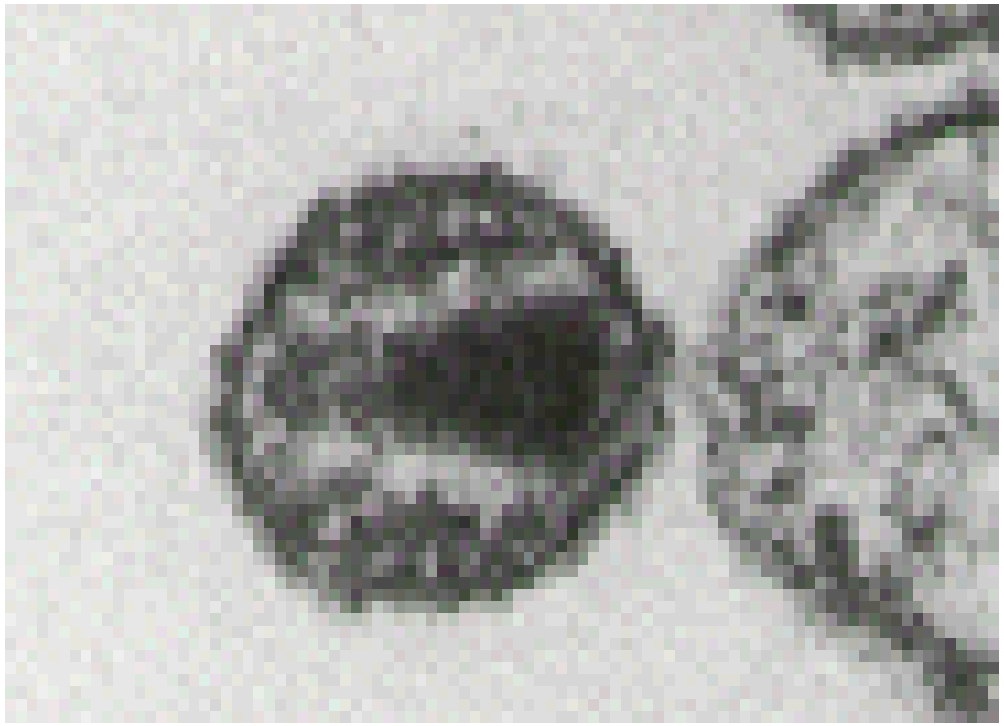
Dissection of HIV-1 Immature Particle Assembly

Alan Rein
HIV Dynamics and Replication Program
National Cancer Institute

Construction of an HIV-1 Particle

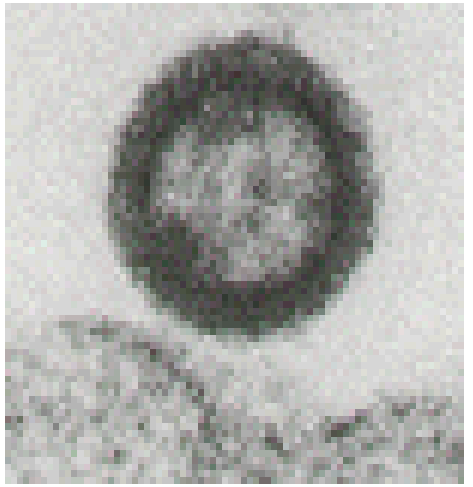
Construction of an HIV-1 Particle



Roughly spherical
~ 120 nm in diameter
Lipid bilayer surrounds the viral RNA and associated proteins

The HIV-1 particle is actually constructed in a sequence of TWO assembly events:

First, assembly of the <u>immature</u> particle:



This particle is assembled from the Gag protein.

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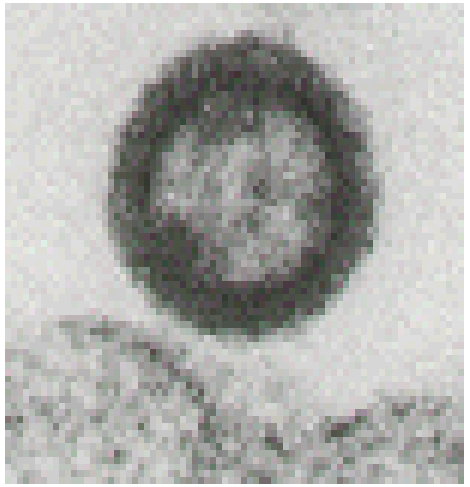
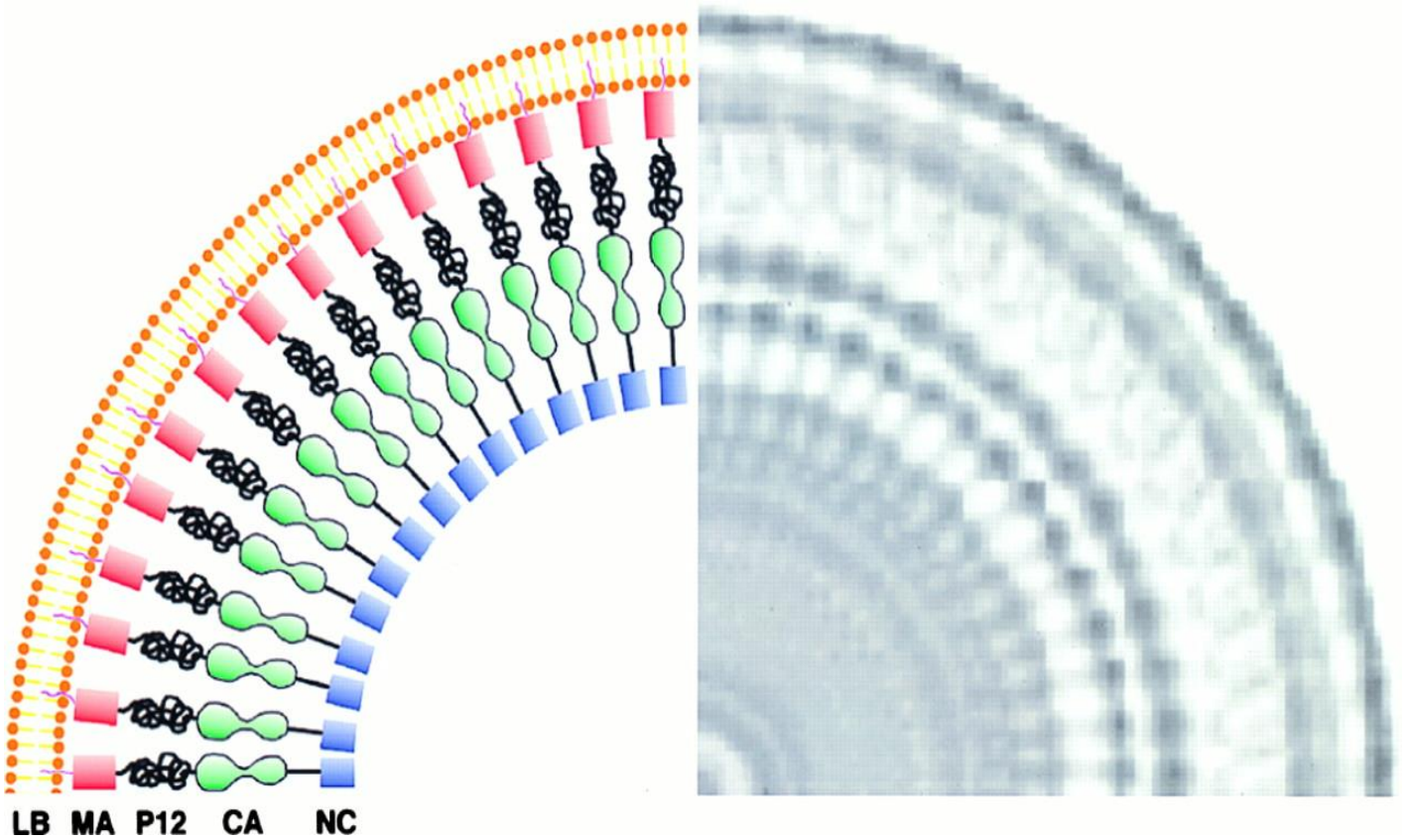


This particle is assembled from the Gag protein.

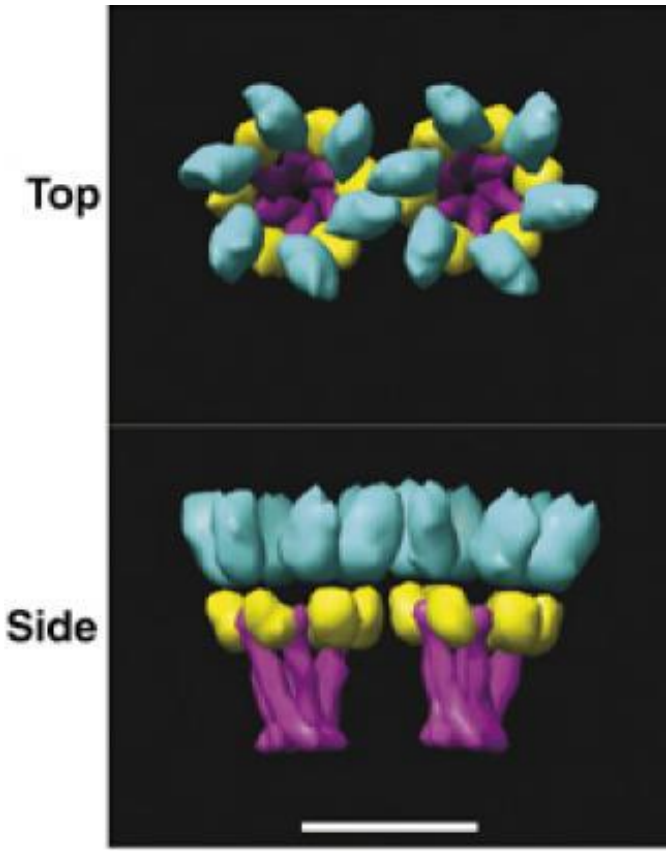
Gag is then cleaved, within the released immature particle, by the viral protease. A subset of the cleavage products then assembles within the particle to form the internal structures of the mature particle.

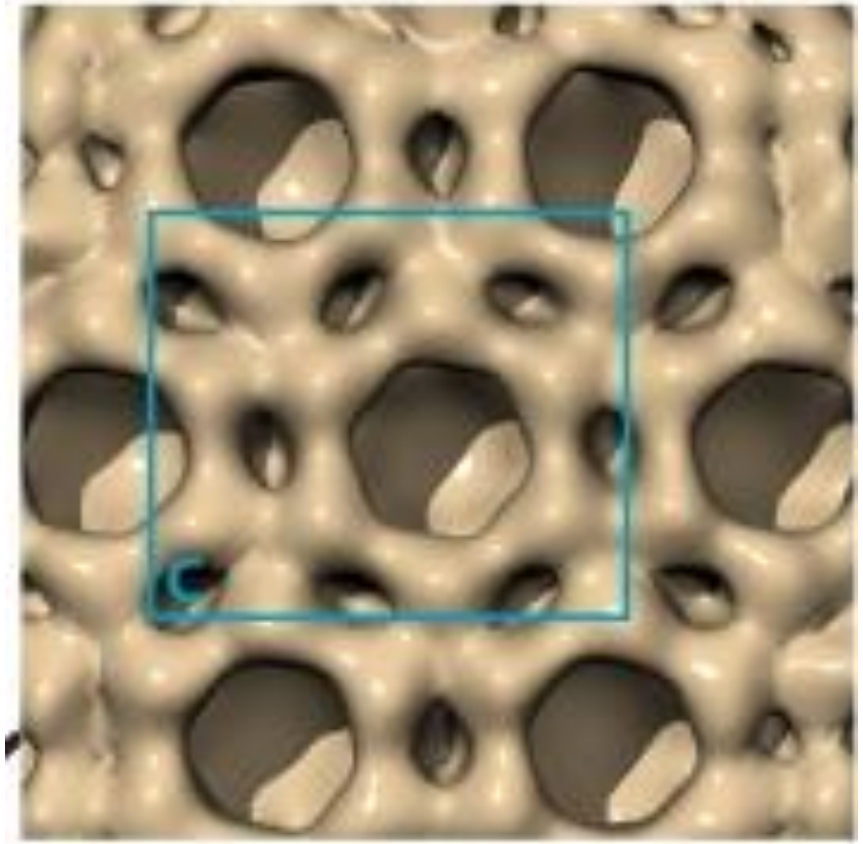
I'm speaking today only about the immature particle. It is composed of ~ 2000 Gag molecules, each with a rodlike conformation, arranged radially in a hexameric lattice.



Yeager et al., PNAS 1998

The Hexameric Lattice of Gag Proteins in Immature HIV



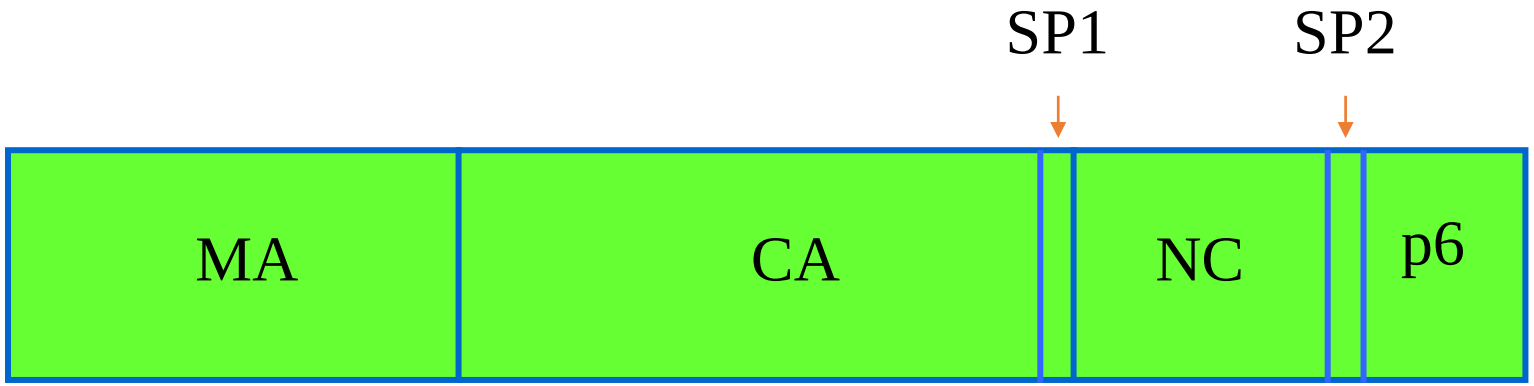


Wright et al., EMBO J., 2007; Briggs et al., PNAS 2009

At maturation, Gag is cleaved into a total of 6 cleavage products;
These are the proteins making up the mature particle.
They include, from N- to C-terminus,

- **MA—functions mainly in interactions with membranes**
- **CA—functions in protein-protein interactions and forms the conical core within the mature particle**
- **NC—functions in interactions with RNA**

…and a couple of others I won't discuss today.



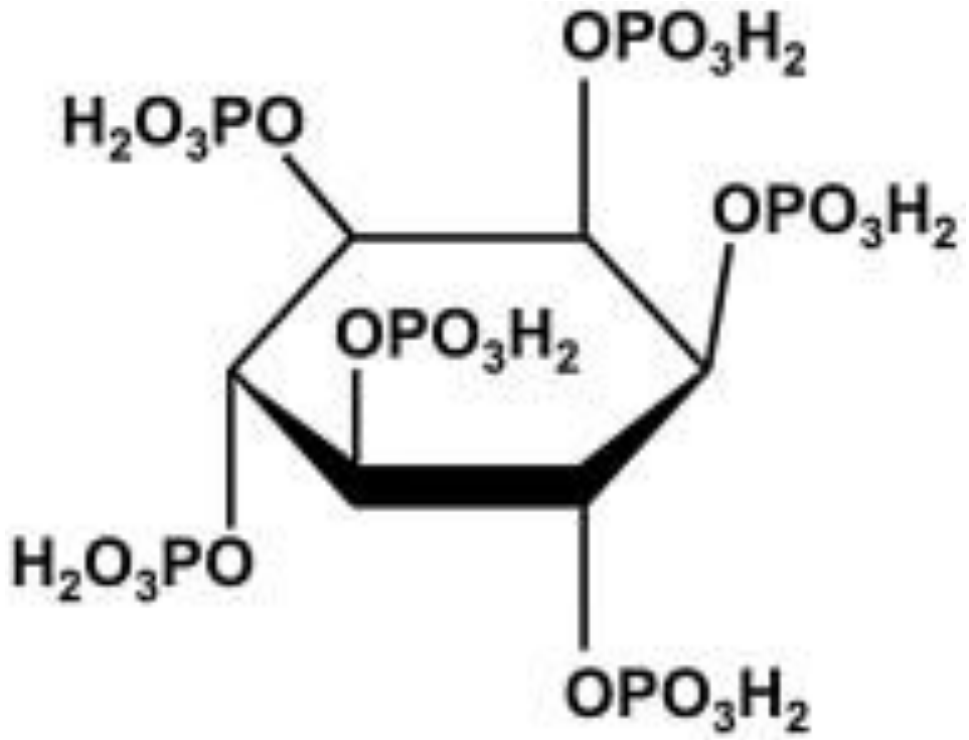
IP6 in Immature HIV Assembly

The experiments I'm presenting were done with recombinant "ΔMA" Gag protein. This is missing most of the MA domain. It also differs from authentic Gag in lacking a fatty acid modification at its N-terminus and an irrelevant domain, "p6", at its C-terminus.

This protein is soluble upon purification from *E. coli*. However, it assembles into virus-like particles, good facsimiles of authentic immature particles, upon addition of a cofactor.

Co-factors can be either NA or IP6.

Inositol Hexakisphosphate (IP6)



(a 6-membered ring with a -12 charge)

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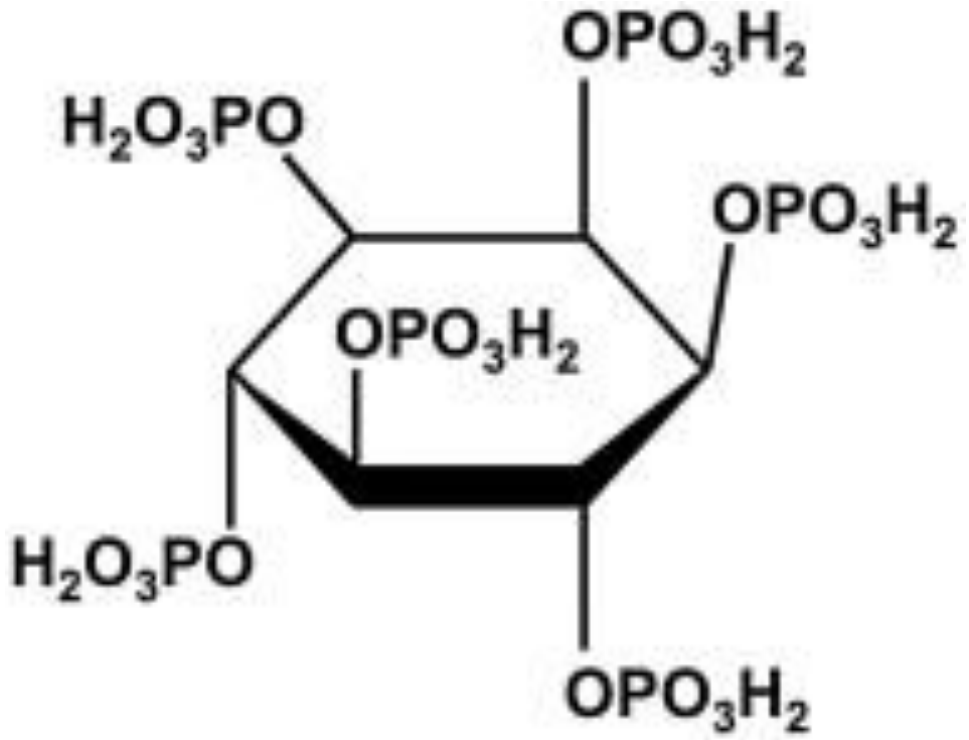
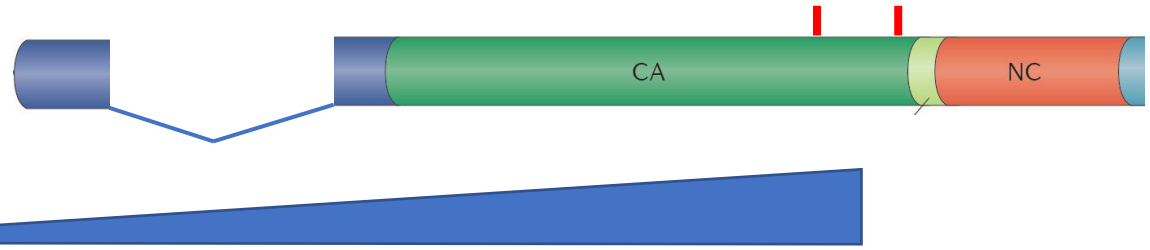
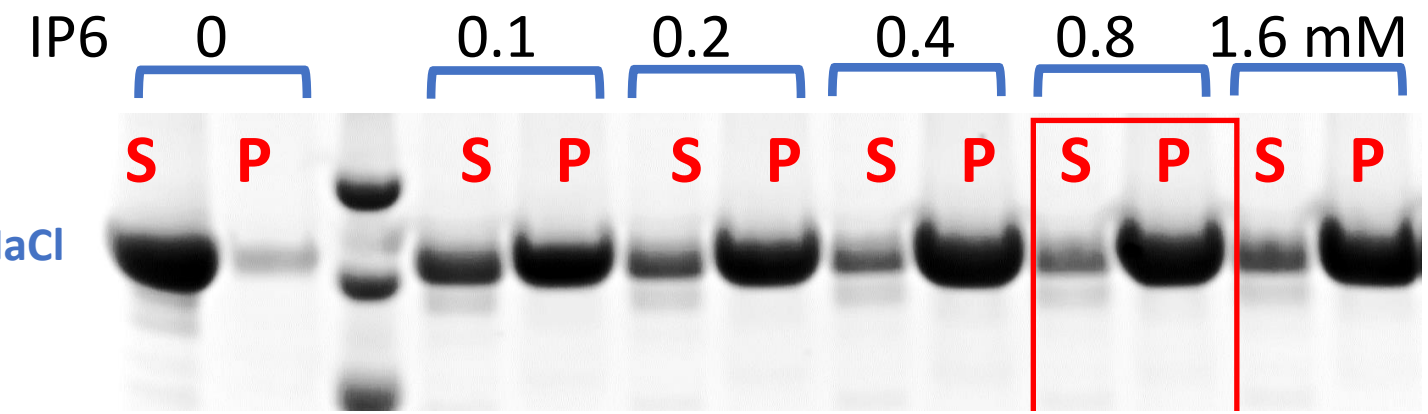


(a 6-membered ring with a -12 charge)

Present in mammalian cells at 10-100 μM

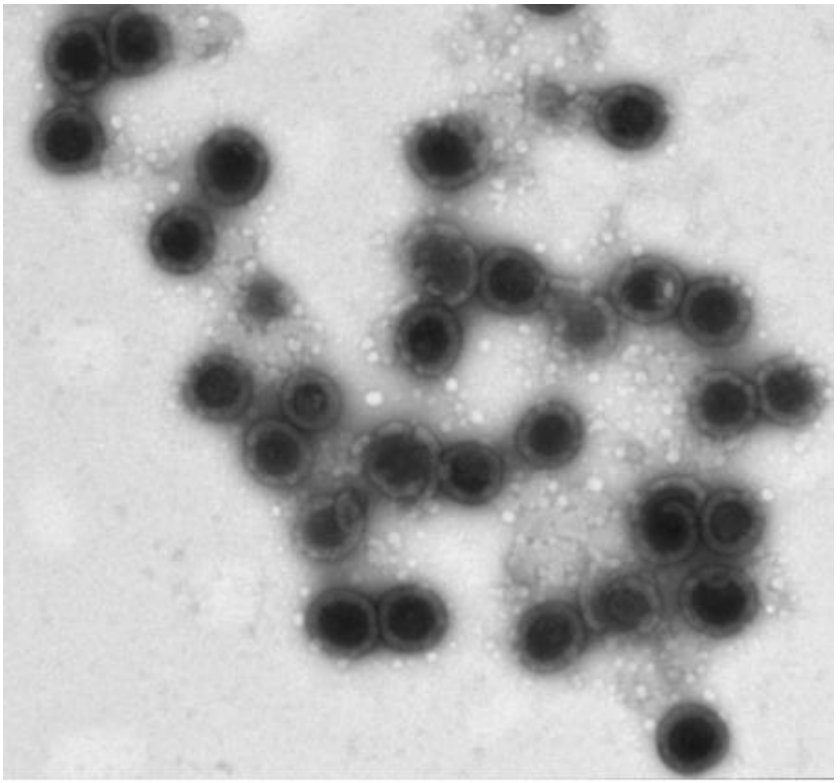
Assembly of Δ MA Gag with IP6





ΔMA Gag is a soluble protein.

Titration of IP6 into it causes it to assemble into VLPs.



20,000 x *g* for 30 min, 4°C

What’s Going On?

How does interaction with IP6 cause Gag to assemble?

In the assembled immature particle, one IP6 is known to be bound by each hexamer in the Gag lattice, between 2 rings of lysines near the C-terminus of the CA domain.

Mallery et al., 2019

In the assembled immature particle, one IP6 is known to be bound by each hexamer in the Gag lattice, between 2 rings of lysines near the C-terminus of the CA domain.

But of course, those lysine rings don't exist in Gag monomers in solution, but are only formed in the hexamers in the assembled lattice.

What's Going On?

How does interaction with IP6 cause Gag to assemble? Where does IP6 bind to Gag?

At relatively low ionic strength, multiple IP6 molecules bind to a single Gag molecule.

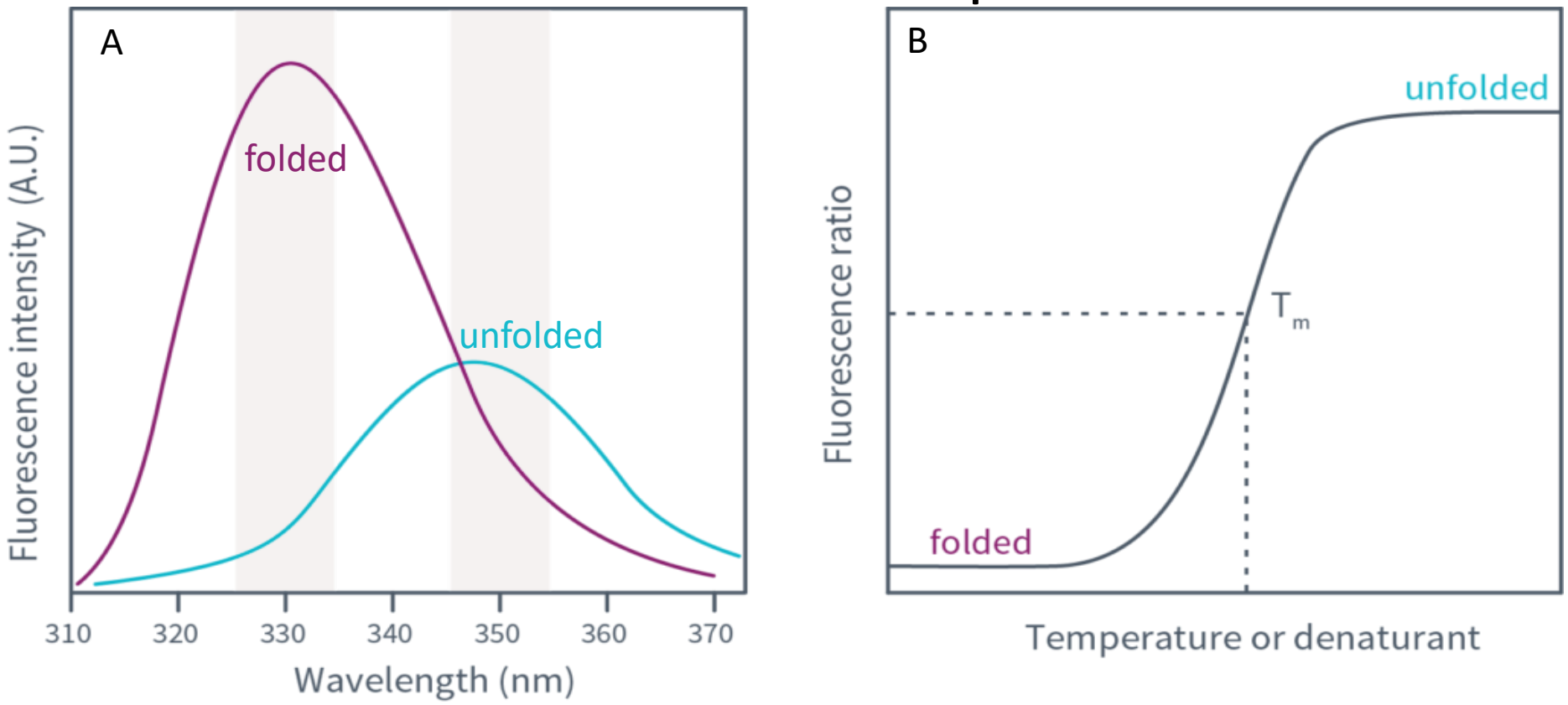
(At 0.5M NaCl, 1 IP6 binds per Gag, but even here the "binding site" could not be localized: both N-terminal and C-terminal regions of Gag are involved.)

Datta et al., JMB 2007

Inositol Hexakisphosphate (IP6)

We are analyzing its contributions to immature assembly in a quantitative assay, using differential scanning fluorimetry.

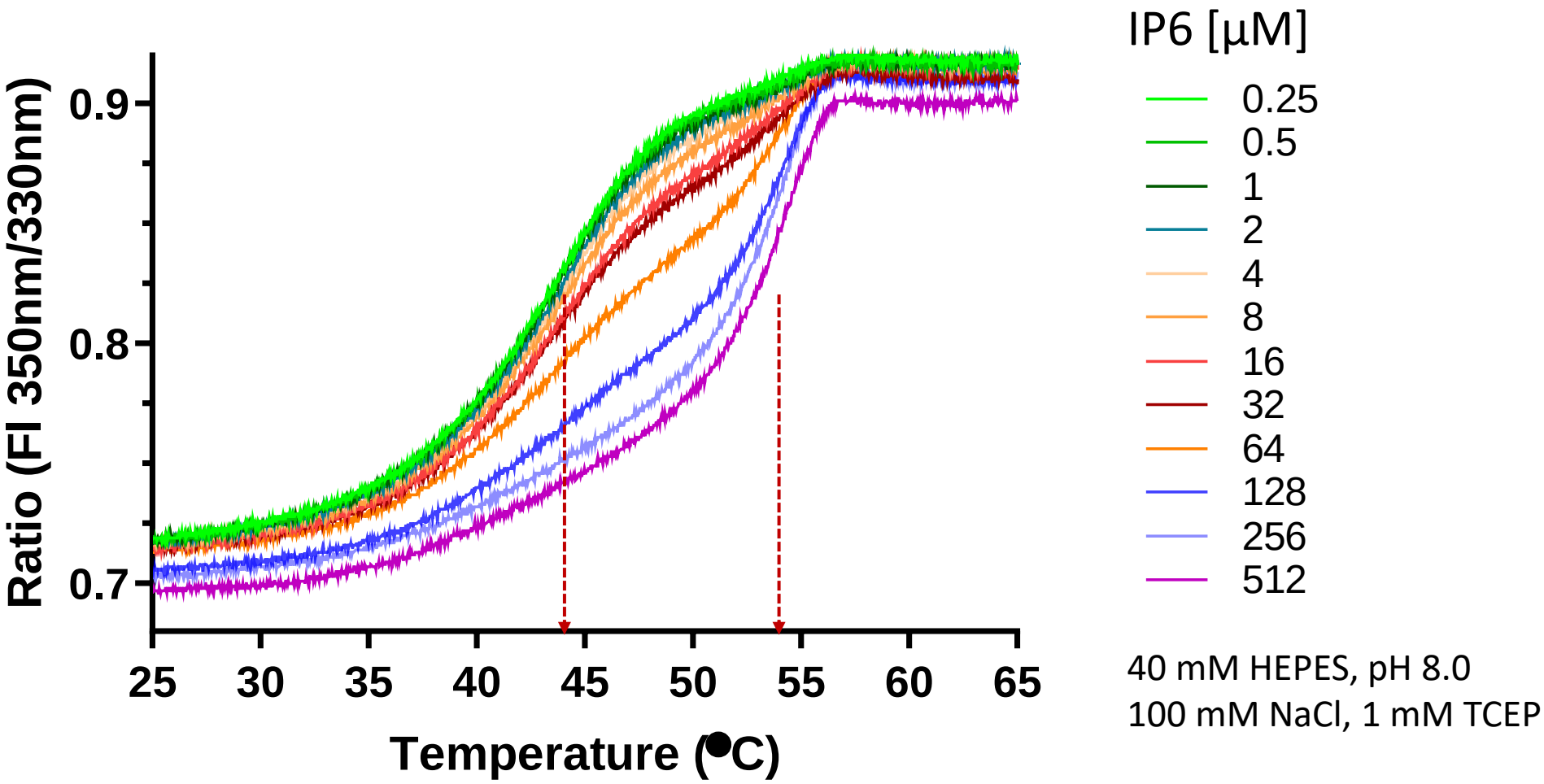
Differential scanning fluorometry: Tryptophan as a reporter of the state of the protein



A) Tryptophan emission spectra are sensitive to the local environment of the fluorophore

B) During denaturation, protein structure is altered – this leads to an alteration in the local environment of the tryptophan(s) and an increase in (fluorescence at 350 nm/fluorescence at 330 nm)

Thermal stabilization of ΔMA Gag by IP6



- Addition of IP6 shows an increase in the Tm of protein unfolding
- Intermediate concentrations of IP6 show bi-phasic profiles

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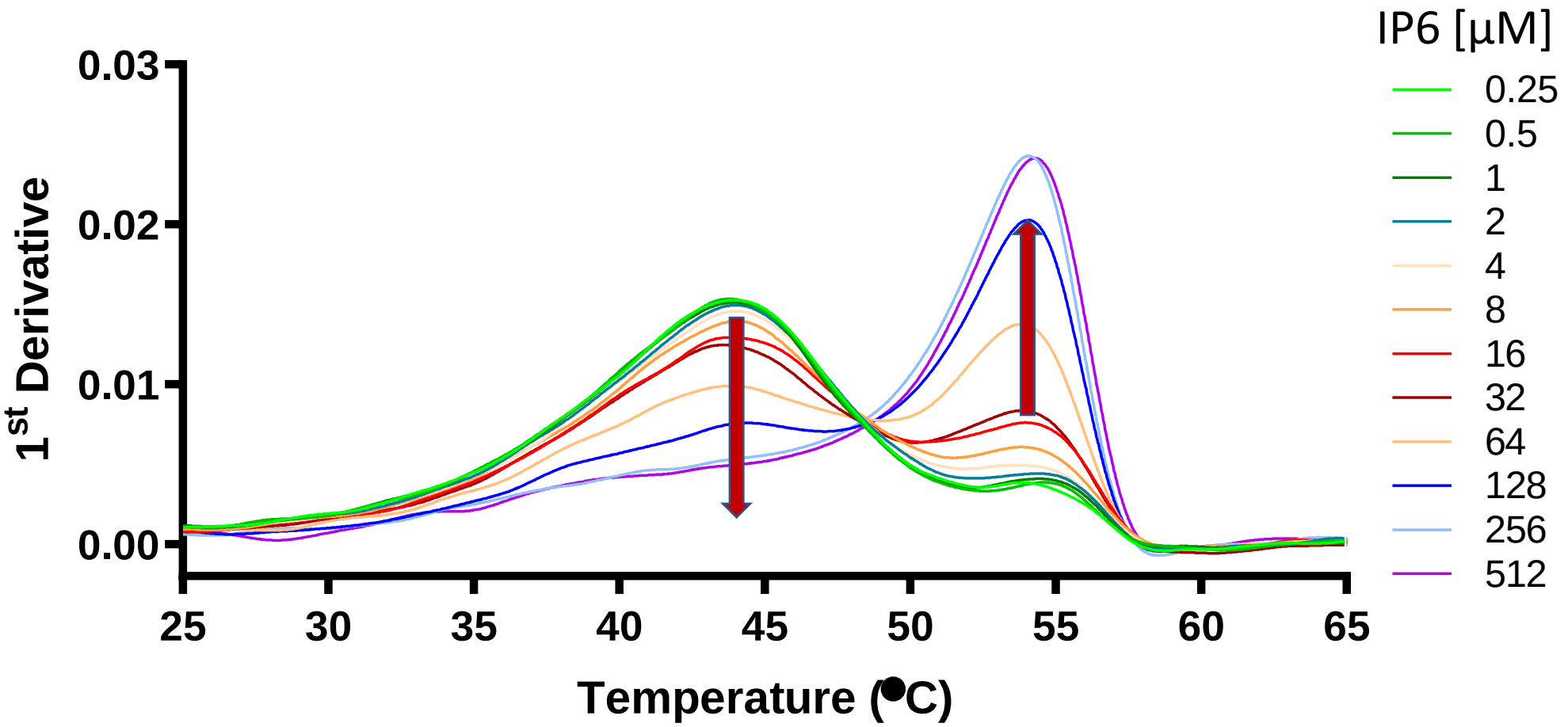

- The Tm of the original peak does not alter, but a second Tm appears ~10°C higher
- All the curves cross over at a single point [isosbestic point] suggesting that the signal is from two species, one more stable than the other

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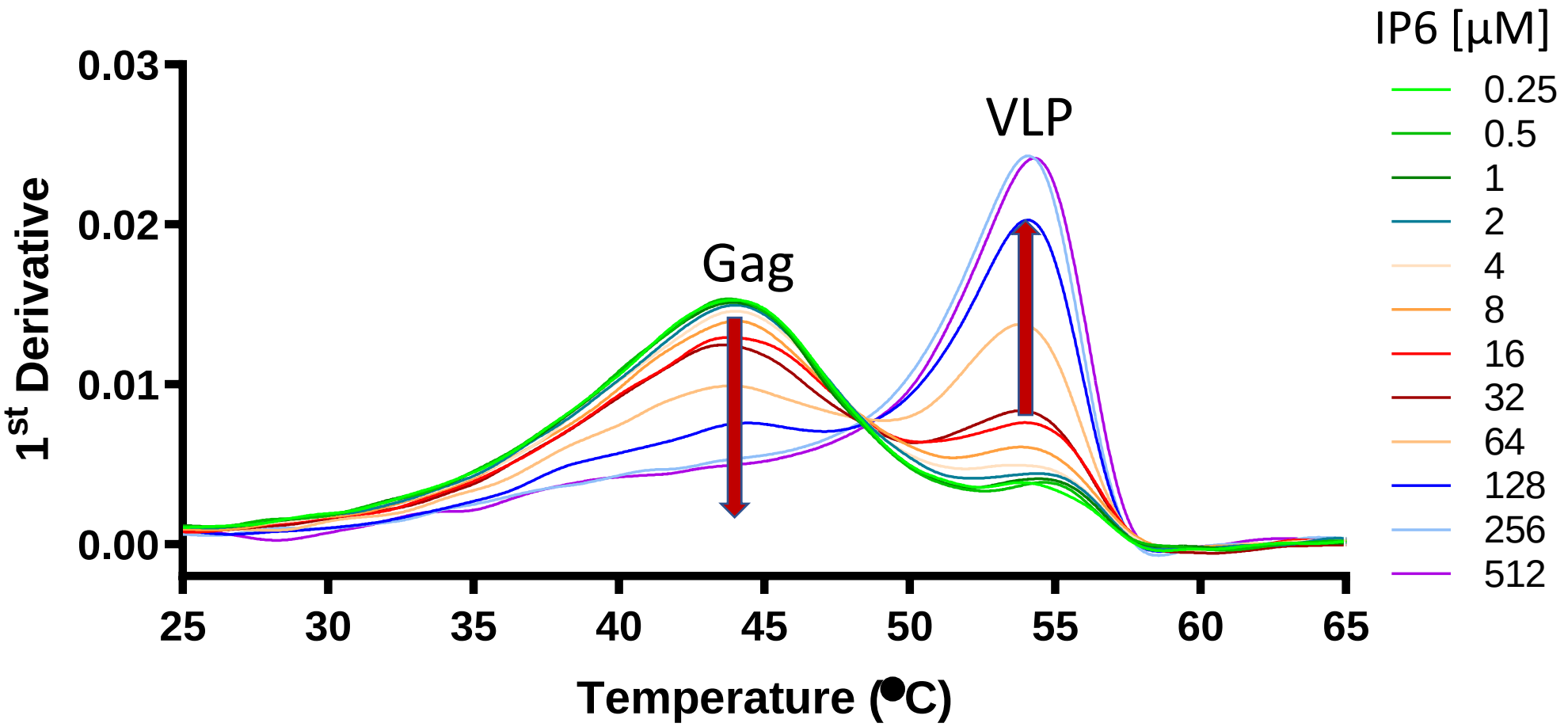

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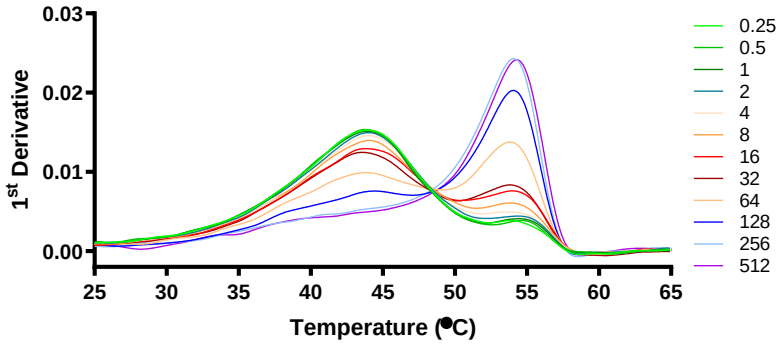
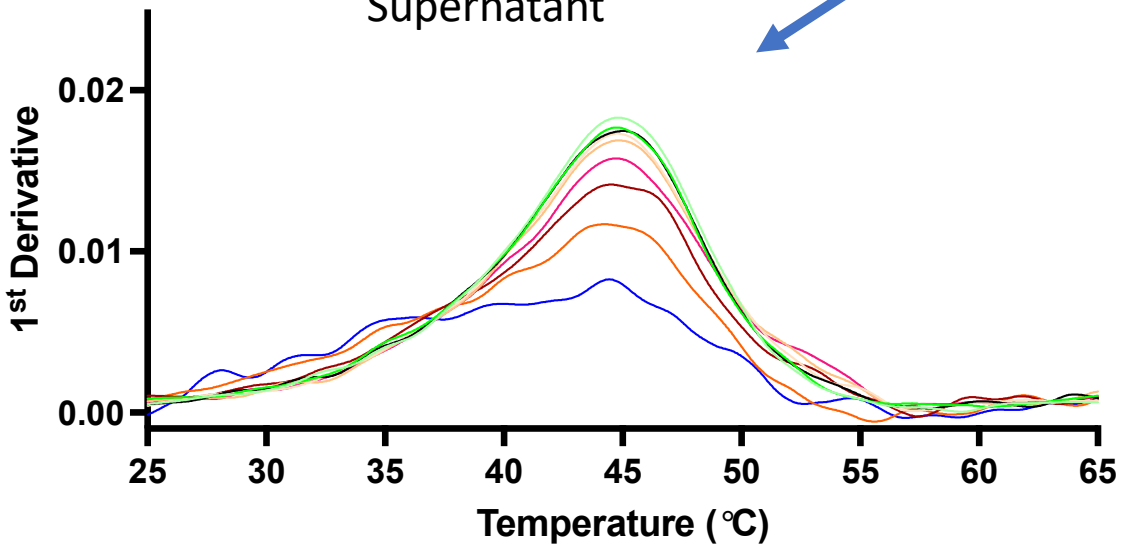
Thermal stabilization of ΔMA Gag by IP6 results from assembly into VLPs

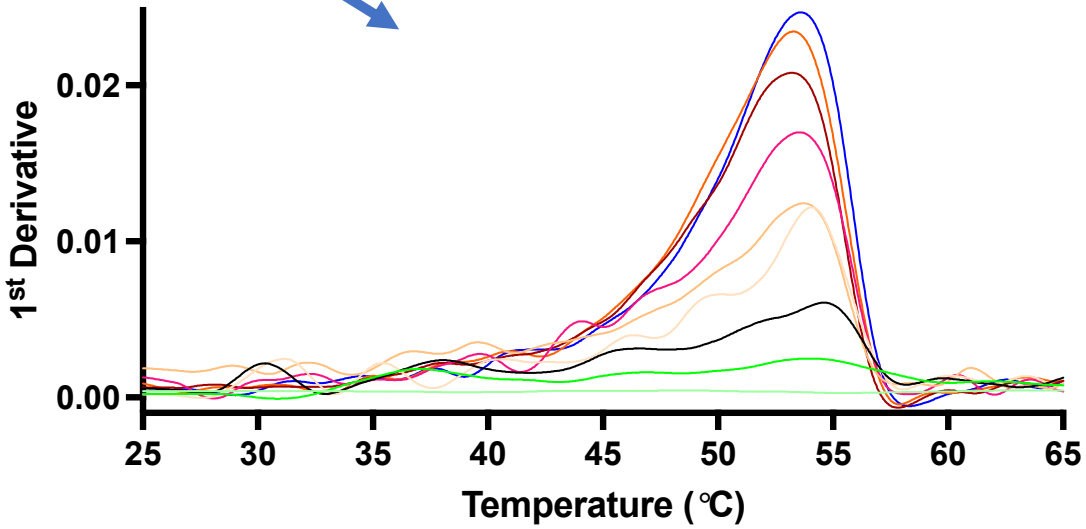


20,000g, 30 min

Supernatant

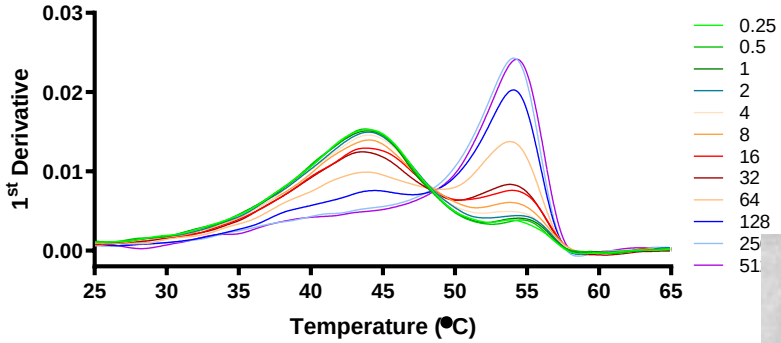


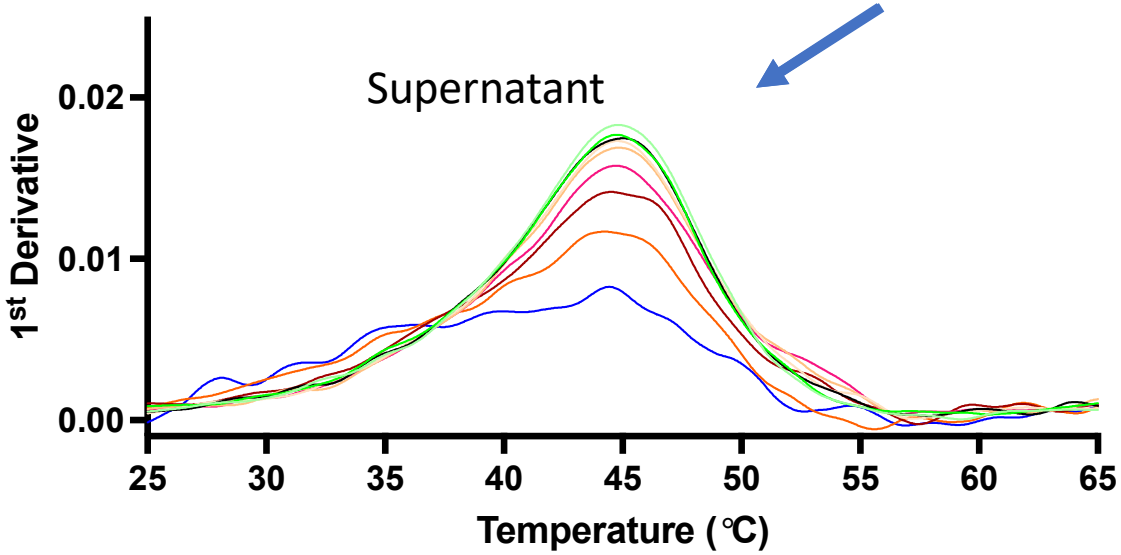
Pellet

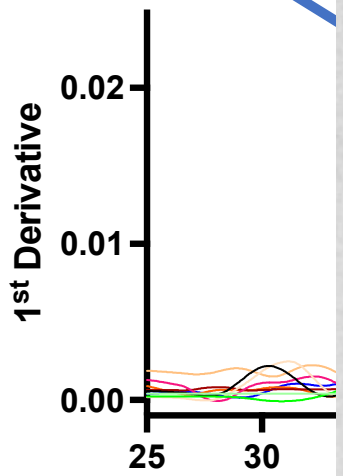


- **There are two species in the tube, one that is pelleted and the other that is not.**

Thermal stabilization of ΔMA Gag by IP6







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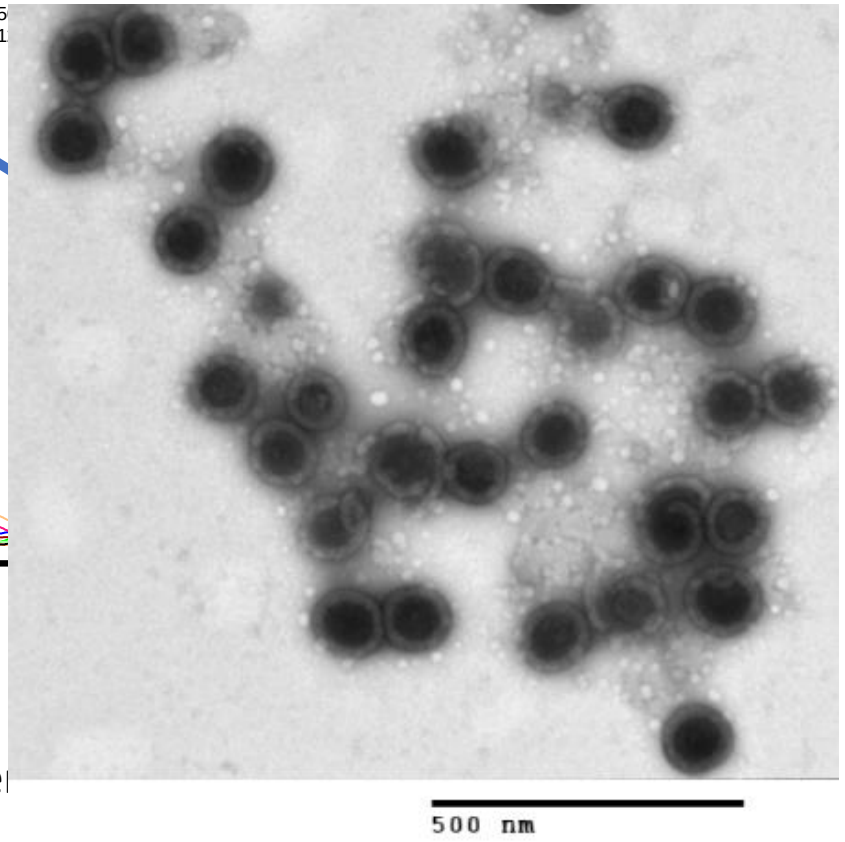
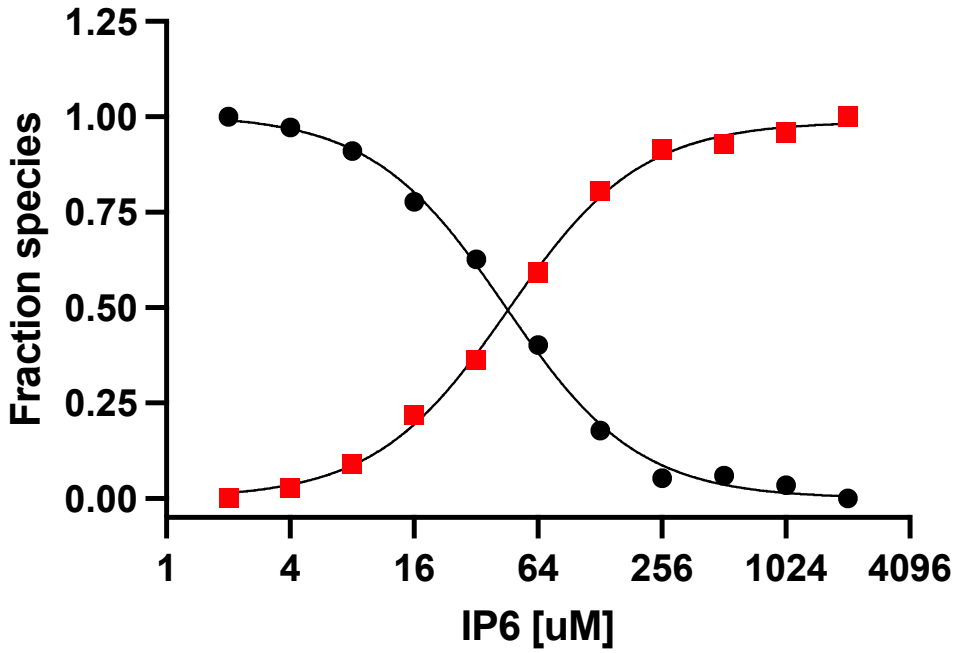


- There are two species in the tube, one that is pelleted and the other

This methodology not only yields the % assembly (with no manipulation or fractionation), but also the thermostability of the assembled species and the “Kd” and cooperativity of the assembly in response to the added co-factor.

IP6-driven assembly of WT Gag

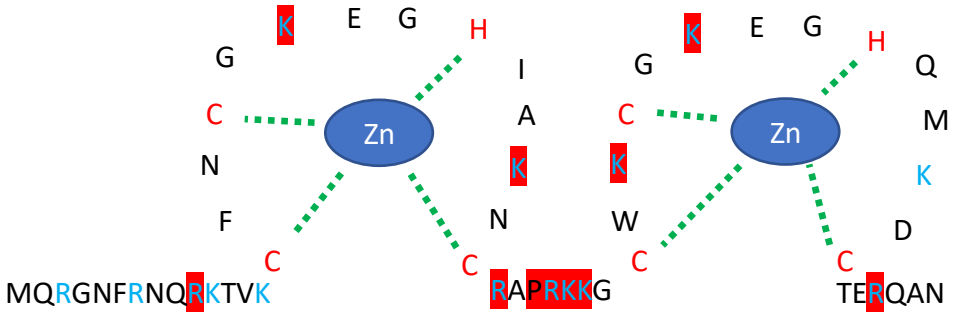


Specific binding with Hill slope		
Best-fit values		
Bmax	1.006	0.9877
h	-1.344	1.343
Kd	44.35	45.74
Tm	54.5 C	

Fitting the weights of the basis spectra used to generate the best fit experimental curves to a specific binding model with Hill slope allows for the extraction of assembly parameters from the raw data.

Basic aa's in NC domain are required for IP6-driven assembly

HIV NC: 14 R's and K's



PI: 10.21 (WT) / charge at pH 7 +11.9

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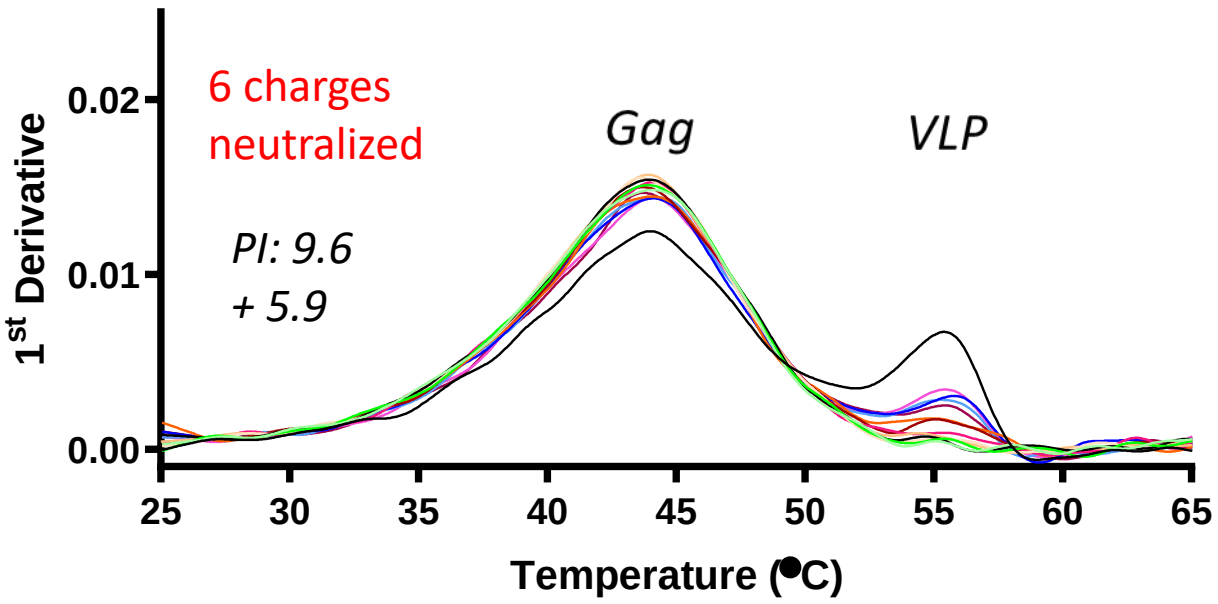

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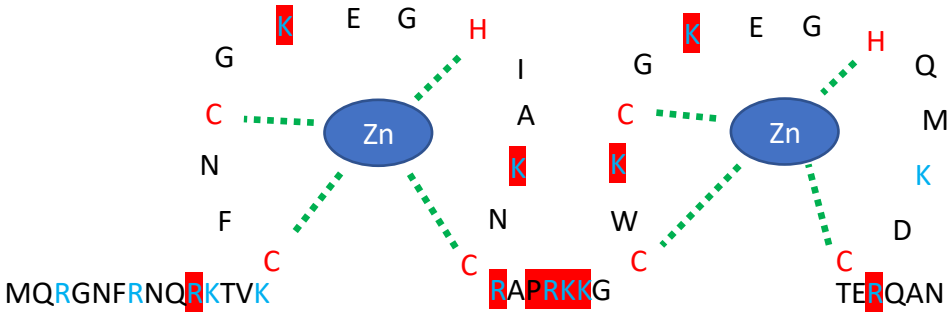

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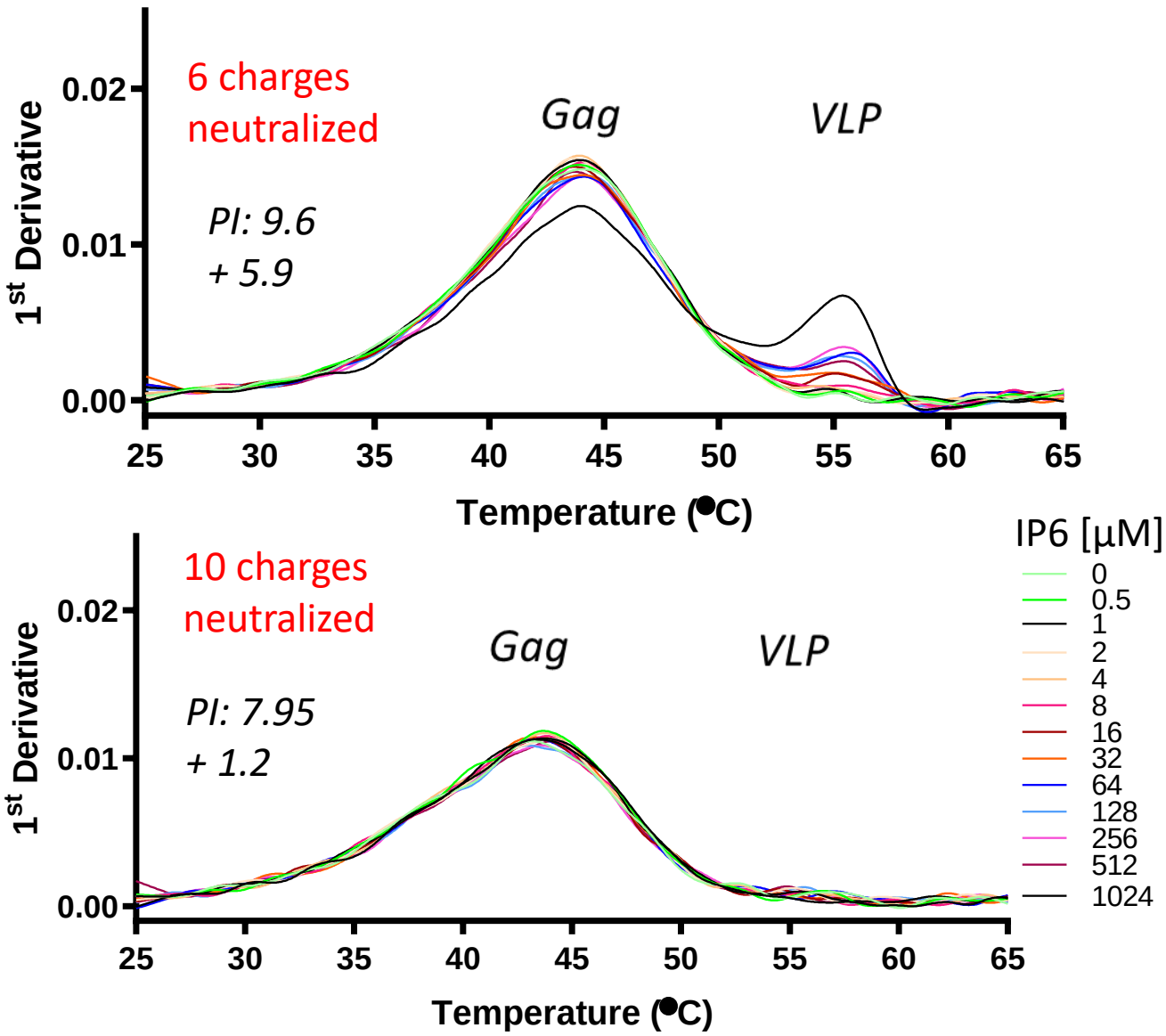

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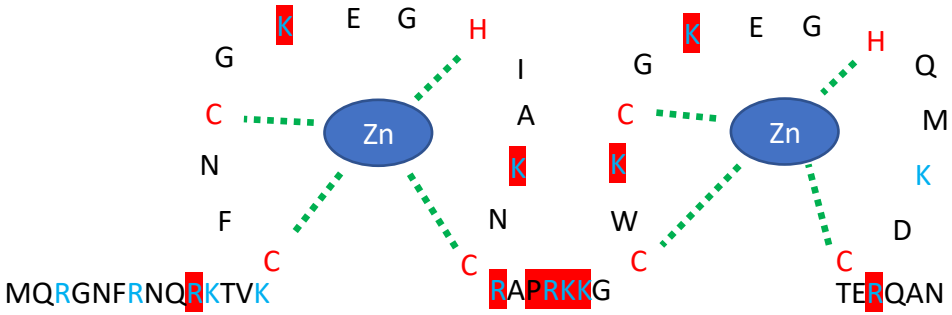

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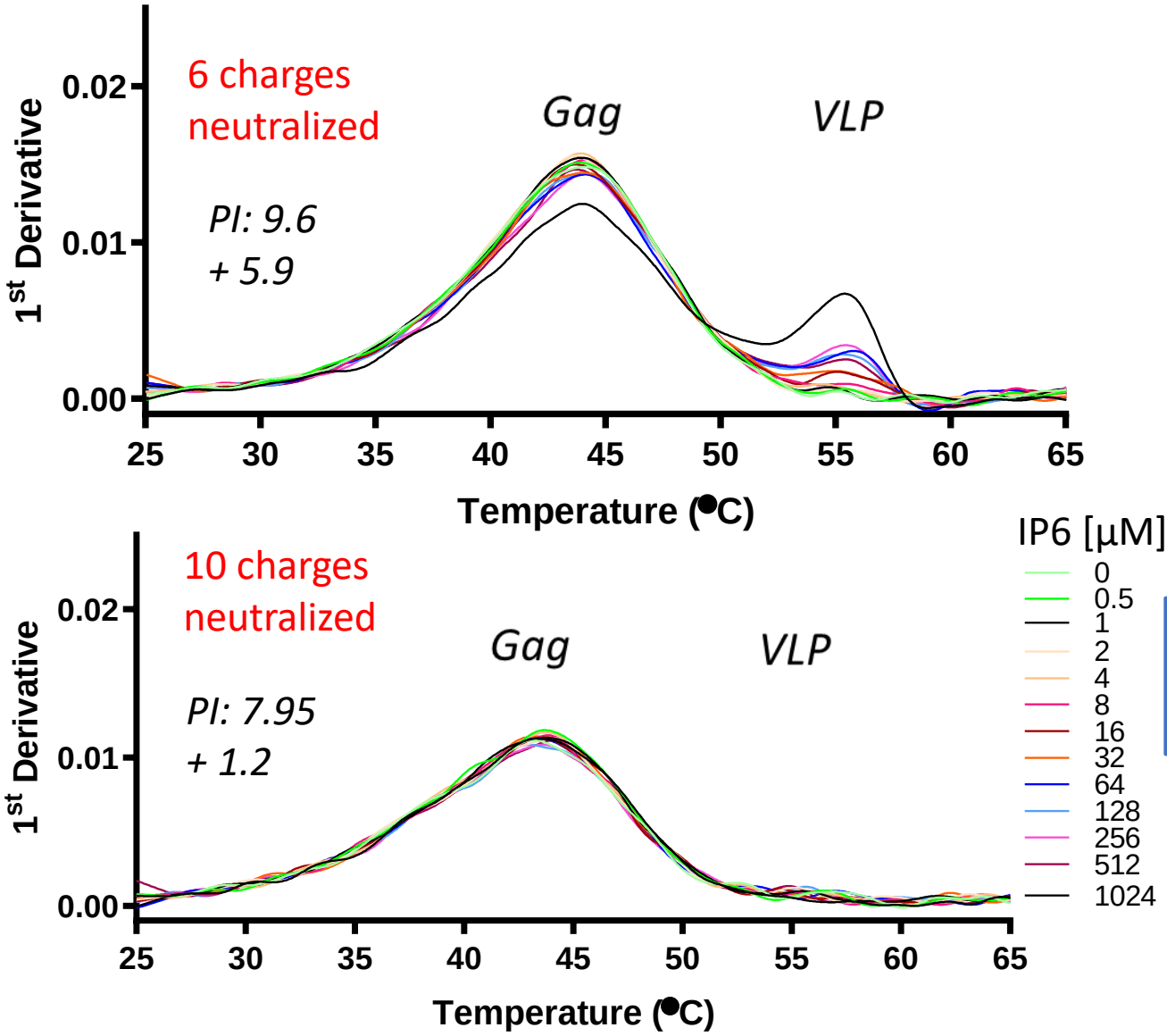

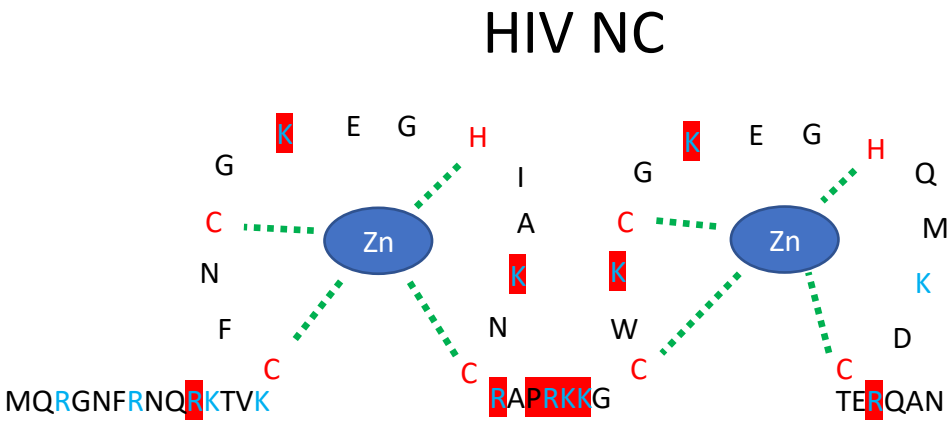

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Neutralizing basic residues in the NC domain eliminates IP6-driven assembly.

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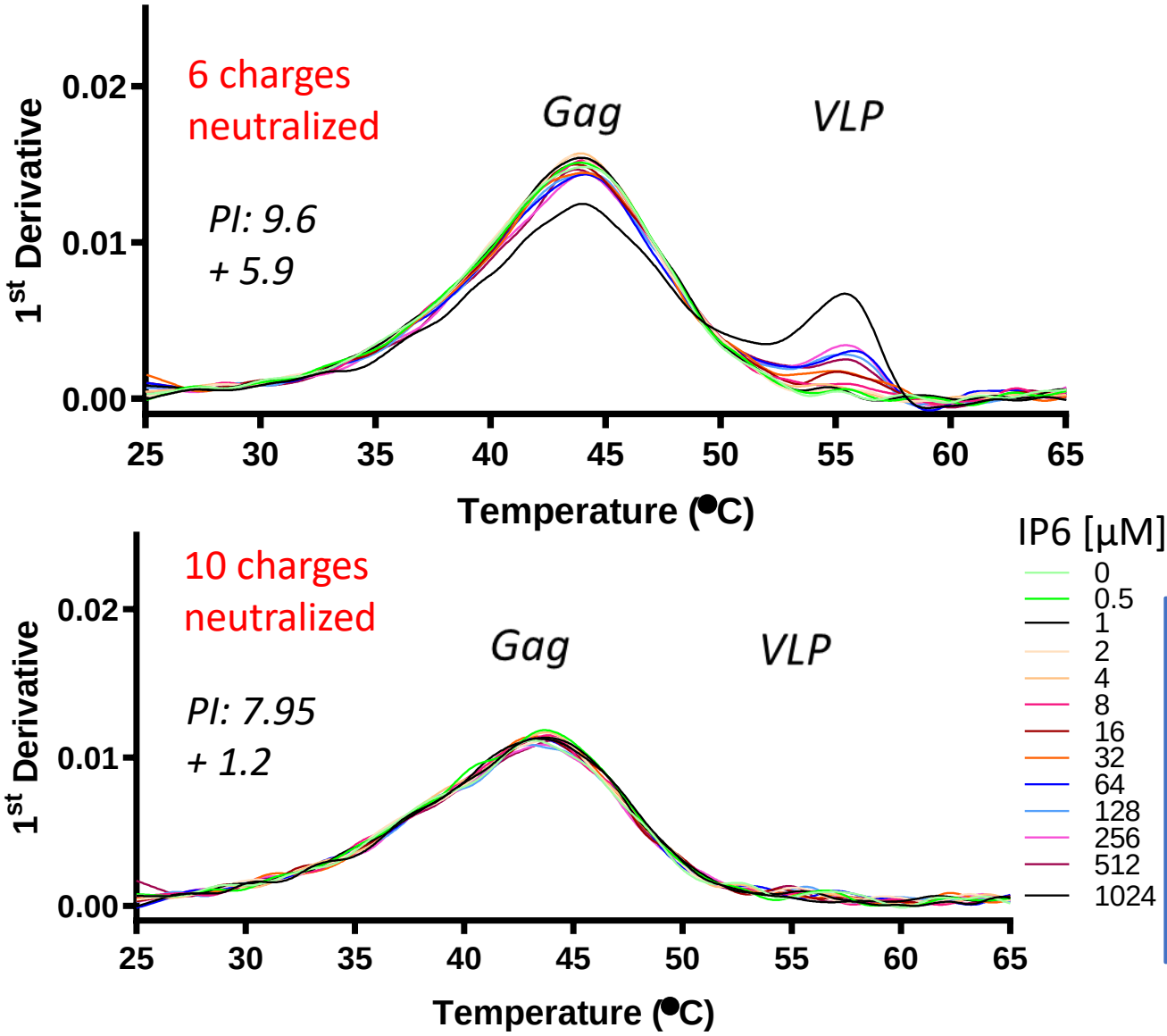

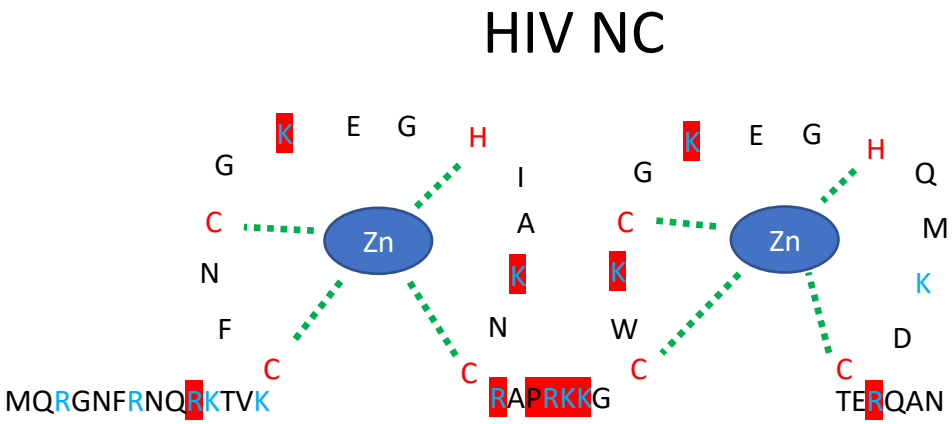

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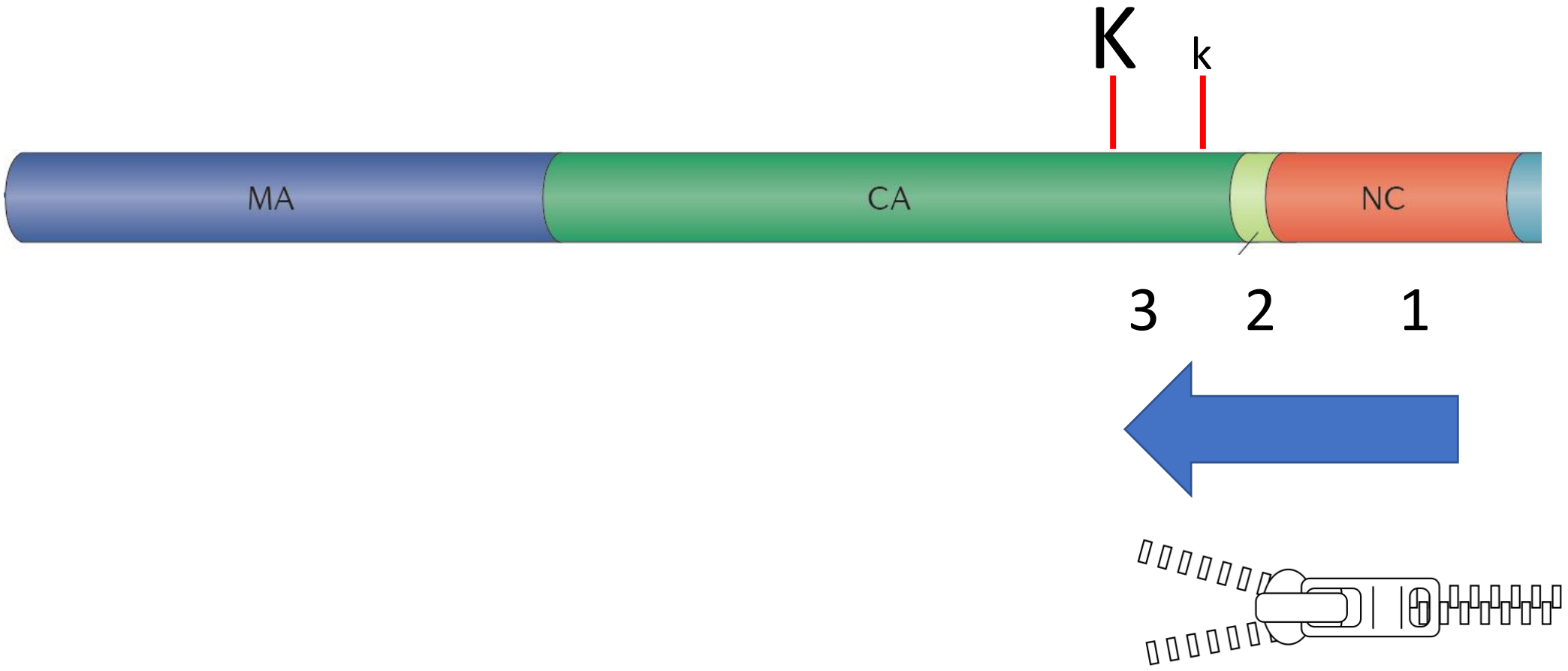
Neutralizing basic residues in the NC domain eliminates IP6-driven assembly. This suggests that these basic aa's normally interact with the negatively charged IP6.

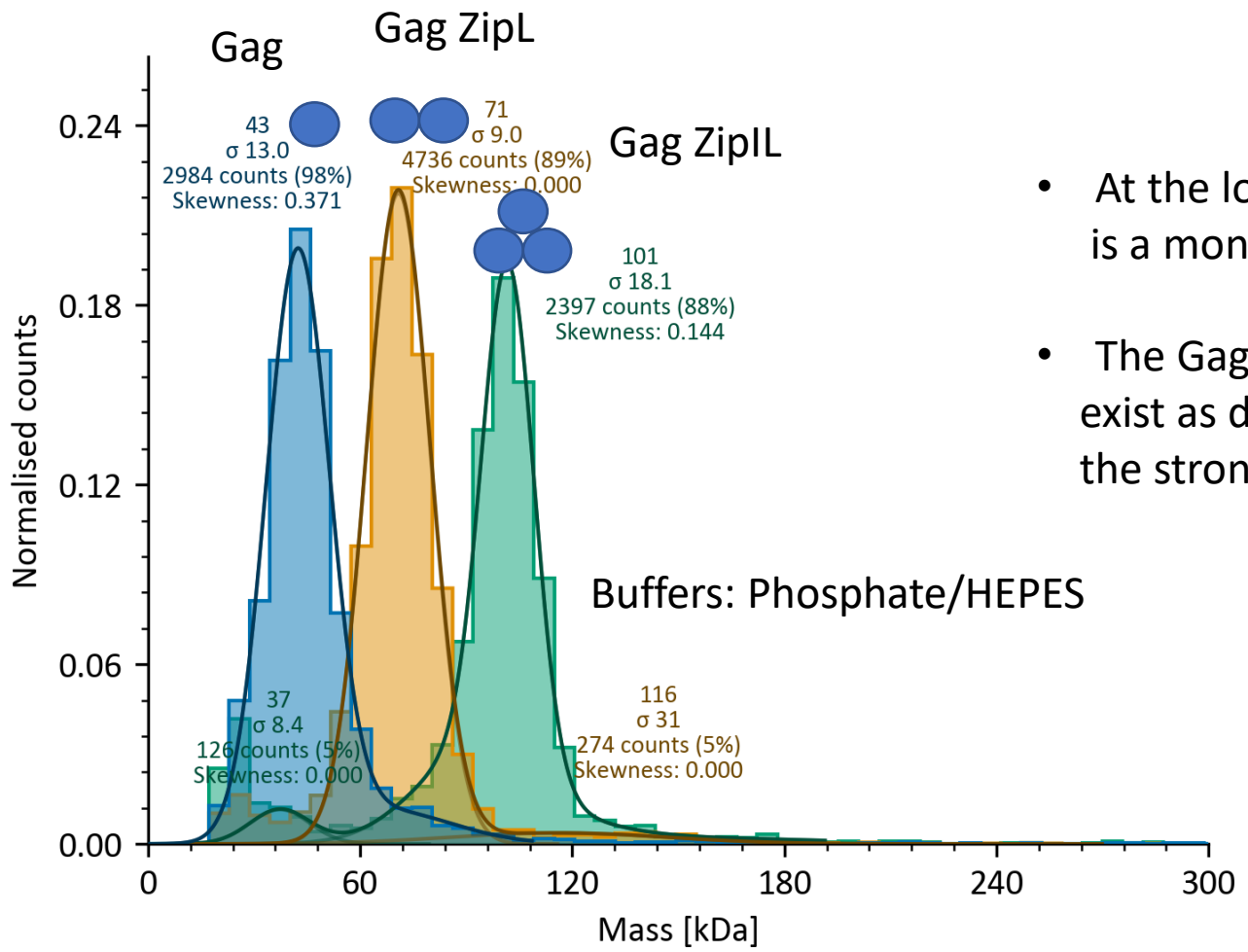
Working Model



Gag Derivatives with Known Oligomeric Status

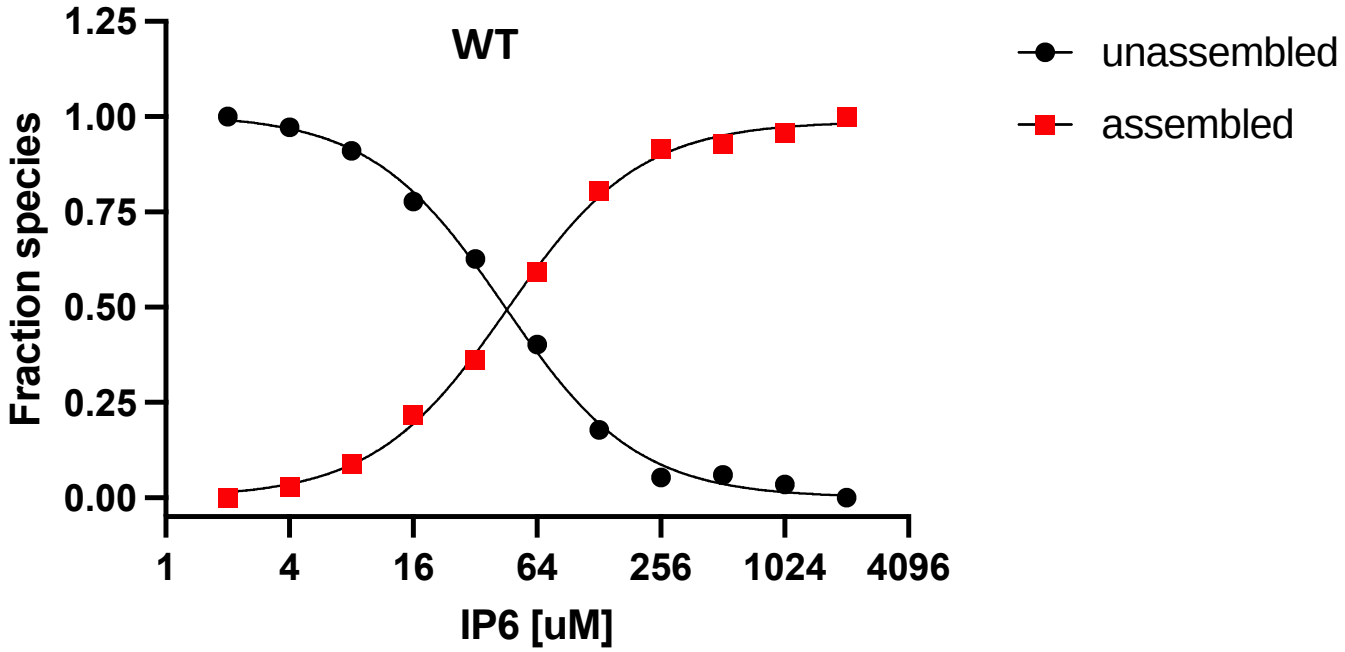
We also have Gag derivatives in which the NC domain is replaced with a "zipper" domain. This zipper domain does not interact with NA. These come in 2 flavors—the "leucine zipper" dimerizing domain; and the "isoleucine zipper" trimerizing domain.

Mass Photometry shows that these proteins have the expected oligomeric status

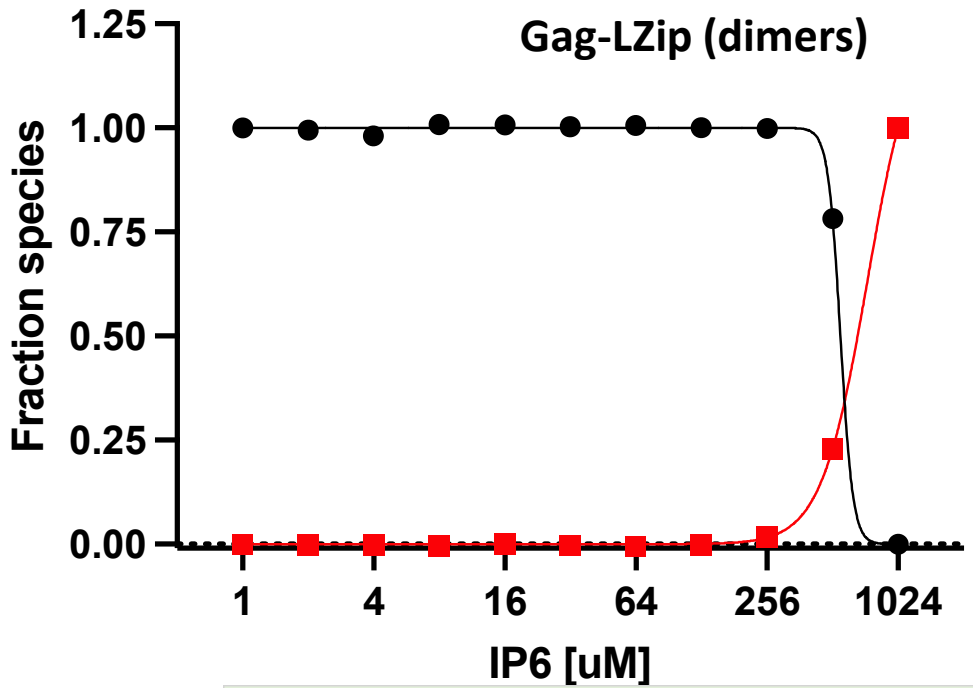


- At the low concentration of 25nM Gag protein is a monomer as expected
- The Gag_Zip-L and Gag_Zip-IL proteins exist as dimers and trimers, respectively, due to the strong zipper interactions

Behavior of Dimeric Gag-LZip is Completely Different from WT Gag



Specific binding with Hill slope		
Best-fit values		
Bmax	1.006	0.9877
h	-1.344	1.343
Kd	44.35	45.74

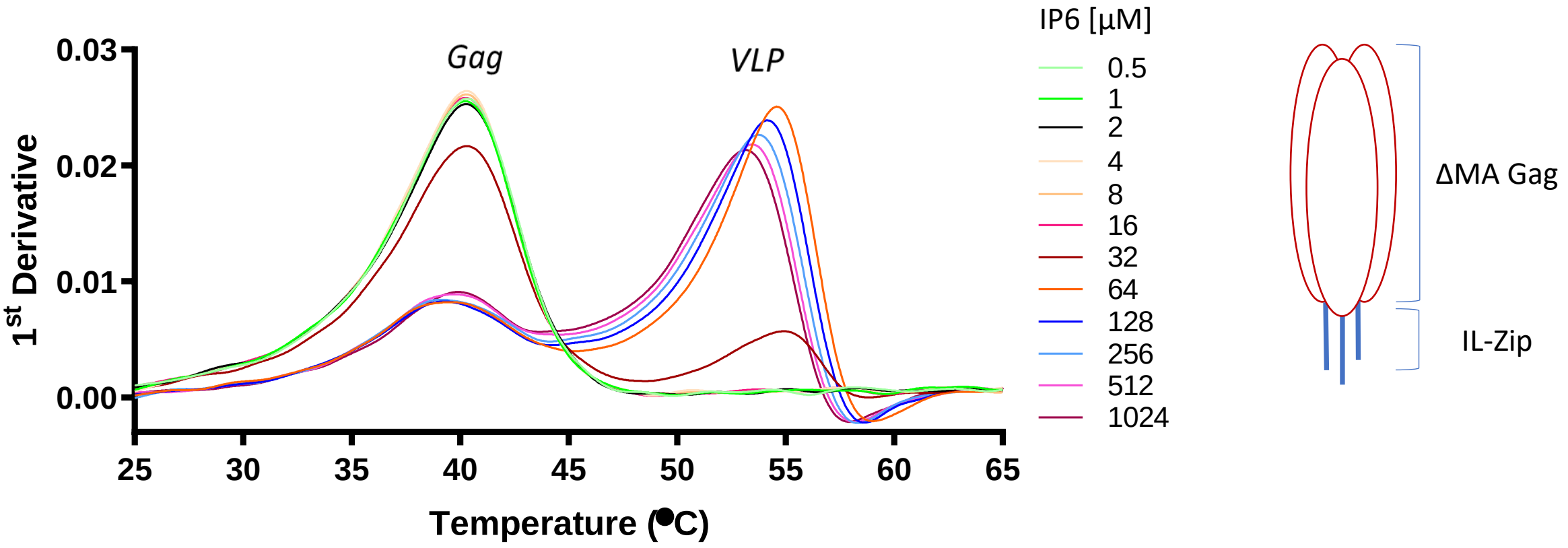


Specific binding with Hill slope		
Best-fit values		
Bmax	0.9998	1.266
h	-15.21	4.096
Kd	556.9	741

- **Assembly starts at a much higher concentration of IP6 (Kd 741 rather than 45), but cooperativity is enhanced (h = 4.0 as vs. 1.3 for WT Gag) once assembly starts.**

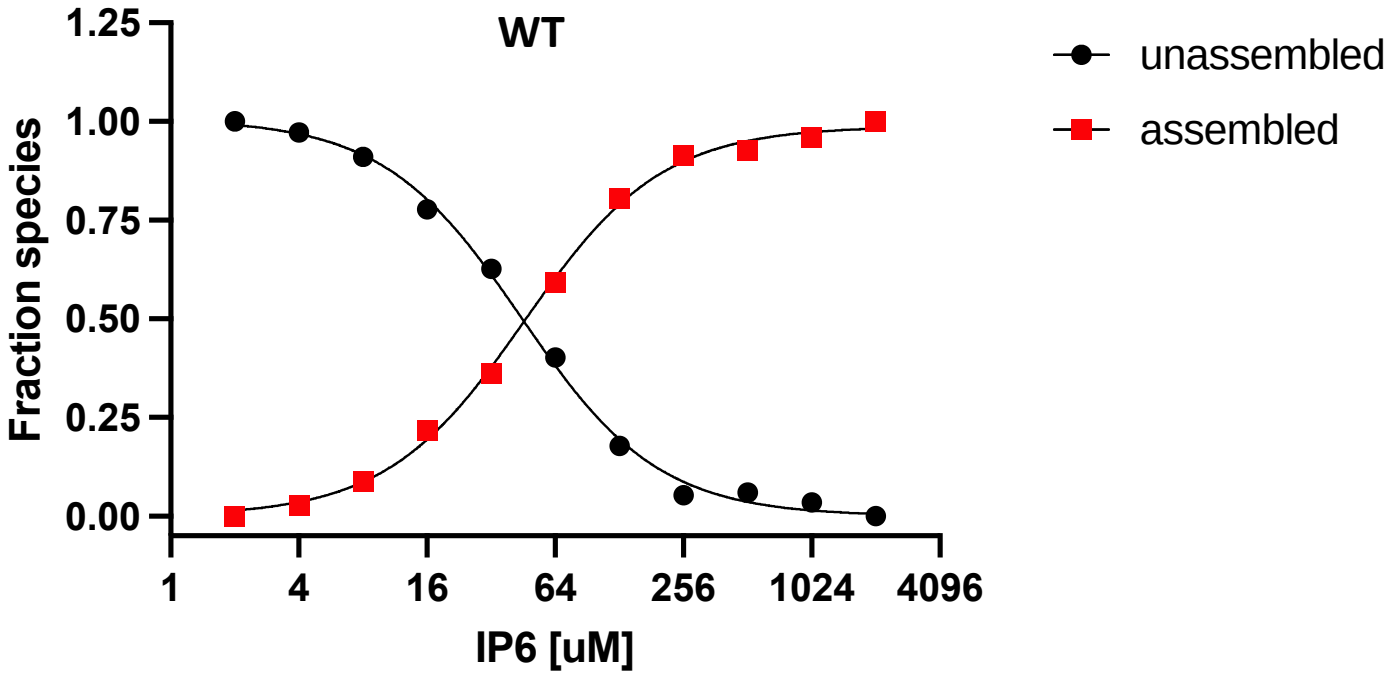
- **Suggests that obligate dimerization inhibits the initiation of assembly.**

Behavior of Trimeric Gag-ILZip



In contrast, Gag in which NC is replaced with this IL-Zip is hyper-responsive to IP6 (even though the charges in the original NC domain are no longer present)

Trimeric Gag (Gag-ILZip) shows greatly enhanced cooperativity in assembly



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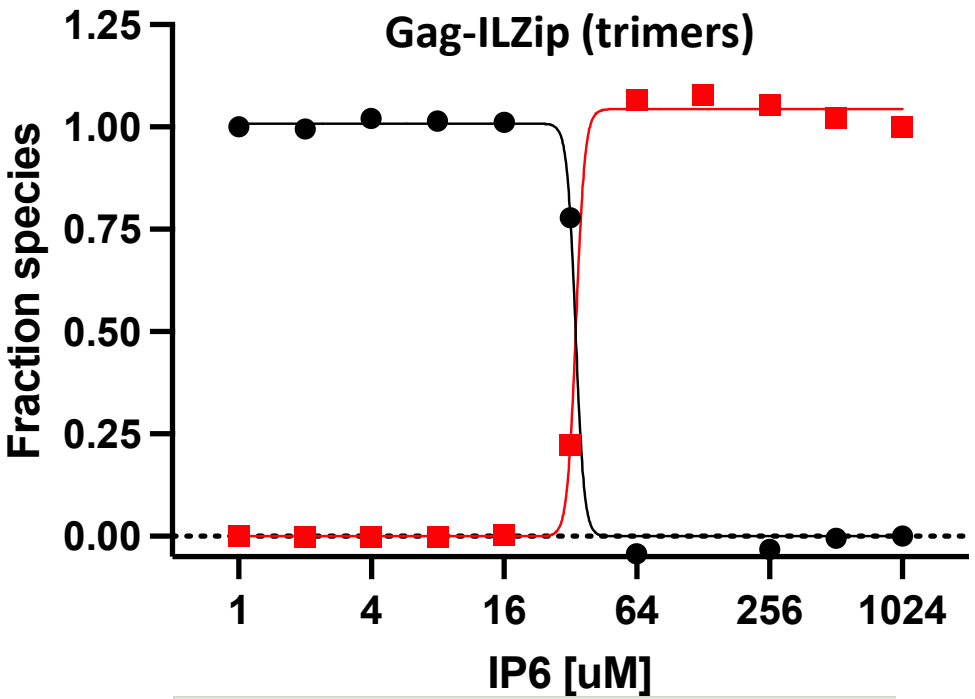

Specific binding with Hill slope		
Best-fit values		
Bmax	1.008	1.044
h	-22.53	21.77
Kd	33.78	33.97

Replacing NC with an IL-Zip domain renders Gag trimeric. This version of Gag is extraordinarily responsive to IP6 addition, with an h (cooperativity) value of 22 rather than ~1.3 as in WT.

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- **Further, Gag molecules in which the NC domain is replaced with a trimeric motif assemble with extremely high cooperativity when exposed to IP6.**
- **In contrast, molecules in which NC is replaced with a dimeric motif are relatively resistant to IP6-driven assembly, needing an IP6 concentration ~ 15-fold > WT Gag to induce assembly.**
- **Taken together the data suggest that IP6-induced assembly requires the intimate association of trimers of Gag molecules at their C-termini.**

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