

A mass spectrum plot with a blue background and white peaks. The x-axis is labeled 'm/z' and has numerical markers at 0, 400, 600, 800, 1000, 1200, and 1400. The y-axis represents relative intensity. The most prominent peak is at approximately m/z 750. Other significant peaks are visible at approximately m/z 400, 600, 800, 1000, and 1200.

THE FLYING IONS

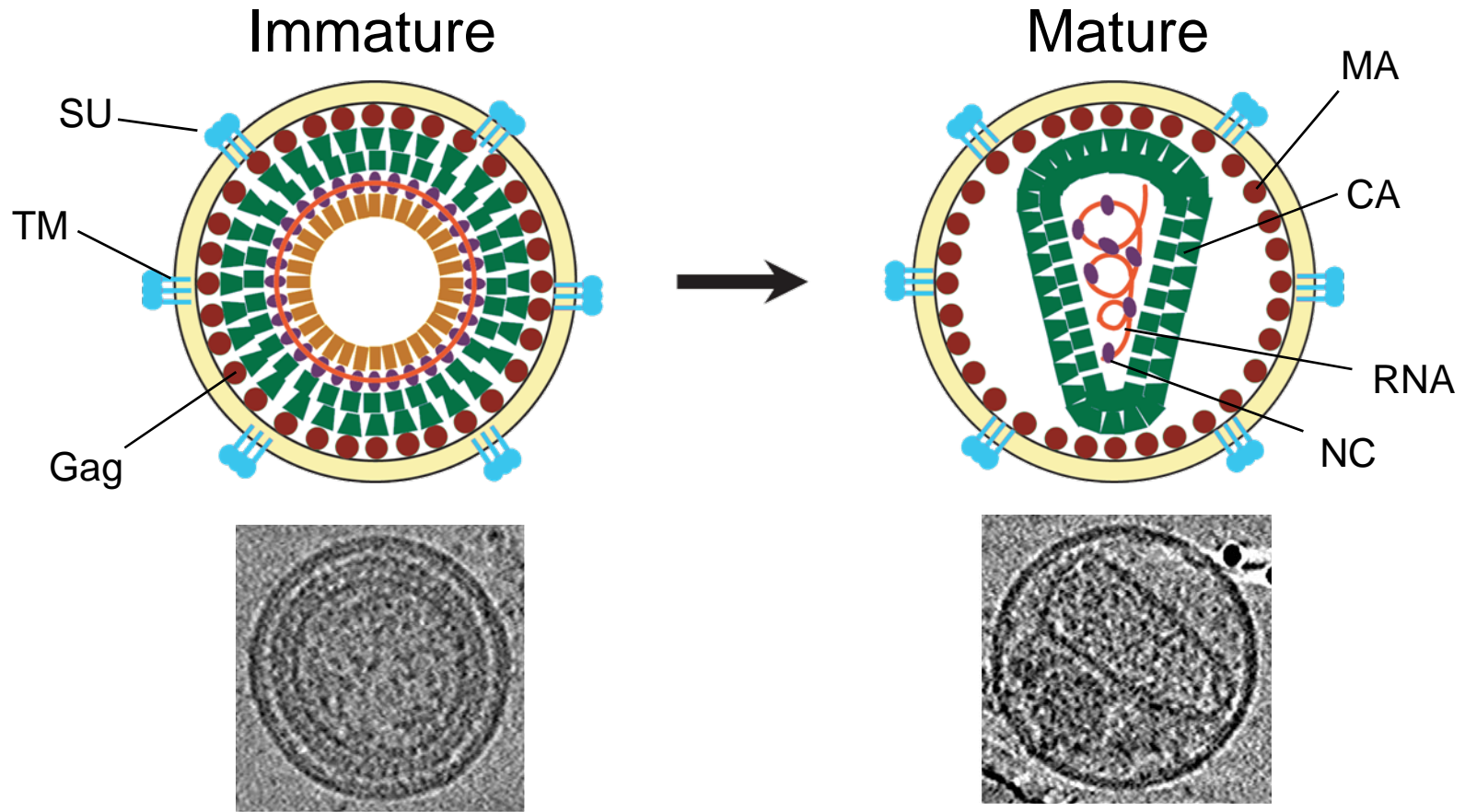
A UAB Mass Spec Interest Group

“Structural Dynamics by MS - A Story of the HIV Capsid”

Eric Monroe, Ph.D.

Tuesday, October 27th, 2009

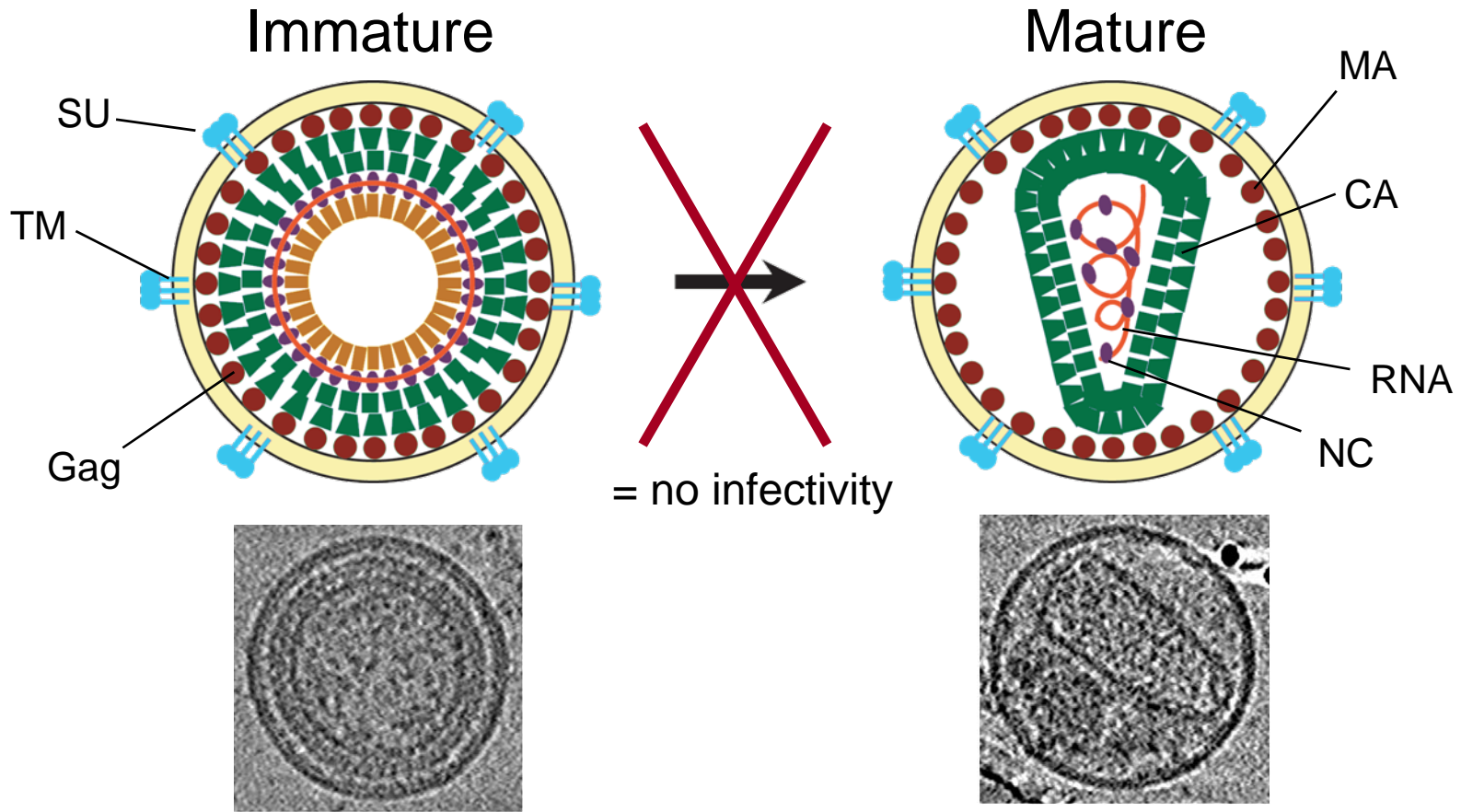
Studying HIV Capsid Maturation



Gag polyprotein



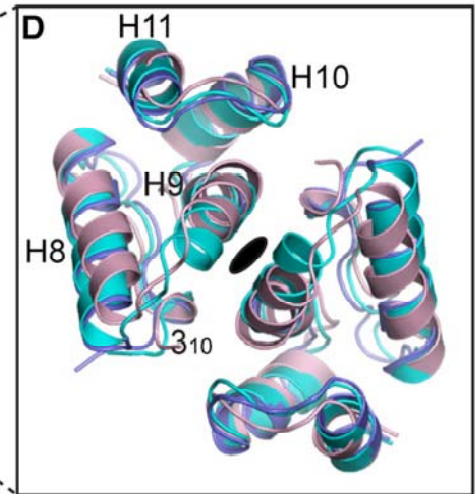
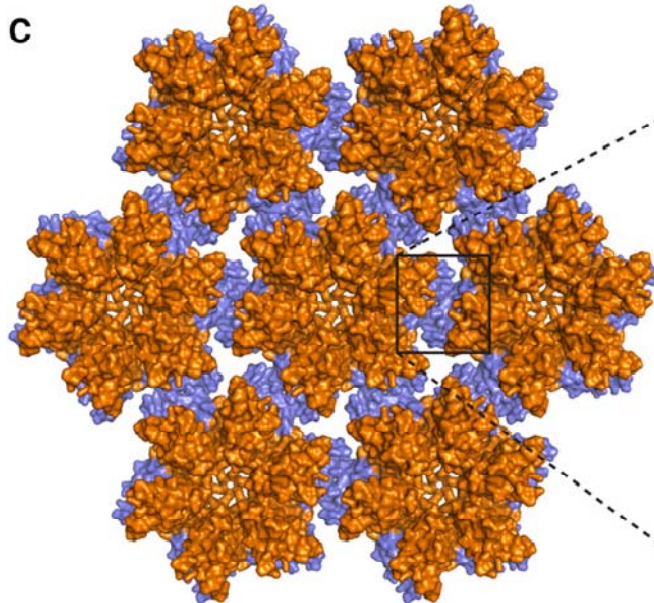
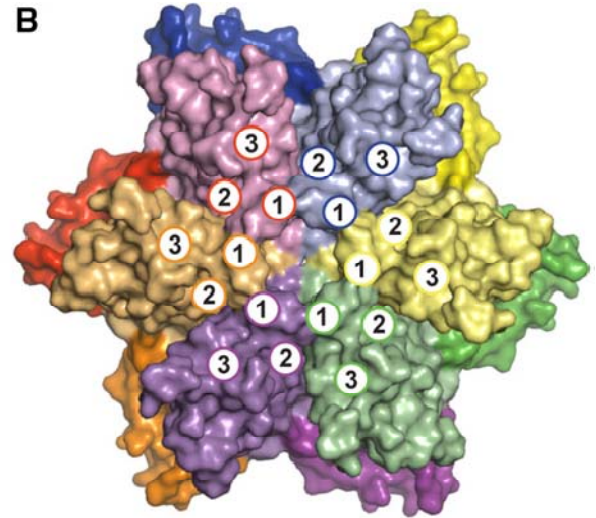
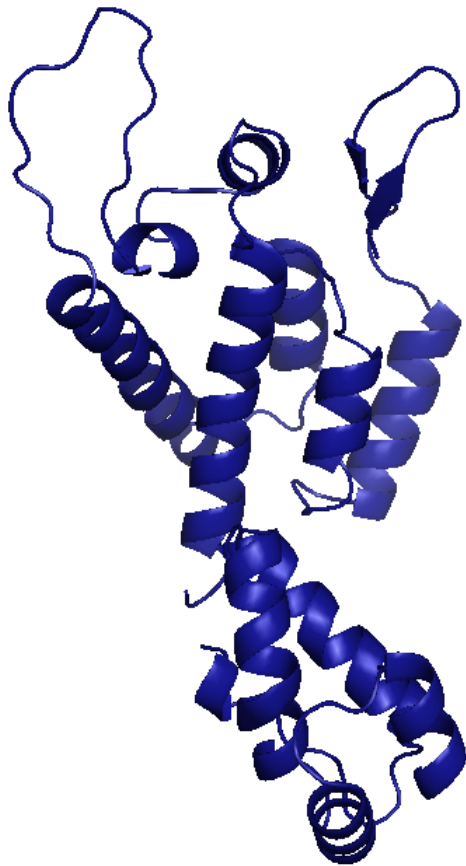
Studying HIV Capsid Maturation



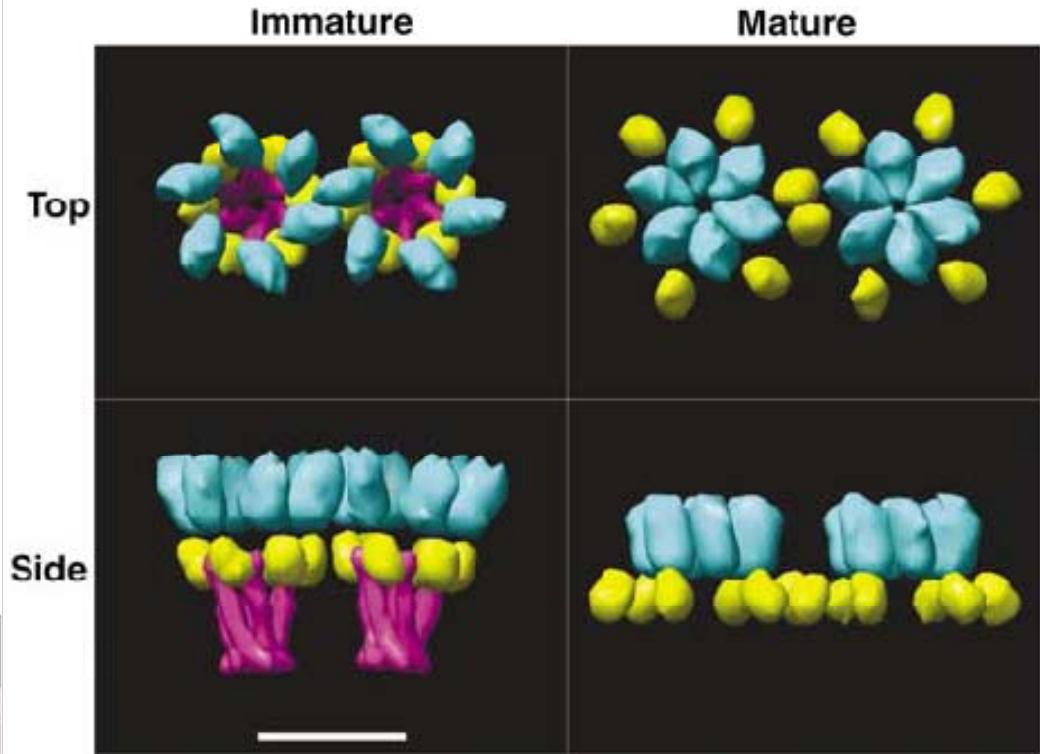
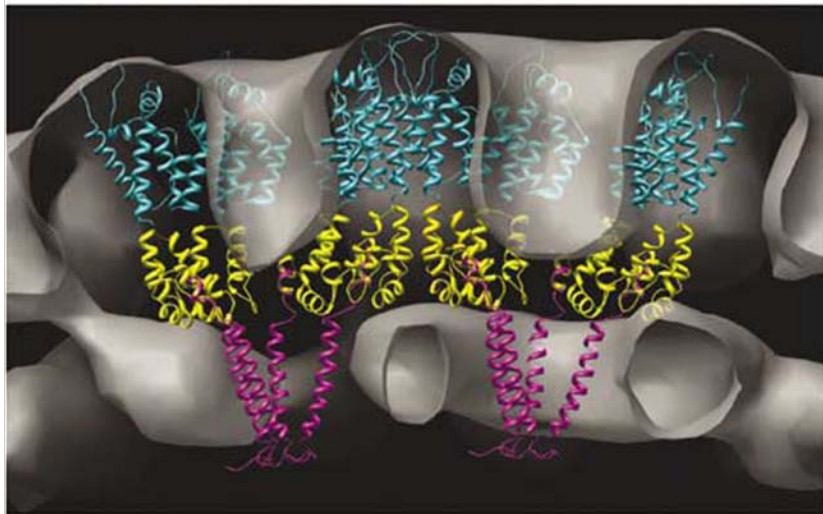
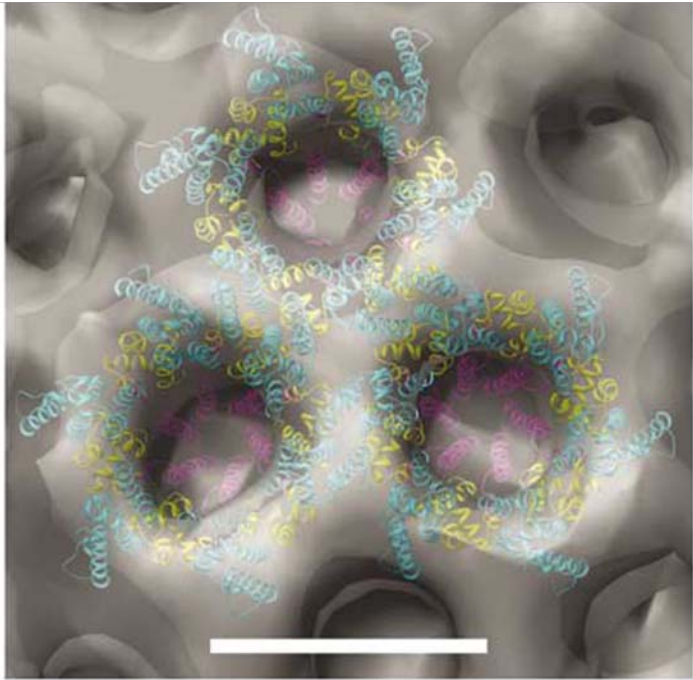
Gag polyprotein



CA mature lattice

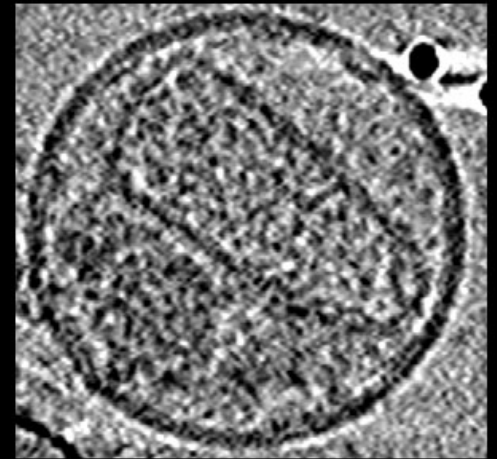
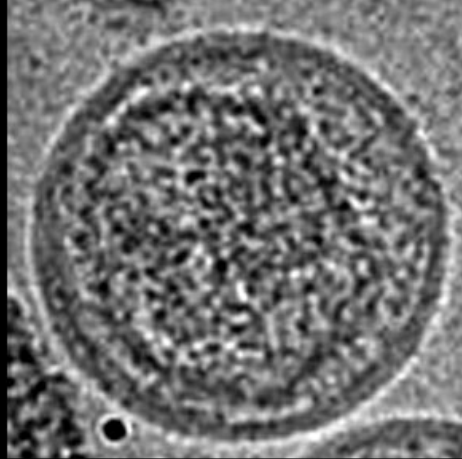


CA immature and comparison

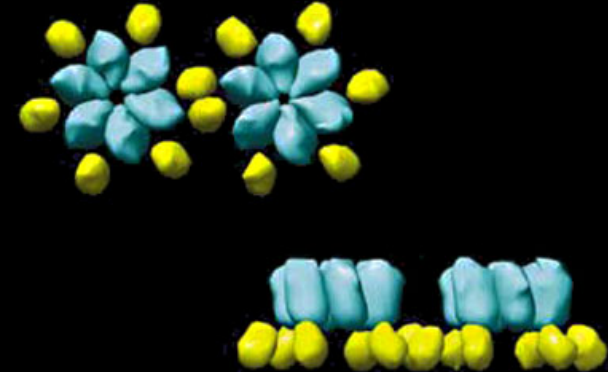


Wright, *EMBO*, 2007

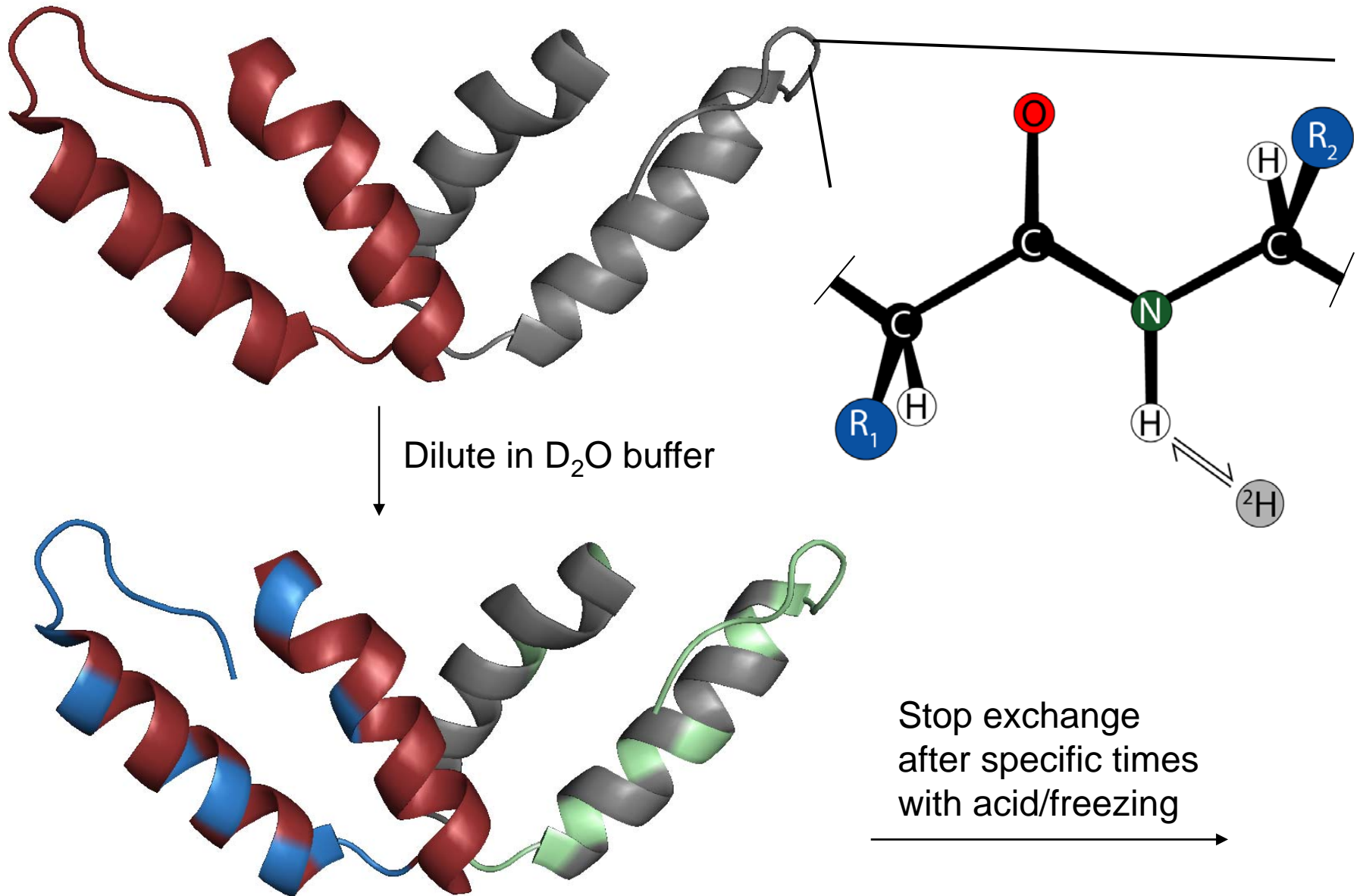
Structural Dynamics of HIV



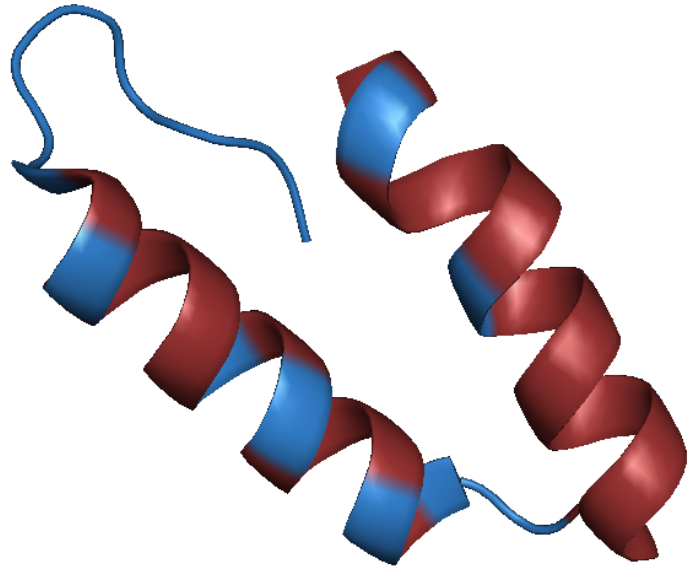
How?



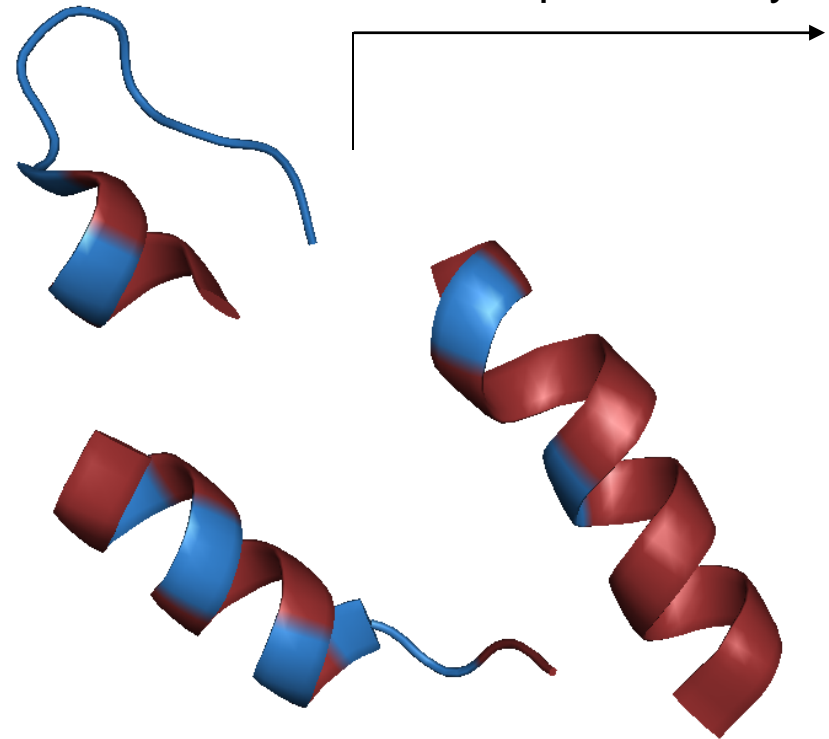
Hydrogen deuterium exchange MS



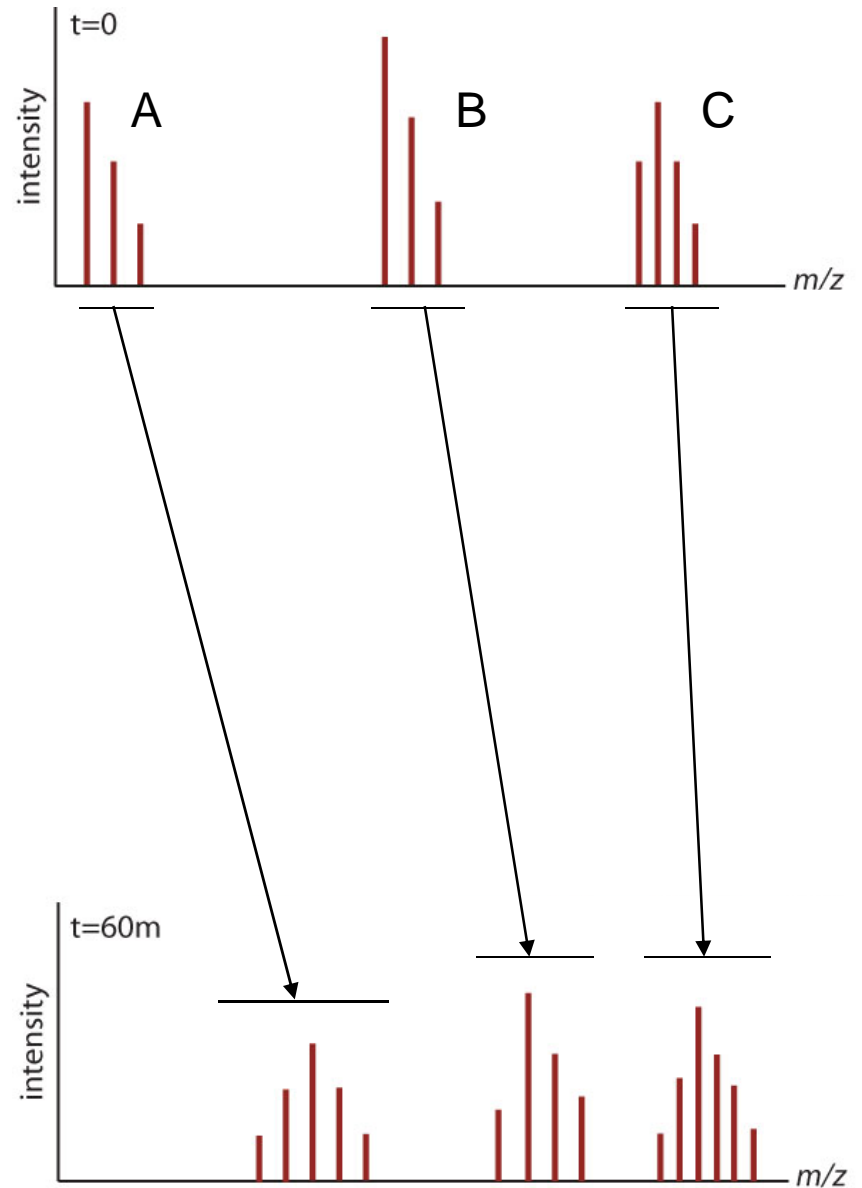
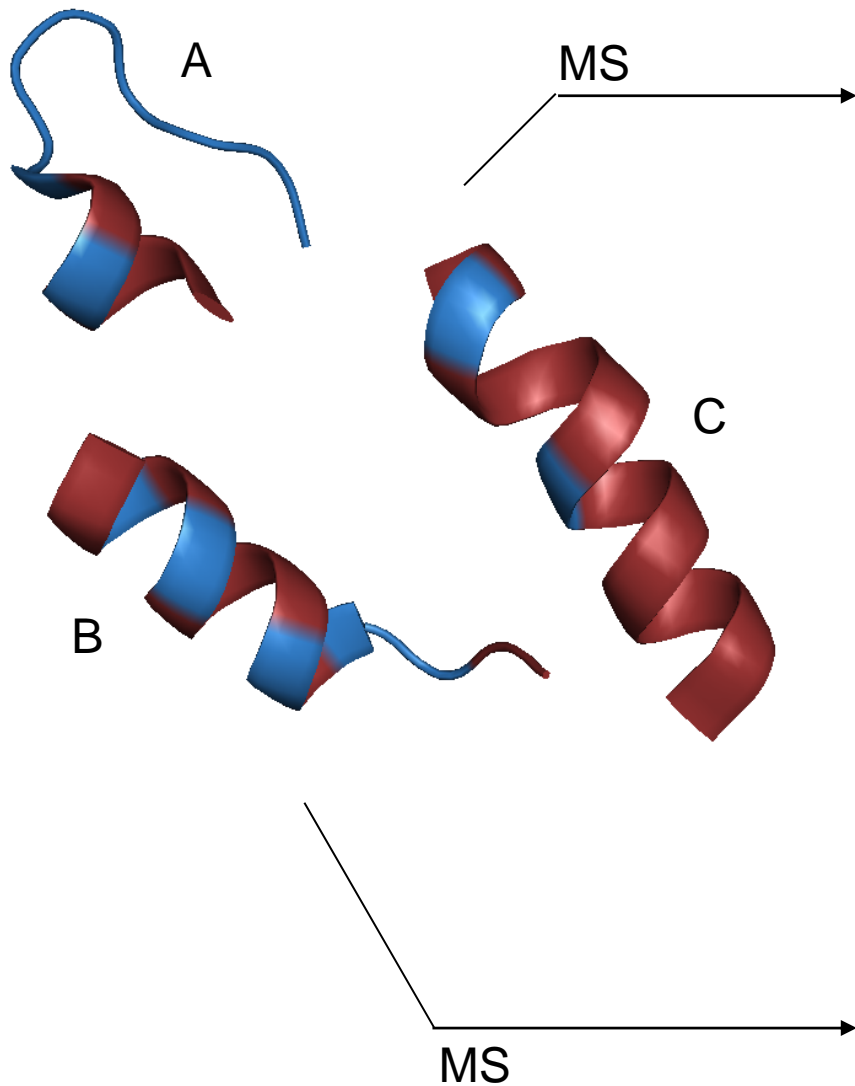
Hydrogen deuterium exchange MS



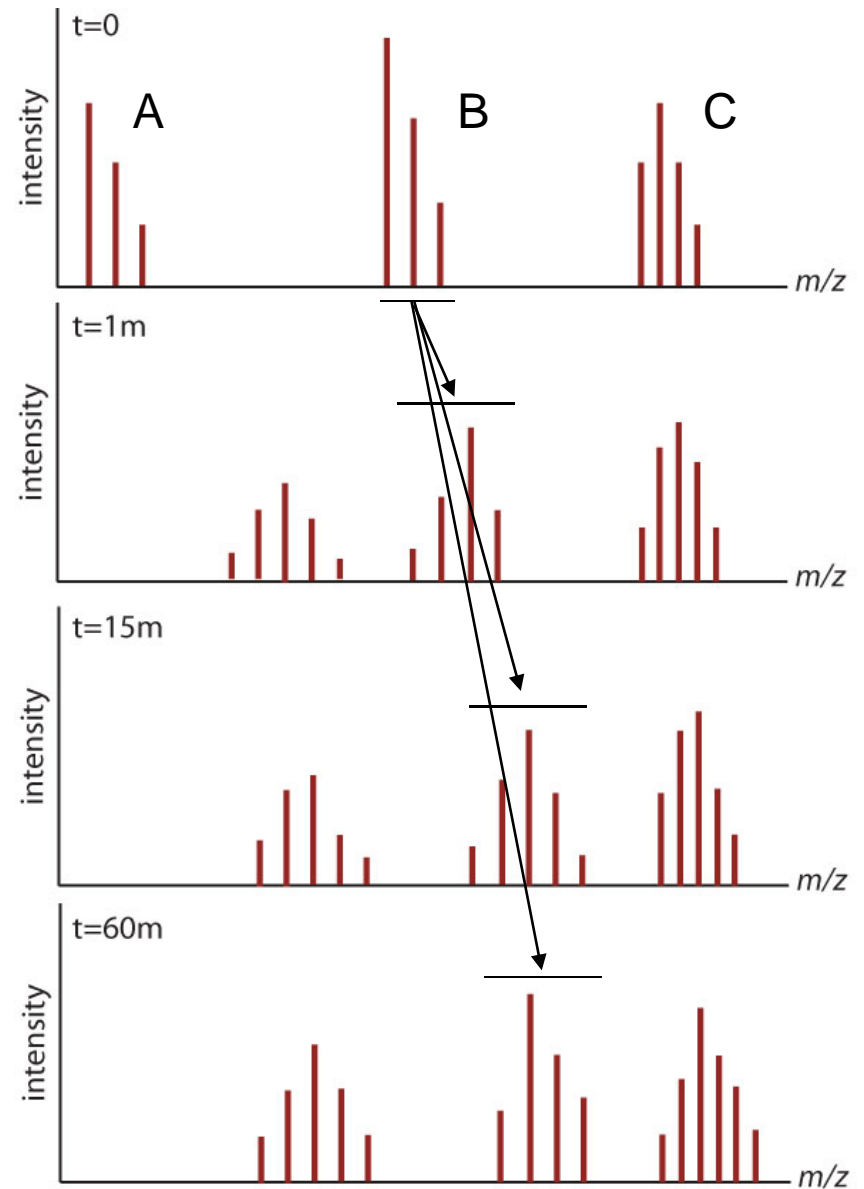
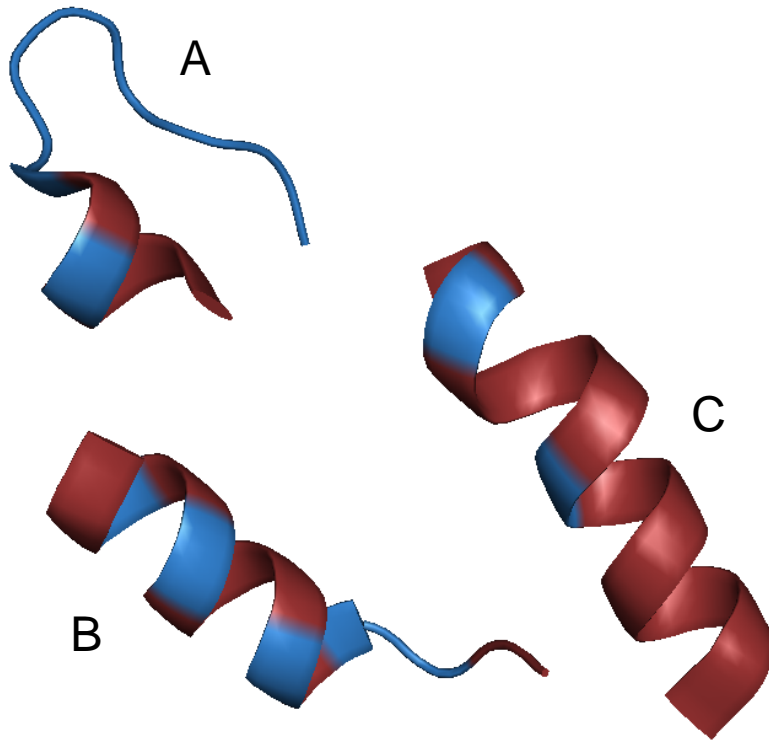
Rapidly thaw and digest with pepsin



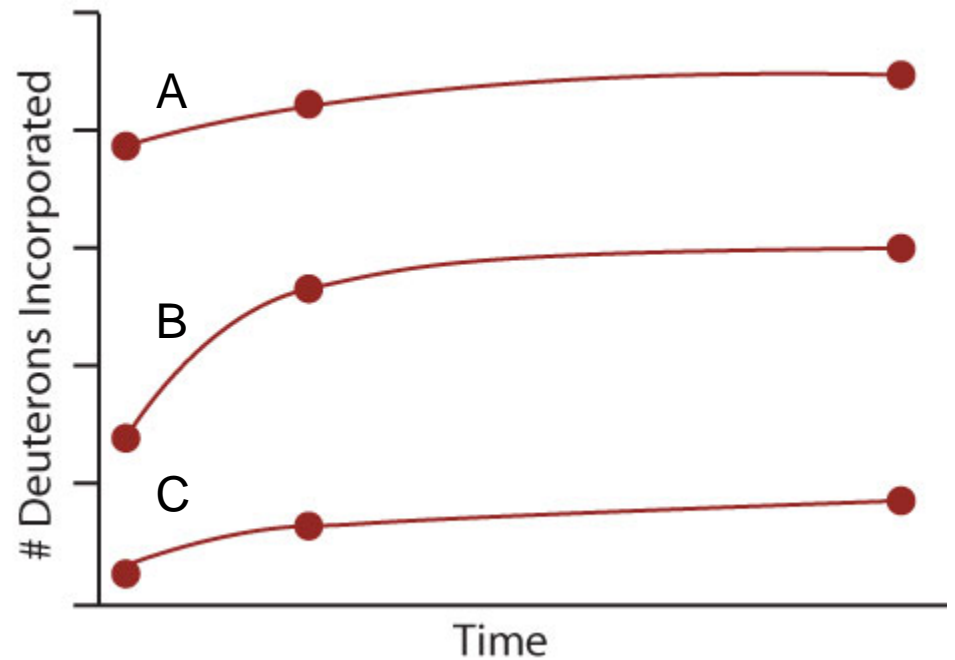
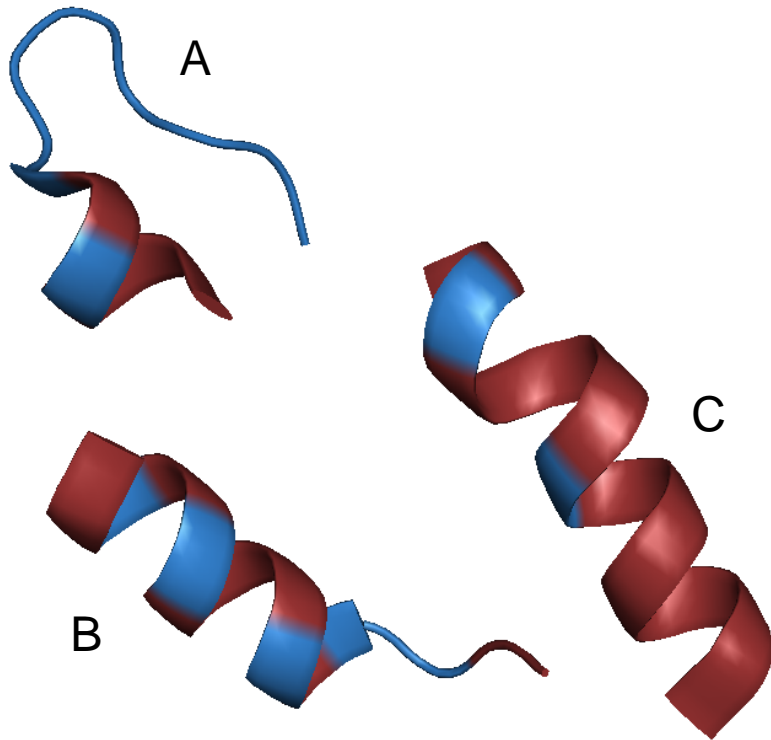
Hydrogen deuterium exchange MS



Hydrogen deuterium exchange MS

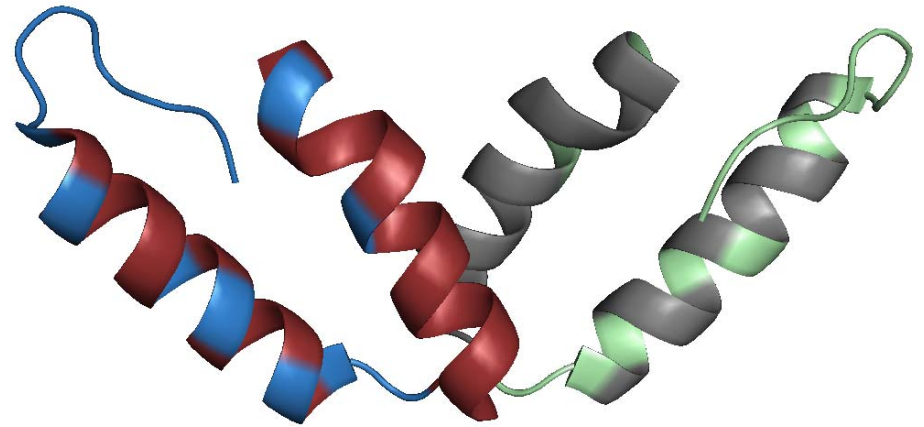
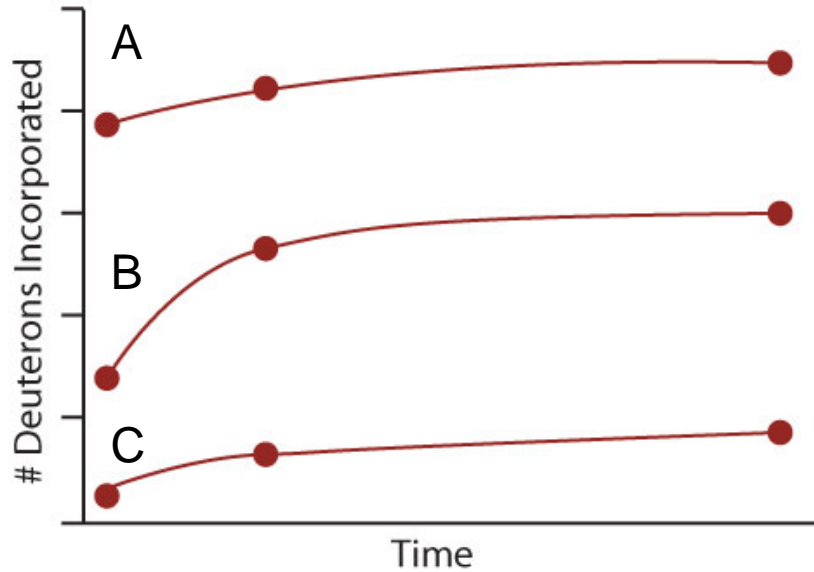


Hydrogen deuterium exchange MS



$$D = N - A e^{(-k_1 t)} - B e^{(-k_2 t)} - C e^{(-k_3 t)}$$

Hydrogen deuterium exchange MS



$$D = N - A e^{(-k_1 t)} - B e^{(-k_2 t)} - C e^{(-k_3 t)}$$

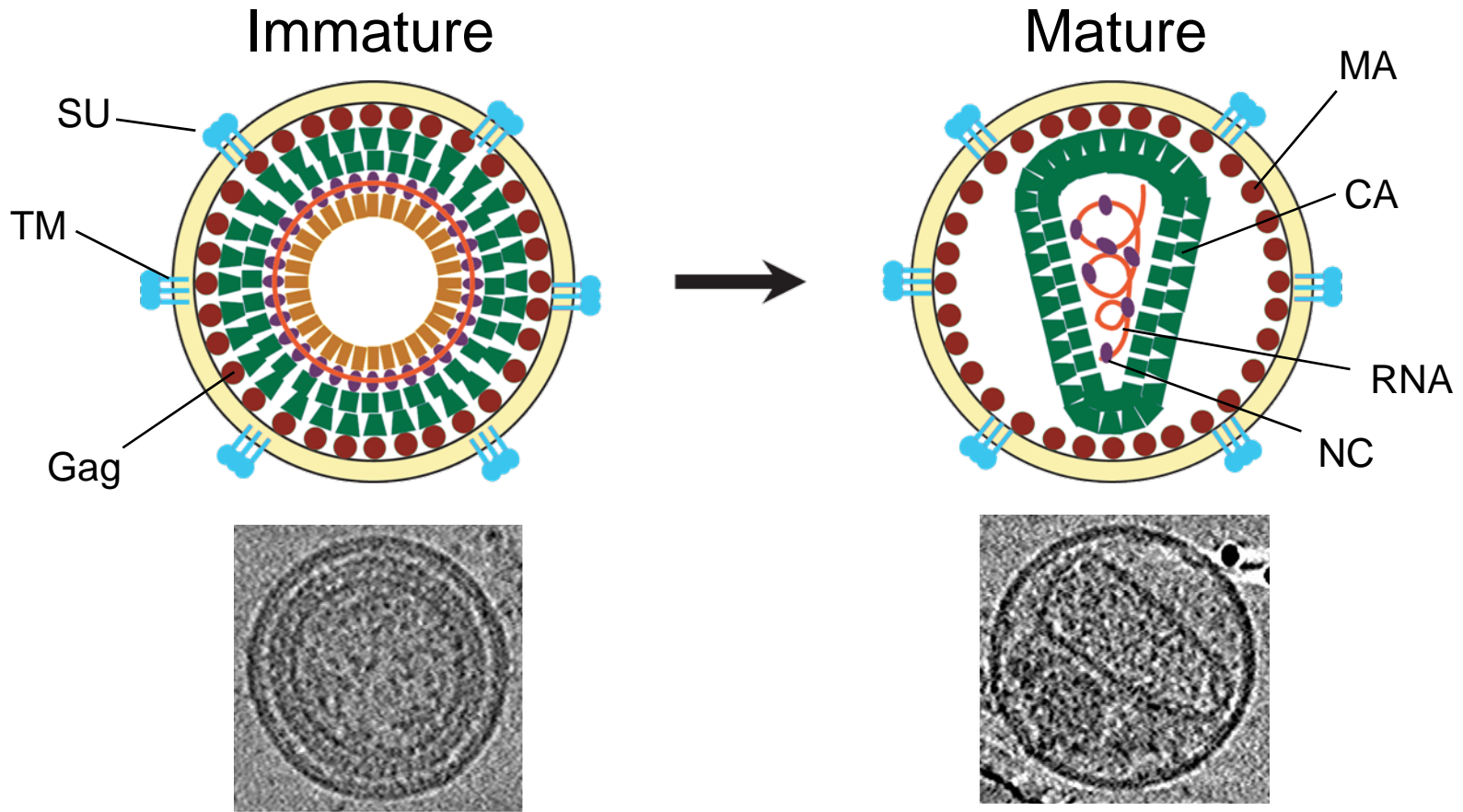
$$k = \ln 2 / t_{1/2}$$

k (min ⁻¹)	t _{1/2} (min)	t _{1/2} (s)	
10	0.07	4.2	
5	0.14	8.3	fast – solvent exposed, no H-bonding
1	0.69	41.6	
0.9	0.77	46.2	
0.5	1.39	83.2	intermediate – structured, conformational fluctuation
0.1	6.93	415.9	
0.09	7.70	462.1	
0.05	13.86	831.8	slow – highly stable regions
0.01	69.31	4158.9	

HDX MS and its uses

- Structure – information is implied
- Real power is in comparisons
- More powerful with high resolution structure of homologous protein or single state of protein known
- Applications: identifying binding sites and intermolecular interfaces or structural changes which result from ligand binding, drug treatment, or maturation...

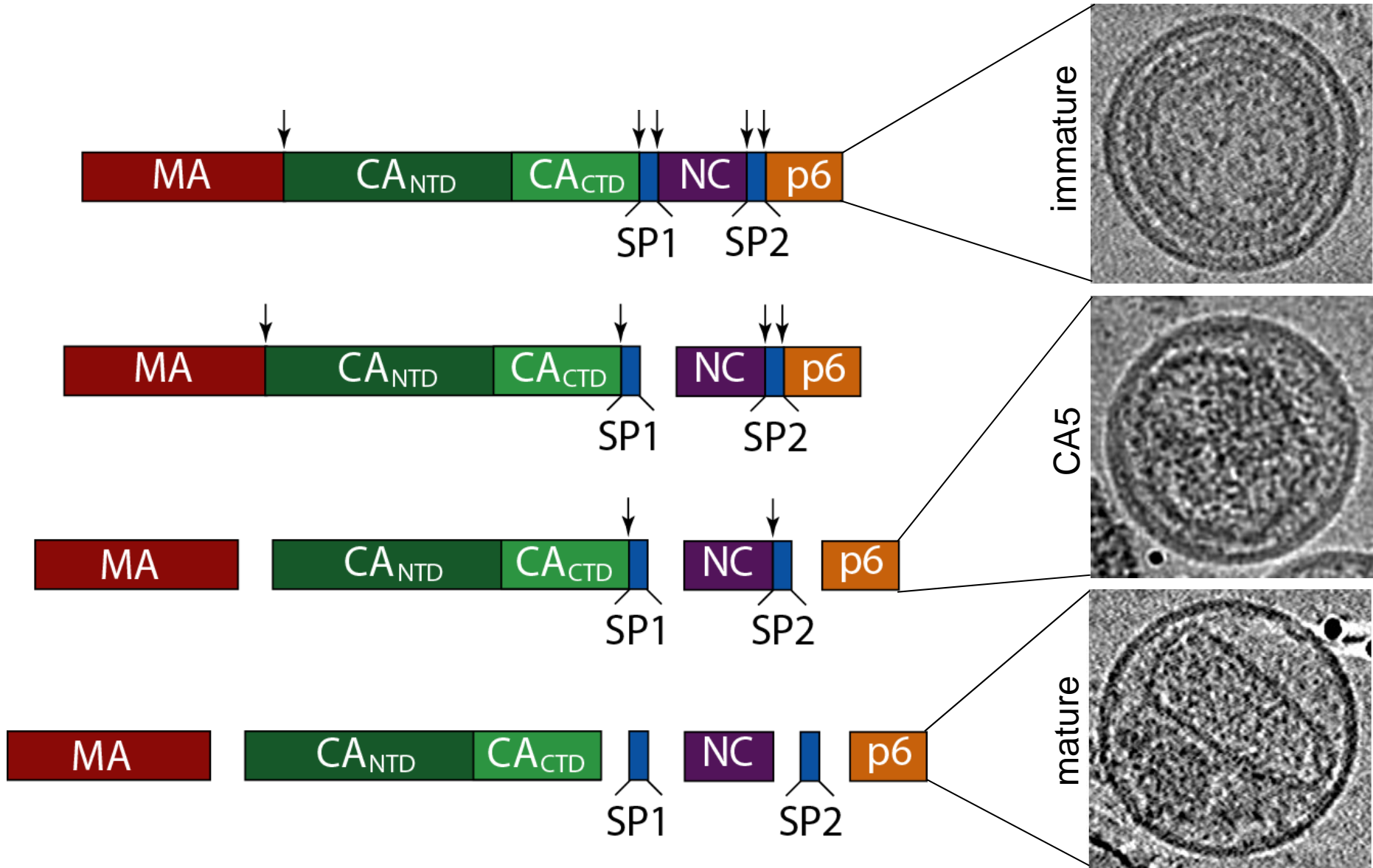
Studying HIV Capsid Maturation



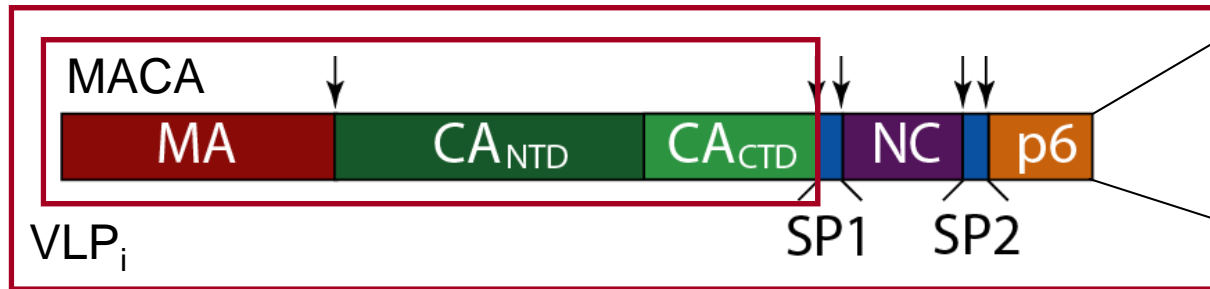
Gag polyprotein



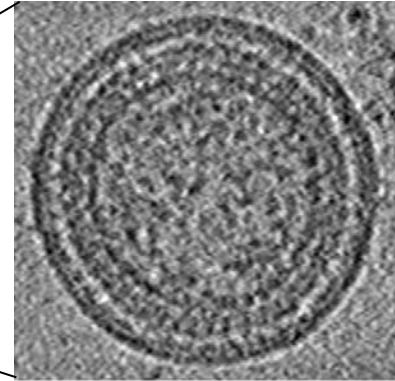
Gag cleavage and maturation



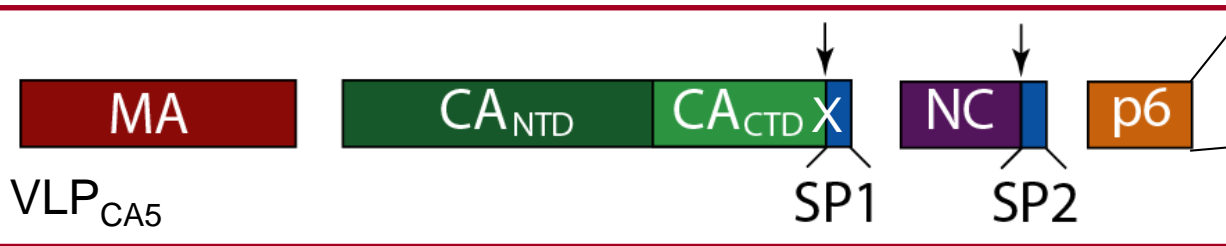
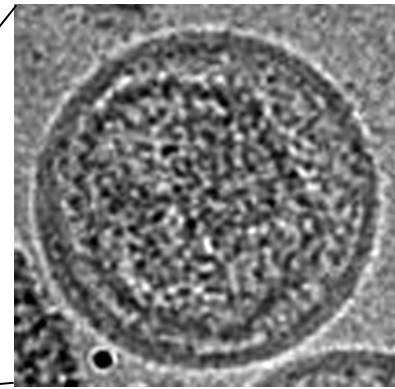
Gag cleavage and maturation



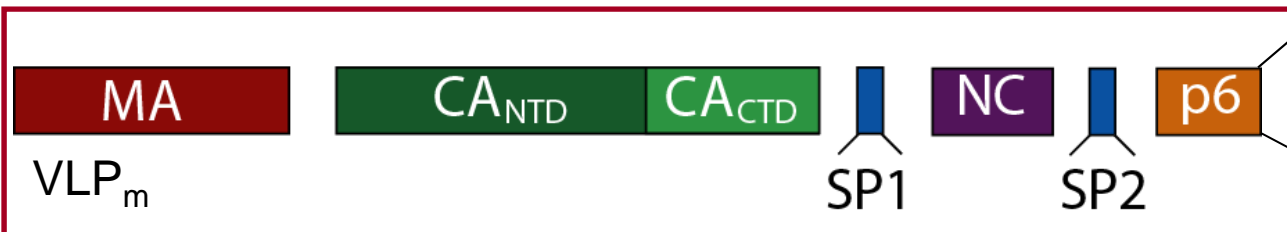
immature



CA5

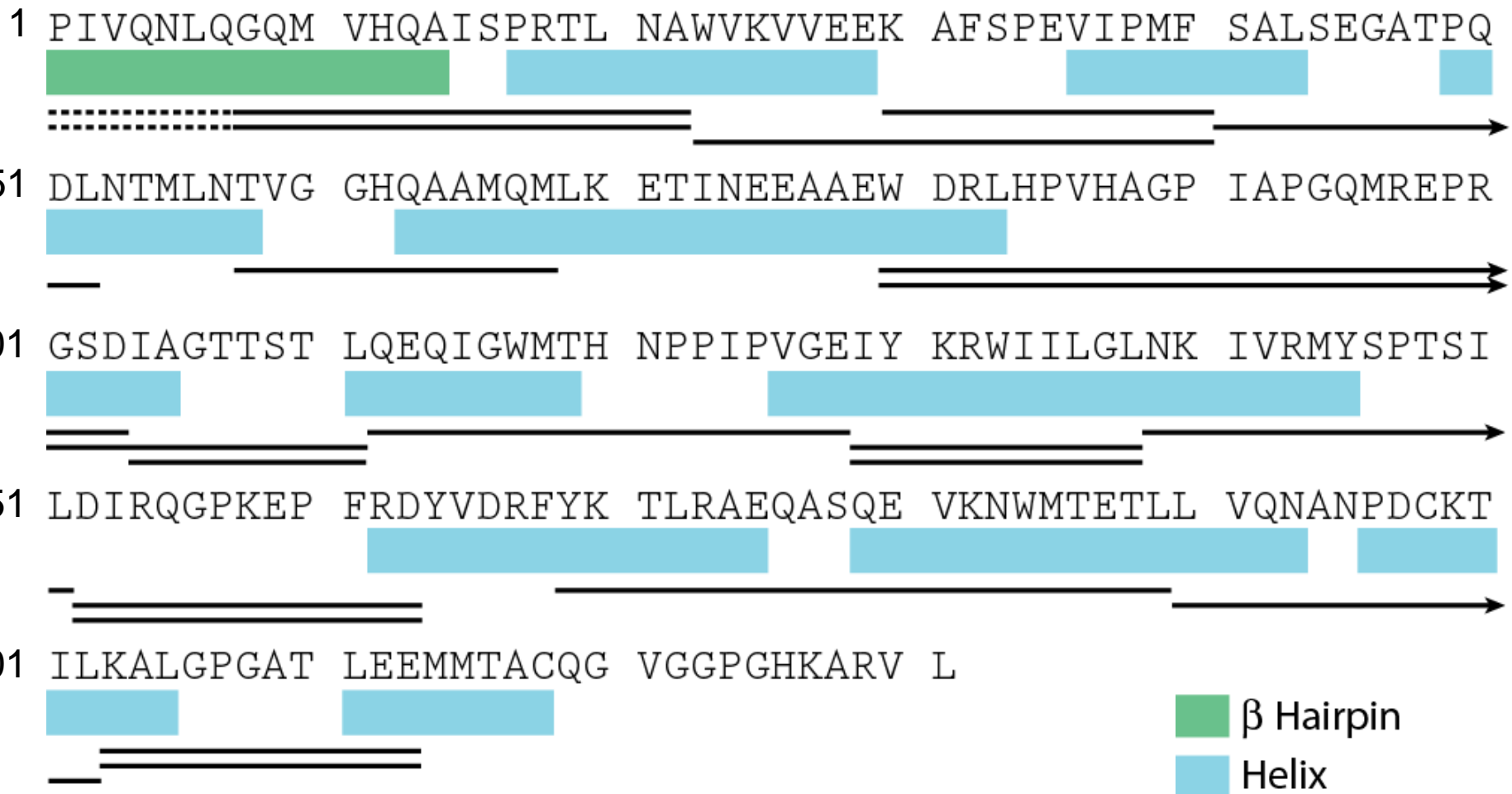


mature

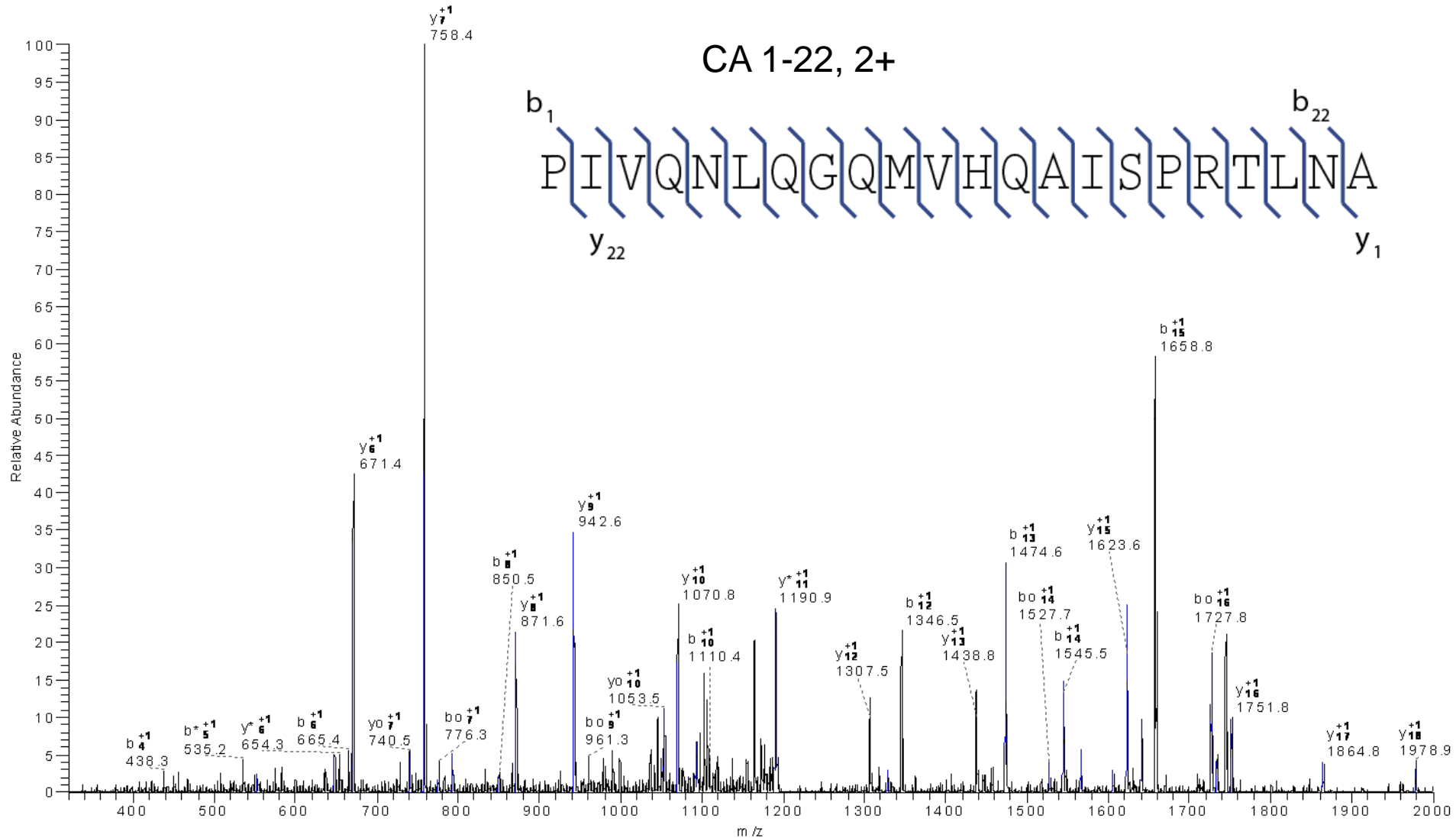


What can I “see”?

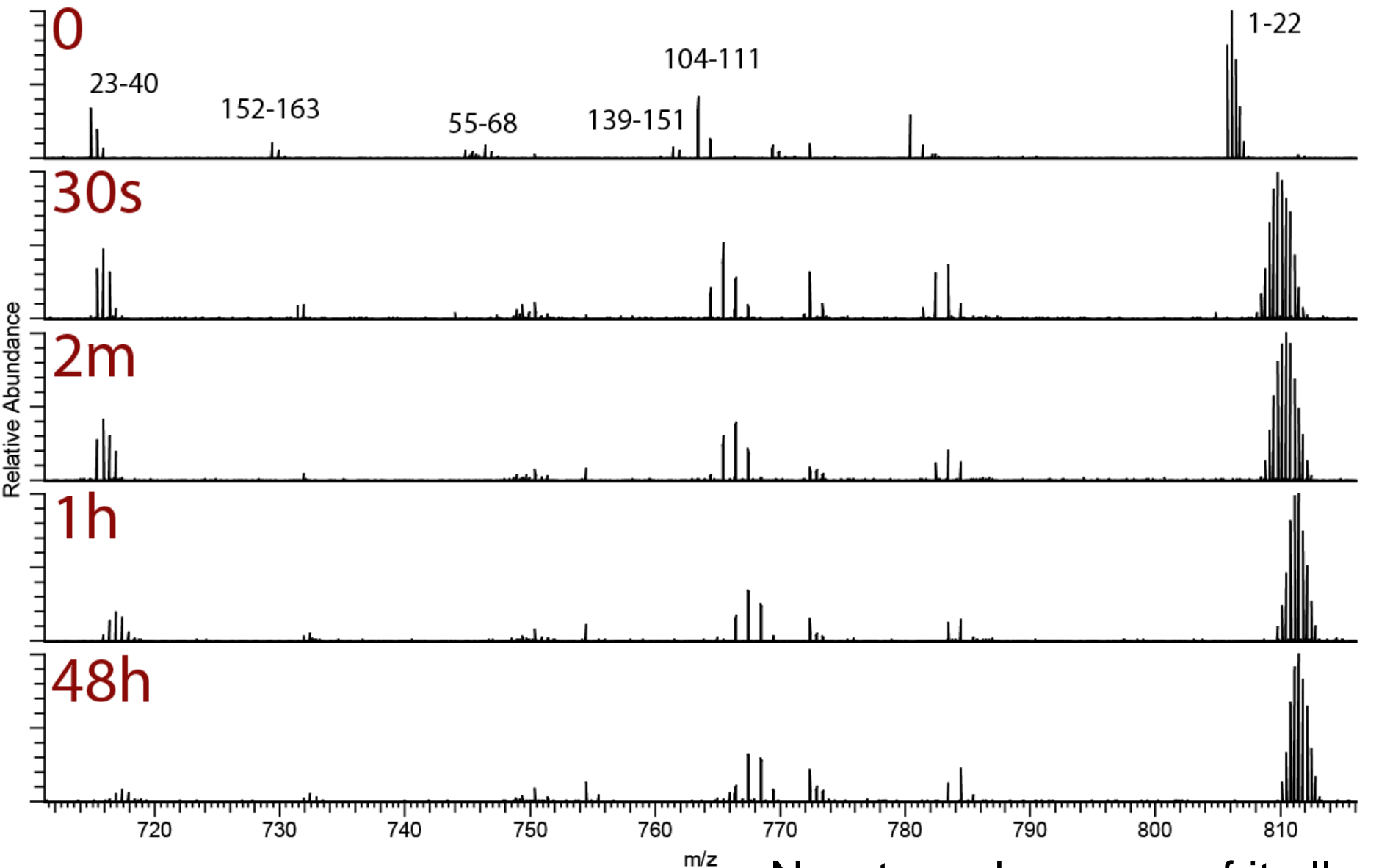
Step 1 – Peptic digestion and MS/MS proteomic sequencing.



Identifying peptides by MS/MS



Mass spectra show exchange



(Actually... > 160 HDX MS runs)

Now to make sense of it all...

Data processing (there's a bit)

HDD - Peptide - Mozilla Firefox

File Edit View History Bookmarks Tools Help

http://hdx.florida.scripps.edu:9090/hd_desktop/start?userId=129

Pandora Radio - Listen to ... HD Desktop Show User HDD - Peptide

HD Desktop - Peptide

double click a datapoint replicate node to load grid

Projects

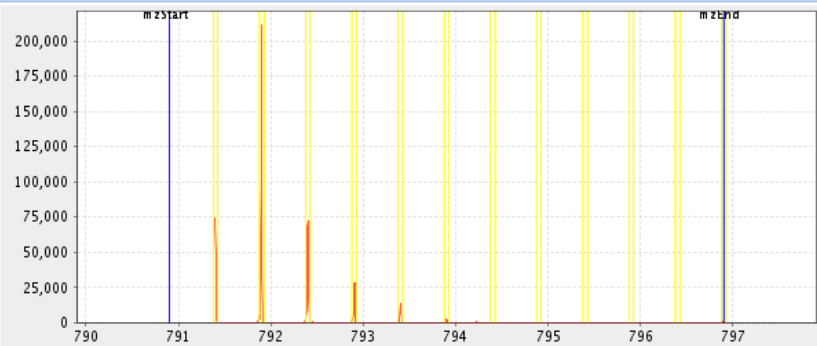
- RSV May 2009
 - RSV VLP and Protein
 - Protein
 - 0
 - 0_1
 - 0_2
 - 0_3
 - 025
 - 050
 - 1
 - 2
 - 4
 - 8
 - 15
 - 30
 - 60
 - 120
 - 480
 - VLP
 - adding new peptides to prev data
 - NMLV-HIV_oldmono
 - NMLV-HIV_old_assembled
 - HIV WT CA old monomer
 - HIV WT CA assembled-old

Grid Data - select a sequence to load spectral view

Id	Sequence	Discarded	z	Start	End	RtStart	RtEnd	MzStart	MzEnd	% Theo	TScore	% D	Centroid
1956	AREEQVTSEQAKF	false	3	1	13	3.05	4.65	507.755	511.921	0	0	4.27594	508.665
1979	WLGLGGGRVSPGPPEC	false	2	14	29	3.05	4.65	790.896	796.896	0	0	5.75056	792.143
1986	VGETTVQRDAKMAPEE	true	2	45	60	3.05	4.65	880.428	887.428	0	0	33.0529	883.026
1989	VGETTVQRDAKMAPEETATPKVTGTSC	false	3	45	71	3.05	4.65	935.957	940	3	926	0.10178	937.616
1959	YHCGTAIGCNC	true	2	72	82	3.05	4.65	570.715	572.72	12	91	10.6341	572.004
1997	AGVGEQQQGGDTPPGAQEQSRAEPGHAGQAPC	false	3	102	138	3.05	4.65	1165.38	1170	6	90	0.0668335	1167.03
1998	AGVGEQQQGGDTPPGAQEQSRAEPGHAGQAPC	false	3	102	139	3.05	4.65	1227.4	1232	4	571	0.0669415	1229.12
1958	RAEPGHAGQAPGALTD	false	3	122	138	3.05	4.65	548.441	552.941	0	0	5.9149	549.433
1981	PVVIKTEGPAWTPLE	false	2	157	171	3.05	4.65	818.451	821	2	496	-0.217014	819.447
1990	PVVIKTEGPAWTPLEPKL	false	2	157	174	3.05	4.65	987.567	994.067	2	75	0.0417921	988.867
1965	PVVIKTEGPAWTPLEPKL	false	3	157	174	3.05	4.65	658.547	663.047	0	0	4.86162	659.6

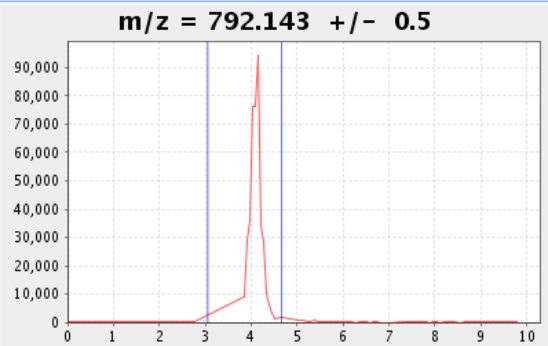
Calc Save PepMap CSV

Details



m/z Start: 791 m/z End: 797

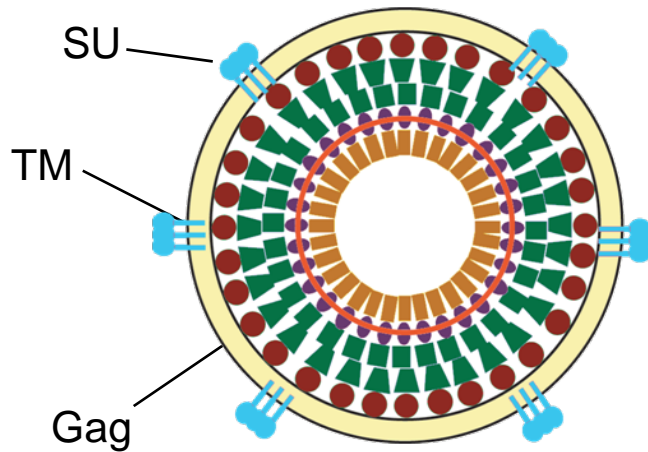
Time Start: 3.05 % Theo: 0.0 %D: 6.0 Score: 0.0
Time End: 4.65 Centroid: 792.143



m/z = 792.143 +/- 0.5

HD Desktop (hdx.florida.scripps.edu)

Immature gag/capsid assembly

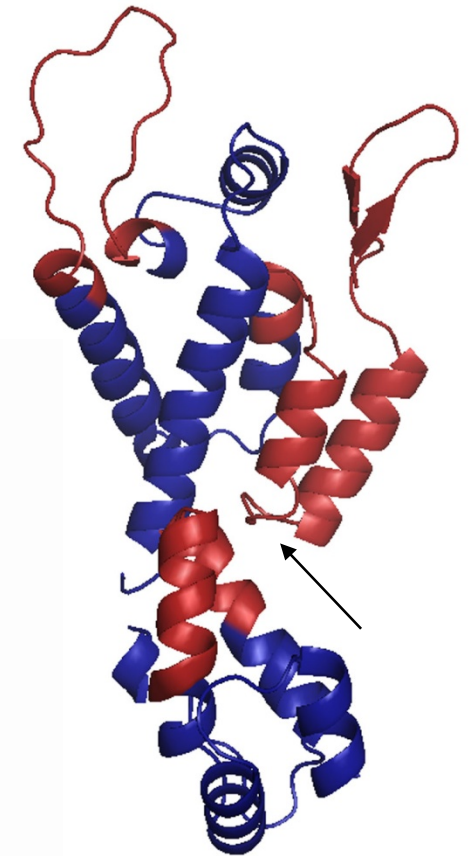
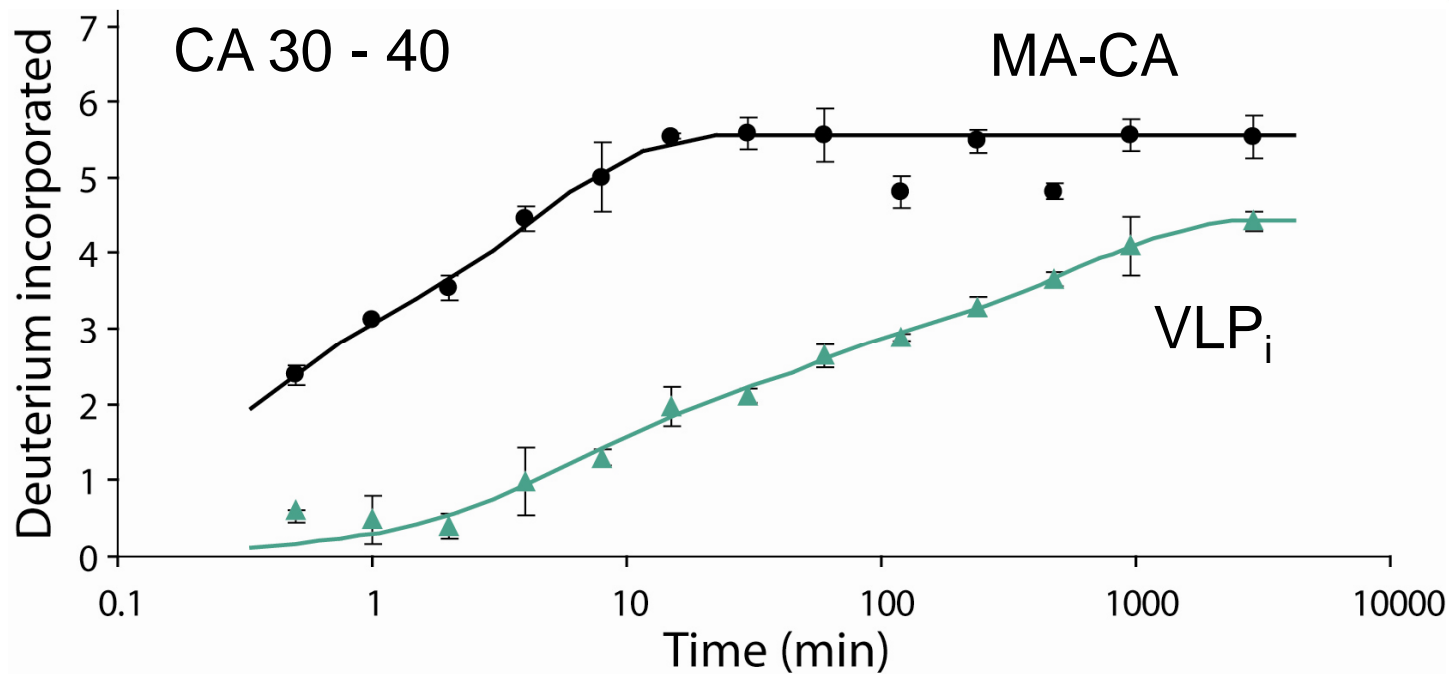


Gag polyprotein



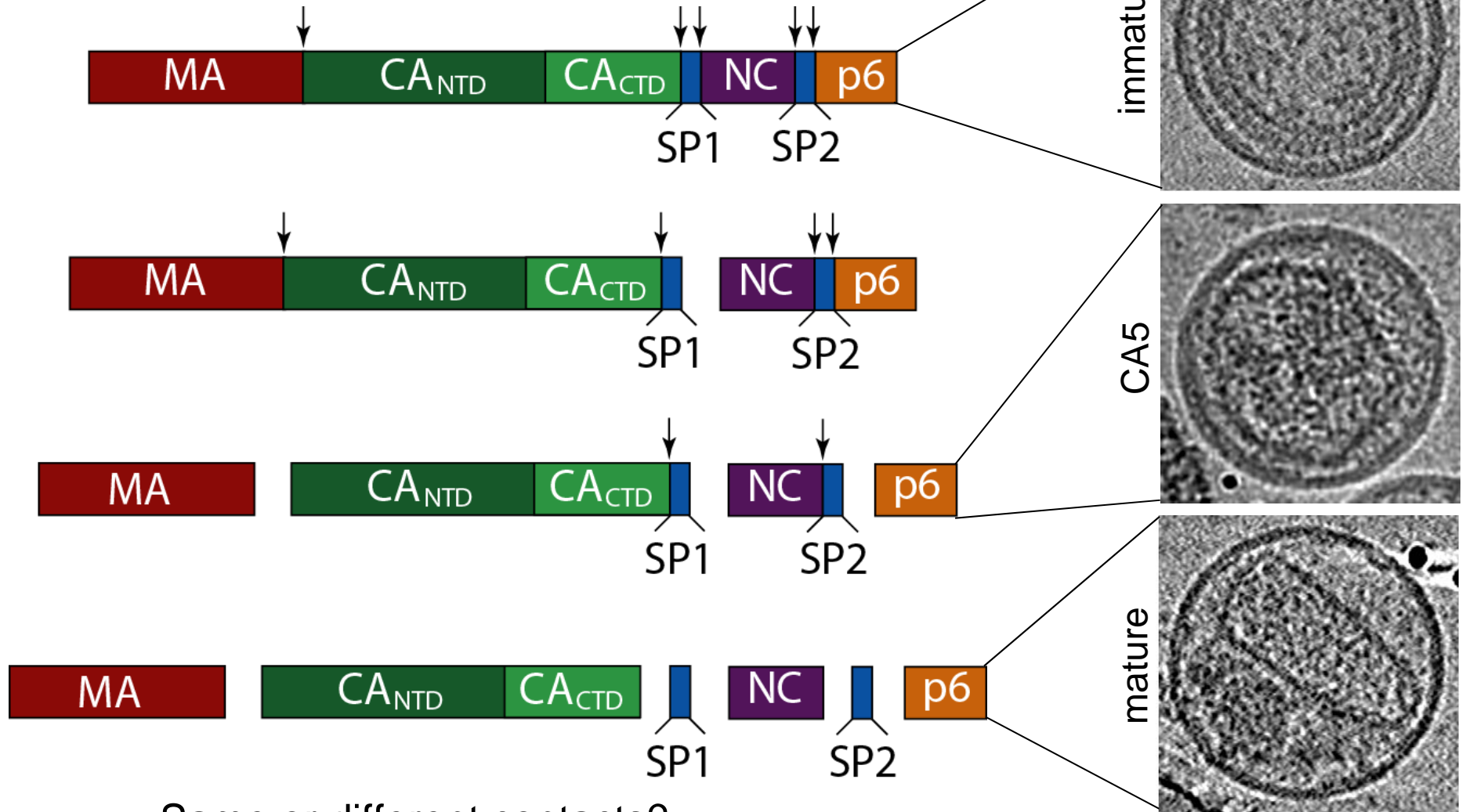
What are the intermolecular contacts?

Identifying assembly contacts



Nearly all match expected contacts from mature lattice structure

HIV CA maturation

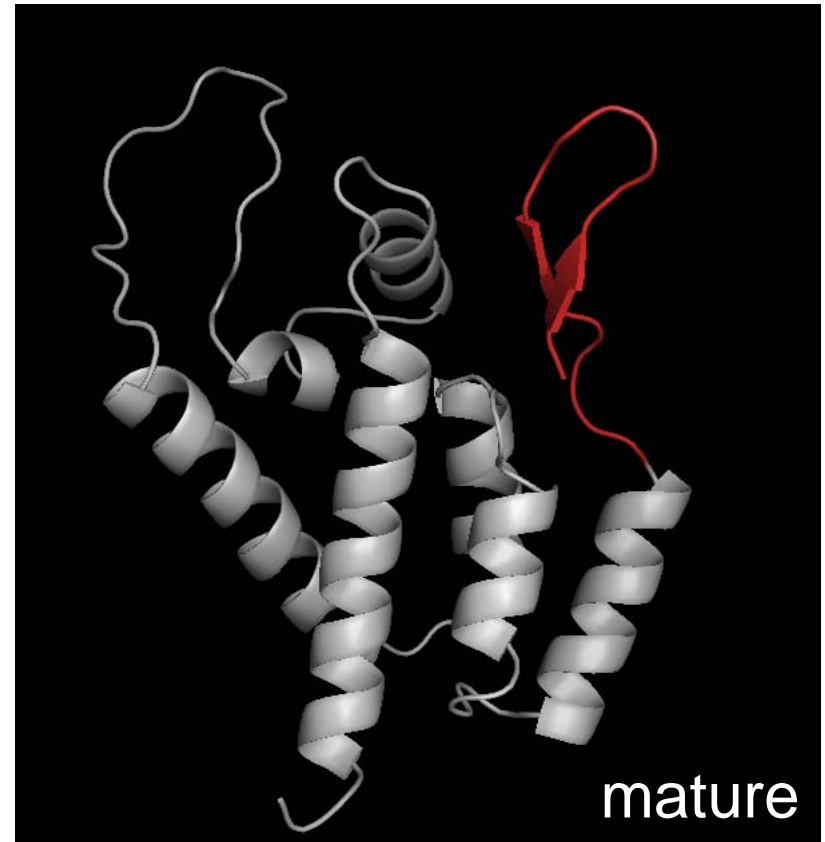
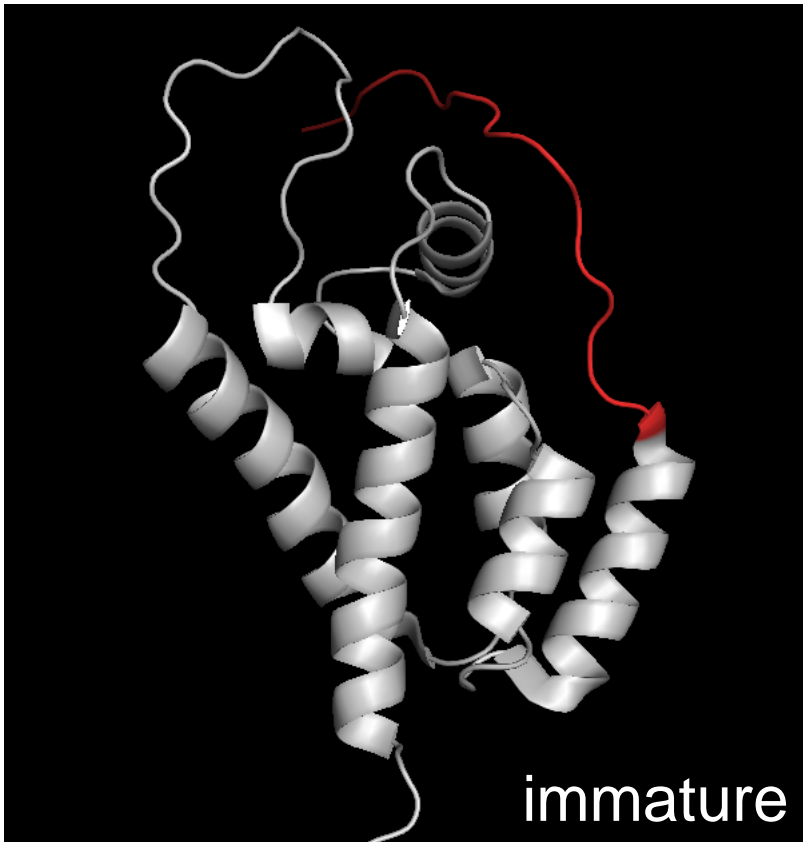


Same or different contacts?
Progression of maturation?
Fall apart and reassemble?

Immature and mature EM images: Sundquist,
Curr Opin Struct Biol (2008) **18**, 203–217

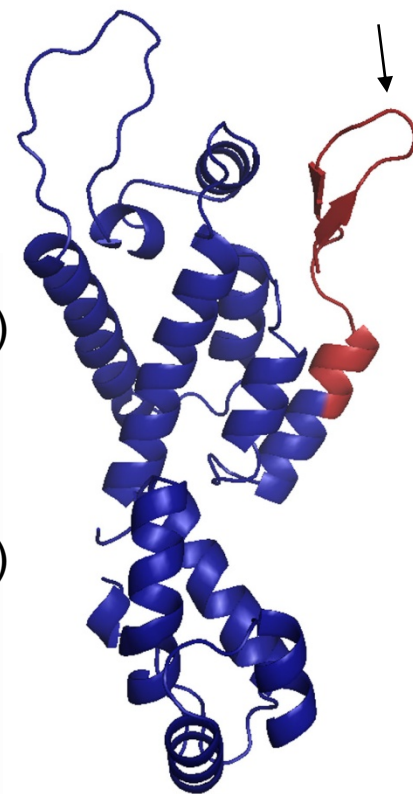
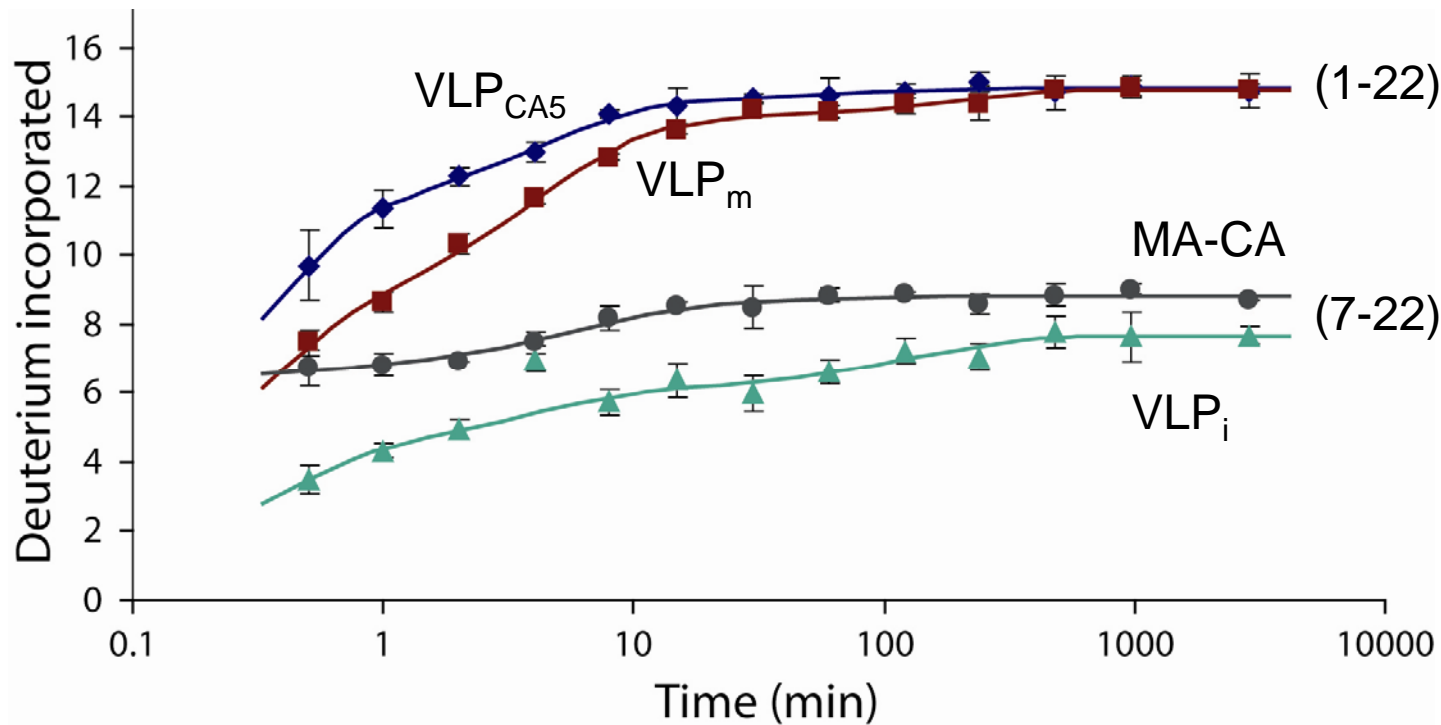
N-terminal hairpin

Hallmark of maturation and required for infectivity
Thought to drive maturation following MA-CA cleavage

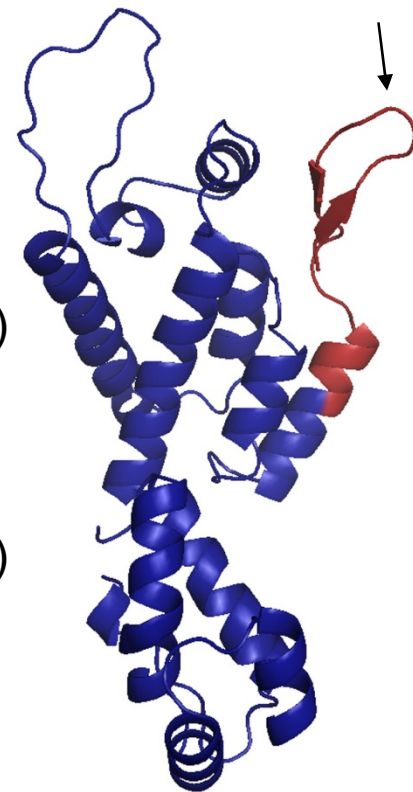
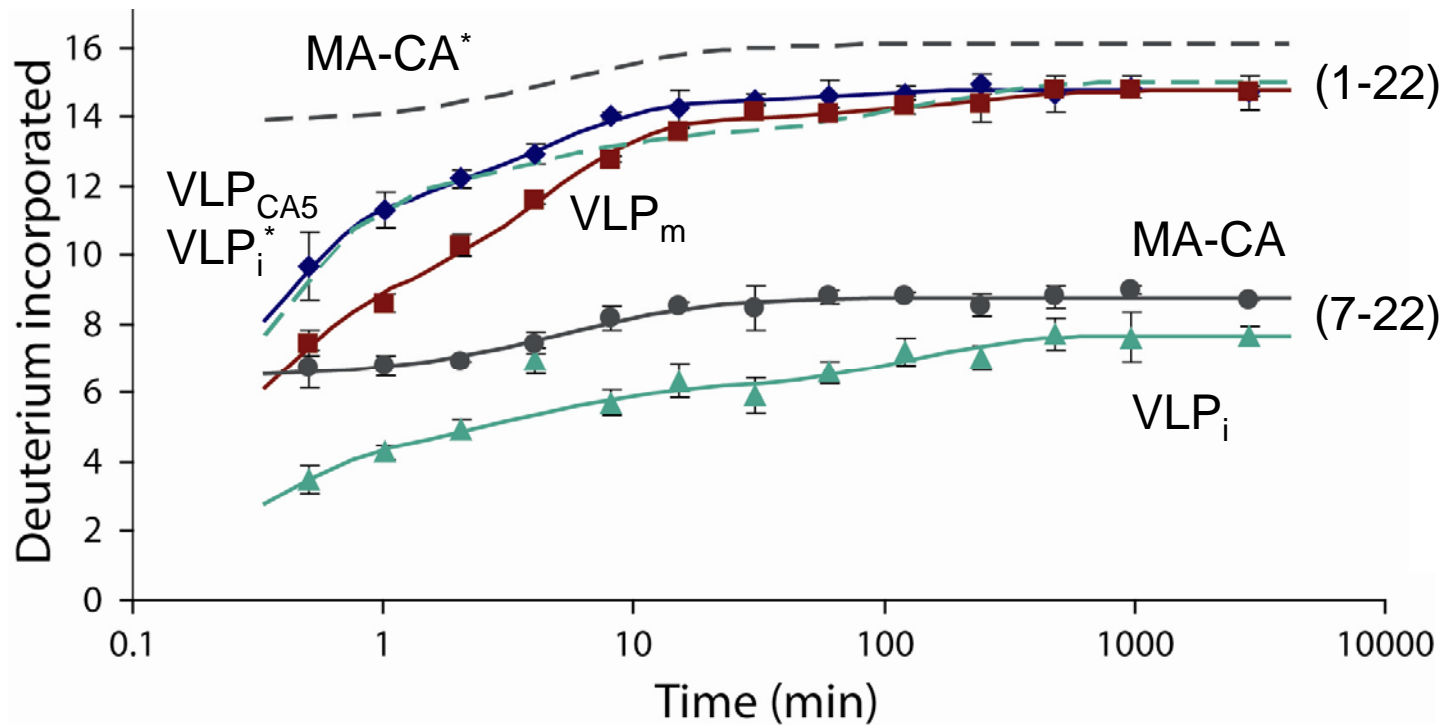


When does the hairpin form?

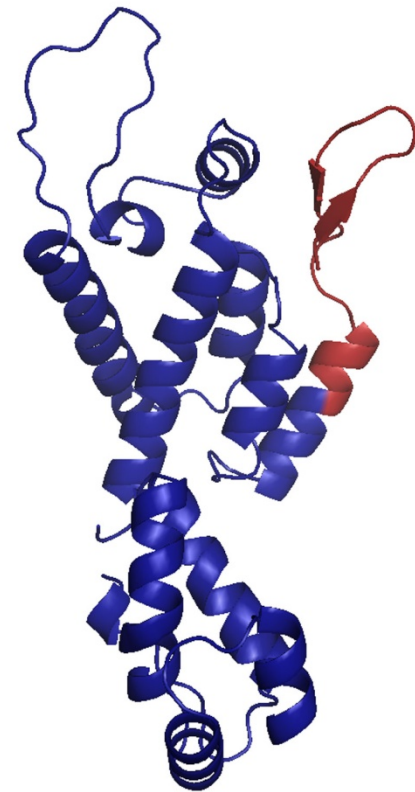
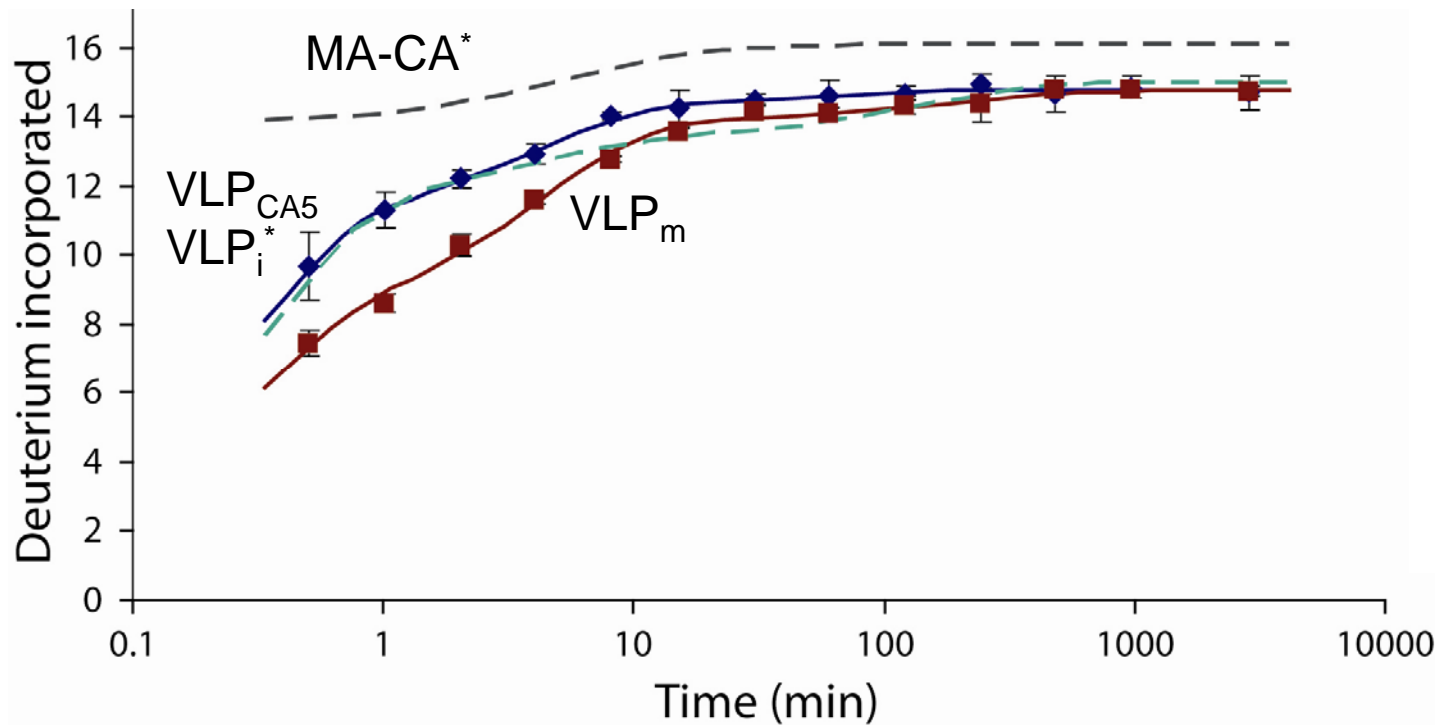
N-terminal of CA



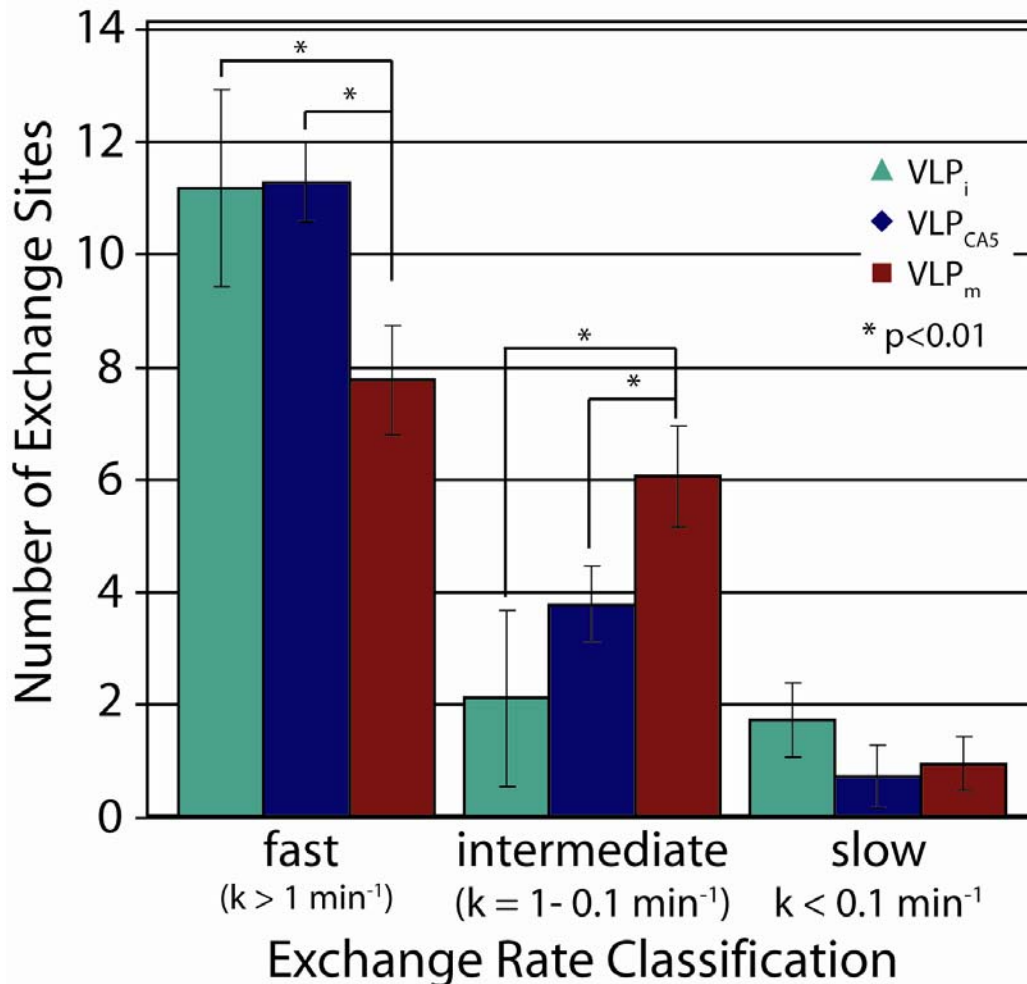
β -hairpin forms after SP1 cleavage



β -hairpin forms after SP1 cleavage

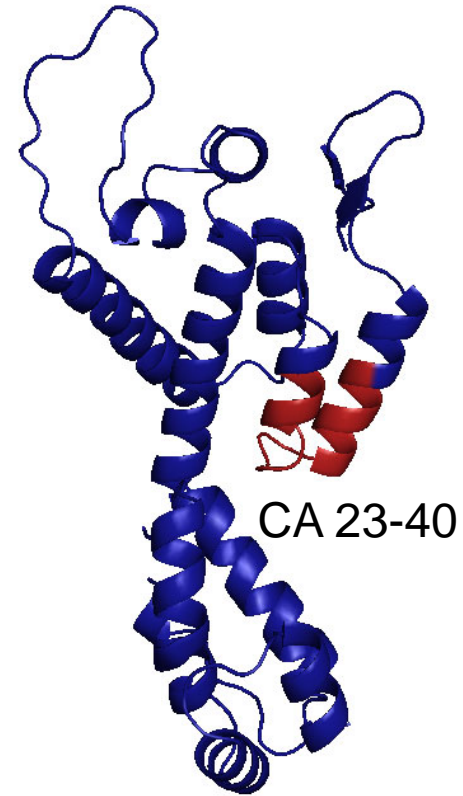
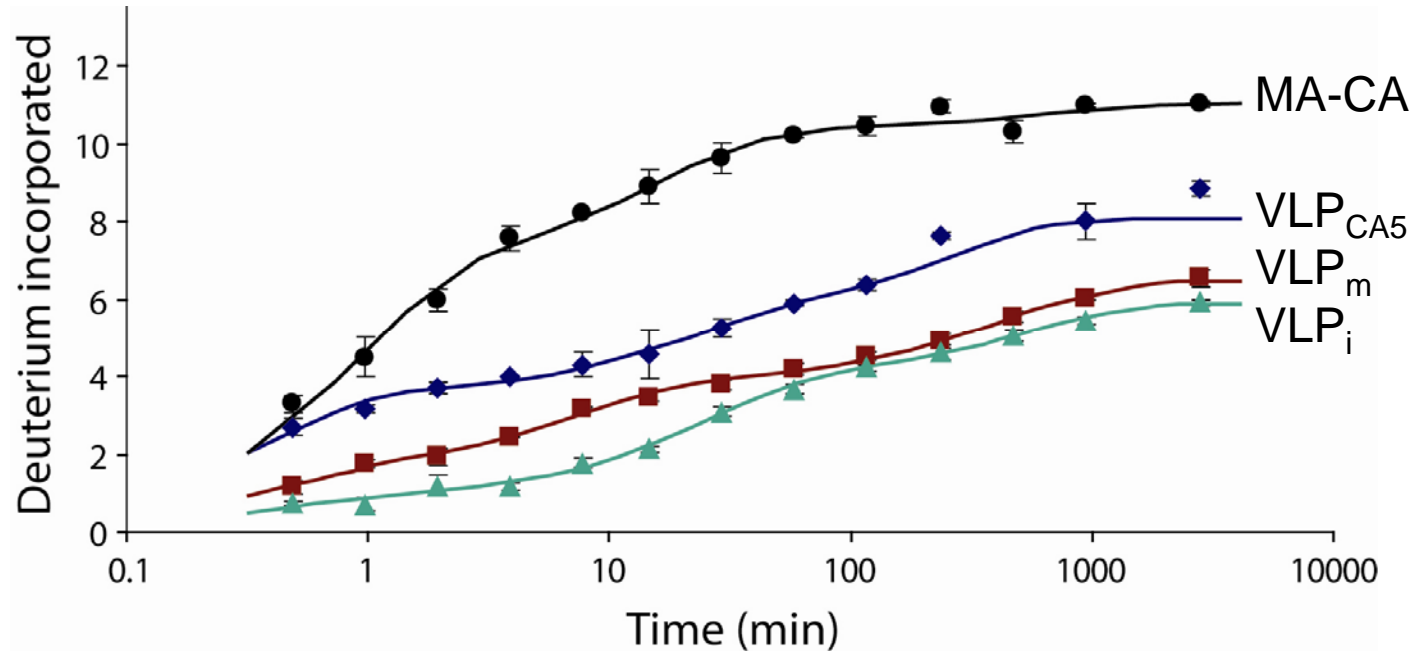


β -hairpin forms after SP1 cleavage



β -hairpin not formed in CA5 structural intermediate
Forms as a **result** of maturation rather than an **initiator**

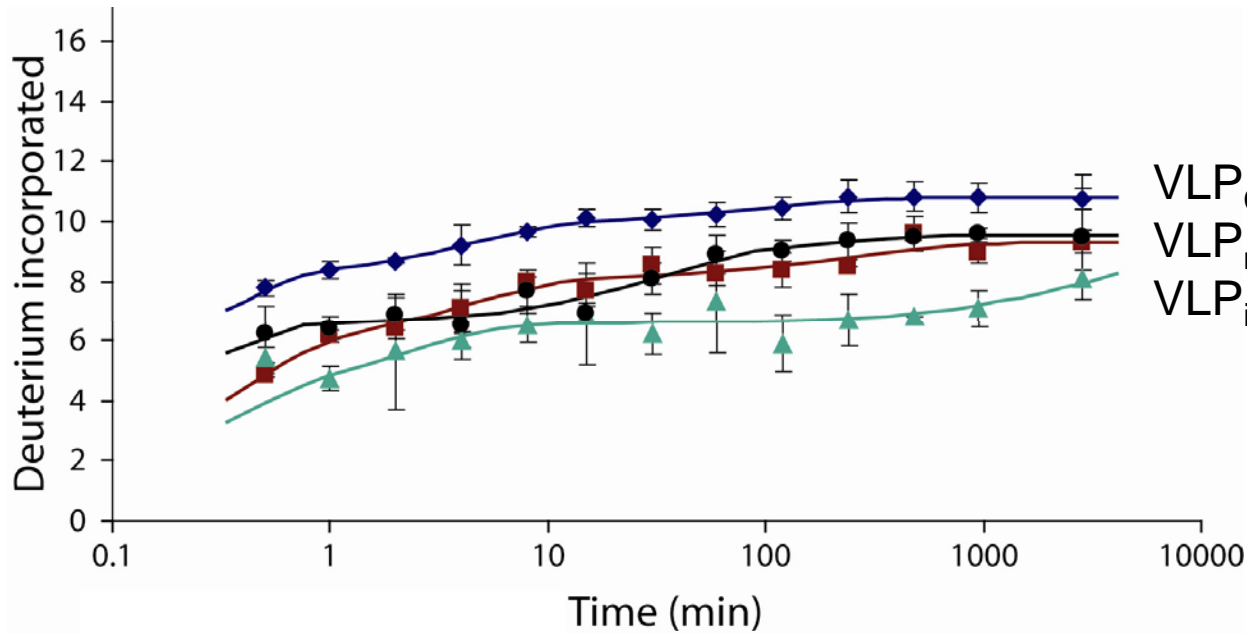
Helix 1 interactions remodel



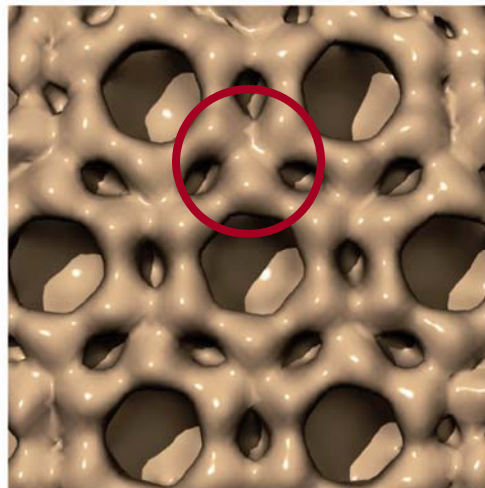
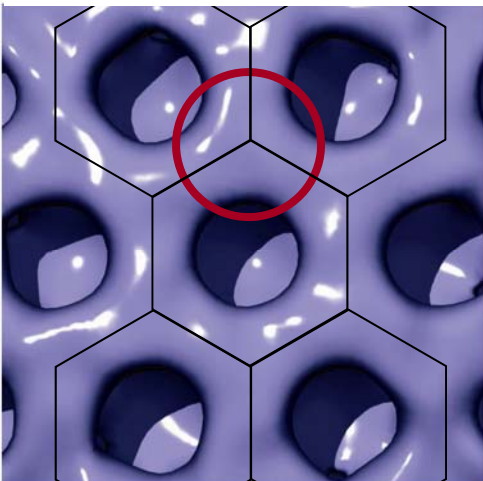
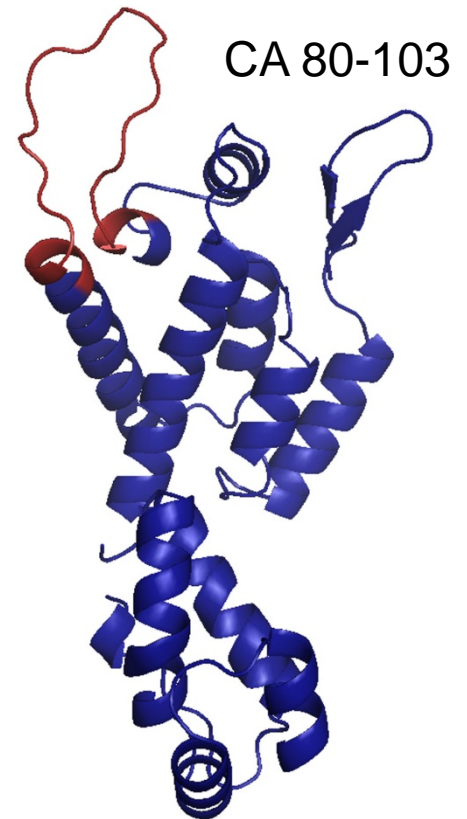
Similar exchange patterns for CA 30-40 (H2 only) so variation due to H1.

Helix 1 orientation changes and transitions during maturation

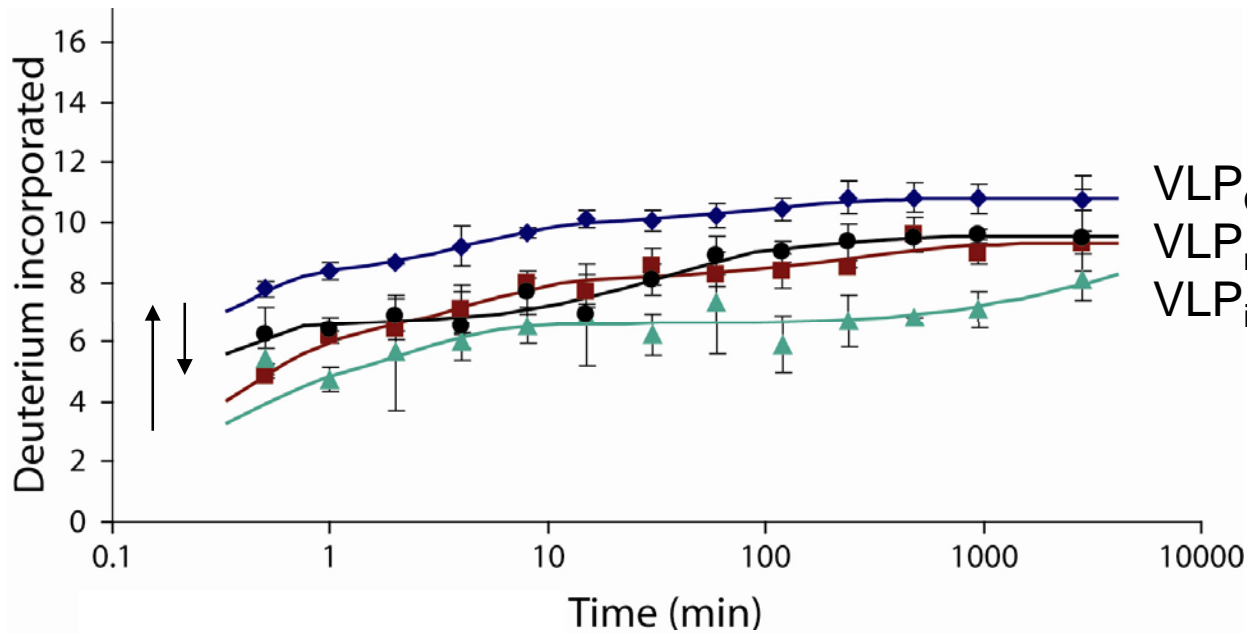
What happens at the 3-fold axis?



VLP_{CA5}
VLP_m/MA-CA
VLP_i

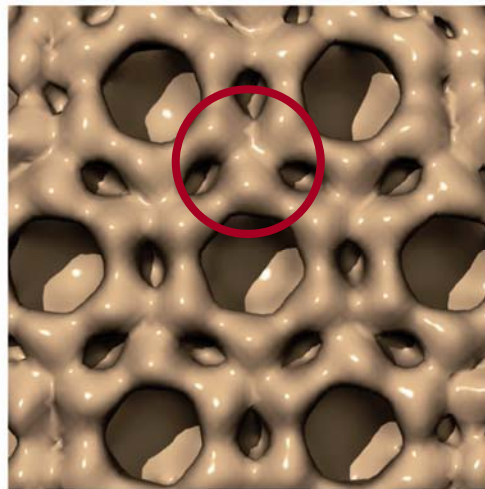
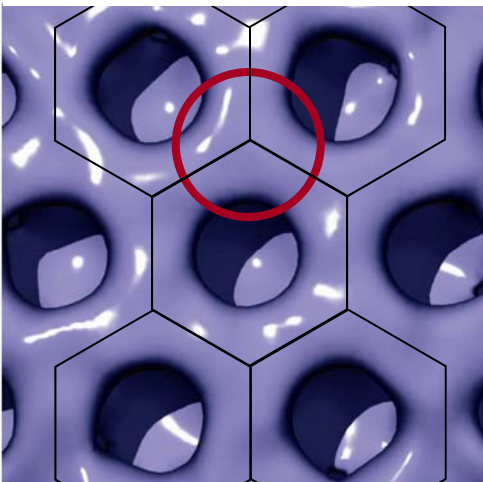
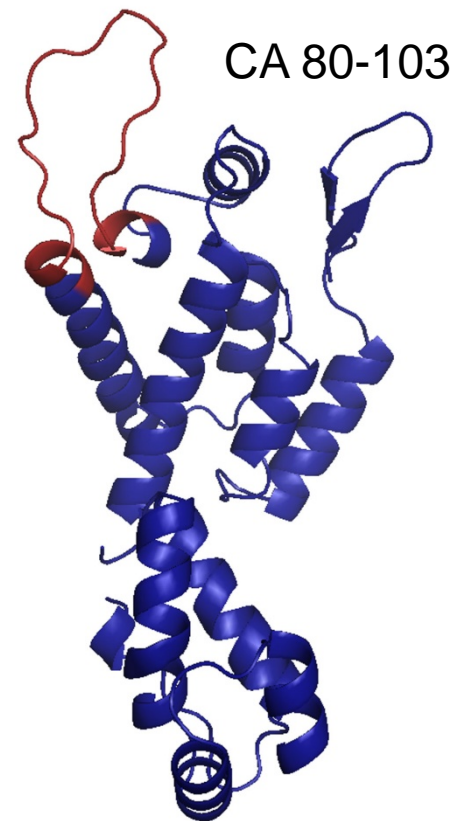


Evidence for 3-fold interaction

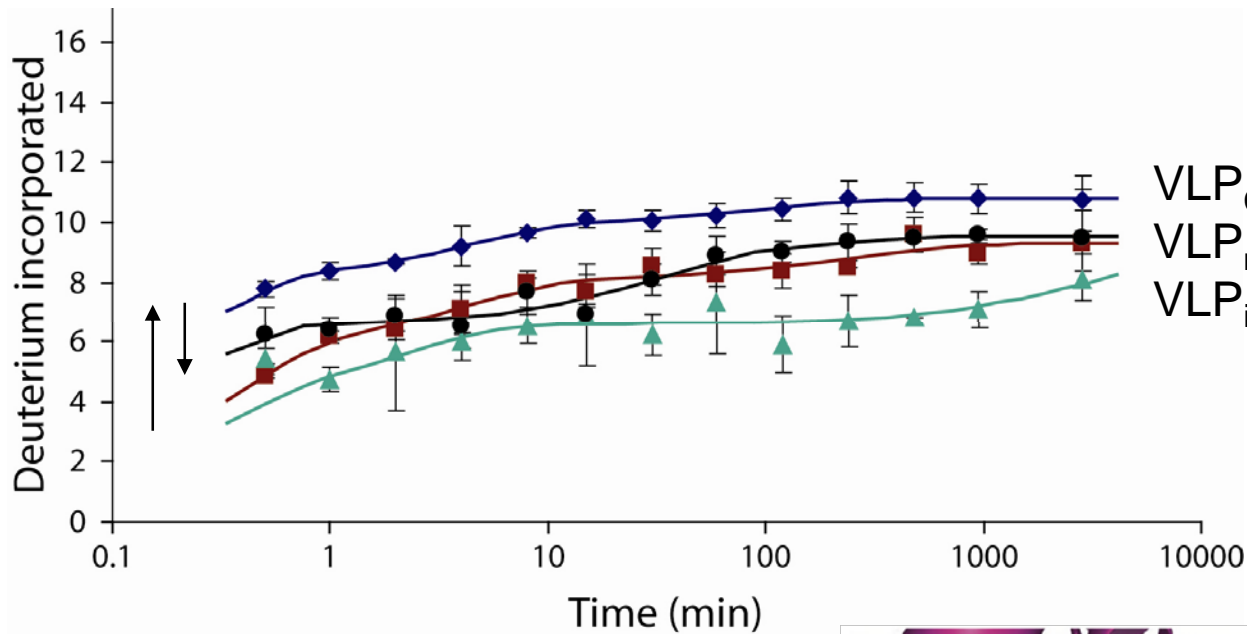


VLP_{CA5}
VLP_m/MA-CA
VLP_i

strain ↗ ↘
no interaction
interaction

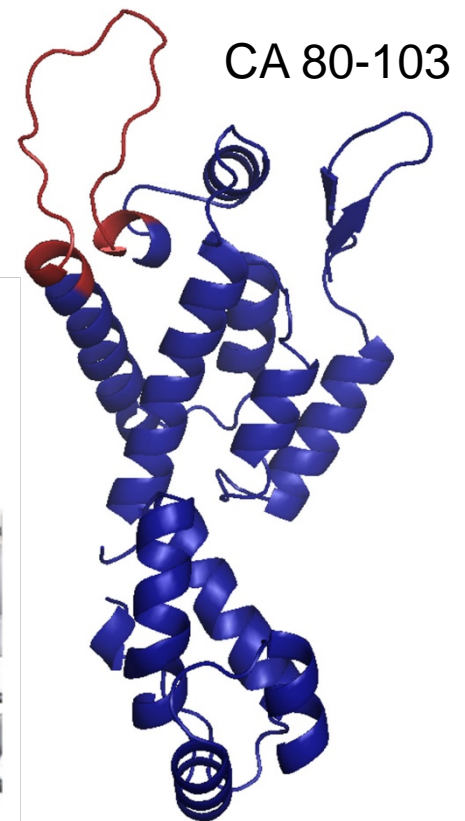


Evidence for 3-fold interaction



VLP_{CA5}
VLP_m/MA-CA
VLP_i

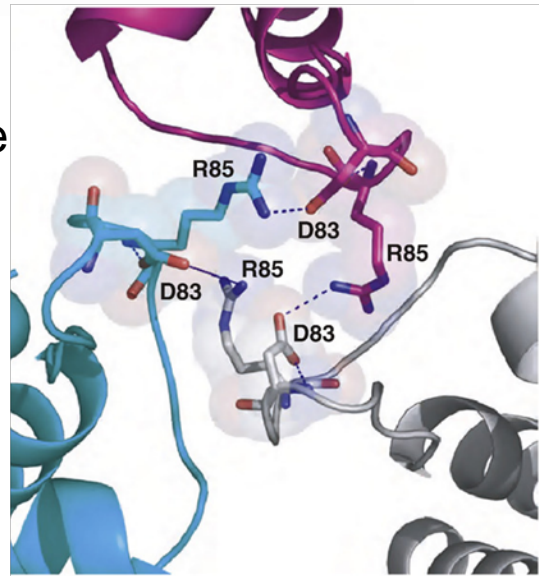
strain ↗
no interaction ↘
interaction ↙



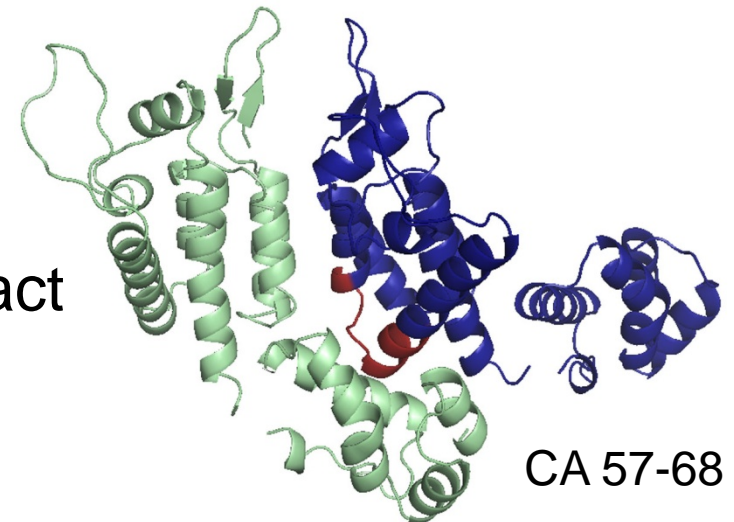
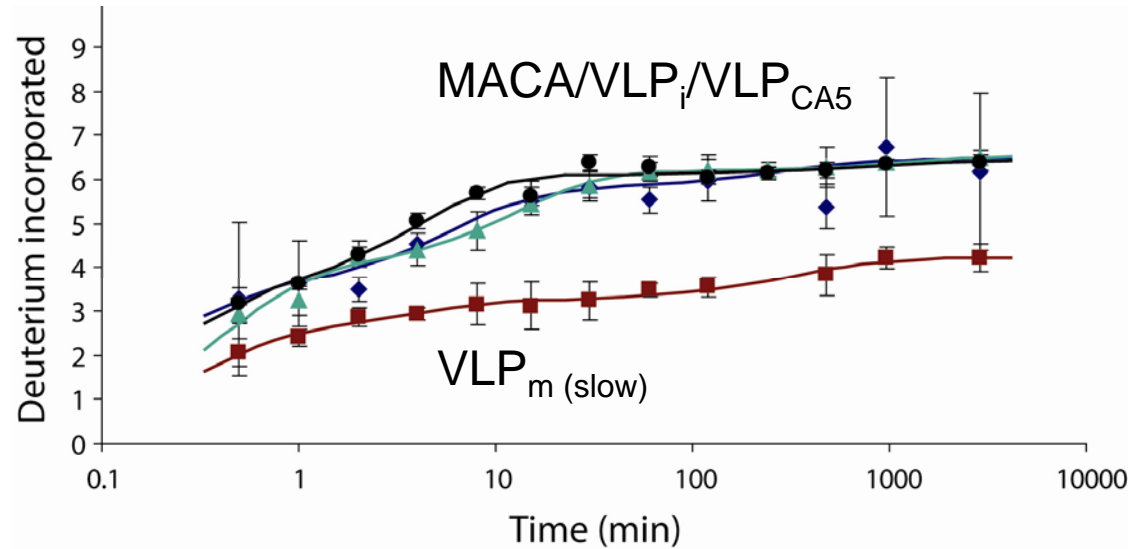
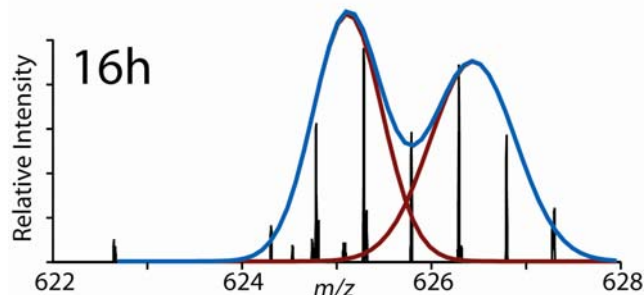
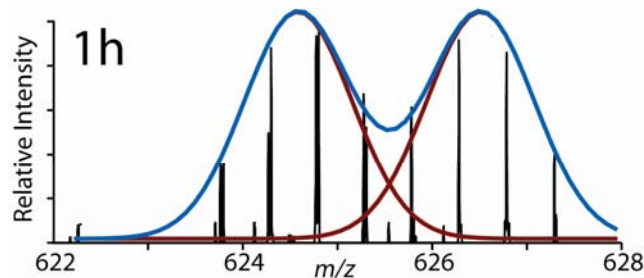
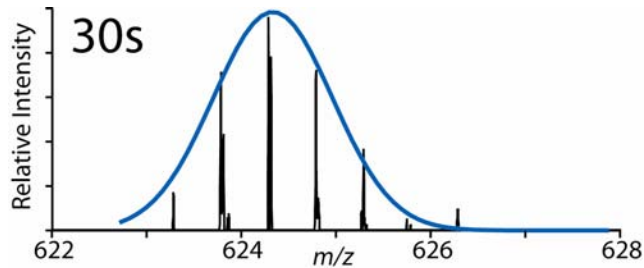
Interaction not present in mature lattice

Similar interaction observed in MLV
(Taylor, J. Mol. Biol. (2008) 376, 1493–1508)

Similar residues well maintained in
HIV CA sequence



NTD-CTD mature interaction

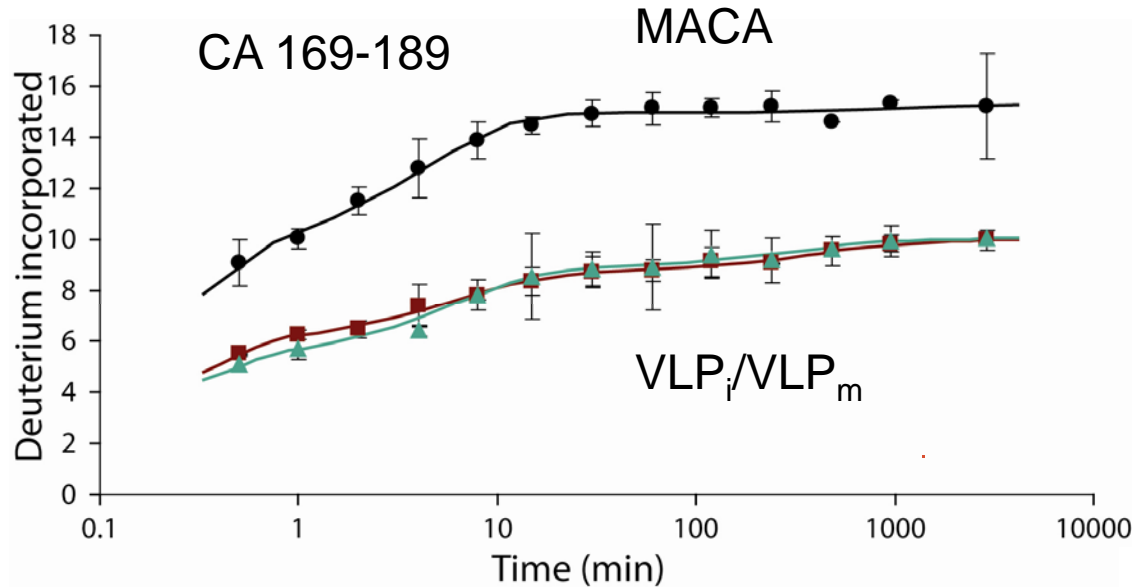


-Previously shown as stabilizing contact in mature lattice

-Only portion of CA in mature core

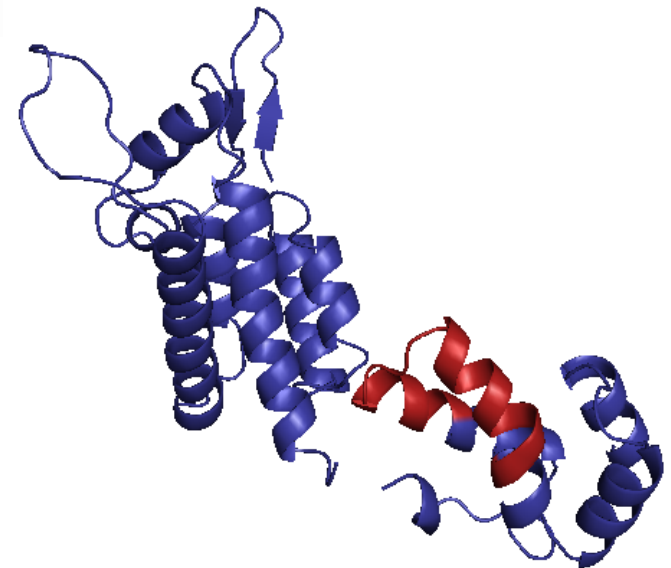
-SP1 keeps CA 'immature' in VLP_{CA5}

CTD side of mature NTD-CTD interface

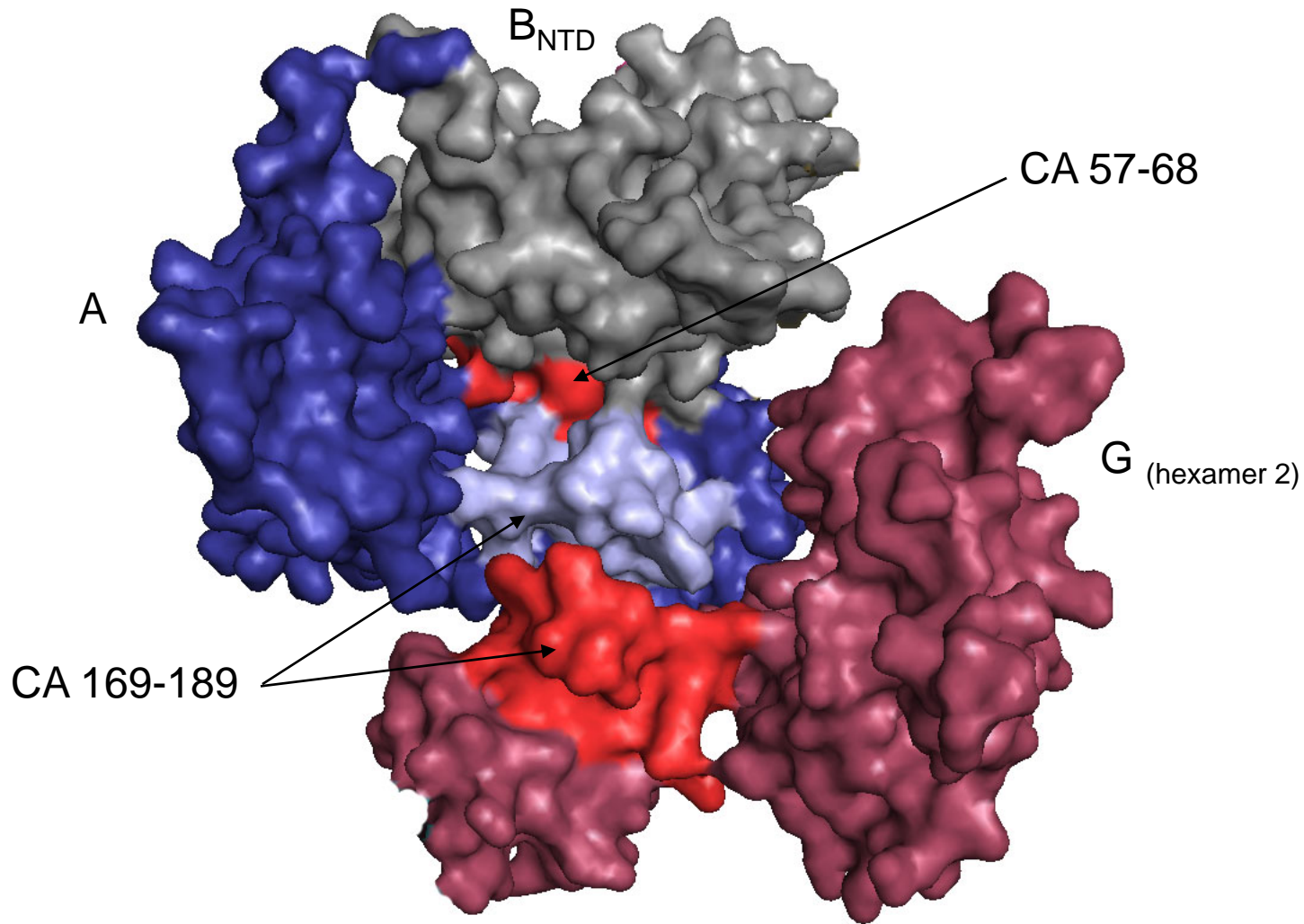


Peptide contains both
CTD portion of mature NTD-CTD
-and-
CTD dimer interface

But no variation...



CTD dimer interface remodeling

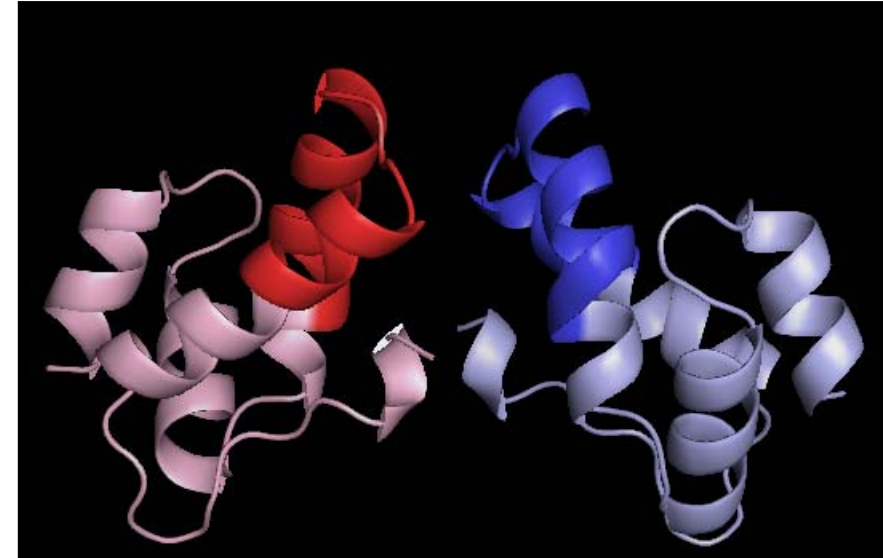
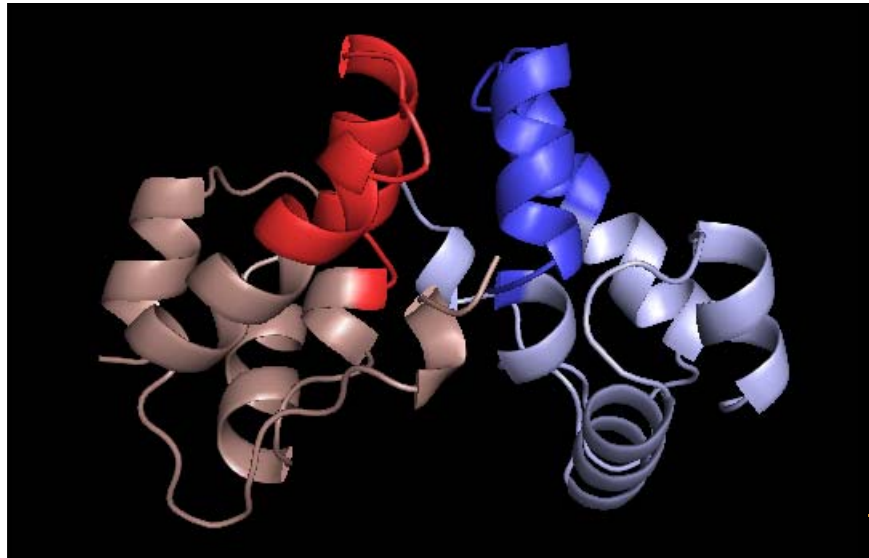


HDX MS unable to discern equivalent contacts

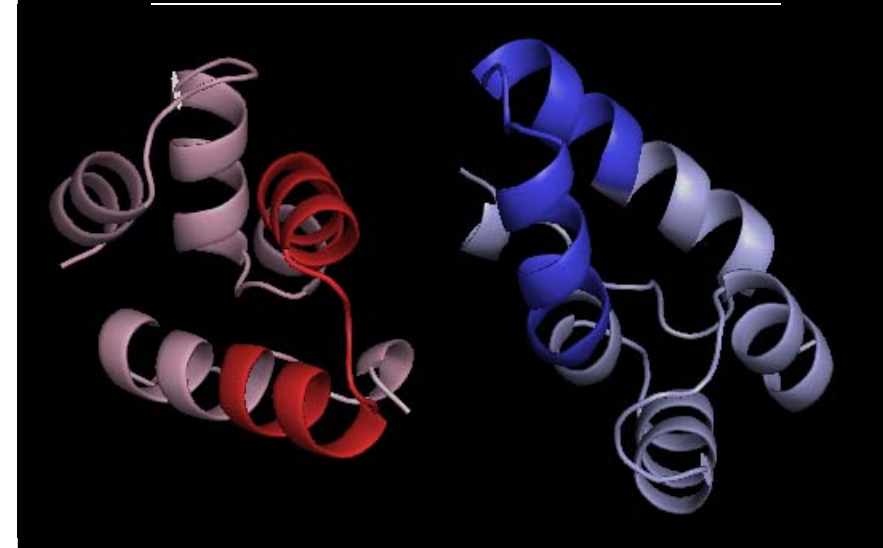
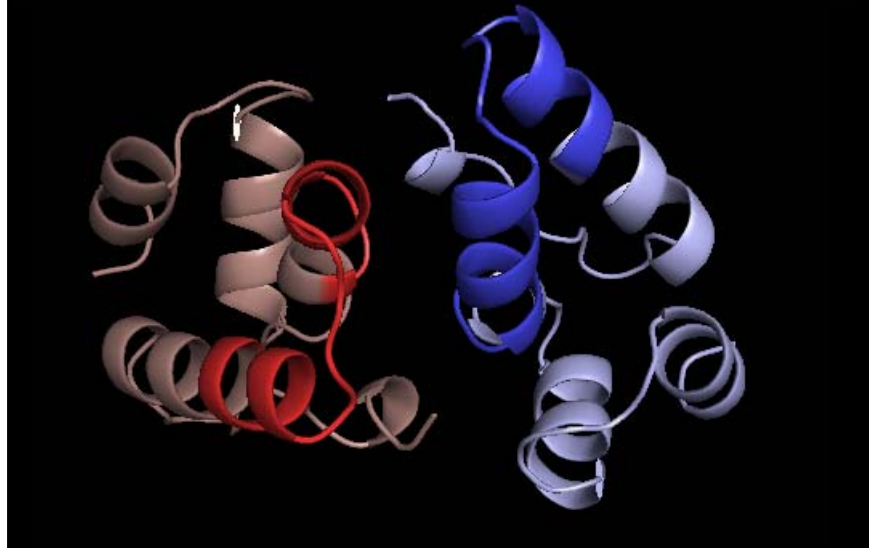
CTD-CTD interface remodels during maturation

Possible interface remodeling

side



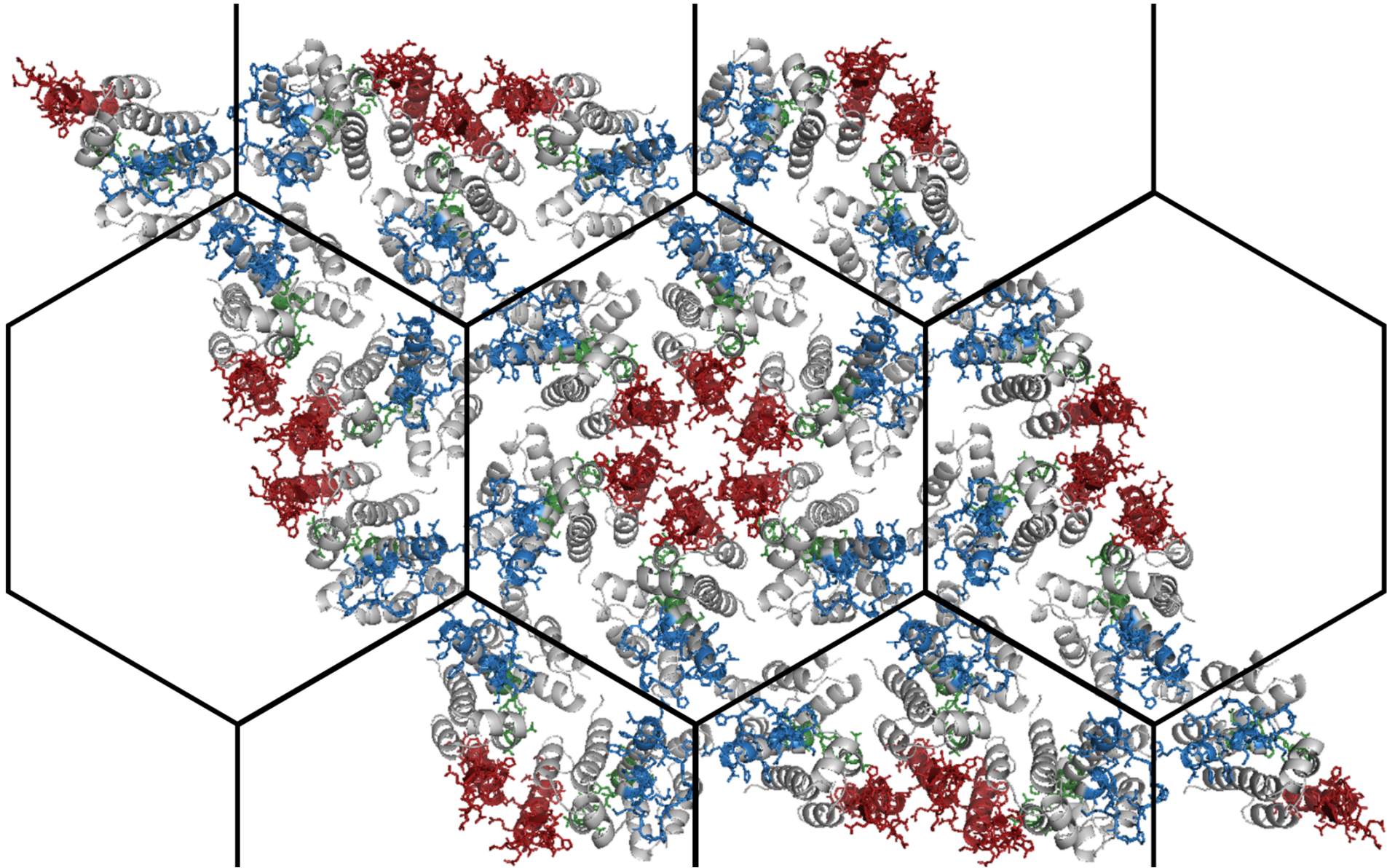
top



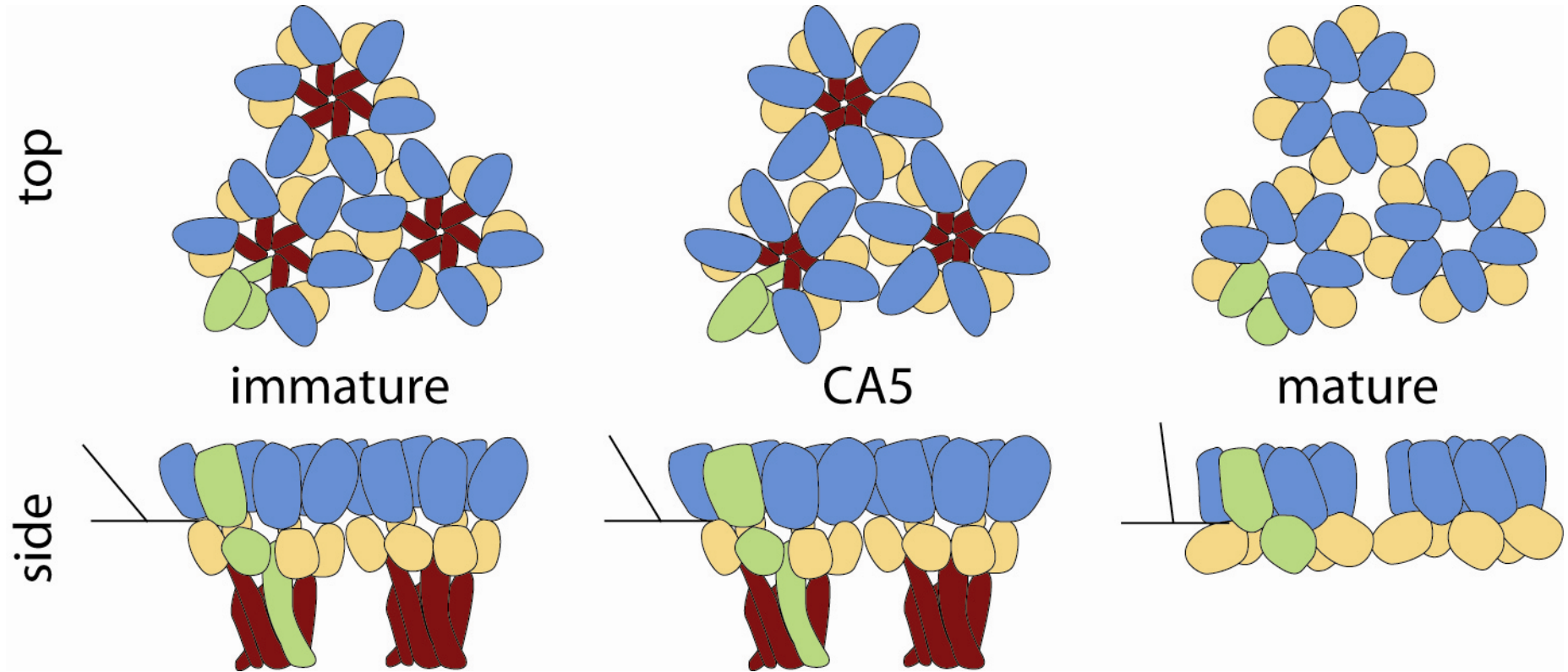
Isolated CTD dimers (1a43)

Mature lattice

Mapping variation of CA maturation



Resulting model of CA maturation



Conclusions

HDX MS can be a powerful tool for examining structure.

Structure is largely implied but the power is in comparison .

HIV capsid maturation proceeds by remodeling of intermolecular interfaces.

- β -hairpin forms only following CA-SP1 cleavage.

- Interaction 3-fold axis that is strained during maturation and absent in mature.

- CTD Dimer interface likely to remodel during maturation.

This model does not require disassembly of lattice or hexamers.

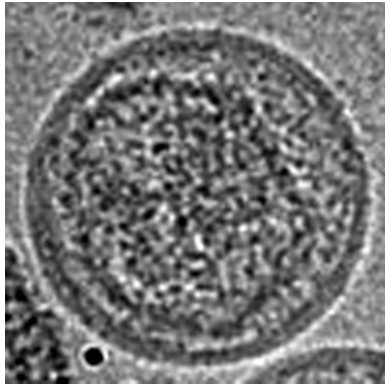
Acknowledgements

immature



Peter Prevelige
Sebyung Kang
Rui Li
Lisa Jones

CA5



Matt Renfrow
Leann Boerma

mature

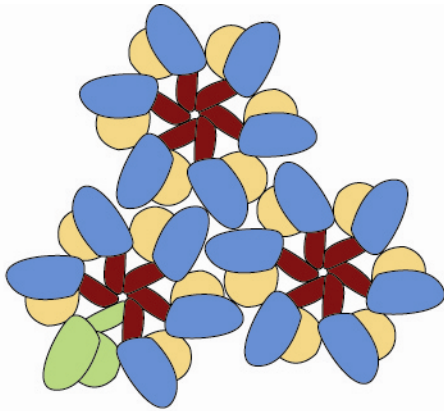


Terije Dokland
Michael Spilman

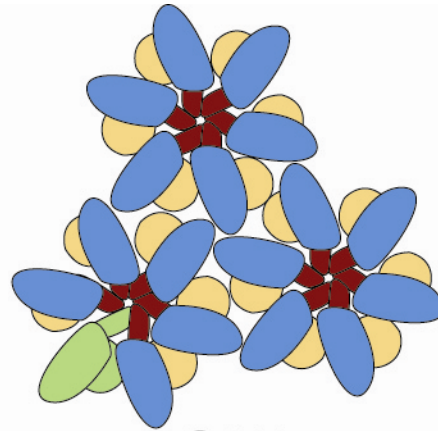
NIH (RO1 and NRSA)
UAB Postdoctoral Scholars Award

Thanks again...

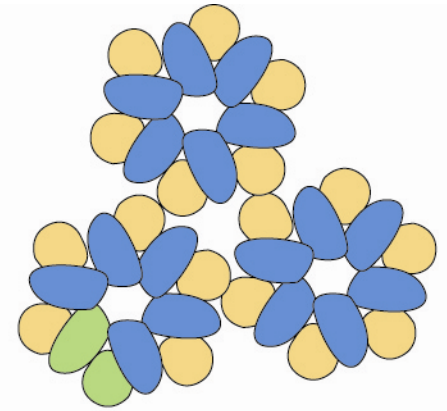
top



immature

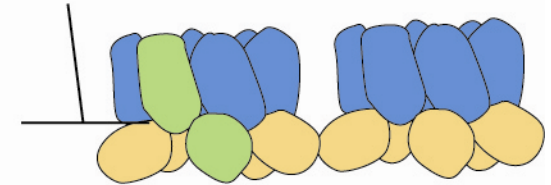
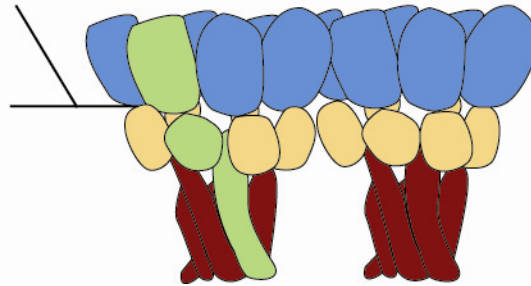
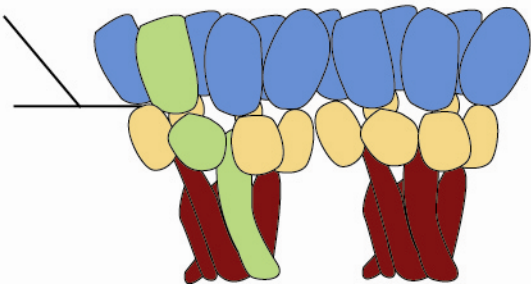


CA5



mature

side



Next Meeting

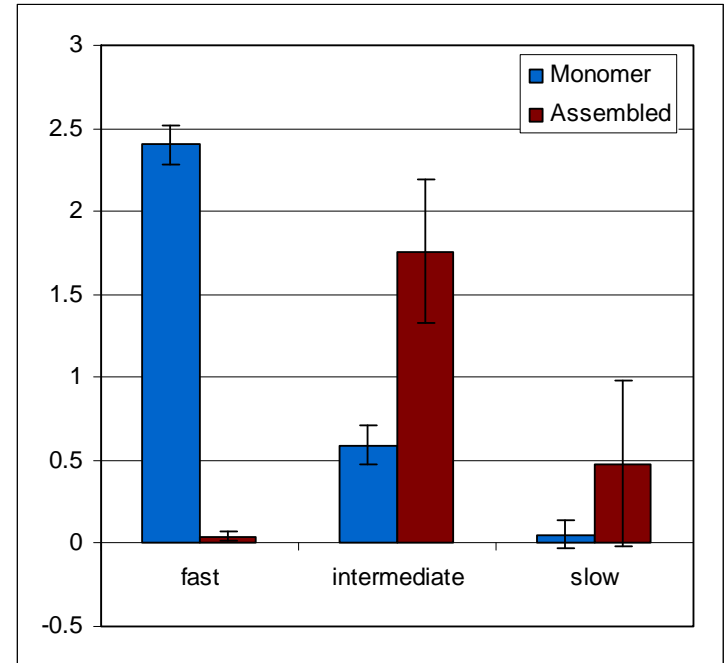
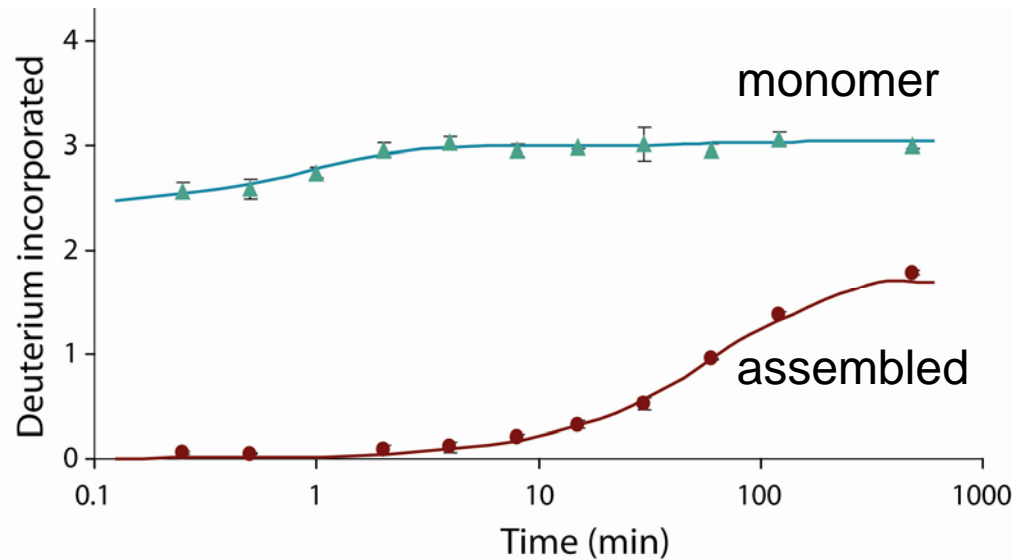


“Lipidomics”

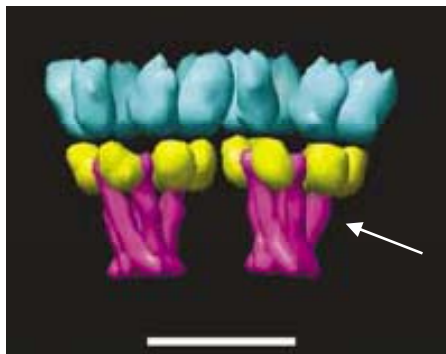
Jeevan Prasain, PhD

Tuesday, November 17th, 2009

Rous sarcoma spacer peptide

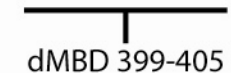


HIV equivalent:



Wright, *EMBO*, 2007

	CA					SP					NC																						
Predictor	A	P	L	T	D	Q	G	I	A	A	M	S	S	A	I	Q	P	L	I	M	A	V	V	N	R	R	E	R	D	G	Q	T	
Jnet																																	
Jhm																																	
PSIPRED																																	
NPS@ SOPMA																																	
NPS@ HNN																																	
NPS@ GOR4																																	
PROF																																	



 dMBD 399-405