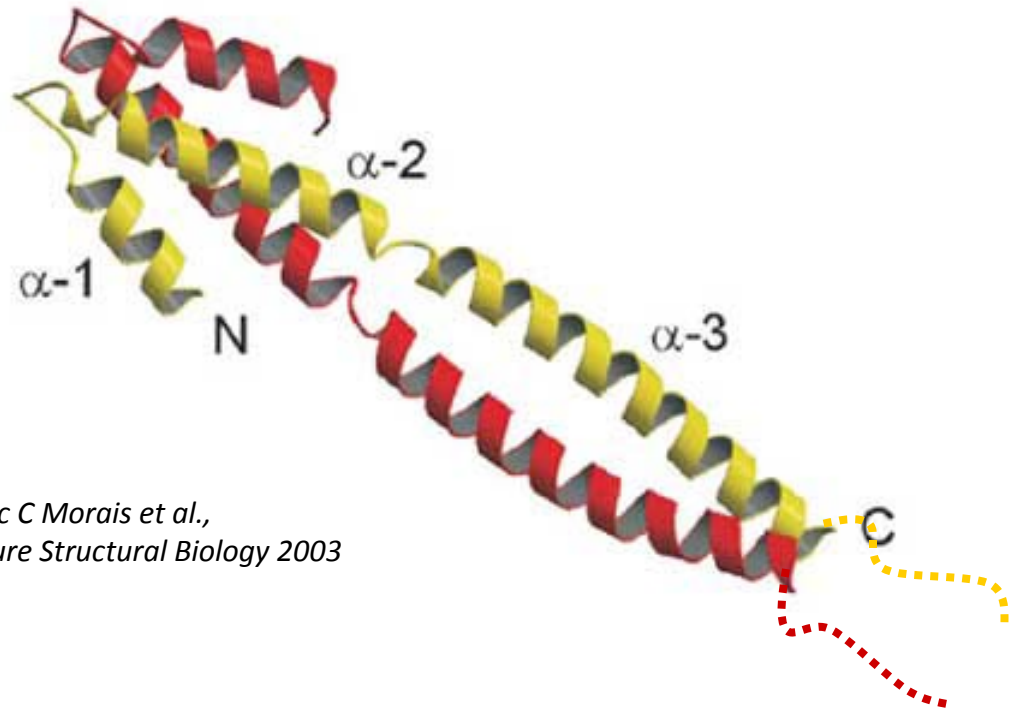
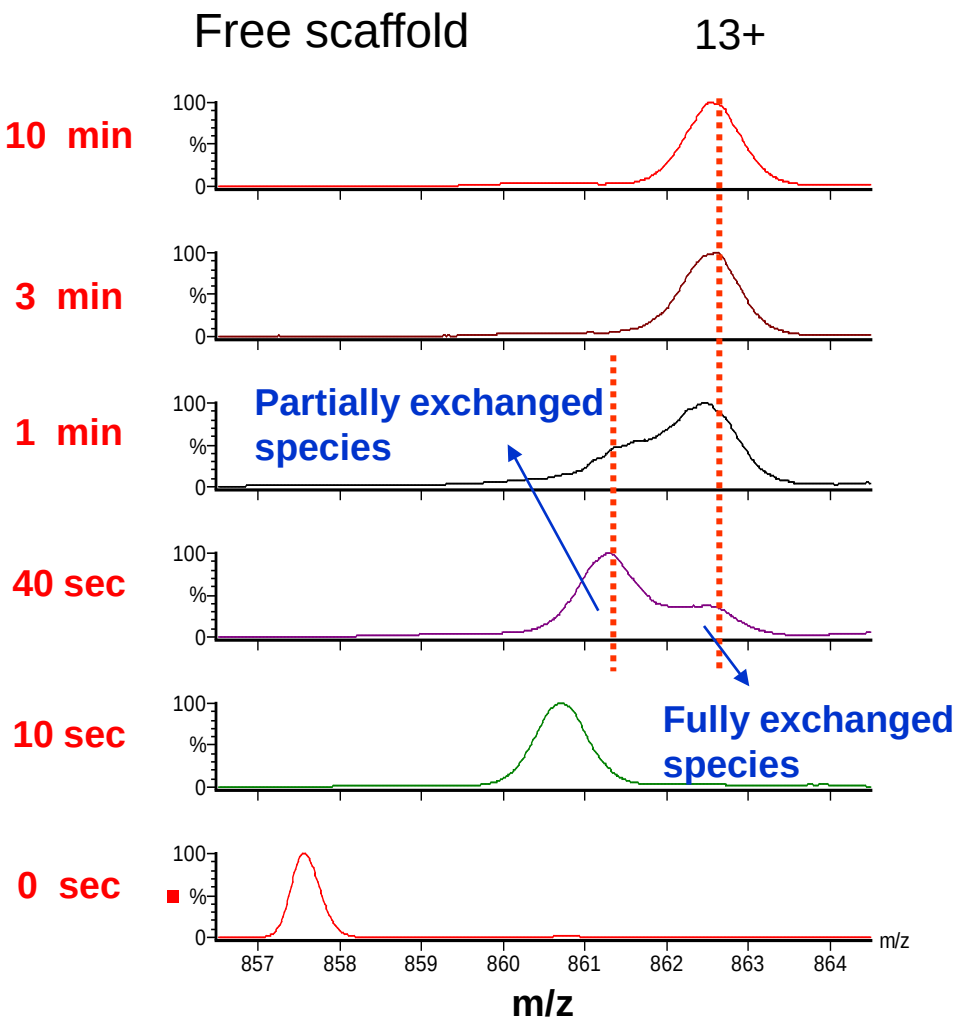
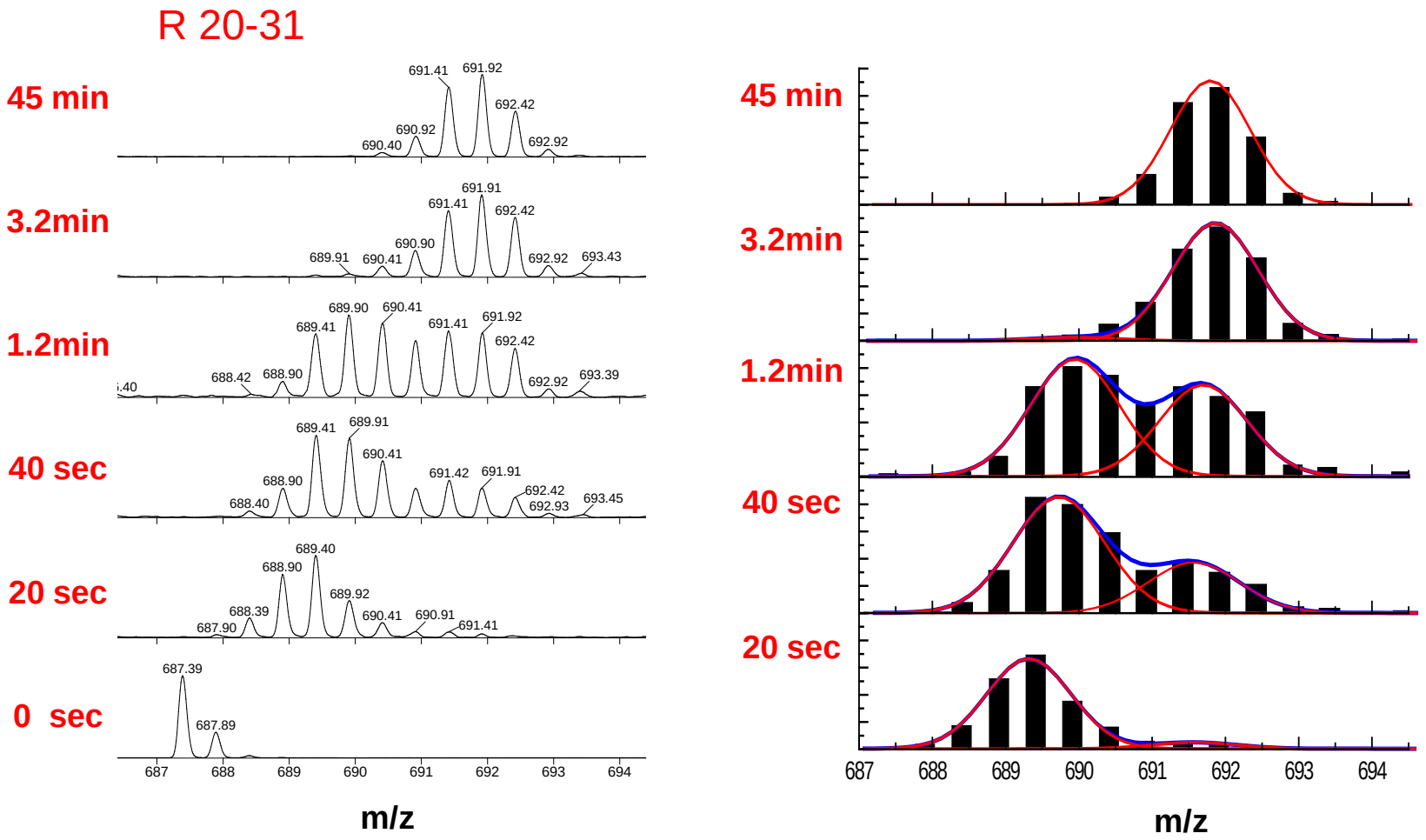
Phi29 Scaffold Has a Helix-Loop-Helix Motif and a Disordered Tail



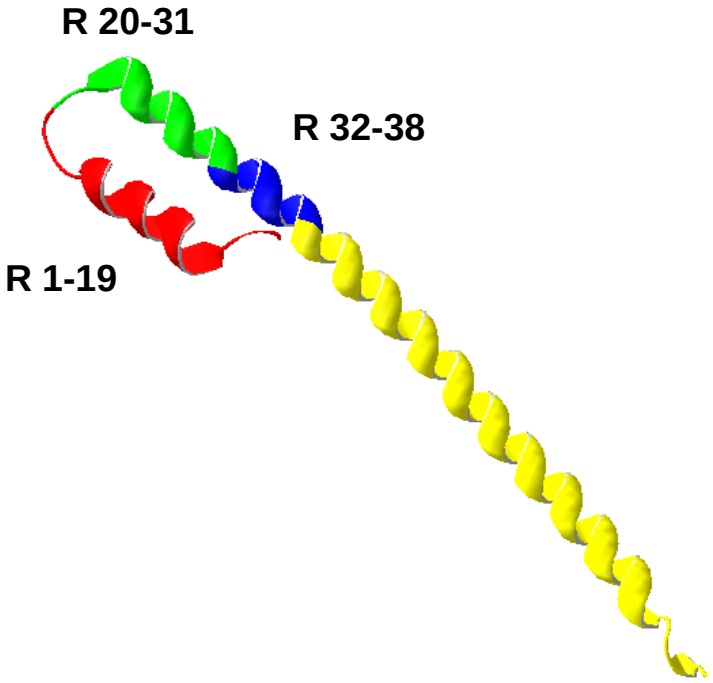
Marc C Morais et al.,
Nature Structural Biology 2003

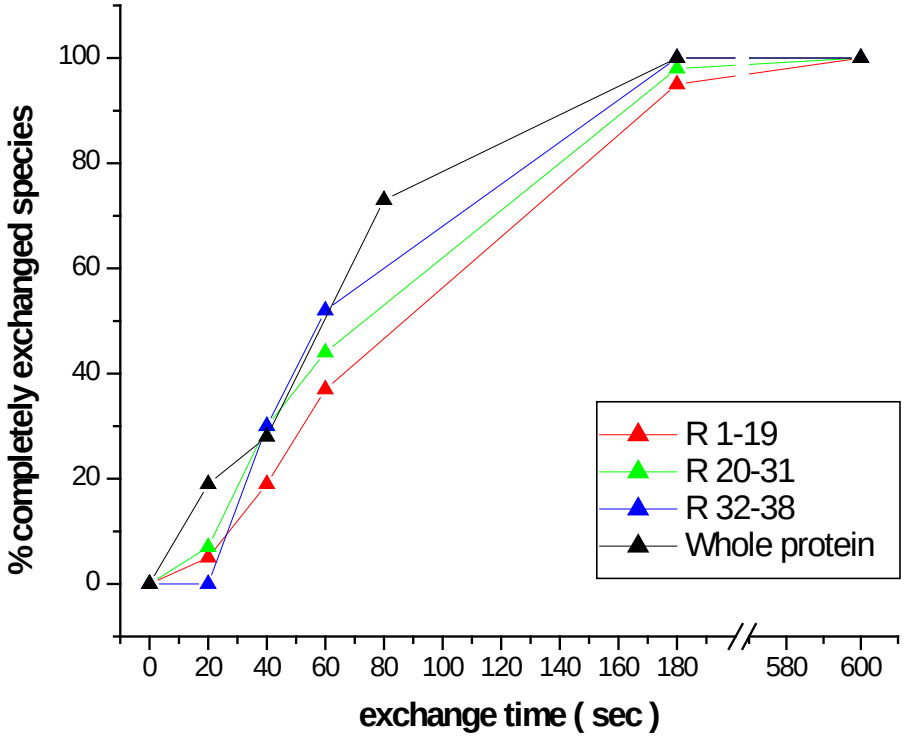

Free scaffold
13+
10 min
3 min
1 min
40 sec
10 sec
0 sec
100
%
0
Partially exchanged species
Fully exchanged species
857
858
859
860
861
862
863
864
m/z
m/z

The Bimodality Maps to N-terminal Helix-Loop-Helix

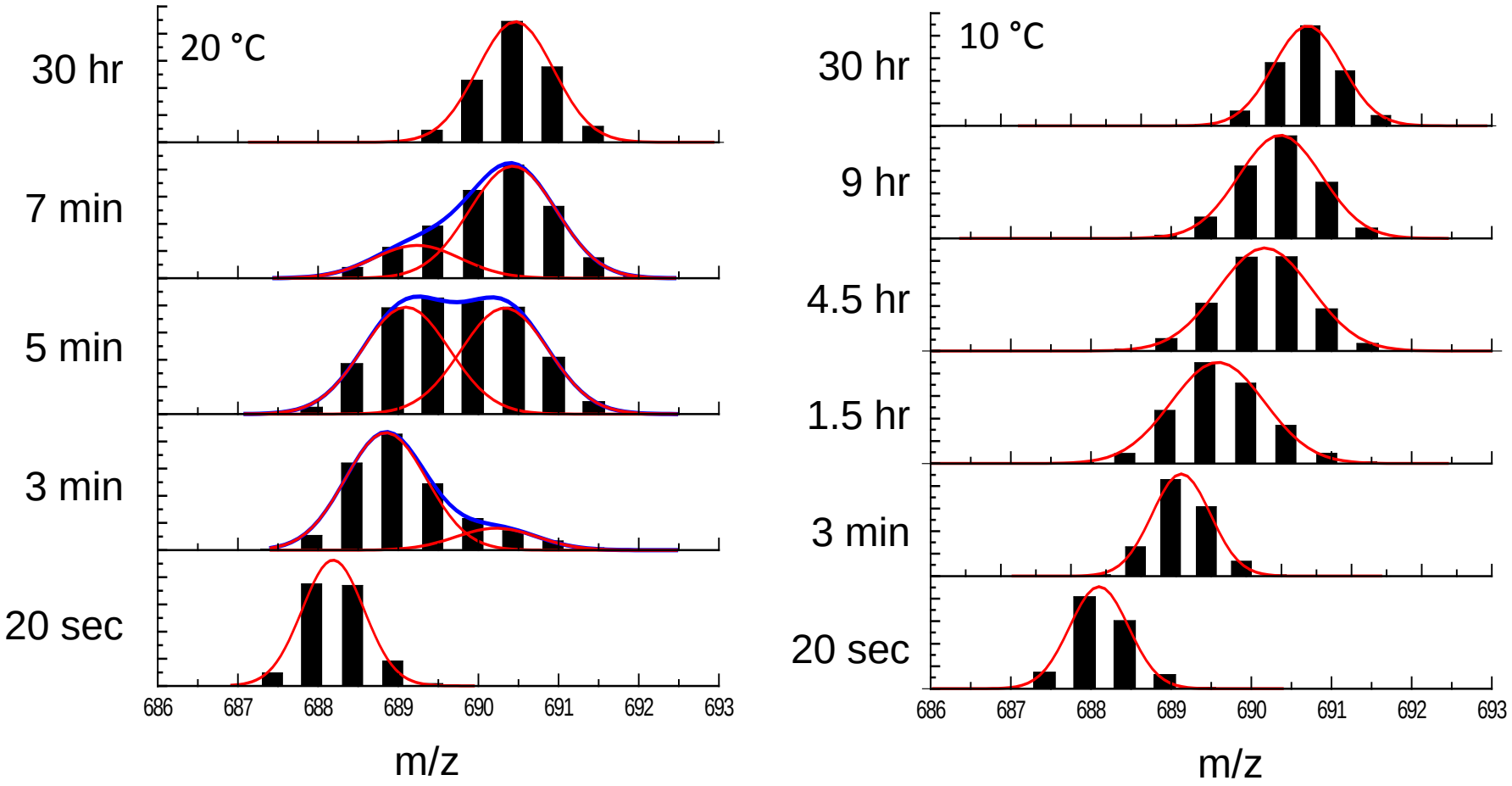


Peptides Derived from H-L-H Region Have Similar Opening Kinetics

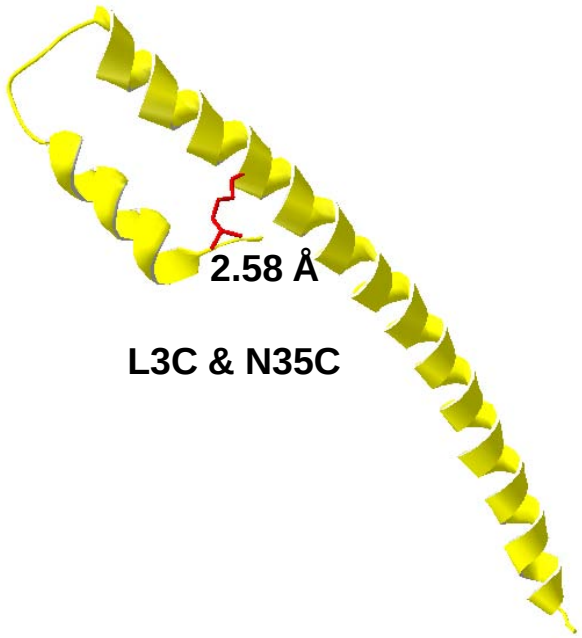




The Cooperative Motions can Be “Frozen” by Lowering the Temperature



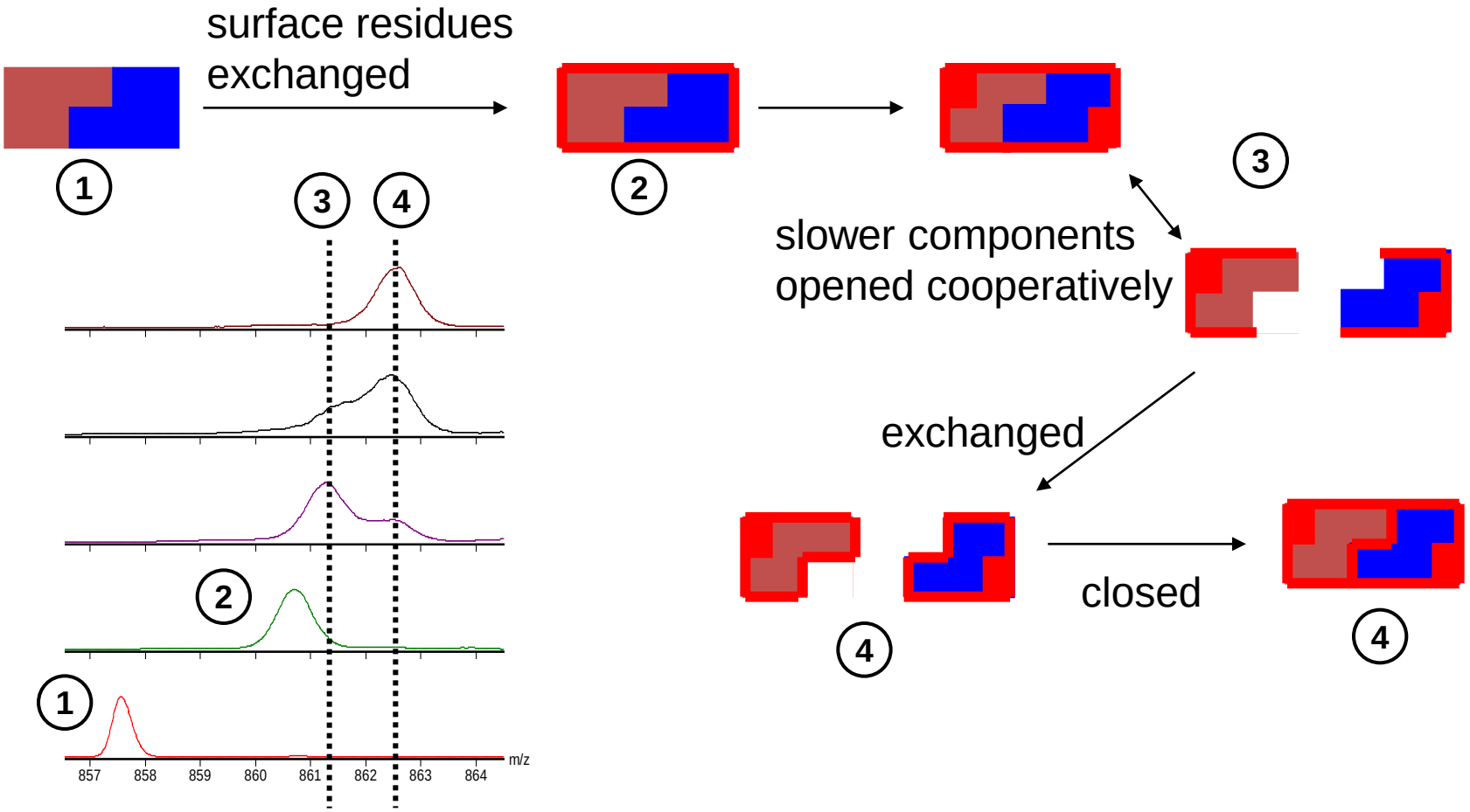
Does Bimodality Originate from Opening of the Interface between Helices 1 & 2 ?



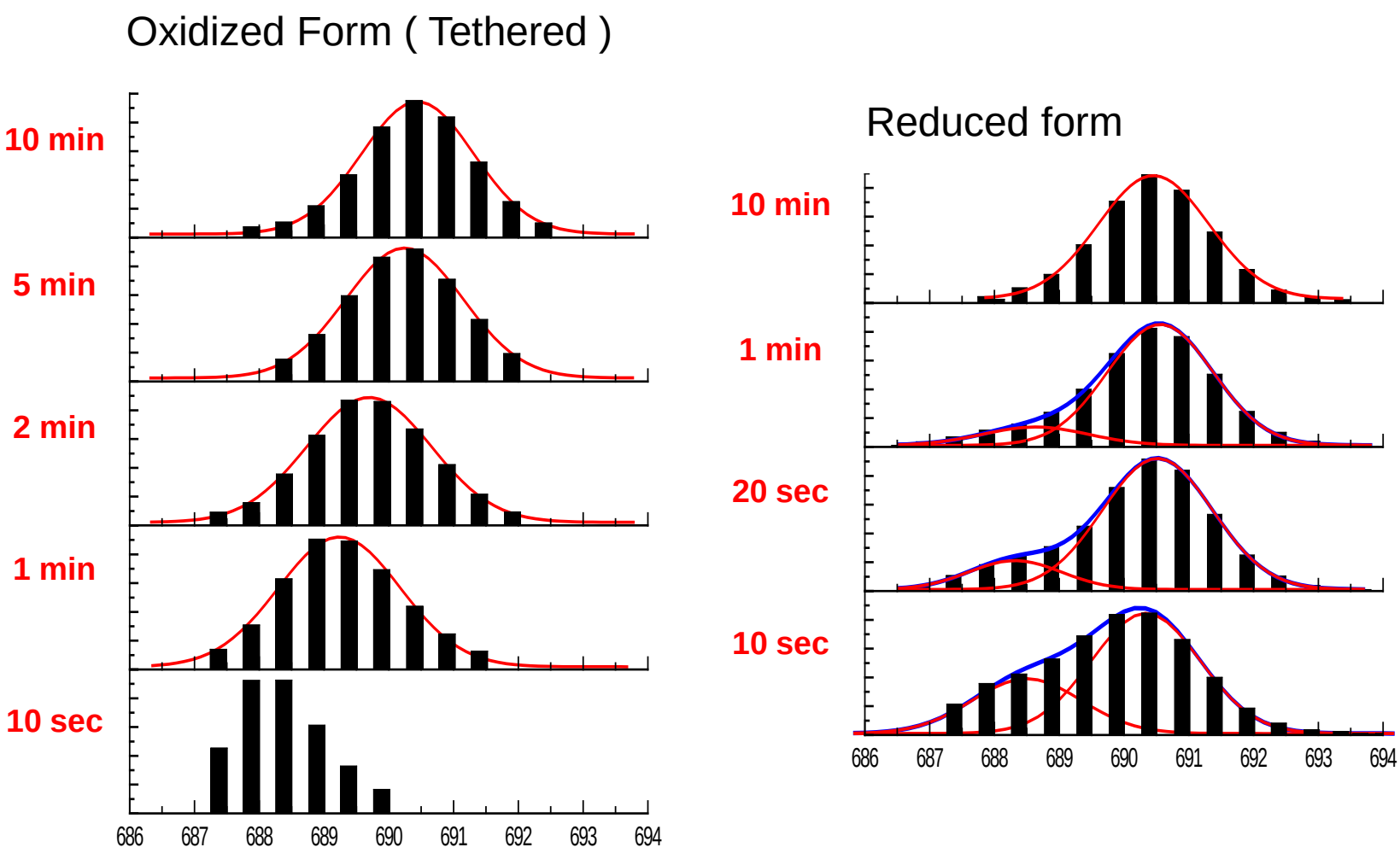
Tethered Form

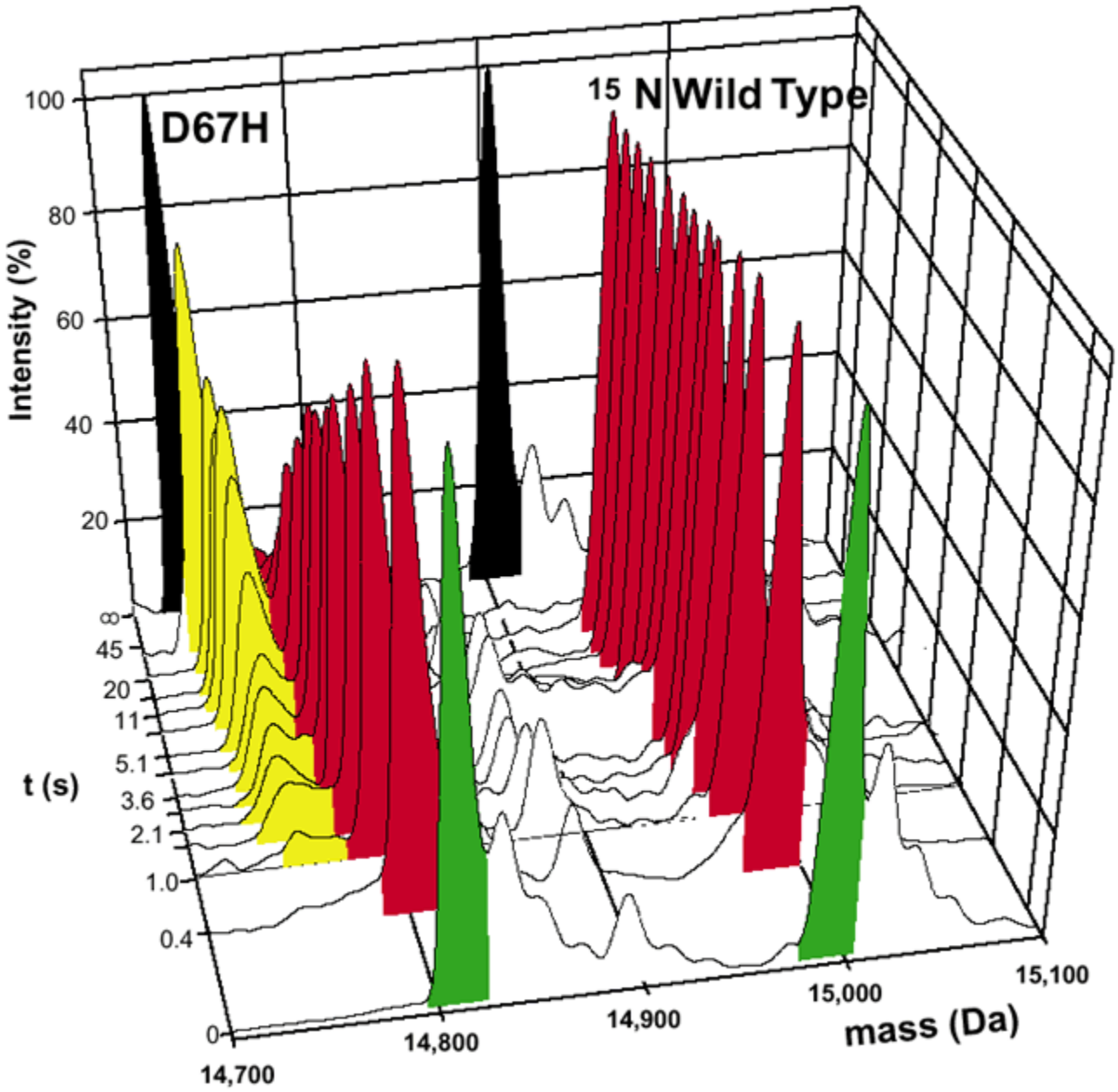
What does Bimodality Indicate?

A group of residues open cooperatively & completely exchange before close again.



The Tethered Form Cannot Open Cooperatively

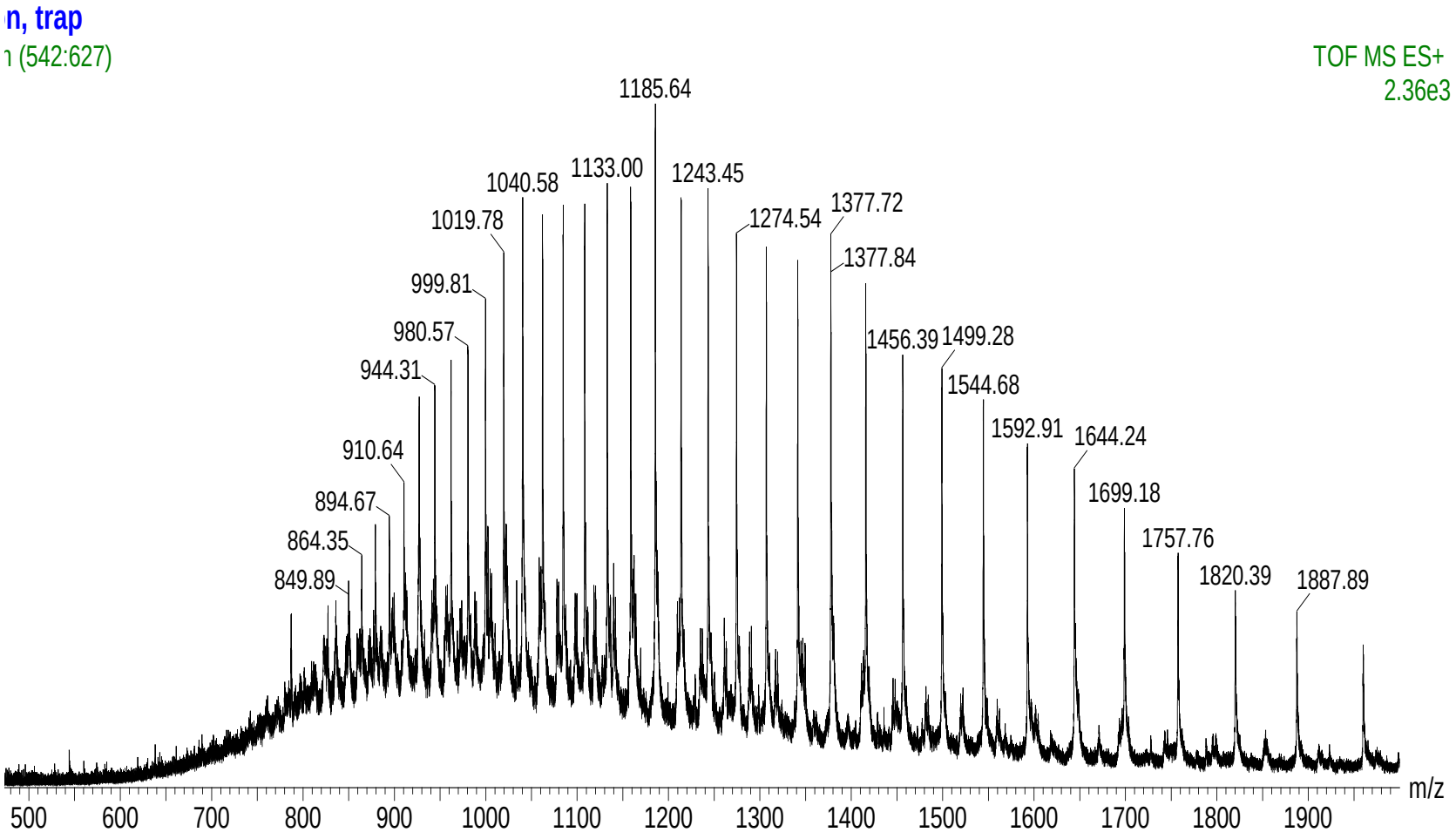



D67H
15 N Wild Type
Intensity (%)
100
80
60
40
20
0
t (s)
∞
45
20
11
5.1
3.6
2.1
1.0
0.4
14,700
14,800
14,900
15,000
15,100
mass (Da)

Non-covalent or Native Mass Spectrometry

We can ionize intact protein complexes using ESI !!

How Do We Determine Charge State?



For any peak:

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

and MW is constant so:

$1185.6 = (MW + nH^+)/n$
$1133.0 = (MW + (n+1)H^+) /(n+1)$

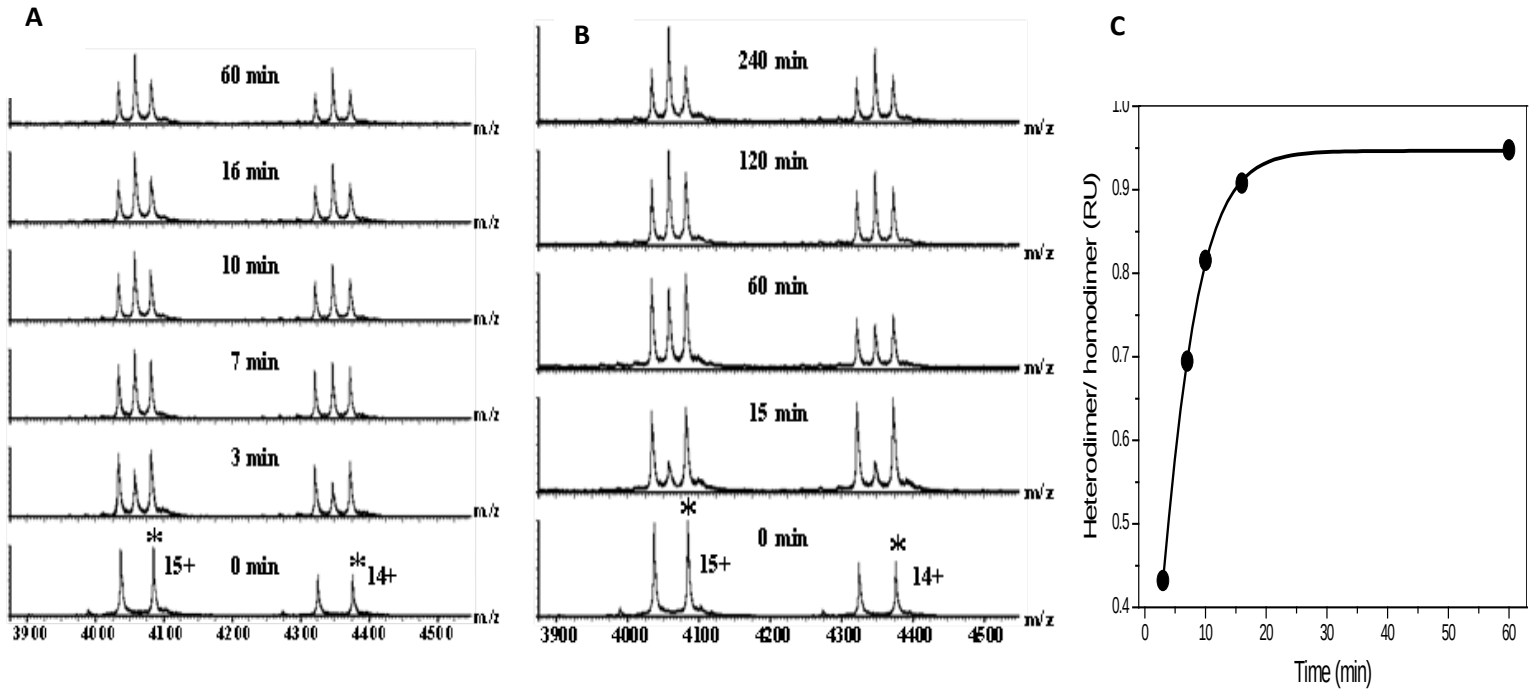
$n(1185.6) - nH^+ = (n+1)1133.0 - (n+1)H^+$

$n = (1133.0 - H^+) / (1185.6 – 1133.0)$

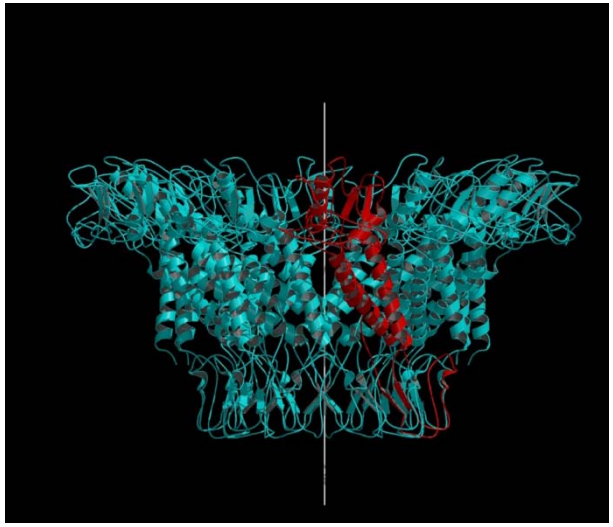
$n = 21$

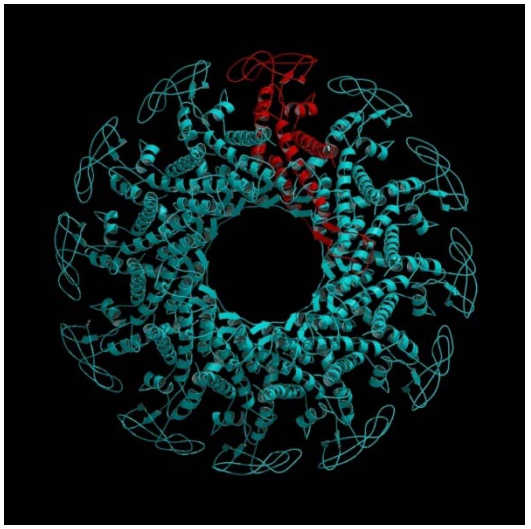
$mass = 21 * (1185.6-21) = 24{,}444$

Subunit Exchange in NAD Synthetase



Portal Motor Packages DNA Into Phage Head





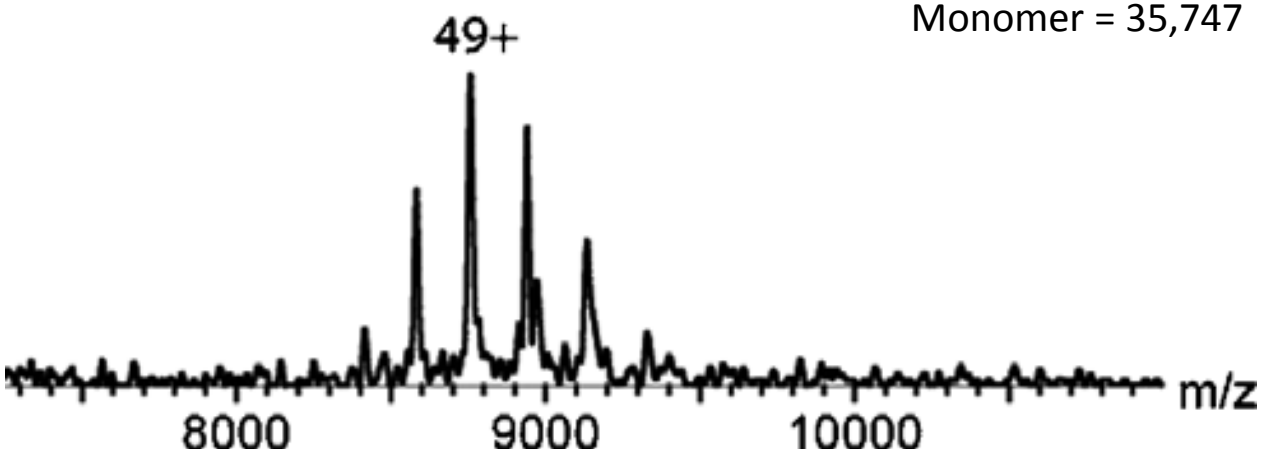
Native Mass Spectrometry Can Determine the Stoichiometry of Macromolecular Complexes

Phi-29 Portal Complex

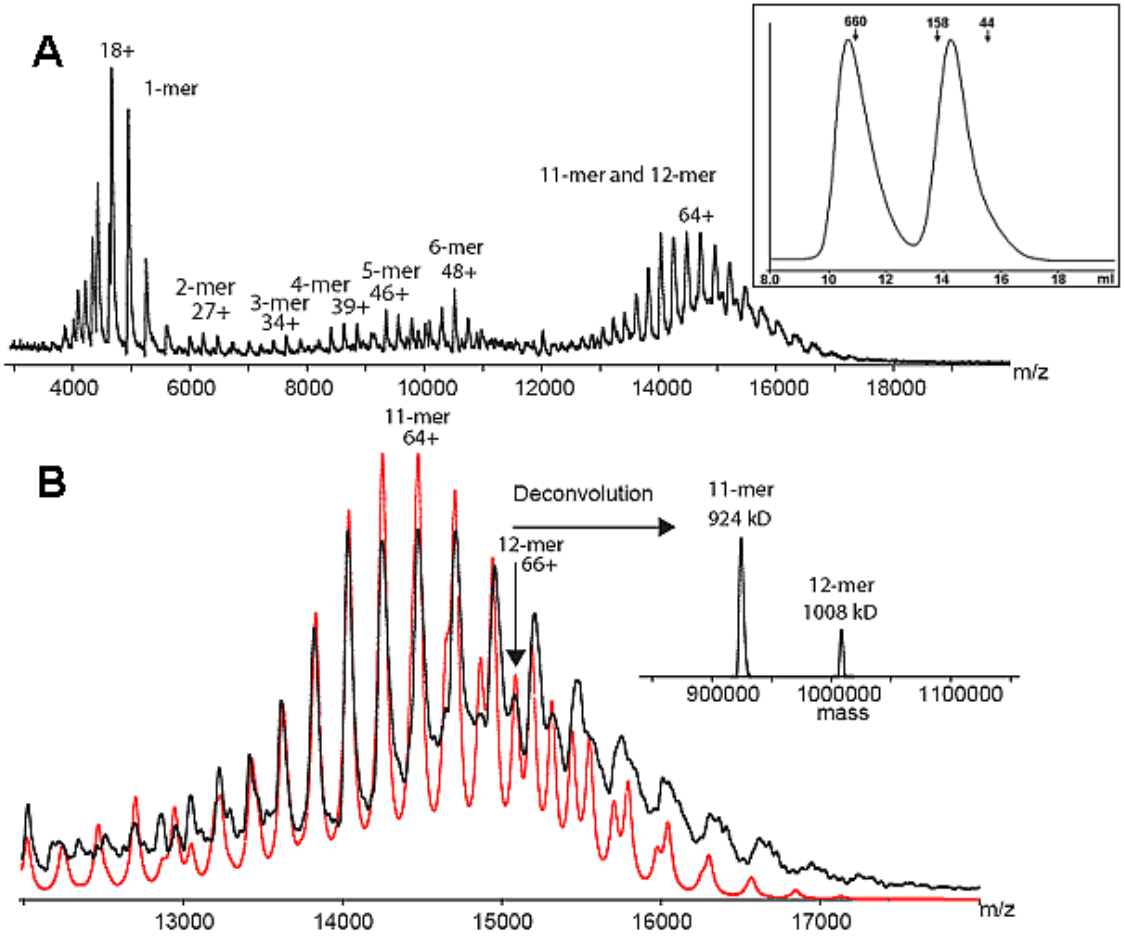
m/z = 8938
m/(z+1) = 8756
m/(z+2) = 8581

m = 429,052

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

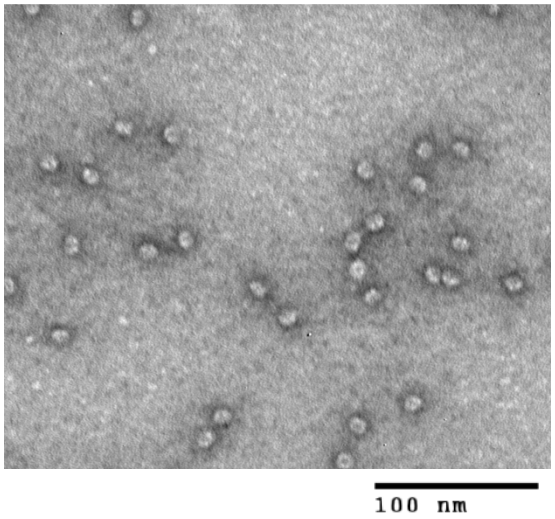


Detection of Intermediates and Sub-populations

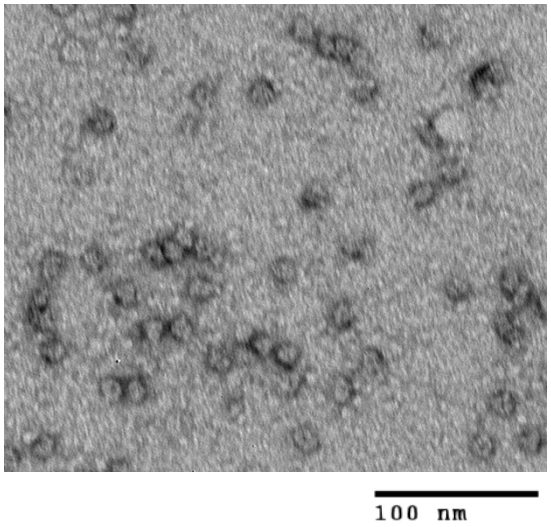


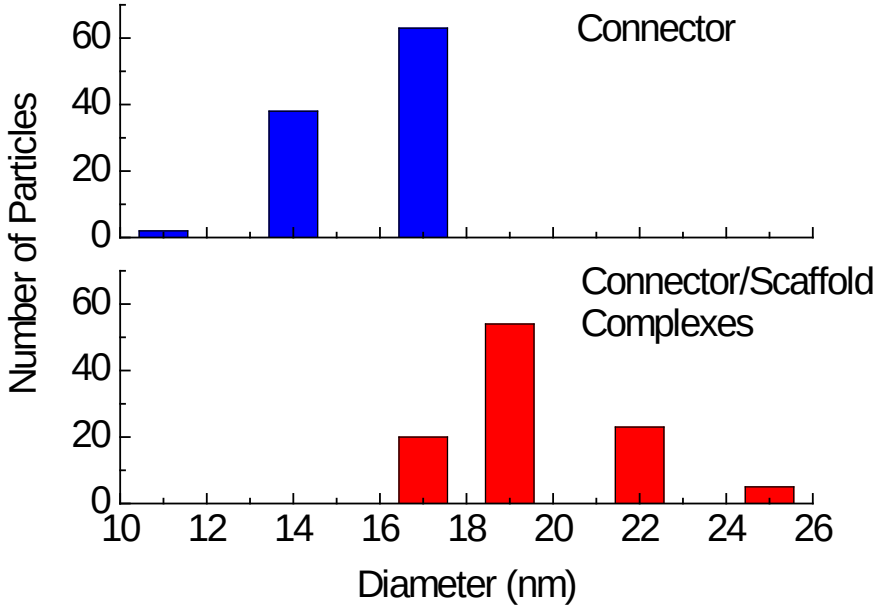
Complexes Formed *in Vitro* Larger Diameters

Connector Dodecamers



Complexes

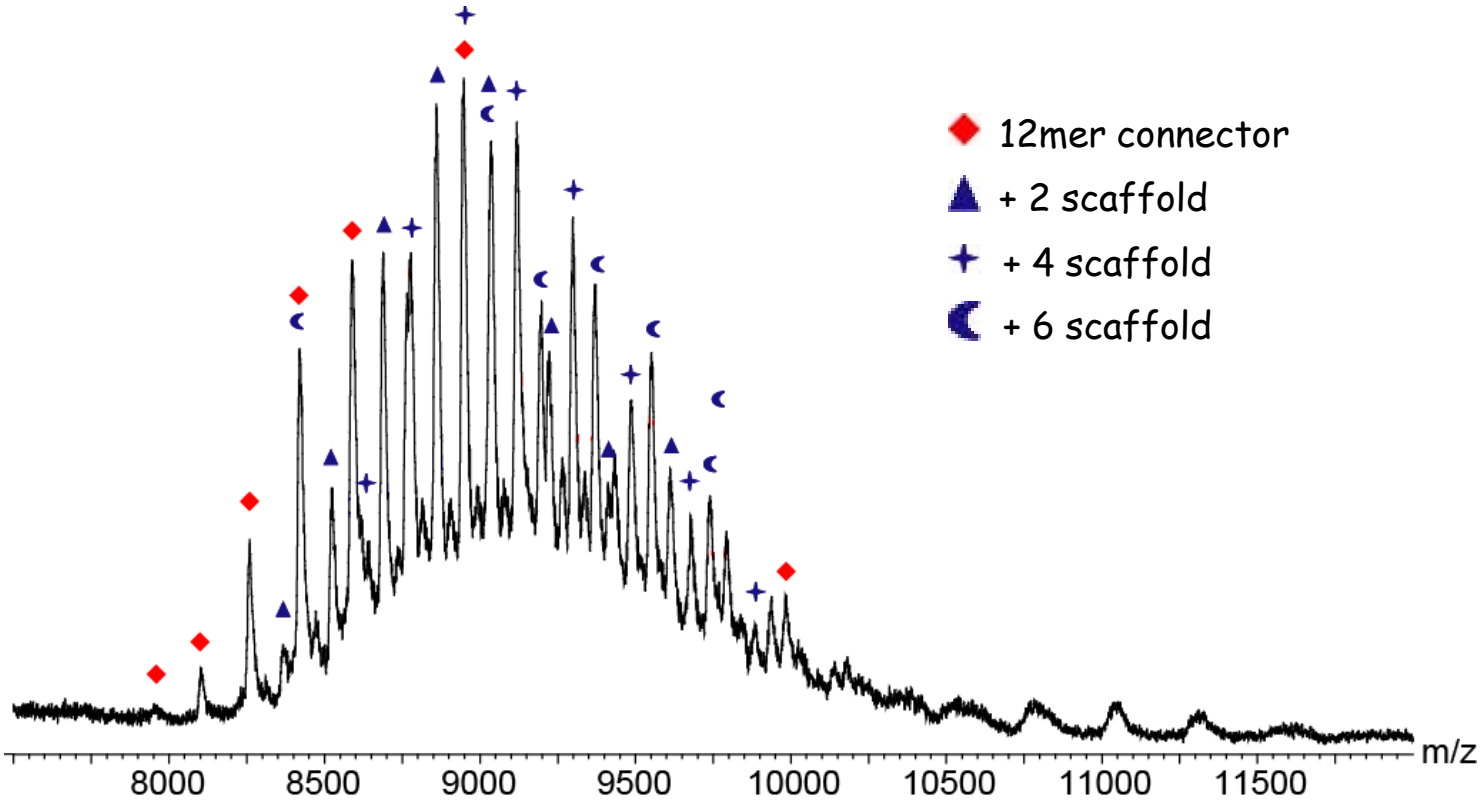




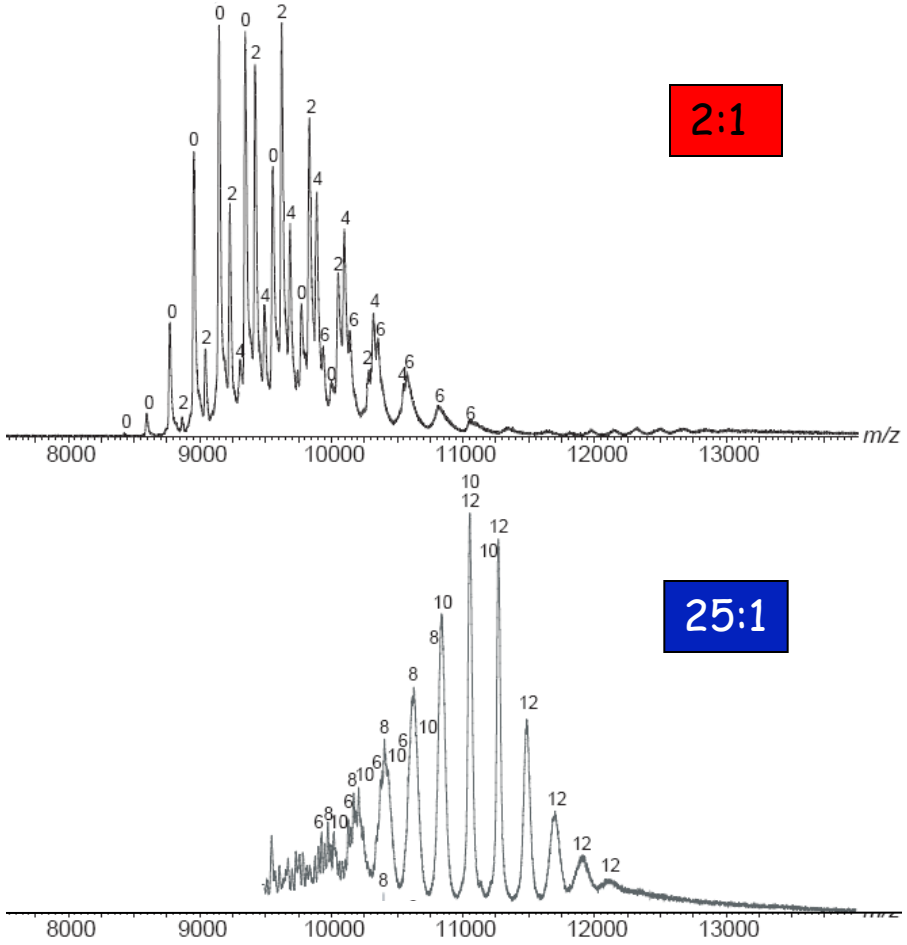
Negative-stained EM

Native Mass Spectrometry Demonstrates Scaffold Binds as a Dimer

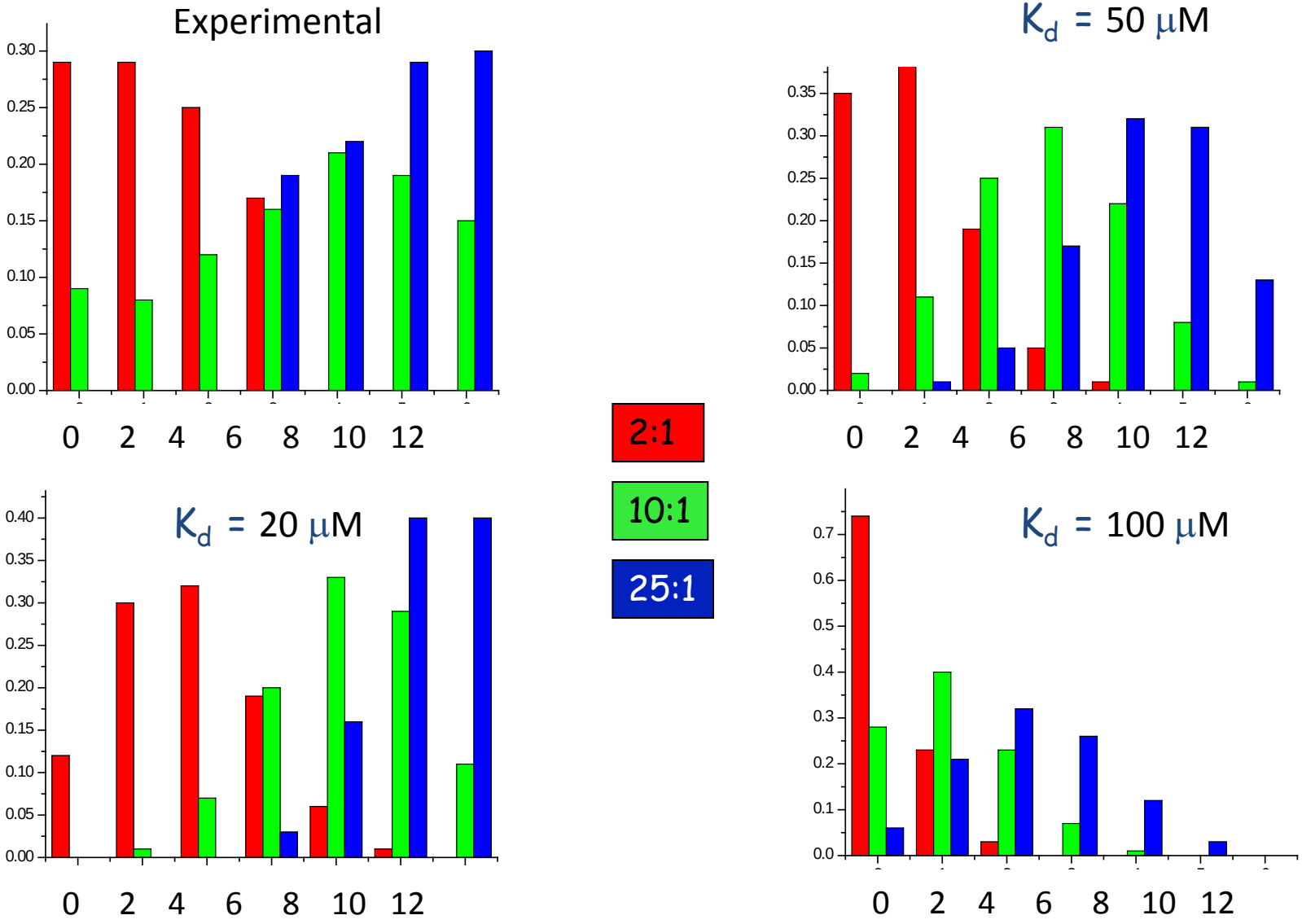
Scaffolding :Connector at 2:1 Input Ratio



Increasing the Scaffolding to Connector Ration Increases the Scaffolding Saturation



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



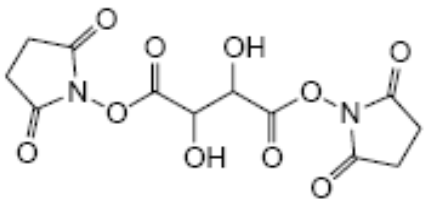
Identification of the Connector/Scaffolding Interface by Chemical Cross-Linking

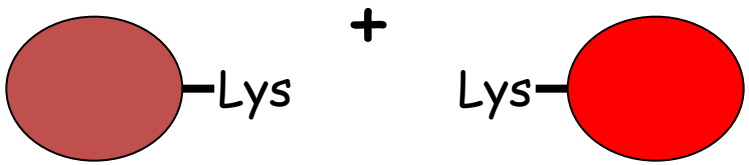
To Locate the Interface on Connector Protein

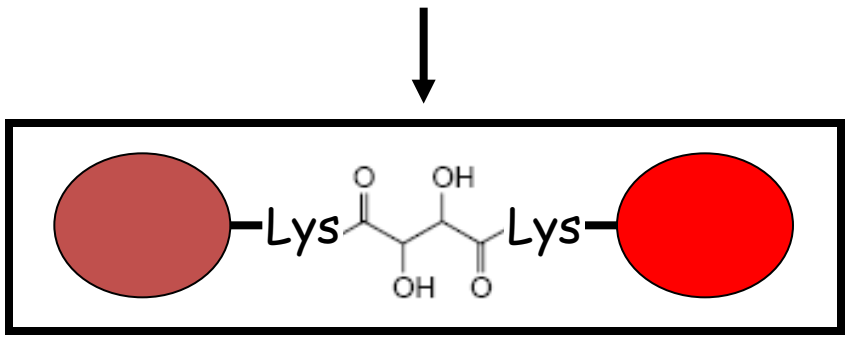
To Obtain Distance Constraint of Interactions

Lysine Reactive DST Cross-Linker

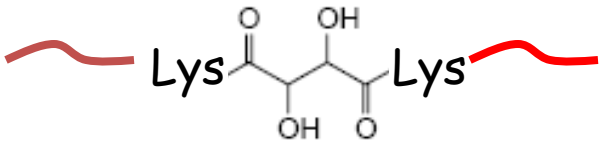
DST: Spacer Arm 6.4 Å





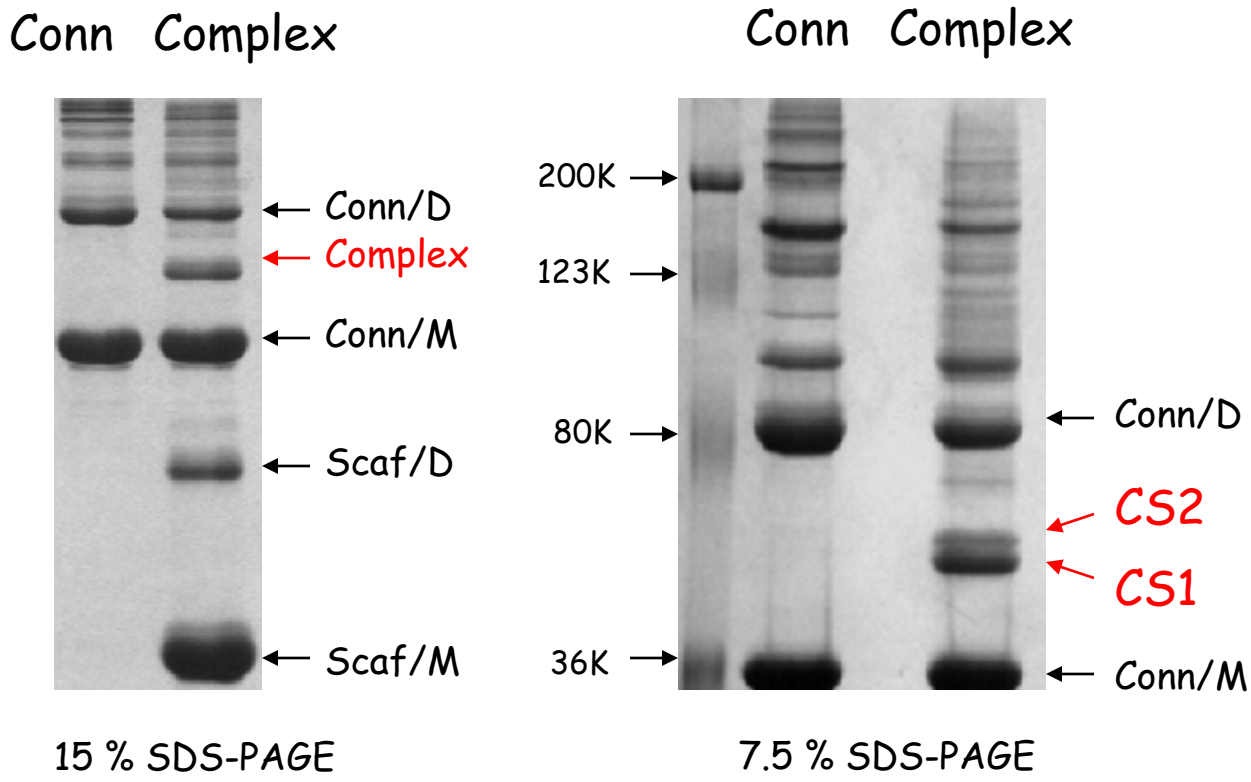


Trypsin Digestion

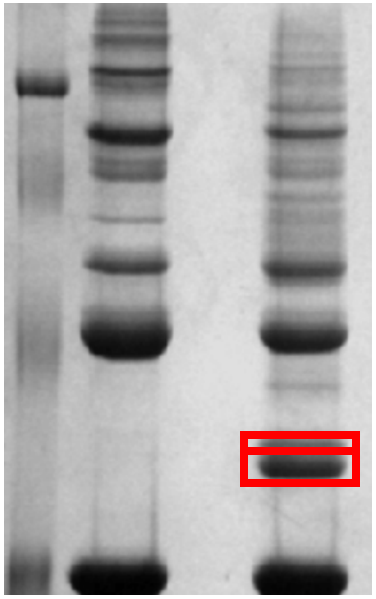


Mass: A+B+114 Da

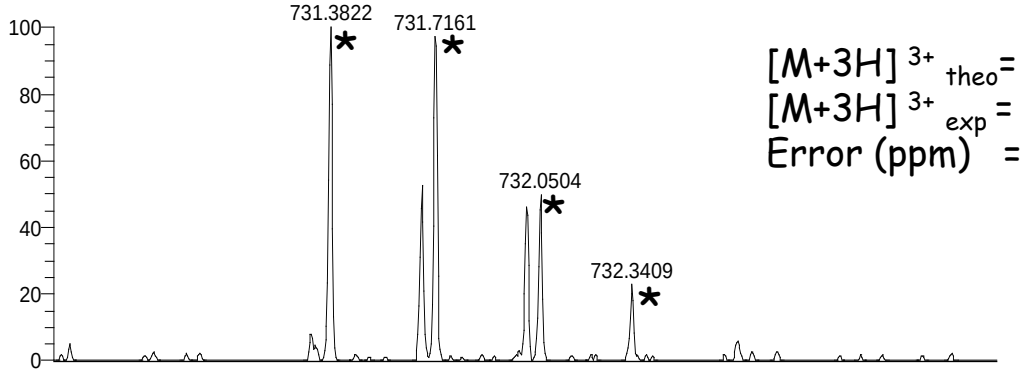
DST Cross-Linking Profiles



Identification of Scaffolding/Connector Interfaces by Chemical Cross-linking

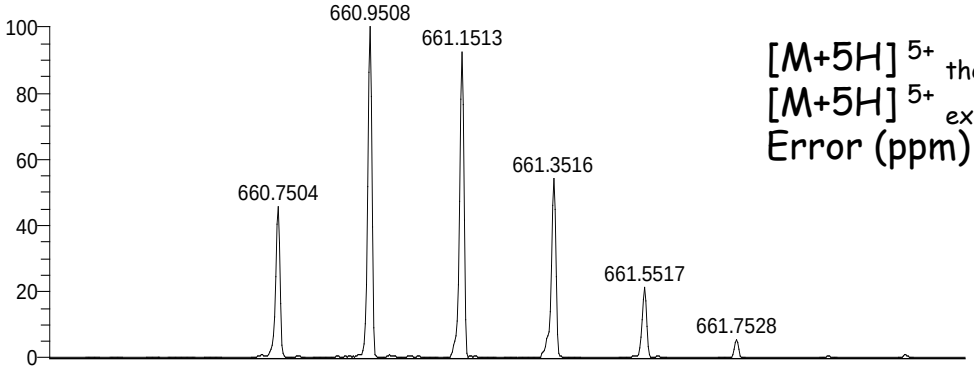


Scaffolding 83-98 Cross-Linked to Connector 4-5/19-20



$[M+3H]^{3+}_{theo}$ = 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

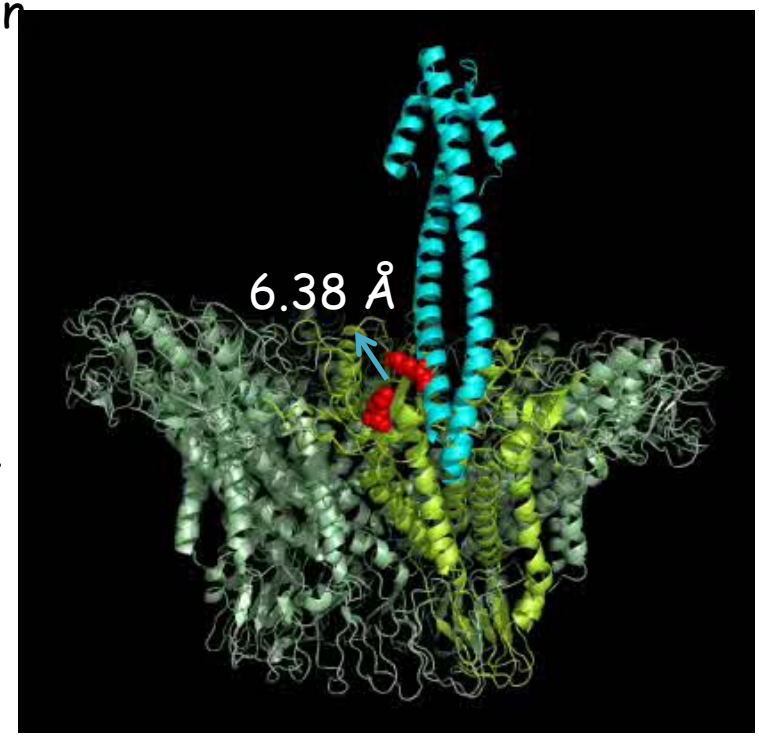
1st step: ZDOCK
(optimize shape complementarity, deslovation electrostatics)
- Use entire scaffolding dimer
- Use connector dimer
- Block interior surface of connector
- Generate #2000 models

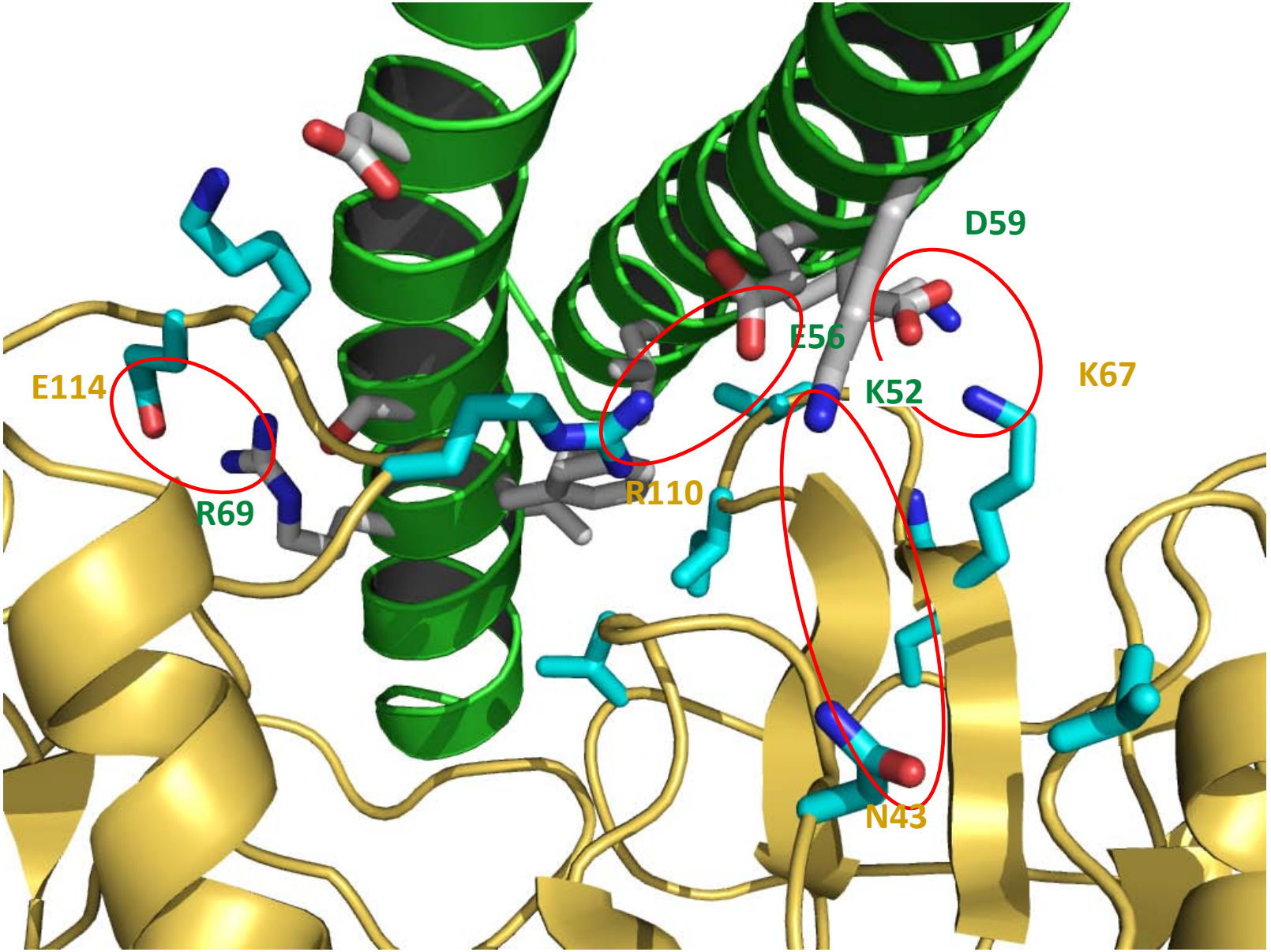
2nd step: filter with
SF66-Conn102 cross-link distance constraint
- Use 8 Å constraint, 26 models

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

Select model #25

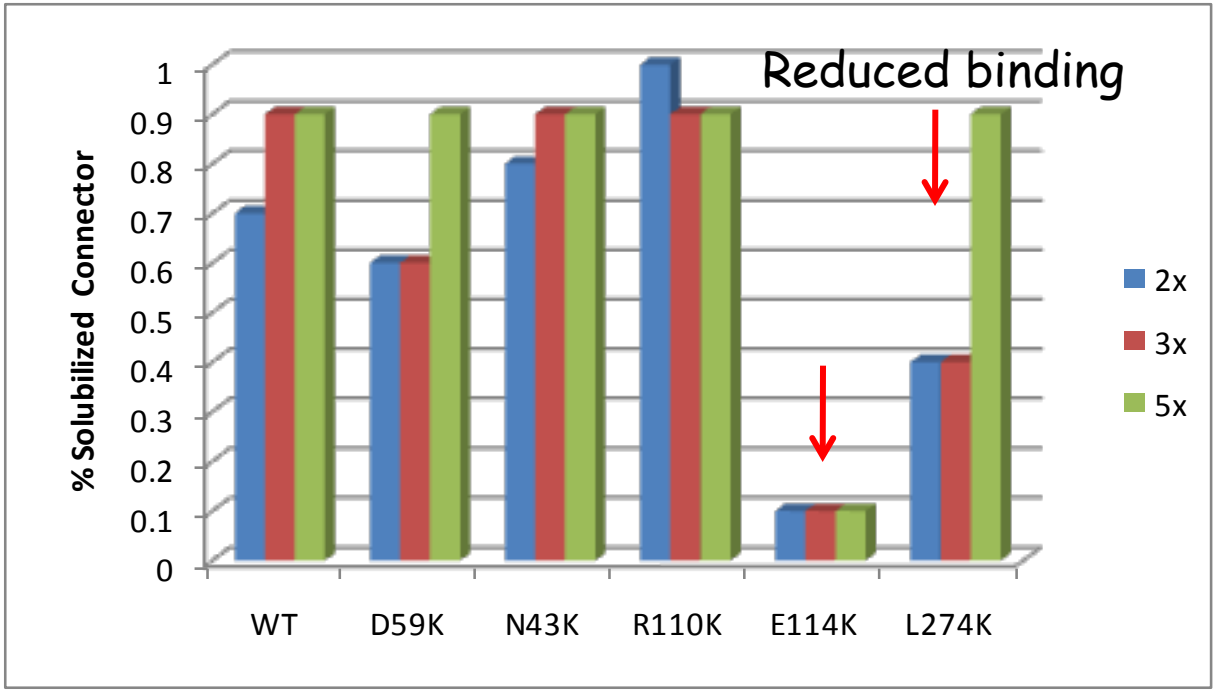
3rd step: model verification by mutagenesis




D59
E56
K52
K67
E114
R69
R110
N43

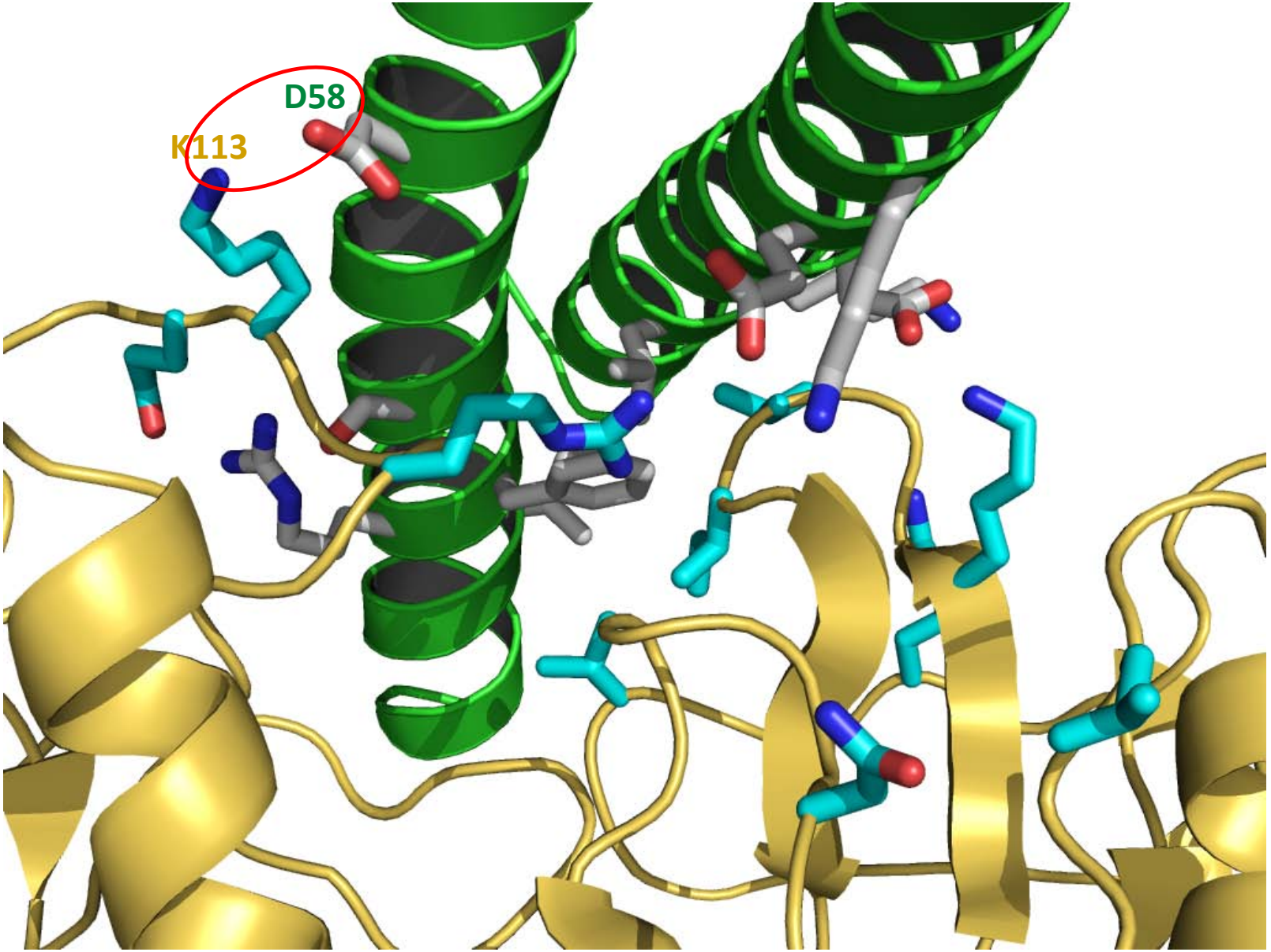
Based on the Model Pairs of Lysines Were Introduced to Alter Complex Stability and Enable Cross-linking

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



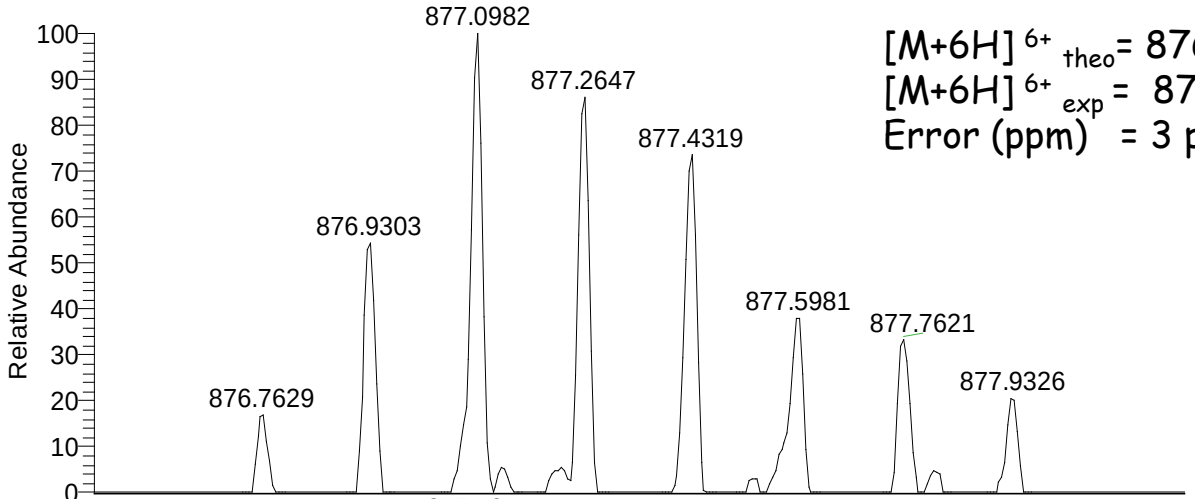
Loss of Function
is Easy !

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.

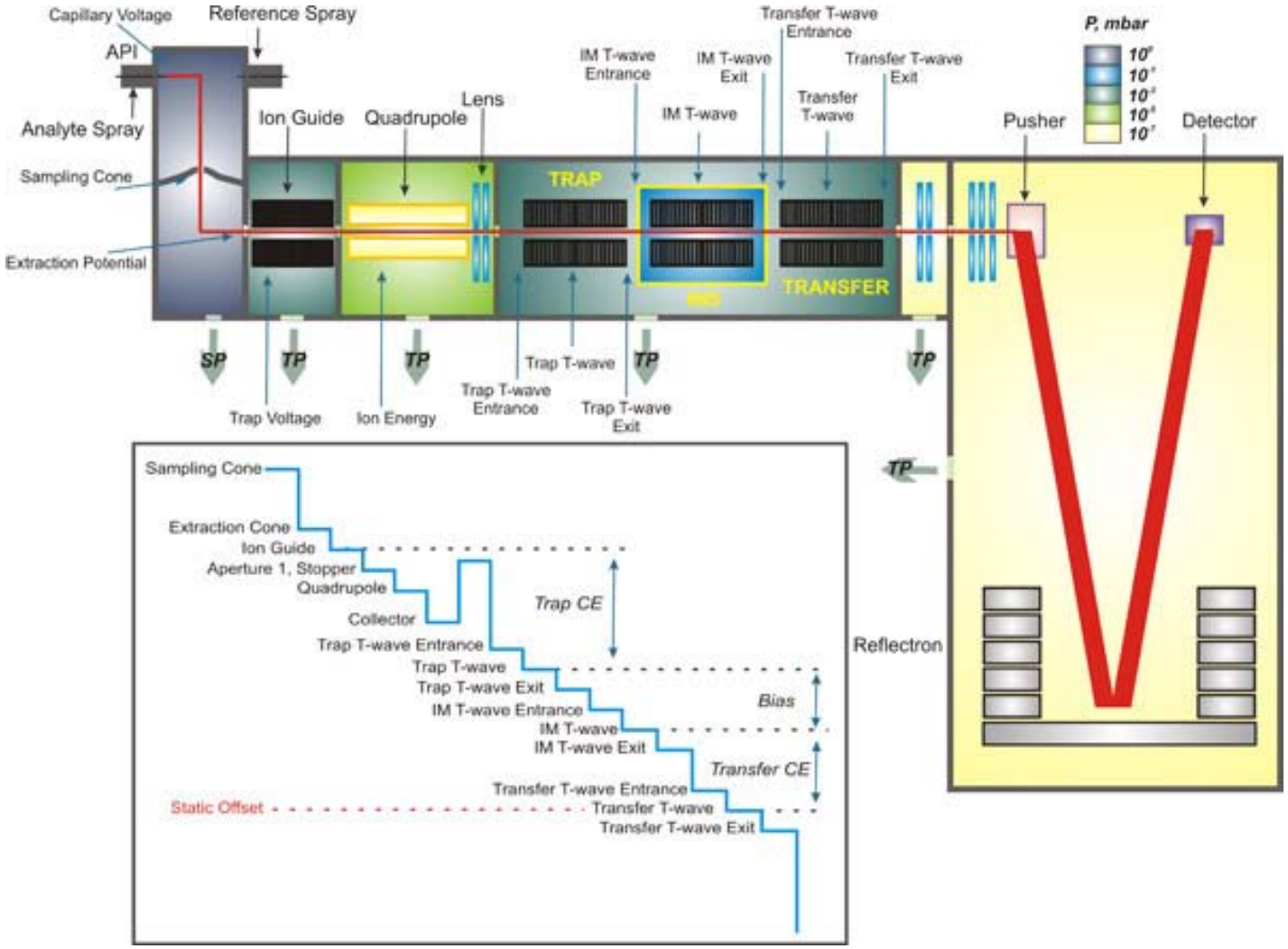
Obtaining Shape Information

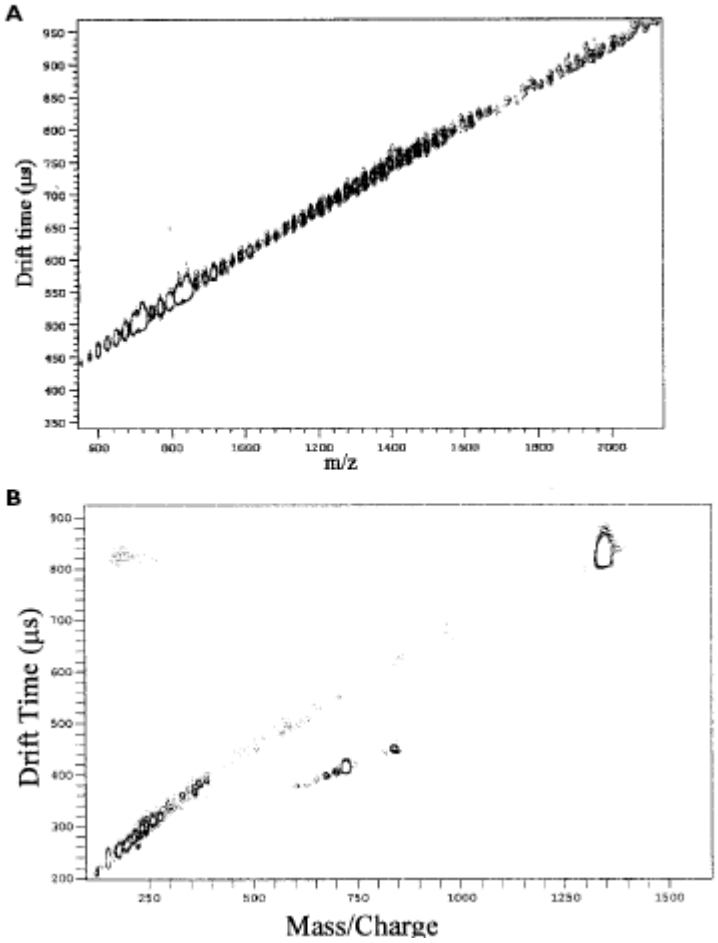
Put a device that separates ions by shape in front of MS

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.

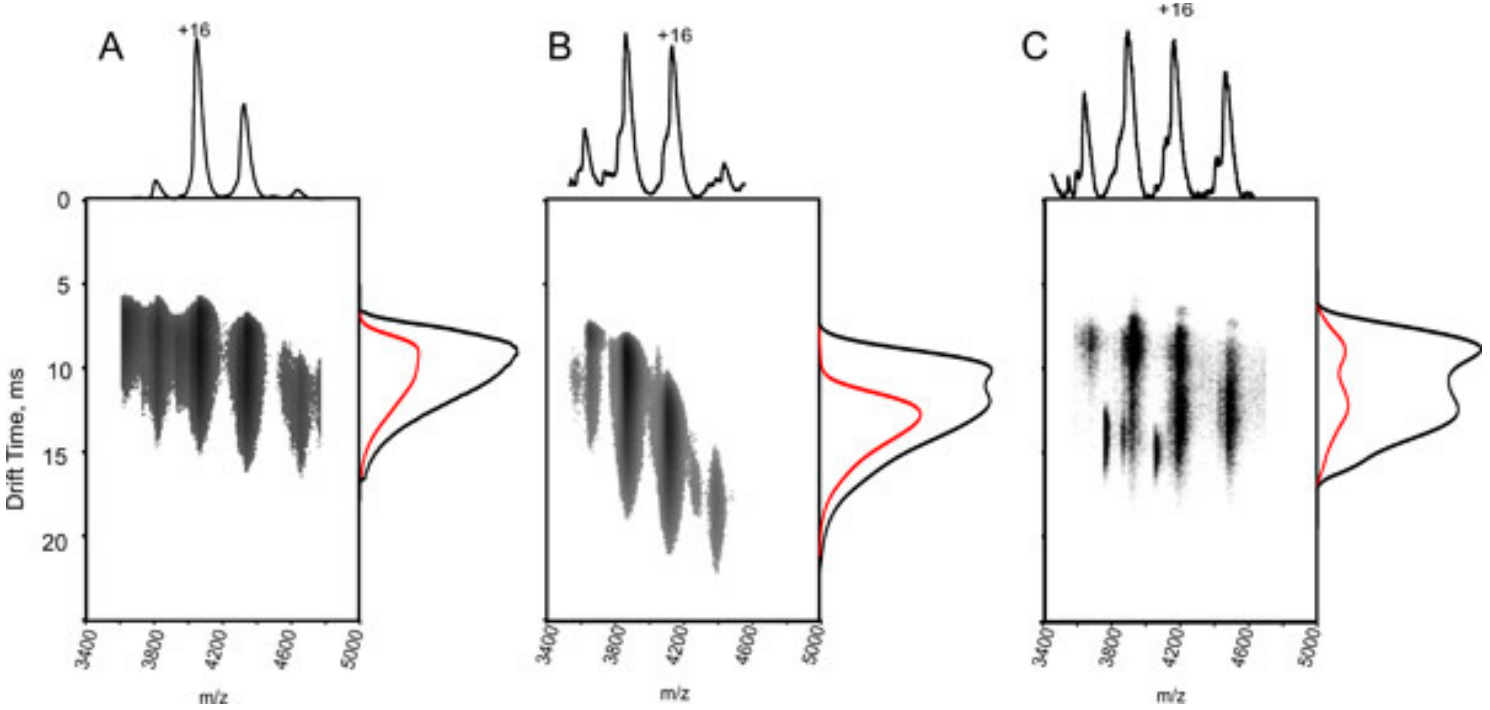
Block Diagram of Waters Synapt IMS-ToF




A
Drift time (µs)
m/z
B
Drift Time (µs)
Mass/Charge

Hemoglobin Tetramer Denaturation

native

partially denatured

partially denatured