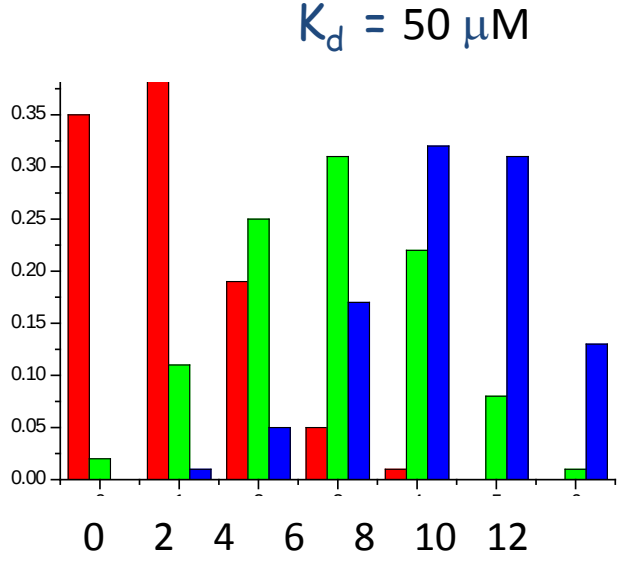


# Increasing the Scaffolding to Connector Ration Increases the Scaffolding Saturation



# Scaffolding Binds Non-cooperatively with a $K_d$ of ~20 μM

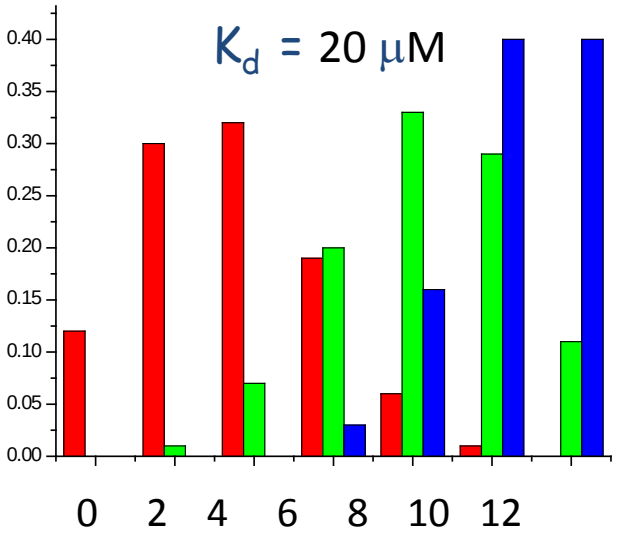


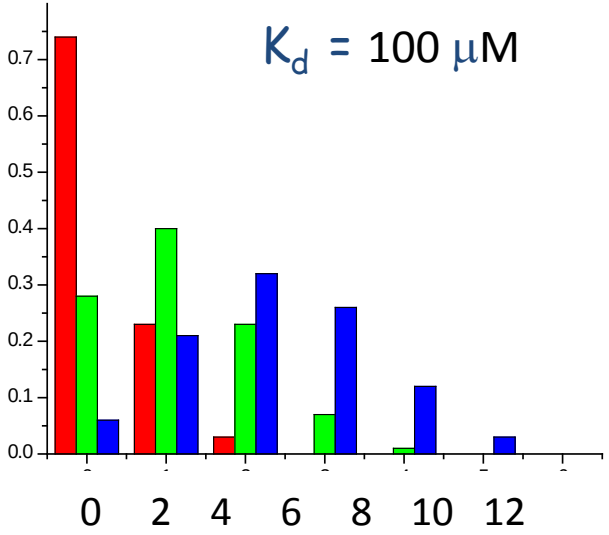




2:1

10:1

25:1



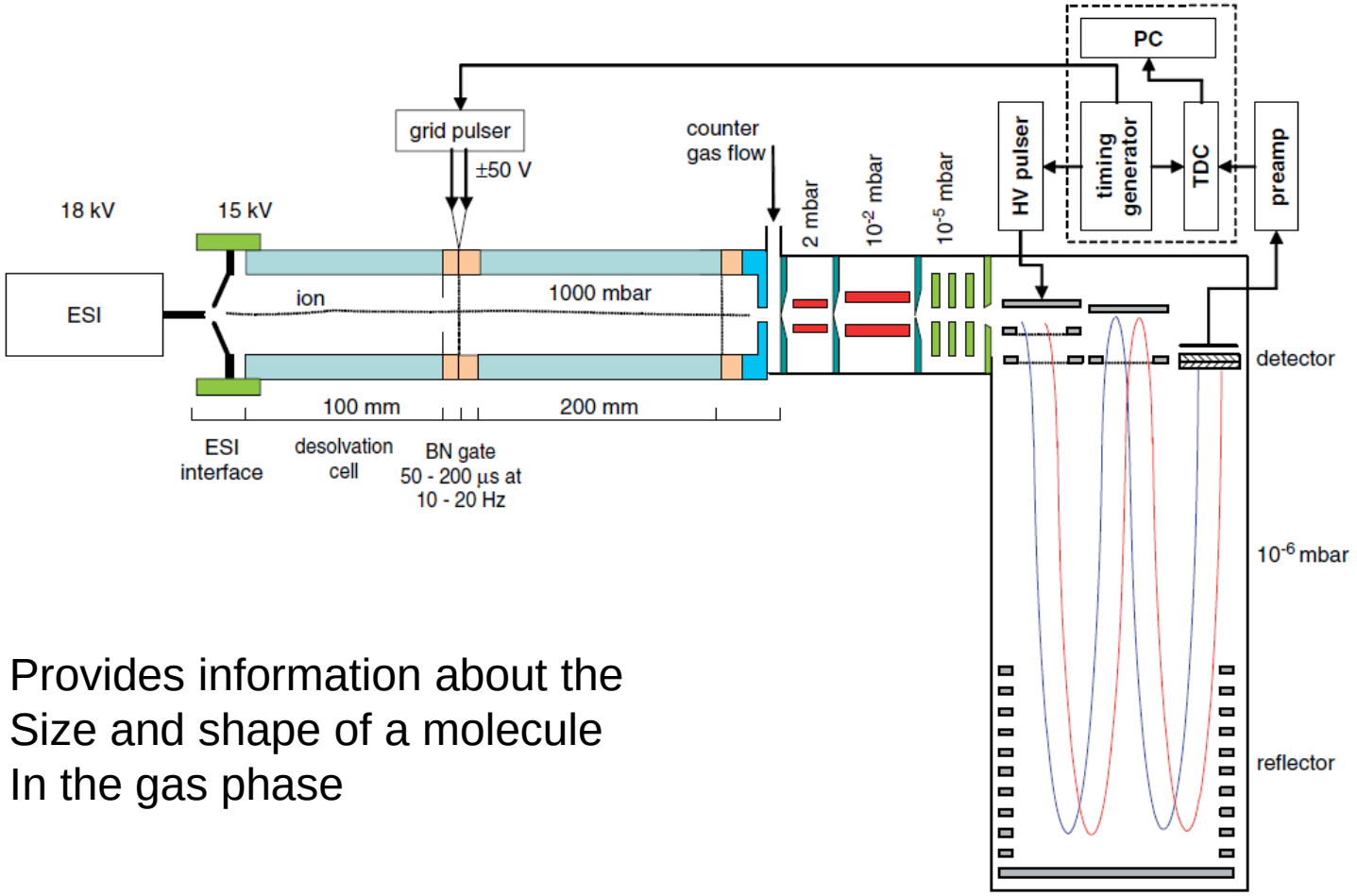
# Obtaining Shape Information

Put a device that separates ions by shape in front of MS (Imagine a size exclusion column)

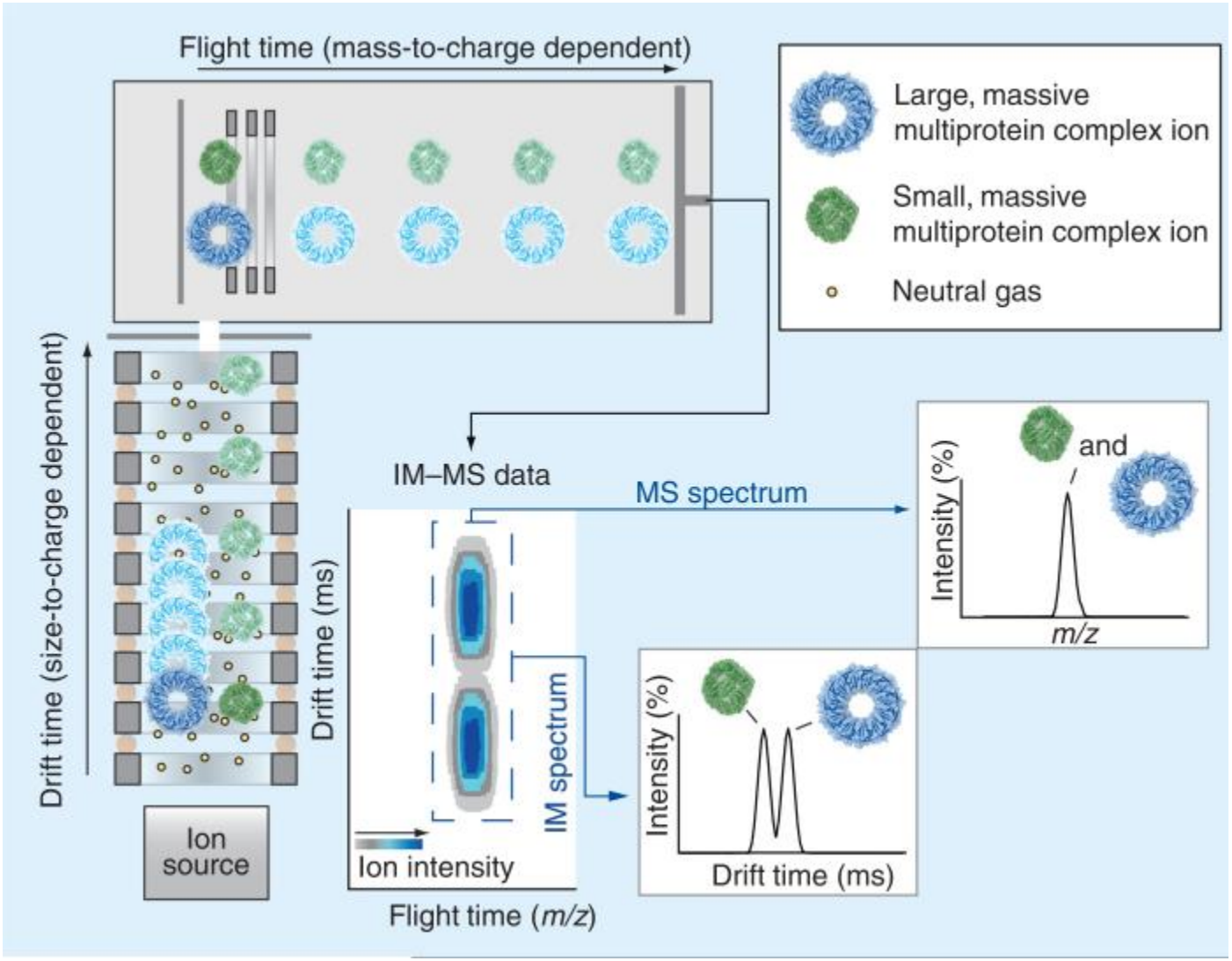
One such device is a “drift tube”

This is a “tube” filled with gas. The progress of the molecules is retarded as they are buffeted by gas.

# Ion Mobility Mass Spec (IMS)



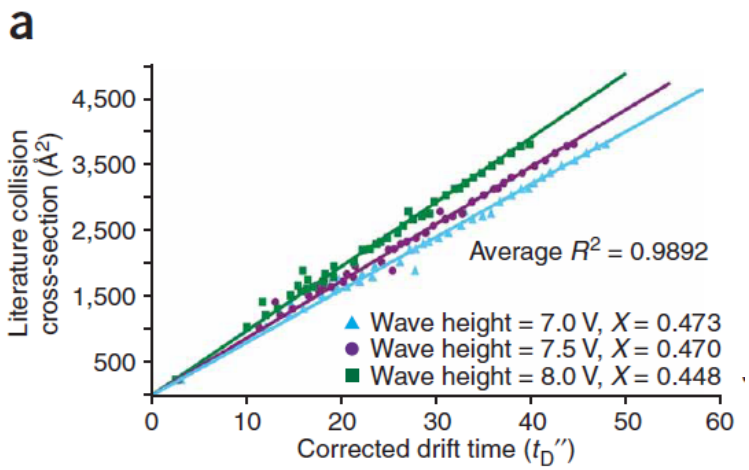

Provides information about the
Size and shape of a molecule
In the gas phase

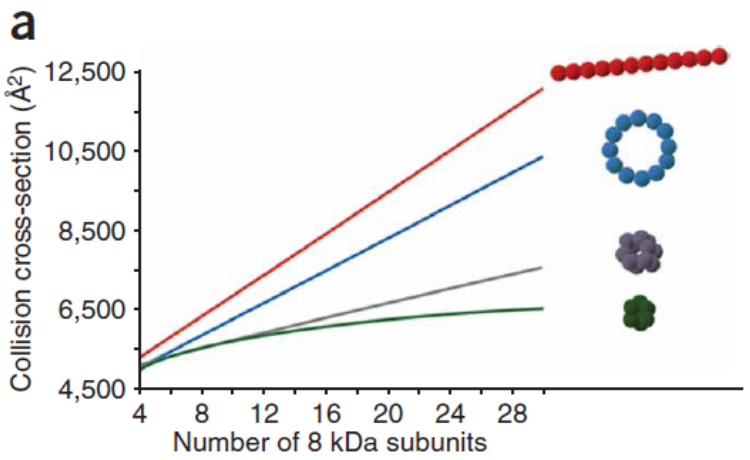

Flight time (mass-to-charge dependent)
Large, massive multiprotein complex ion
Small, massive multiprotein complex ion
Neutral gas
Drift time (size-to-charge dependent)
Ion source
IM–MS data
MS spectrum
Drift time (ms)
IM spectrum
Ion intensity
Flight time (m/z)
Intensity (%)
and
m/z
Intensity (%)
Drift time (ms)

How do we derive collision cross section from drift time?

What good is it?

- another dimension of separation
- provide shape information



# Transthyretin (TTR)

Serum carrier of throxine and retinol

Tetramer of 55 KDa subunits

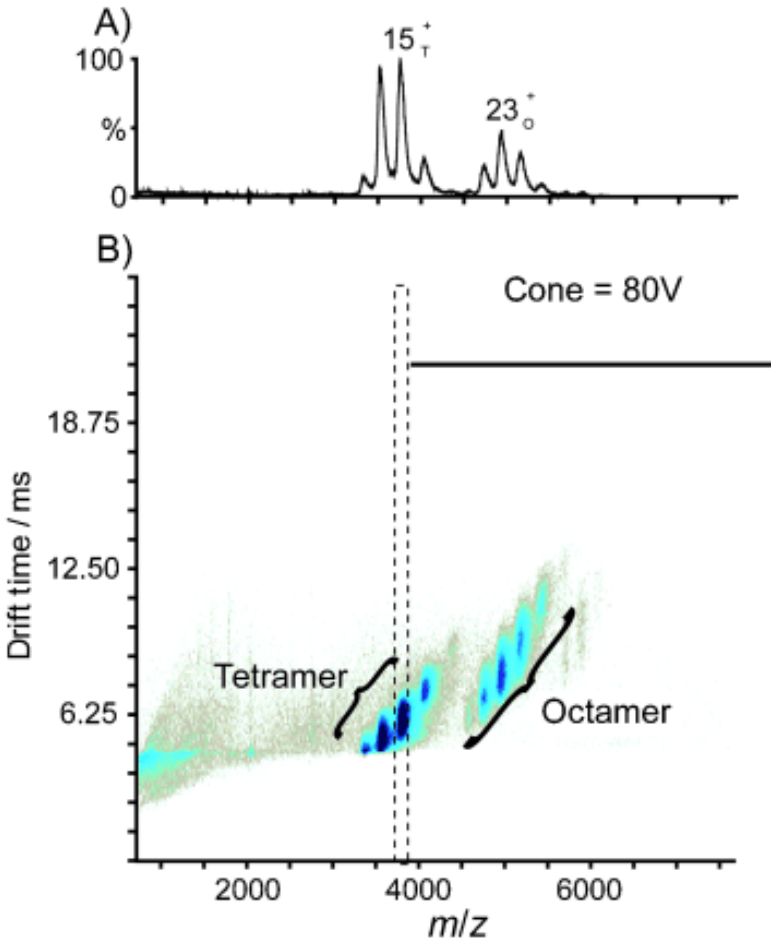
Misfolding results in systemic amyloidosis

Aggregation requires partial unfolding of monomer

Thought that stabilizing tetramer would be therapeutic

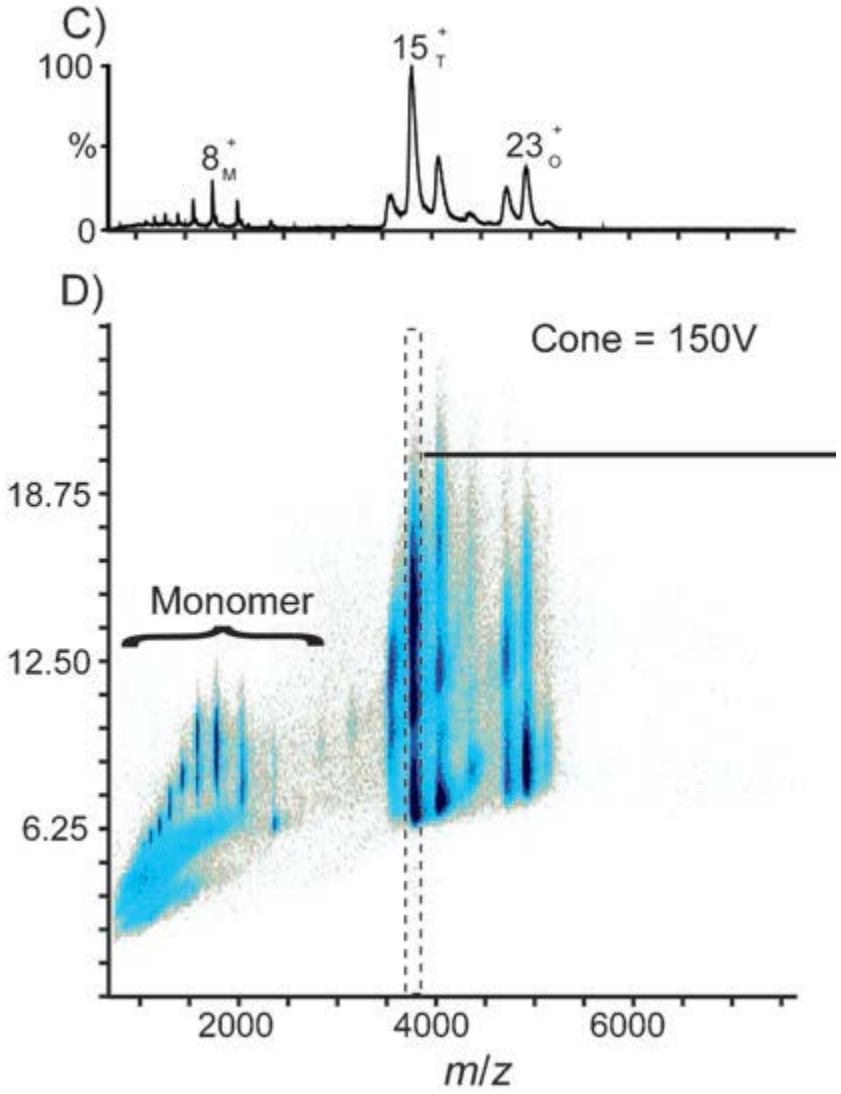
Can one partially unfold and remain tetrameric?

# IMS Mass Spectrum of Equilibrium Mixture of TTR Tetramer and Octamer




Separation of tetramer and octamer

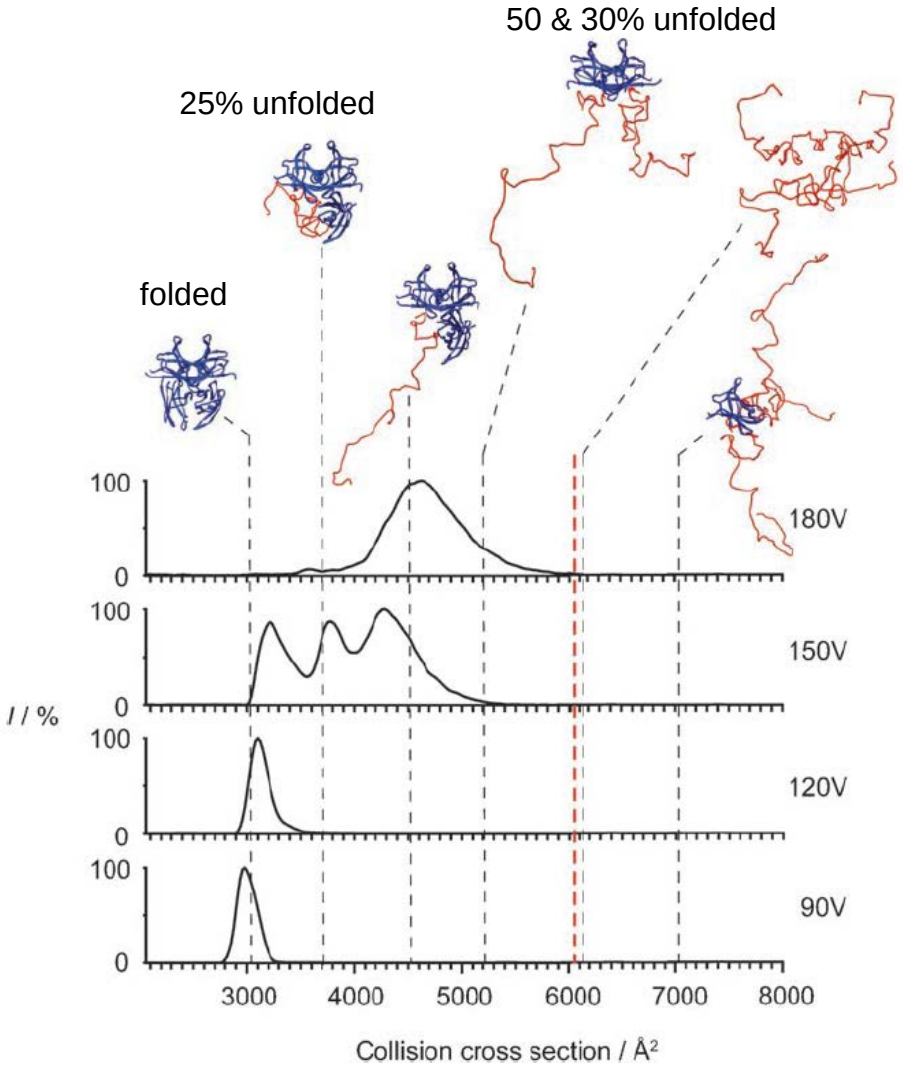
Narrow drift time – uniform states




Increase cone voltage

Increased and Broadened drift time

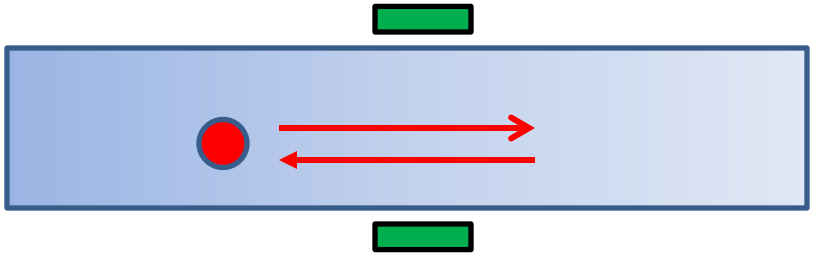
# Select Tetrameric Species Data and Model Cross-sectional Area

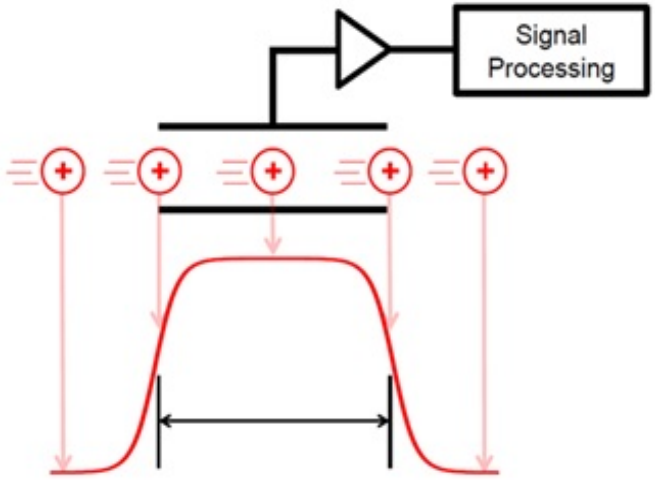


# Direct Measurement of Charge on Complexes (CDMS)

# Basis of CDMS

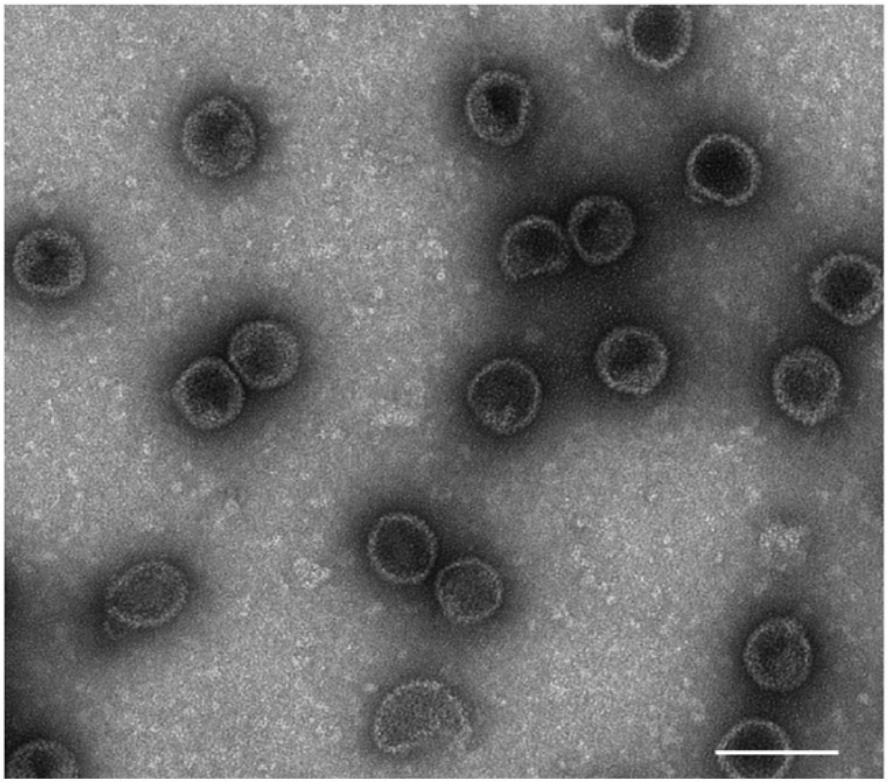
Ion Trap






- Analyze individual ions for 95 ms
- Frequency – mass/charge
- Induced current - charge

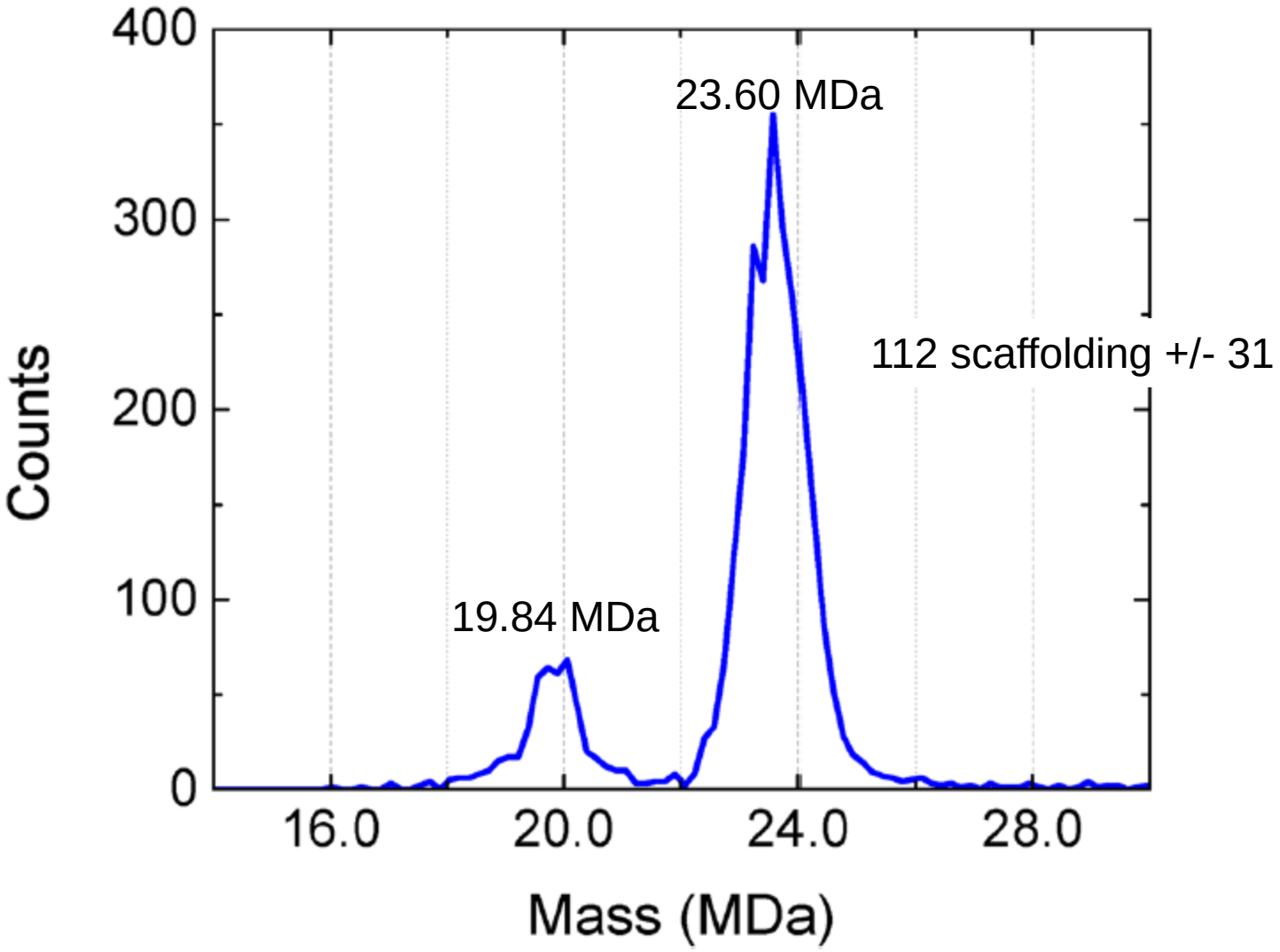
What is the dynamic range of a single molecule experiment?



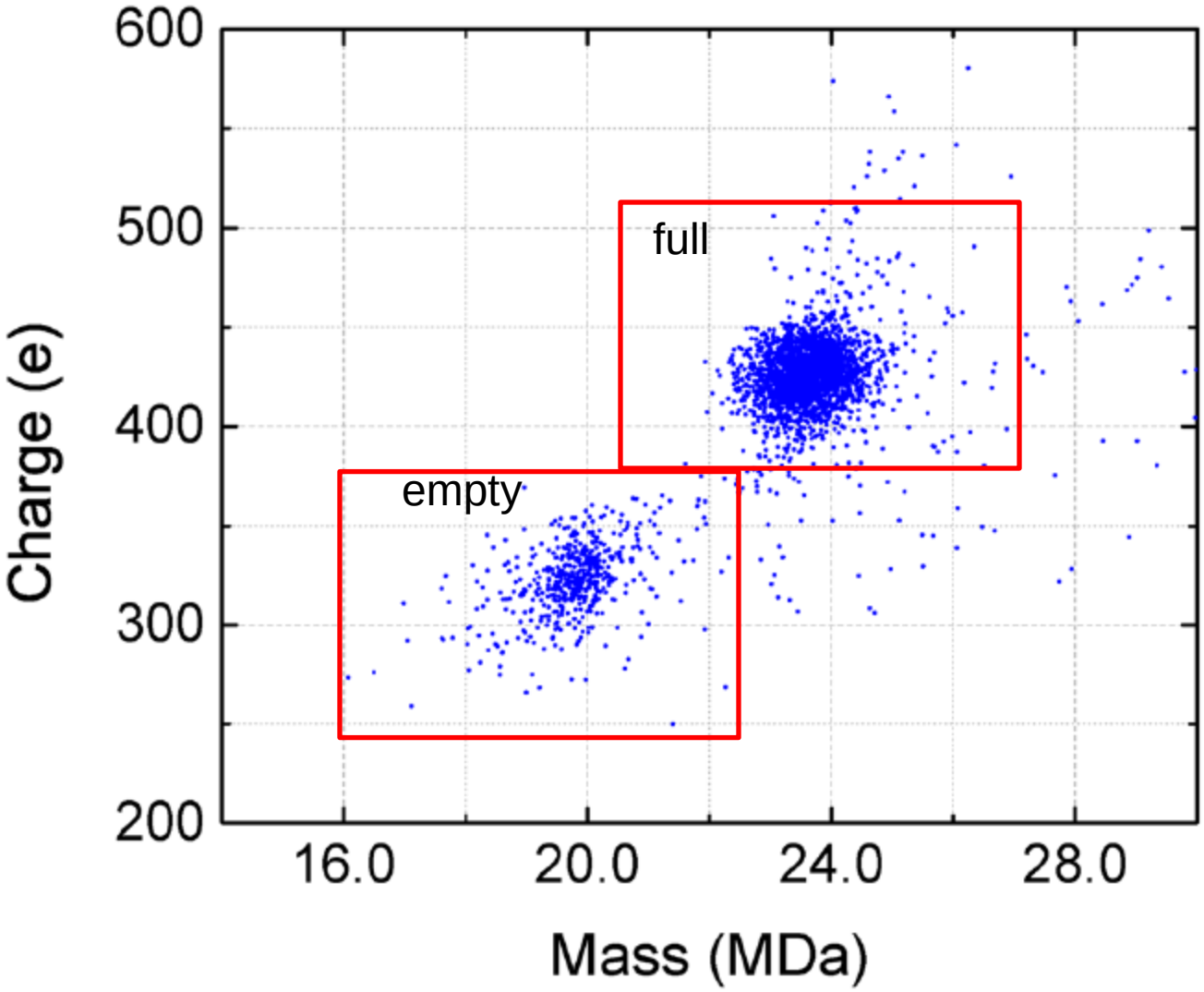
420 molecules of 46,500 Da
= 19.58082 Mda

Unknown number of scaffolding protein of mol wt = 35,000 Da

Bacteriophage Capsids



Breakdown of Charge Residue Model?

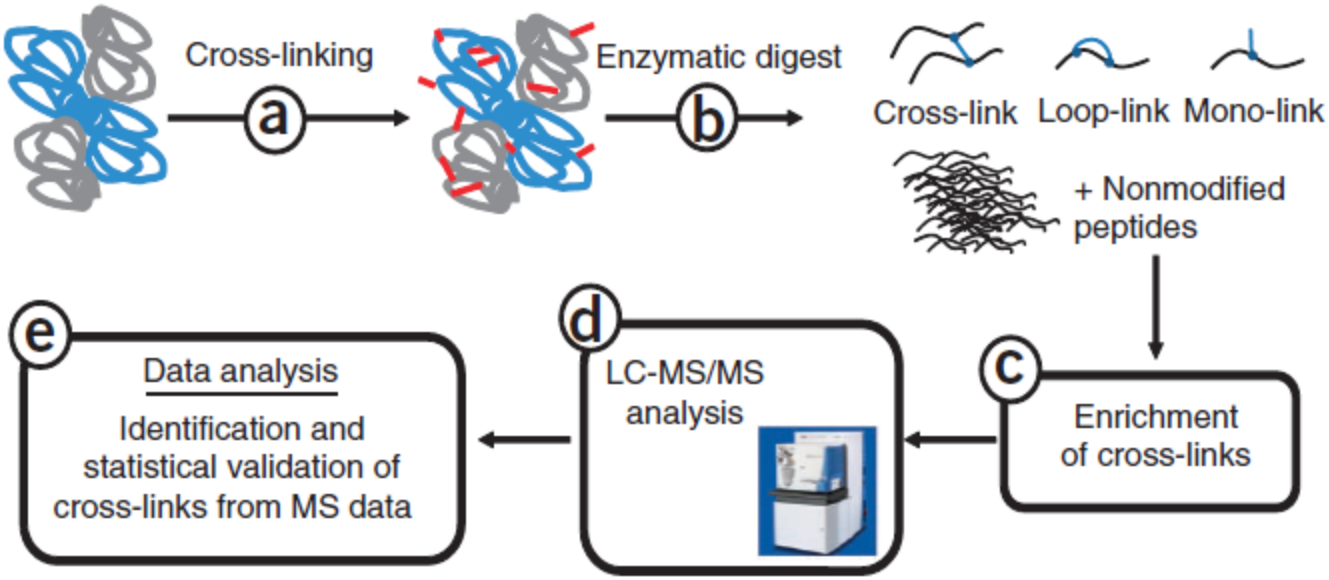


# Identification of the Connector/Scaffolding Interface by Chemical Cross-Linking

To Locate the Interface on Connector Protein

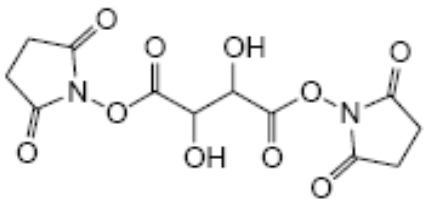
To Obtain Distance Constraint of Interactions

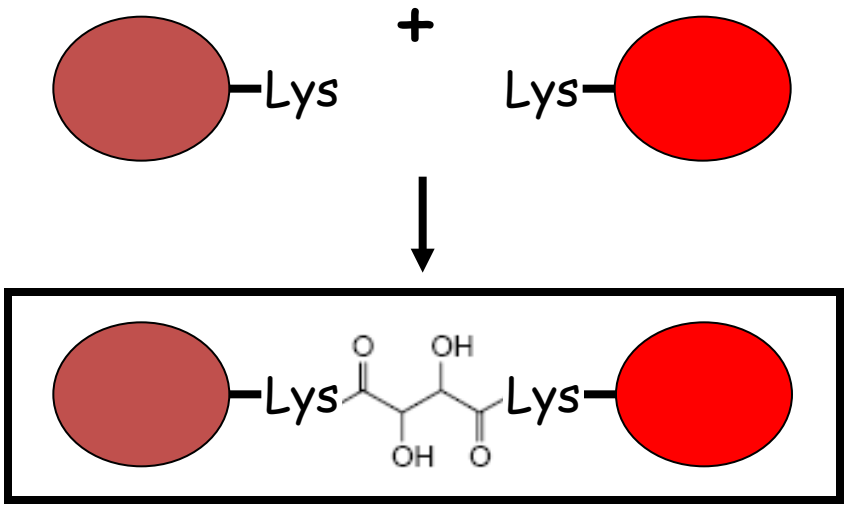
Schematic of Crosslinking Experiments.



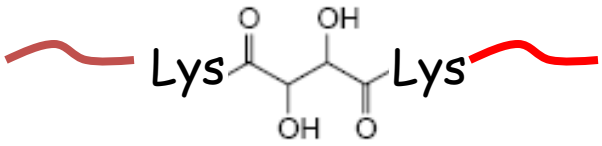
# Lysine Reactive DST Cross-Linker

DST: Spacer Arm 6.4 Å



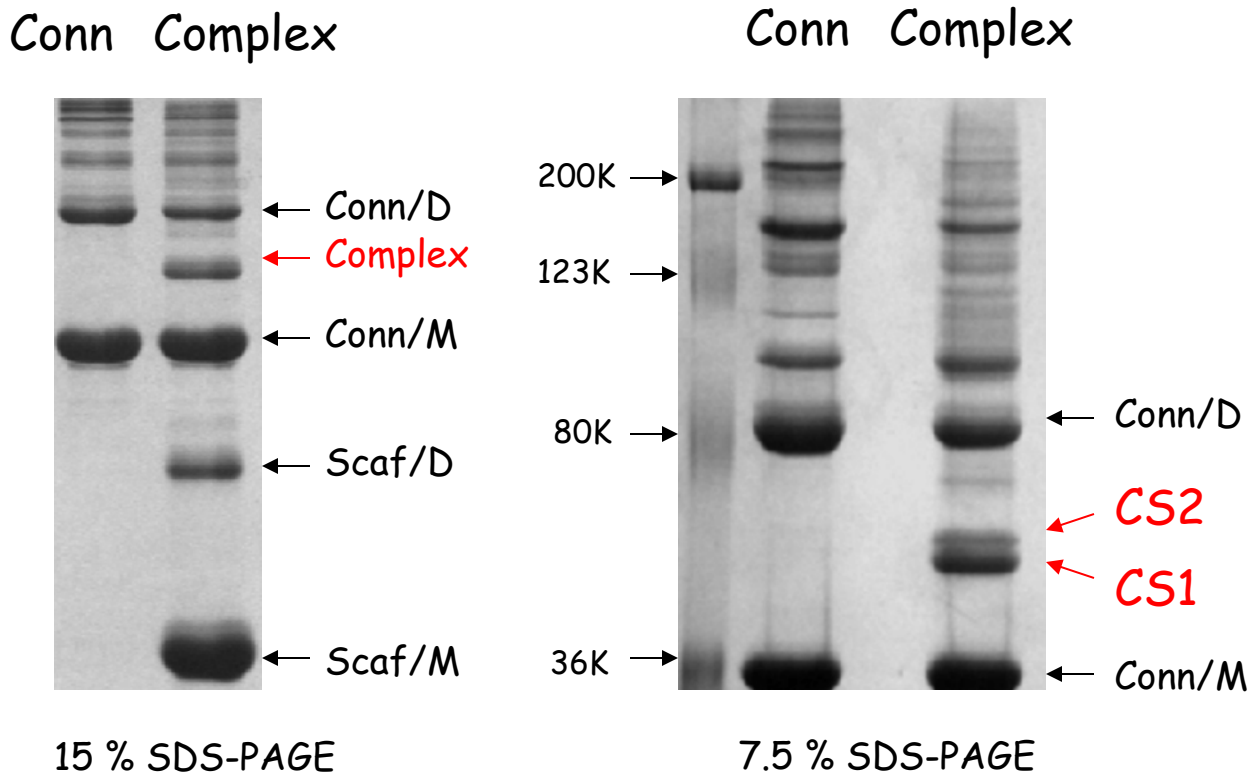




Trypsin Digestion

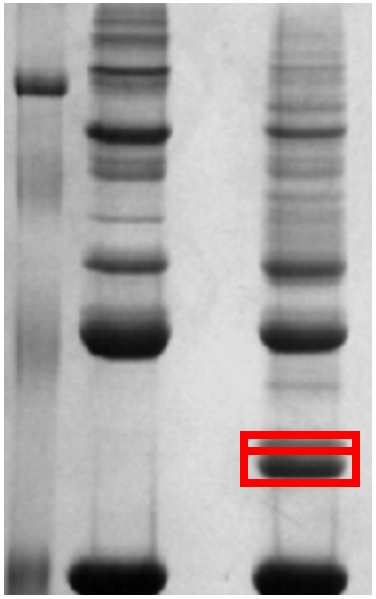



Mass: A+B+114 Da

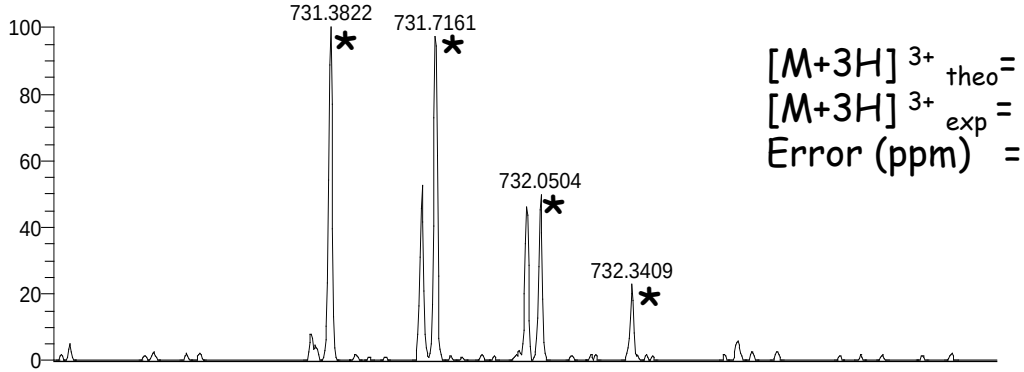
# DST Cross-Linking Profiles



# Identification of Scaffolding/Connector Interfaces by Chemical Cross-linking

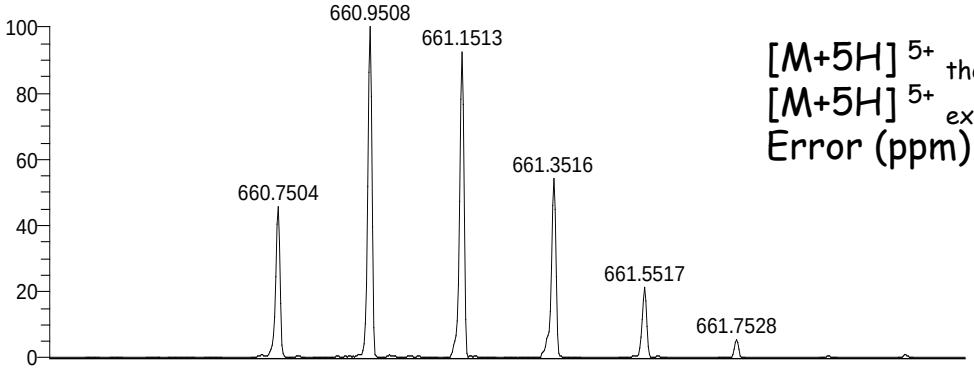


## Scaffolding 83-98 Cross-Linked to Connector 4-5/19-20




$[M+3H]^{3+}_{theo}$ = 731.3813
$[M+3H]^{3+}_{exp}$ = 731.3822
Error (ppm) = 1.2 ppm

## Scaffolding 53-68 Cross-Linked to Connector 94-105




$[M+5H]^{5+}_{theo}$ = 660.7490
$[M+5H]^{5+}_{exp}$ = 660.7504
Error (ppm) = 2 ppm

Peptides were sequenced by MS/MS.

# Docking Model of Connector/Scaffolding Complexes

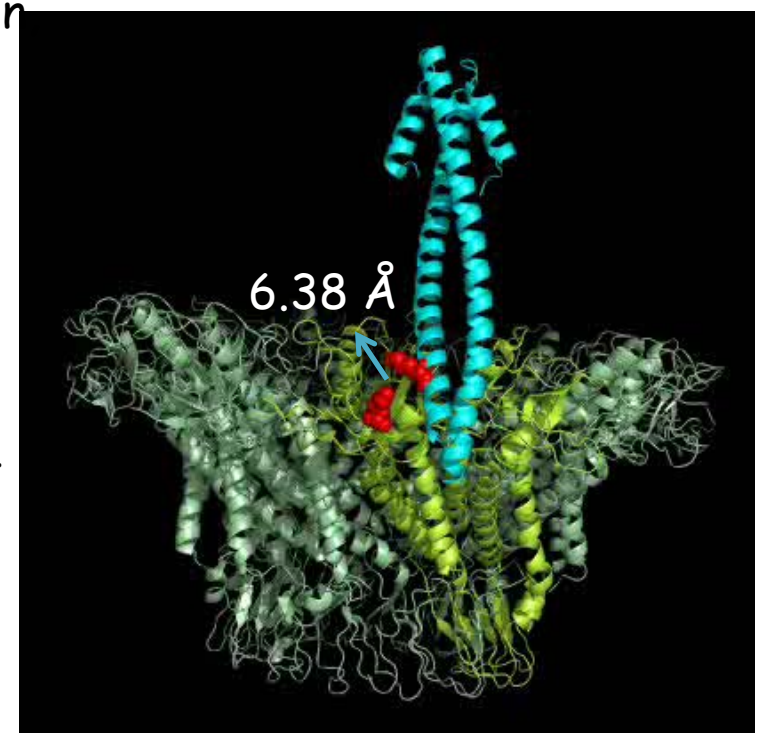
1st step: ZDOCK
( optimize shape complementarity, deslovation electrostatics)
- Use entire scaffolding dimer
- Use connector dimer
- Block interior surface of connector
- Generate #2000 models

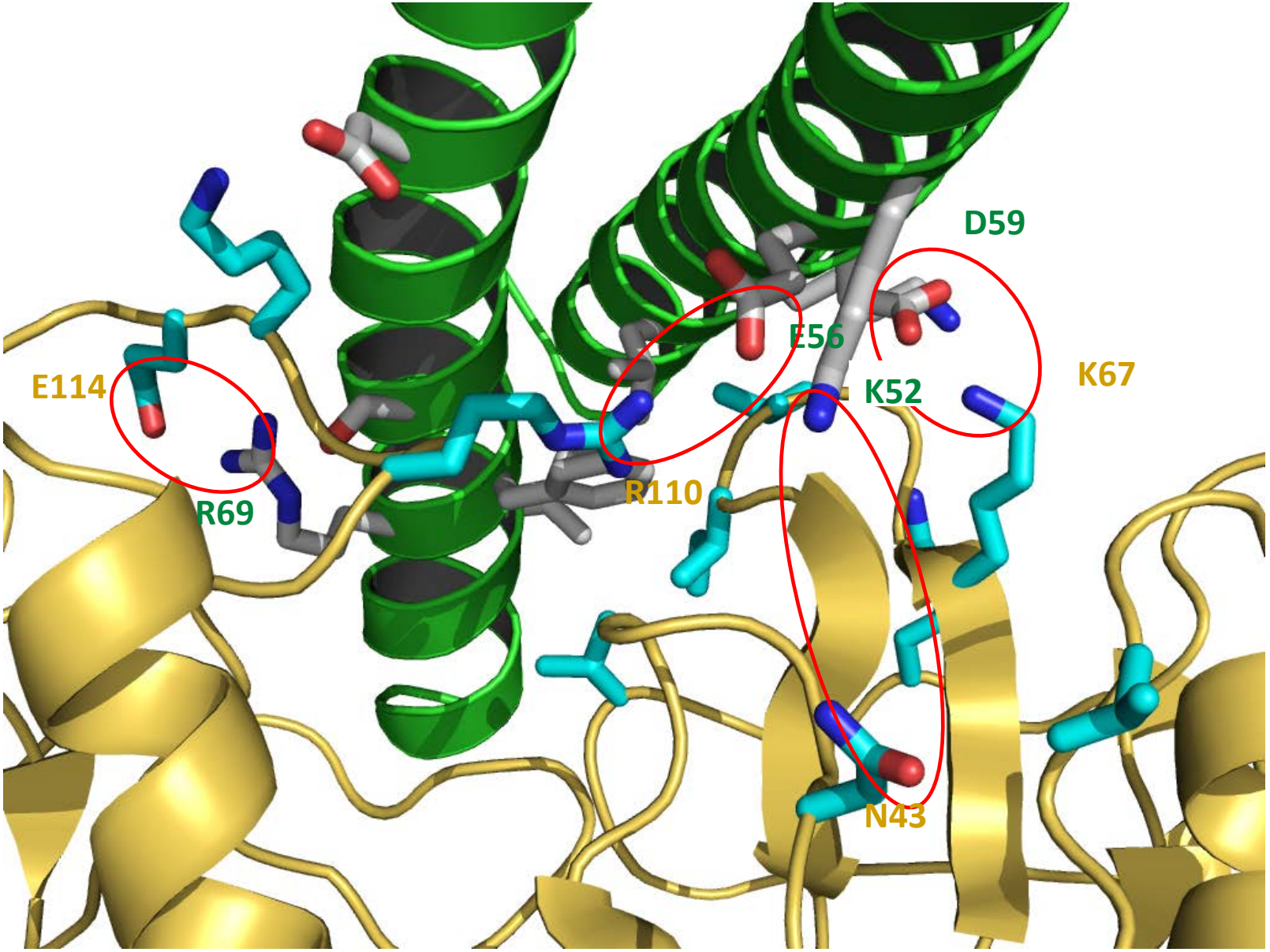
2nd step: filter with
SF66-Conn102 cross-link distance constraint
- Use 8 Å constraint, 26 models

Scaffolding orientation
- Defined by SF83 Conn4/19 cross-link

Select model #25

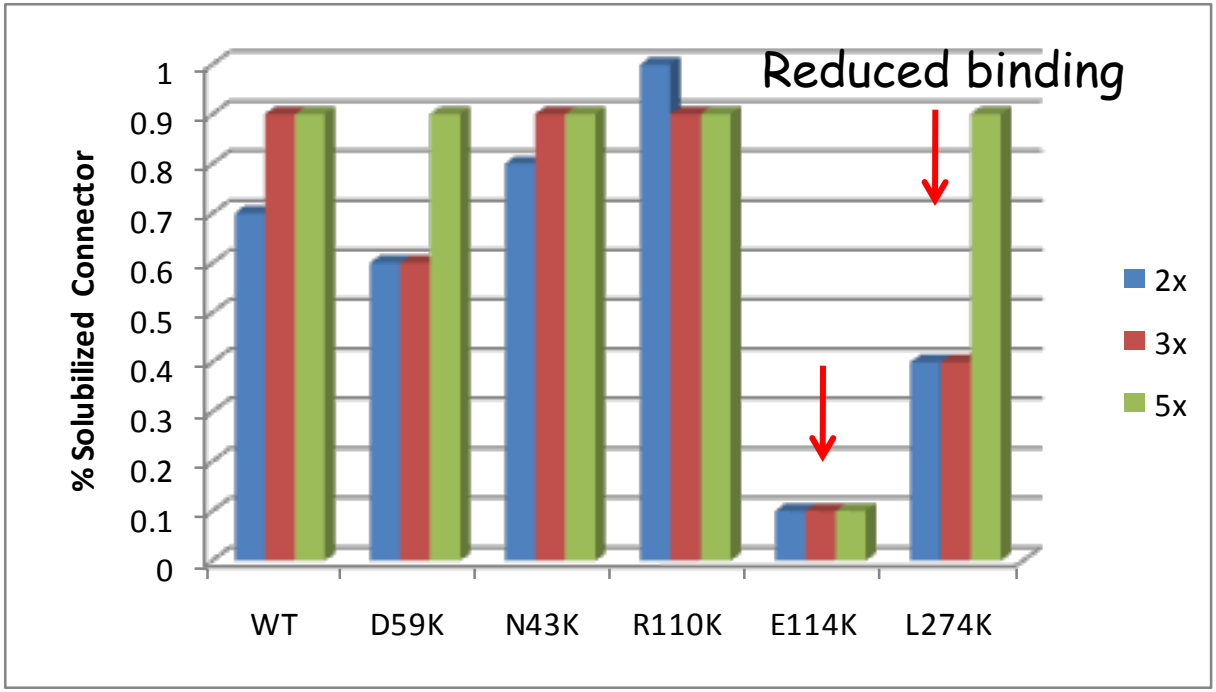
3rd step: model verification by mutagenesis




D59
E56
K52
K67
E114
R69
R110
N43

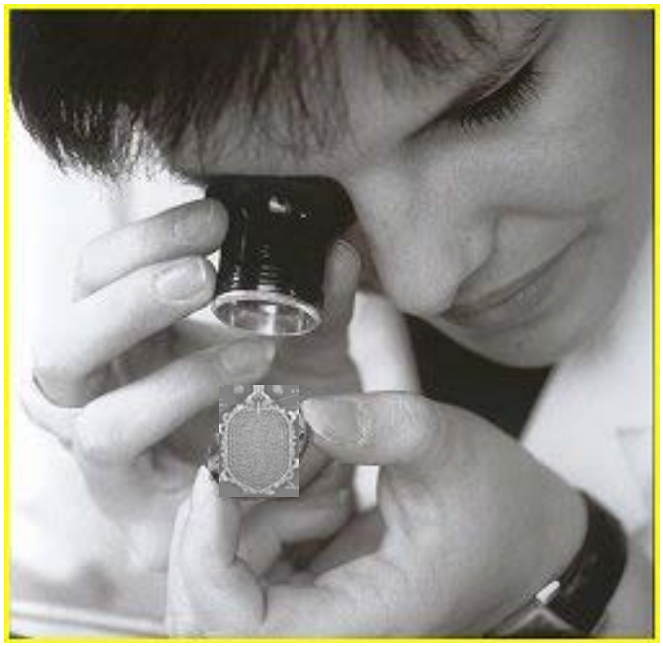
# Based on the Model Pairs of Lysines Were Introduced to Alter Complex Stability and Enable Cross-linking

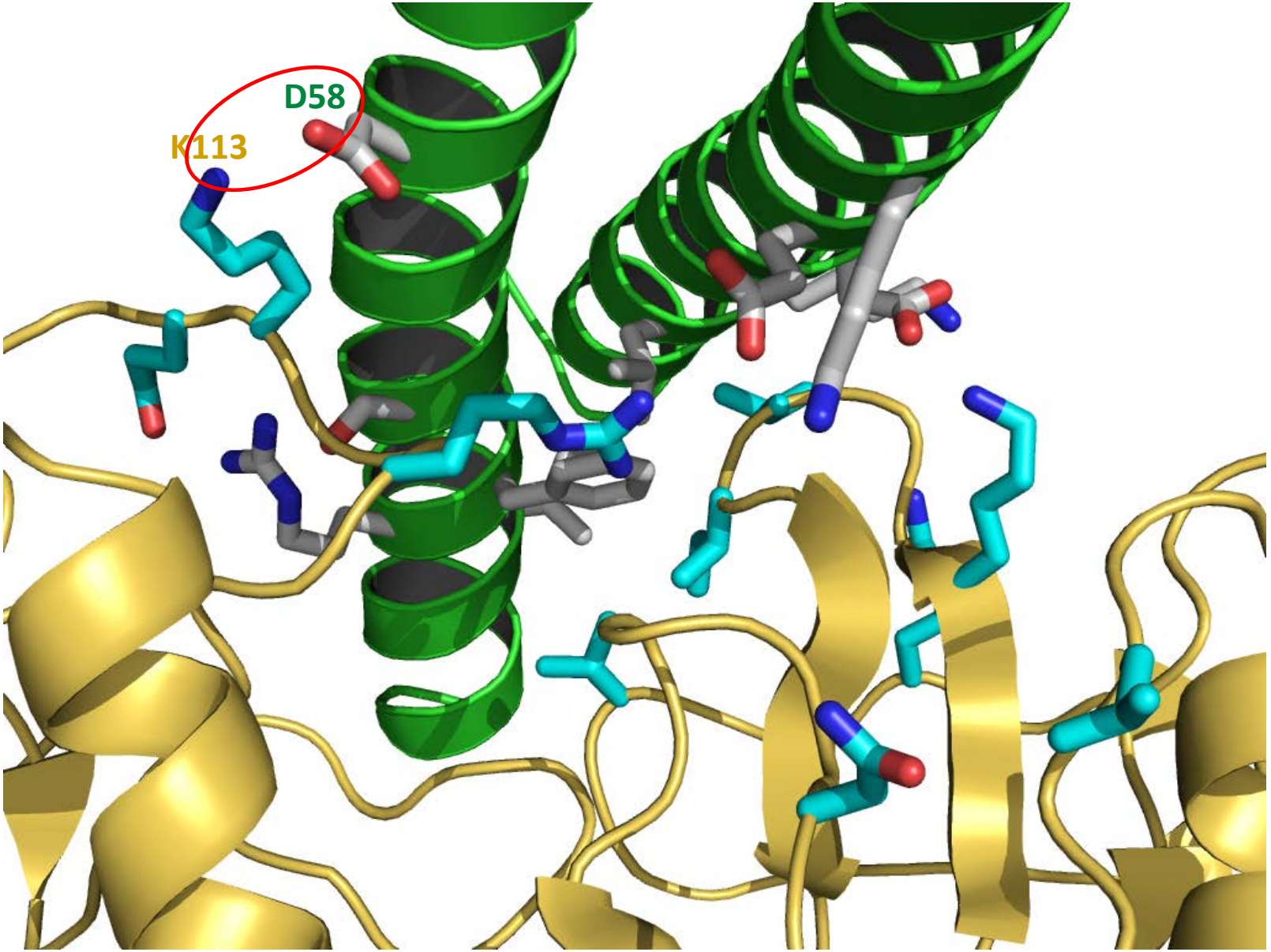
| Connector | Scaffolding | Distance |
|---|---|---|
| K67 | D59 | 2.98 Å |
| N43 | K52 | 6.88 Å |
| R110 | E56 | 4.05 Å |
| E114 | R69 | 3.86 Å |
| L274 | K52 | 9.81 Å |



Loss of Function
is Easy !

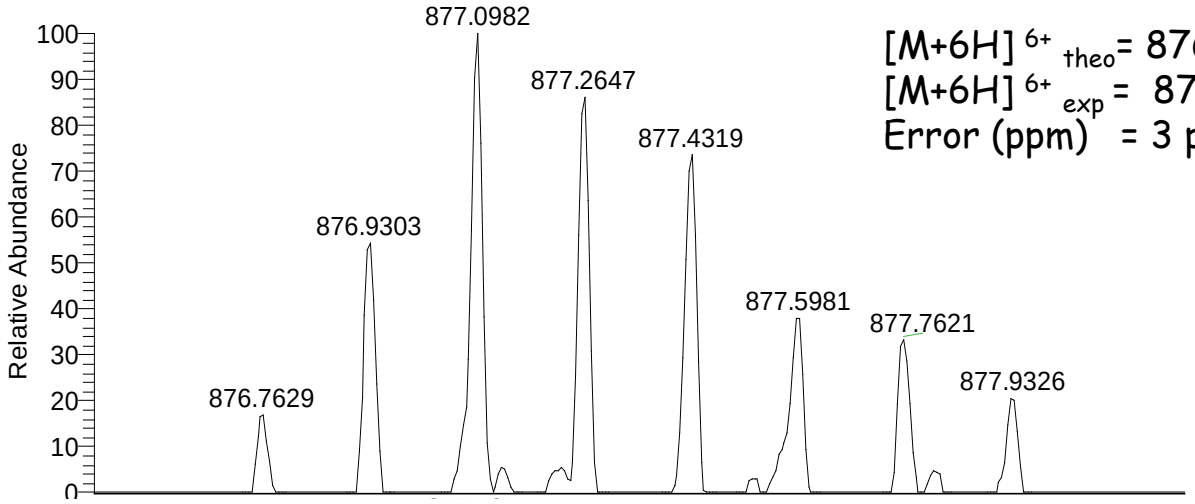
# Gain of Function




D58
K113

# Scaffolding D58K Cross-links to Connector K113 within the Distance Range Predicted by the Model

D58K Scaffolding 48-65 Cross-Linked to
Wt Connector 112-139 using DST (6.4 Å)




$[M+6H]^{6+}_{theo}$ = 876.7669
$[M+6H]^{6+}_{exp}$ = 876.7647
Error (ppm) = 3 ppm

Peptide was sequenced by MS/MS.
Predicted distance D58 to K113 ~3.8 Å.

The Needle in a Haystack is the Challenge for Crosslinking Studies

Strategies

Enrichment via biotin/strepavidin or size exclusion chromotography

Identification

Use of deuterated crosslinkers

Limitation

Lysine C$\alpha$-C$\alpha$ of 30 A

Cys-Cys or zero length would be better