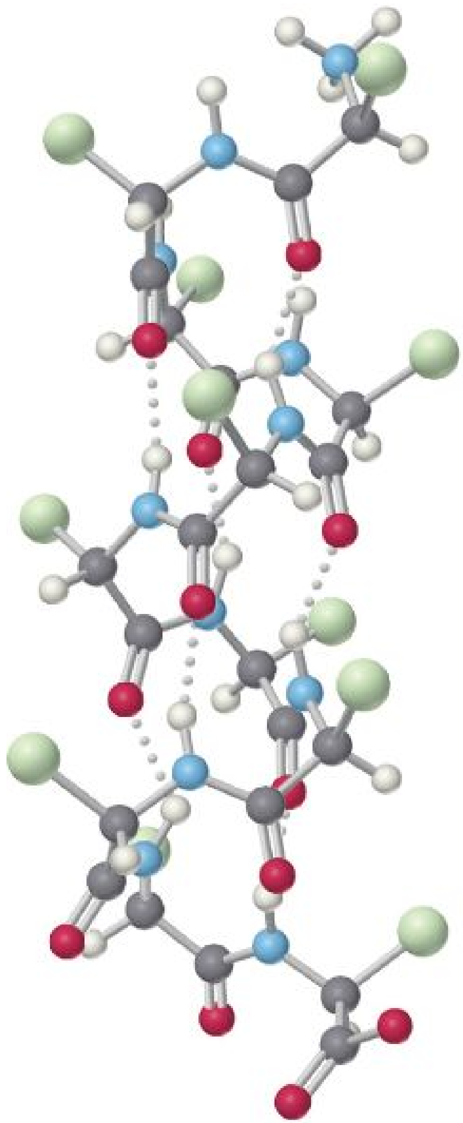
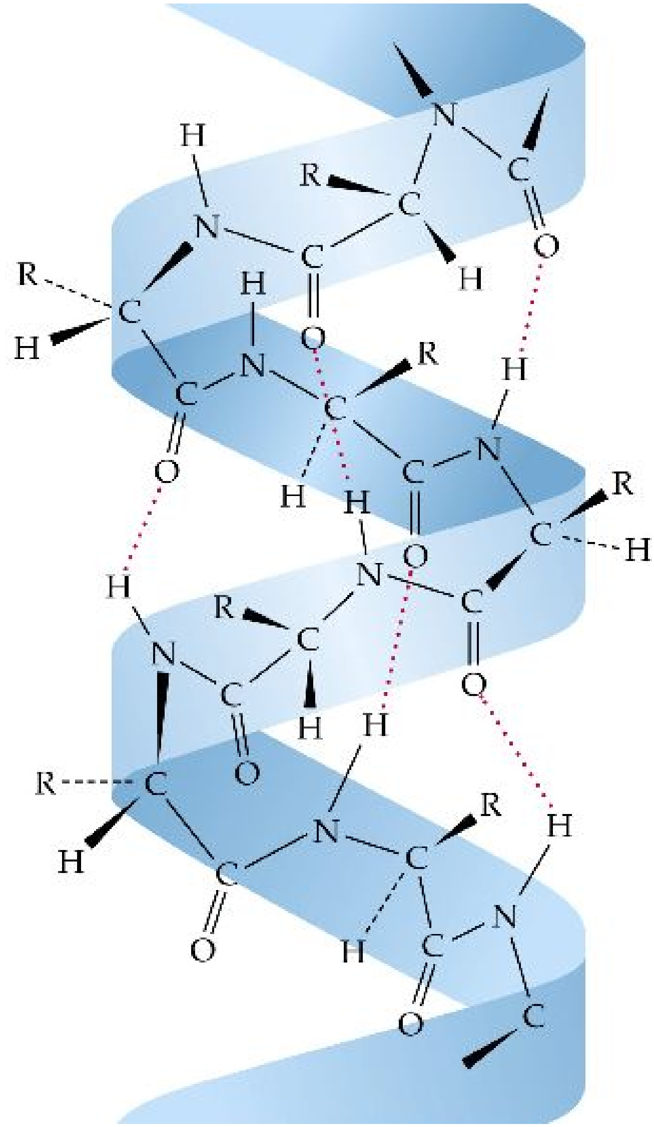
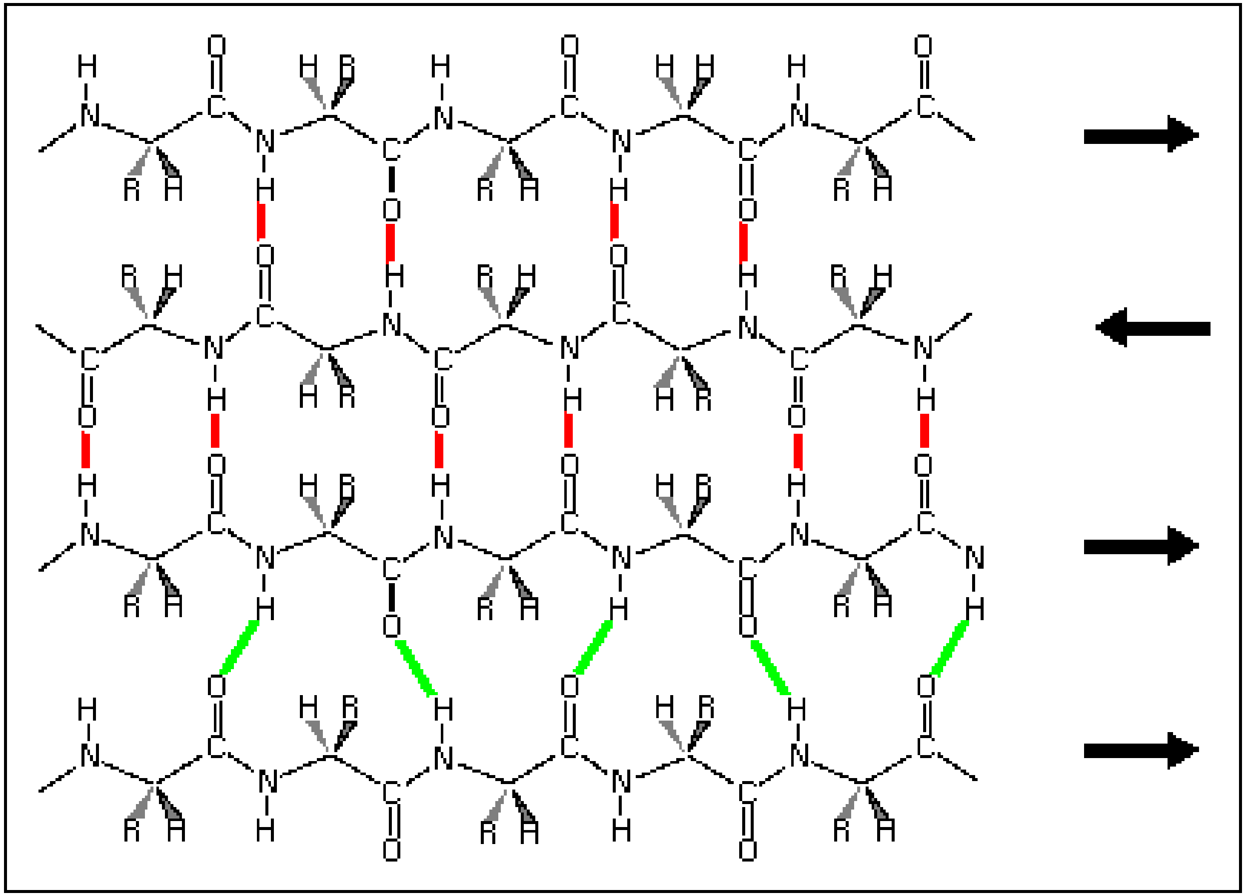
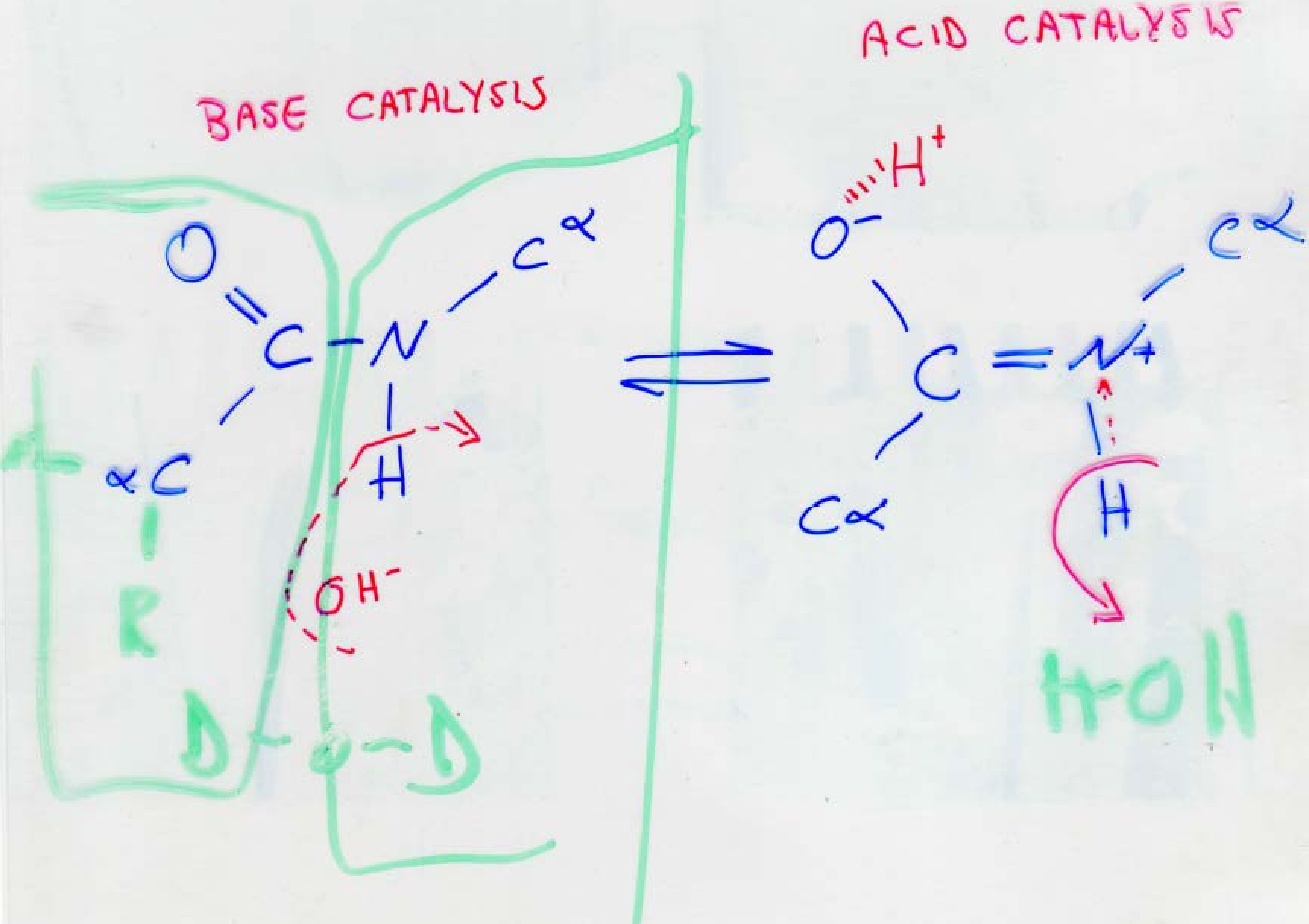
Structure of the α-Helix



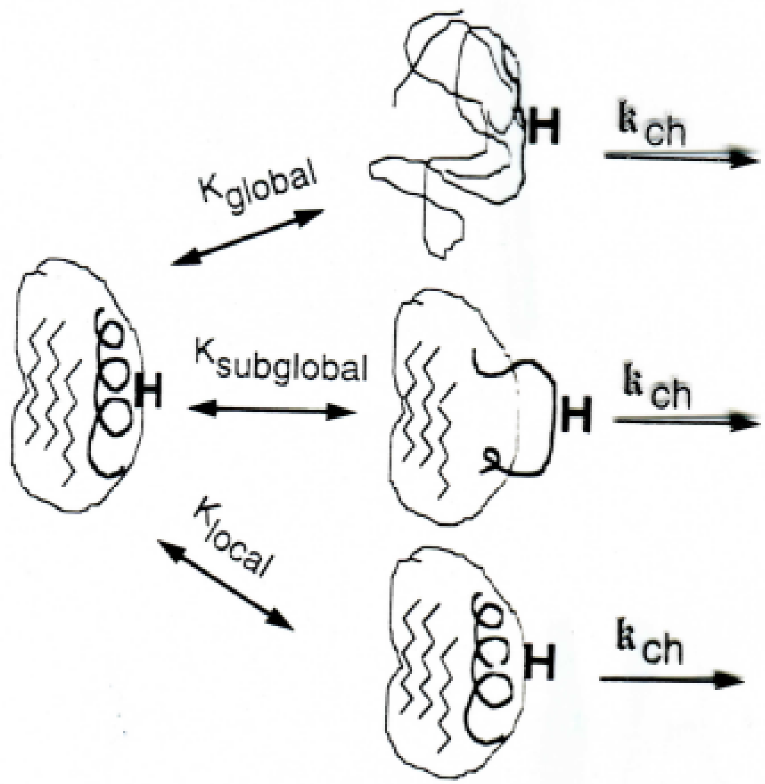


Structure of the β–Sheet




BASE CATALYSIS
ACID CATALYSIS
O
C
N
Cα
αC
H
R
OH⁻
D
D
H⁺
N⁺
H
HOH

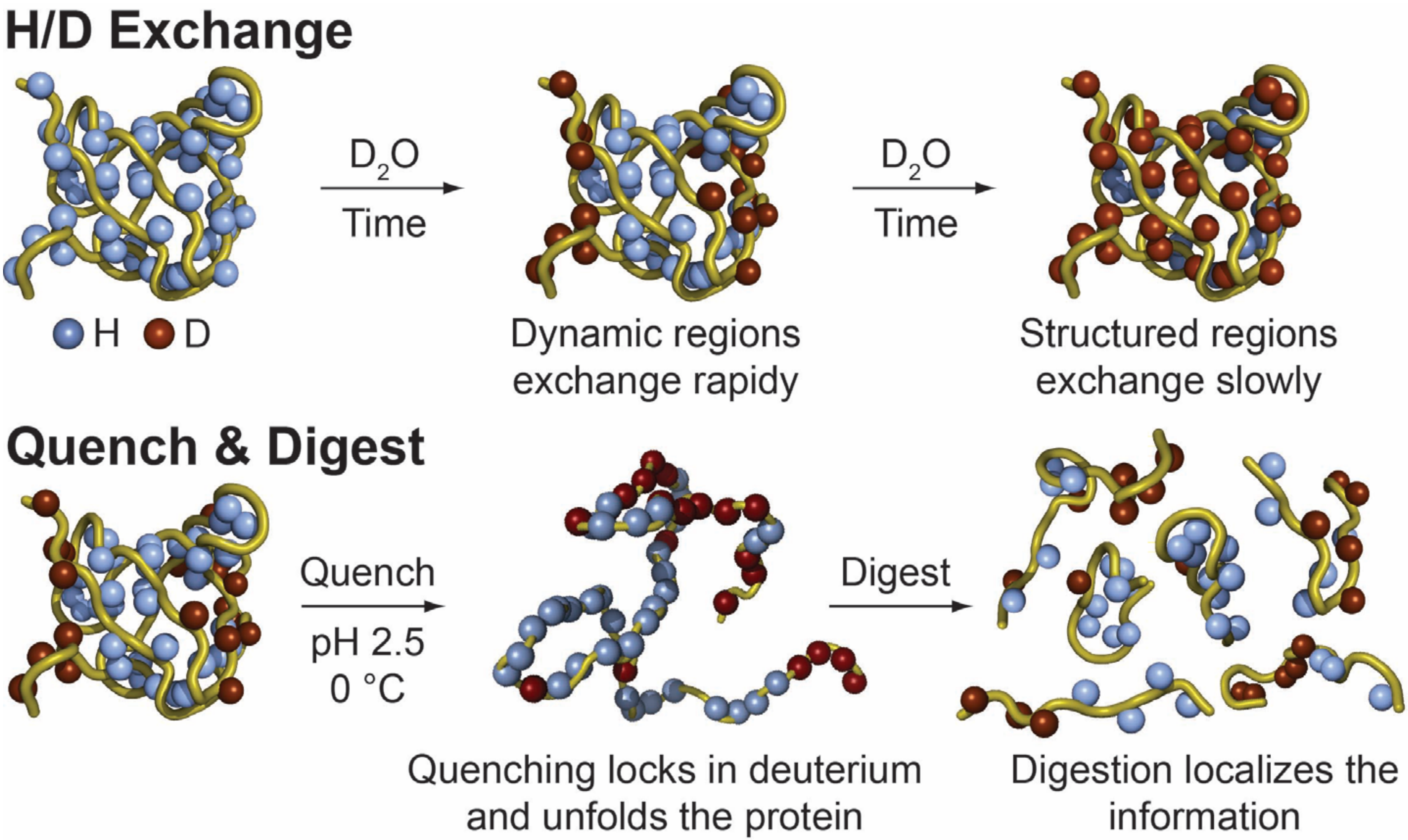
Protein Dynamics



$$k_{ex} = K_{ap} k_{ch}$$

$$\Delta G = -RT \ln K_{ap}$$

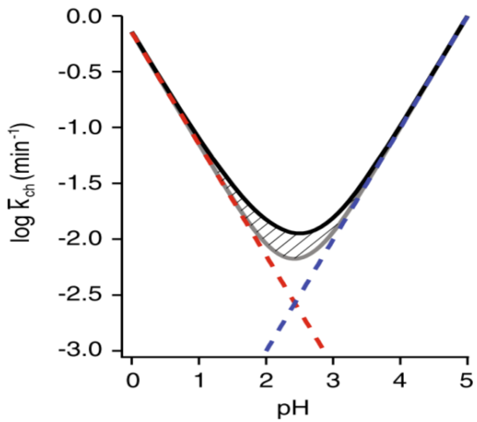
[redacted]


H/D Exchange
D2O
Time
D2O
Time
H
D
Dynamic regions exchange rapidy
Structured regions exchange slowly
Quench & Digest
Quench
pH 2.5
0 °C
Digest
Quenching locks in deuterium and unfolds the protein
Digestion localizes the information

Basics of Quenching HDX

Hydrogen exchange of amide protons is catalyzed by H_2O, OH^-, and H_3O^+, but it's most dominated by base catalyzed reactions.

Ka	6.95E-01
Kb	**1.87E+08**
Kw	5.27E-04



In addition to lowering the pH, chemical kinetics are considerable slowed with decreasing temperature.

$$k = Ae^{\frac{-E_a}{k_B T}}$$

Place of Quenched Reaction	Half Life of Back Exchange
My hand	1 minute
In a Theoretical Lab	4 minutes
In a UAB Lab	7 minutes
On Ice (0C)	1 hour
In the -20C Fridge	14 hours
In the -80C Fridge	7 Years
In Liquid Nitrogen	When Hell Freezes Over

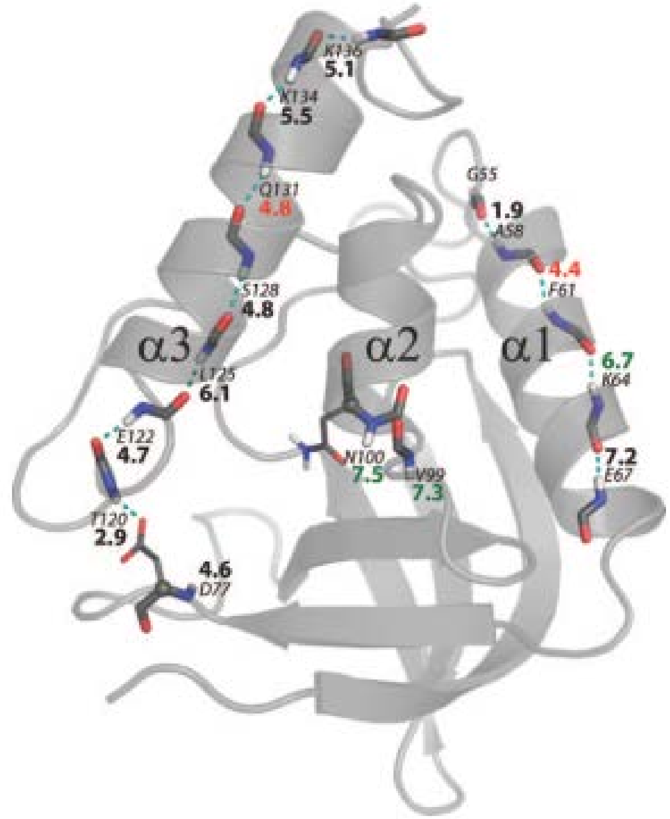
Kinetics of HDX

For exchange to occur:

1. The site needs to be at least transiently exposed to the bulk solvent.

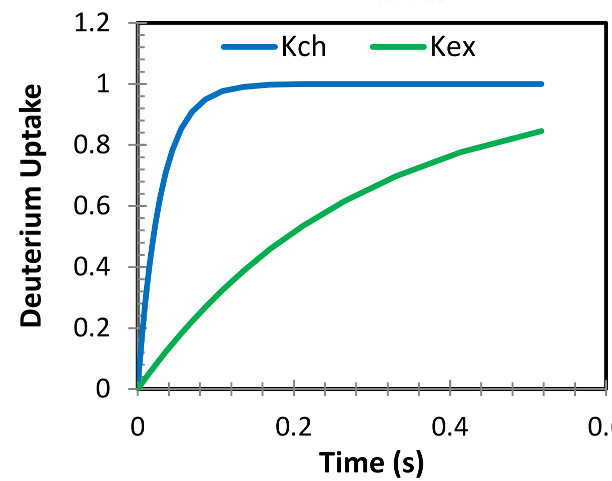
2. The site must be available to form new hydrogen bonds.

If it is either exposed with an unavailable site for hydrogen bonding or buried (most always hydrogen bonded in this case), it is described as <u>**protected**</u>.

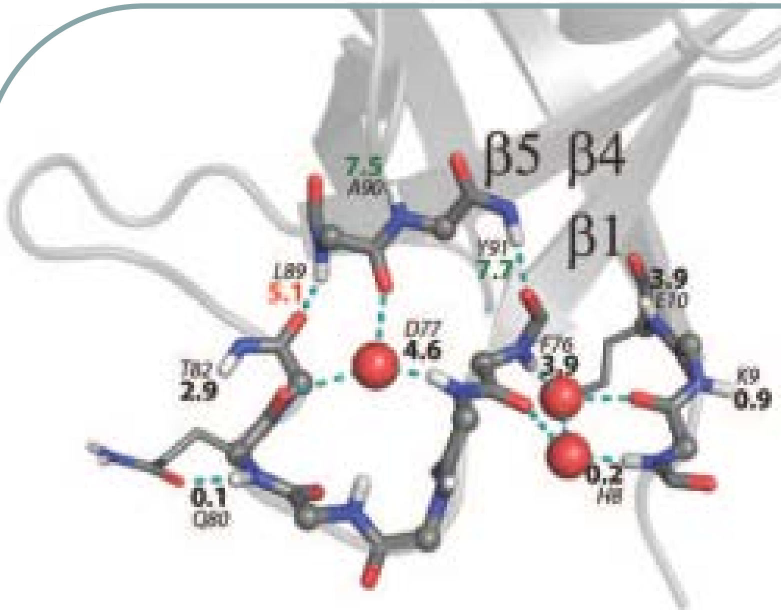


In HDX experiments, the degree of protection (P_f) is empirically determined by the extent the rate of exchange (k_{ex}) is slowed from the predicted chemical rate (k_{ch}) .

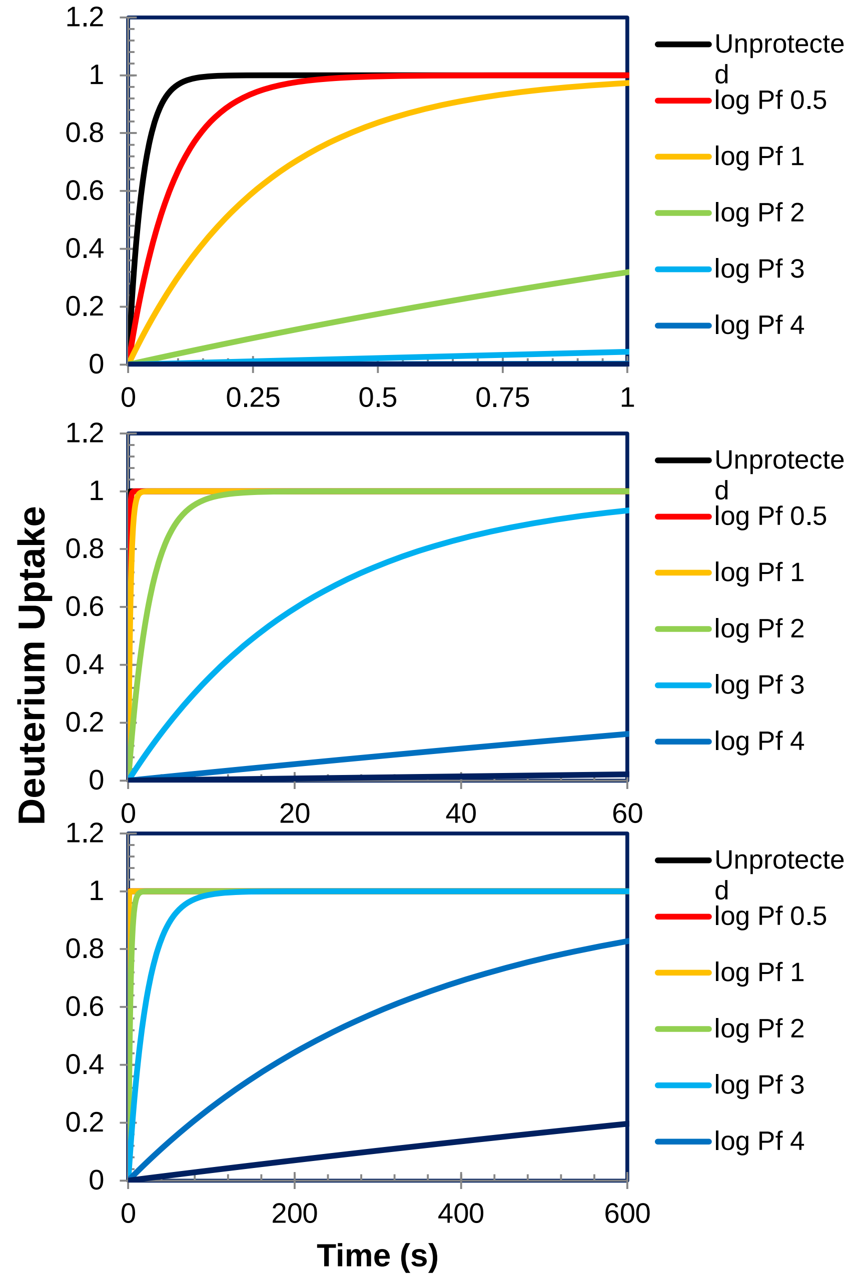
$$P_f = \frac{k_{ch}}{k_{ex}} \longrightarrow \log P_f = \log \frac{k_{ch}}{k_{ex}}$$



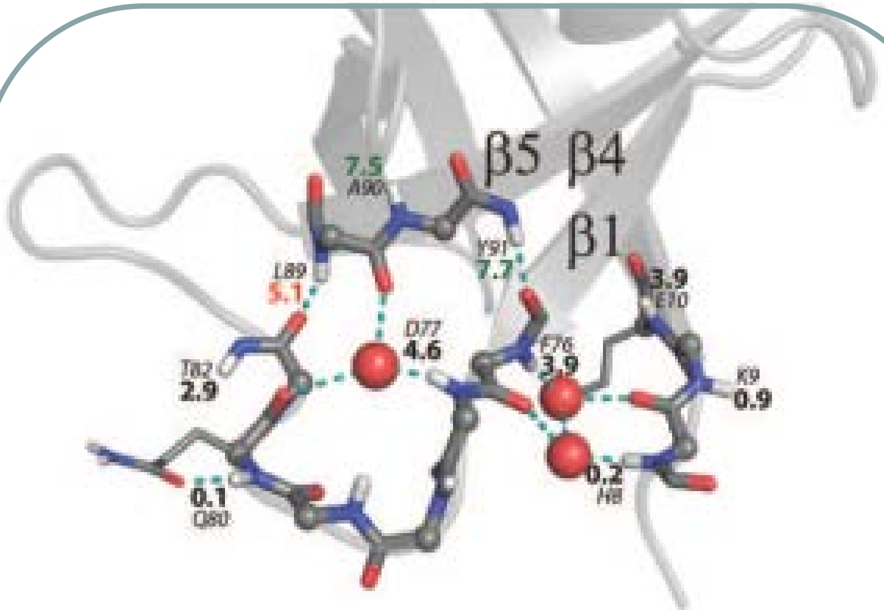
Kinetic Range of Protection



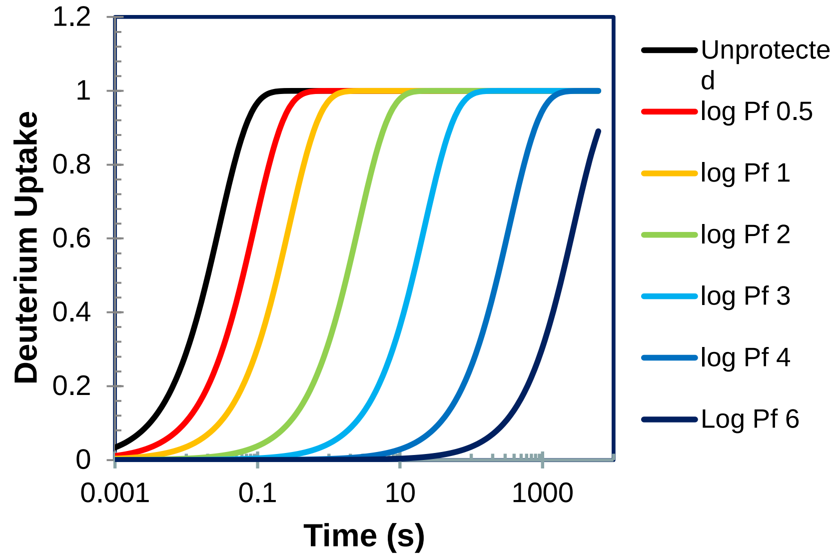
A wide span of different kinetics can be observed for the range of protection that can occur within a single protein.



Kinetic Range of Protection

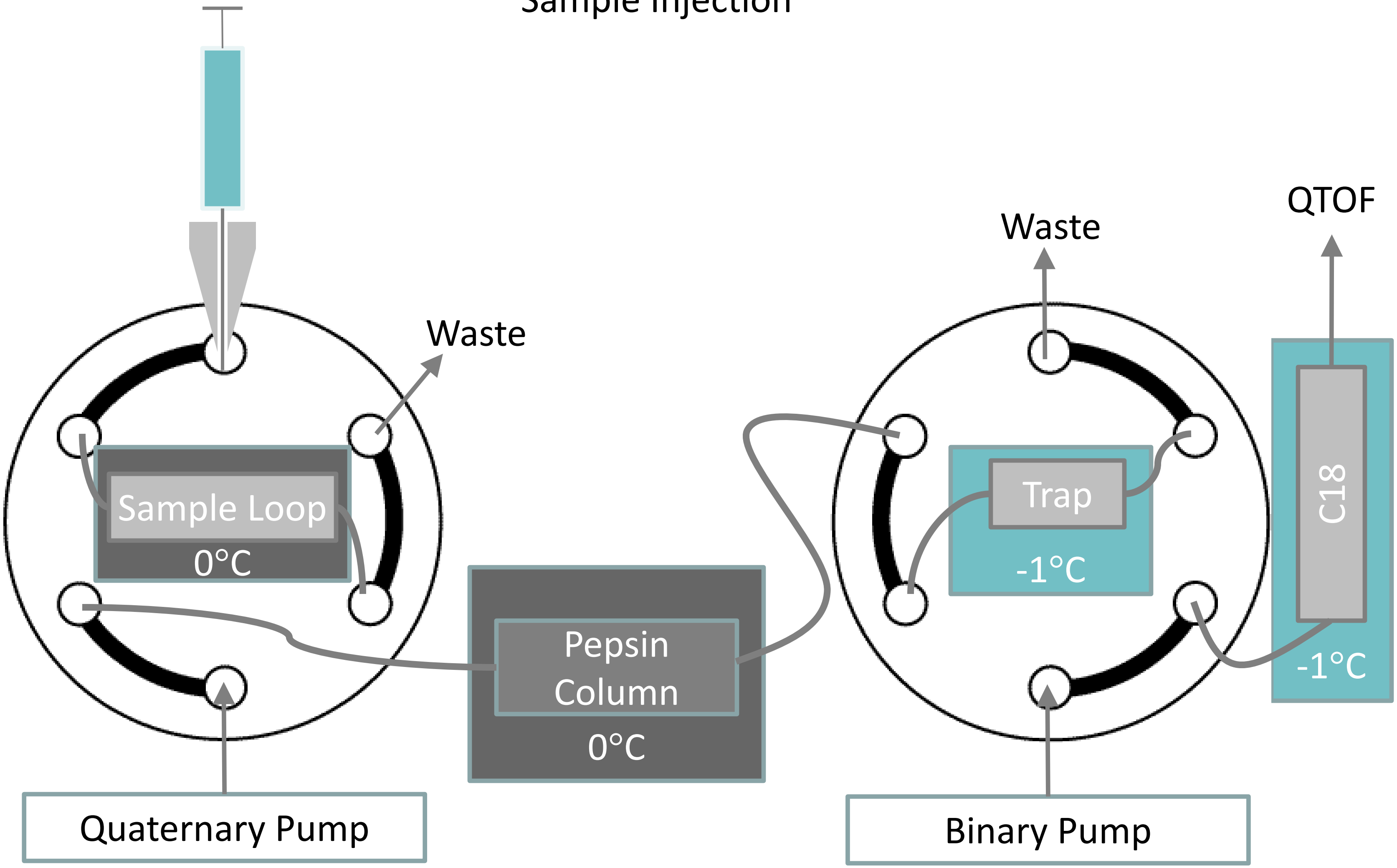


A wide span of different kinetics can be observed for the range of protection that can occur within a single protein.

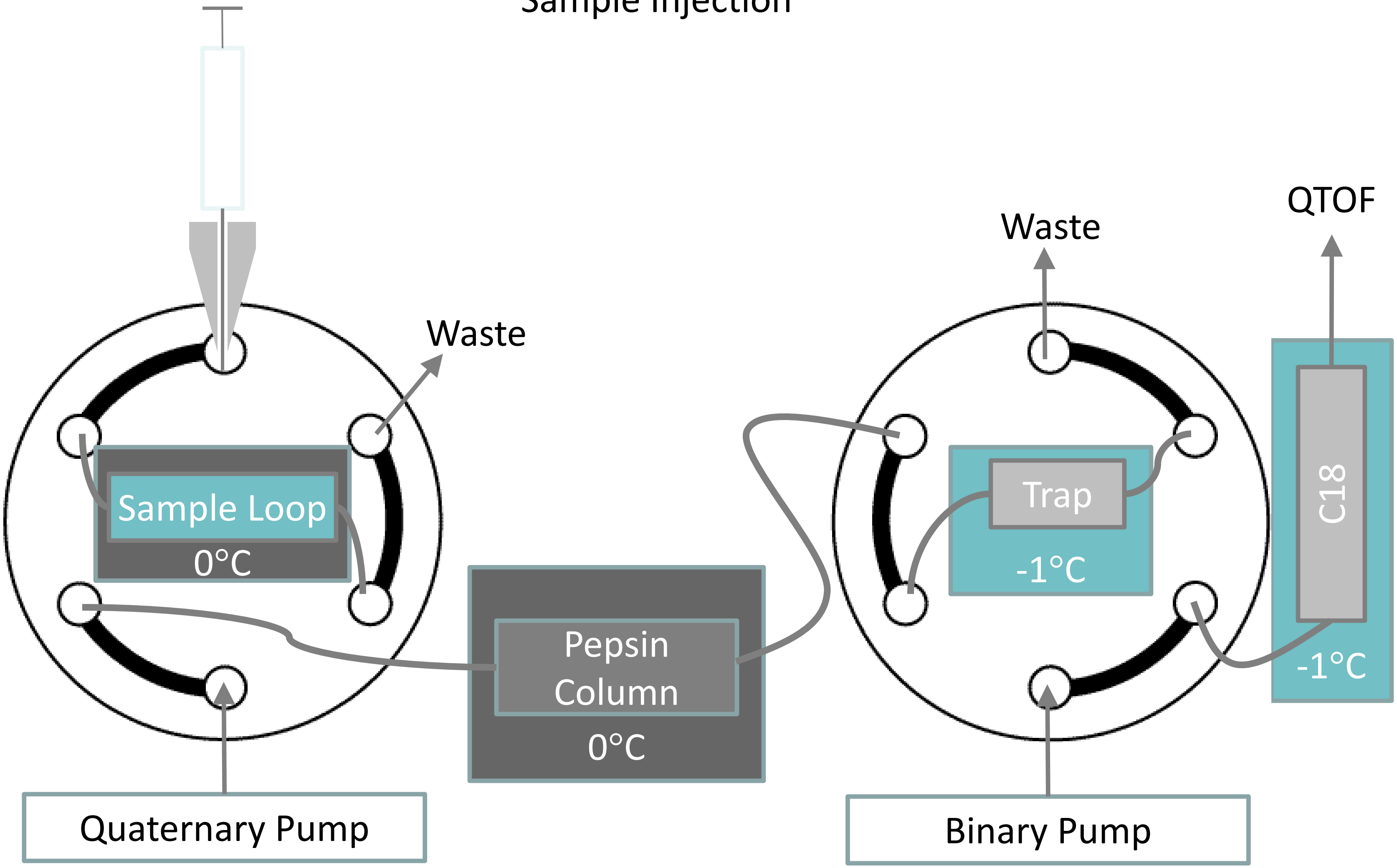


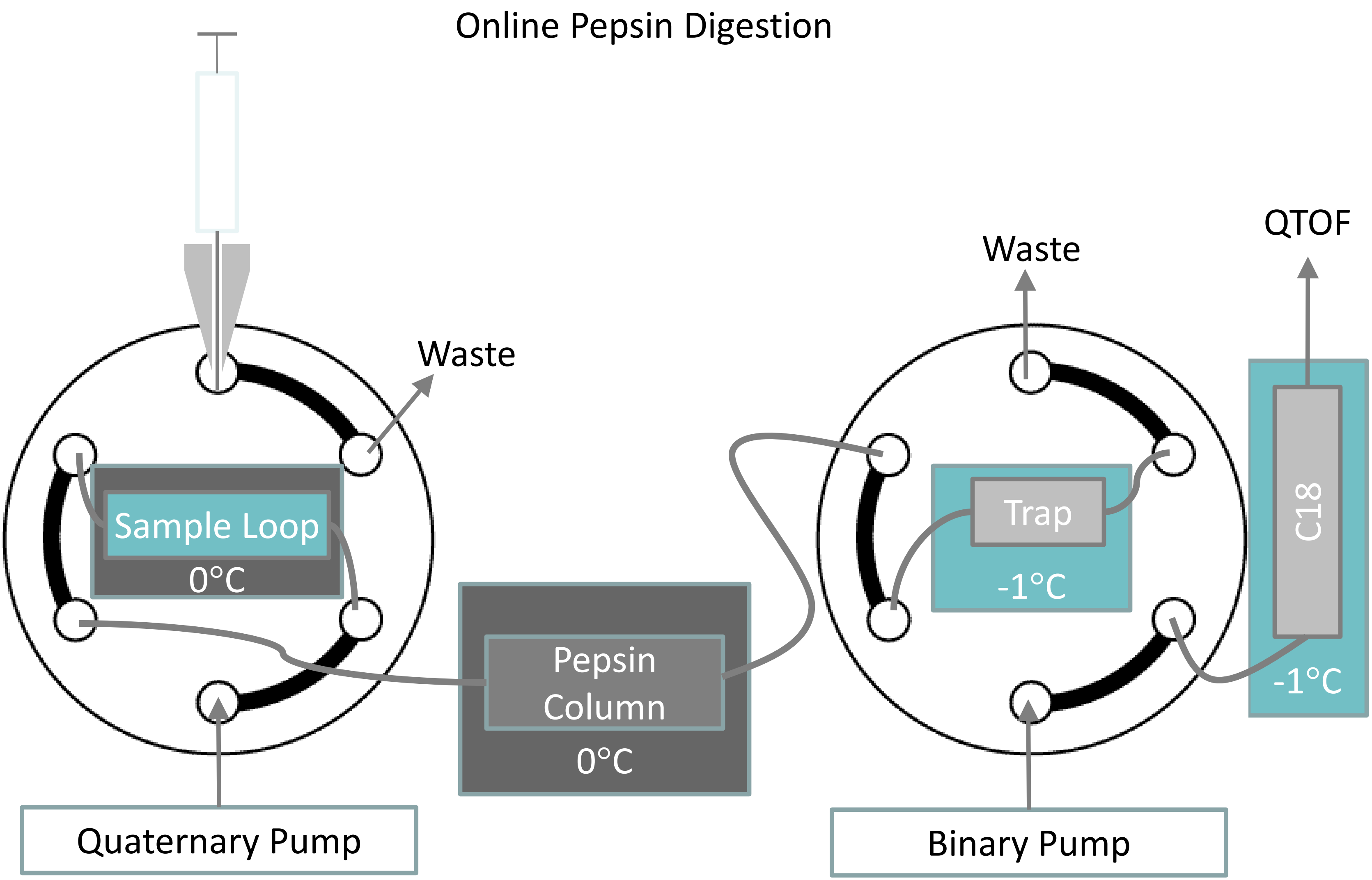
Because of this, the data is more reasonably visualized on the log scale.

Sample Injection

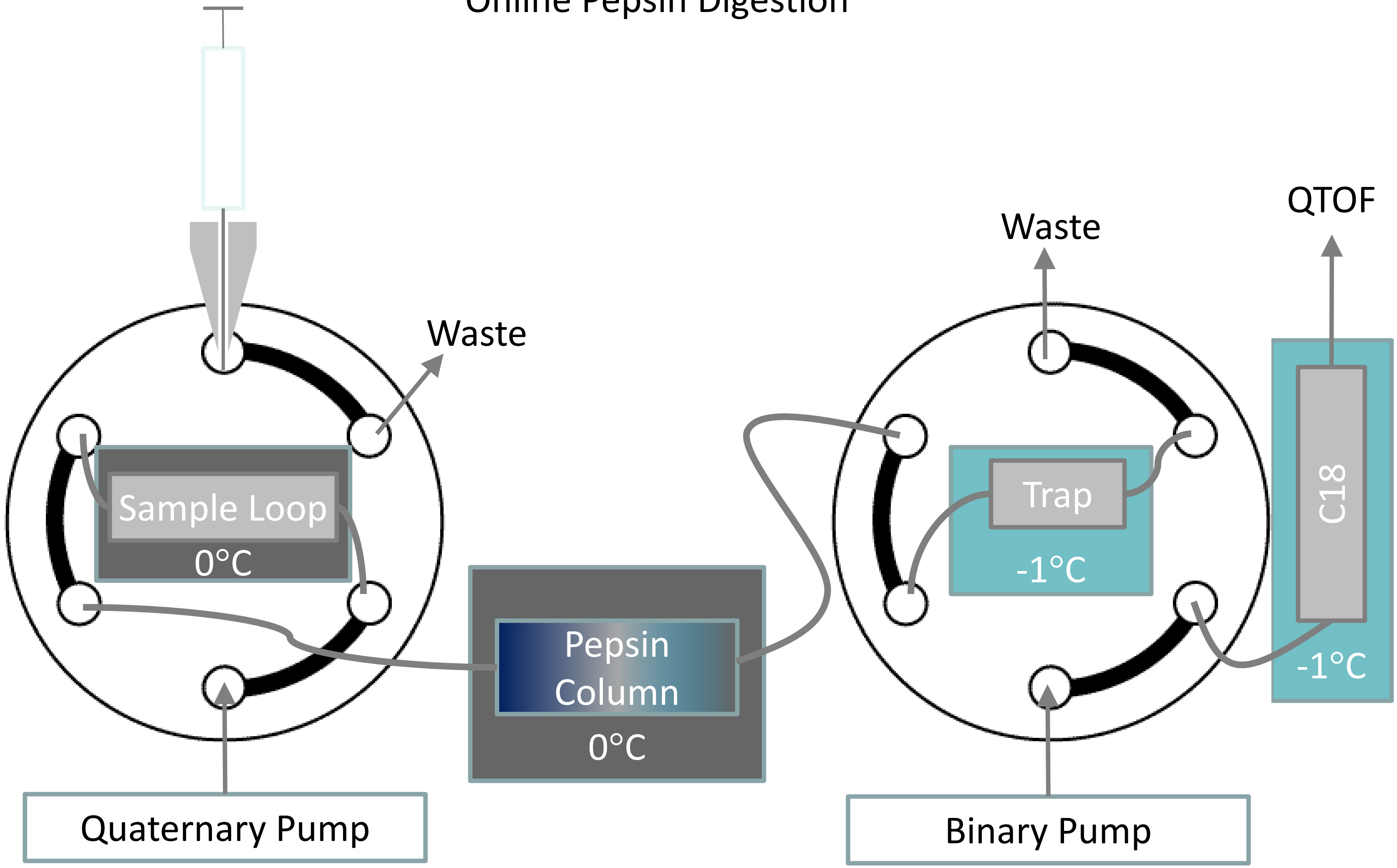


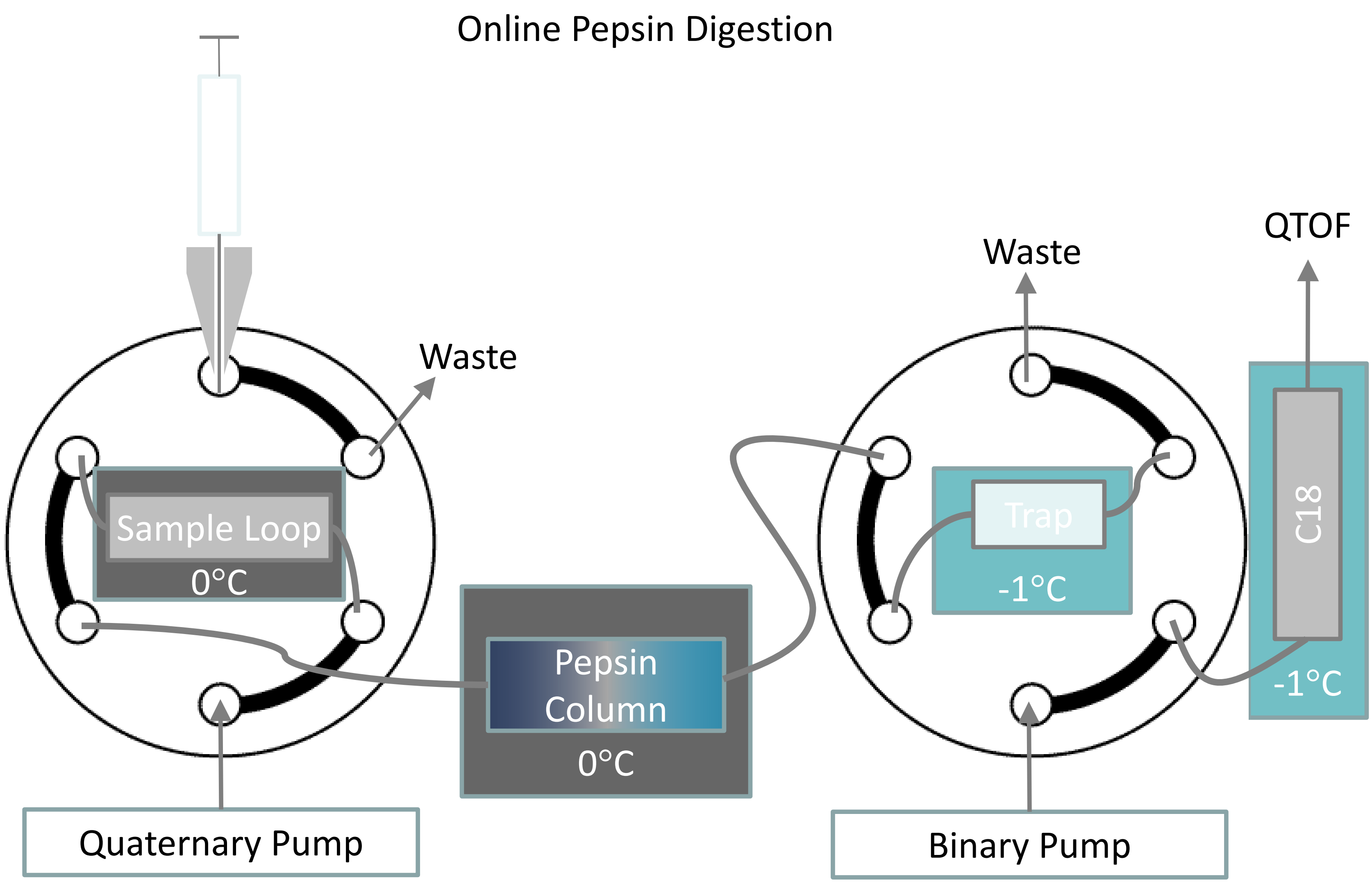
Sample Injection

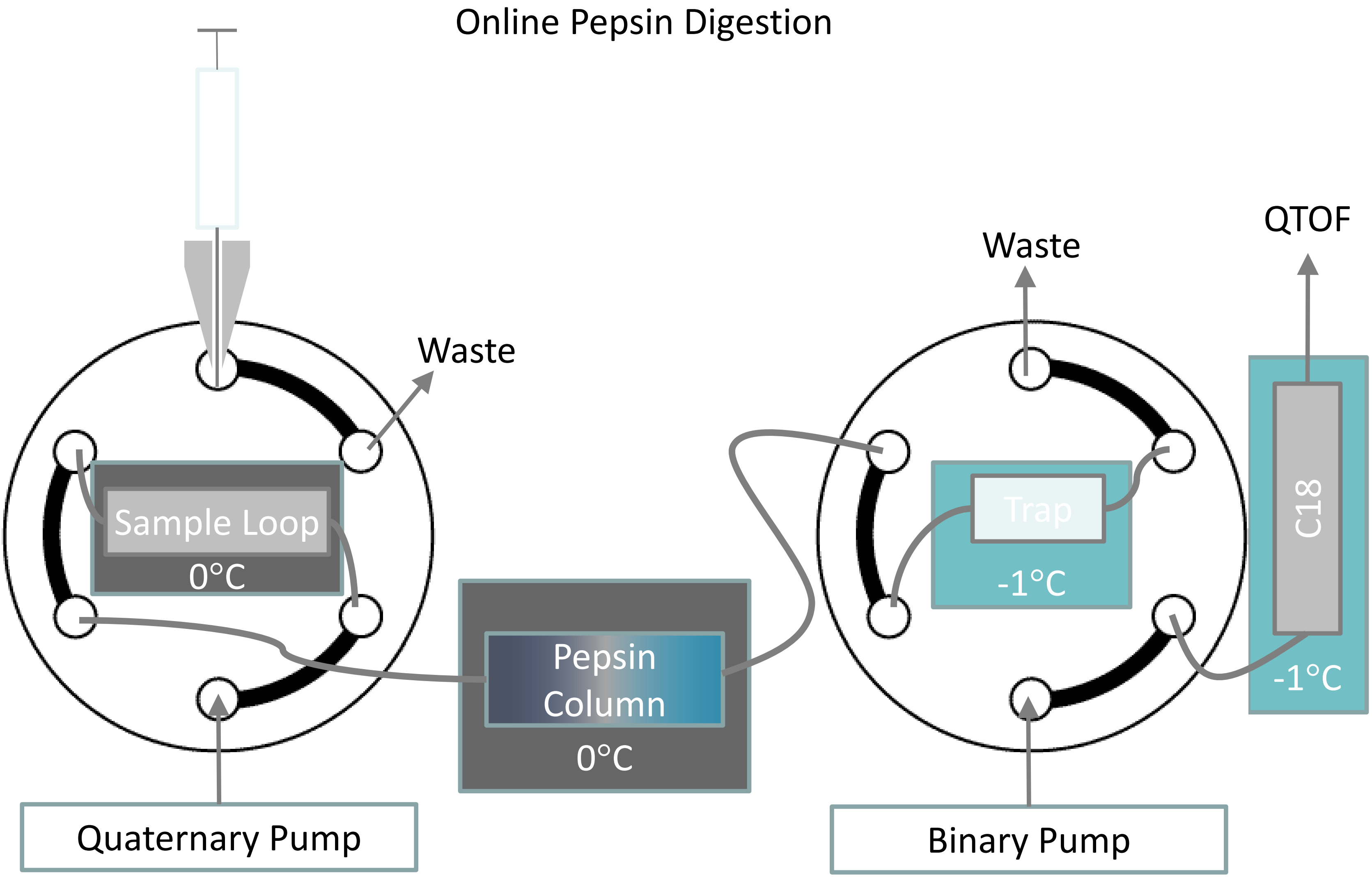



Online Pepsin Digestion
Waste
Sample Loop
0°C
Quaternary Pump
Pepsin
Column
0°C
Waste
QTOF
Trap
-1°C
C18
-1°C
Binary Pump

Online Pepsin Digestion

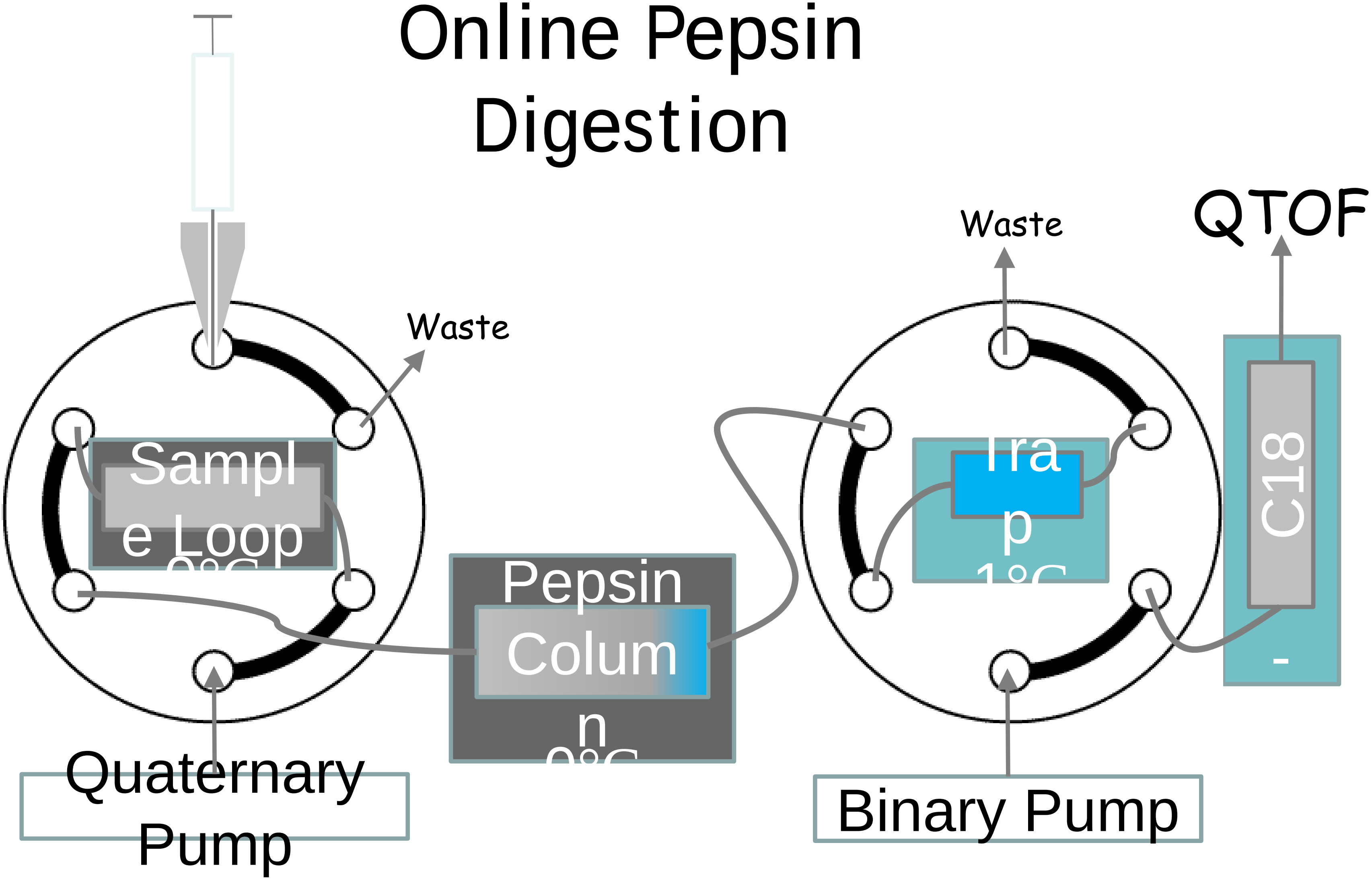



Online Pepsin Digestion
Waste
Sample Loop
0°C
Pepsin
Column
0°C
Quaternary Pump
Waste
QTOF
Trap
-1°C
C18
-1°C
Binary Pump

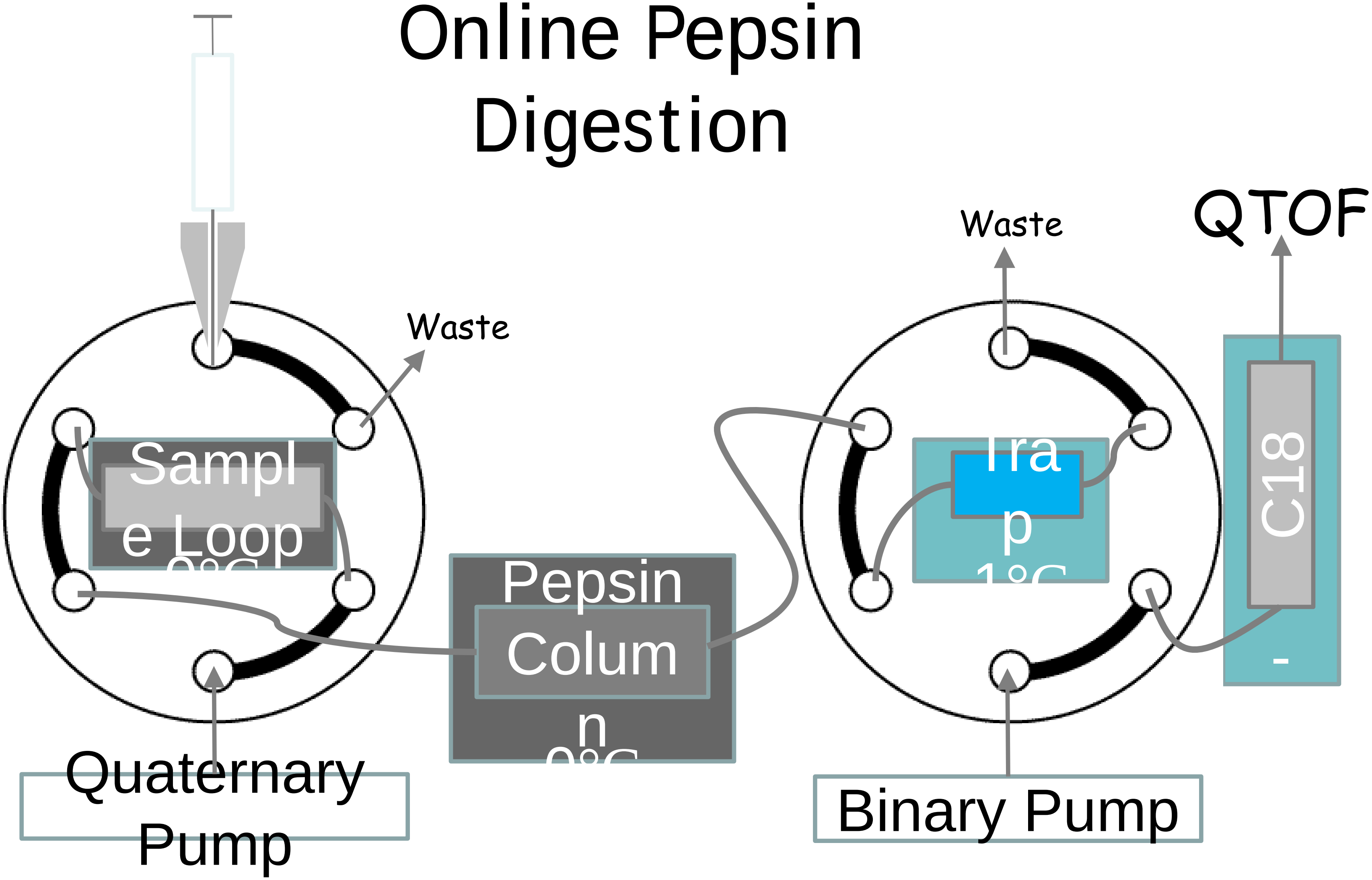


Online Pepsin Digestion
Waste
Sample Loop
0°C
Pepsin
Column
0°C
Quaternary Pump
Waste
QTOF
Trap
-1°C
C18
-1°C
Binary Pump

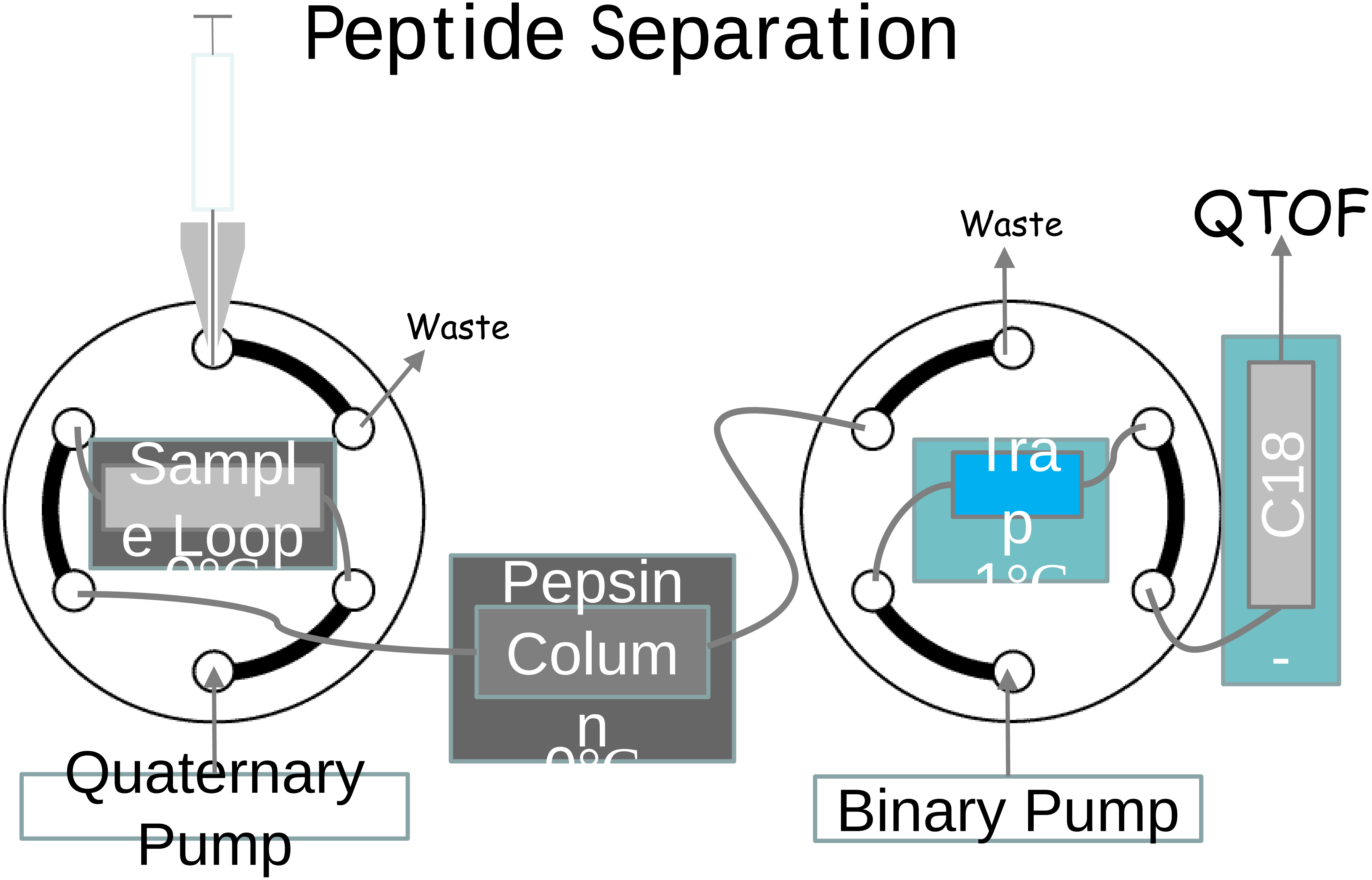
Online Pepsin Digestion



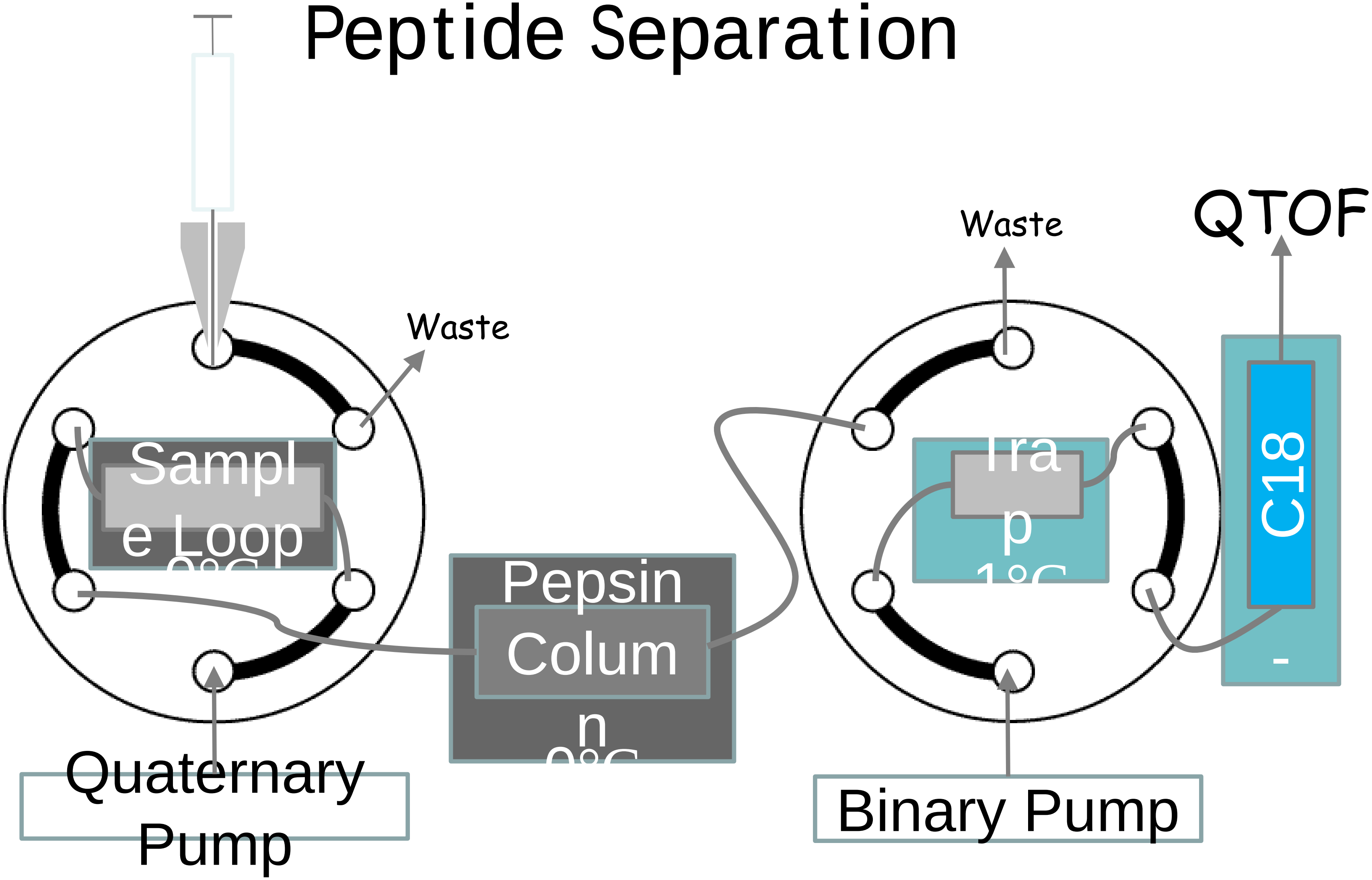
Online Pepsin Digestion



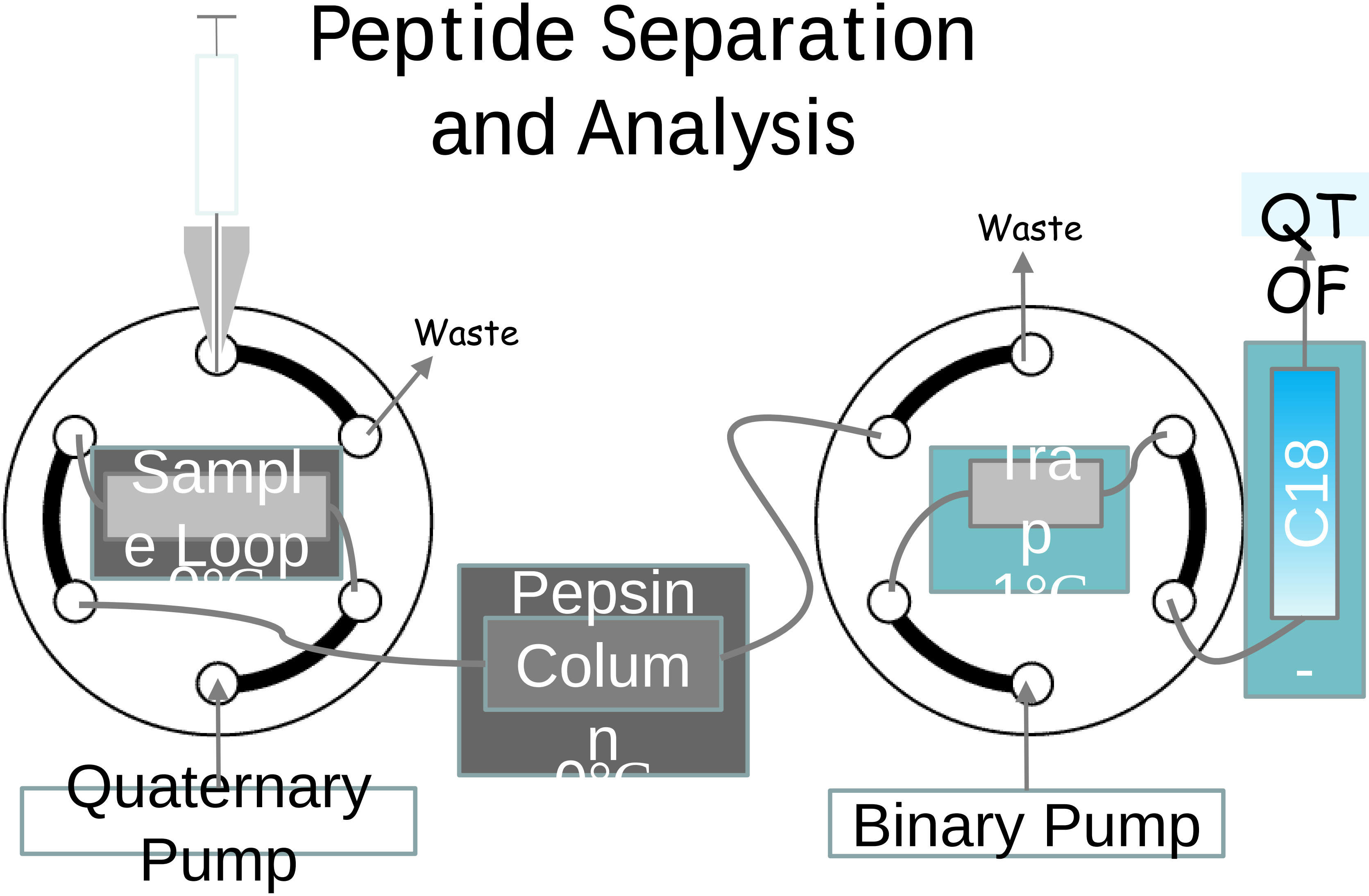
Peptide Separation



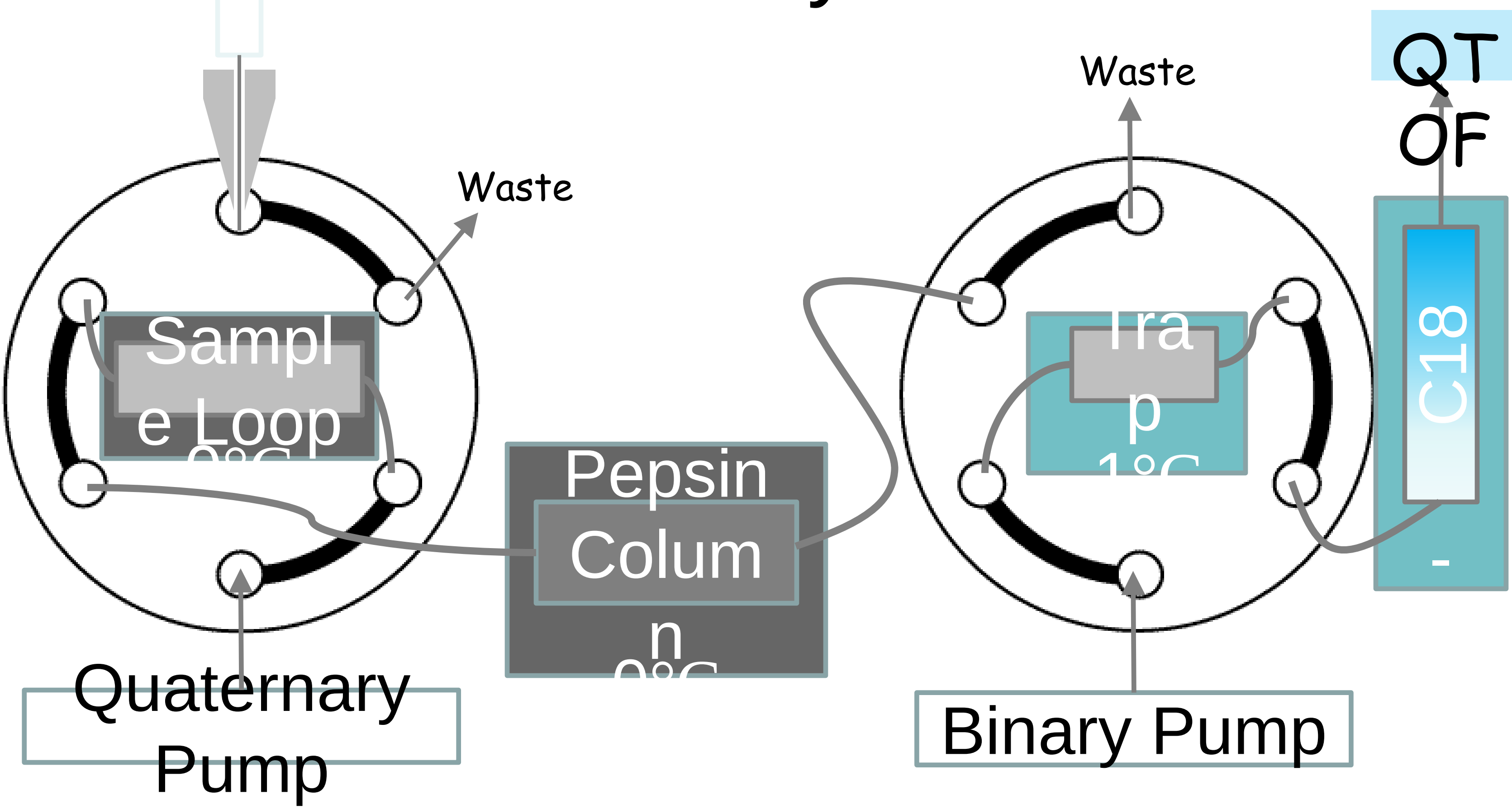
Peptide Separation



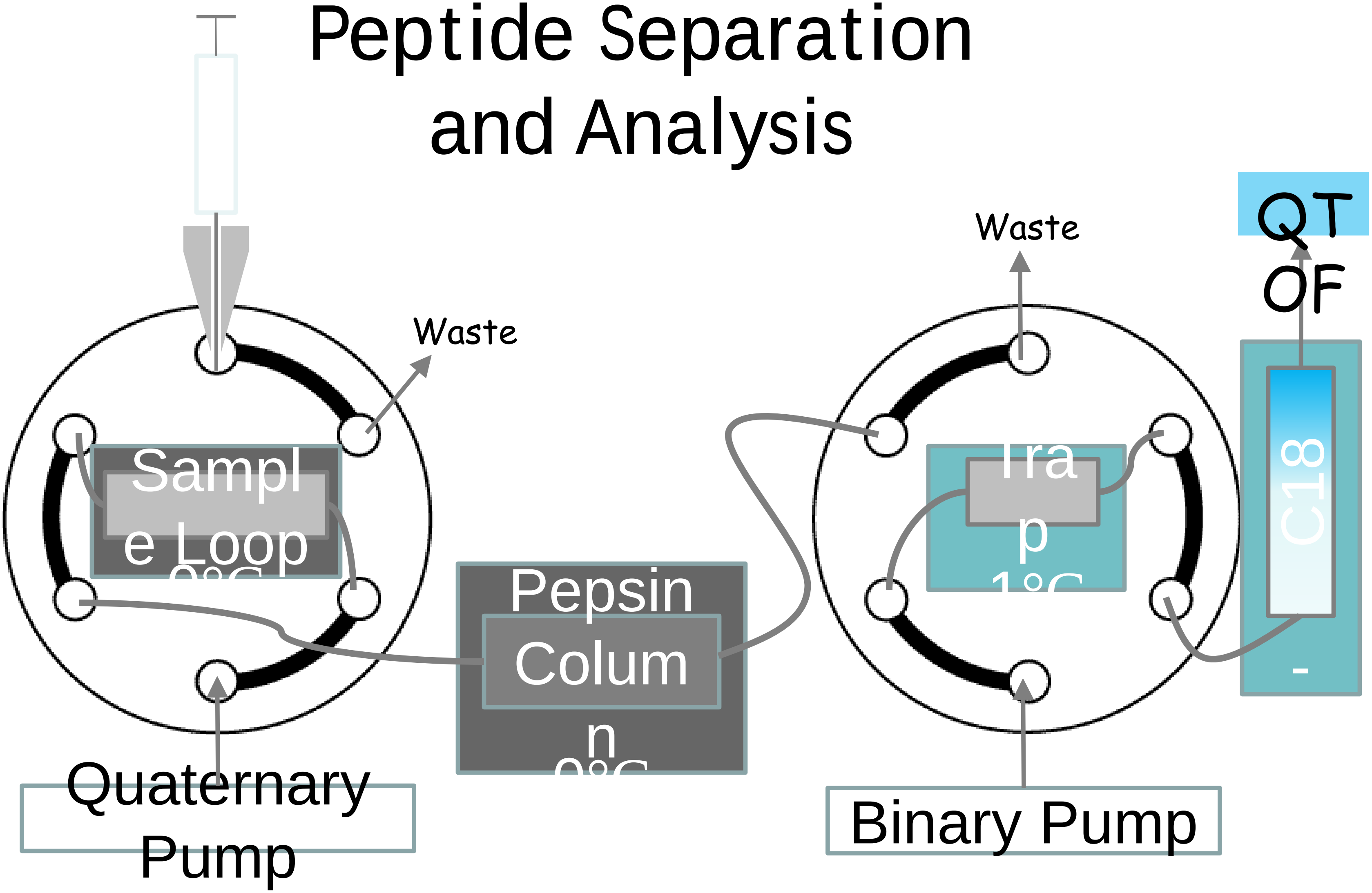
Peptide Separation and Analysis



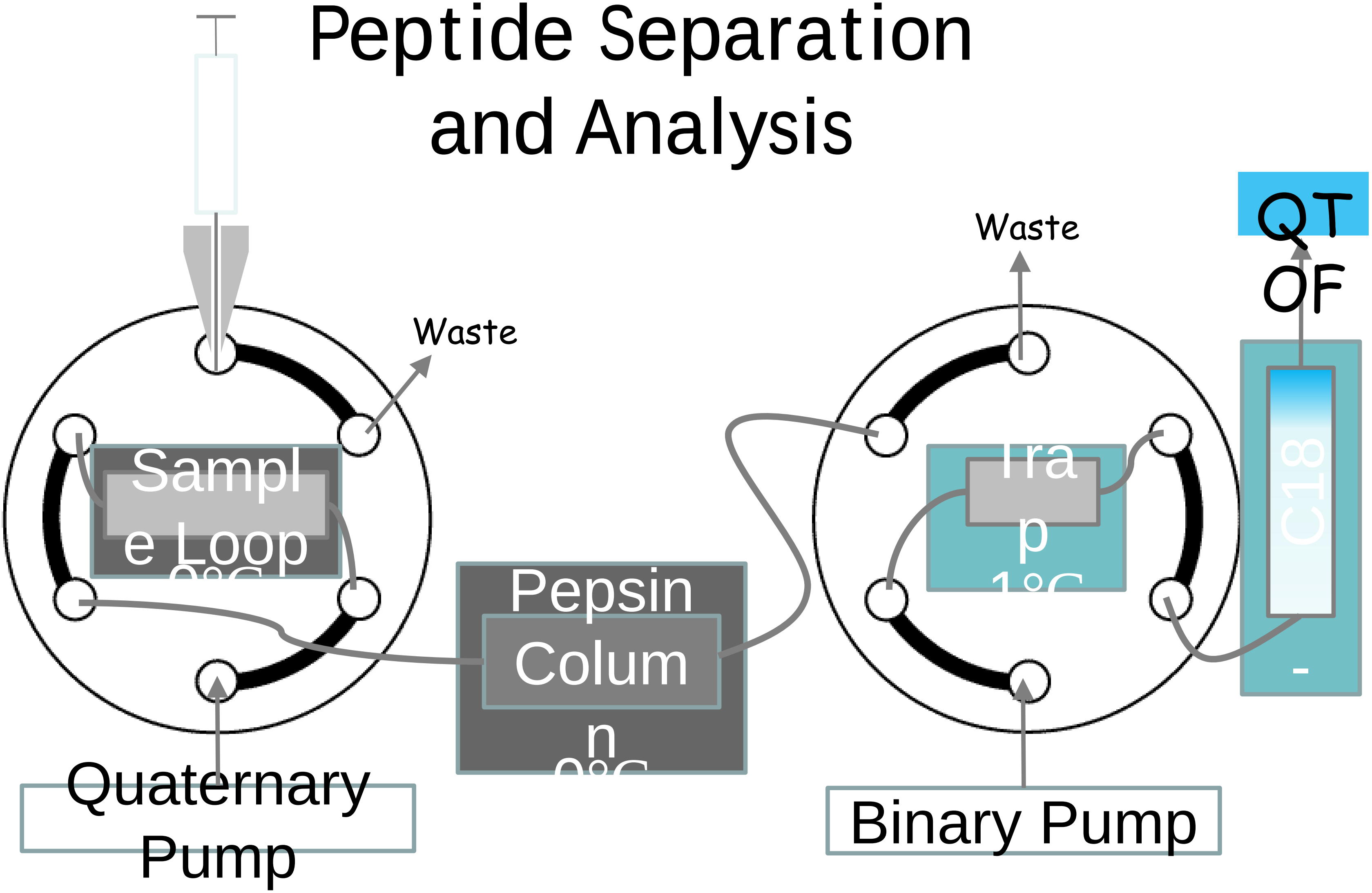
Peptide Separation and Analysis



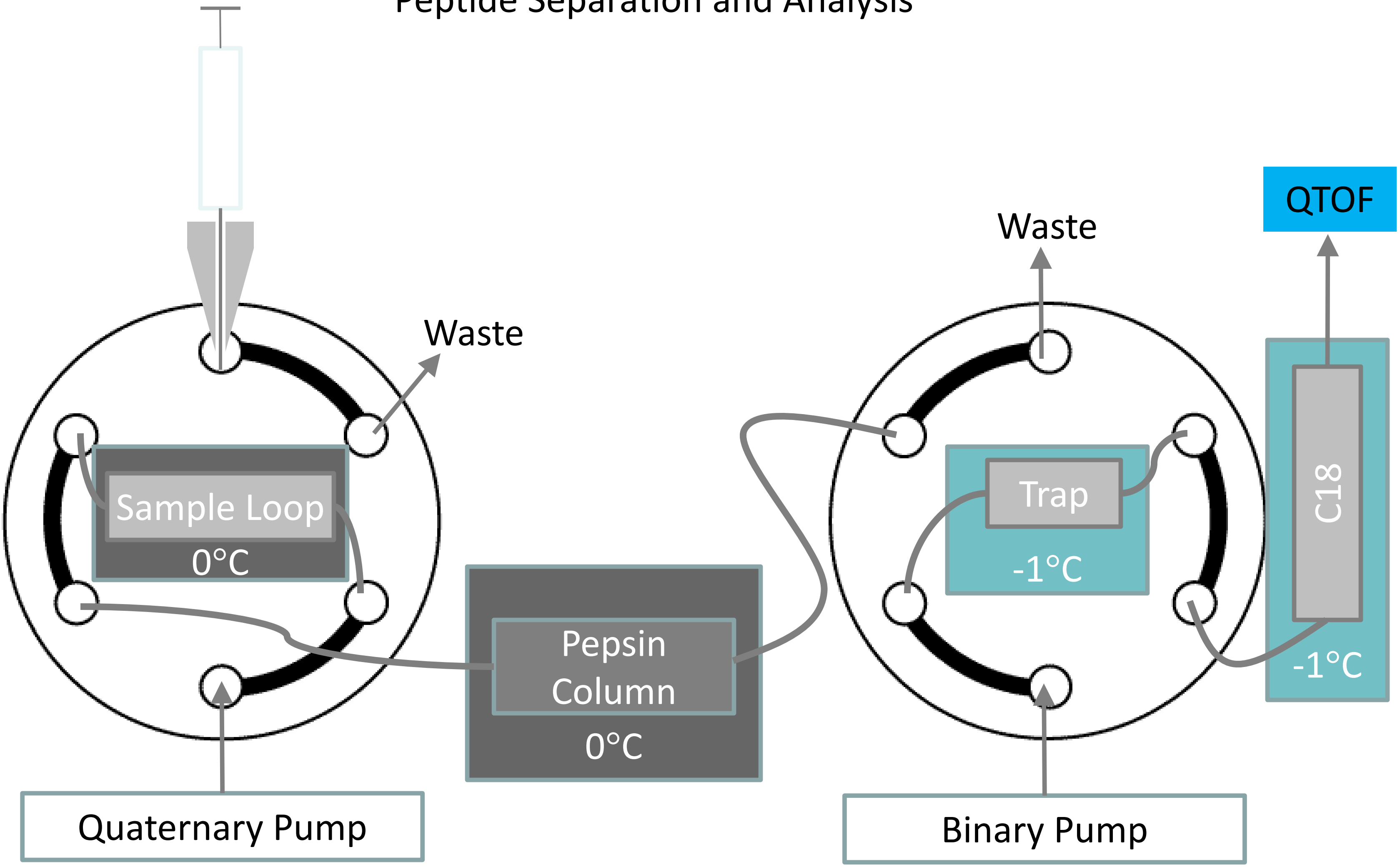
Peptide Separation and Analysis



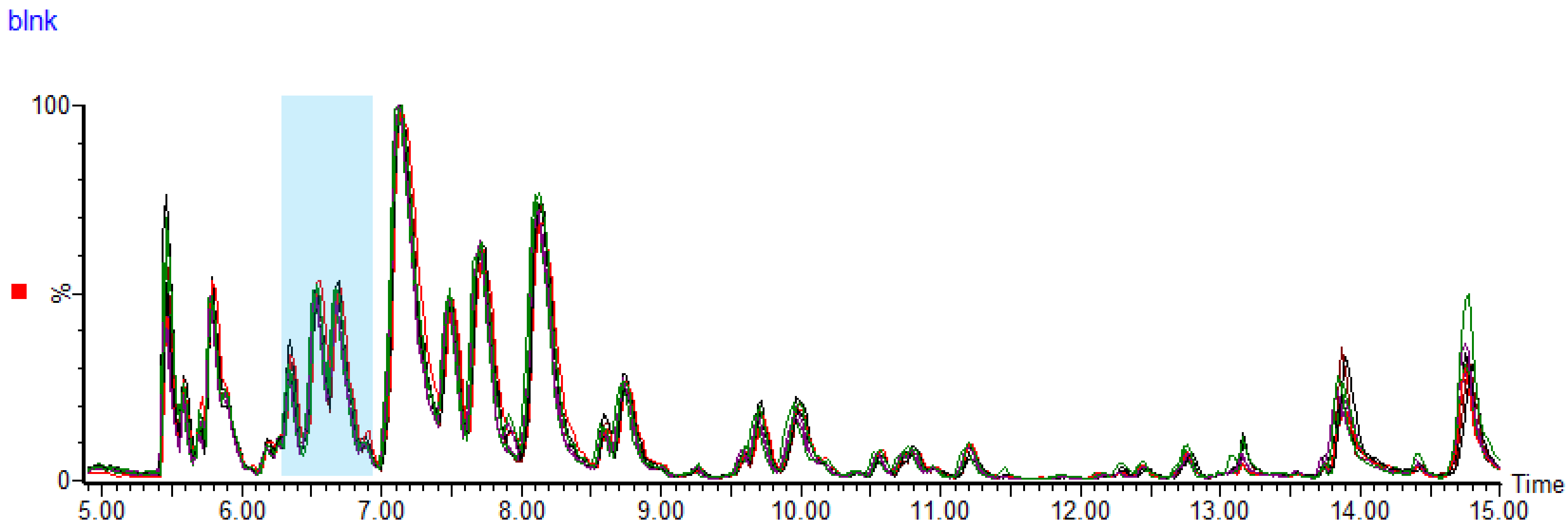
Peptide Separation and Analysis

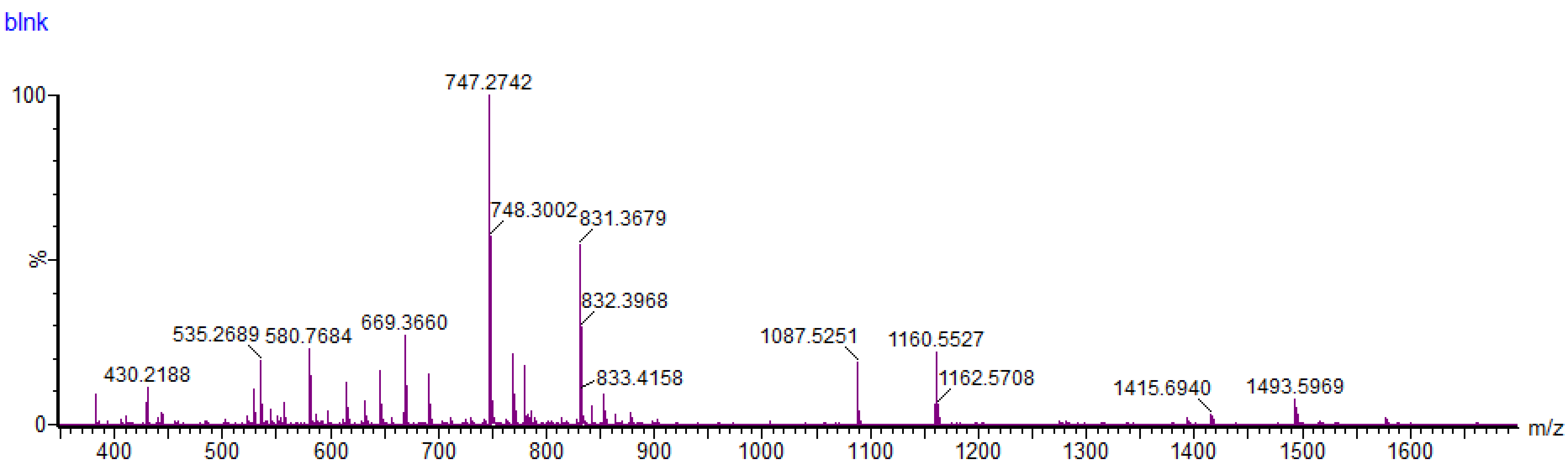


Peptide Separation and Analysis



Reproducibility and Peptide Diversity

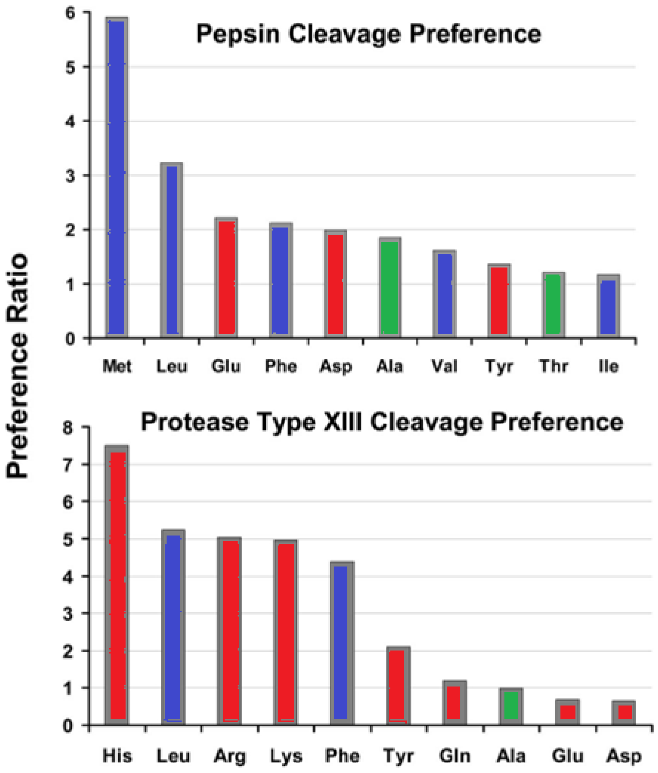




HuTrim5a Coverage Map

```
         5         10        15        20        25        30        35        40        45        50        55        60
1   ASWEH PQFEK GAENL YFQSE FGTAT MEEVA REYQV KLQAA LEMLR QKQQE AEELE ADIRE
         65        70        75        80        85        90        95        100       105       110       115       120
61  EKASW KTQIQ YDKTN VLADF EQLRD ILDWE ESNEL QNLEK EEEDI LKSLT NSETE MVQQT
         125       130       135       140       145       150       155       160       165       170       175       180
121 QSLRE LISDL EHRLQ GSVME LLQGV DGVIK RTENV TLKKP ETFPK NQRRV FRAPD LKGML
         185       190       195       200       205       210       215       220       225       230       235       240
181 EVFRE LTDVR RYWVD VTVAP NNISC AVISE DKRQV SSPKP QIIYG ARGTR YQTFV NFNYC
         245       250       255       260       265       270       275       280       285       290       295       300
241 TGILG SQSIT SGKHY WEVDV SKKTA WILGV CAGFQ PDAMC NIEKN ENYQP KYGYW VIGLE
         305       310       315       320       325       330       335       340       345       350       355       360
301 EGVKC SAFQD SSFHT PSVPF IVPLS VIICP DRVGV FLDYE ACTVS FFNIT NHGFL IYKFS
         365       370       375       380       385       390       395
361 HCSFS QPVFP YLNPR KCGVP MTLCS PSSLE HHHHH H
```

Aspergillus Protease Complements Pepsin



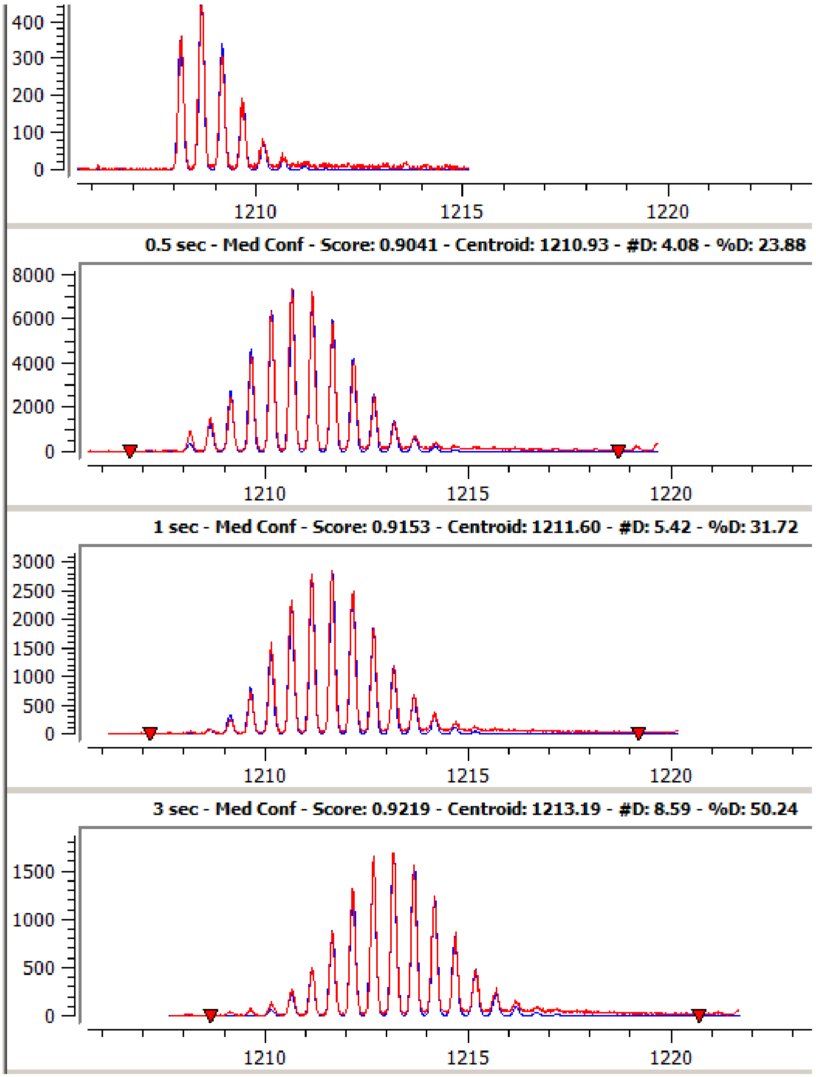
FRACTION BURIED

0-0.2

0.2-0.4

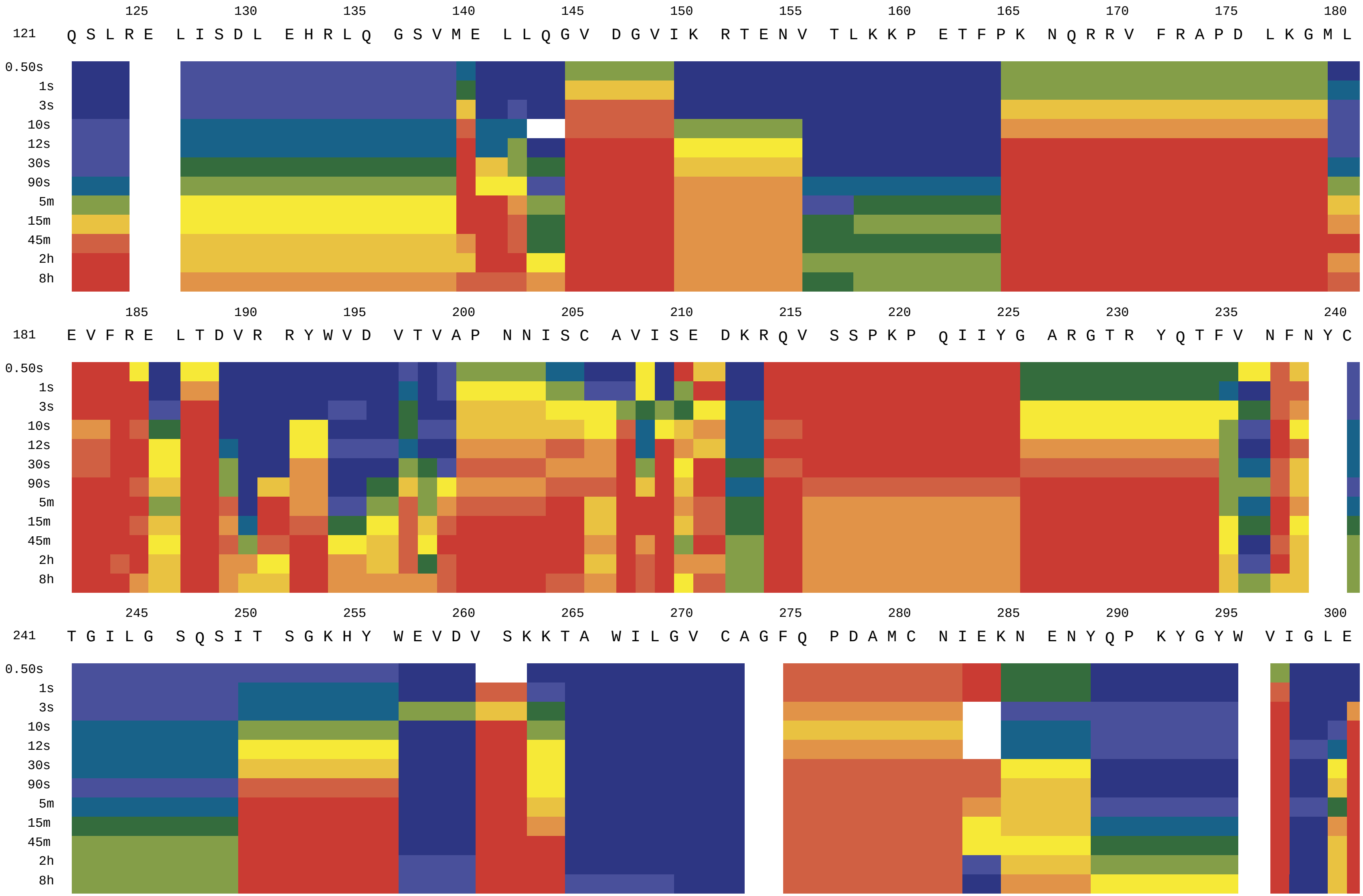
0.4-0.6

Data Analysis using HDExaminer

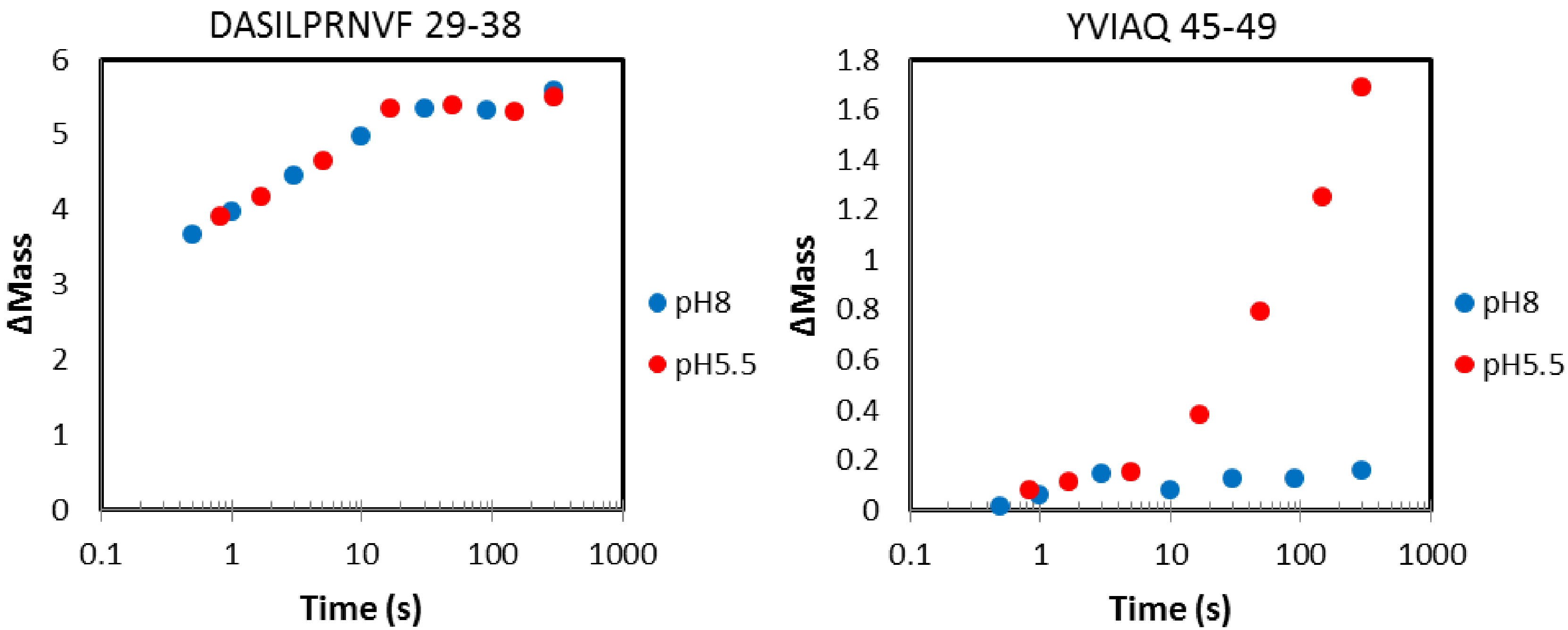


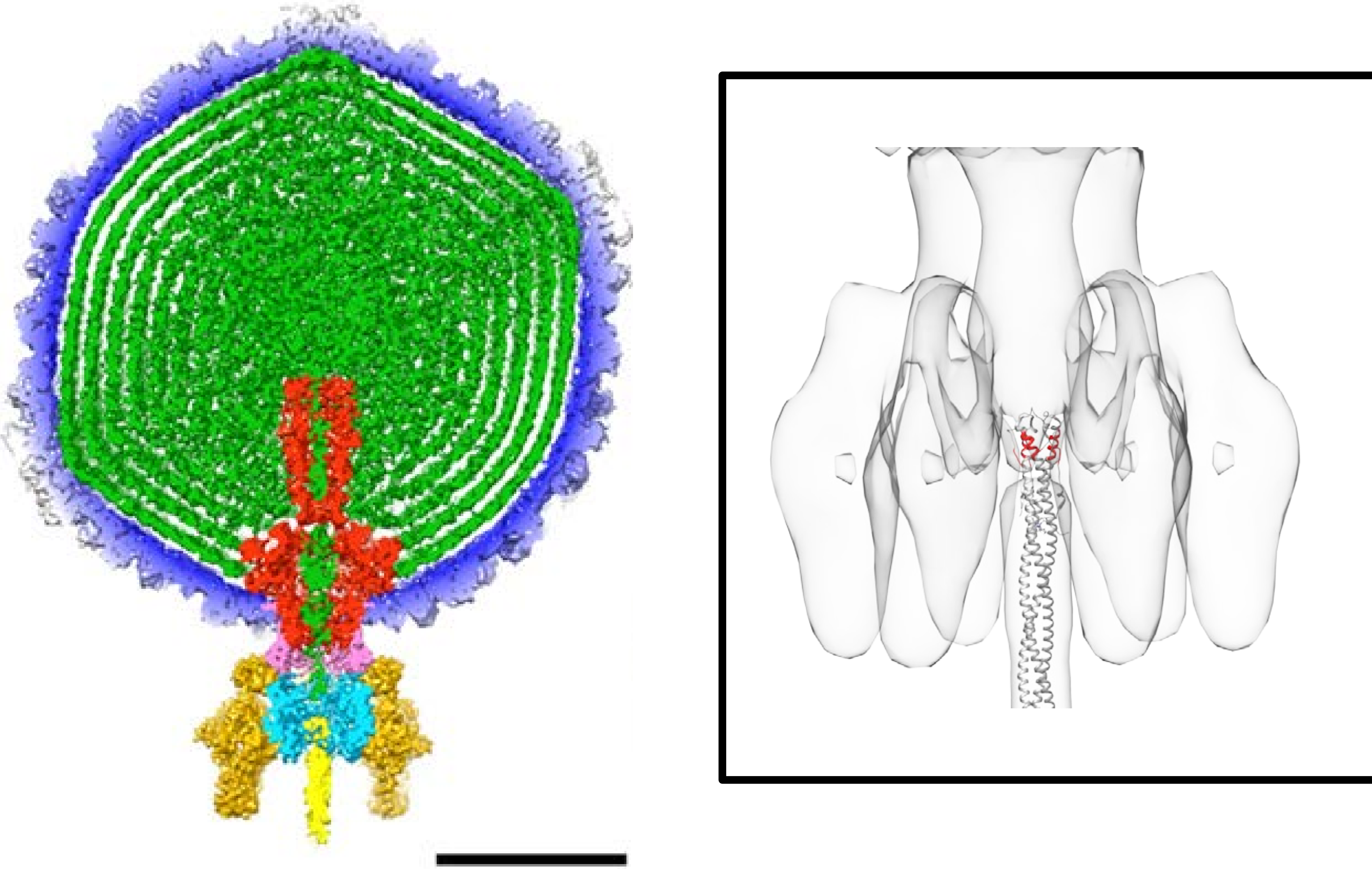
- Calculate isotope distribution using known chemical composition
- Calculate theoretical spectra for peptide +1, +2, +3 …. Deutrons
- Fit experimental spectra with Gaussian distributed sum of calculated spectra
- Assess quality of fit, calculate percent exchange

Heat Map Showing Exchange Kinetics

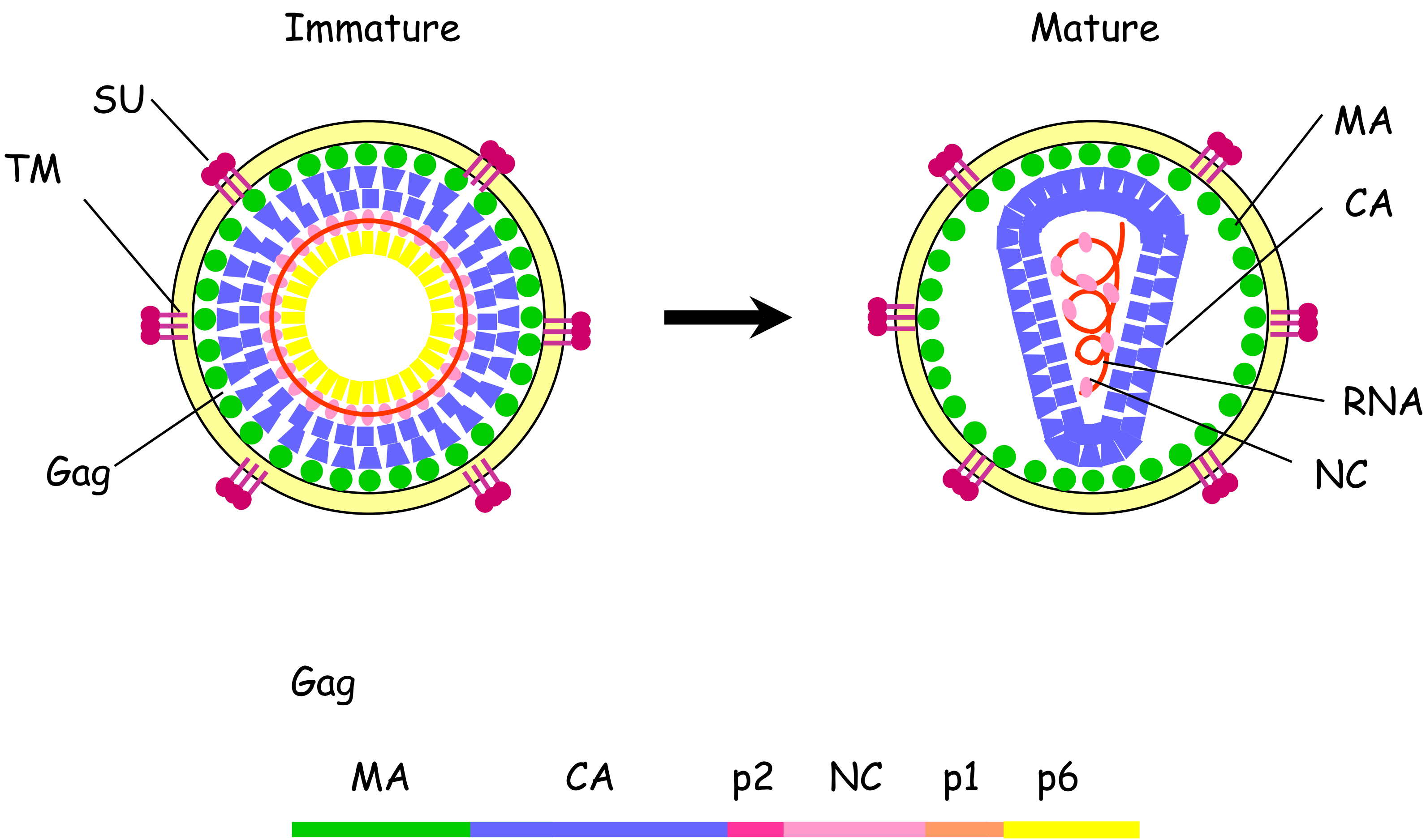


Effect of pH on Tail Needle

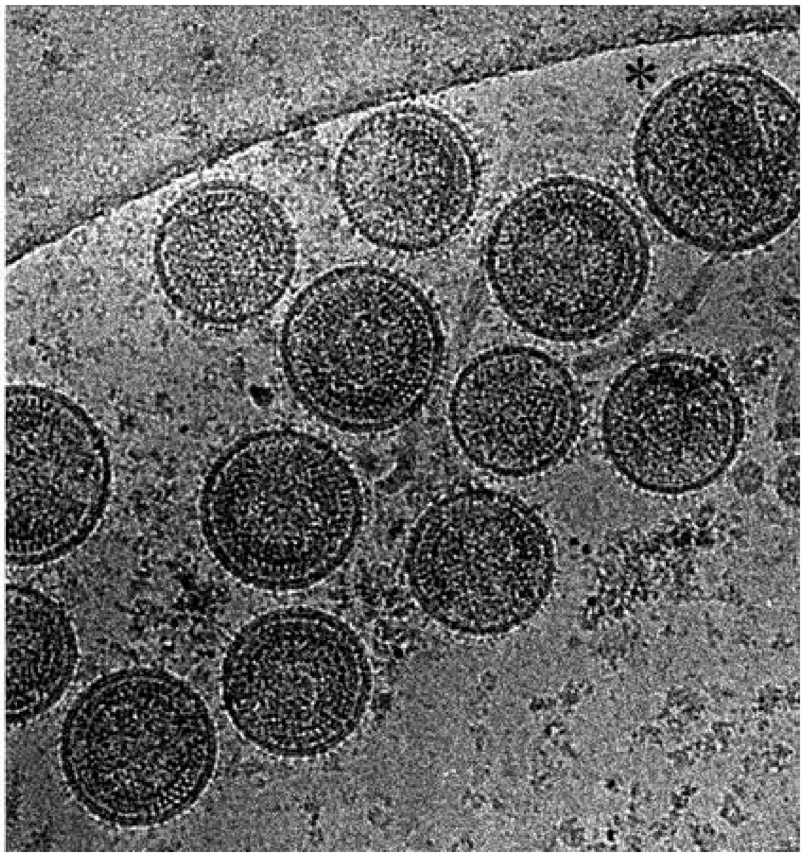




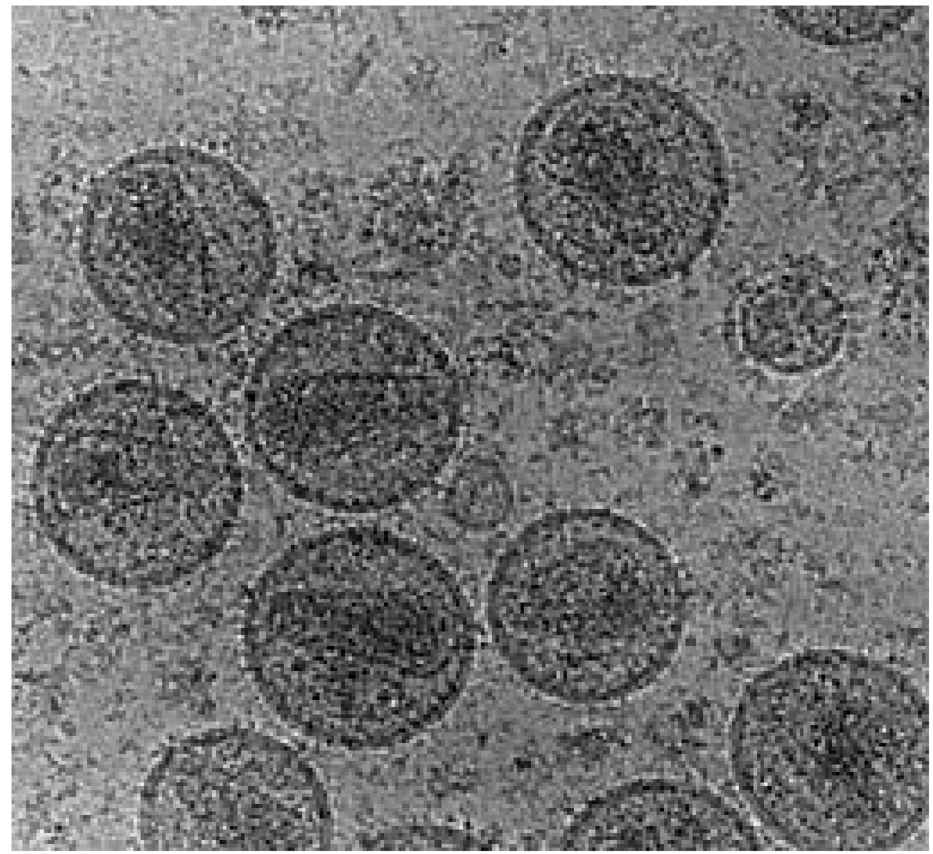
HIV Assembly & Maturation



Immature and Mature Virions are Pleomorphic

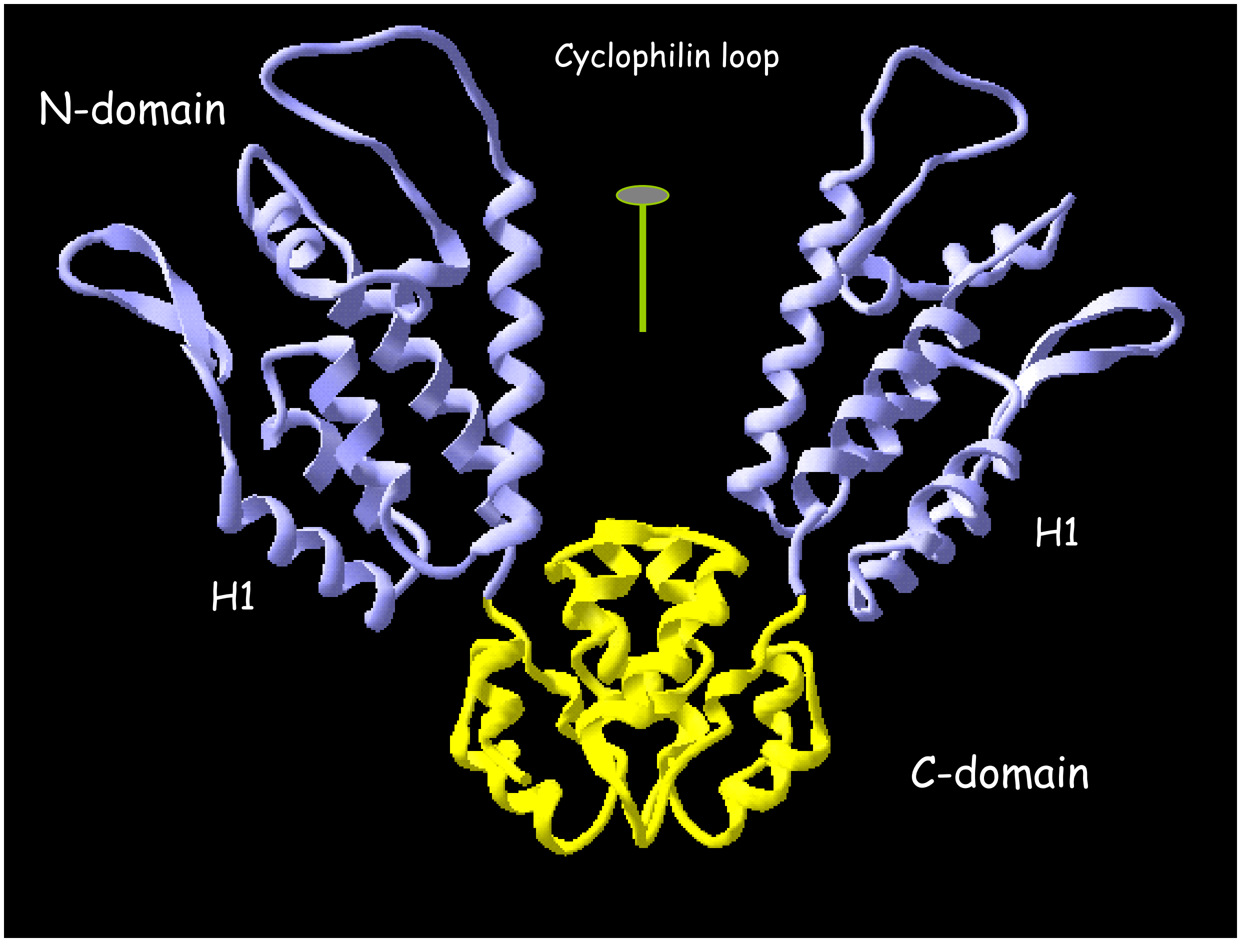


Immature

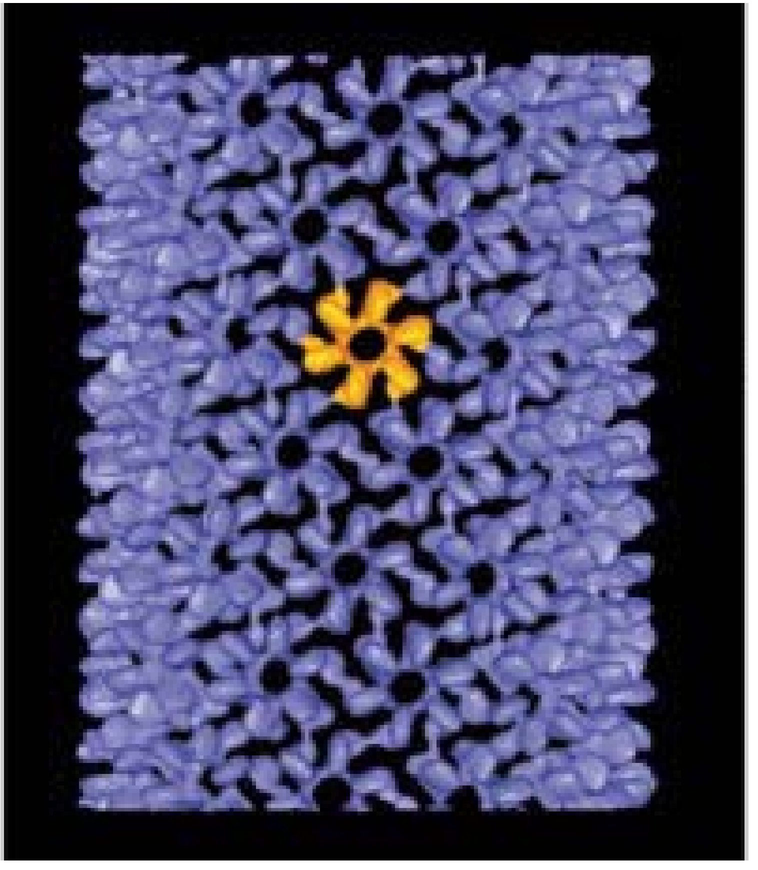


Mature

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

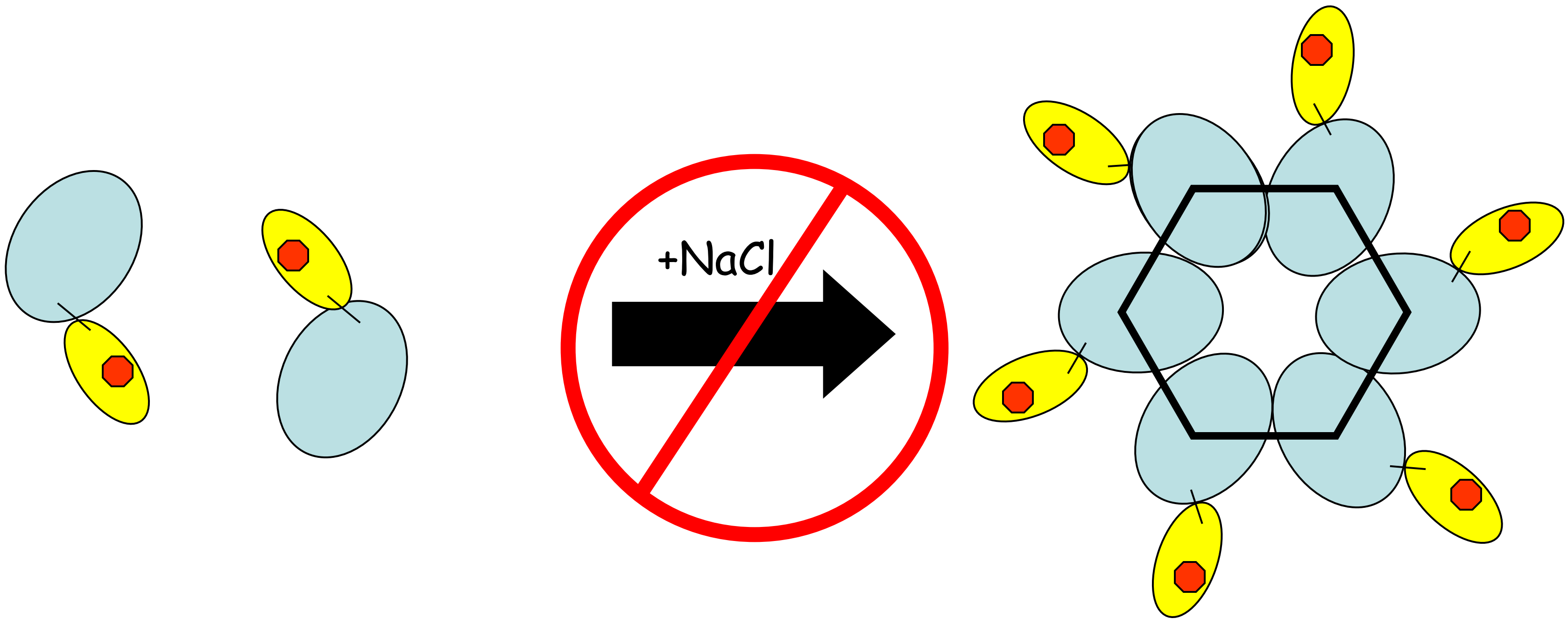


CA Cylinders are Based on a Hexamer Lattice



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

CA does not Form Hexamers without the C-Domain (dimer) Interaction



H/D Exchange Experimental Protocol

CA Dimers and Tubes

- Exchange with D_2O
- Quench with low pH over time

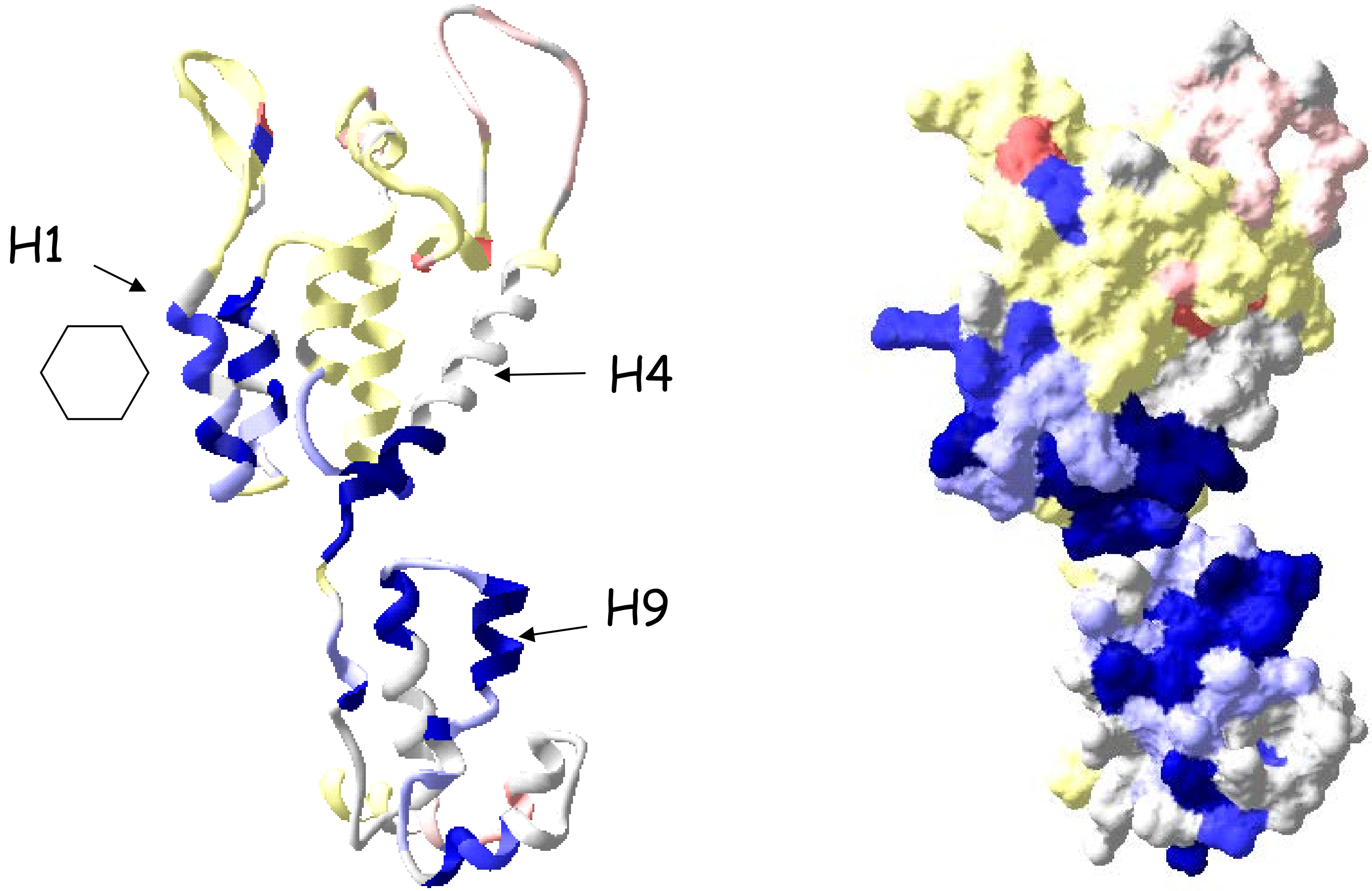
Pepsin digestion

- Fragment size 10-20 residues

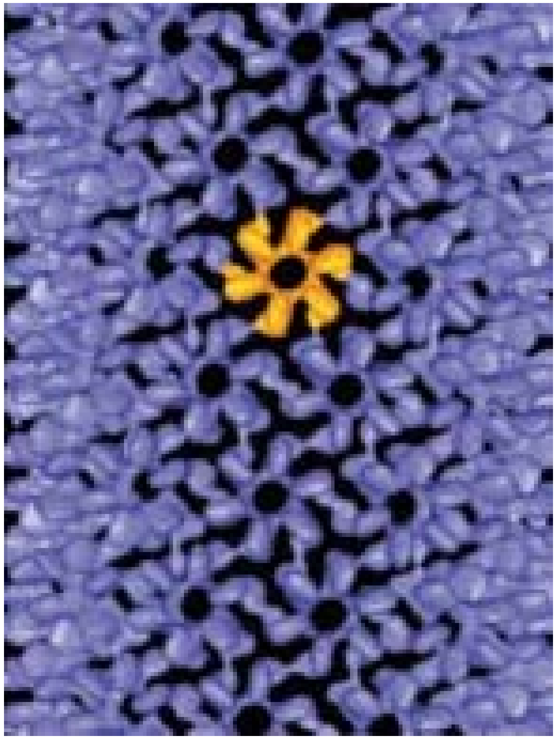
Mass spectrometry

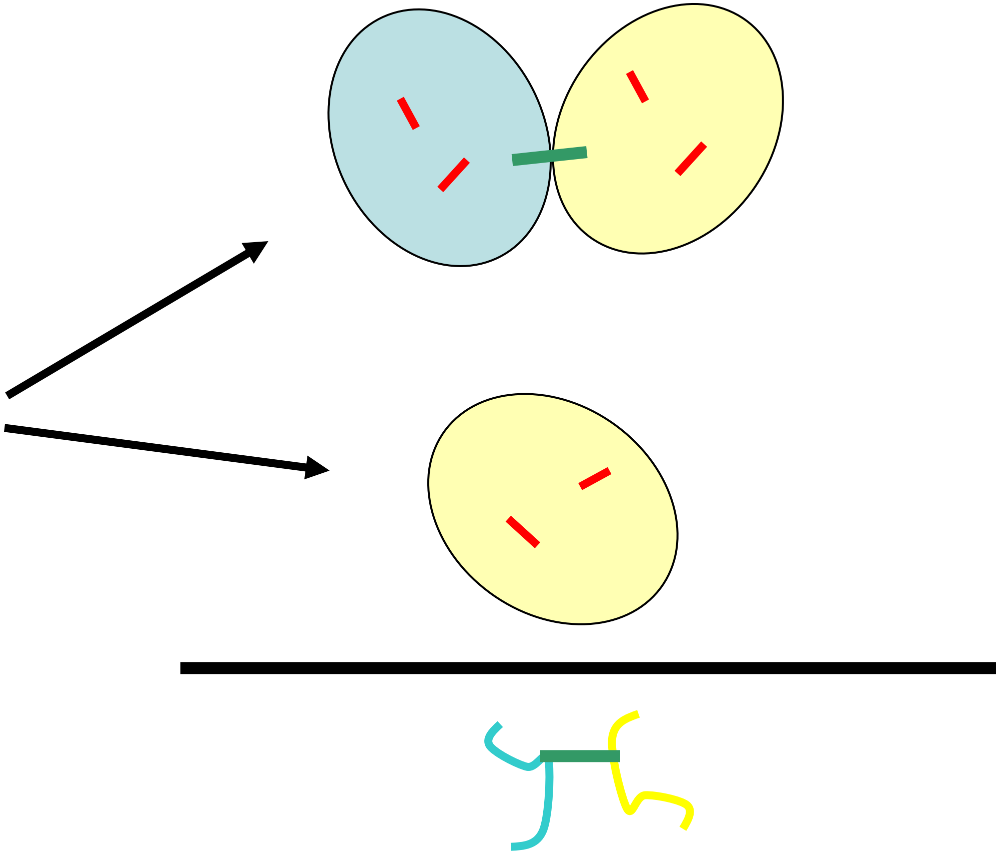
- Assignment of peptides
- Quantifying exchange

Changes in Exchange Rates due to CA Assembly



Crosslinking of Intact Tubes will Allow Identification of Intersubunit Interfaces

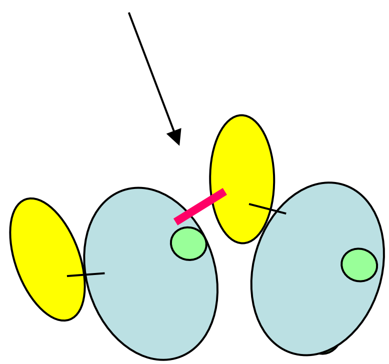


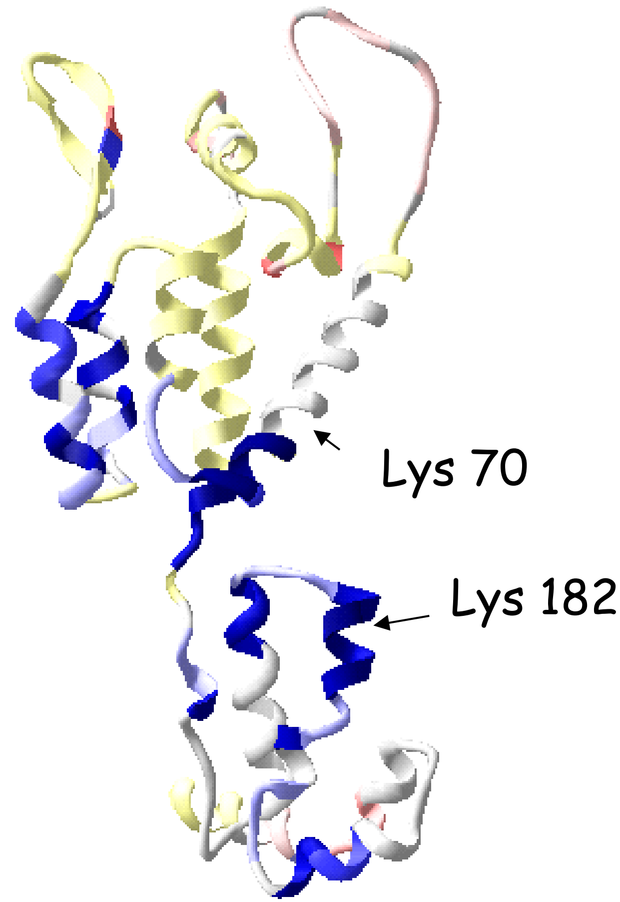


Lysine 70 was Cross-Linked to Lysine 182

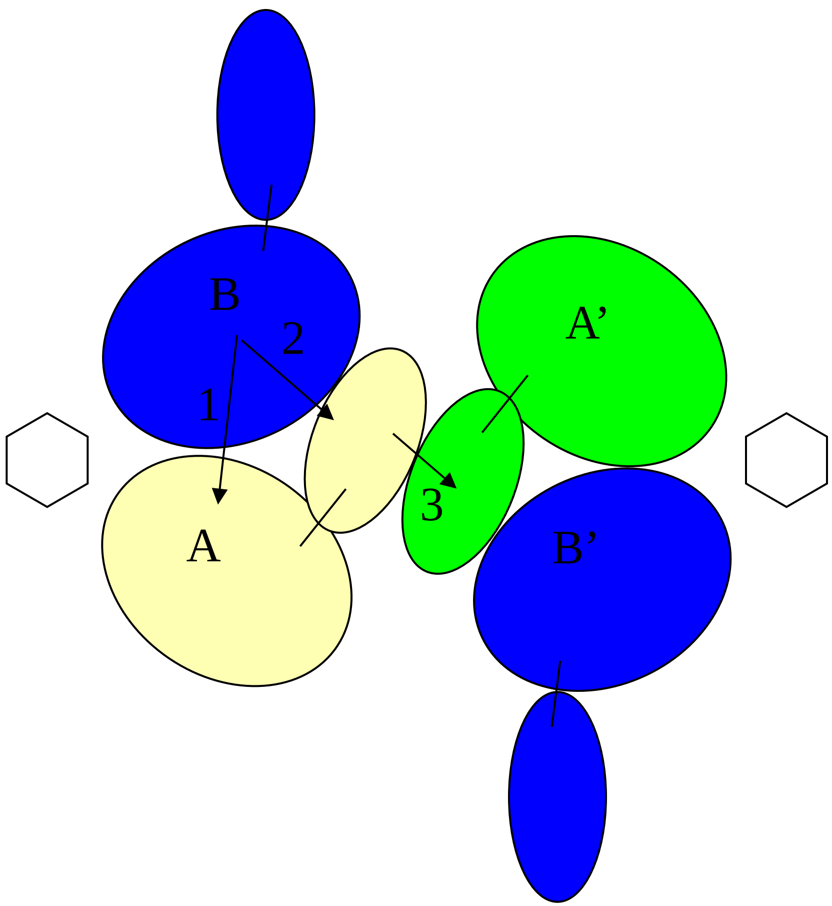
31 to 131	=	10 948.9
171 to 199	=	3 375.9
+DST	=	114.0
Expected mass	=	**14 438.8**
Observed mass	=	**14 439**

Lys70 cross-linked to 182





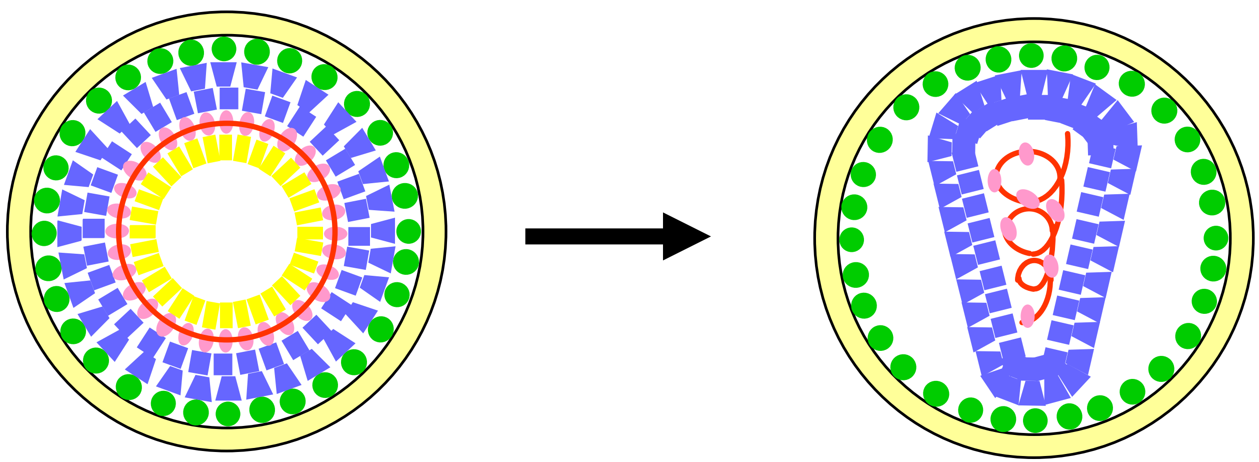
A Model for An N-domain to C-domain Interaction in CA Assembly



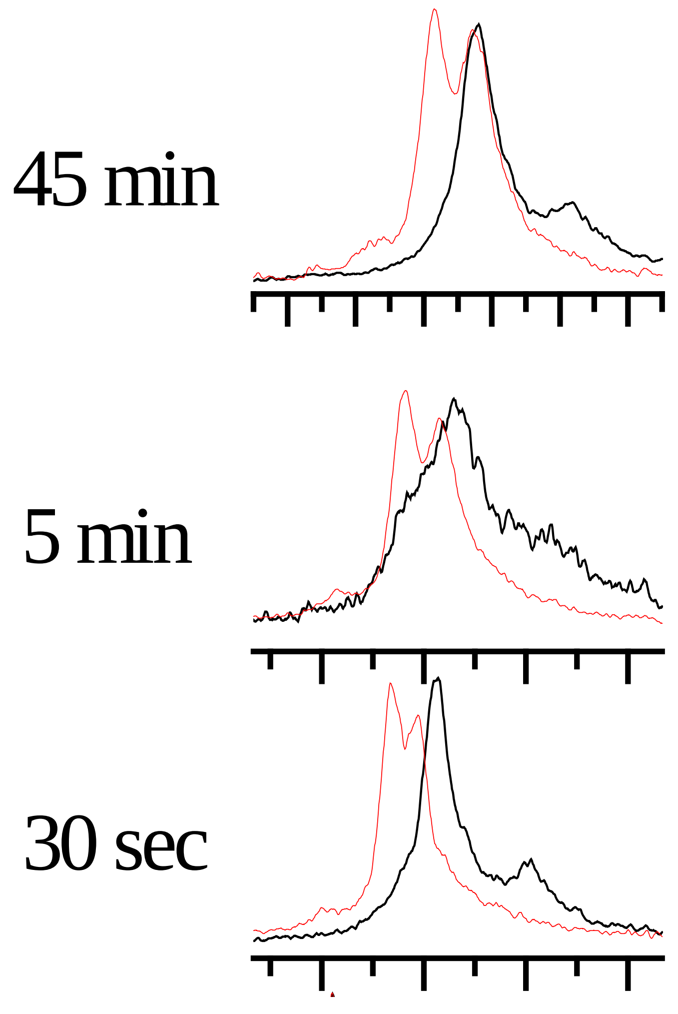
Hydrogen Deuterium Exchange Studies on Virus-Like Particles

Immature viral-like particles

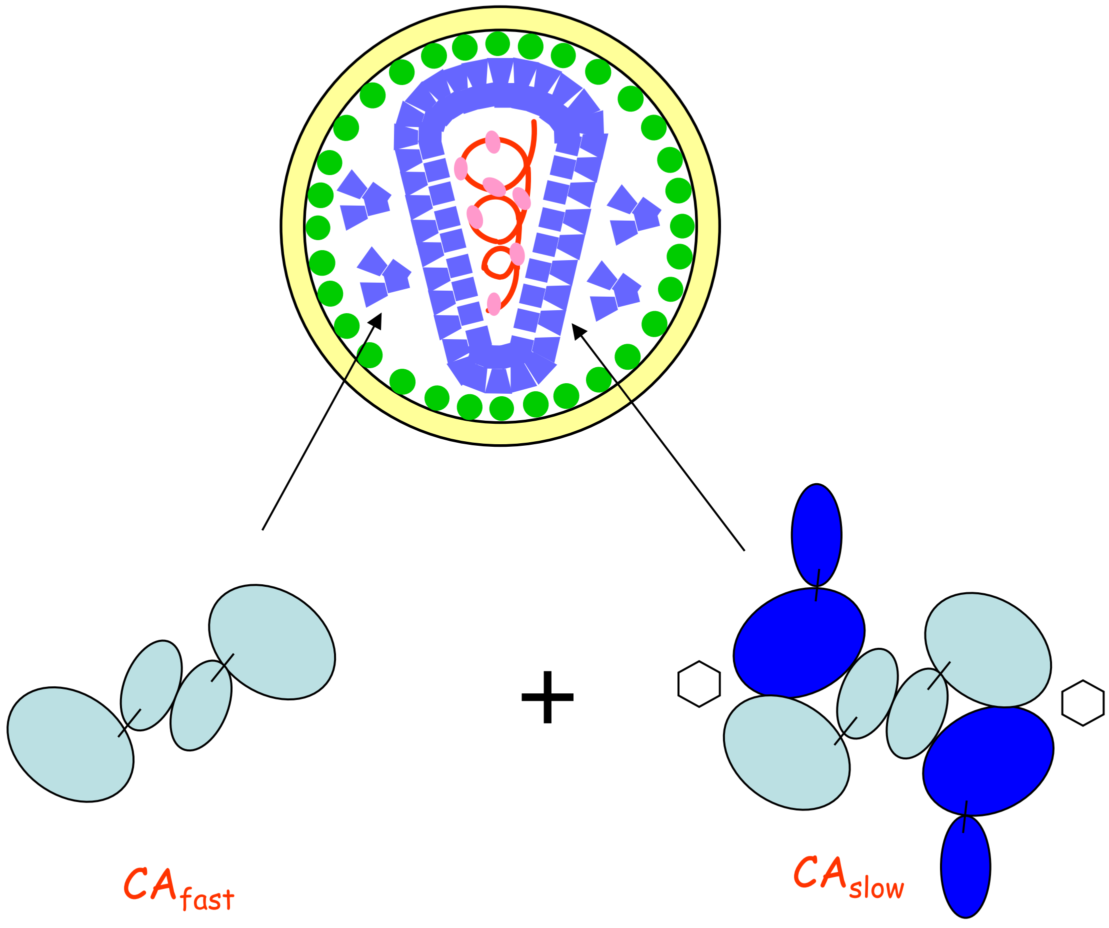
Mature viral-like particles



CA_{fast} is Partially Protected at Early Times

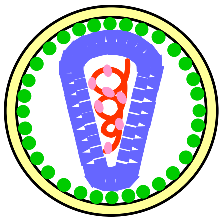


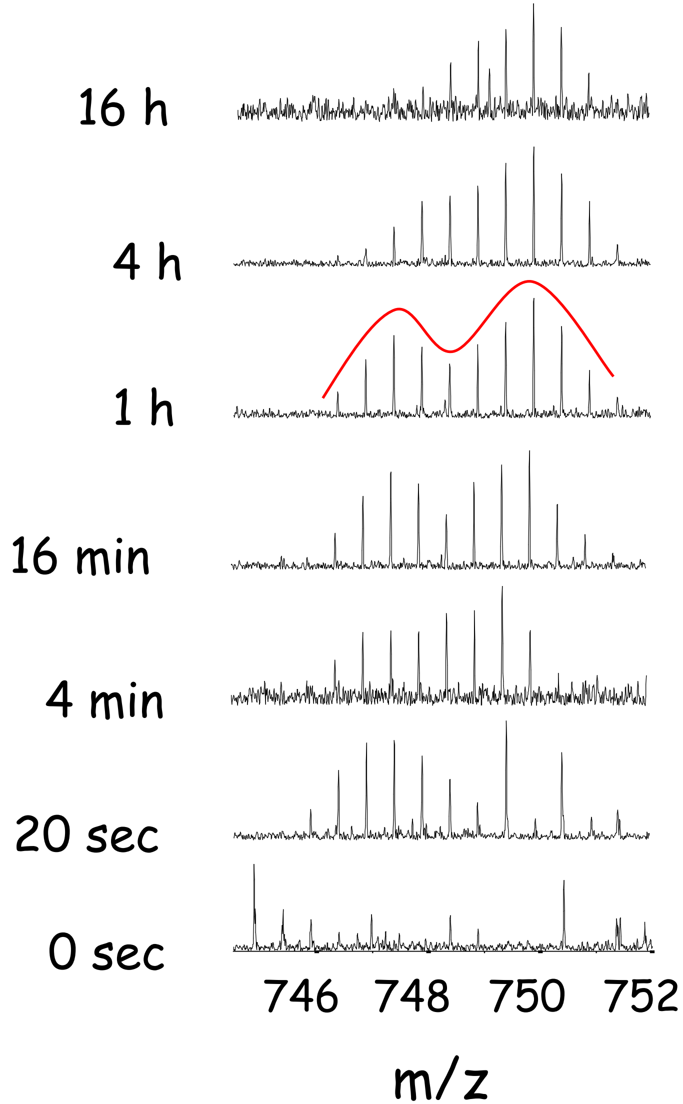
CA in Mature Virus-Like Particles is 50% Free and 50% Core-Incorporated



The N:C Interface is Bimodal in Mature Virions

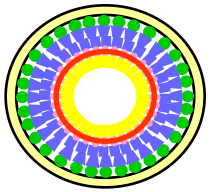
Mature virus-like particles

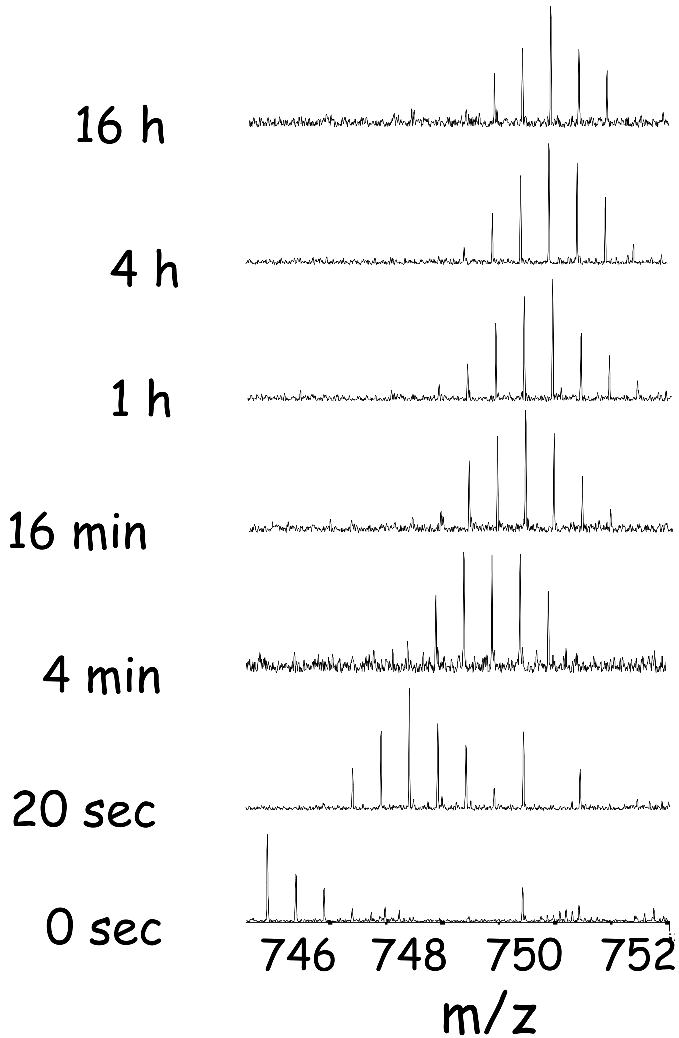




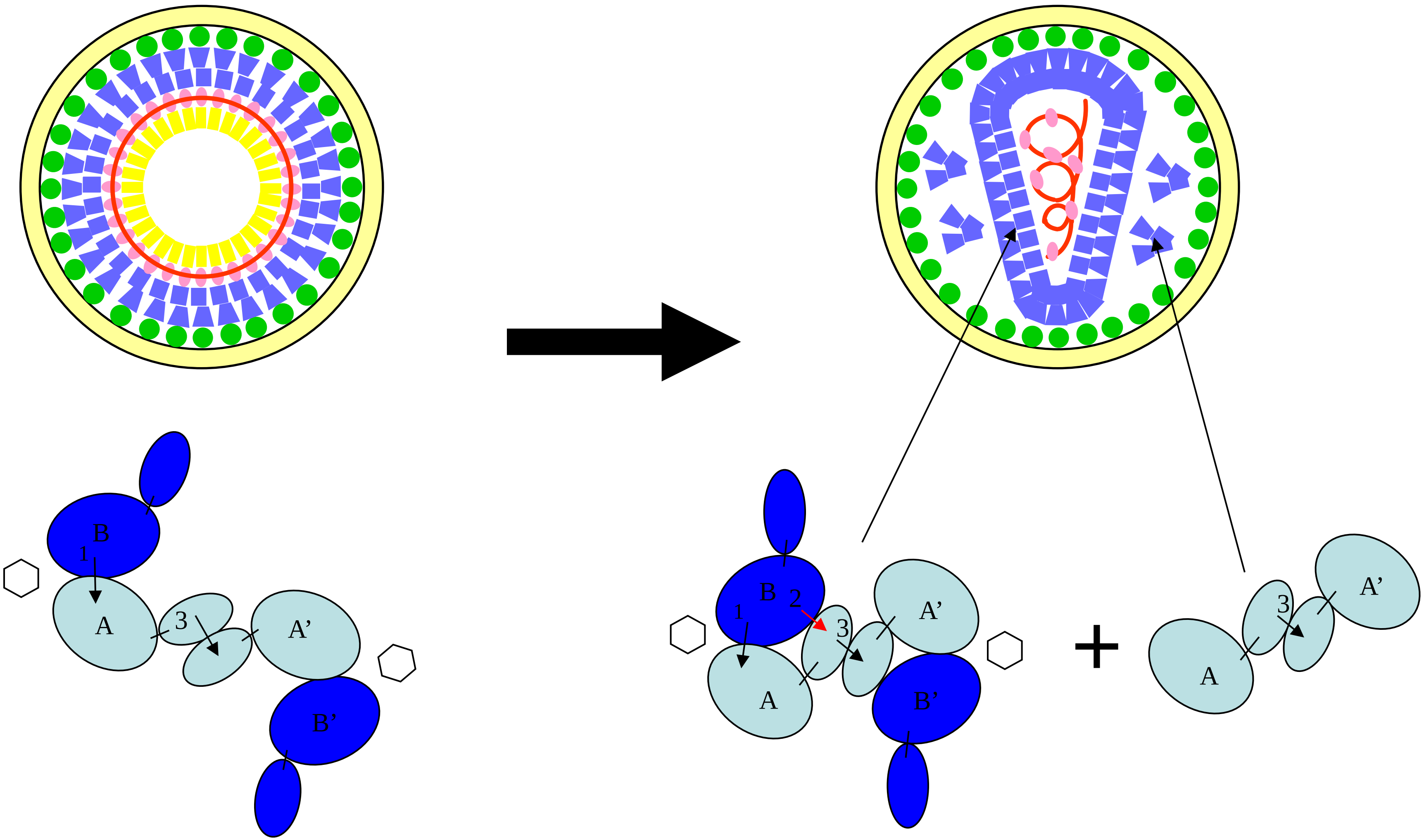
The N:C Interface is Not Formed in Immature Virions

Immature virus-like particles

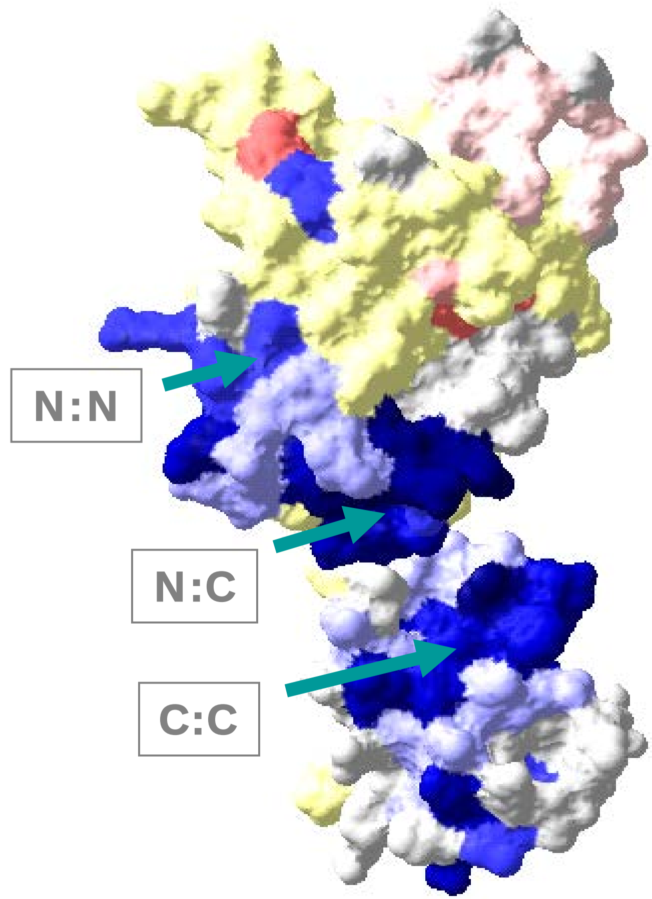




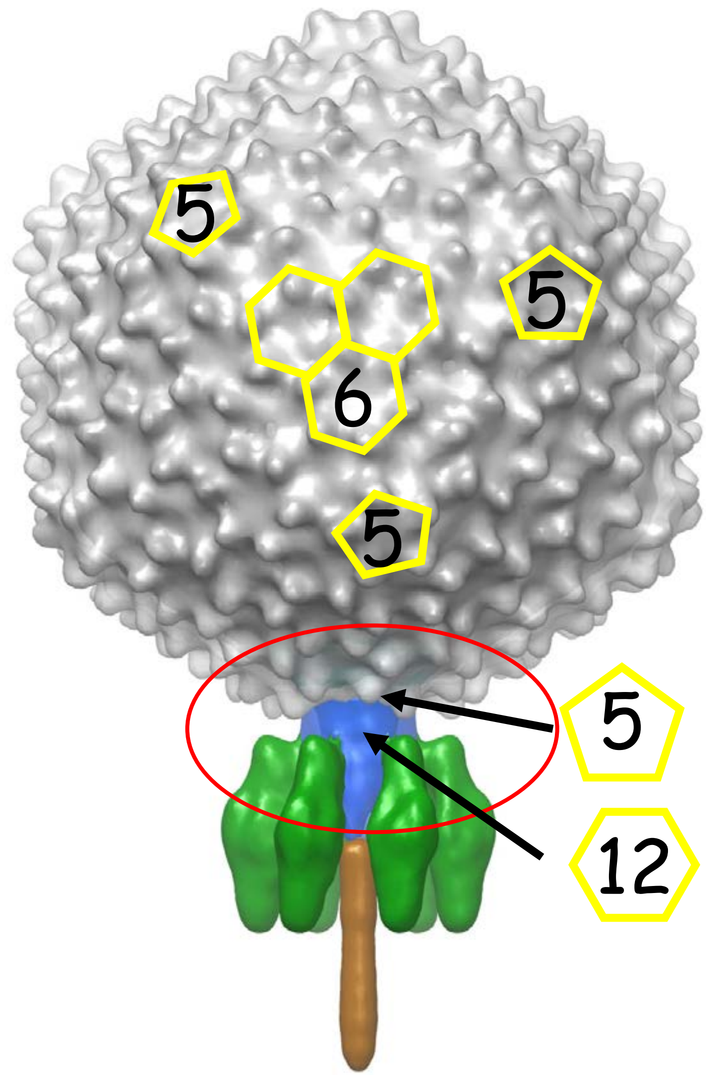
The N-domain:C-domain Interaction is Crucial for Viral Maturation



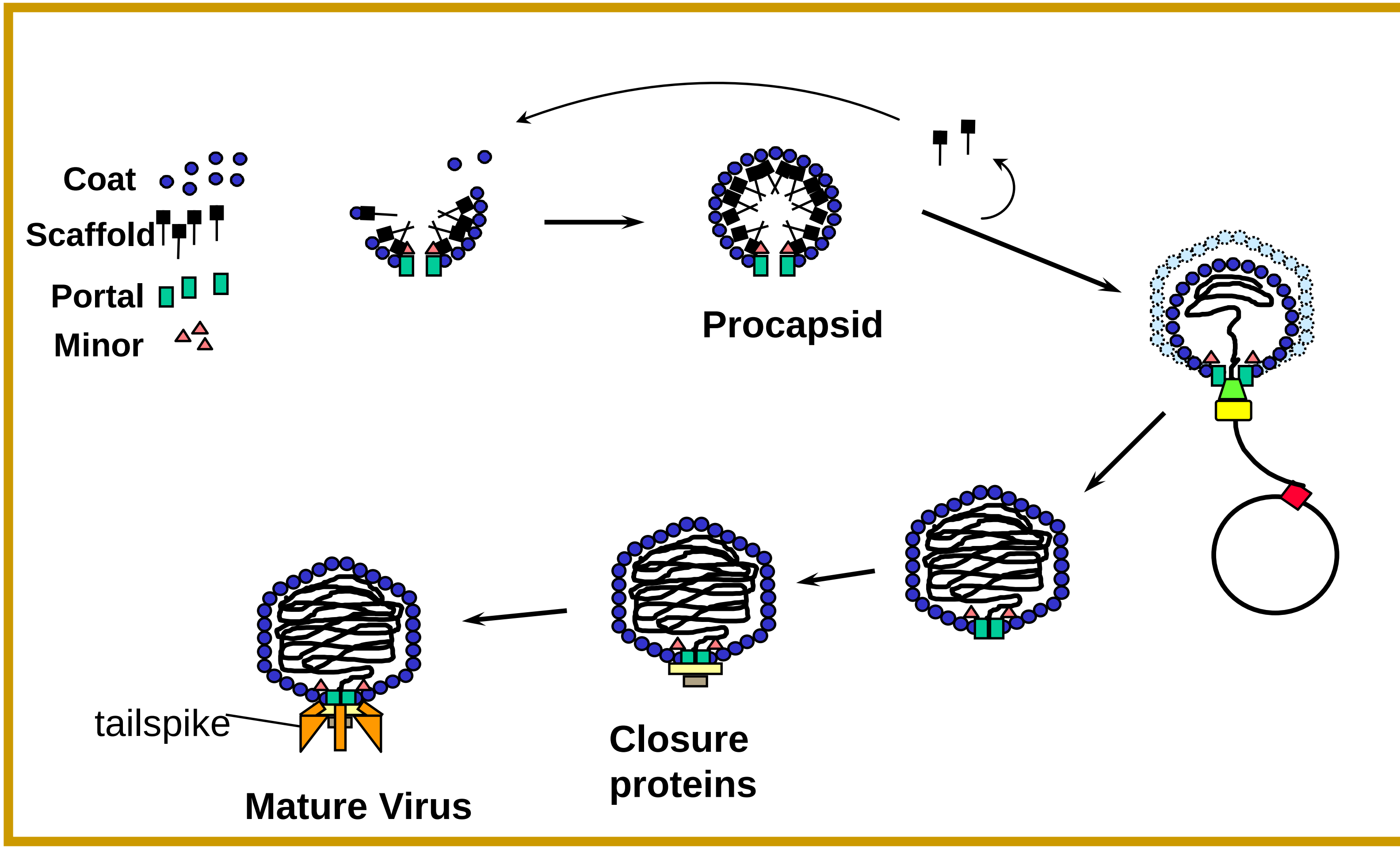
Three Sites of Interaction During Assembly and Maturation



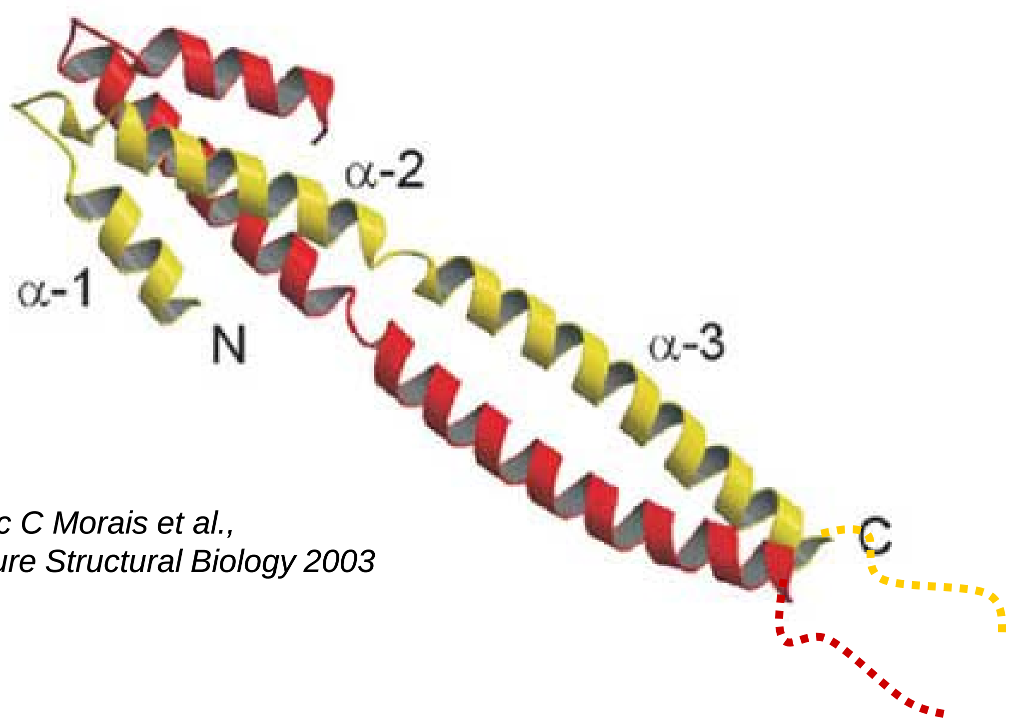
dsDNA Containing Phage Morphology



The dsDNA Phage Assembly Pathway

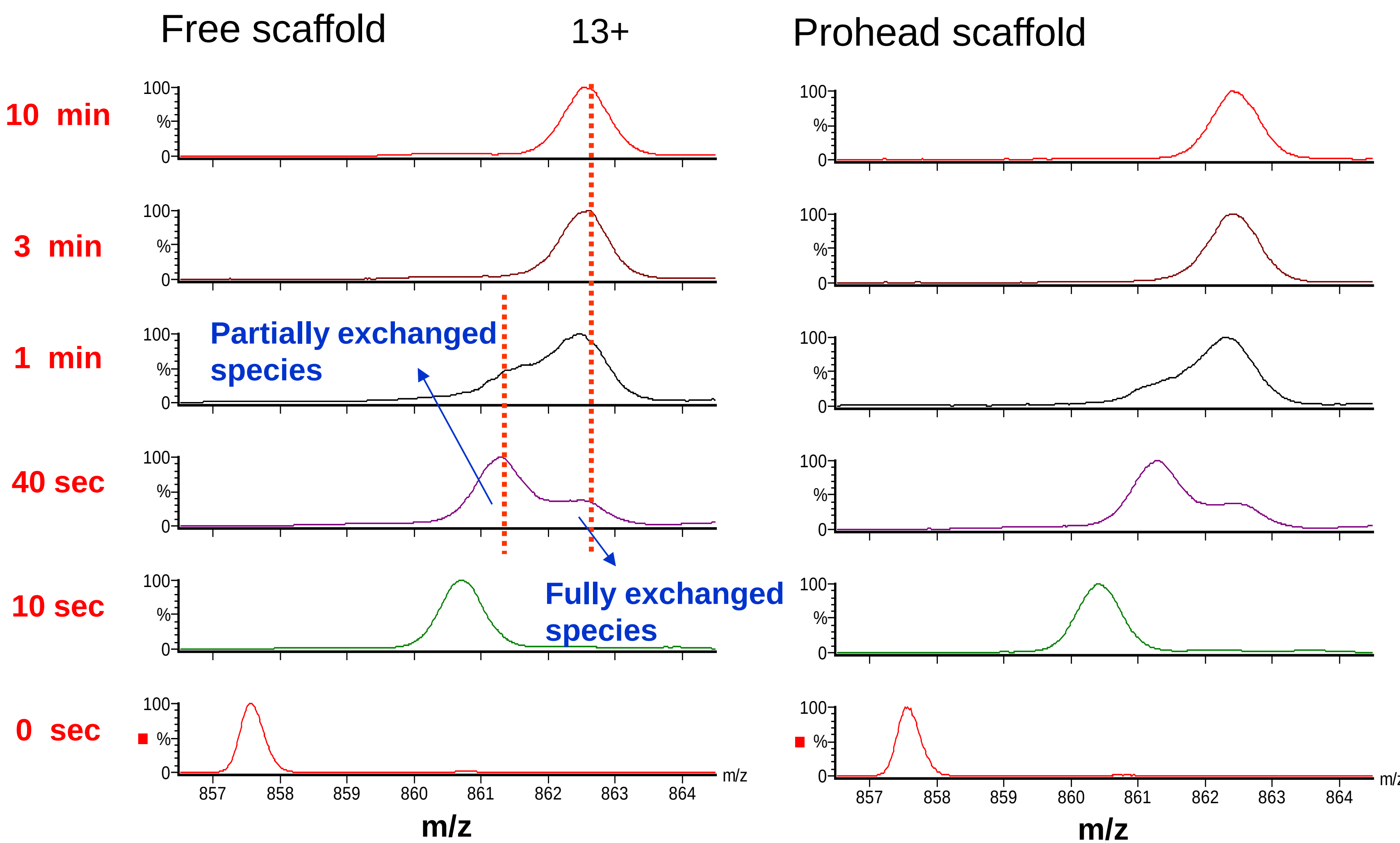


Phi29 Scaffold Has a Helix-Loop-Helix Motif and a Disordered Tail



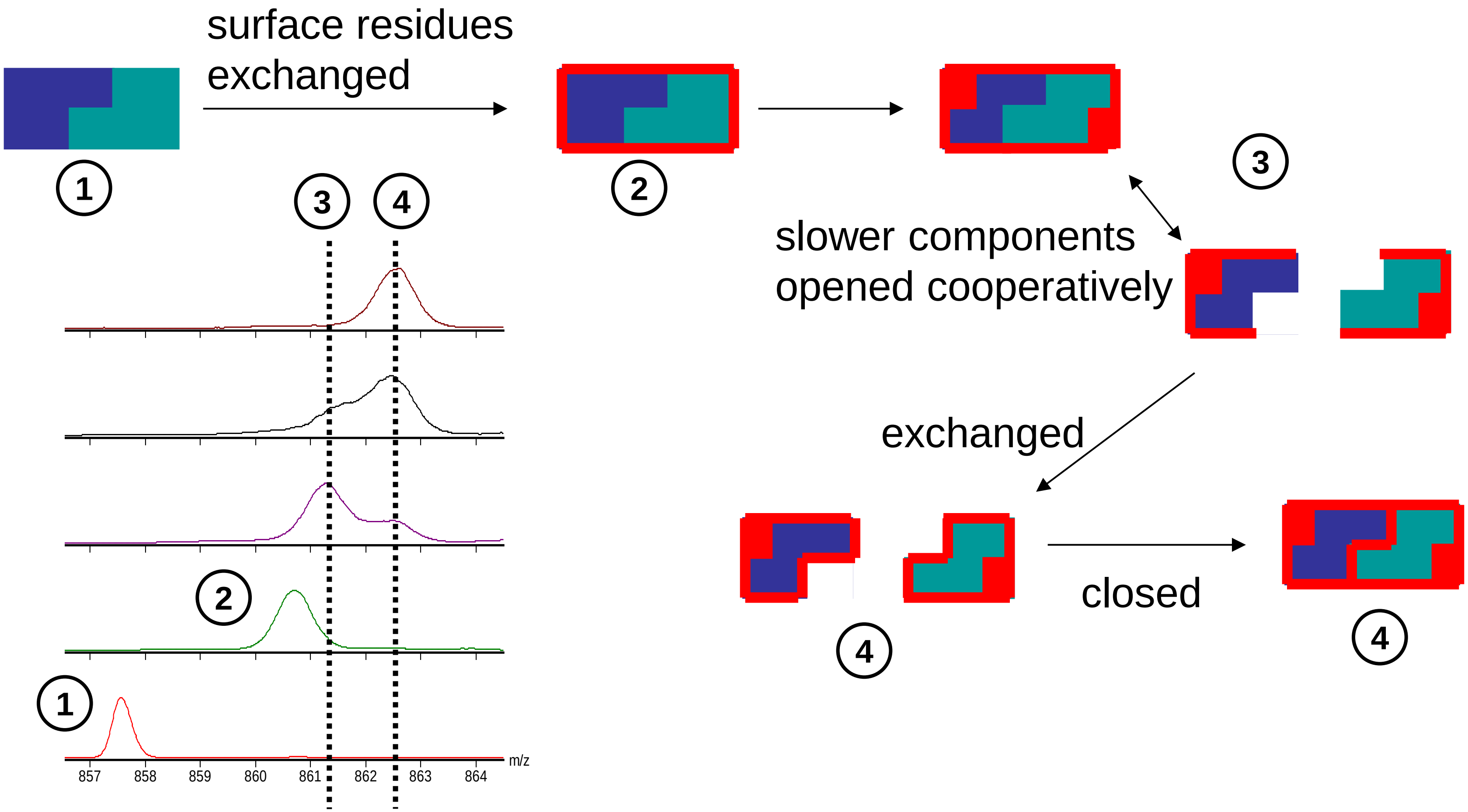
Marc C Morais et al.,
Nature Structural Biology 2003

Both Free and Bound Scaffold Exchange Bimodally

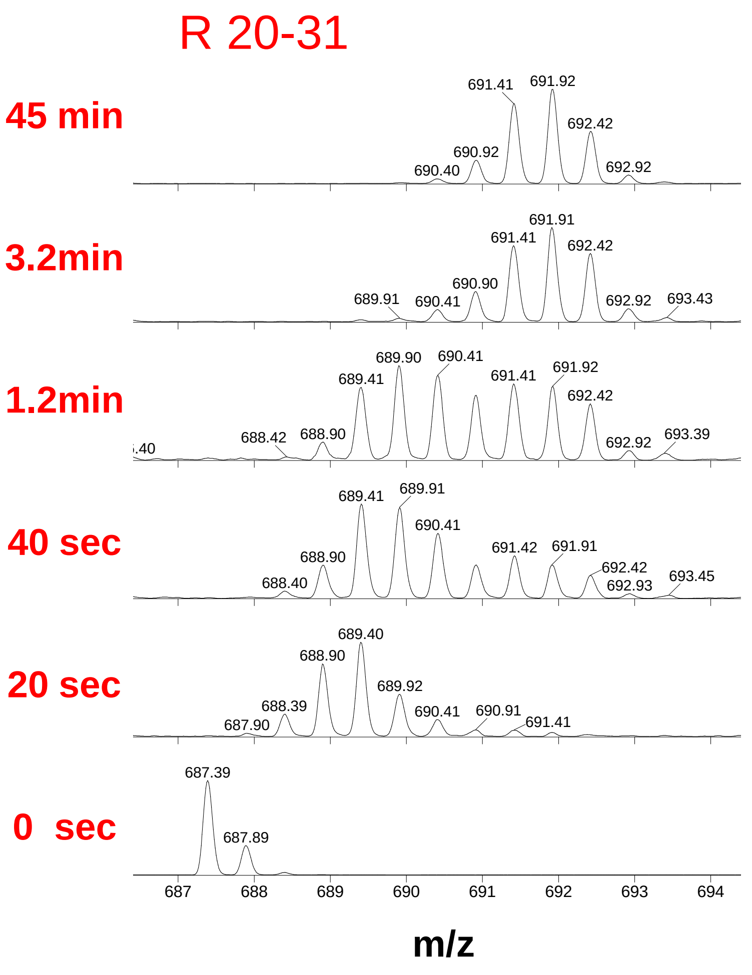


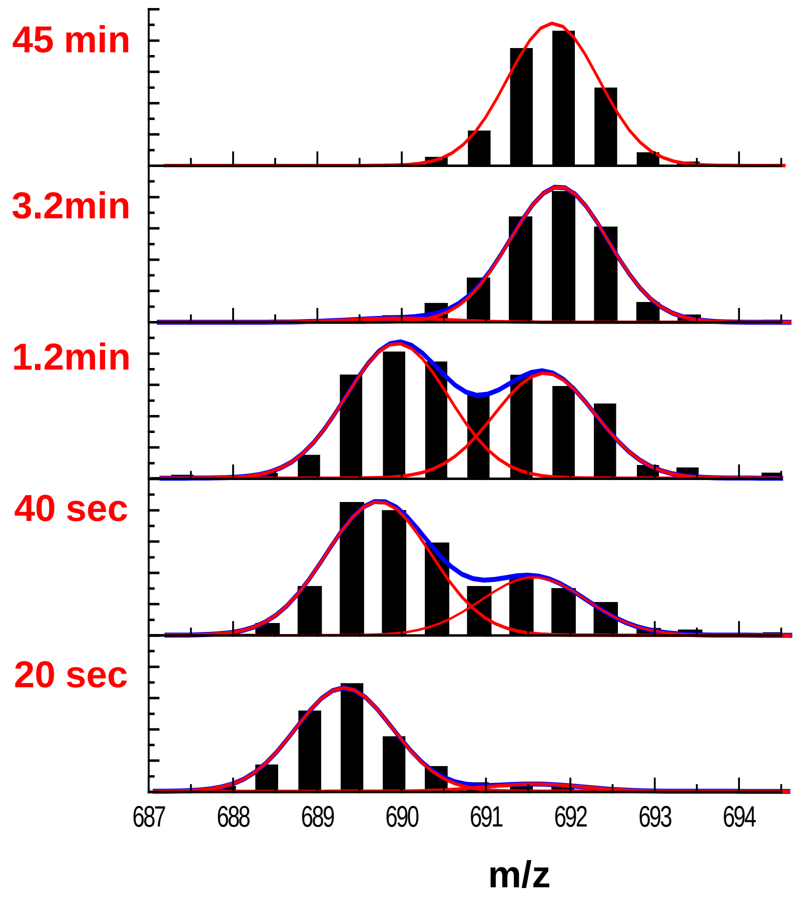
What does Bimodality Indicate?

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

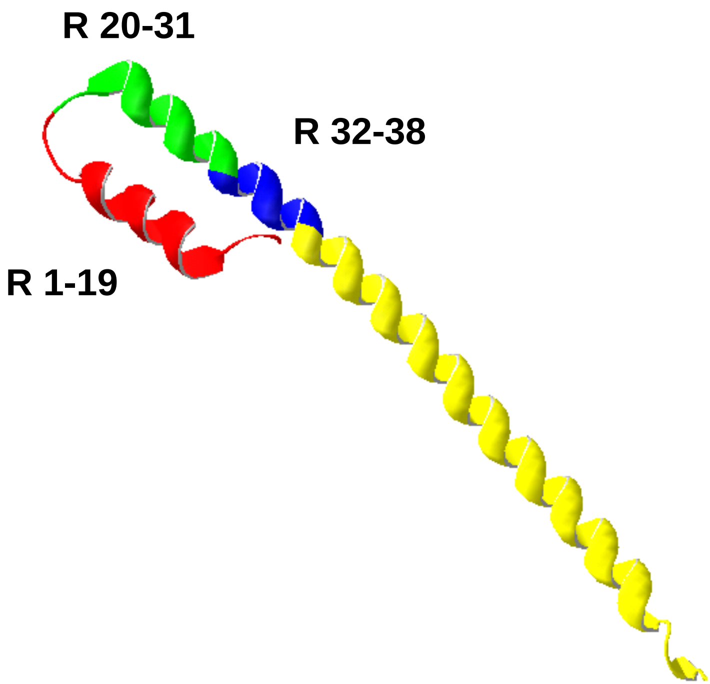


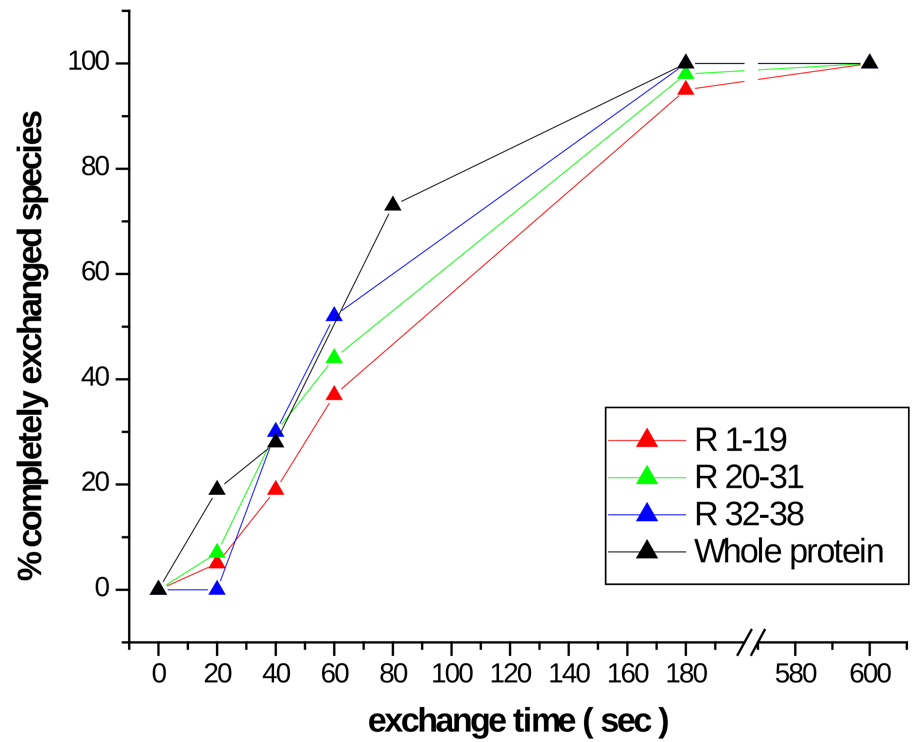
The Bimodality Maps to N-terminal Helix-Loop-Helix



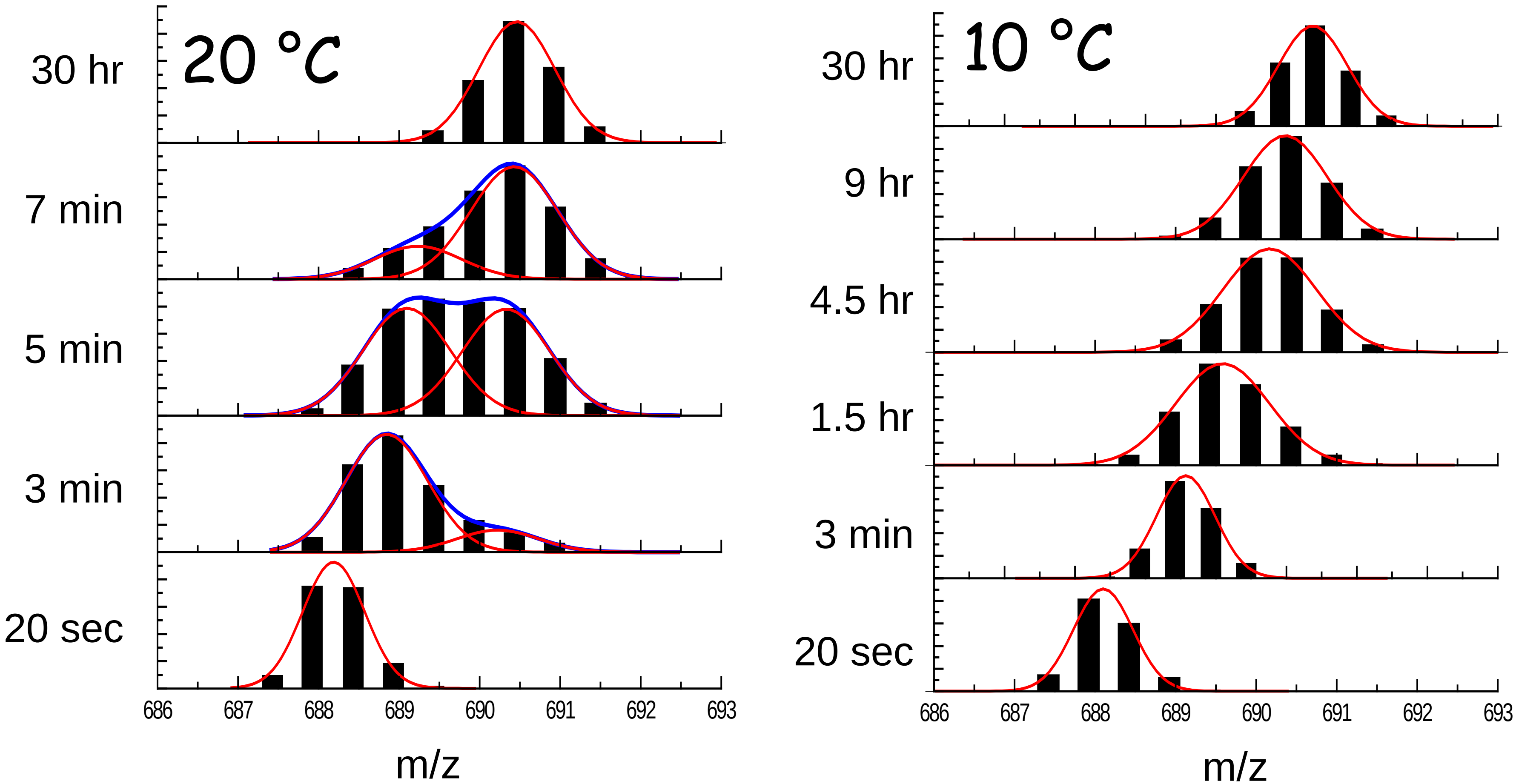


Peptides Derived from H-L-H Region Have Similar Opening Kinetics

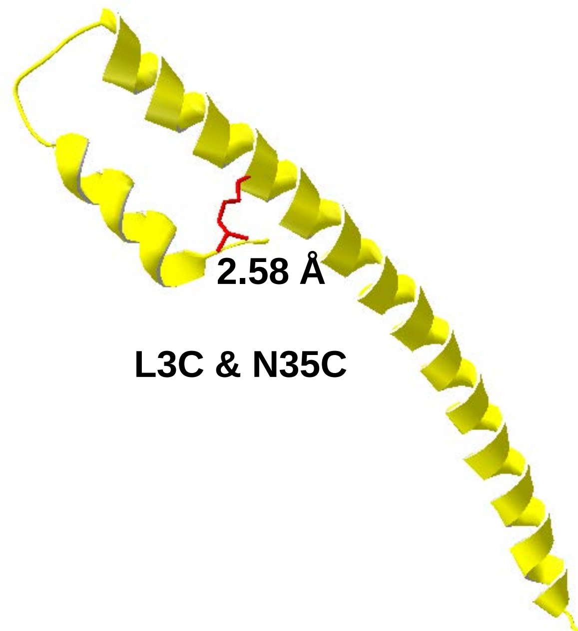




The Cooperative Motions can Be "Frozen" by Lowering the Temperature



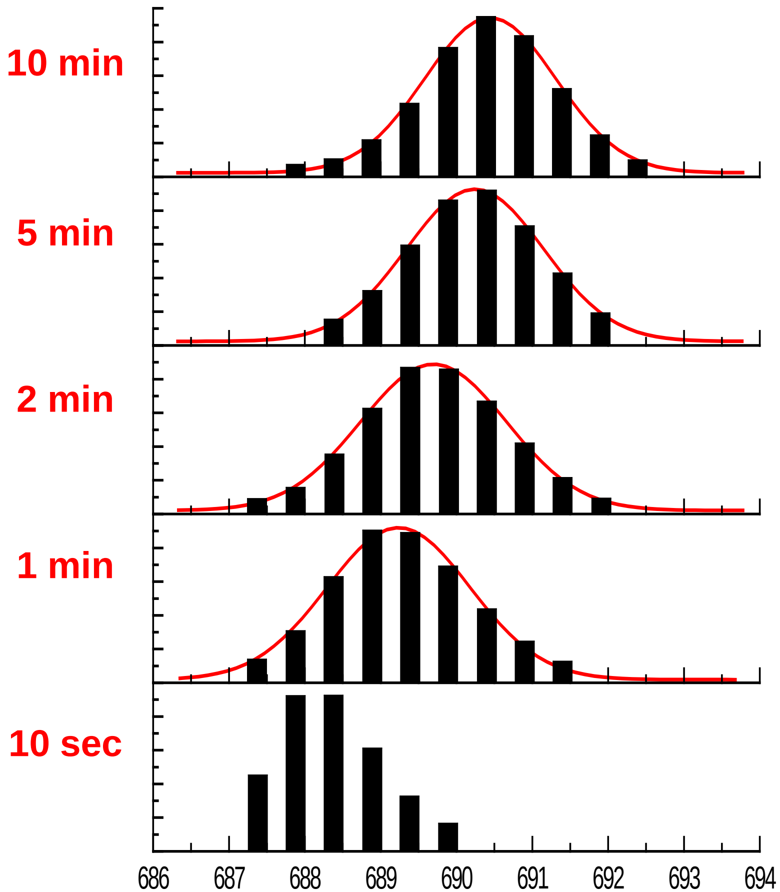
Does Bimodality Originate from Opening of the Interface between Helices 1 & 2 ?



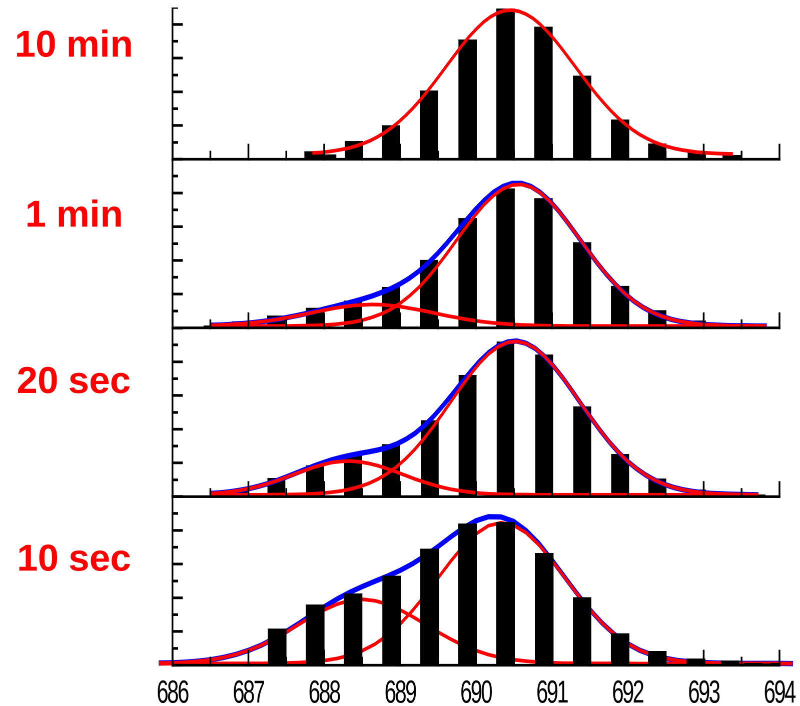
Tethered Form

The Tethered Form Cannot Open Cooperatively

Oxidized Form (Tethered)

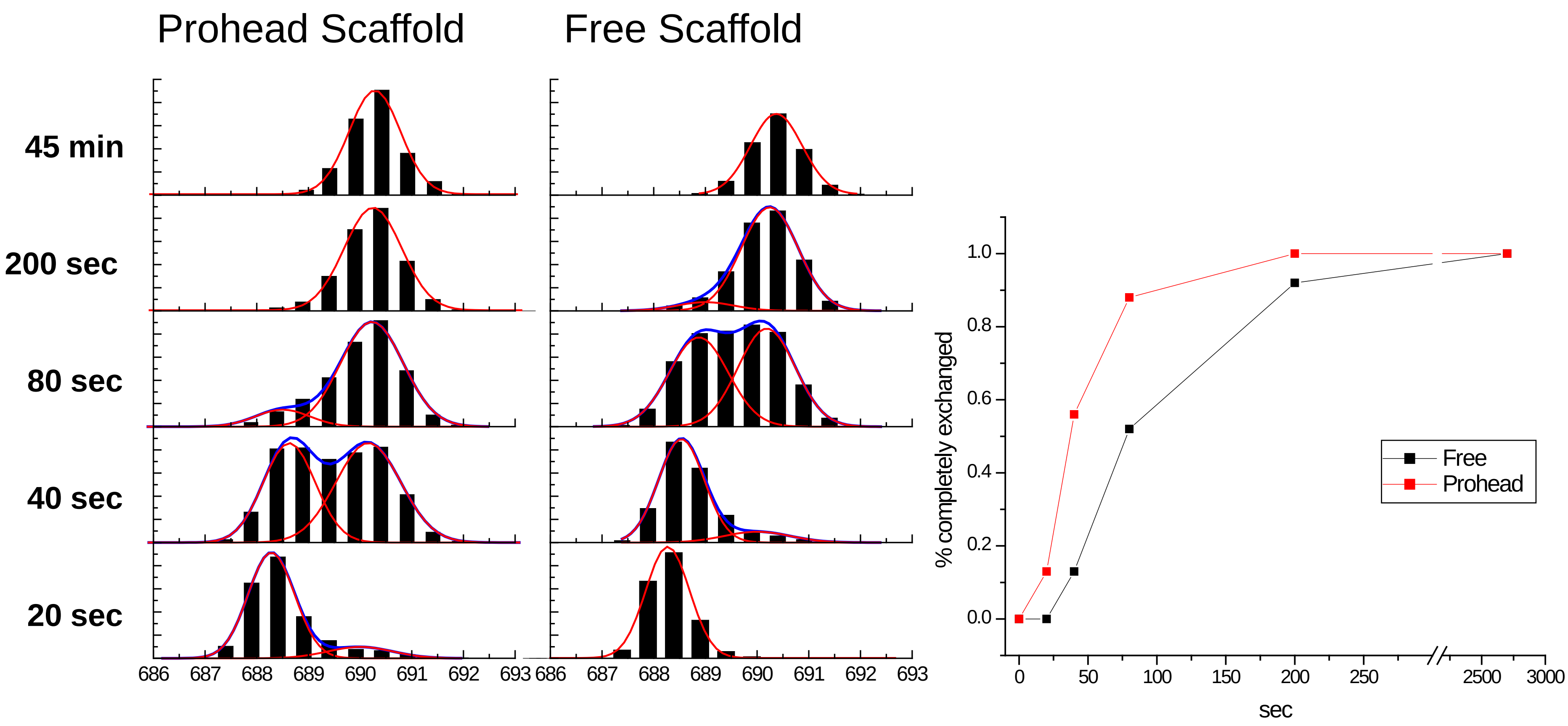


Reduced form

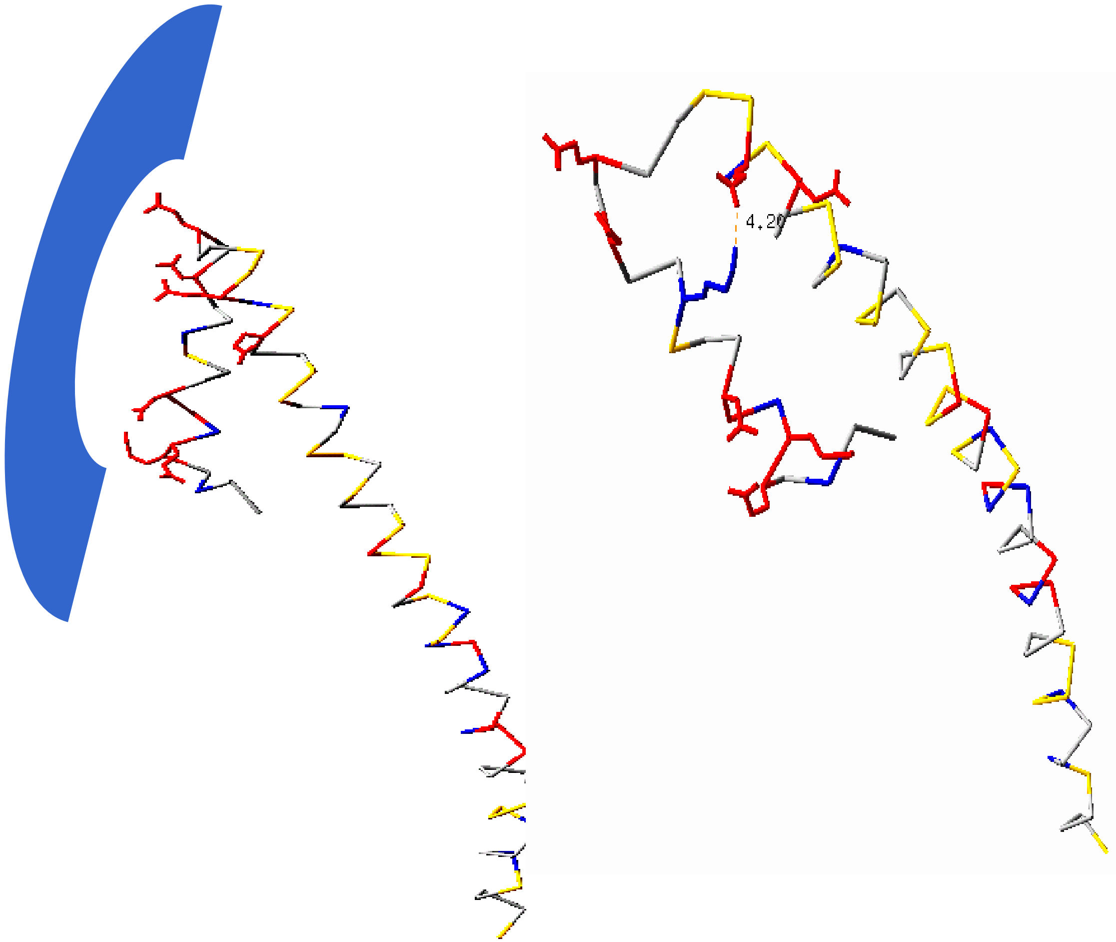


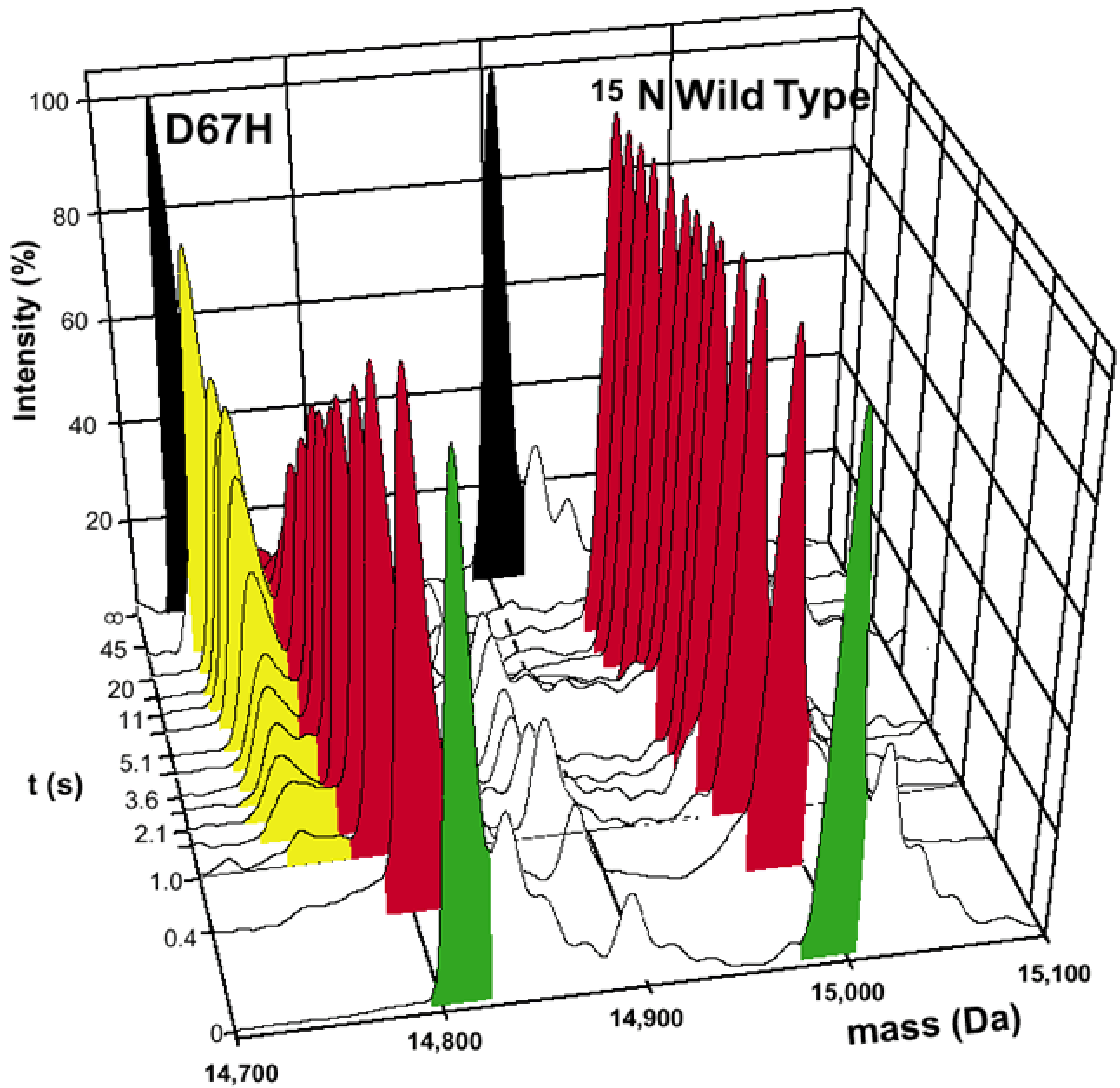
- Does Binding Alter the Exchange Kinetics?

Prohead Bound Scaffold has Faster Kinetics



Model : The Interactions Stabilizing H-L-H are Weakened when Contact with Capsid





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)