Proteins?

Protein function

Protein folding

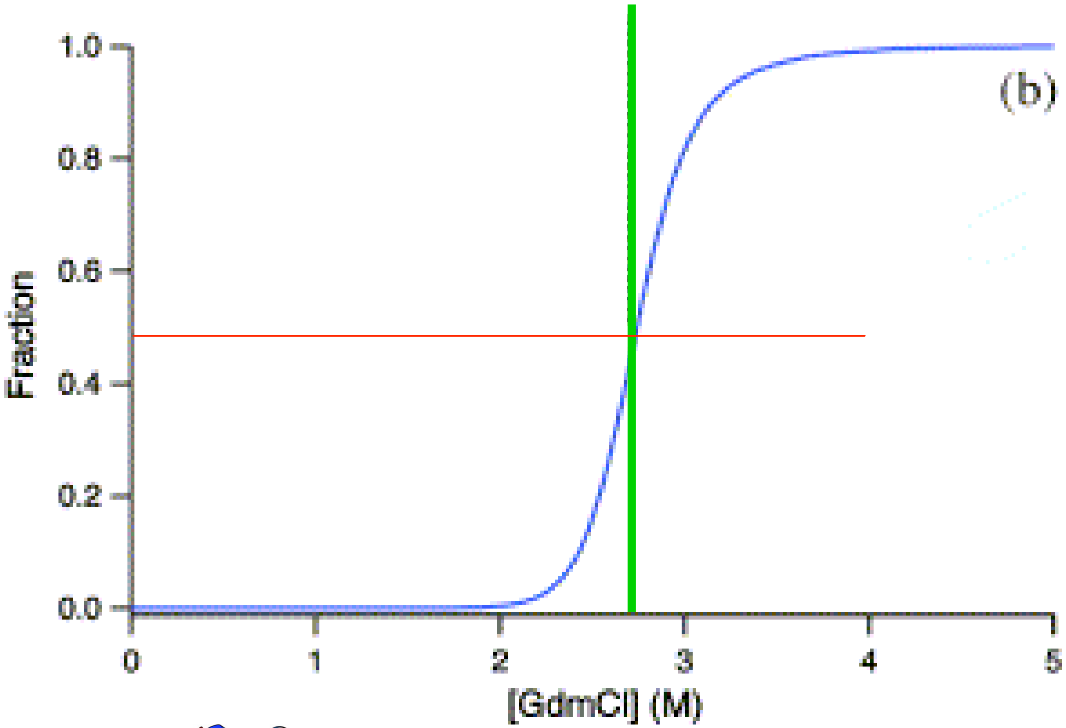
Protein folding diseases

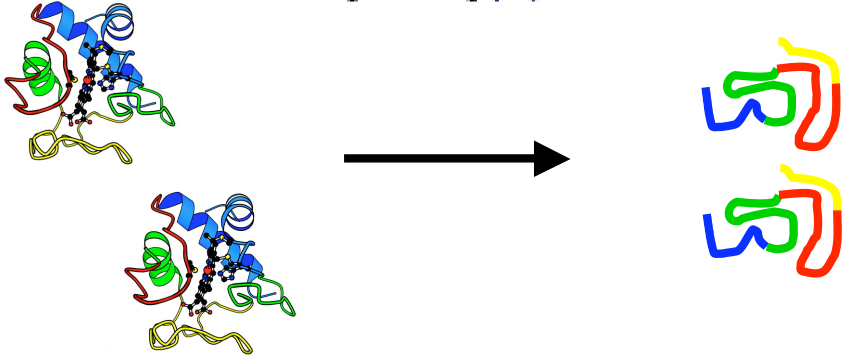
Protein interactions

Macromolecular assemblies

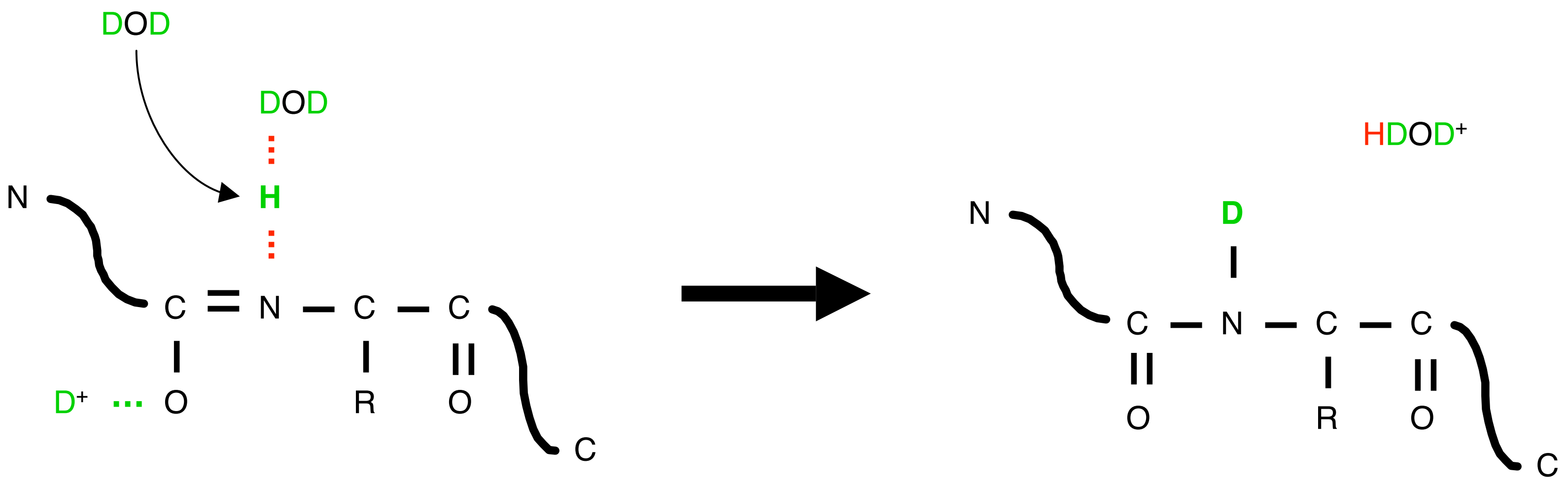
The end product of Genes

Protein Unfolding

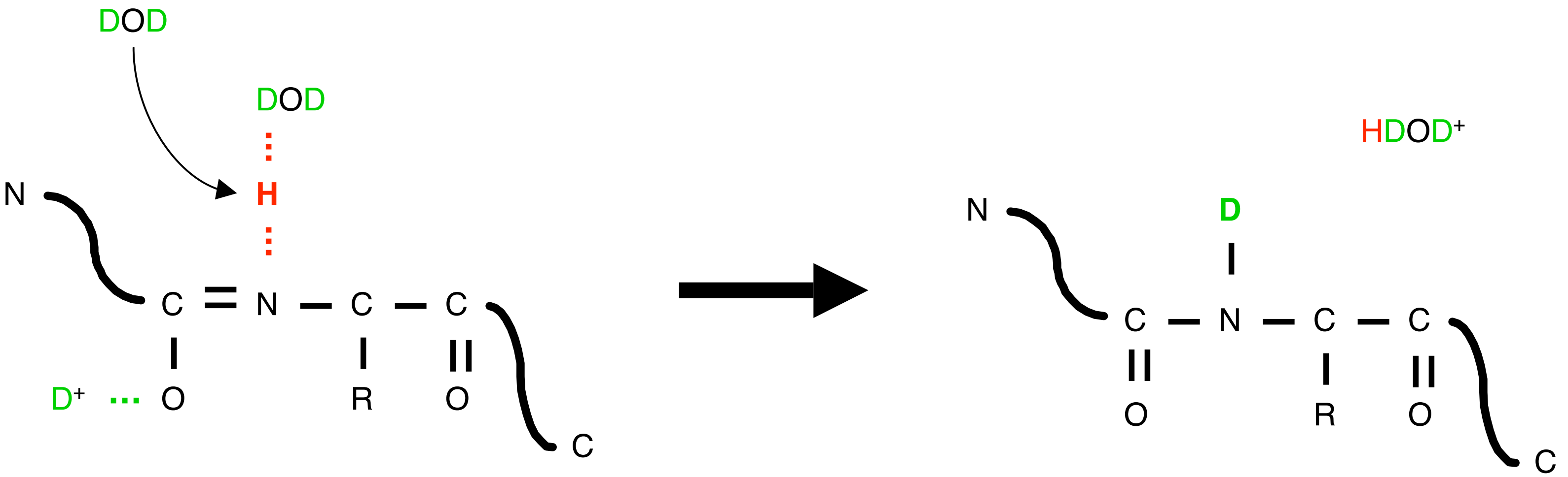




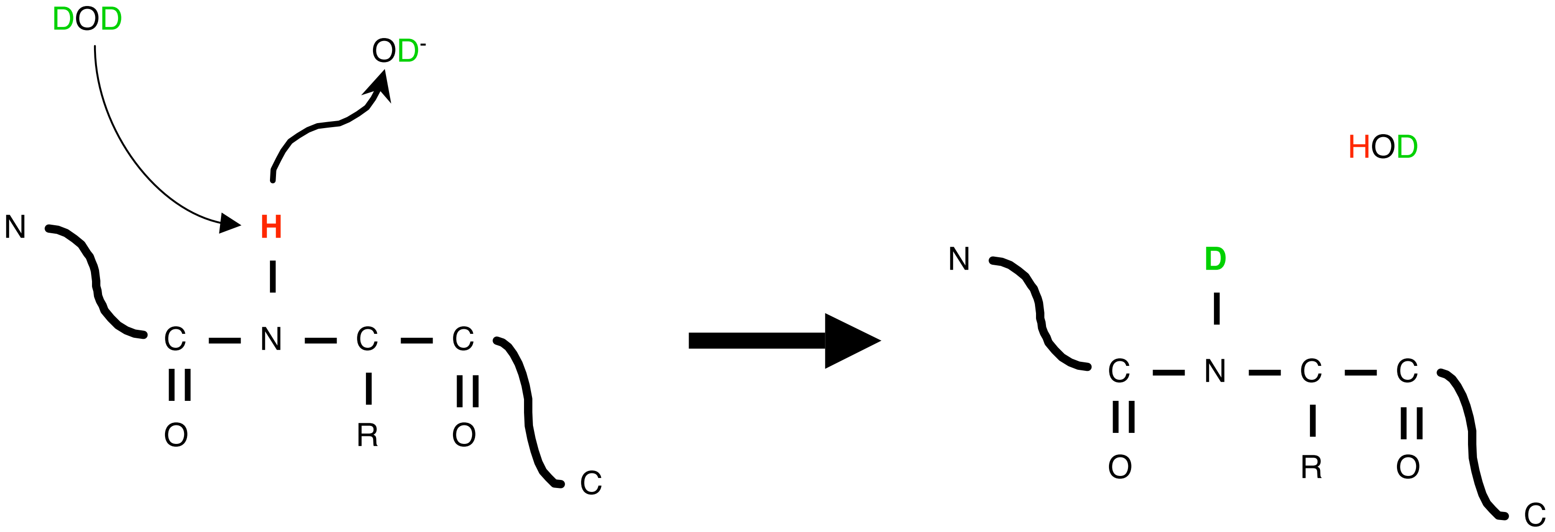
Acid Catalysis



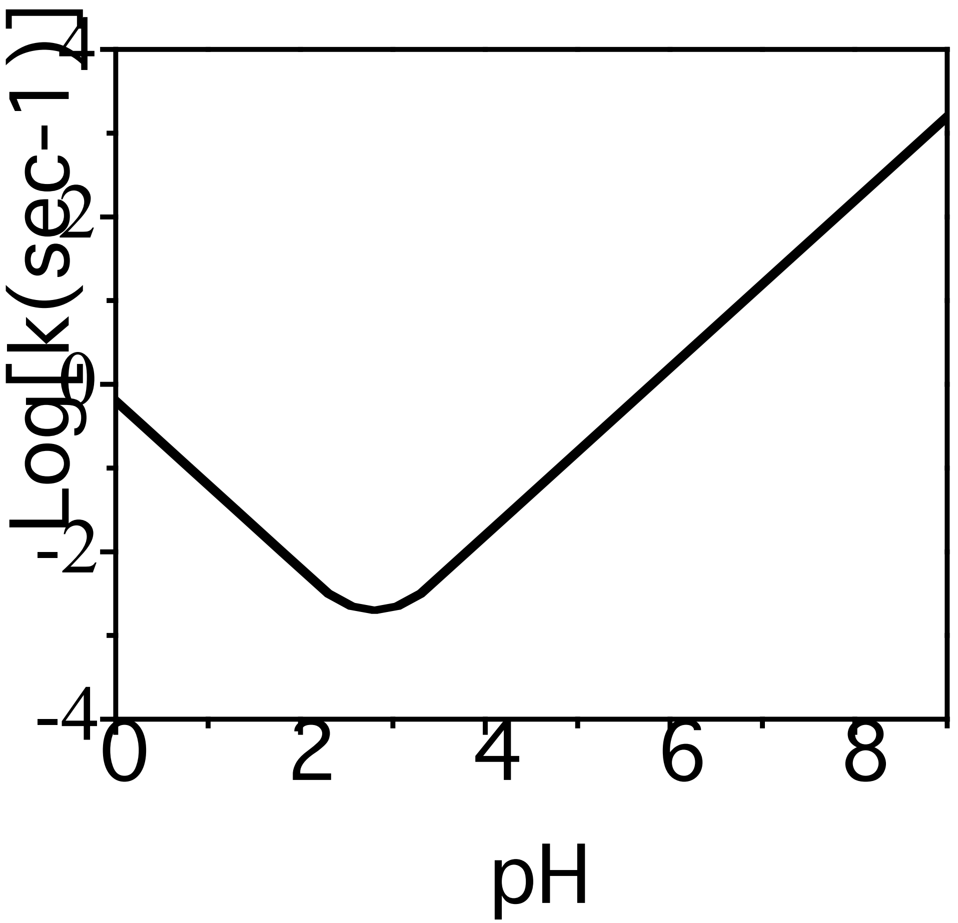
Acid Catalysis



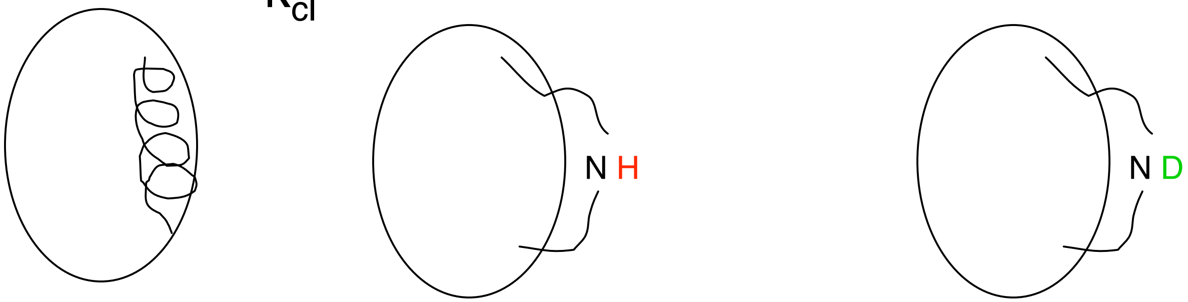
Base Catalysis



Amide Proton Exchange is pH dependent



$$\text{Folded} \underset{k_{cl}}{\overset{k_{op}}{\rightleftharpoons}} \text{Open} \xrightarrow{k_{ch}} \text{hydrogen exchange}$$



EX$_1$ mechanism: $k_{ch} > k_{cl}$

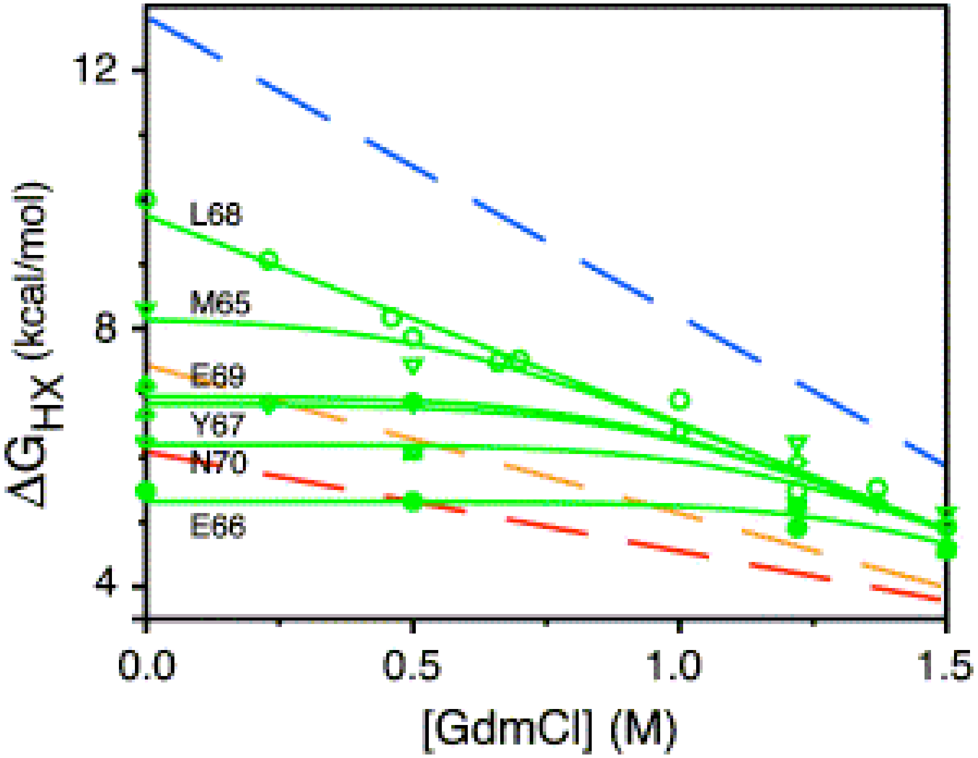
$$k_{ex} = k_{op}$$

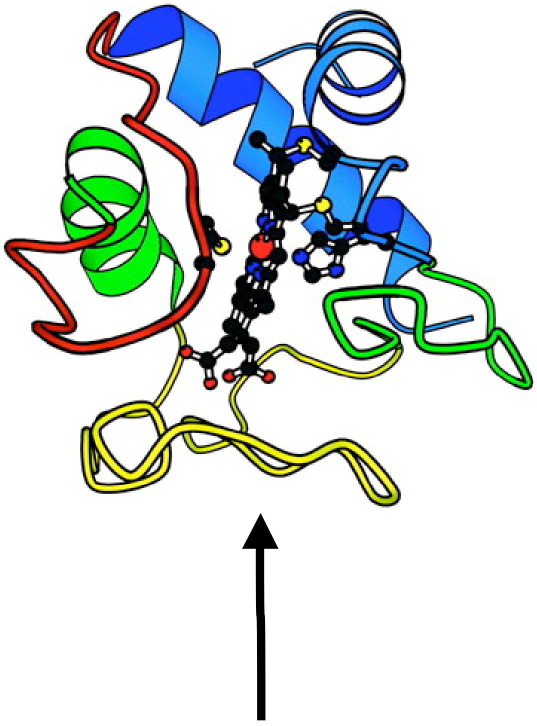
EX$_2$ mechanism: $k_{ch} < k_{cl}$

$$k_{ex} = K_{(op/cl)} \cdot k_{ch}$$

“pH dependent”

Cooperative Unfolding

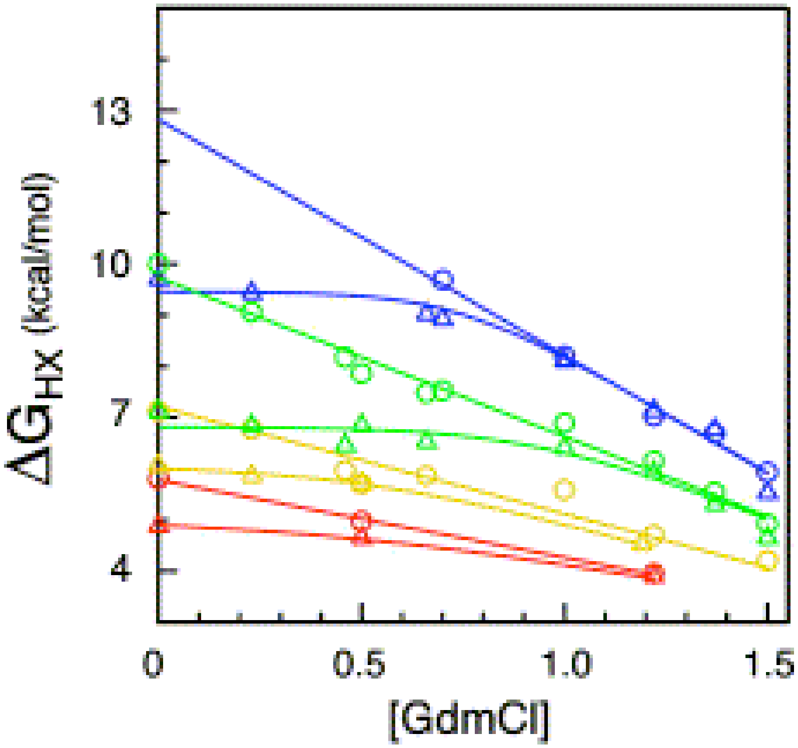


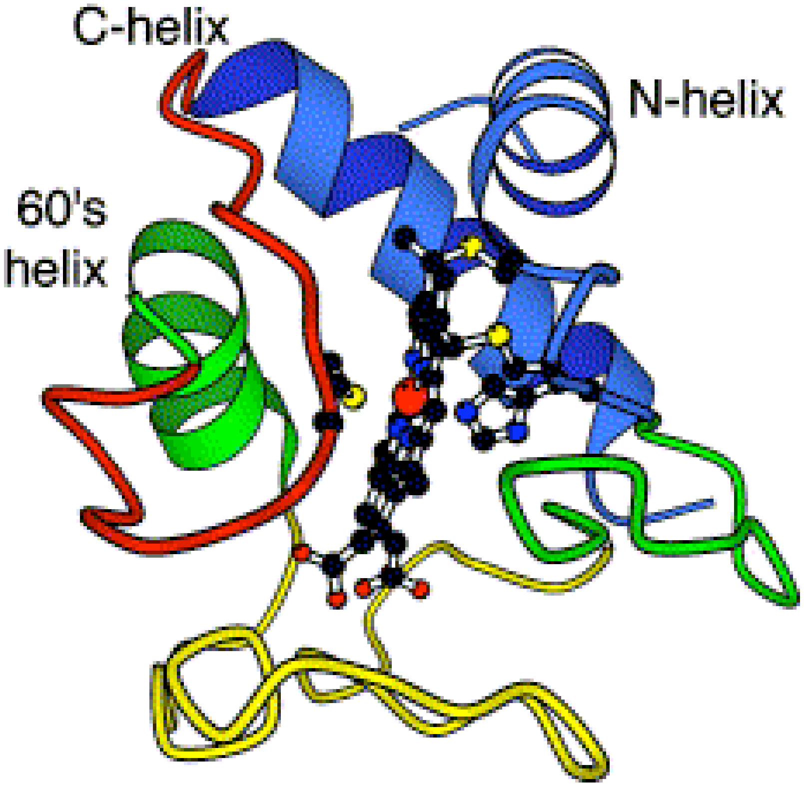


Cytochrome C

Englander, *et. al.* 2002

Cooperative Unfolding

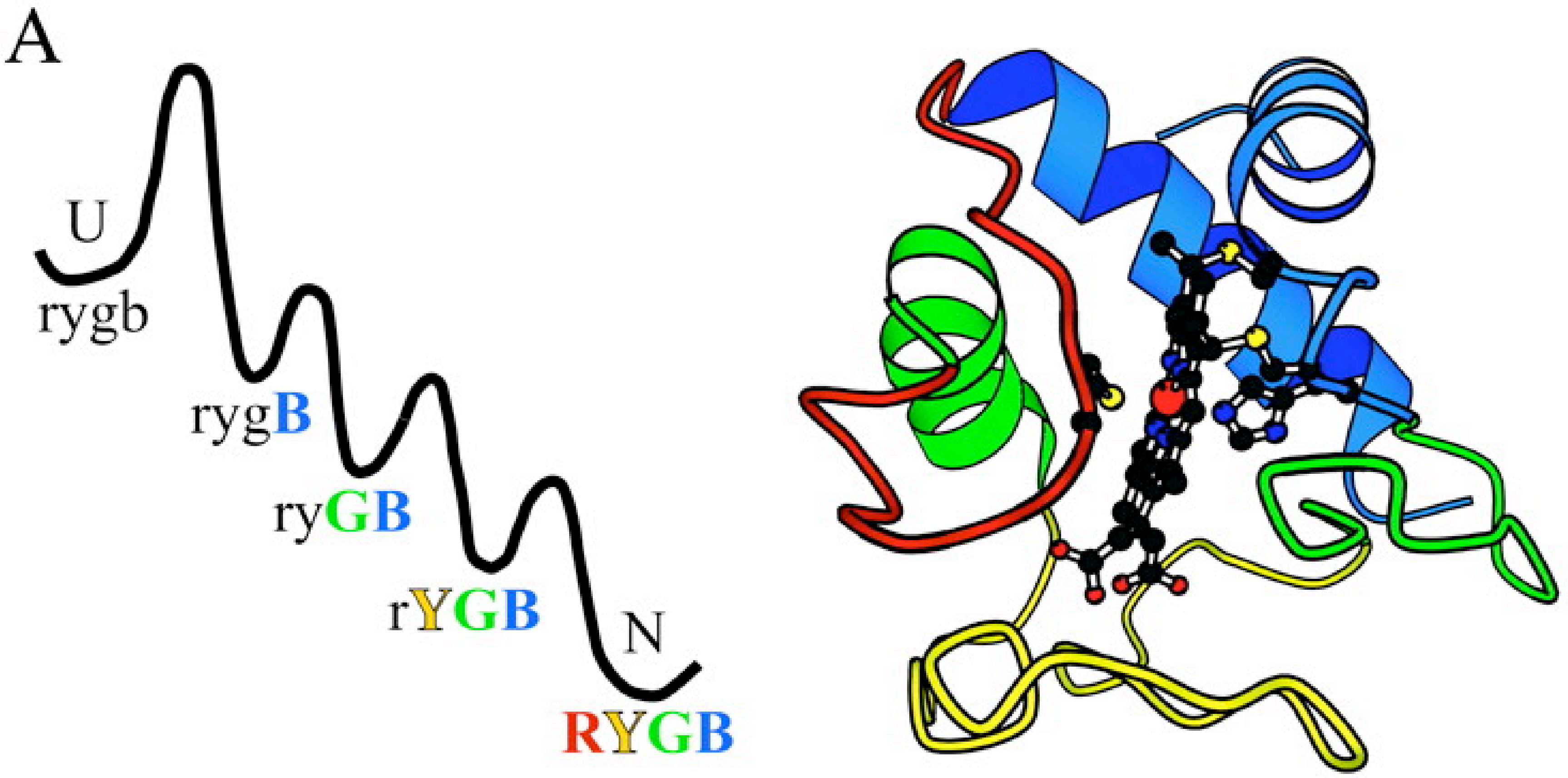




Cytochrome C

Englander, *et. al.* 2002

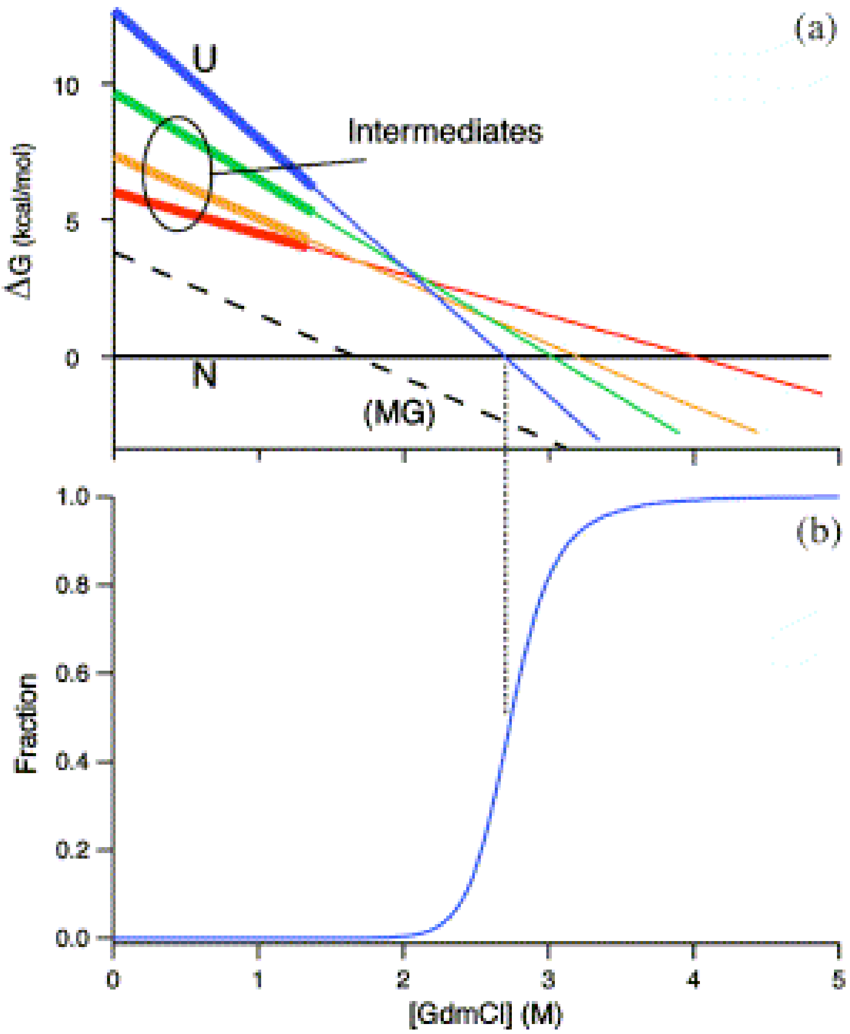
Cytochrome C Folding Pathway



U ⇌ rygB ⇌ ryGB ⇌ rYGB ⇌ N

Hoang, *et. al.* 2002

Multi-state vs Two State Protein Unfolding



U ⇌ rygB ⇌ ryGB ⇌ rYGB ⇌ N

Englander, *et. al.* 2002

NMR H/D exchange

Individual amide proton exchange rates

Sensitive to subtle protein dynamics

Used extensively to study protein folding

Problems

Need pure sample

Need high concentrations

Only small proteins

Mass Spec H/D exchange?

David Smith, (Zhang *et al 1993)*

Sample need not be pure

Low sample concentrations

Large proteins

Macromolecular complexes

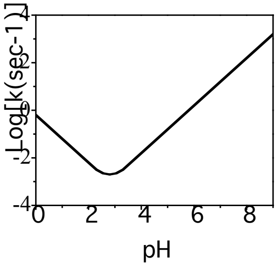
Problems

Digestion coverage

Exchange rate is averaged over the whole peptide

Buffer intolerance

H/D Exchange Experimental Protocol



Protein

- Exchange with D_20
- Quench with low pH over time

Pepsin digestion (Optional)

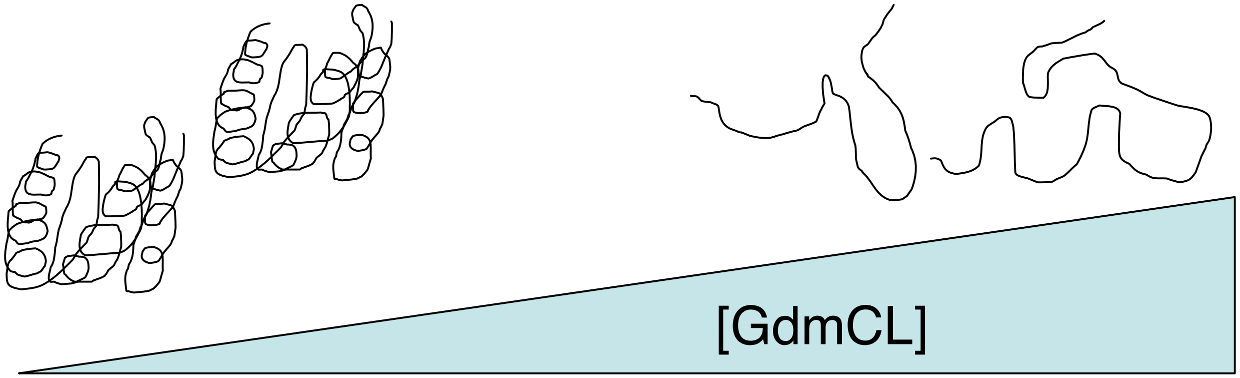
- Fragment size 10-20 residues

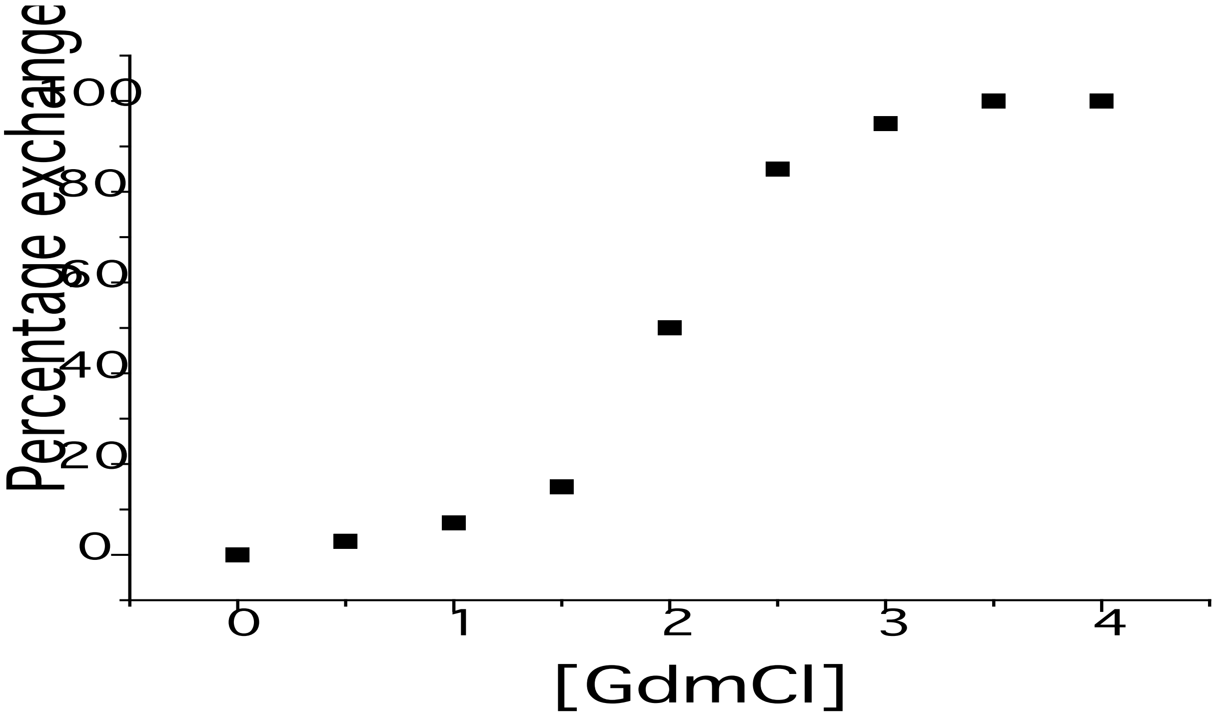
Mass spectrometry

- Assignment of peptides
- Quantifying exchange

Protein stability

“pulsed labeling H/D exchange”





MALDI Analysis of Pulse labeled proteins

“SUPREX”

SUPREX; Stability of Unpurified Proteins from Rates of H/D Exchange

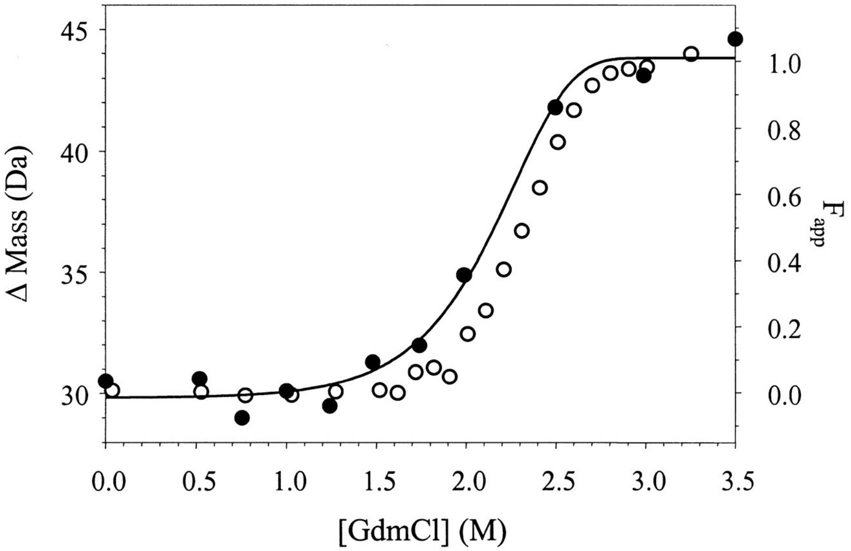
- Unpurified proteins
- Rapid analysis
- High throughput
- Protein stability in the cell

Ghaemmaghami, *et. al. 2000*

SUPREX

Suprex = ●

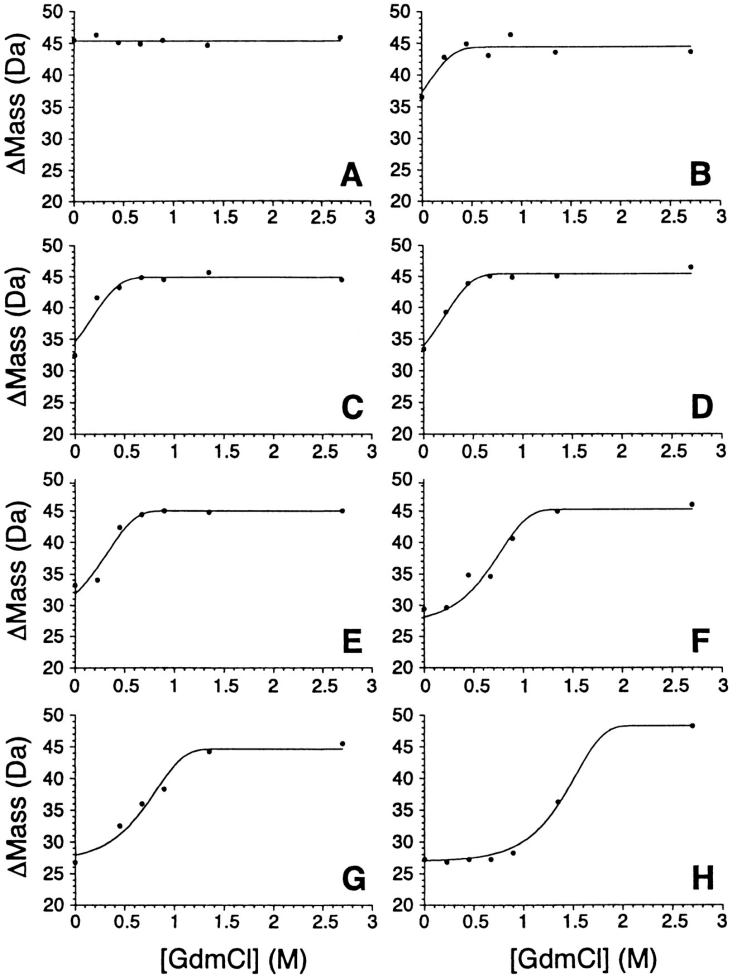
CD = ○

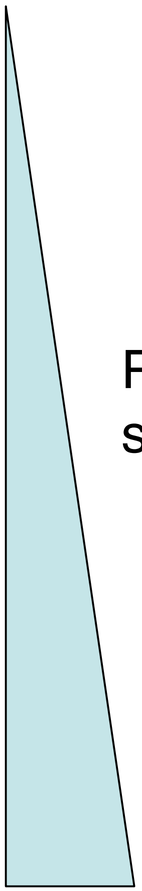


4-oxalocrotonate tautomerase

Powell, *et. al. 2000*

SUPREX analysis of mutations and protein stability



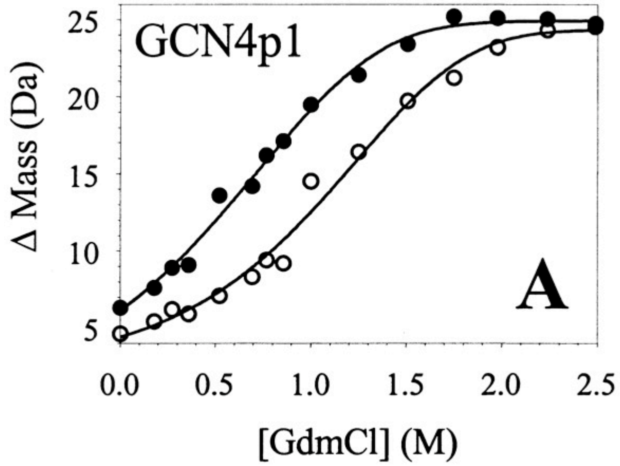


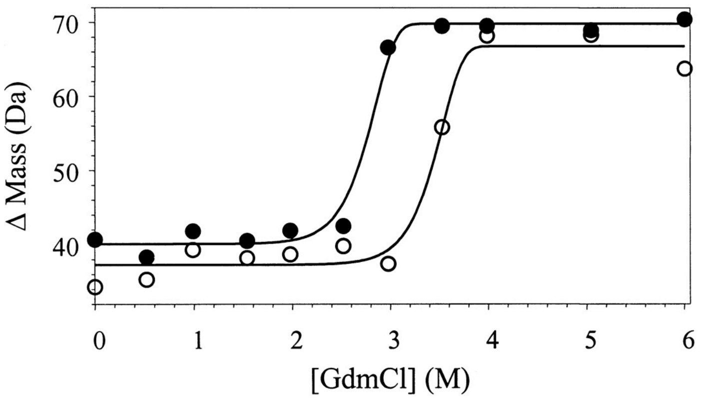
Protein stability

Maltose binding protein

Ghaemmaghami, *et. al. 2000*

Association detected by SUPREX



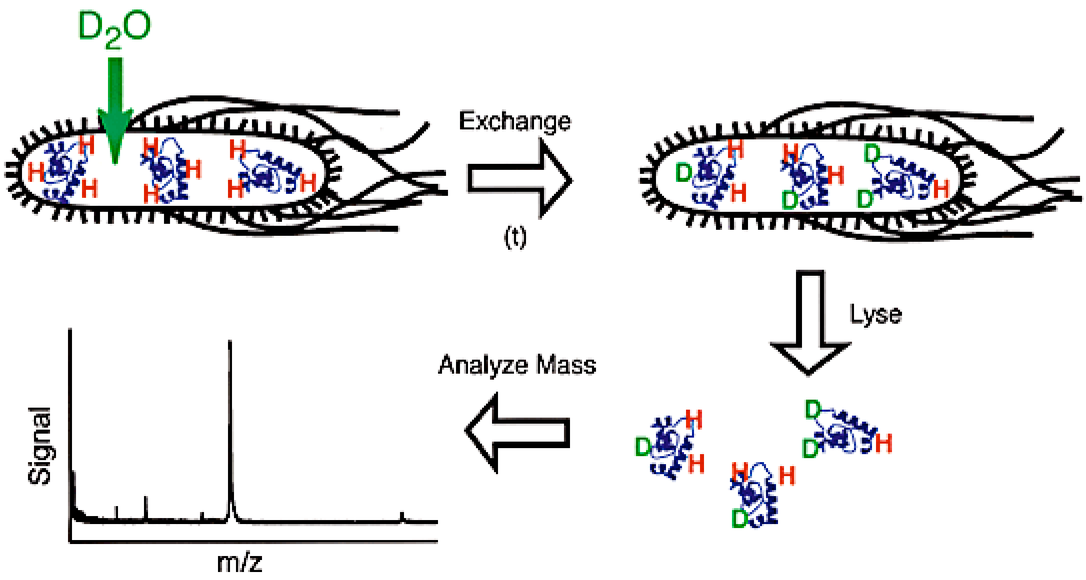


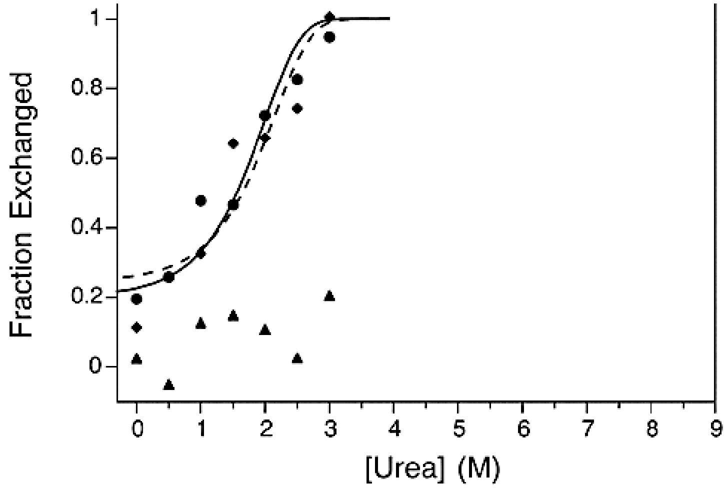
10 µM = ●
56 µM = ○

Trp repressor (TrpR)
- L-tryptophan = ●
+ L-tryptophan = ○

Powell, *et. al. 2000*

Protein Stability in Cells





Ghaemmaghami, *et. al. 2001*

MALDI Analysis of Pulse labeled proteins

“SUPREX”

- Unpurified proteins
- Rapid analysis
- High throughput
- Protein stability in the cell
- ?

Continous H/D Exchange

Protein folding

Protein interfaces

Quantitatively determine rates

?

H/D Exchange Experimental Protocol

CA Tubes or Dimers

- Exchange with D_20
- Quench with low pH over time

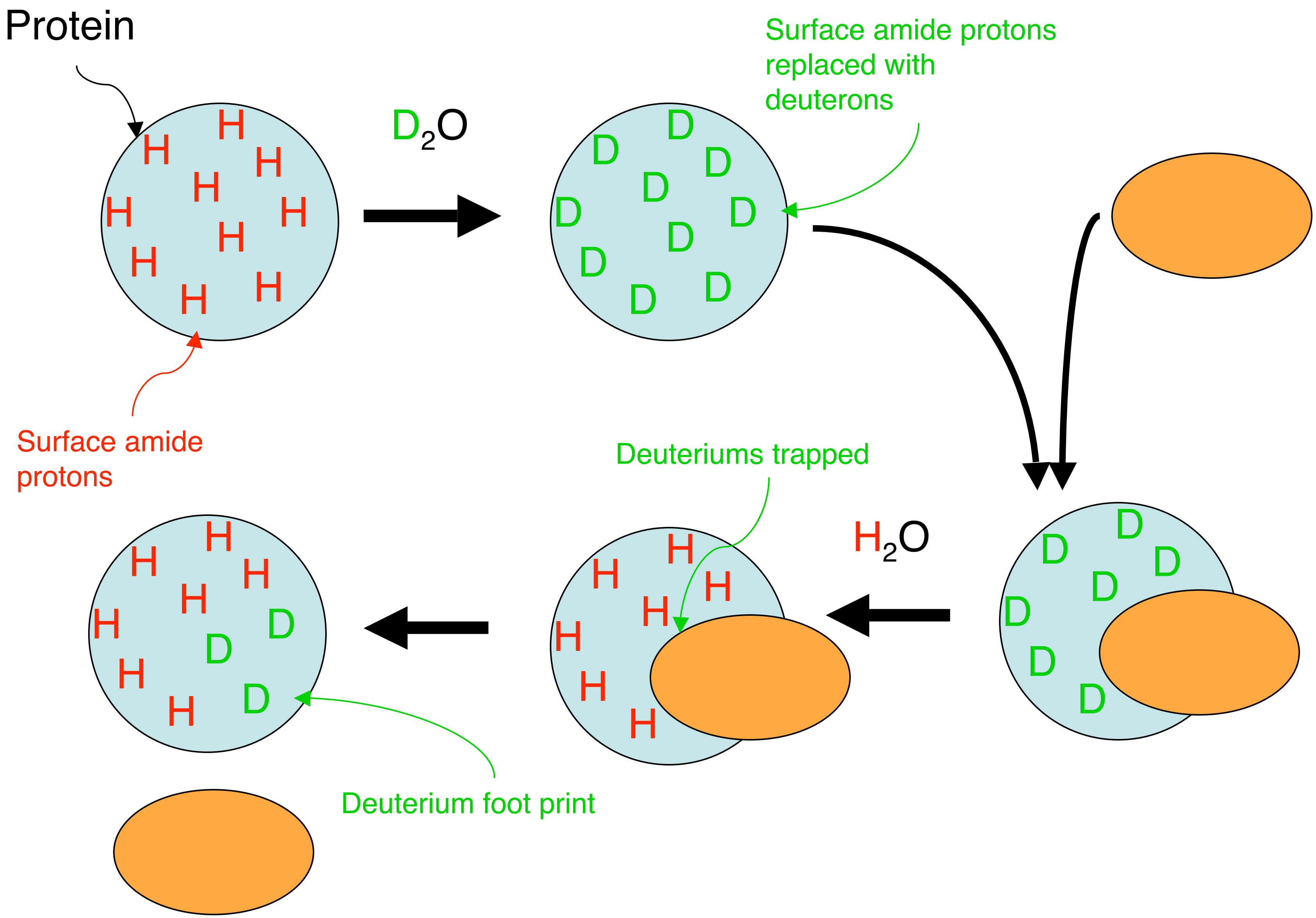
Pepsin digestion

- Fragment size 10-20 residues

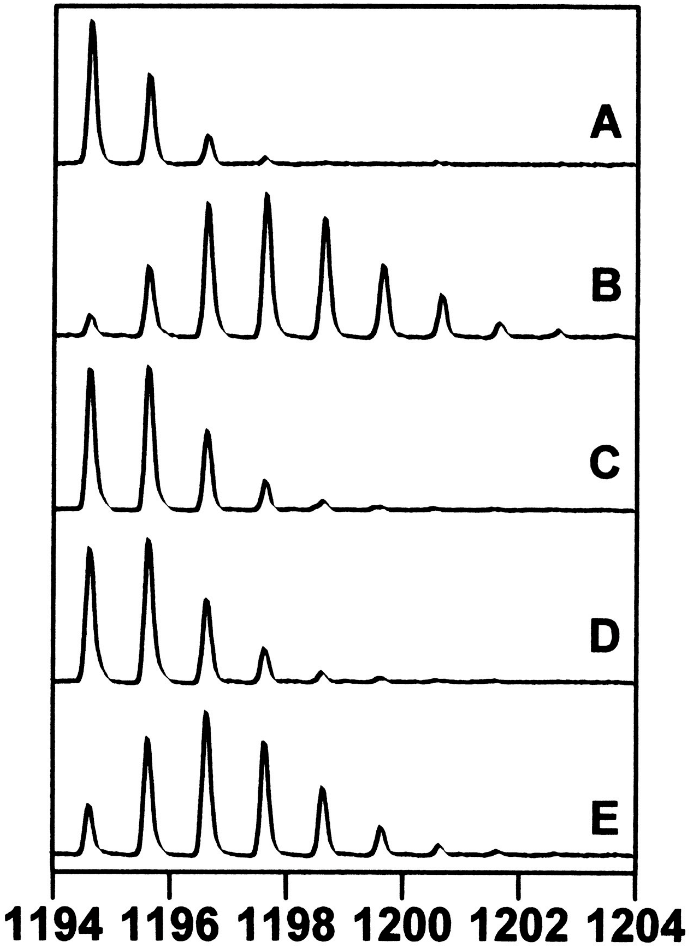
Mass spectrometry

- Assignment of peptides
- Quantifying exchange

Identification of protein interaction interfaces



Detection of PKI Interaction with PKA



Protonated

Deuterated

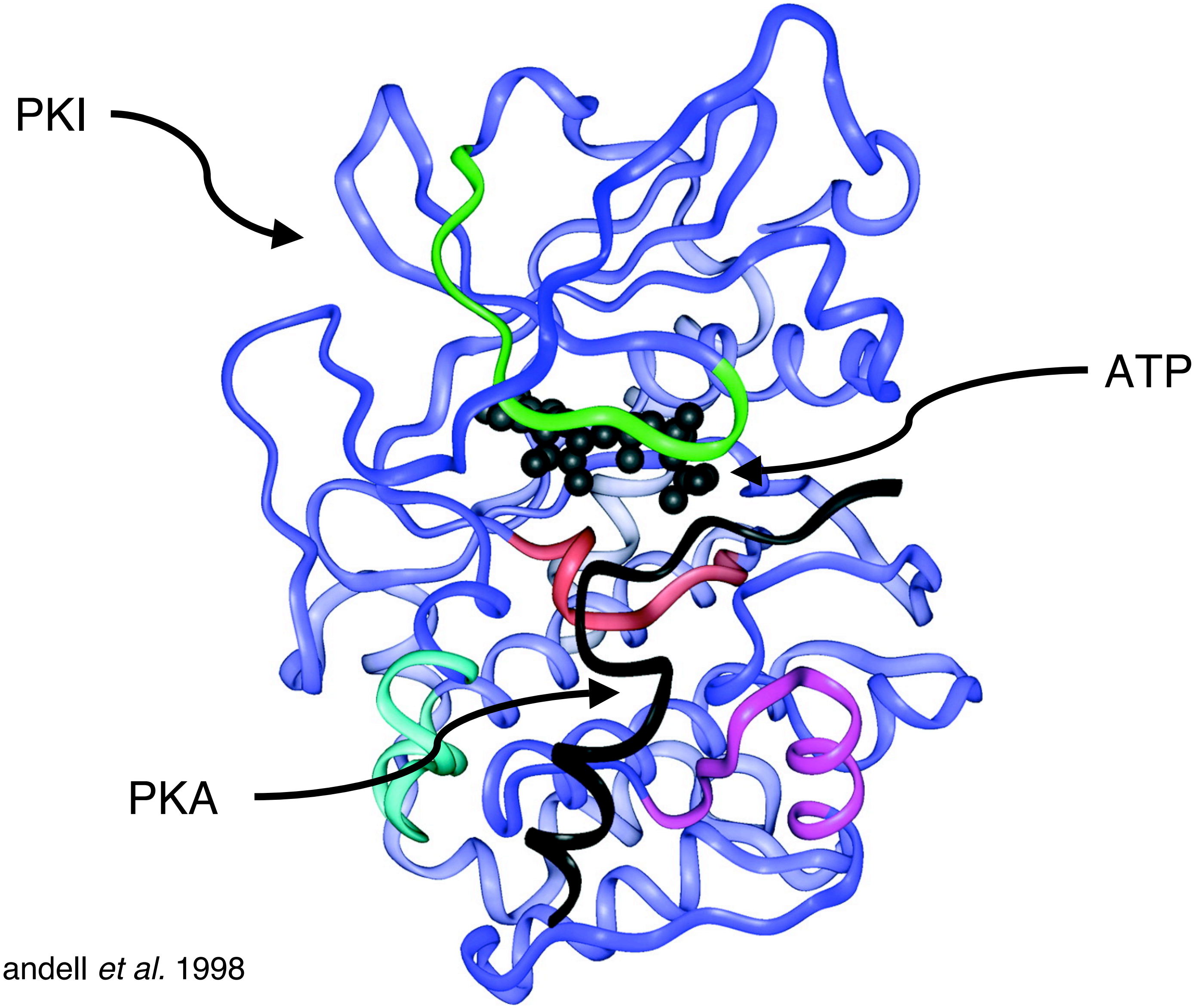
Off exchange (10 min)

+ ATP

+ ATP & PKI

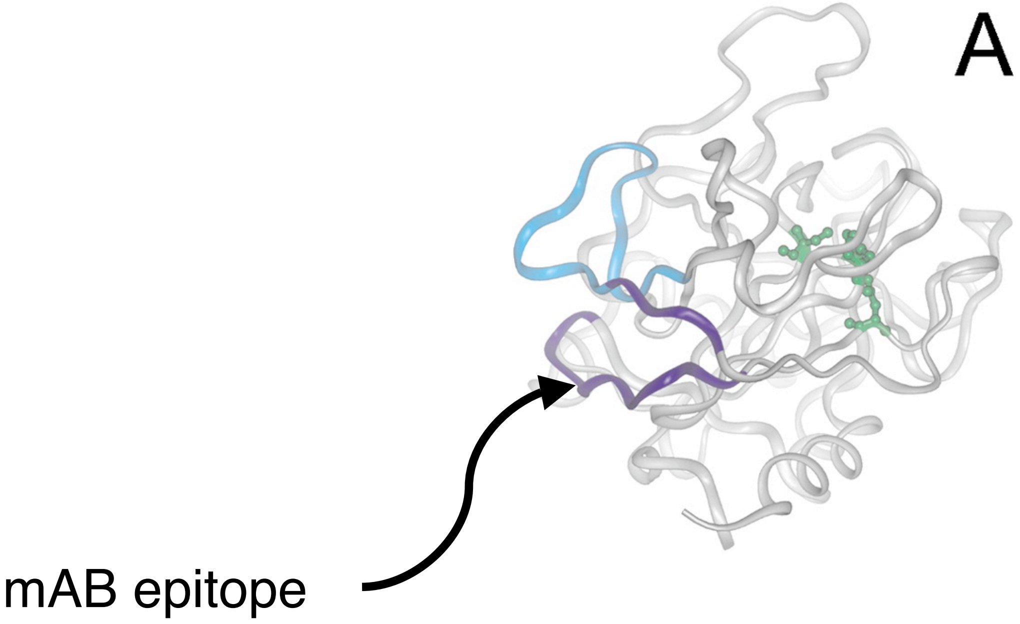
Mandell *et al.* 1998

PKI, PKA, and ATP



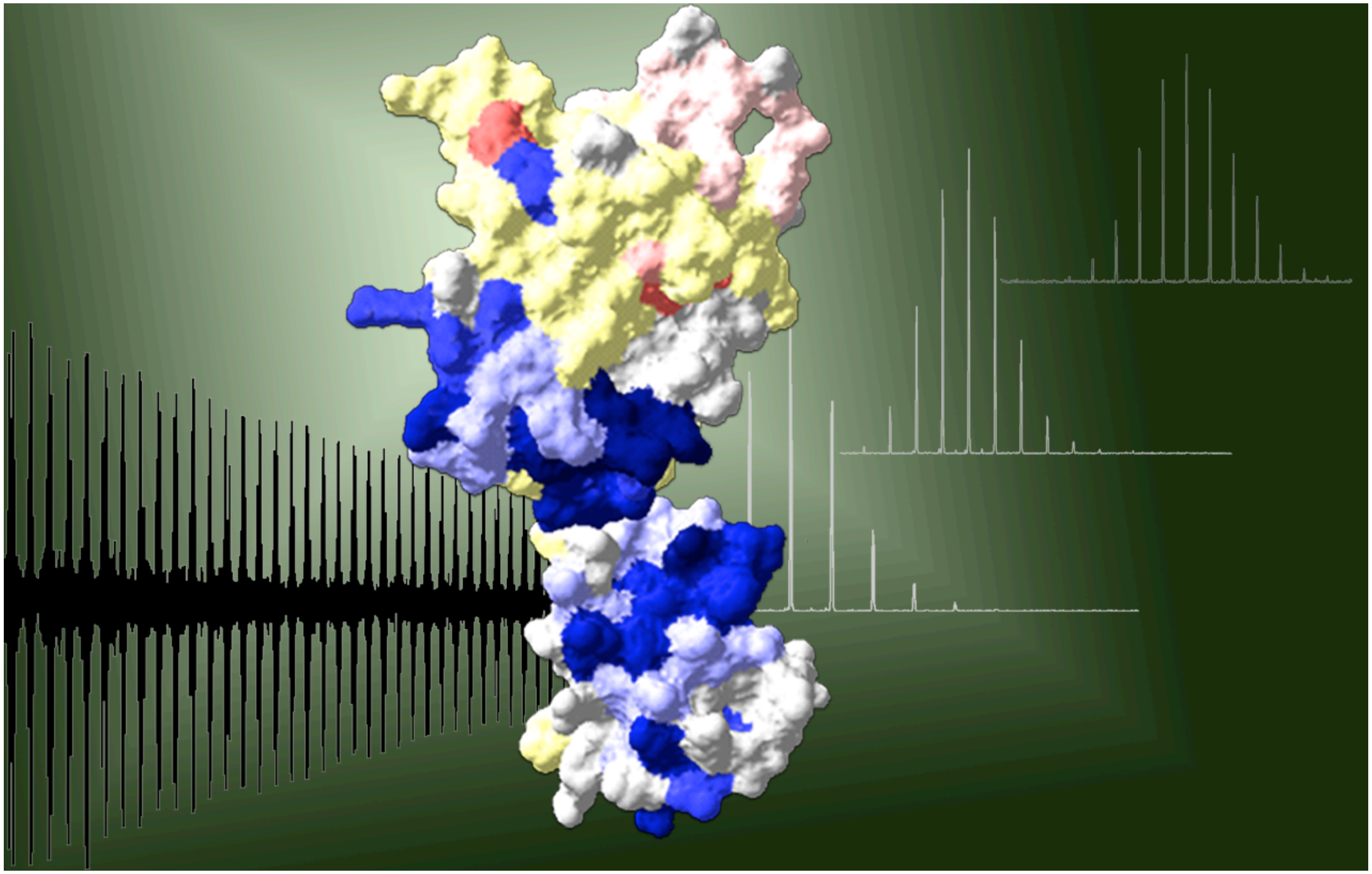
Mandell *et al.* 1998

Epitope mapping of a monoclonal antibody against thrombin by H/D-exchange



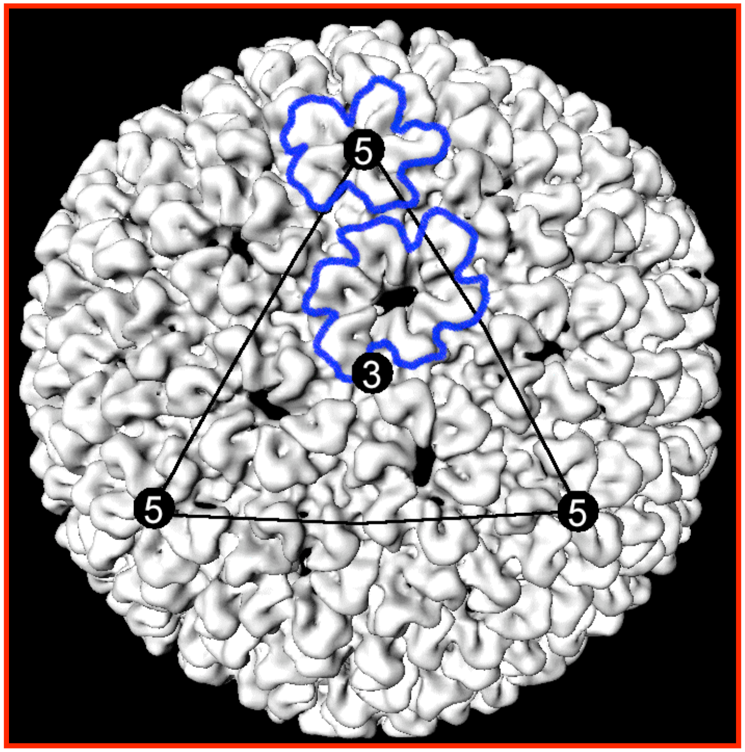
mAB epitope

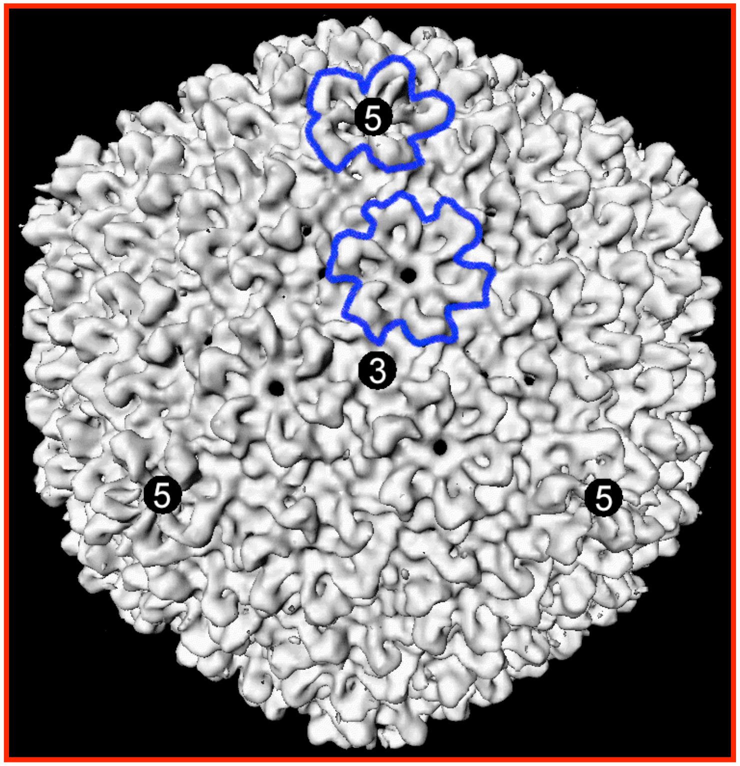
Novel Approaches for Understanding Virus Assembly and Dynamics.



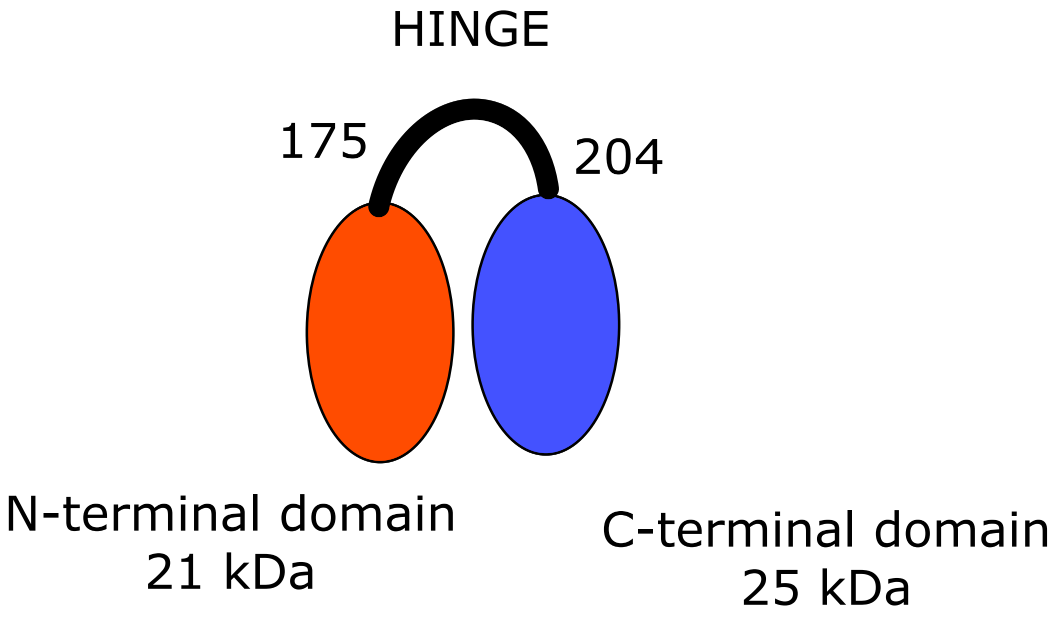
Lanman *et al.* 2003

Image Reconstructions of Procapsid and Mature Virion





The Coat Protein Subunit has a Two Domain Structure



HINGE

175

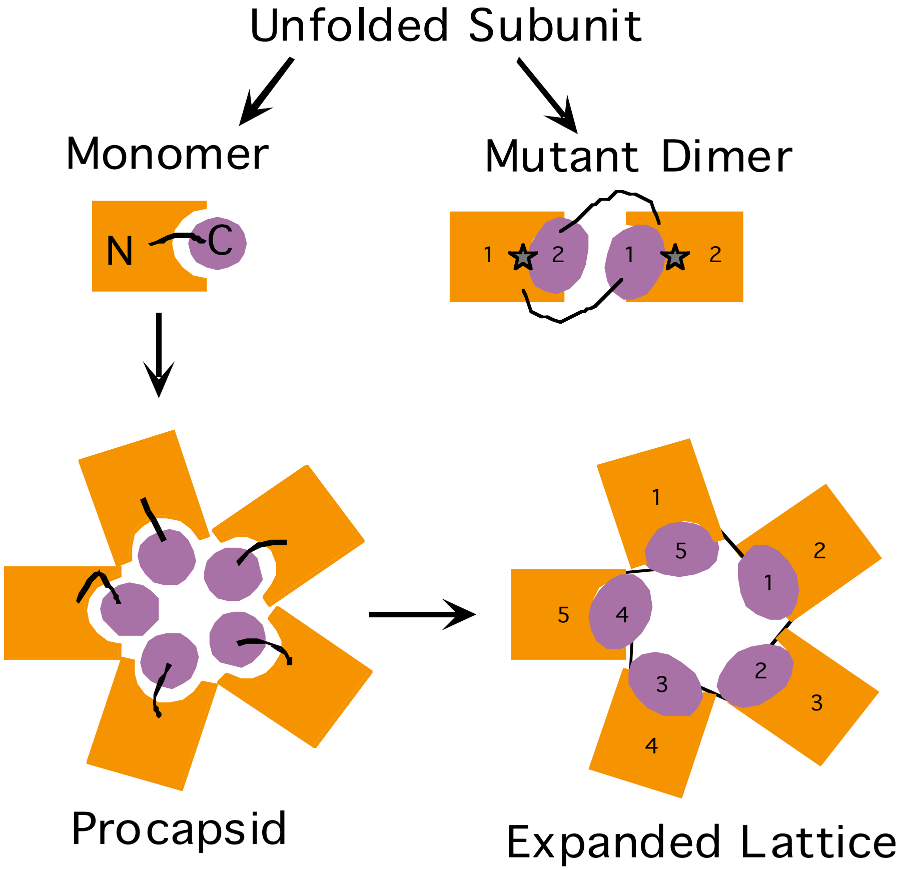
204

N-terminal domain
21 kDa

C-terminal domain
25 kDa

Lanman, *et. al. 1999*

A domain-shuffling model for capsid expansion


Unfolded Subunit

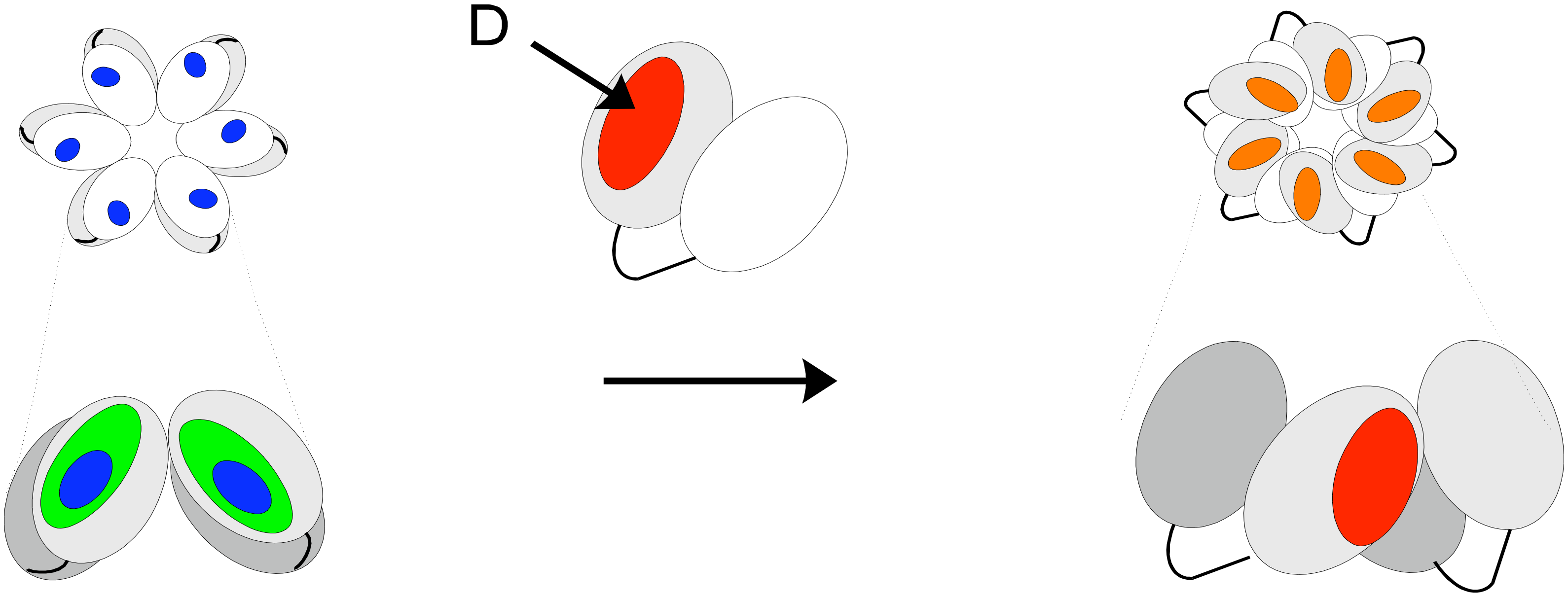
Monomer

Mutant Dimer

Procapsid

Expanded Lattice

The Model Predicts Trapping of Deuterium during Expansion



Procapsid

Transition State

Expanded

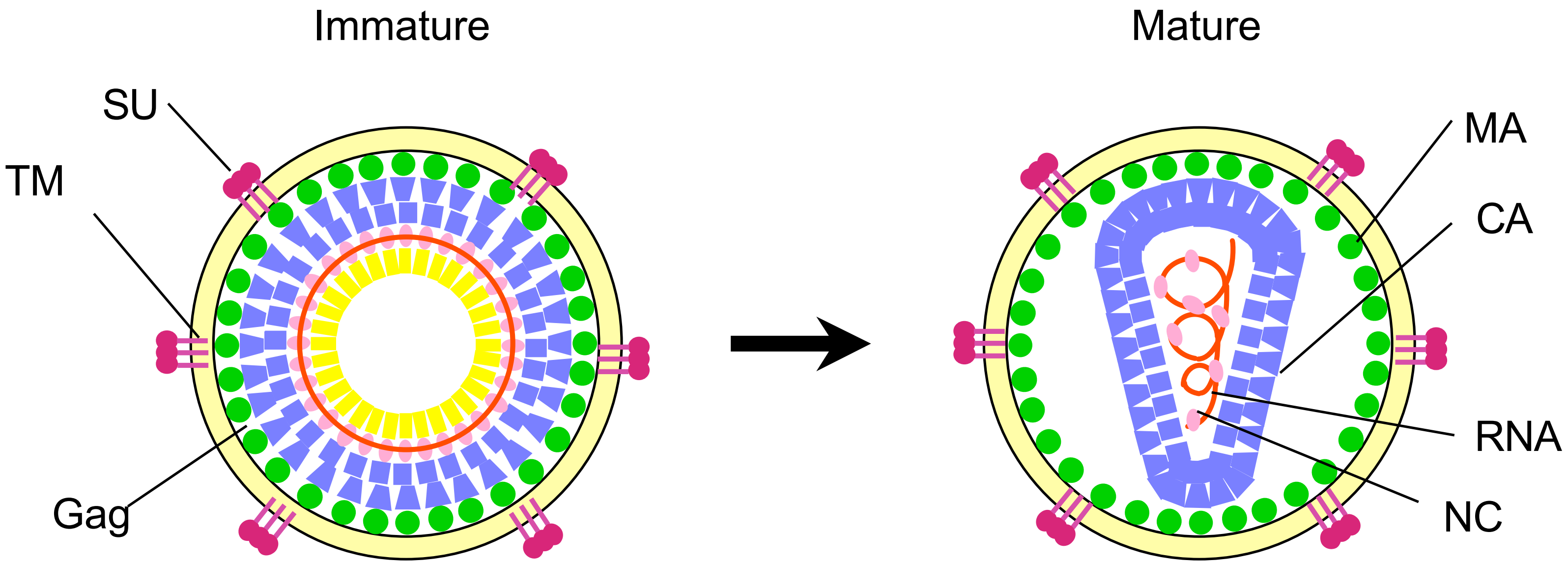
Changes in Exchange Protection during Assembly & Maturation

MALNEGQIVT LAVDEIIETI SAITPMAQKA KKYTPPAASM QRSSNTIWMP VEQESPTQEG 60
WDLTDKATGL LELNVAVNMG EPDNDFFQLR ADDLRDETAY RRRIQSAARK LANNVELKVA 120
NMAAEMGSLV ITSPDAIGTN TADAWNFVAD AEEIMFSREL NRDMGTSYFF NPQDYKKAGY 180
DLTKRDIFGR IPEEAYRDGT IQRQVAGFDD VLRSPKLPVL TKSTATGITV SGAQSFKPVA 240
WQLDNDGNKV NVDNRFATVT LSATTGMKRG DKISFAGVKF LGQMAKNVLA QDATFSVVRV 300
VDGTHVEITP KPVALDDVSL SPEQRAYANV NTSLADAMAV NILNVKDART NVFWADDAIR 360
IVSQPIPANH ELFAGMKTTS FSIPDVGLNG IFATQGDIST L SGLCRIALW YGVNATRPEA 420
IGVGLPGQTA 430

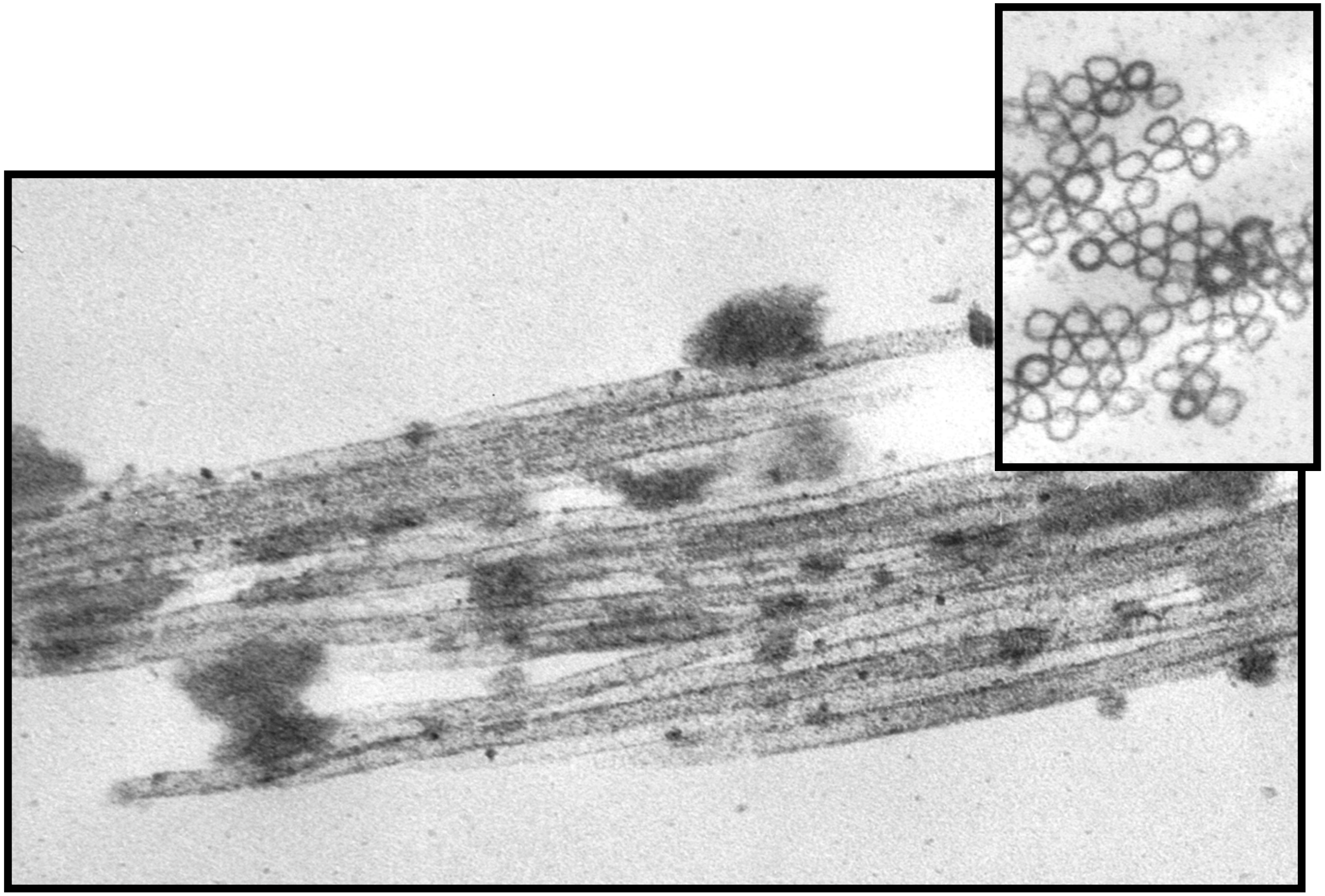
🟩 monomer protection
🟦 procapsid shell protection
🟥 expanded shell protection
🟪 deuterium retention after expansion

Tuma, *et. al. 2000*

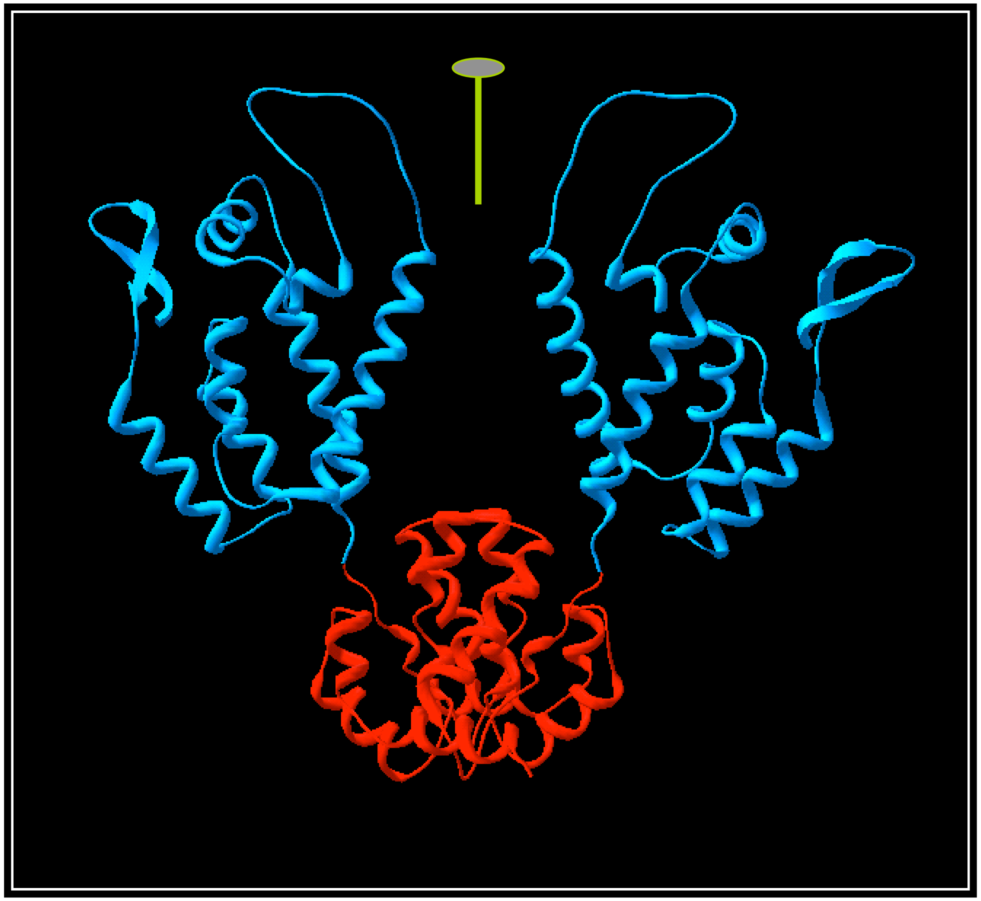
HIV Assembly & Maturation



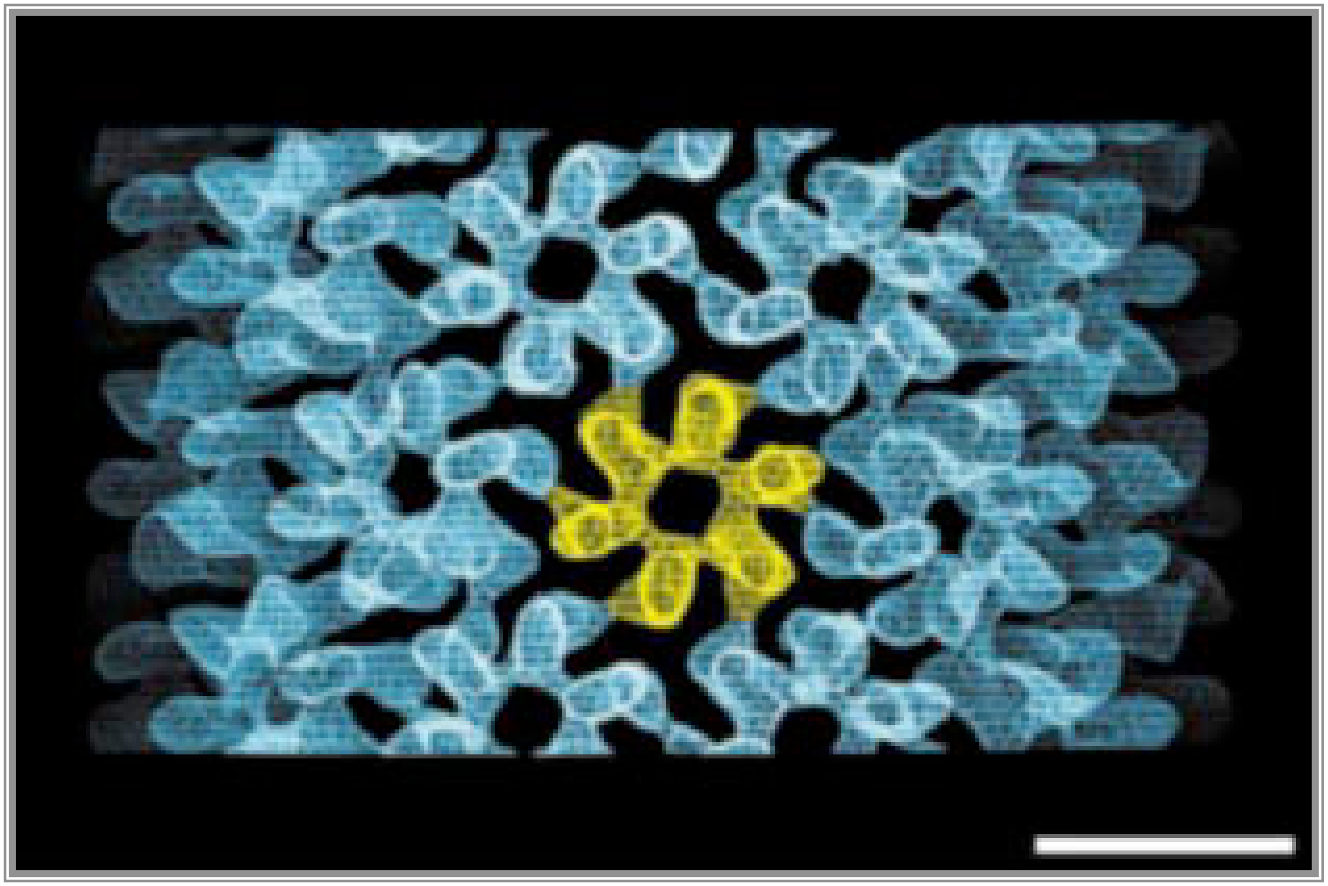
Thin-Section EM Analysis of Assembly Products



CA is Comprised of Distinct N- and C-Terminal Structural Domains

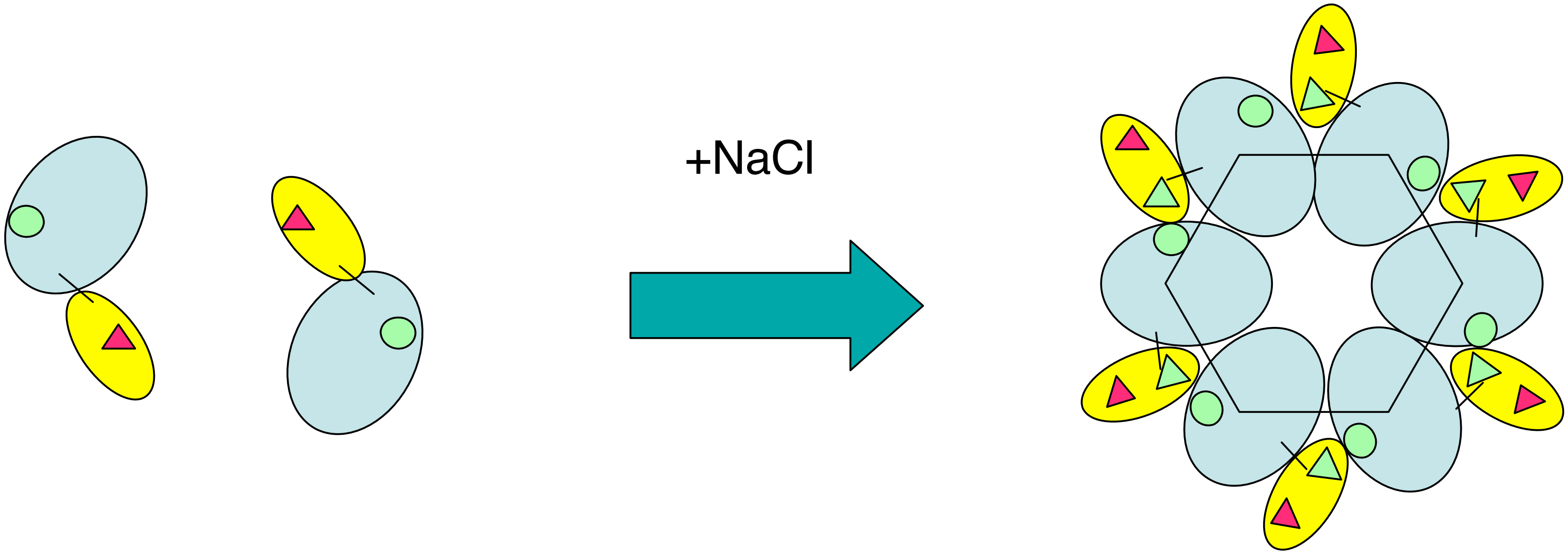


CA Cylinders are Based on a Hexamer Lattice

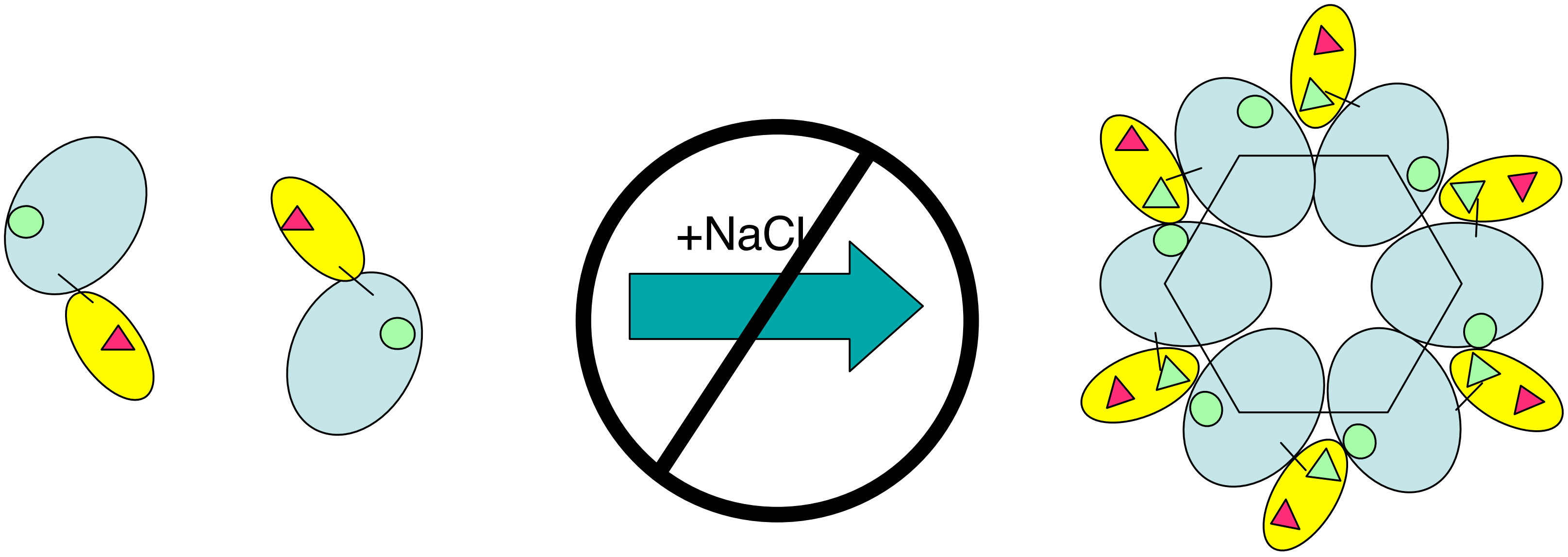


From Li et al, Nature 407:409 (2000)

Hypothesis: The CA that does not form dimers, because of a mutation at the dimer interface should form hexamers at high NaCl concentrations

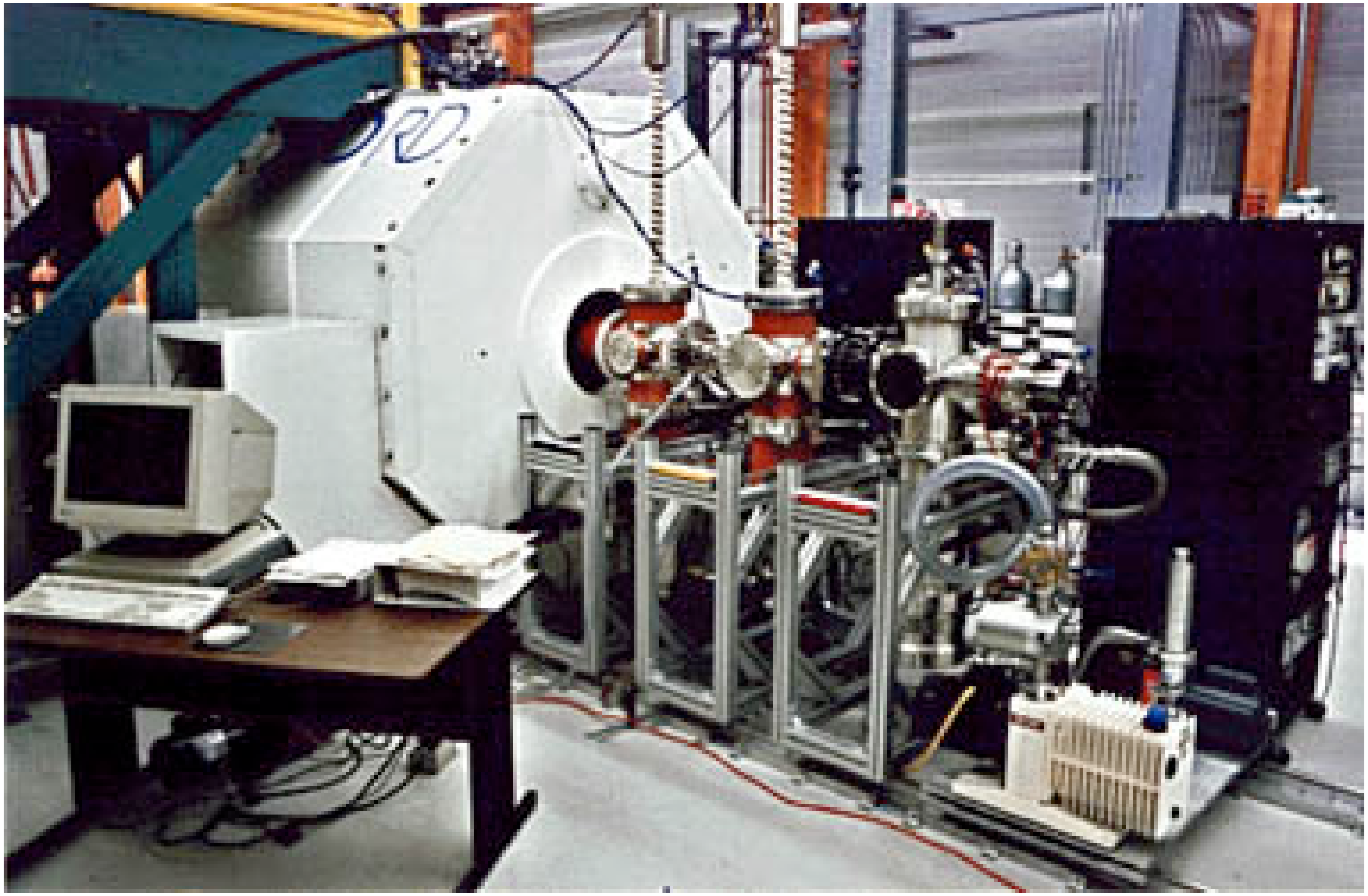


Hypothesis: The CA that does not form dimers, because of a mutation at the dimer interface should form hexamers at high NaCl concentrations



CA does not form hexamers with out C-domain (dimer) interaction.

H/D exchange analyzed with FT-MS



H/D Exchange Experimental Protocol

CA Tubes or Dimers

- Exchange with D_20
- Quench with low pH over time

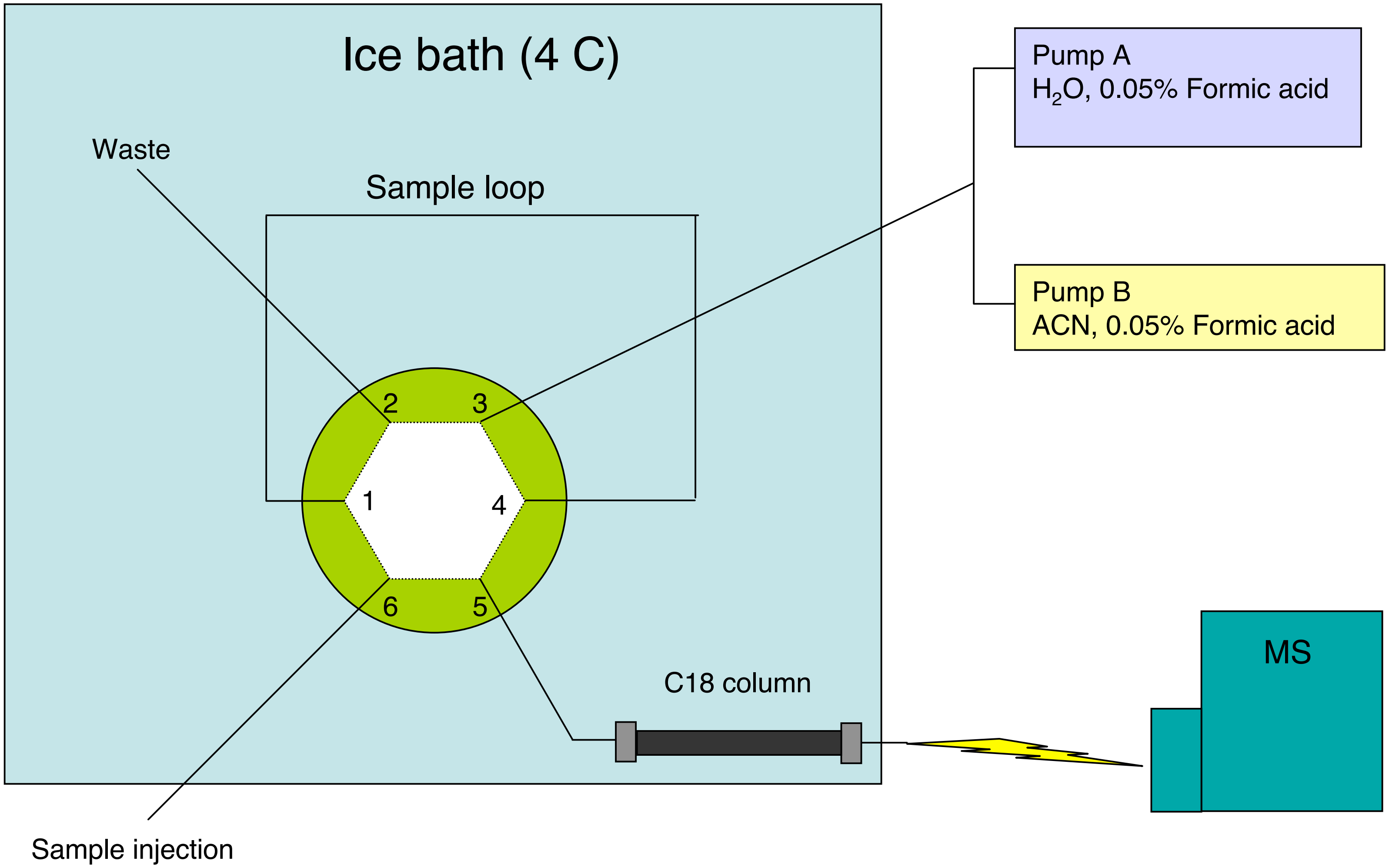
Pepsin digestion

- Fragment size 10-20 residues

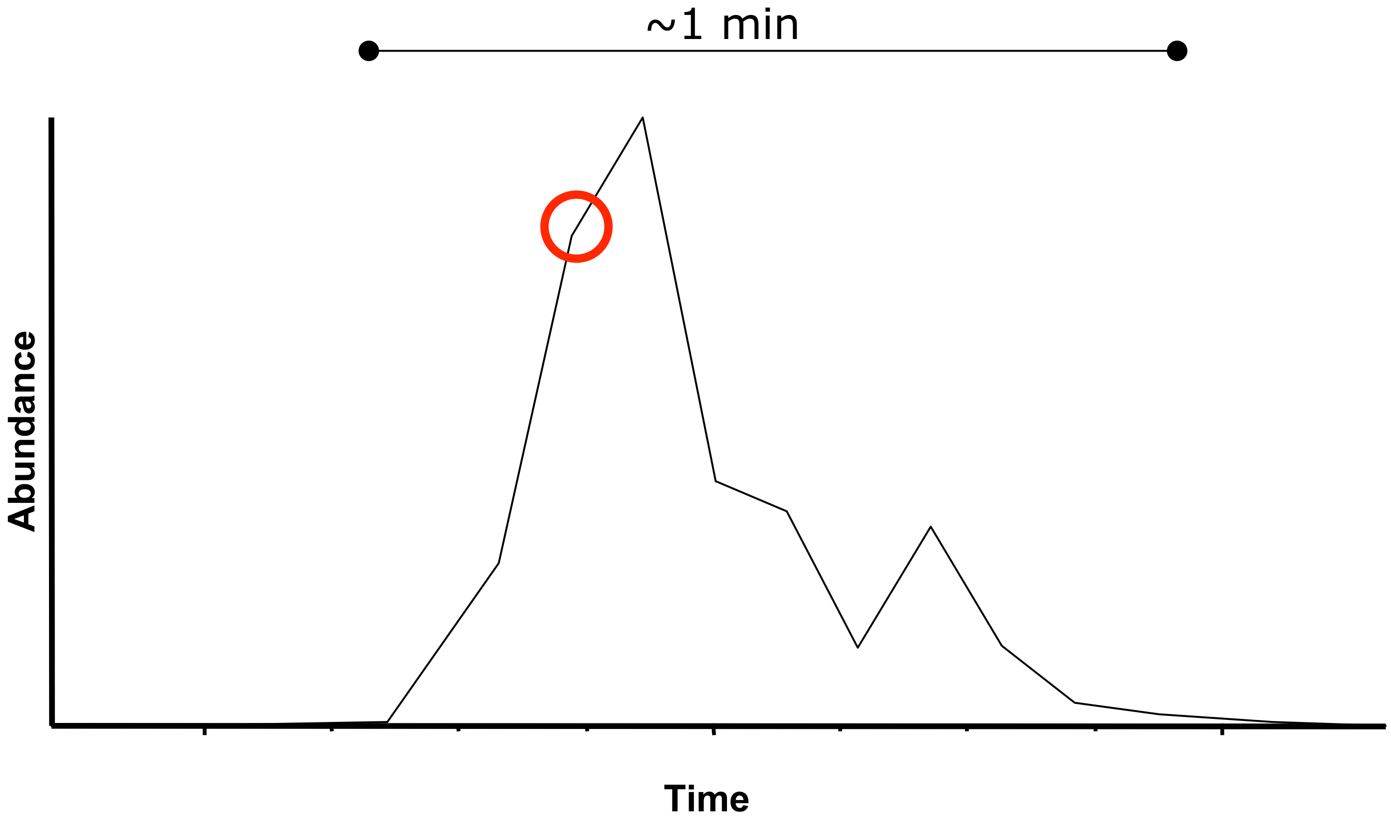
Mass spectrometry

- Assignment of peptides
- Quantifying exchange

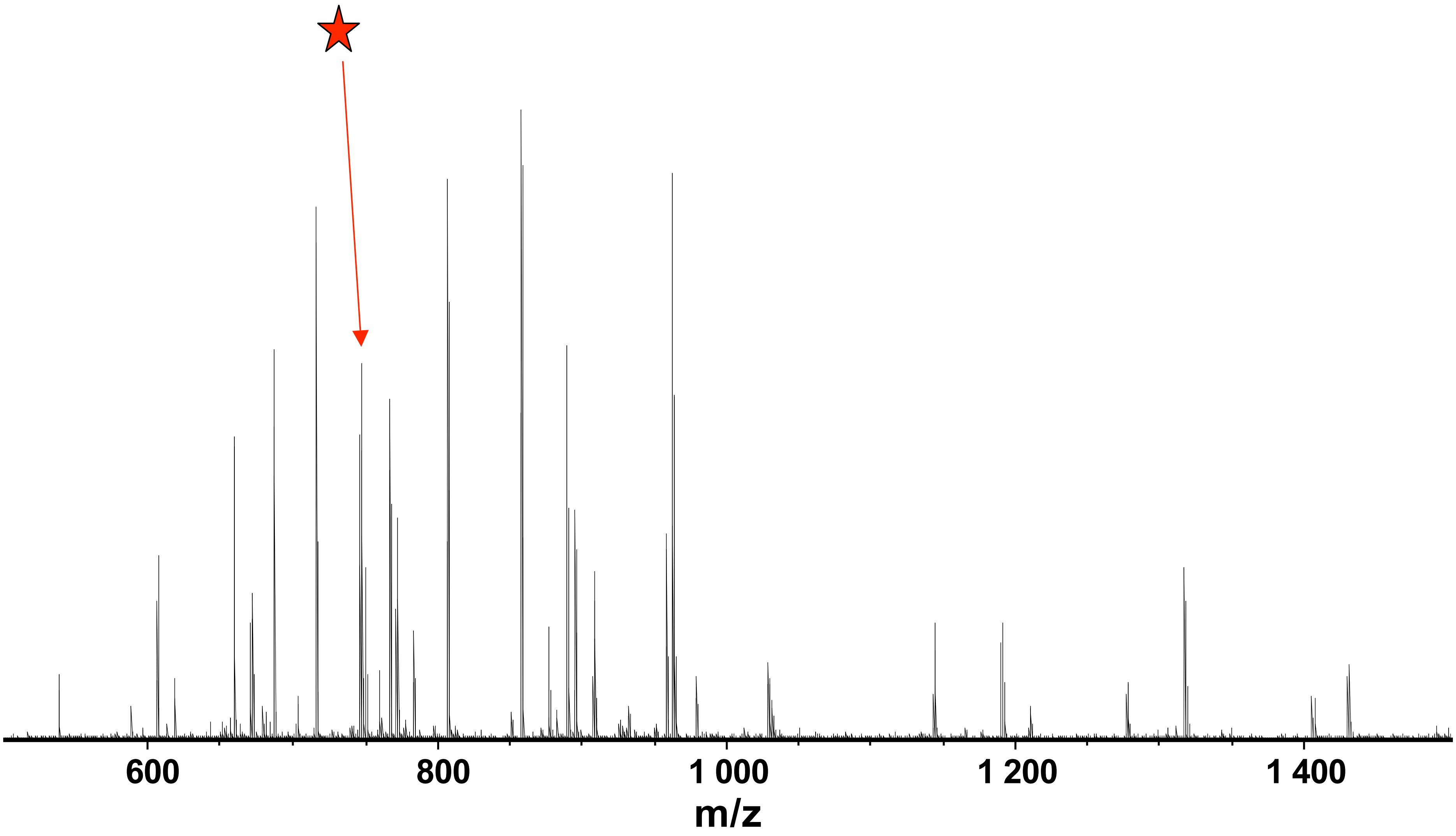
ESI-MS Analysis of Peptides



Elution of Digest from C4 Column



Representative FT-MS Scan

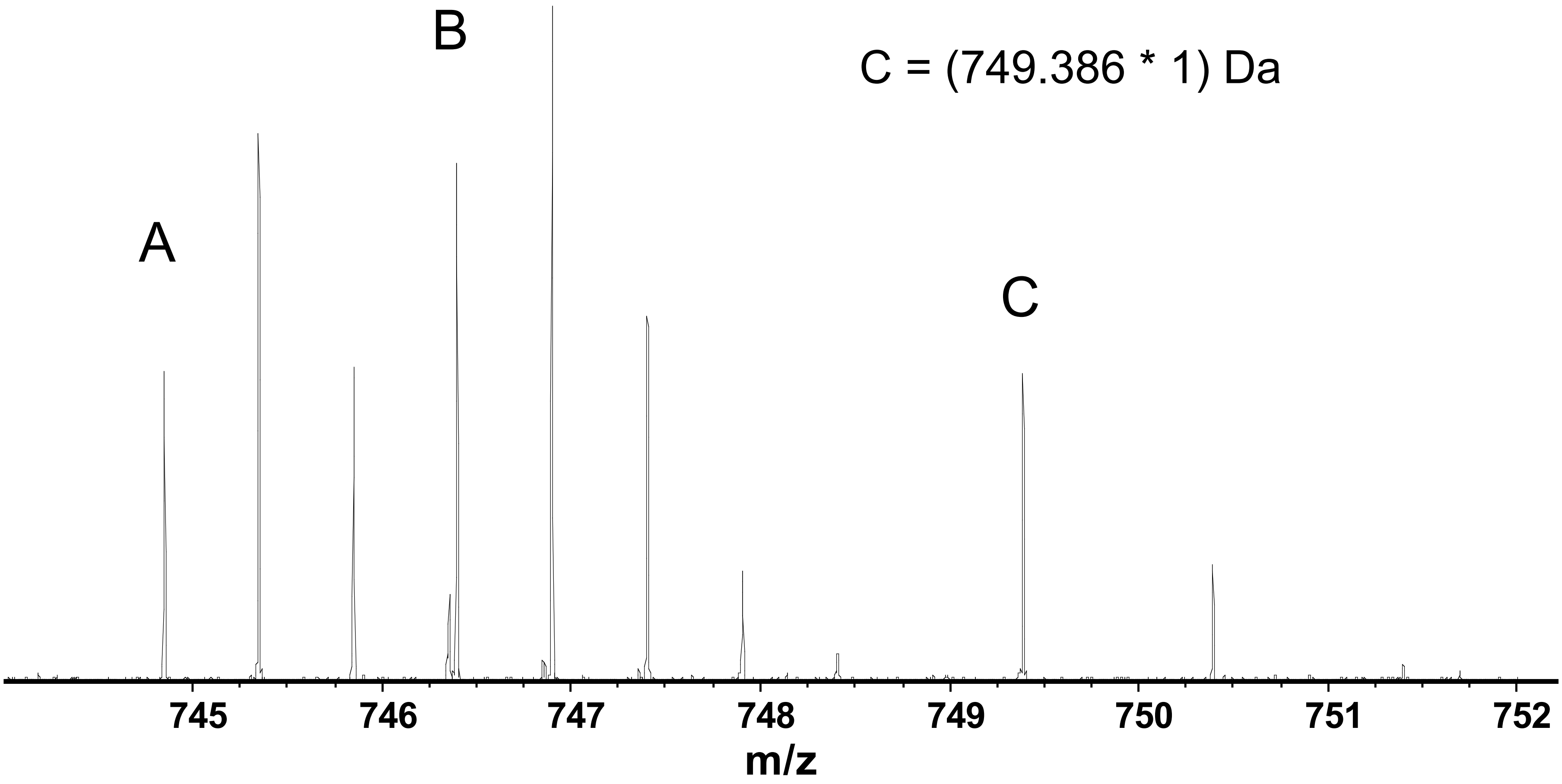


Expanded View of 744-752 m/z Region

A = (744.842 * 2) Da

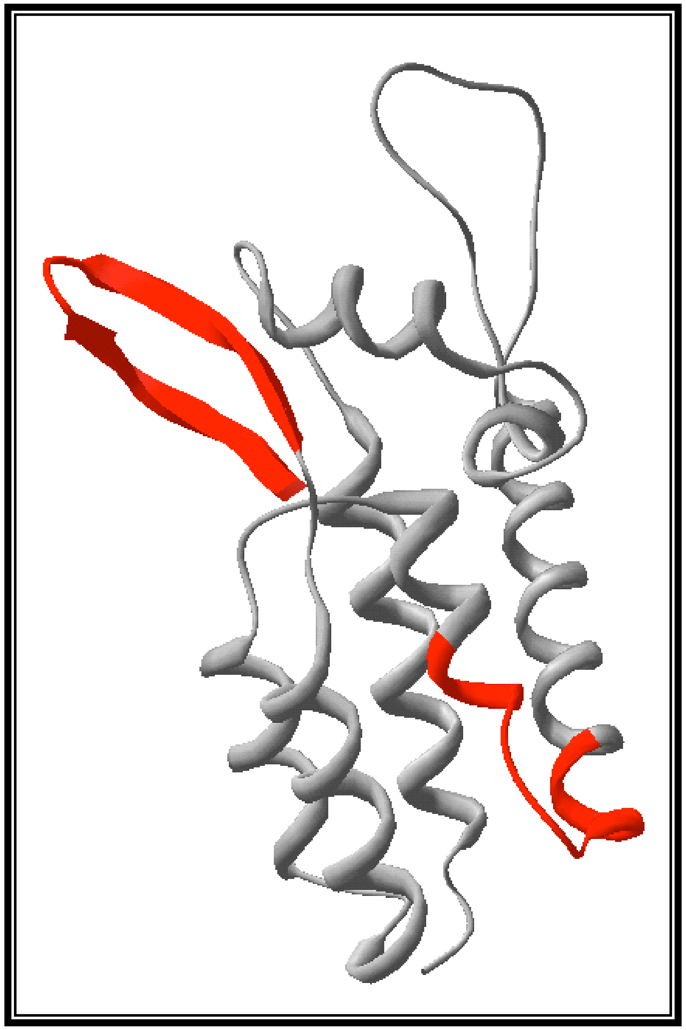
B = (746.390 * 2) Da

C = (749.386 * 1) Da

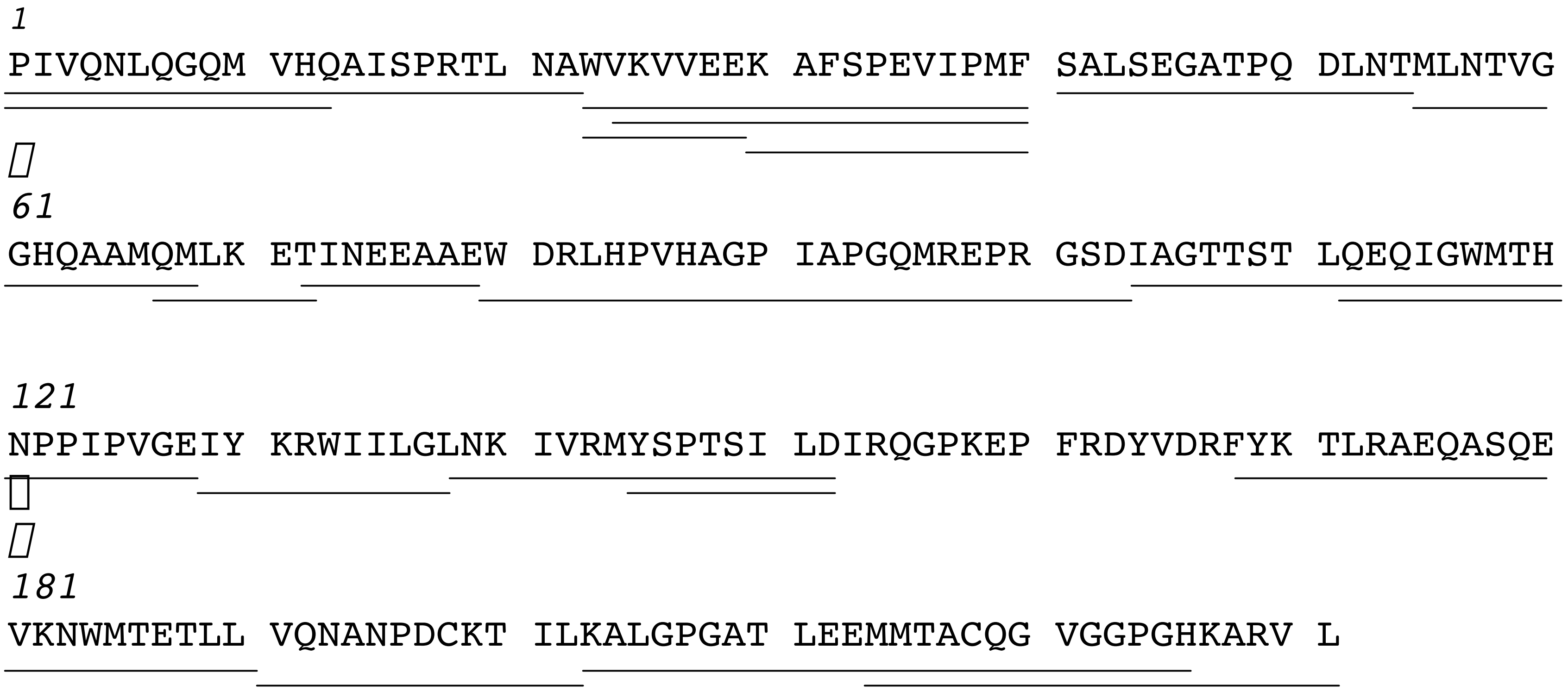


With High Resolution Peptides of Similar m/z can be Analyzed

744.842 within 1 ppm
746.390 within 0.5 ppm



Peptides Covering 95% of the Sequence have Been Assigned



1
PIVQNLQGQM VHQAISPRTL NAWVKVVEEK AFSPEVIPMF SALSEGATPQ DLNTMLNTVG

61
GHQAAMQMLK ETINEEAAEW DRLHPVHAGP IAPGQMREPR GSDIAGTTST LQEQIGWMTH

121
NPPIPVGEIY KRWIILGLNK IVRMYSPTSI LDIRQGPKEP FRDYVDRFYK TLRAEQASQE

181
VKNWMTETLL VQNANPDCKT ILKALGPGAT LEEMMTACQG VGGPGHKARV L

Expanded View of 744-752 m/z Region

A = (744.842 * 2) Da

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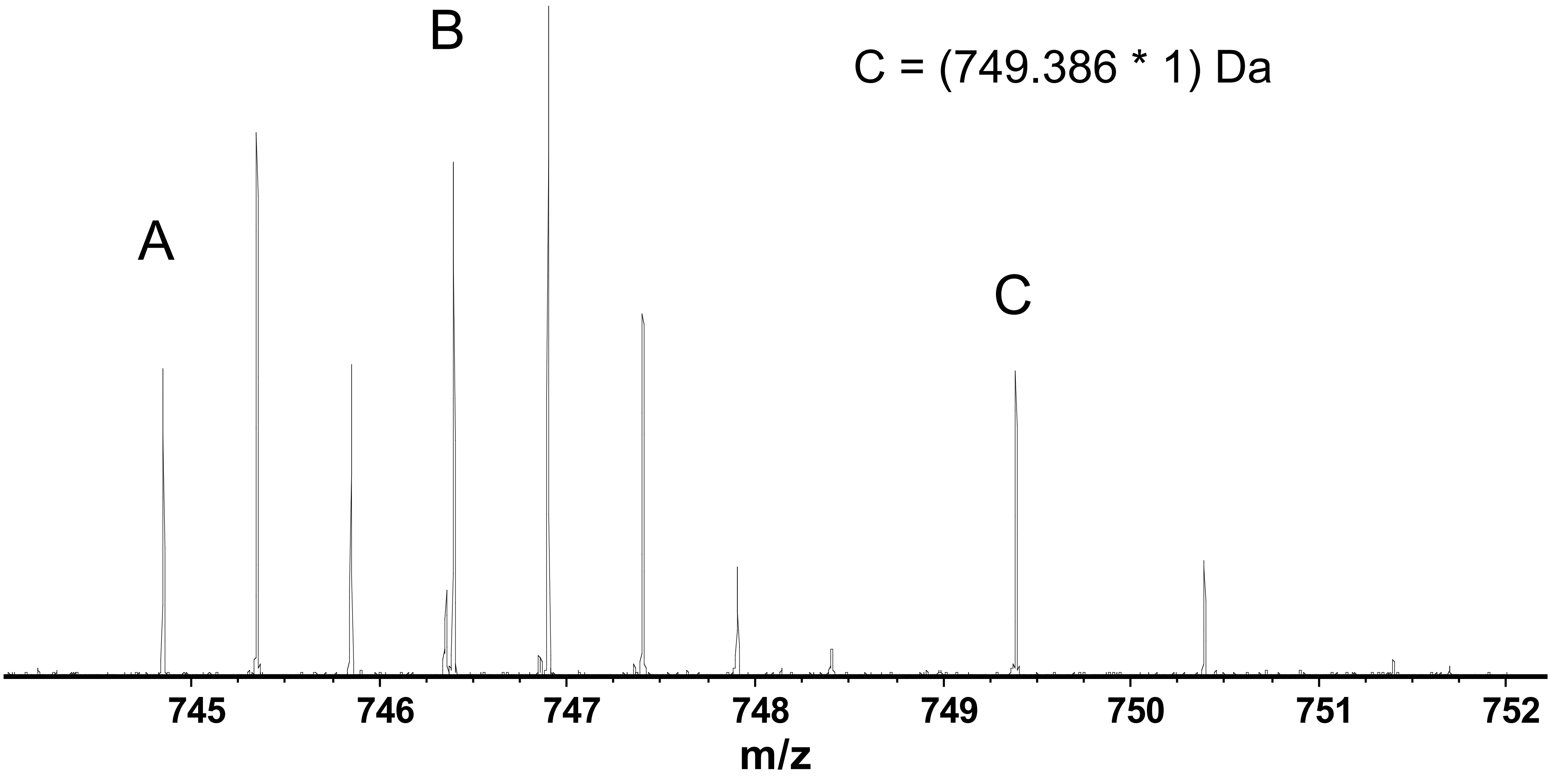
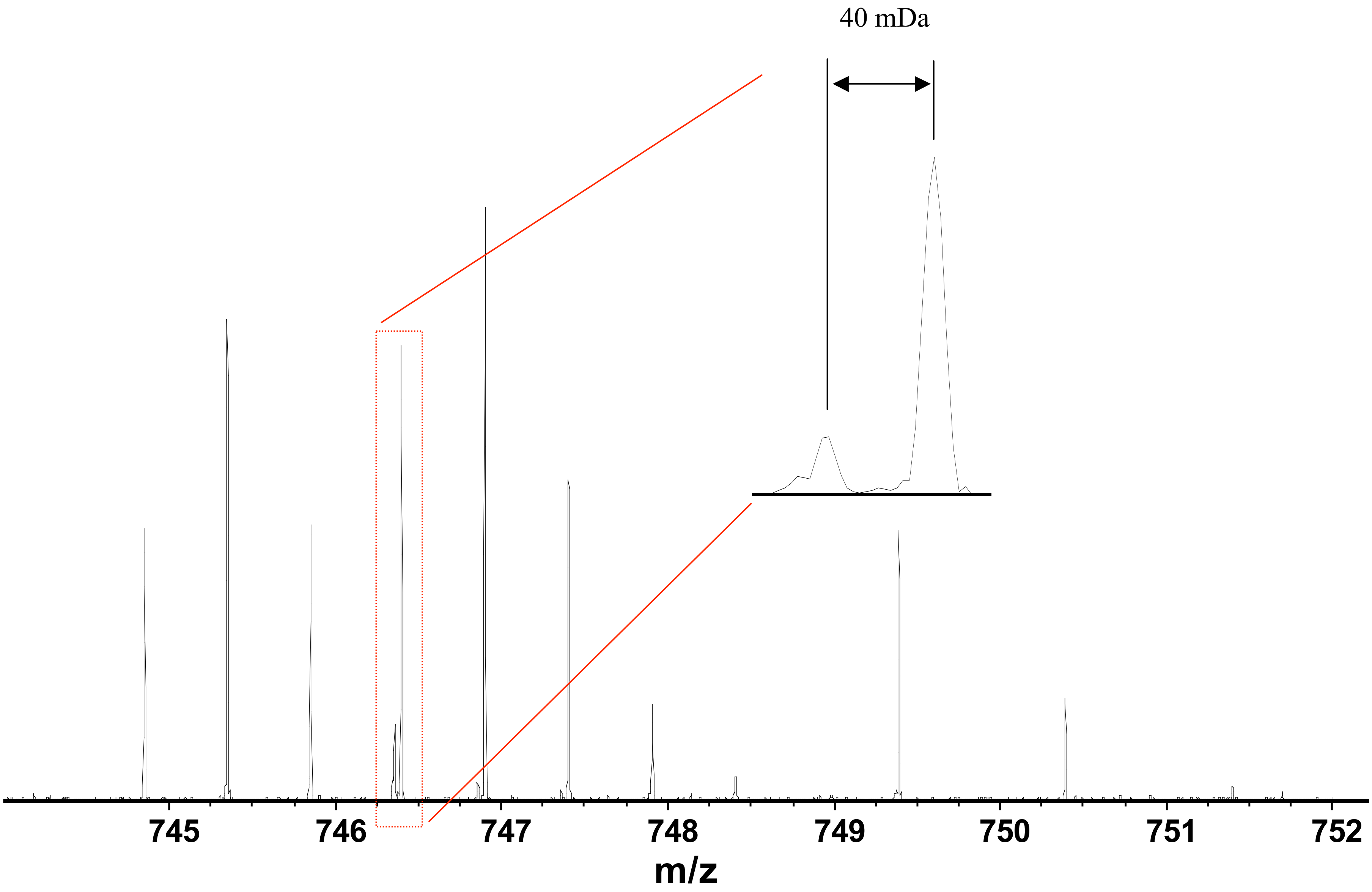
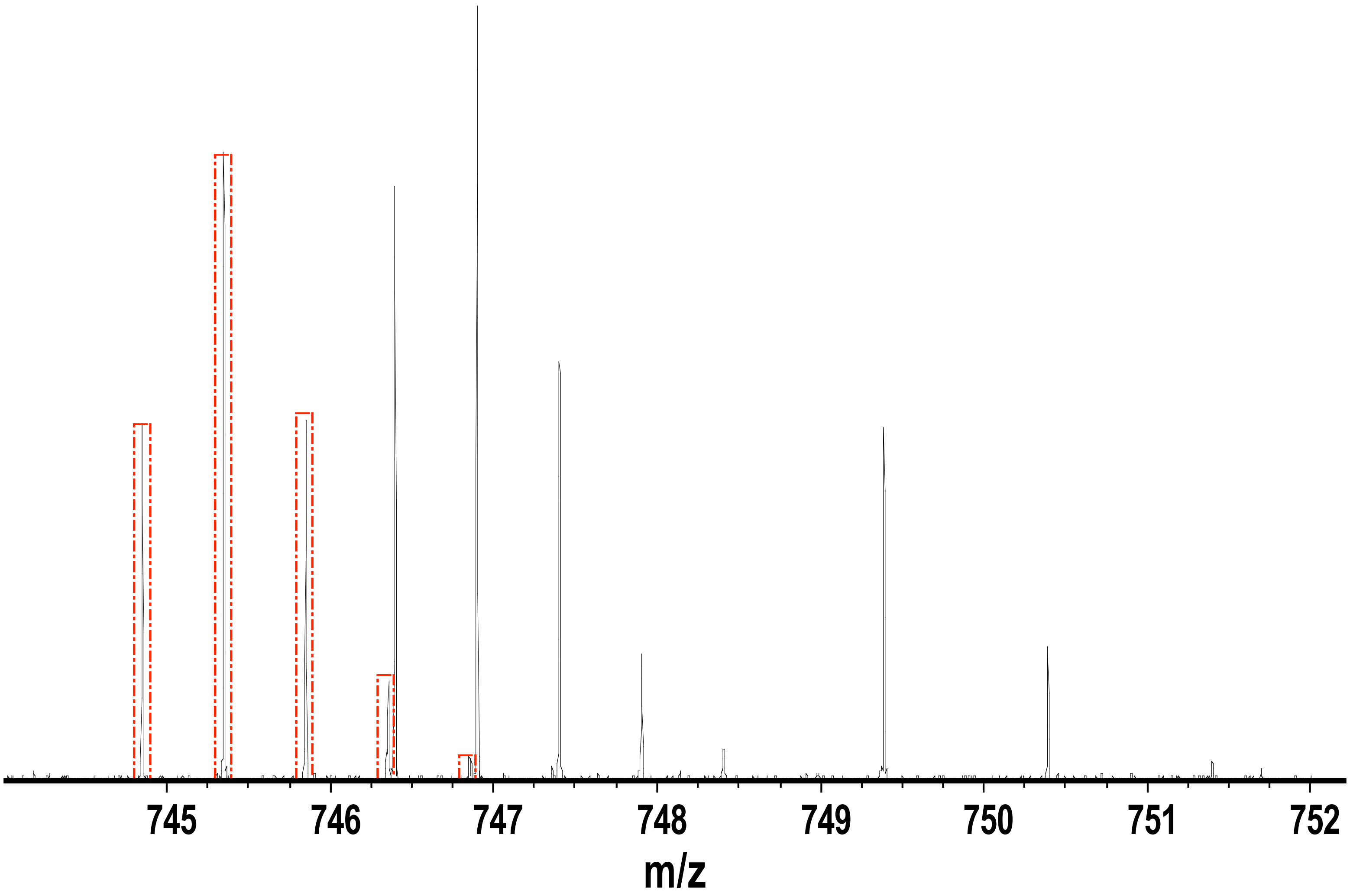


Extremely High Resolution is Achievable with FT-MS



The Related Peaks are Analyzed to Determine the Distribution

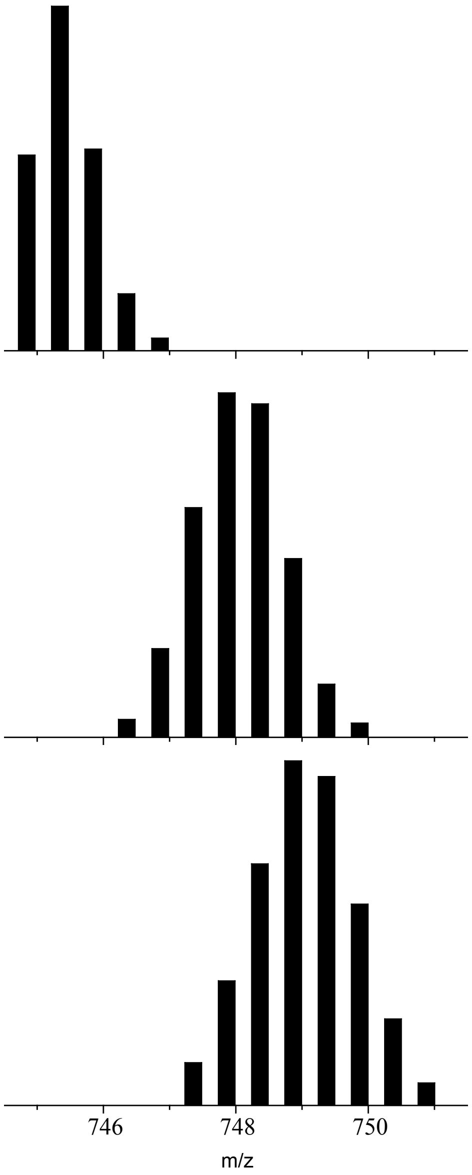


Isotopic distributions during H/D exchange

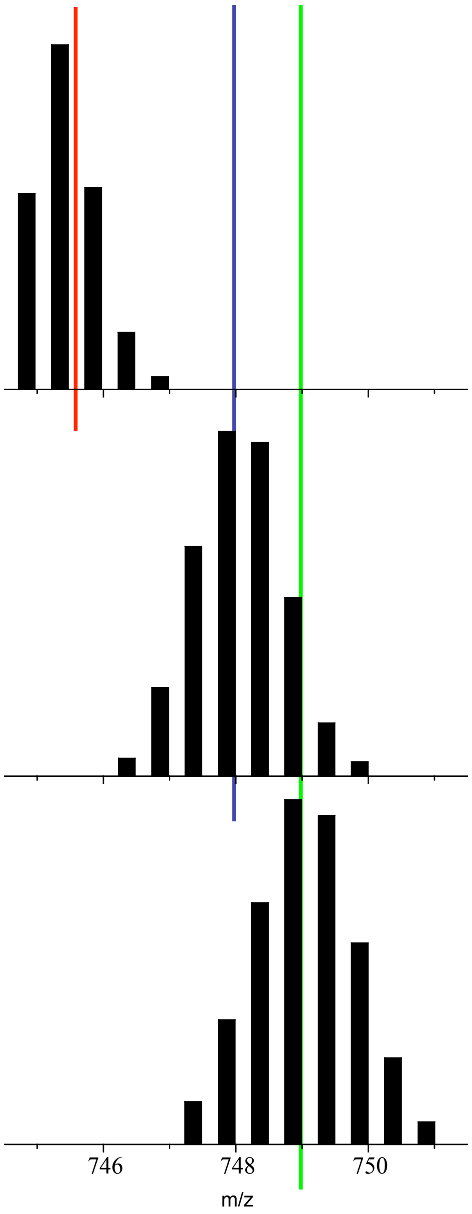
0 min

0.5 min

2 h



The Centroids of the Distribution are Calculated



0 min

Centroid = 745.5

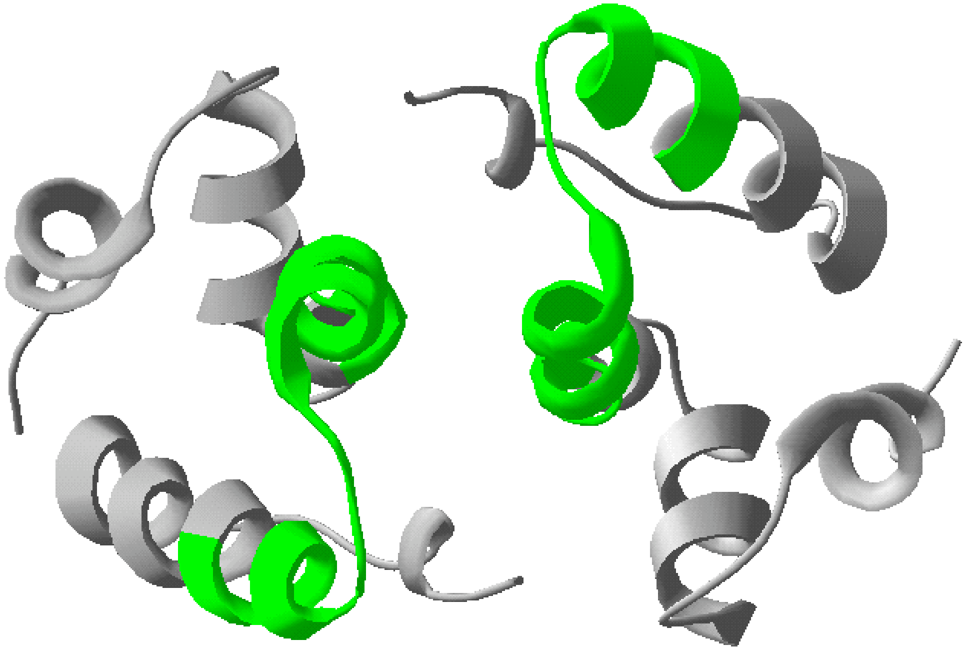
0.5 min

Centroid = 748

2 h

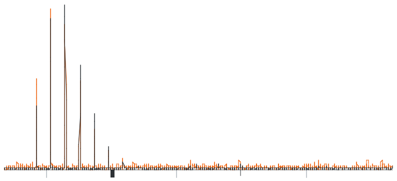
Centroid = 749

Deuterium Incorporation at the Dimer Interface

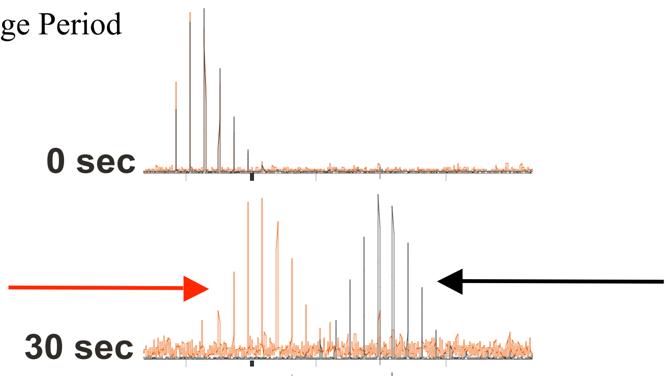


Deuterium Incorporation Causes a Mass Increase

H/D Exchange Period



Deuterium Incorporation Causes a Mass Increase



H/D Exchange Period

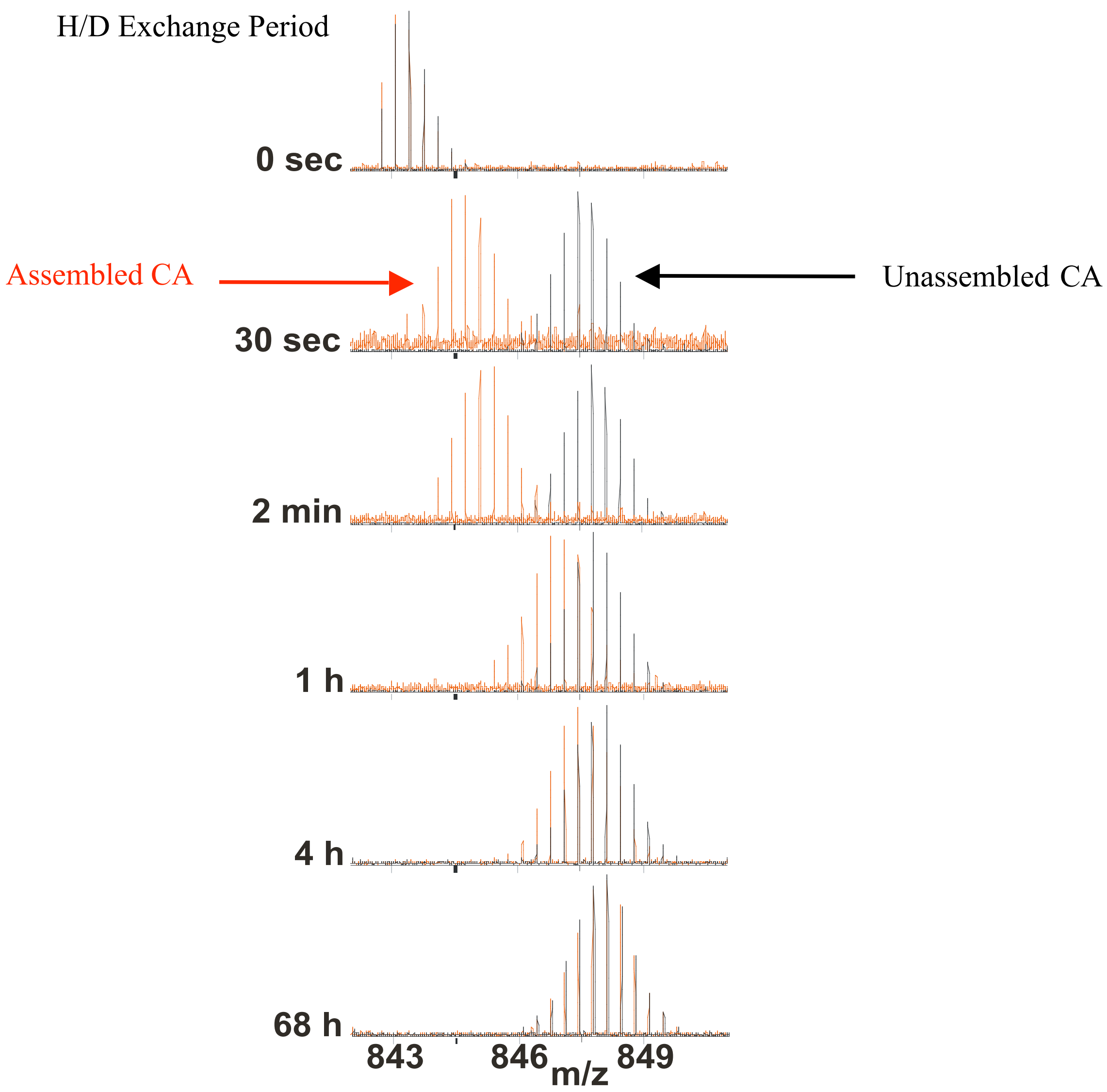
0 sec

Assembled CA

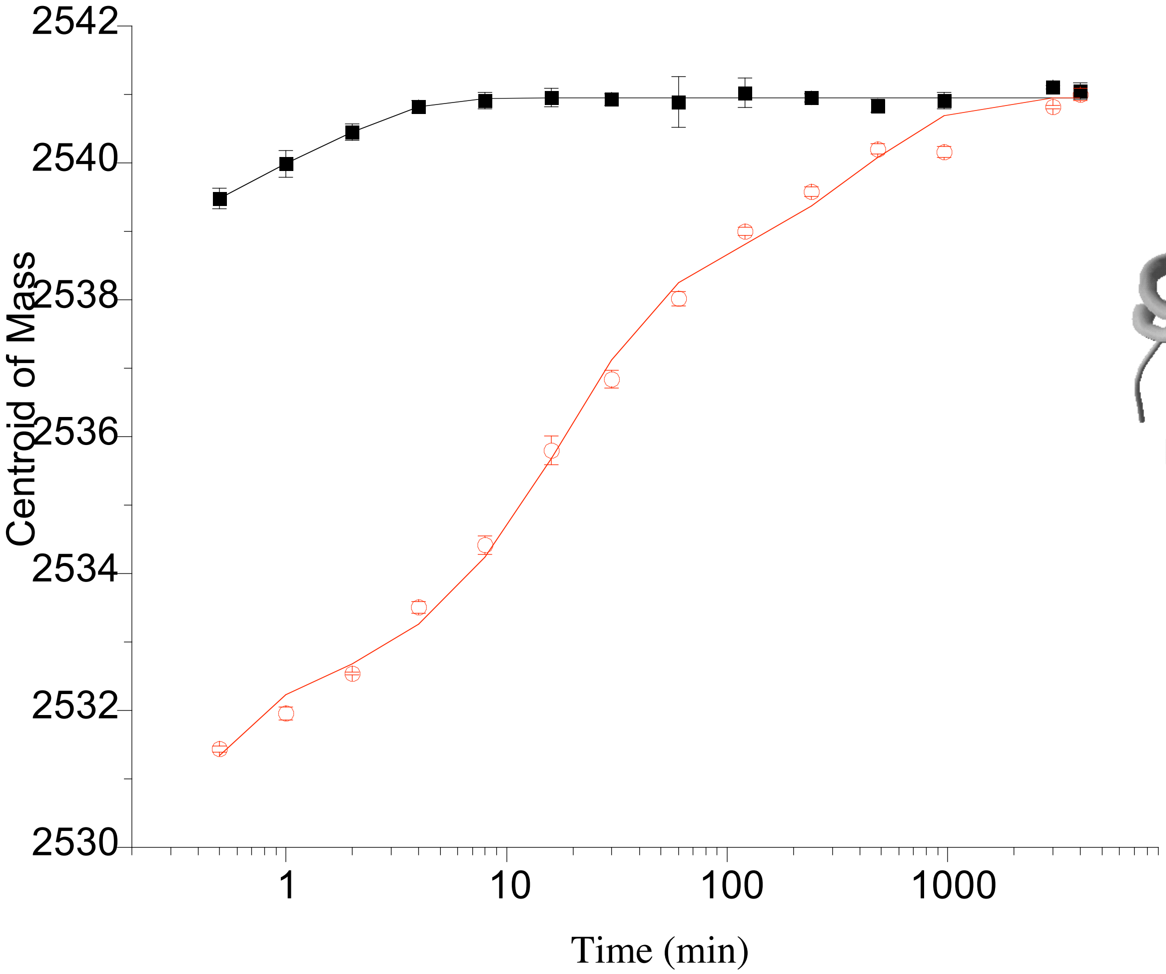
Unassembled CA

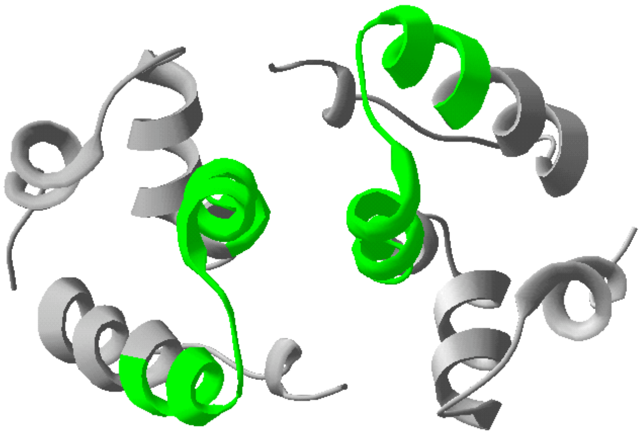
30 sec

Deuterium Incorporation Causes a Mass Increase

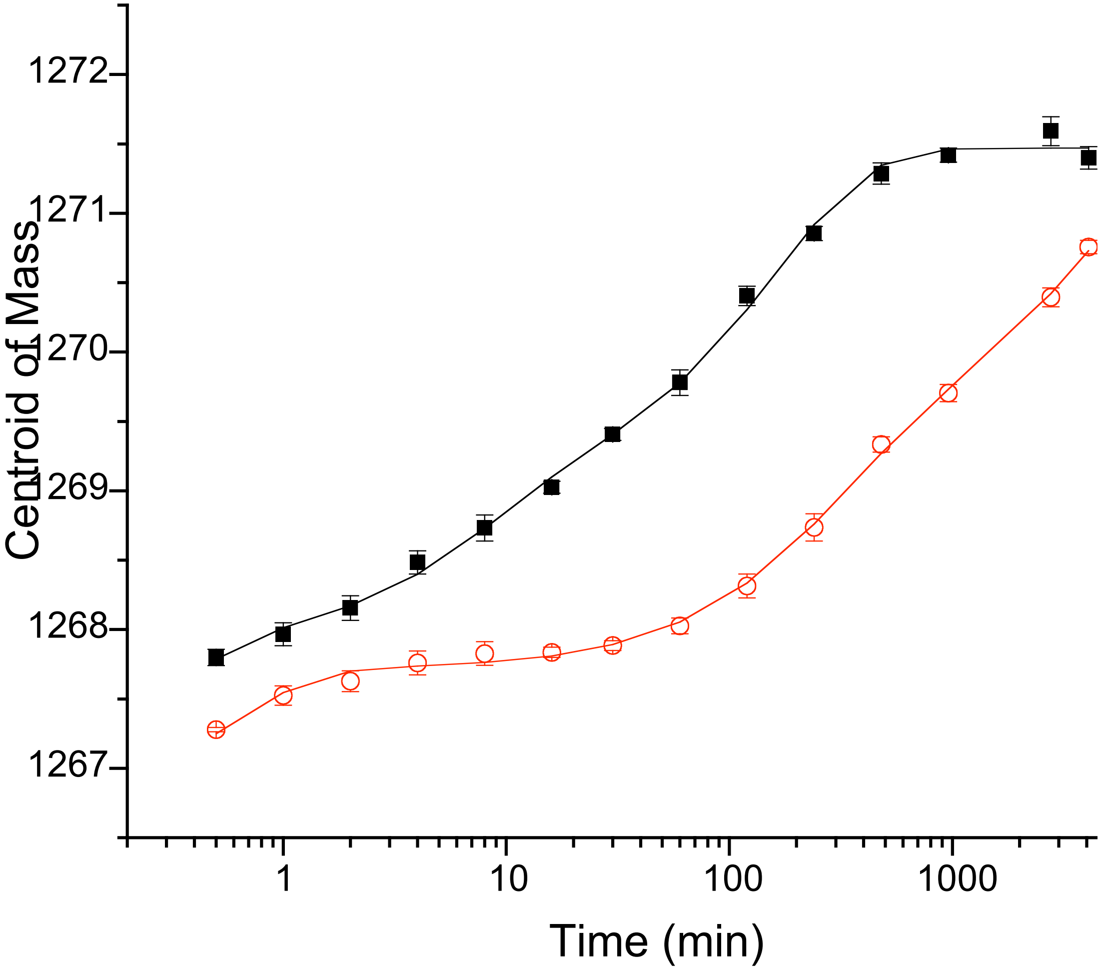


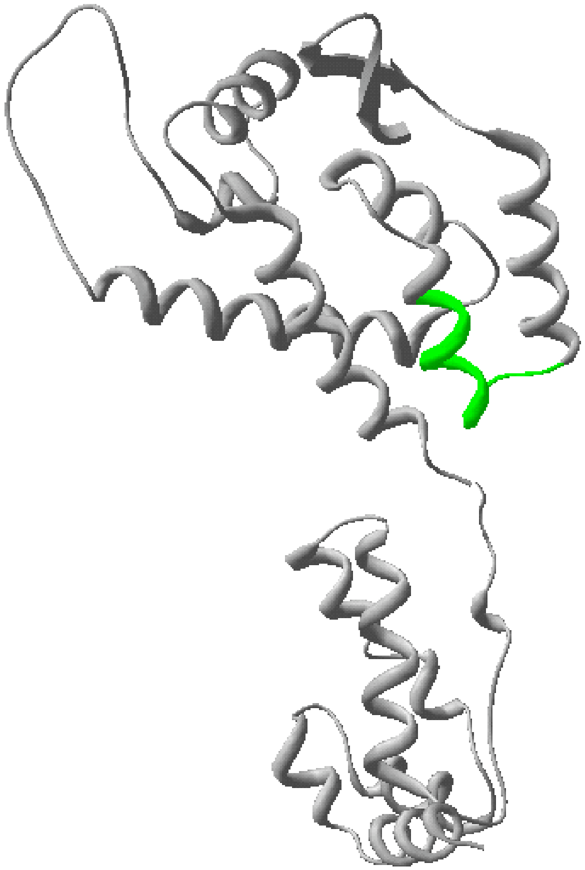
The Dimer Interface is Protected Upon Assembly



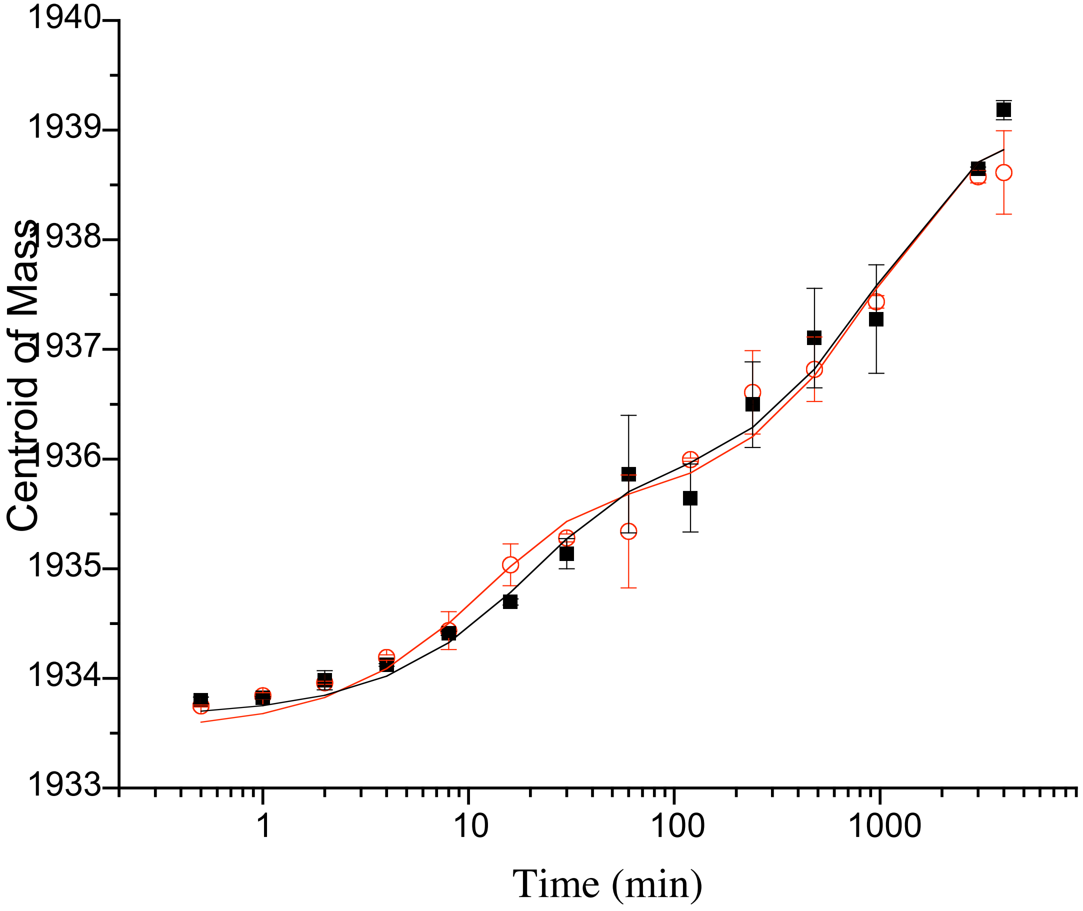


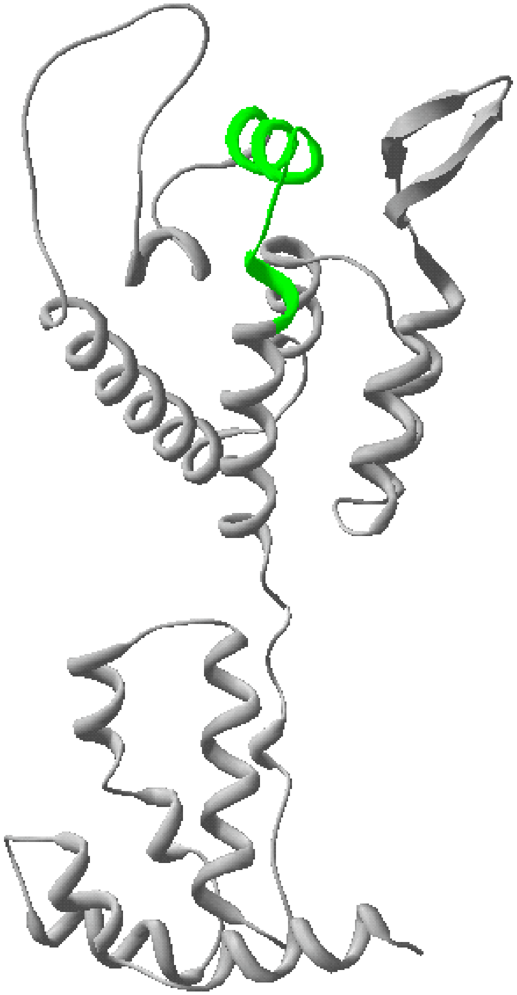
Helix II Shows Protection Upon Assembly



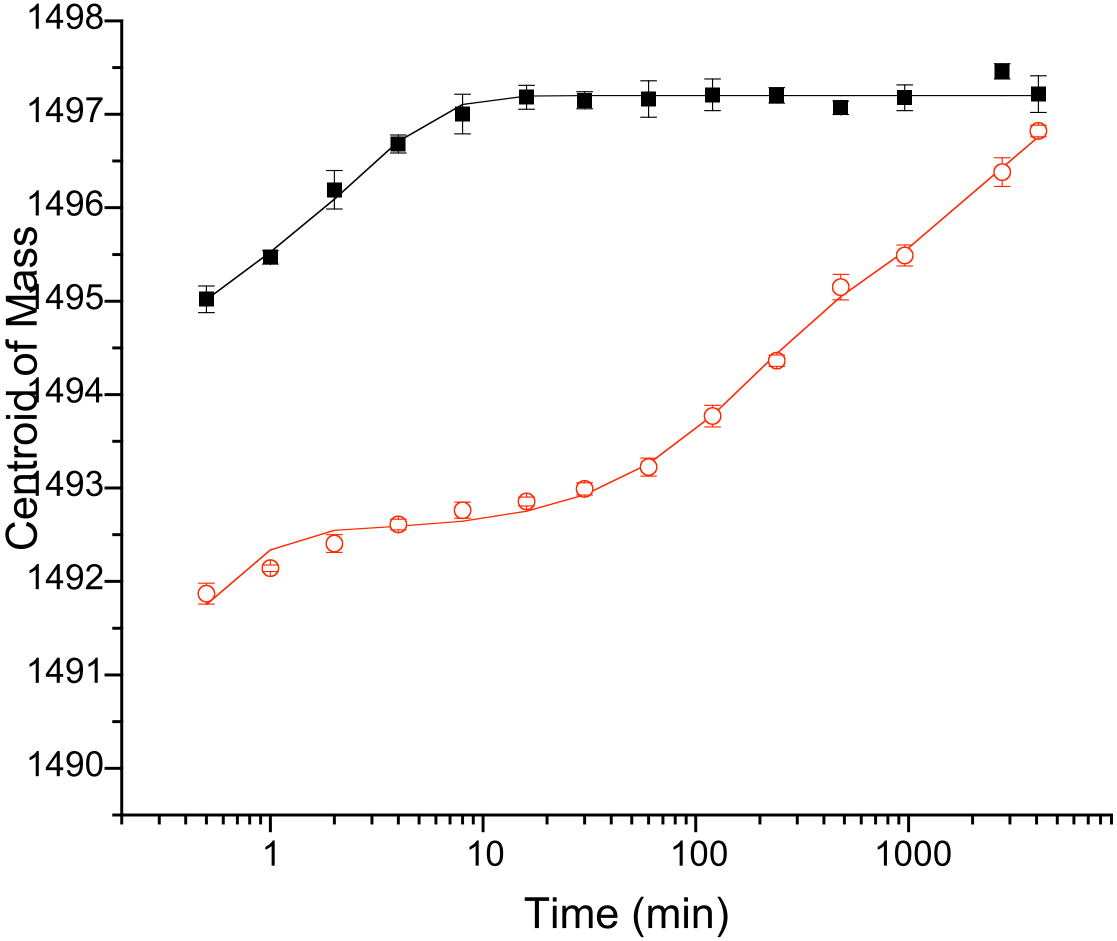


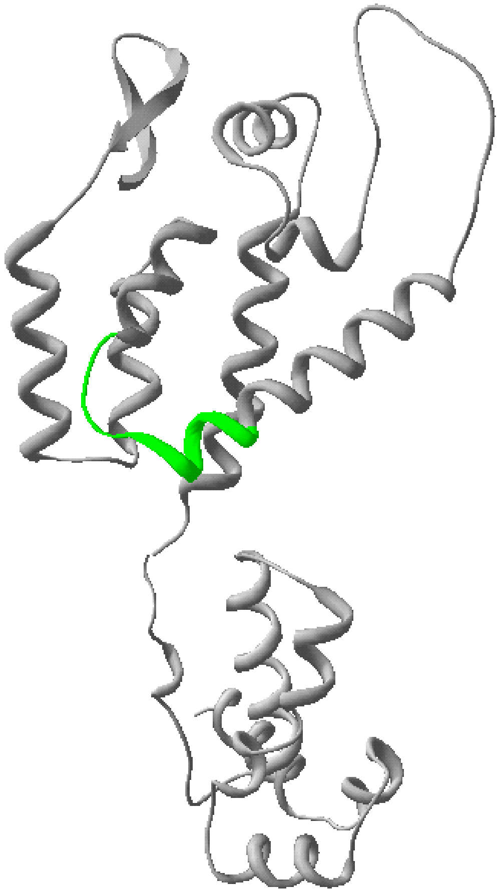
Helices VI and VII Show Little Protection



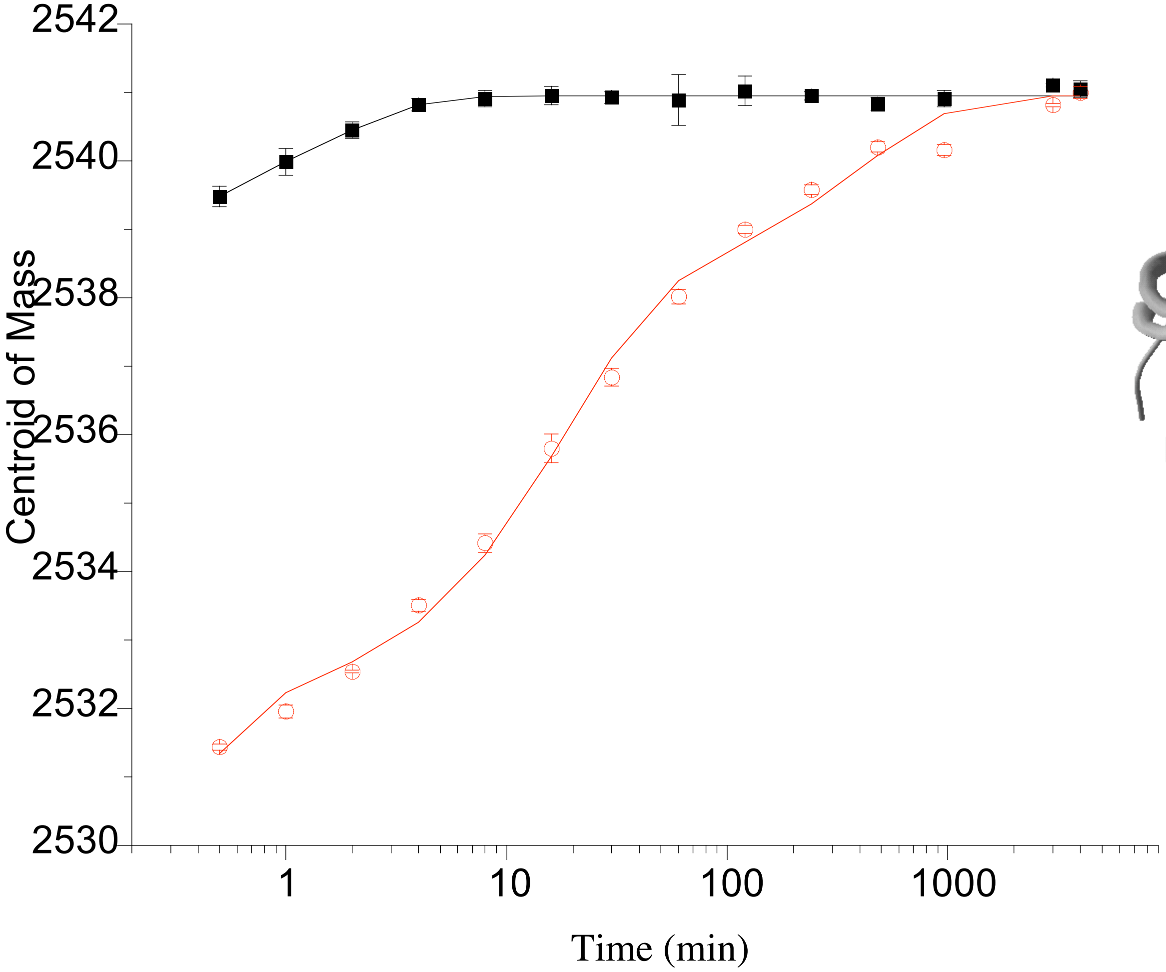


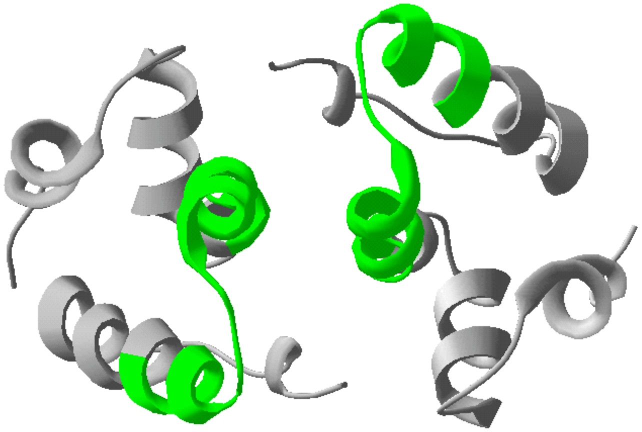
The Bottom of Helix III becomes Protected



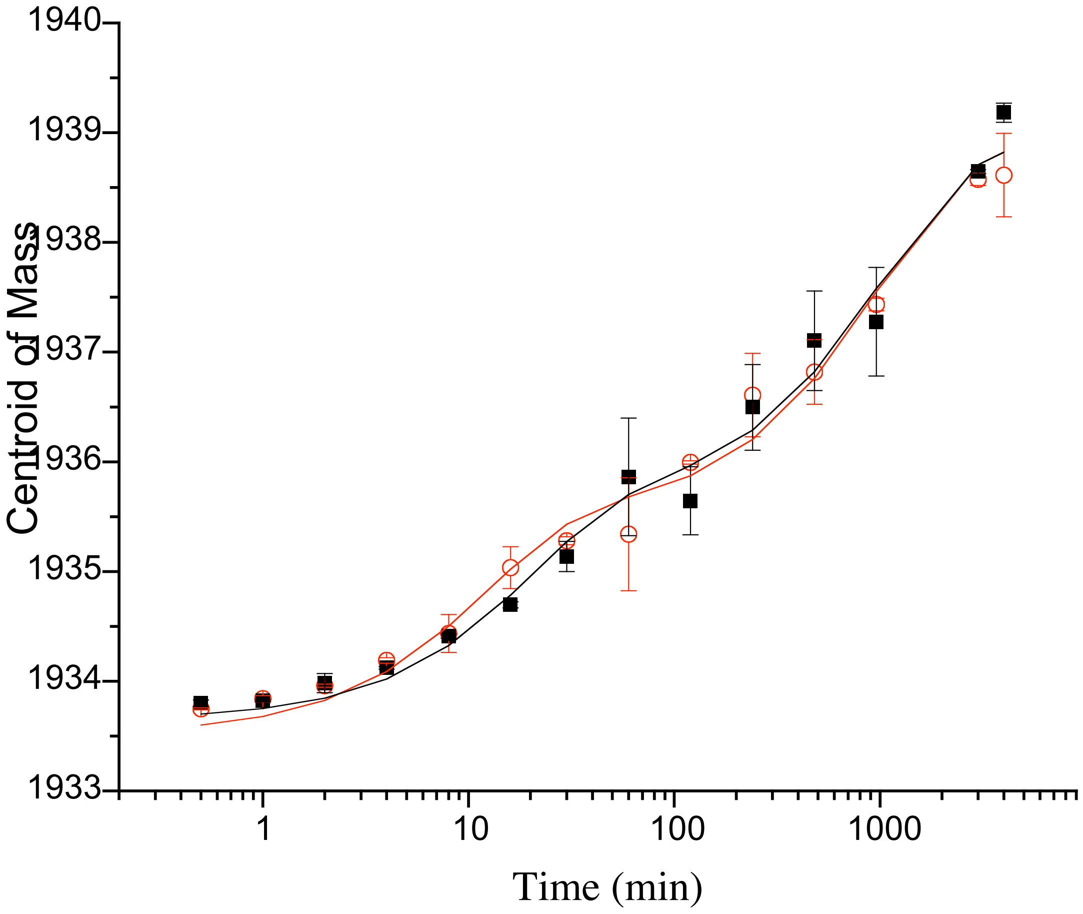


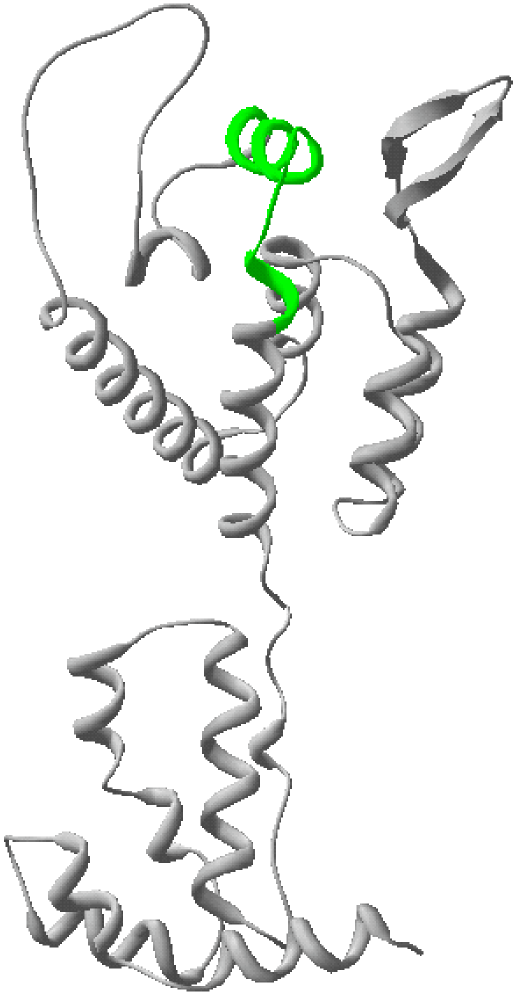
The Dimer Interface is Protected Upon Assembly



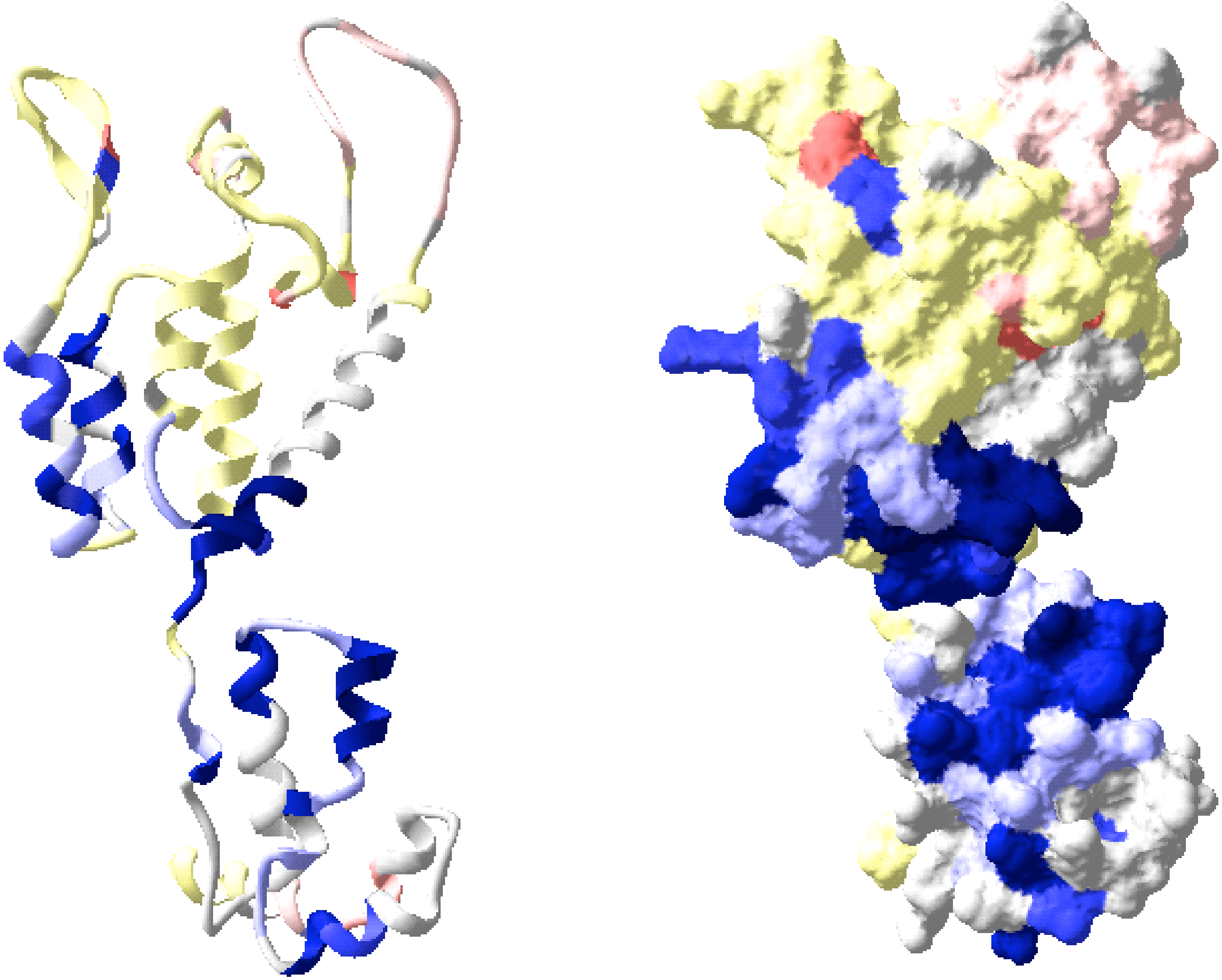


Helices VI and VII Show Little Protection



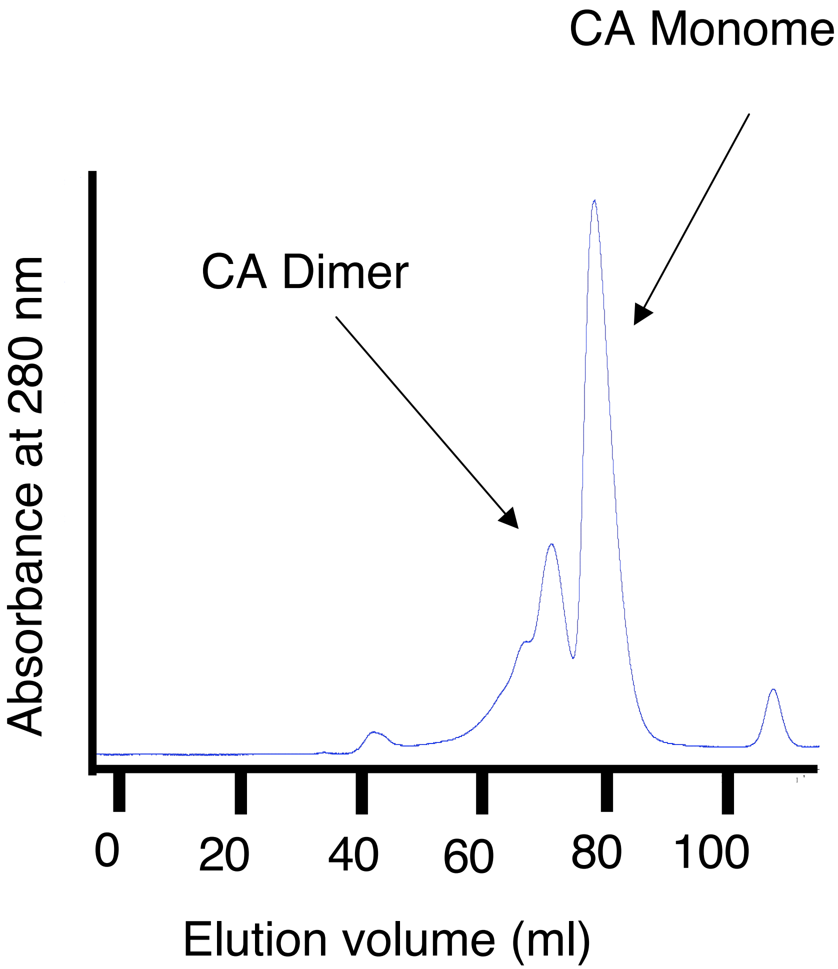


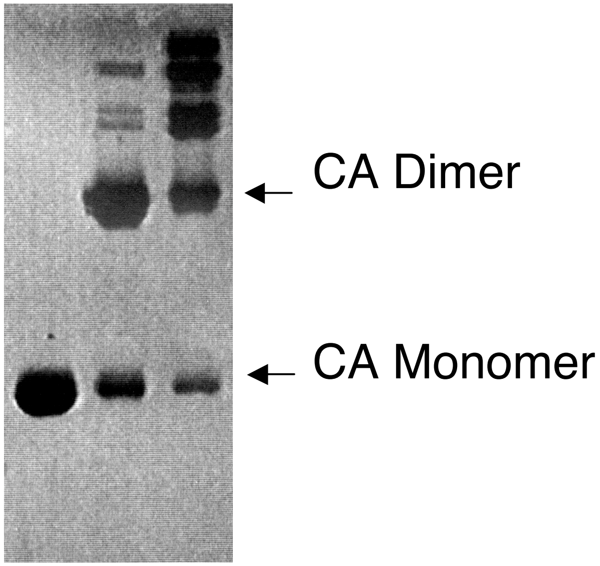
Changes in Exchange Rates due to CA Assembly



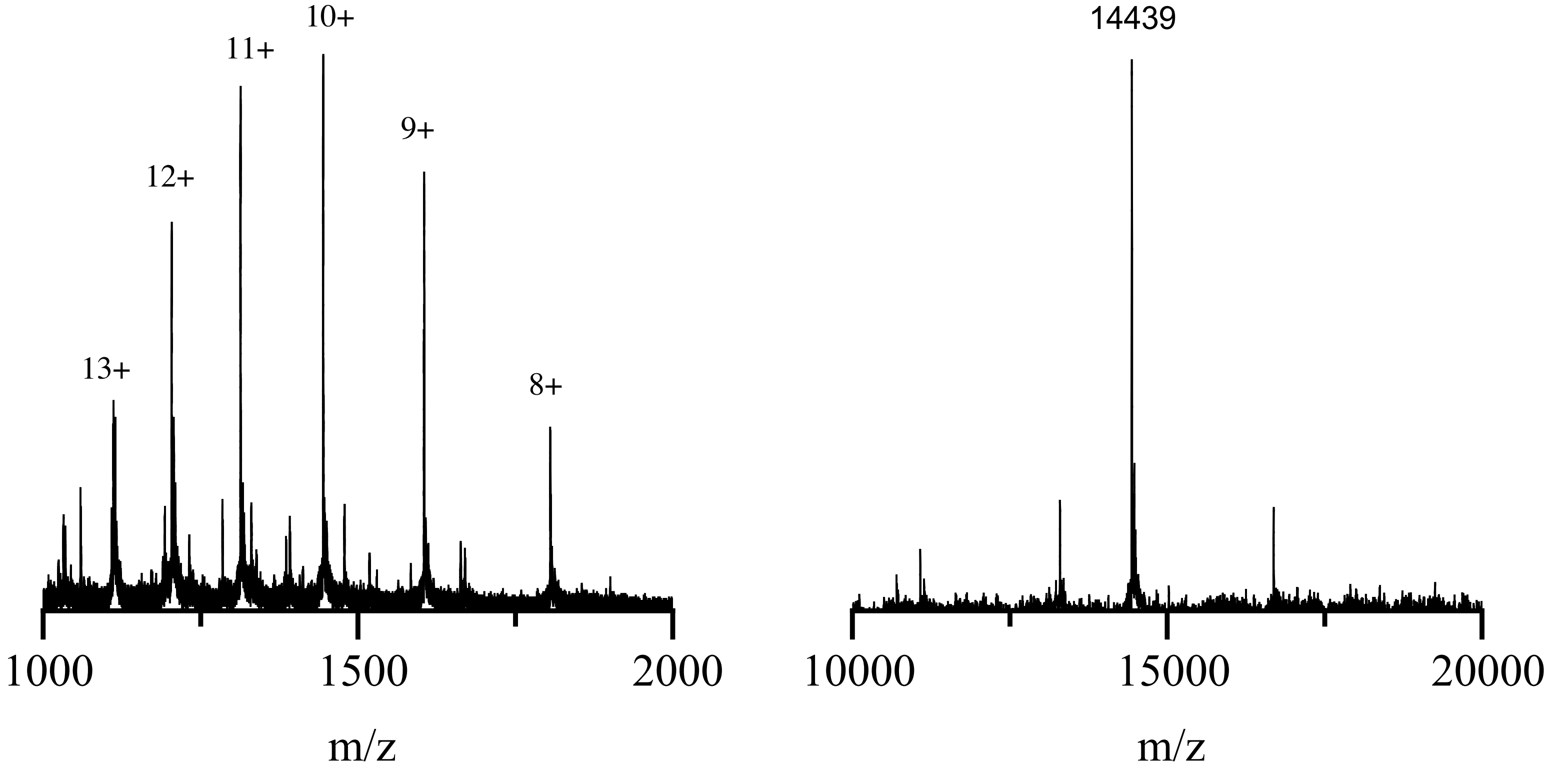
Lanman, *et. al. 2003*

SEC separation of cross-linked CA monomer and dimer





Mass spectra of the cross-linked species



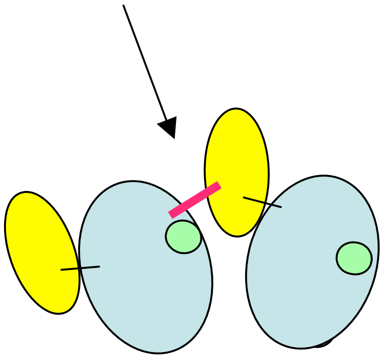
Lysine 70 was cross-linked to Lysine 182

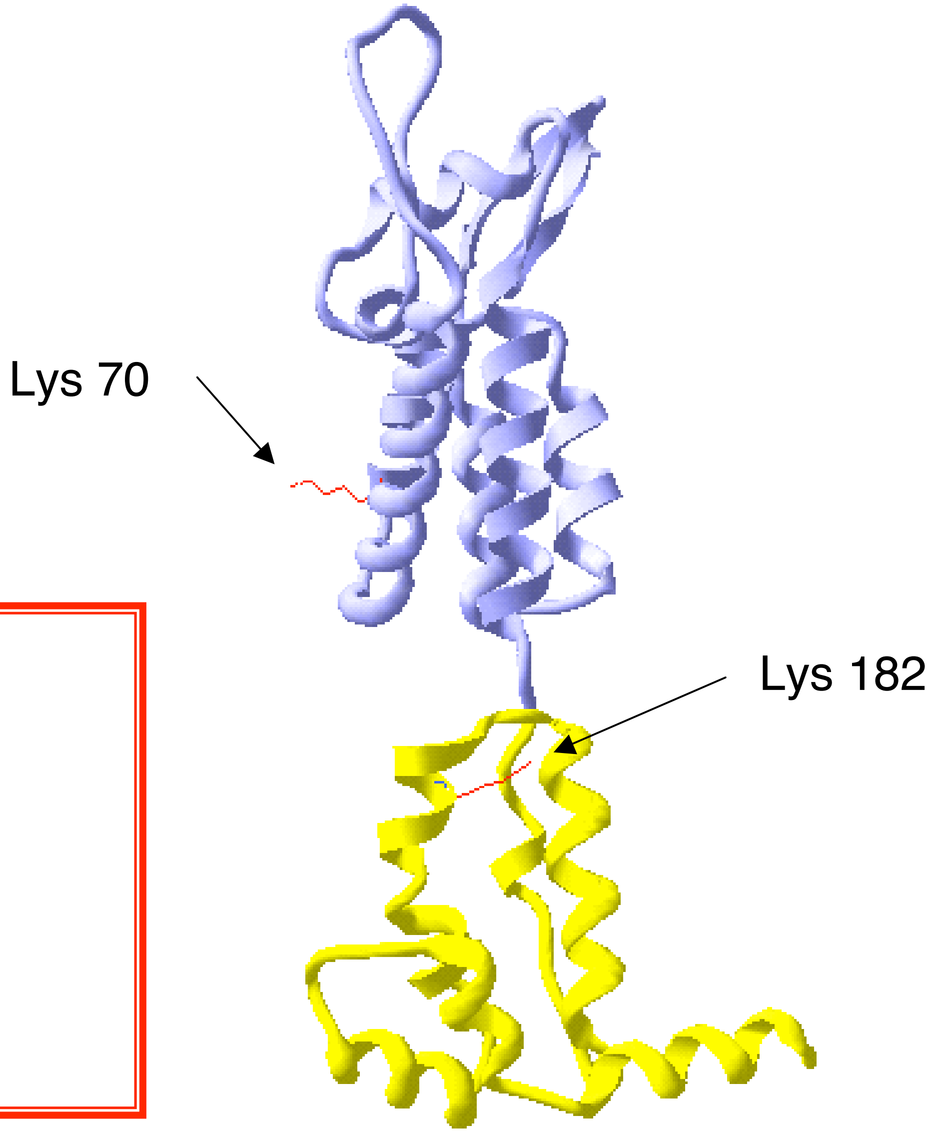
31 to 131 = 10948.9
171 to 199 = 3375.9
+DST = 114.0

Expected mass = 14438.8

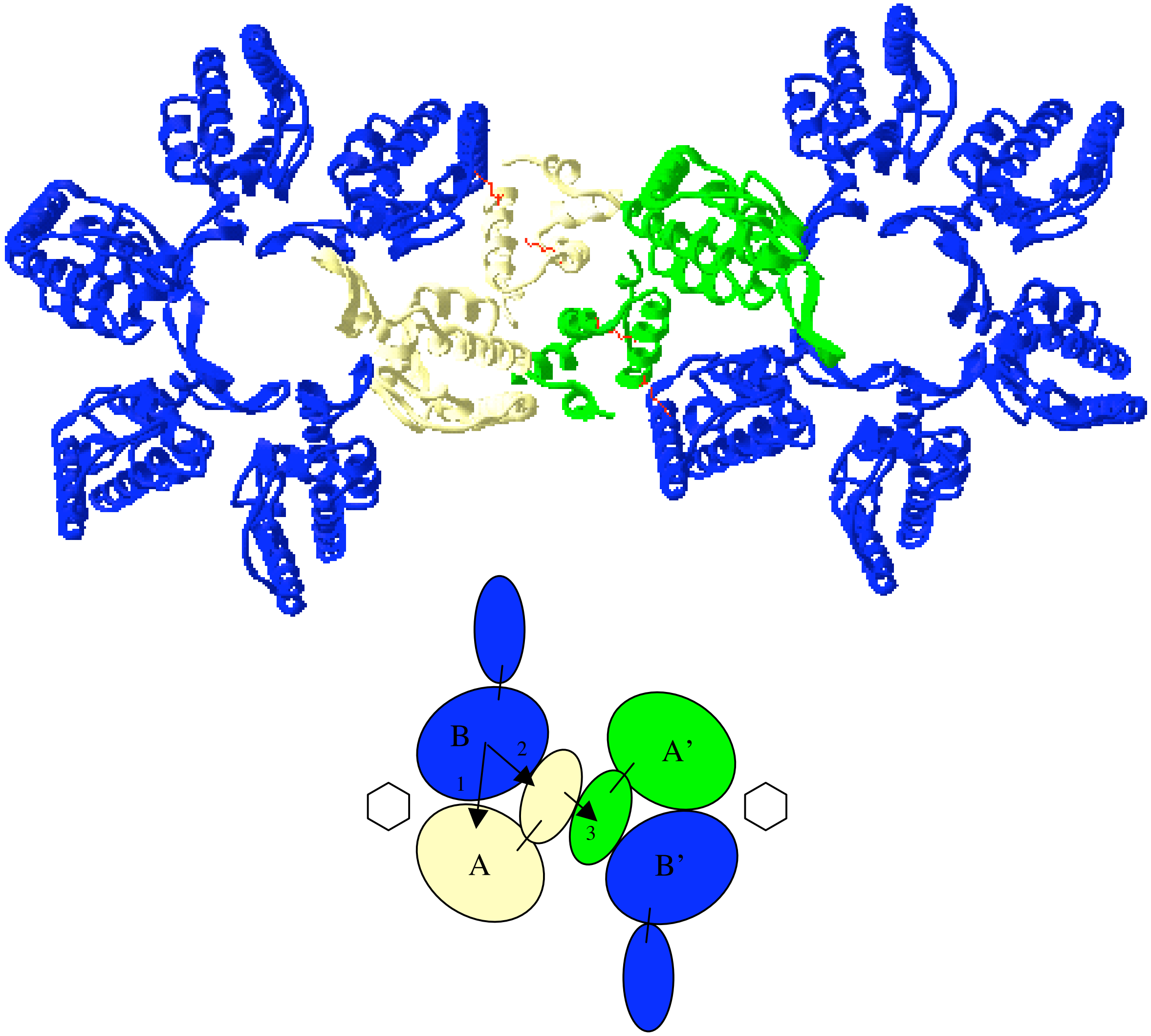
Observed mass = 14439

Lys70 cross-linked to 182

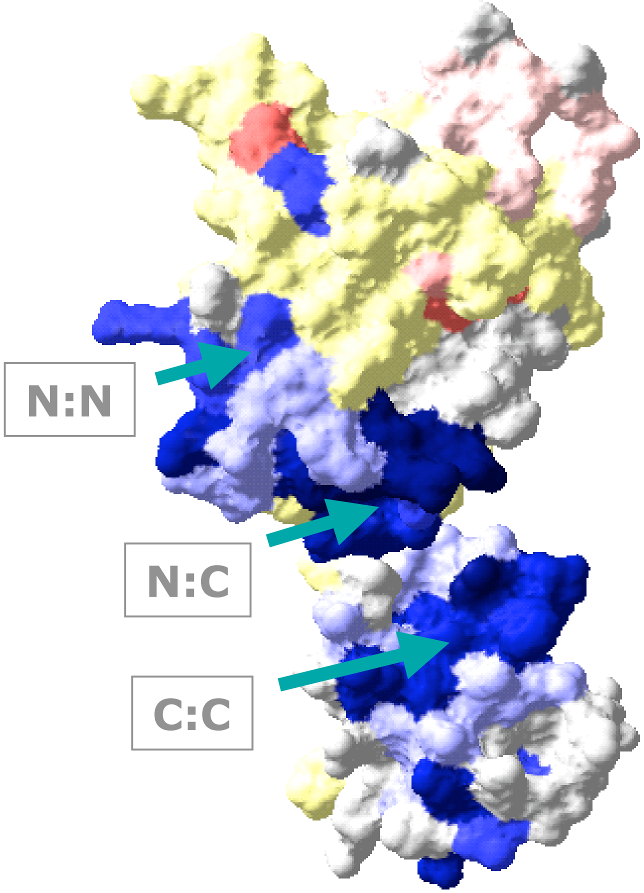




An N-domain to C-domain Interaction in CA Assembly



Three Sites of Interaction During Assembly



Advantages of Hydrogen/Deuterium Exchange

- Small quantities required (10^{-12} mole)
- Needn’t be pure
- No symmetry constraints
- Can provide time resolved or dynamic information

The future of H/D exchange

Multi protein macromolecular complexes

Cooperativity in large proteins or macromolecular complexes

Exchange rates for individual amide protons

Protein dynamics during motor motions