How is genomic RNA of HIV selectively packaged?

Attempt at simple theory

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CENTER FOR RNA BIOLOGY

Acknowledgements

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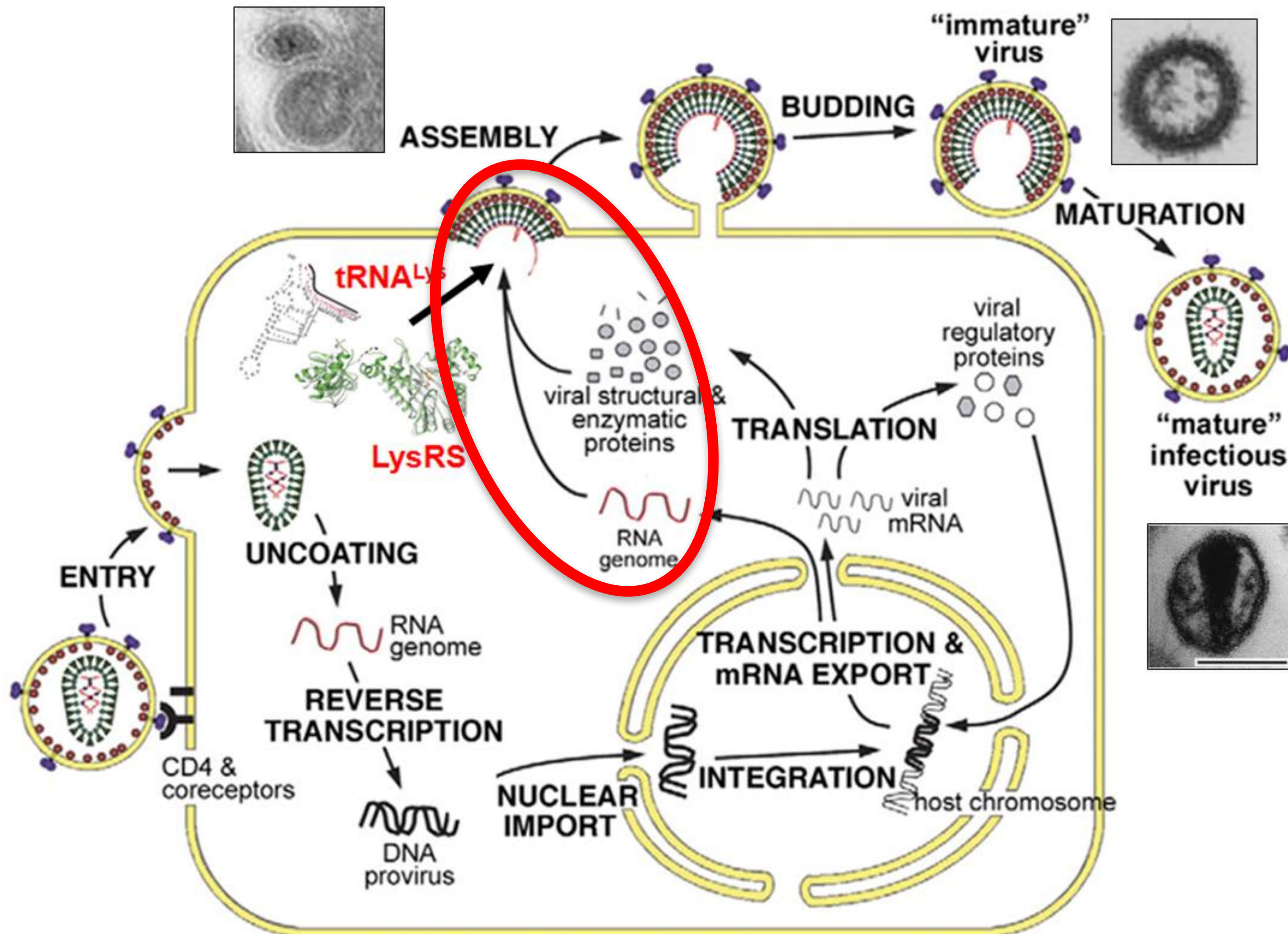
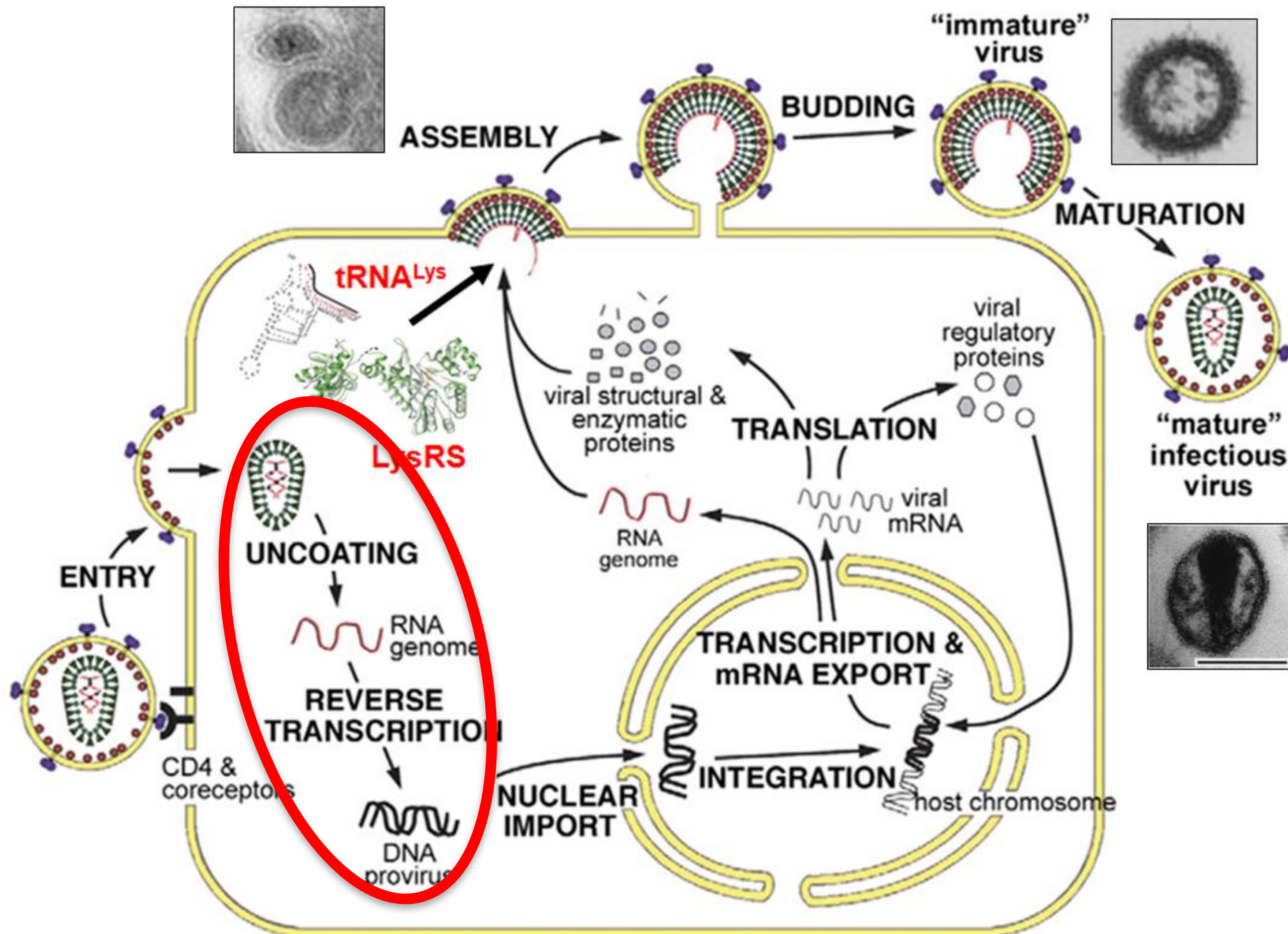
How is gRNA selected for packaging?



Ganser-Pornillos, Yeager, and Sundquist. *Curr. Opin. Struct. Biol*. **18**:203–217(2008).

How mature HIV capsid "uncoats"?



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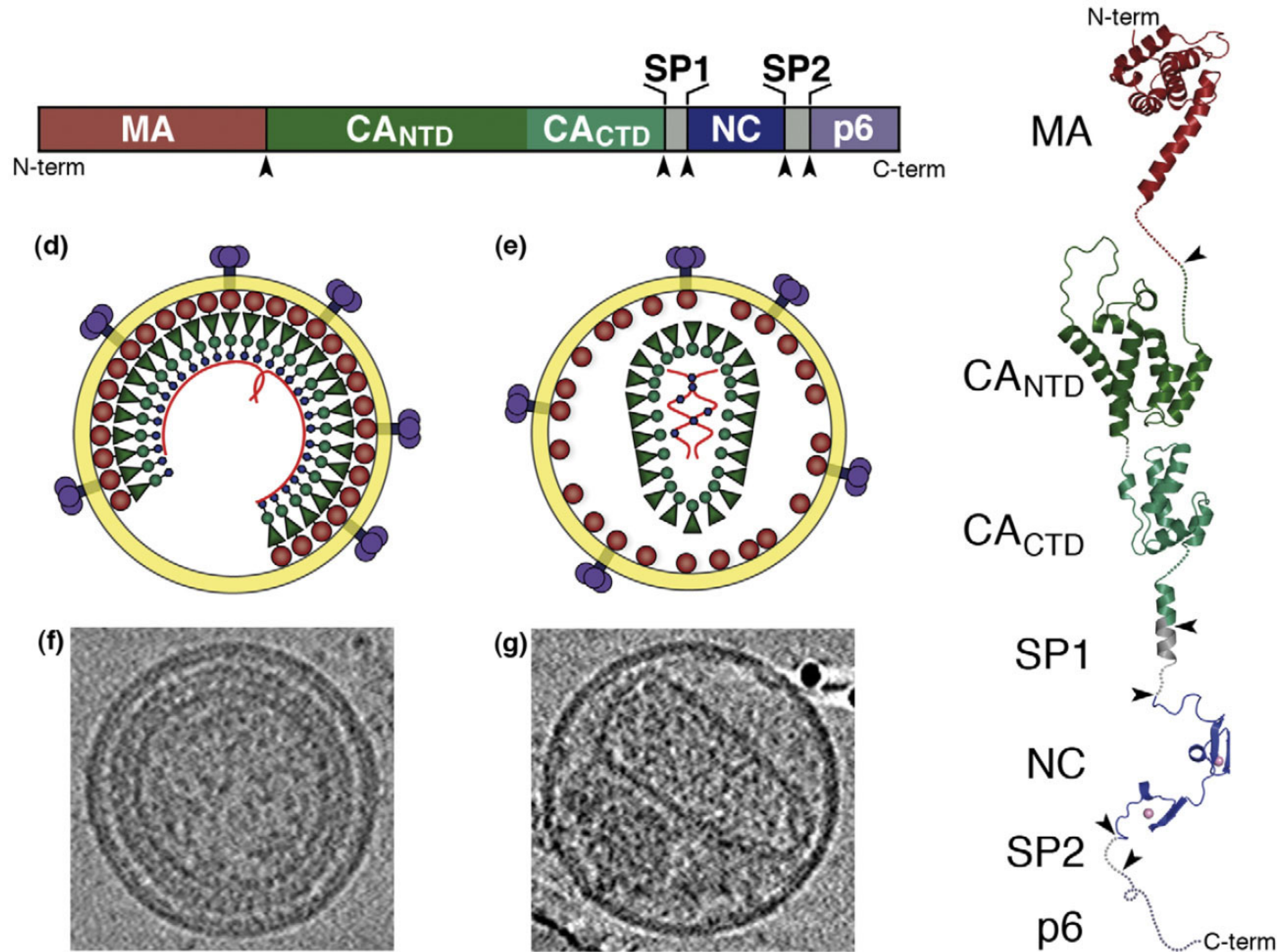
Immature and mature HIV-1 capsid are completely different



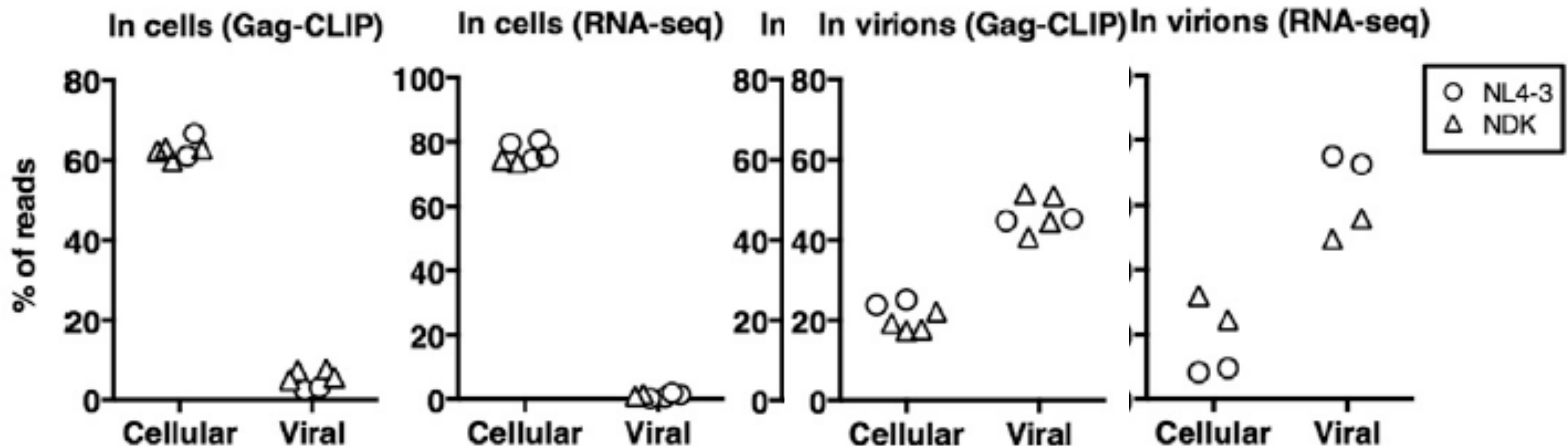
- **Immature capsid is made of full length Gag and has RNA and PM as part of its structure;**
- **Mature capsid is made of CA only, has different 2D crystalline arrangement, different set of CA-CA contacts, and is RNA and PM independent.**

Problem of selective gRNA packaging

- HIV-1 Gag bind to packaging (Psi) RNA signal of gRNA about as strongly as to any random RNA in physiological salt.
- In the absence of gRNA virions assemble on any RNA (but at higher [Gag]).
- There is a huge excess of non-gRNA in the cytoplasm.
- There seems to be a critical [Gag*] in cytoplasm below which assembly does not happen, even though Gag is present both in the RNA and on cytoplasm in comparable amounts.
- Unclear role of gRNA dimerization in its selective packaging: gRNA dimers are packaged preferentially, but in vitro NC and Gag binding to dimeric vs monomeric Psi-RNA are not very different.

Selective gRNA packaging happens at the step of assembly nucleation

Global changes in the RNA binding specificity of HIV-1 Gag regulate virion genesis.
Kutlay&Bieniasz, Cell, 2014

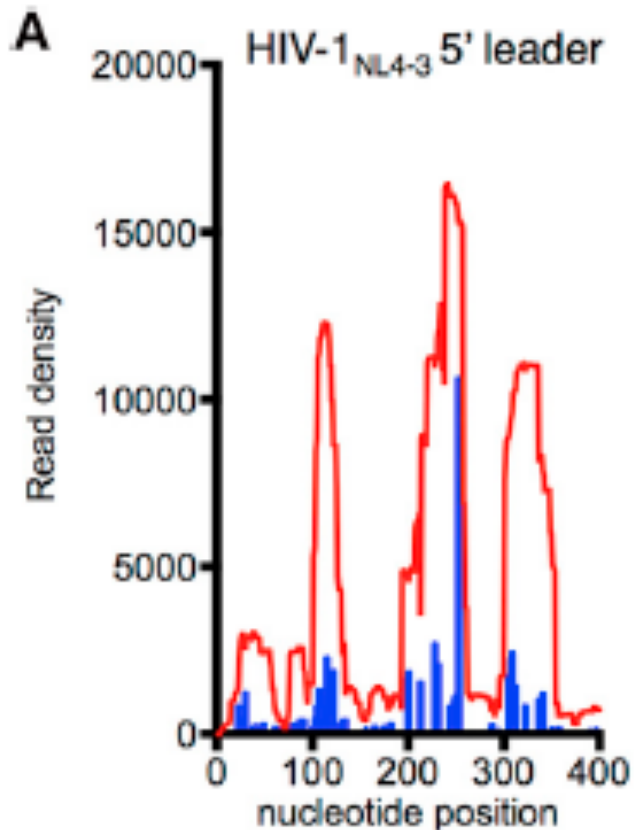


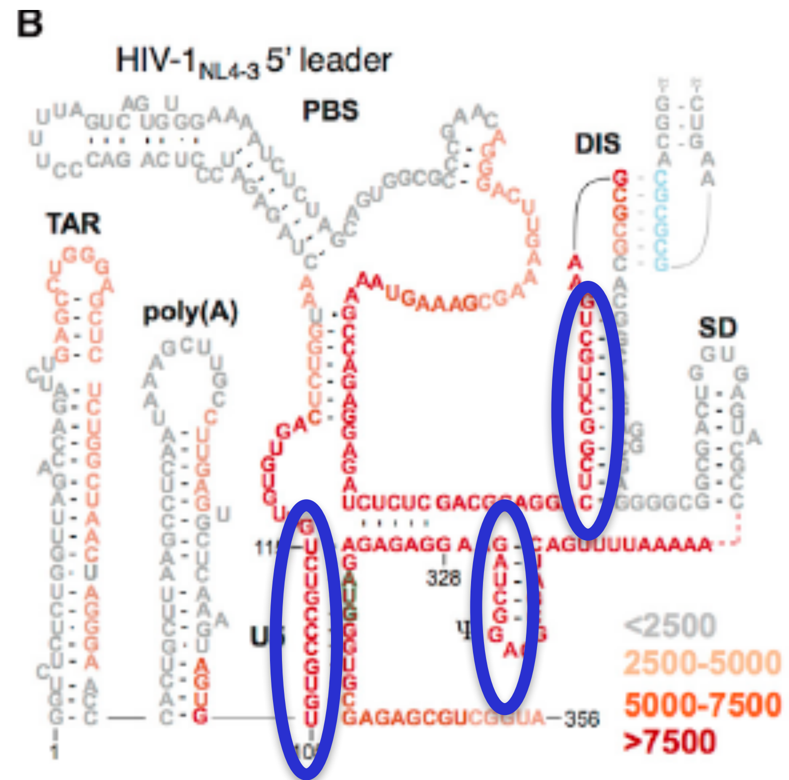
<u>Selective gRNA packaging in virions happens in two steps:</u>

(i) Selective (<~10-fold) Gag-gRNA binding in cytoplasm;
(ii) selective incorporation of Gag-gRNA into virions on PM (~100-fold)

Gag binds Psi-gRNA region at three specific sites

Kutlay&Bieniasz, Cell, 2014

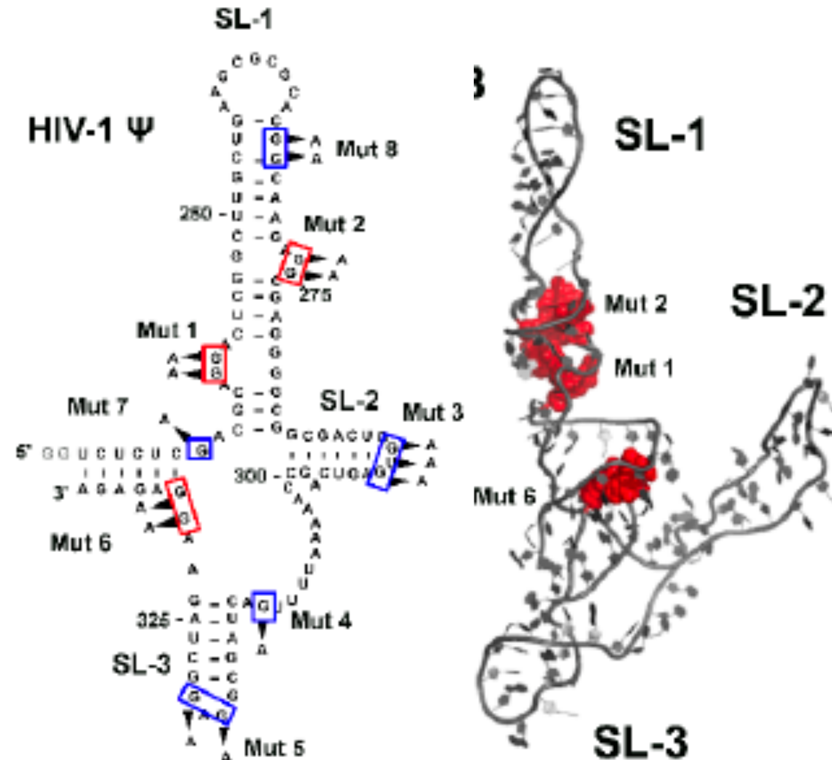




Three specific sites for Gag in Psi RNA are nearly identical to in vitro observed NC sites (Summers), and Gag sites (Marquet)

100 nt Psi RNA has three strong adjacent binding sites for NC

Specific Gag binding sites on 100 nt HIV-1 Psi RNA
(Erik Olson et.al. Viruses, 2016)



Preliminary mass spec results show one Psi RNA being bound with 3 Gag molecules.

- Dimer of Psi RNA will have six (or four) strong adjacent NC binding sites
- Dimer of Psi RNA does not bind Gag stronger then the monomer (weak Gag-Gag contacts)

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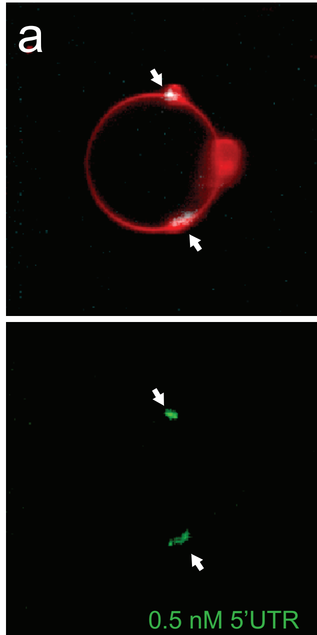
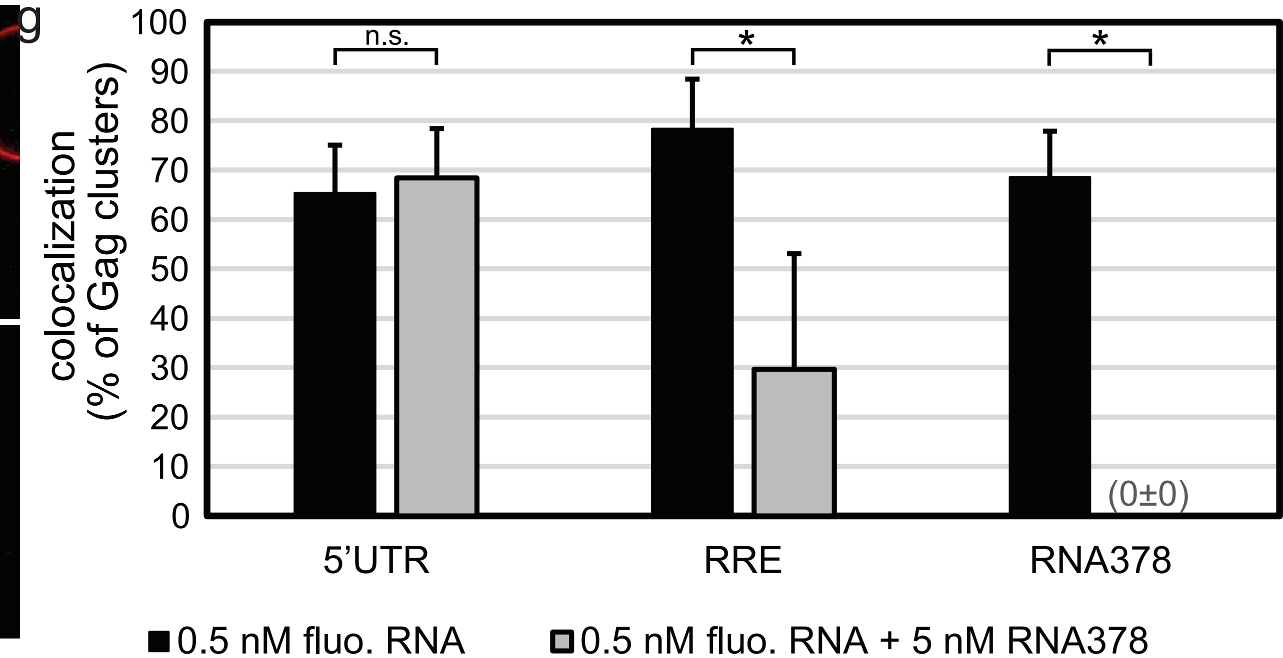
Selective g… vitro in th…





Membrane – red
Gag – **white (@ 100nM)**
gRNA – green

Membrane with PM composition

Three ~370nt RNAs: 5'UTR – contains Psi;
RRE – slightly specific;
RNA378 – non-specific

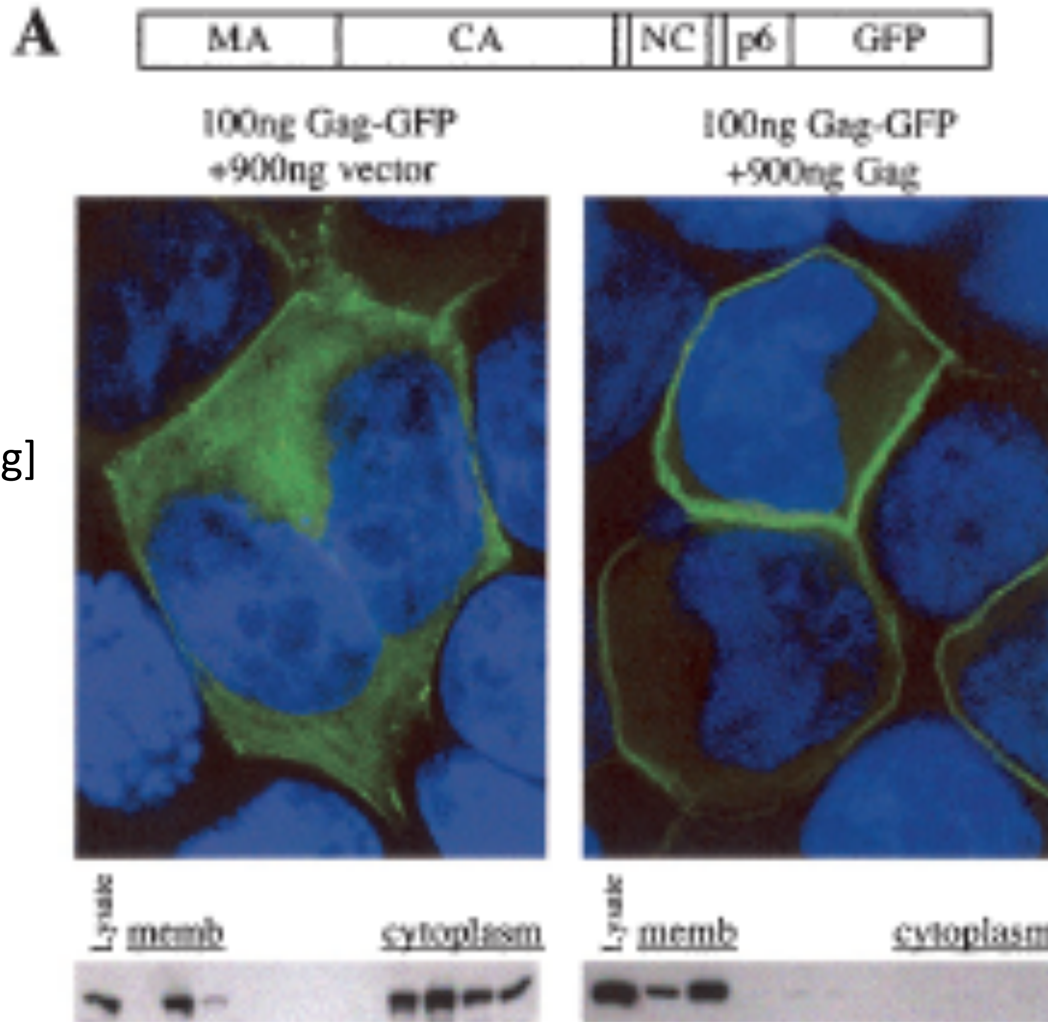
So, there is a hope to understand the selective gRNA packaging in physics terms

Individual Gag interactions with RNA and PM are of comparable strength

Low [Gag] lead to Gag monomer or small oligomers equally distributes between cytoplasm and PM

High [Gag] lead to Gag multimerization on PM

Low [Gag]

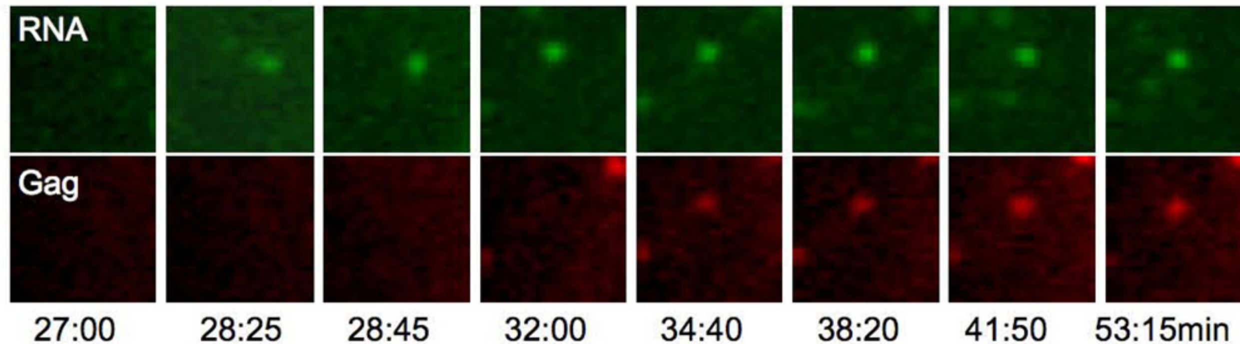


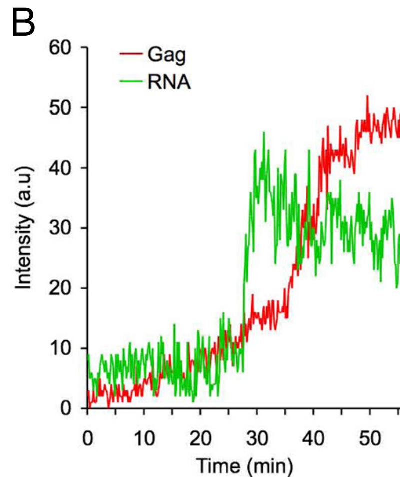
High [Gag]

HIV-1 MA inhibits and confers cooperativity on Gag/PM interactions. Bieniasz et.al. 2004

gRNA is picked in the cytoplasm by a few Gag molecules and brought to PM. Assembly on PM after nucleation takes ~10 min.

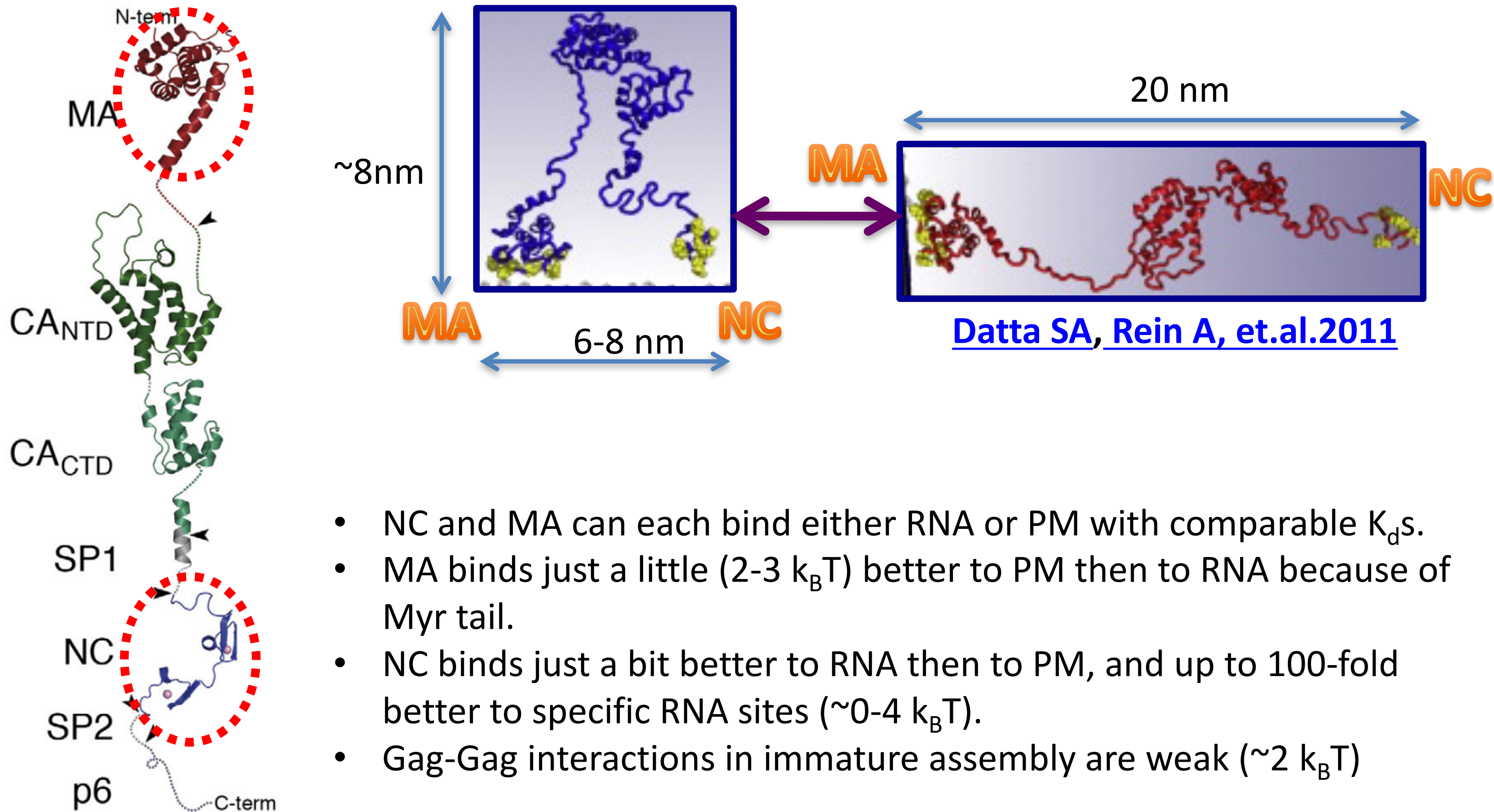
Jouvenet et al, *PNAS*, (2009).





- Cytoplasmic Gag/gRNA binding at low [Gag] & [gRNA] (<1 uM)
- No cytoplasmic Gag assembly or multimerization @ these low [Gag];
- No Gag assembly on PM prior to gRNA/Gag complex arrival;
- Poor Gag-RNA assembly on PM prior to gRNA dimerization that happens on PM
- Slow (~10 min) Gag multimerization on PM.
- Gag comes into assembly from cytoplasm, not from PM

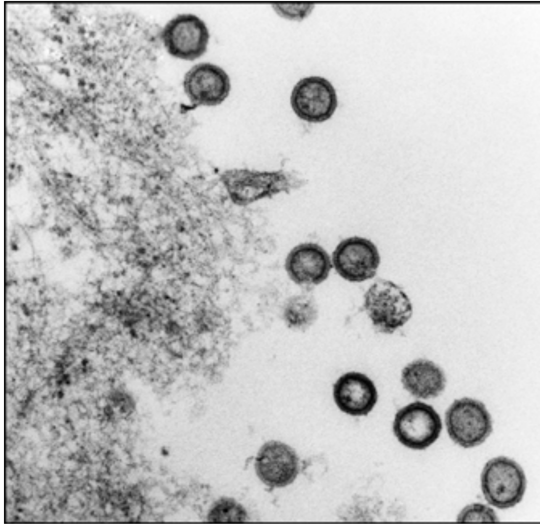
HIV-1 Gag is highly flexible and has two cationic domains.



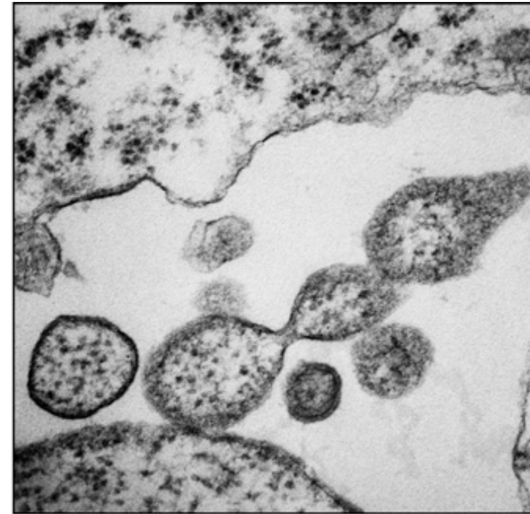
- NC and MA can each bind either RNA or PM with comparable K_ds.
- MA binds just a little (2-3 k_BT) better to PM then to RNA because of Myr tail.
- NC binds just a bit better to RNA then to PM, and up to 100-fold better to specific RNA sites (~0-4 k_BT).
- Gag-Gag interactions in immature assembly are weak (~2 k_BT)

Gag-Gag interactions in immature assembly are very weak (~2 $k_B T$)

WT Gag

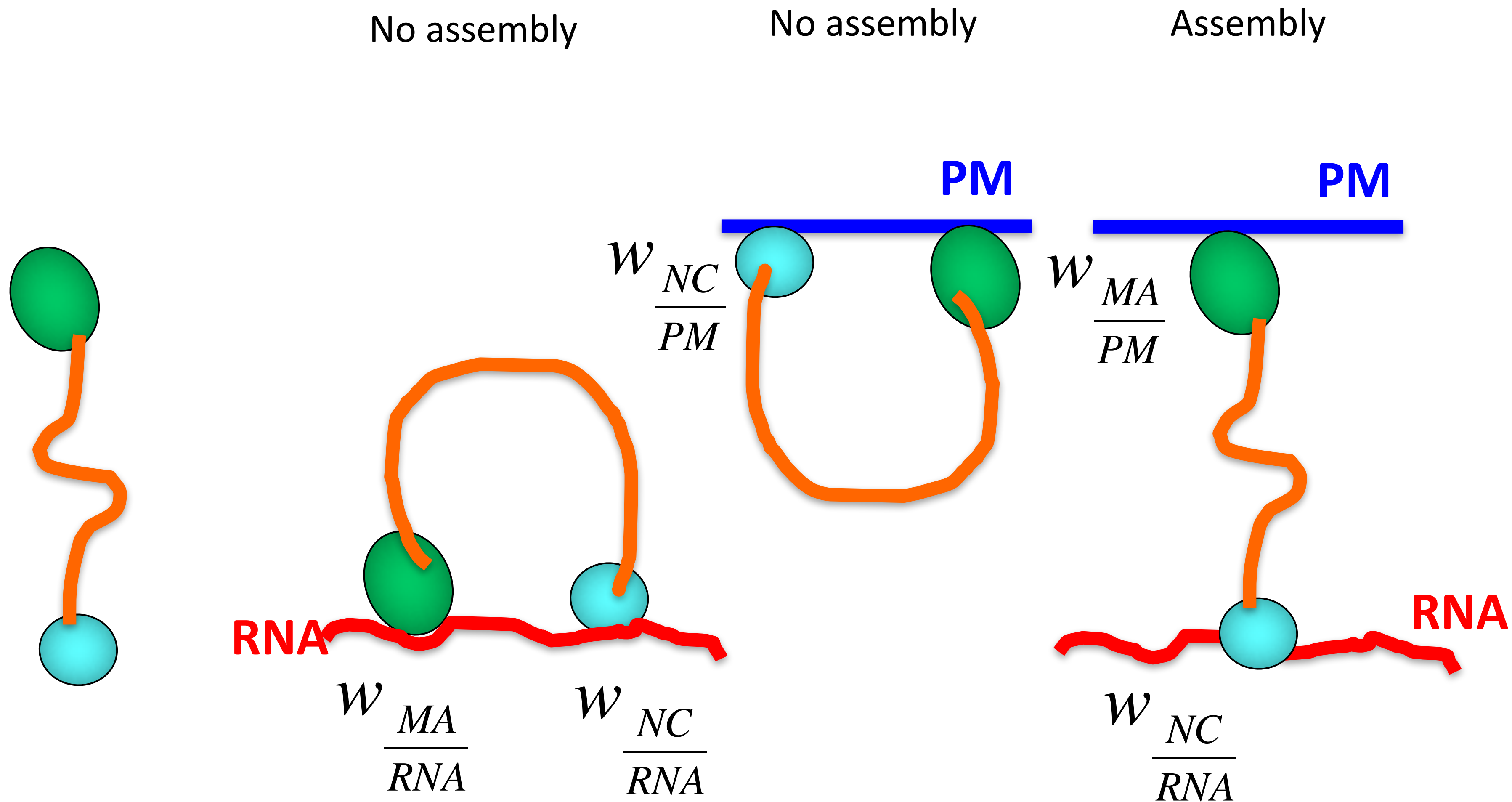


WM Gag dimerization site mutant



- Gag with all interaction sites mutated still assemble into imperfect macroscopic structures containing PM, Gag and RNA.
- Binding of WM Gag to RNAs is just 2-3 fold weaker then of WT Gag. $k_B T * Ln(3) \sim 1\ k_B T$.

Simplest model of three Gag binding states

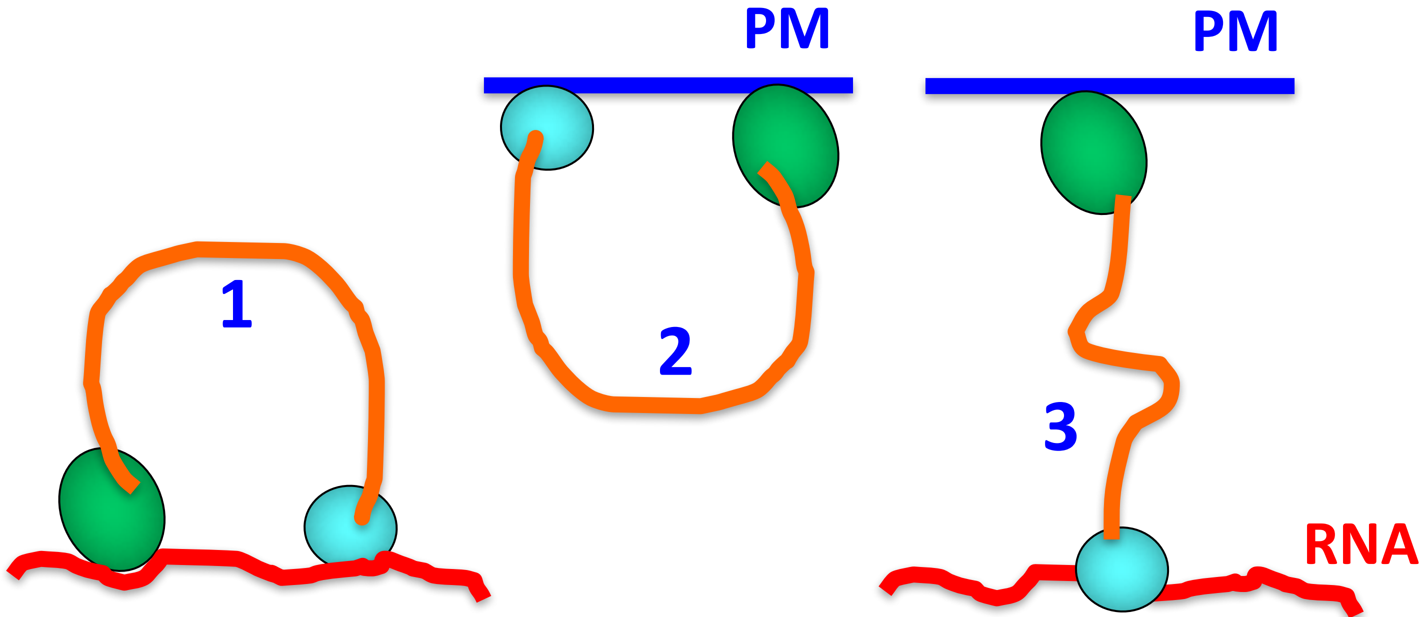


Conditions:

[RNA sites] &[PM sites]>>[Gag]; binding of Gag to RNA and PM is strong; all Gag is bound.

Free energy of states of flexible Gag

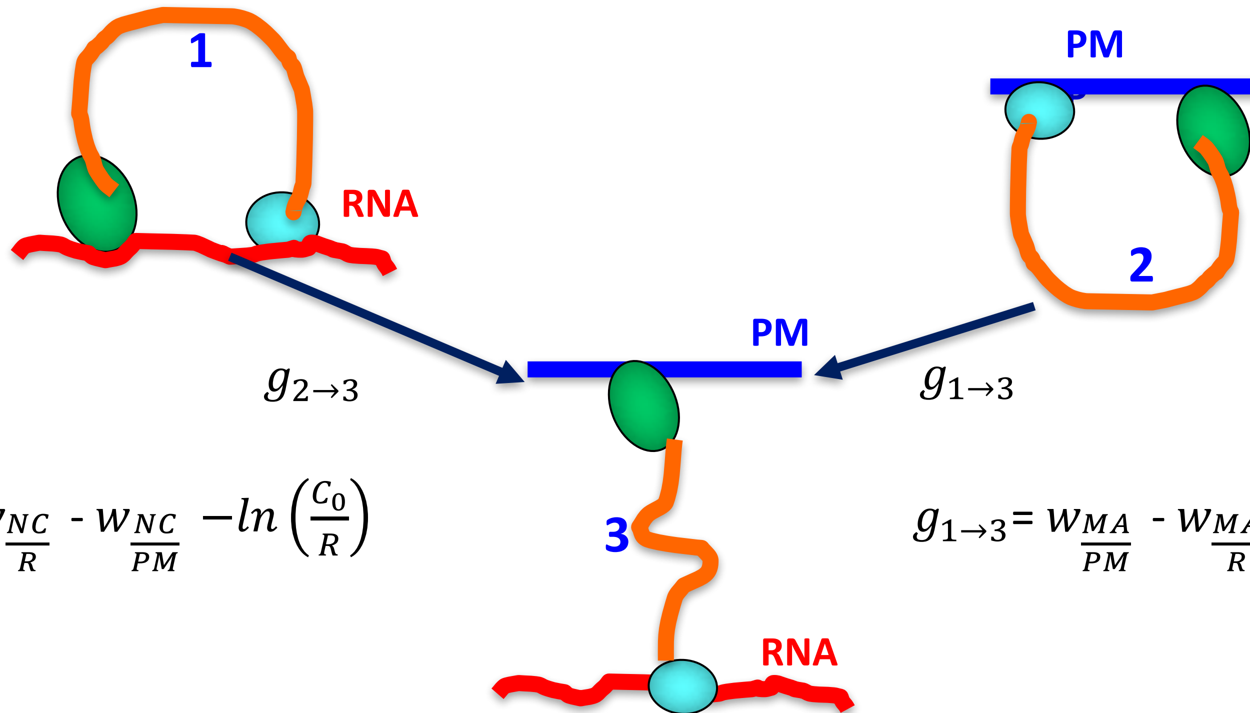
$$g_2 = w_{\frac{NC}{PM}} + w_{\frac{MA}{PM}} - ln\left(\frac{C_0}{Gag}\right)$$



$$g_1 = w_{\frac{NC}{R}} + w_{\frac{MA}{P}} - ln\left(\frac{C_0}{R}\right)$$

$$g_3 = w_{\frac{NC}{PM}} + w_{\frac{MA}{PM}} - ln\left(\frac{C_0}{Gag}\right) - ln\left(\frac{C_0}{R}\right)$$

Free energies of state transitions



$$g_{2\rightarrow3} = w_{\frac{NC}{R}} - w_{\frac{NC}{PM}} - ln\left(\frac{C_0}{R}\right)$$

$$g_{1\rightarrow3} = w_{\frac{MA}{PM}} - w_{\frac{MA}{R}} - ln\left(\frac{C_0}{Gag}\right)$$

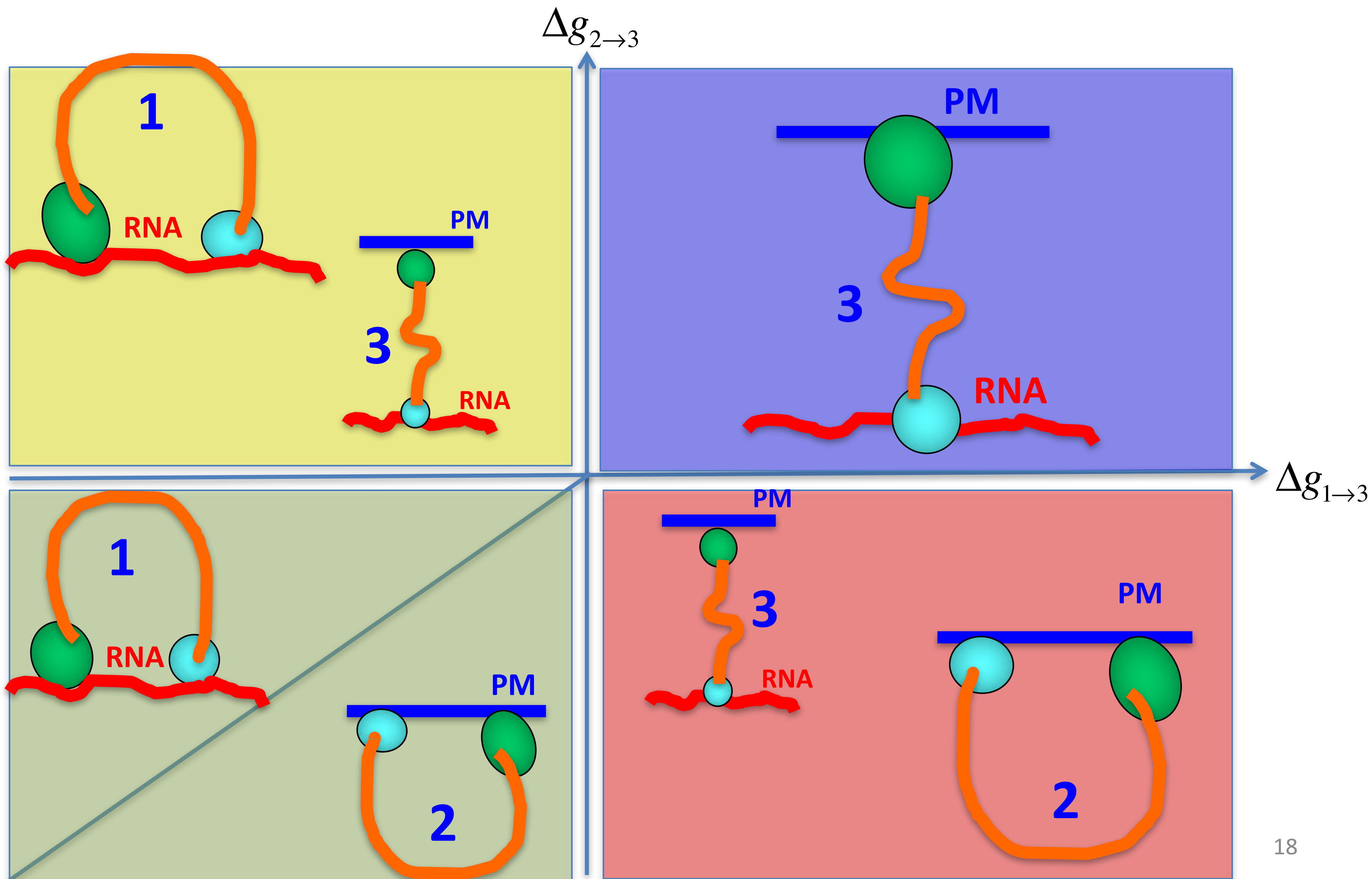
R=[RNA]=100μM ; [Gag]=100 nM; C_0=10^{-2}M;

$w_{\frac{NC}{R}} - w_{\frac{NC}{PM}} \sim 0 - 2kBT$

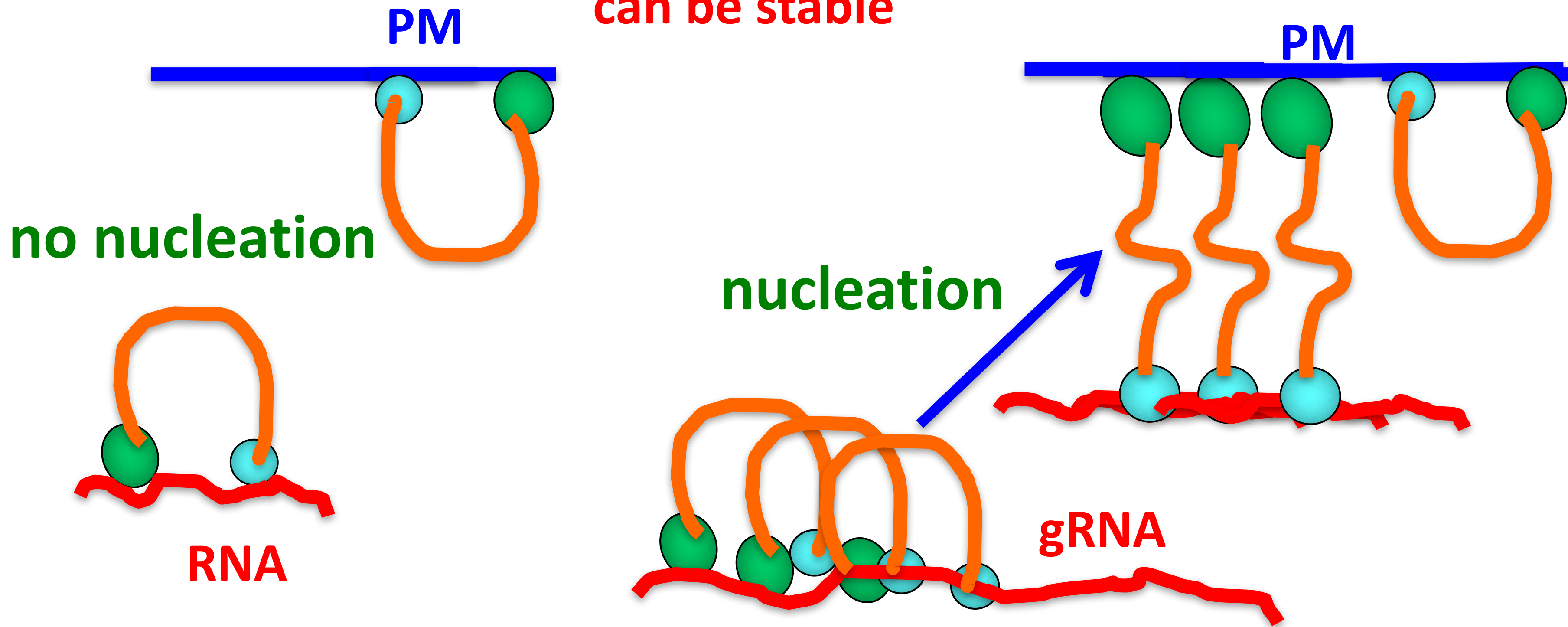
$ln\left(\frac{C_0}{R}\right) \sim 5$kBT ; $ln\left(\frac{C_0}{Gag}\right) \sim 11k_BT$

$w_{\frac{MA}{PM}} - w_{\frac{MA}{R}} \sim 2 - 3kBT$

Phase diagram of single Gag states



One extended Gag is unstable, but few brought by the same RNA can be stable



Single Gag extended between RNA and PM is unstable at low [Gag]:

$$\Delta g_{\frac{MA}{R \to PM}} = w_{\frac{MA}{PM}} - w_{\frac{MA}{R}} - \ln\left(\frac{C_0}{Gag}\right) < 0$$

~2k$_B$T ~10k$_B$T

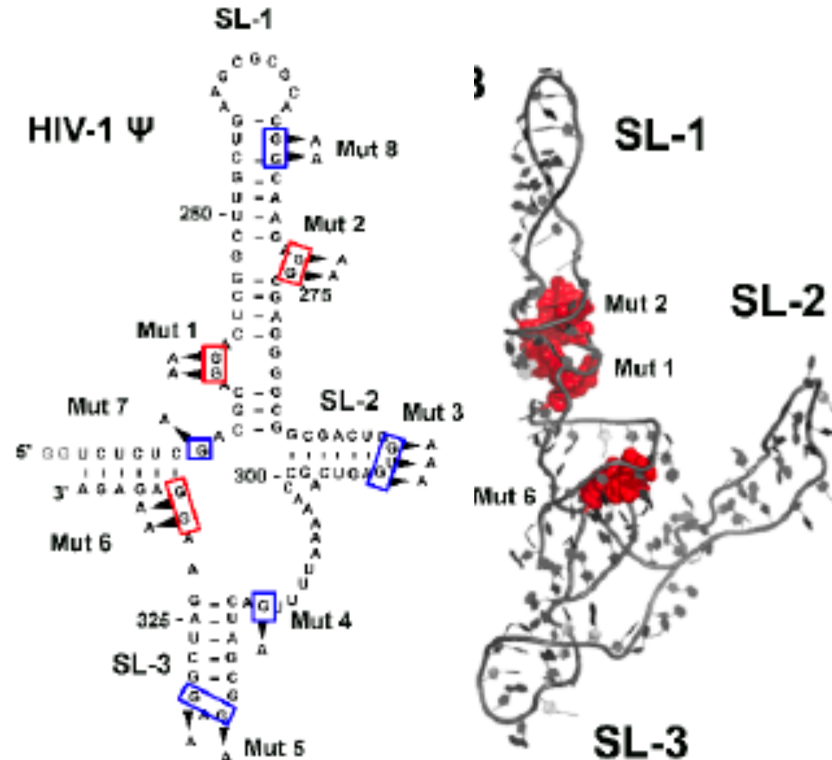
But several Gags attached to RNA - PM together (nucleus) can be stable

$$\Delta g_{nucl,n} = w_{\frac{MA}{PM}} - w_{\frac{MA}{R}} + w_{CA-CA} \cdot \left(1 - 1/n^{1/2}\right) - \frac{1}{n} \cdot \ln\left(\frac{C_0}{Gag}\right) > 0$$

~2k$_B$T ~1-2k$_B$T ~10/n k$_B$T

100 nt Psi RNA has three strong adjacent binding sites for NC

Specific Gag binding sites on 100 nt HIV-1 Psi RNA
(Erik Olson et.al. Viruses, 2016)



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- Dimer of Psi RNA will have six (or four) strong adjacent NC binding sites
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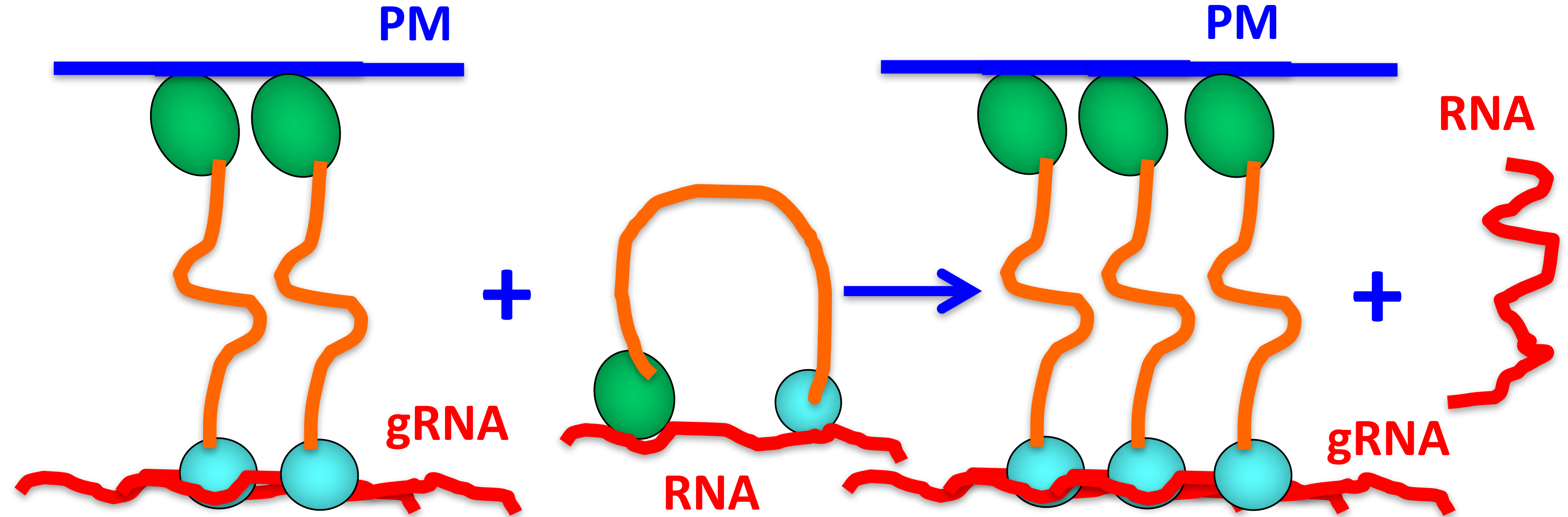
Few extended Gags form stable nucleus that grows via accumulation of Gag from cytoplasm that is strongly driven by release of RNA from it.



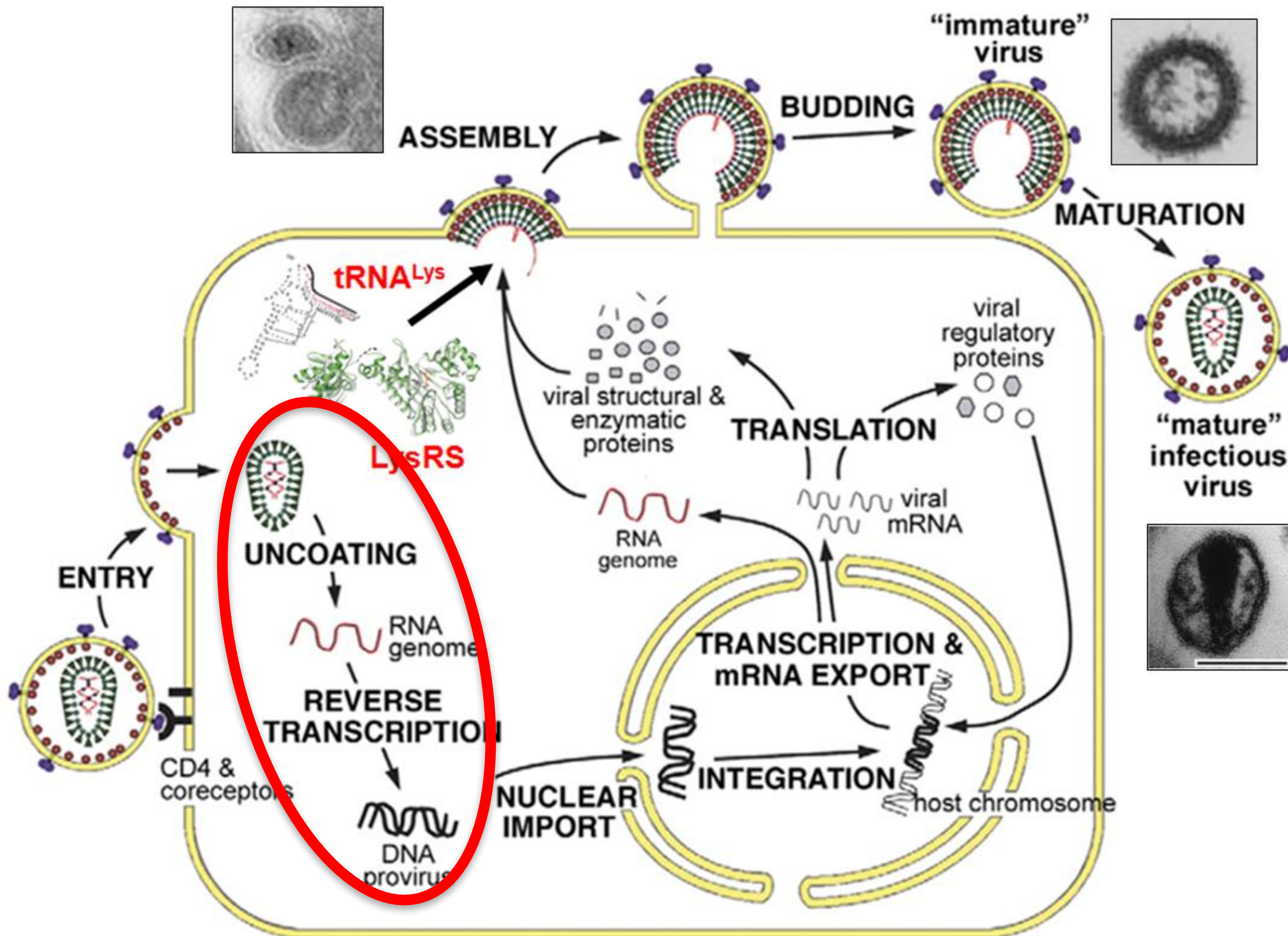
$$\Delta g_{growth} = \overbrace{w_{\frac{MA}{PM}} - w_{\frac{MA}{R}}}^{+3k_BT} + \overbrace{w_{CA-CA}}^{+2k_BT} - \overbrace{\ln\left(\frac{C_0}{Gag}\right)}^{-10k_BT} + \overbrace{\ln\left(\frac{C_0}{R}\right)}^{+5k_BT} \;>\!\sim 0$$

Driven assembly

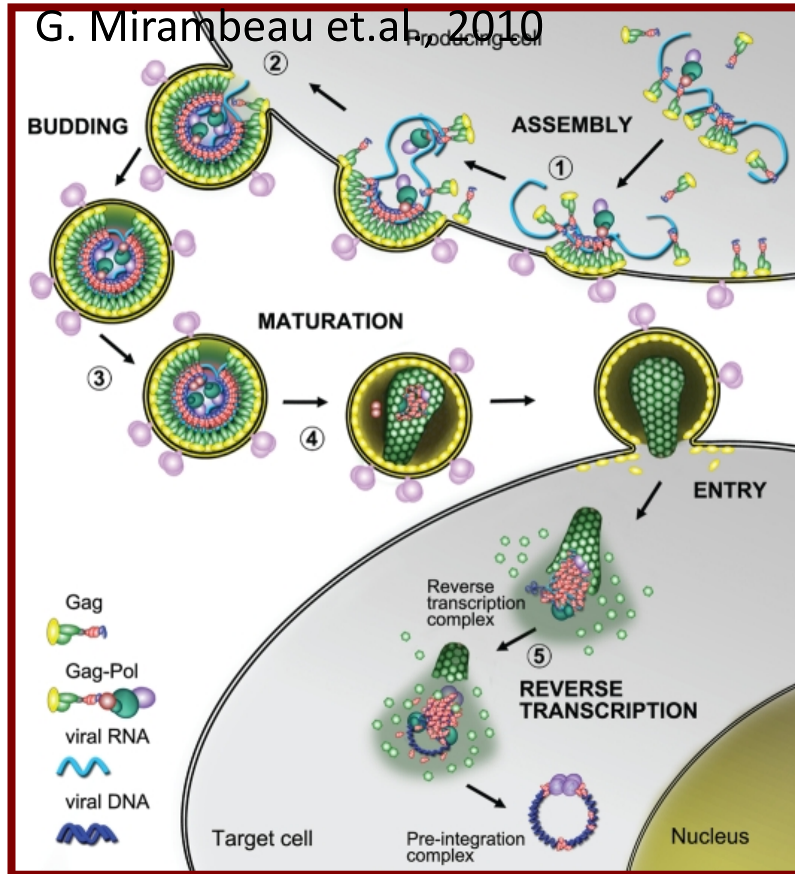
Conclusions

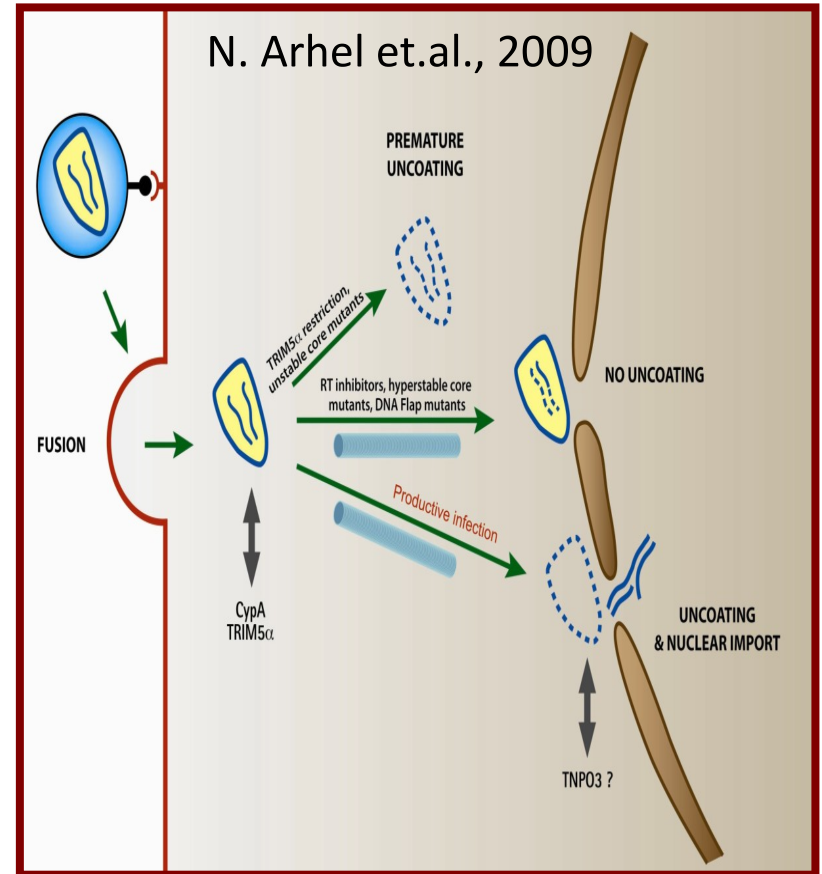
- Psi RNA signal initiates the assembly by binding several Gag molecules to nearby specific NC sites at once, thereby allowing these Gags to simultaneously attach their MA domains to PM without dissociating from Psi RNA.
- At low [Gag] the non- specific RNA cannot initiate assembly, as one extended Gag molecules is unstable, leading to assembly nucleation only on Psi RNA.
- Virion growth after assembly nucleation happens by cytoplasmic Gag joining. It is driven by cellular RNA release from those Gag (entropic assembly).
- gRNA dimerization happens at the stage of assembly nucleation, as the dimer of Psi RNA binds twice as many Gag molecules as monomer, and this higher Gag oligomer attaches stronger to PM for assembly to proceed.
- Gag-Gag interactions are weak (~2 k_BT) compared to the entropy of RNA release upon Gag joining the assembly (~10k_BT). Thus, Gag-Gag interactions contribute moderately to virion assembly and selective gRNA packaging.
- Other retroviruses, most likely, select their genomes differently, as flexibility of Gag and competitive binding of its MA and NC to RNA or PM are essential feature of HIV, but not of many other retroviruses.

How mature HIV capsid "uncoats"?



Does reverse transcription (RTion) happen before or after mature HIV capsid uncoating?





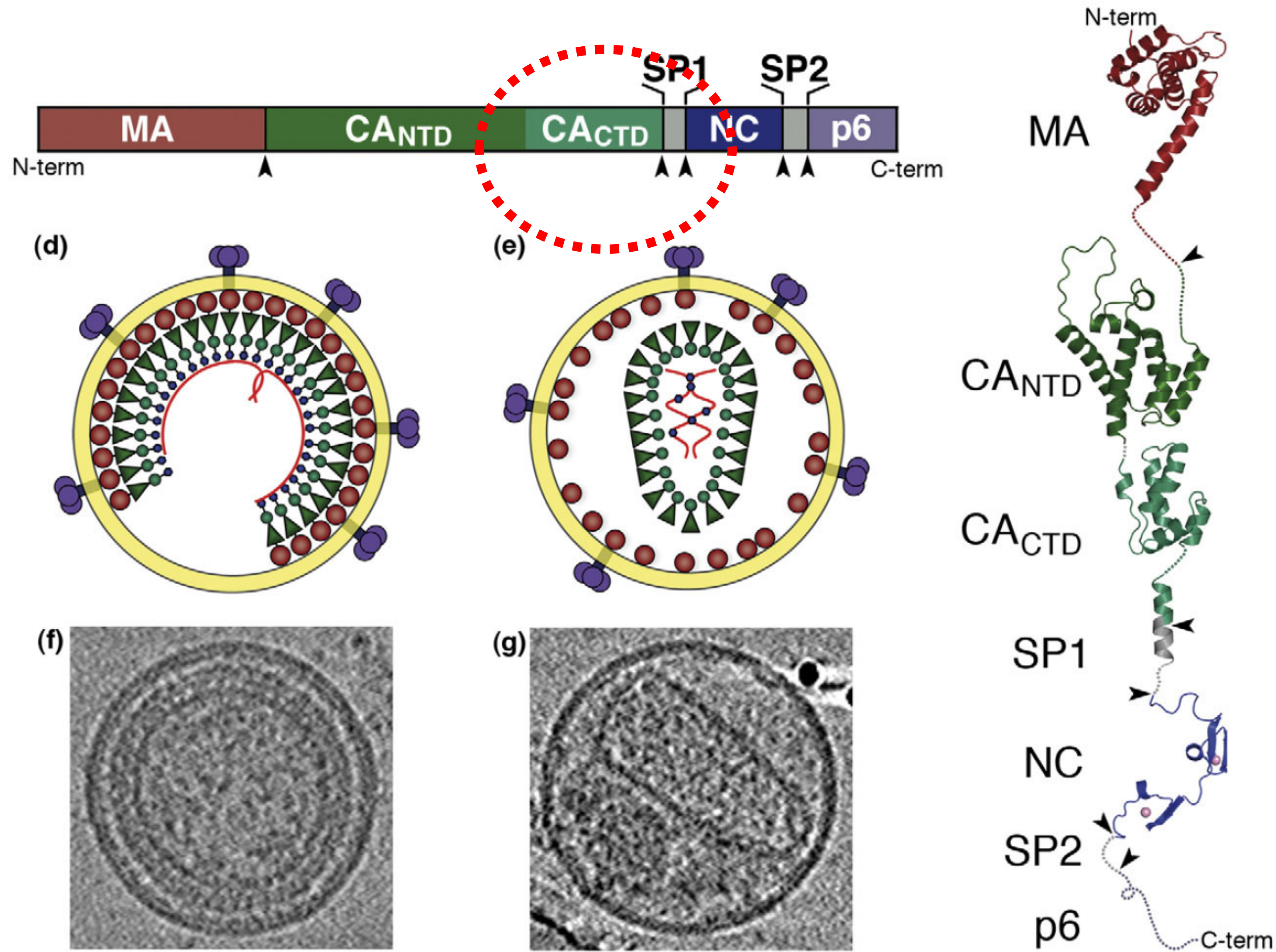
Model I

RTion happens in cytoplazm after uncoating - traditional view

Model II

RTion has to complete for uncoating. Intact capsids observed with full length v-dsDNA by the nuclear pore.

Immature and mature HIV-1 capsid

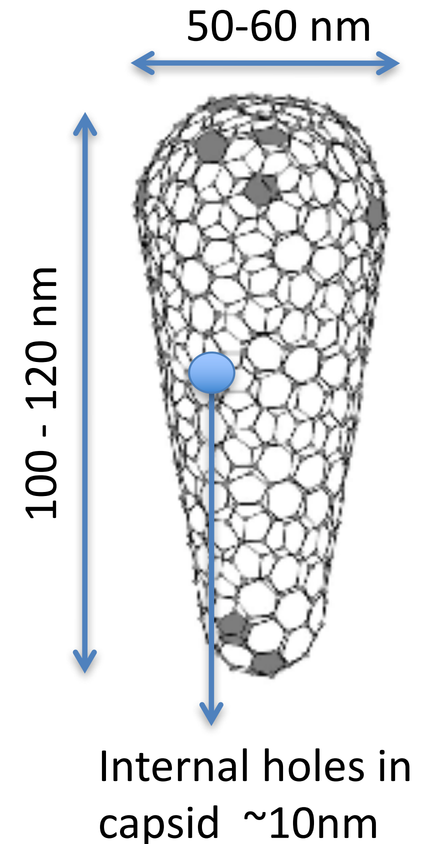


Ganser-Pornillos et al, *Curr Opin Struct Biol* **18**:203-17 (2008).

NC protein is processed from Gag and aggregates with vRNA inside mature capsid prior to RTion

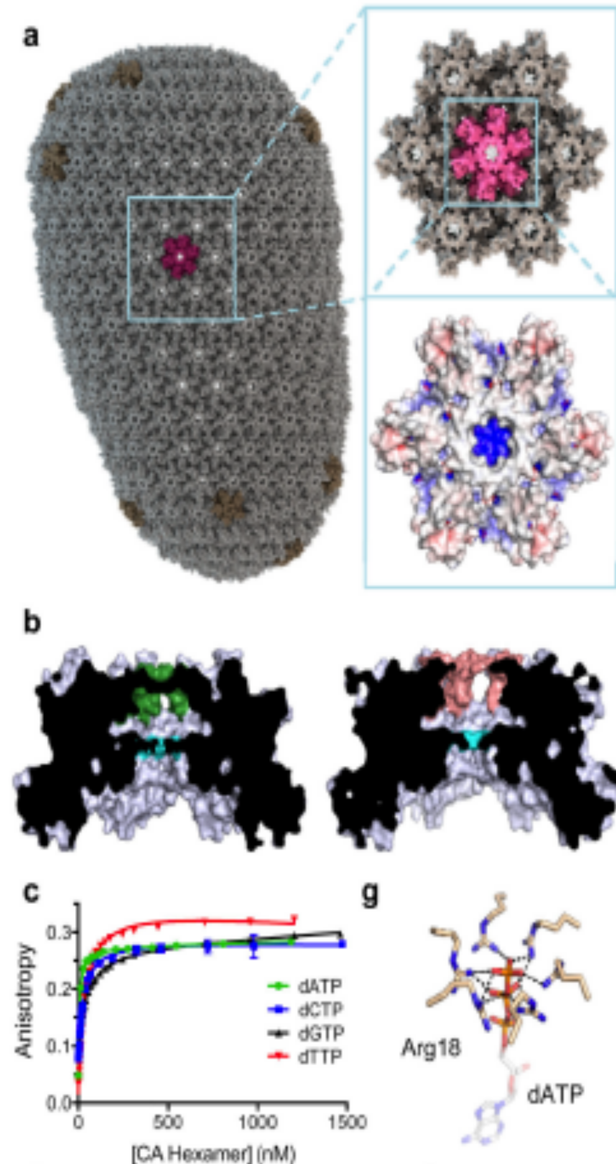
RTion inside mature capsid is possible

•~8 nm holes in capsid make it transparent to dNTPs and RT inhibitors, but not to larger molecules;
•Endogenous RTion happens in mature virions;
•RTion up to full-length vDNA detected in mature capsids;
•No host cell factors are needed for RTion or uncoating;
•Higher or lower capsid stability lead to RTion defect.



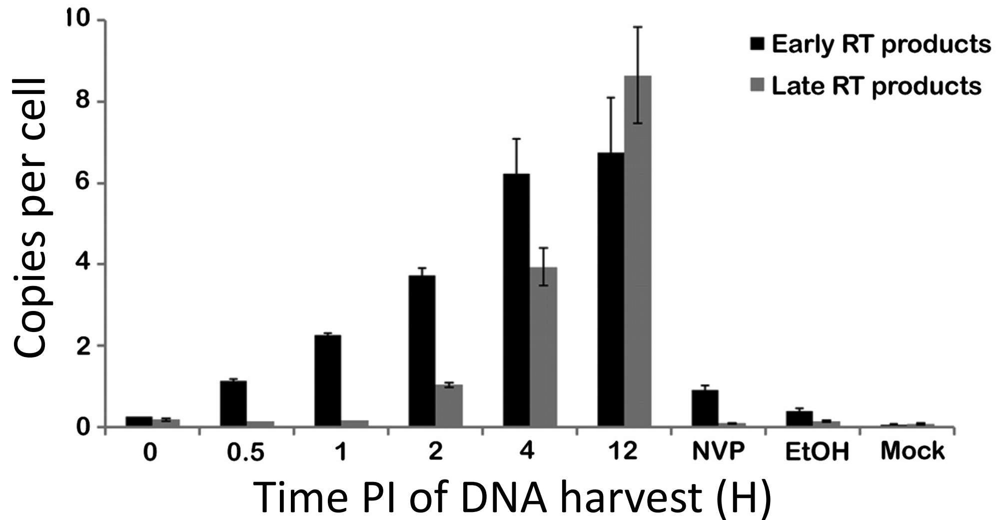
HIV capsid has dynamic pores that import nucleotides for RTion

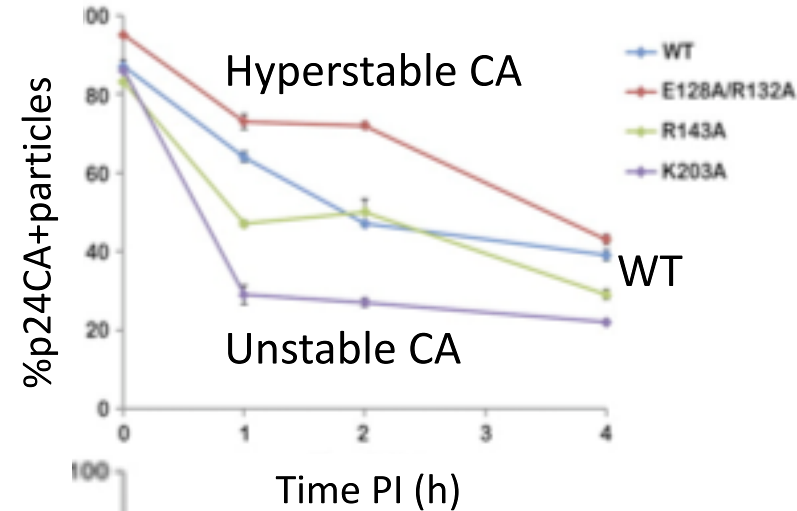
- HIV mature capsid has pores at the hexamer center that are surrounded by six Arg and strongly bind nts;
- Size of the pore is regulated by pH. The pore is open at pH<7 and closed at pH>7.
- Movement of the Beta turn of NTD CA regulates pore opening and closing;
- Kinetics of nts on and off is very fast, i.e. close to diffusion limit;
- These pores are strongly conserved in most retroviruses;
- Mutation of Arg lead to slowed on kinetics, poor RTion and infectivity, but increases the capsid stability;
- This pore regulates the in capsid RTion rate .

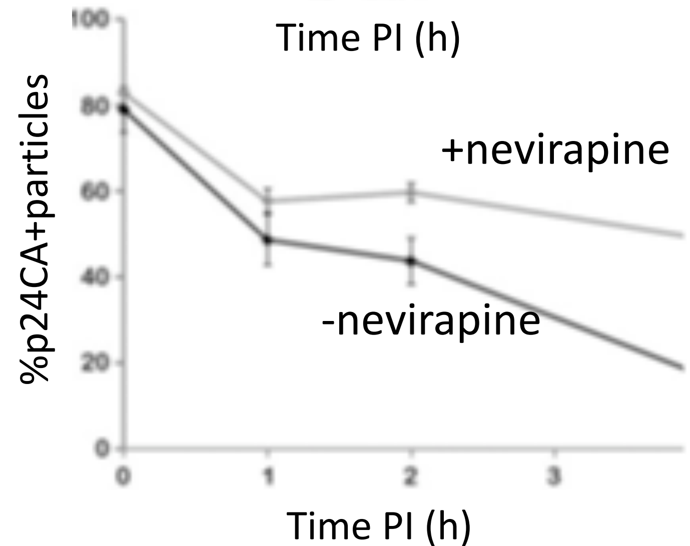


RTion and capsid uncoating are inter-dependent

Hulme, Perez and Hope. PNAS, 2011

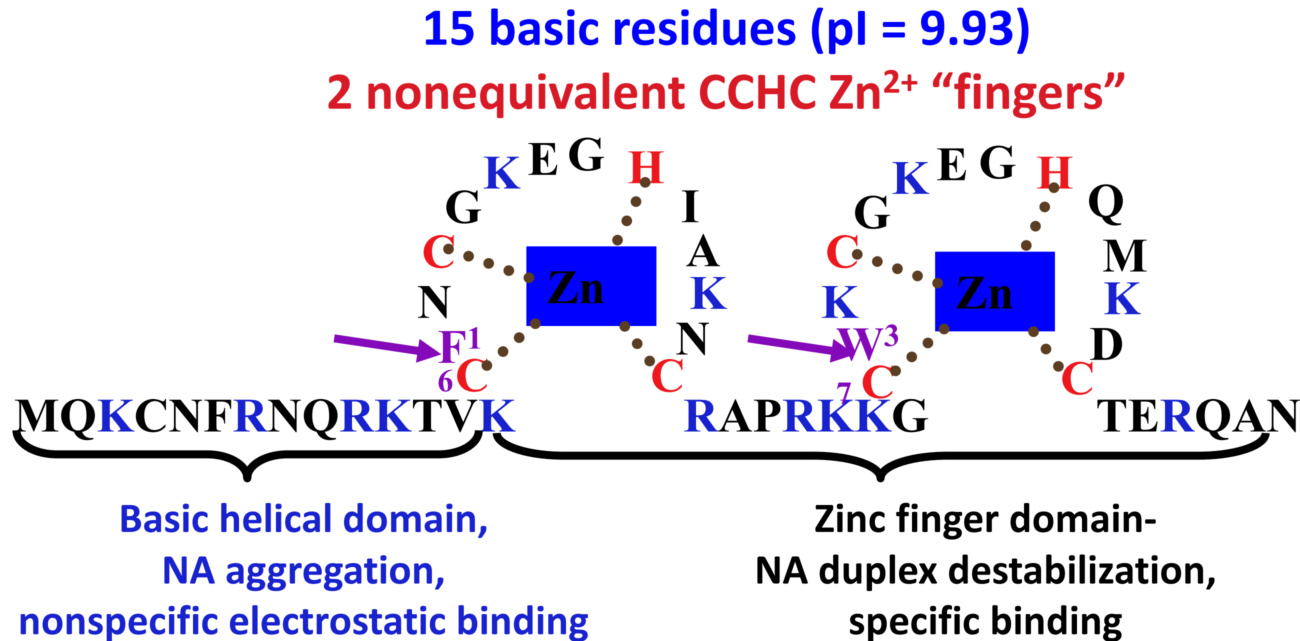






- Faster uncoating of *in vitro* less stable CA mutants;
- NVP stalls both RTion and uncoating
- Time of late RT products formation correlates with uncoating

Could NC control mature HIV capsid uncoating?



NC binds NA as a mobile cation with effective charge ~+3.5

NC concentration inside mature HIV capsid is ~10 mM

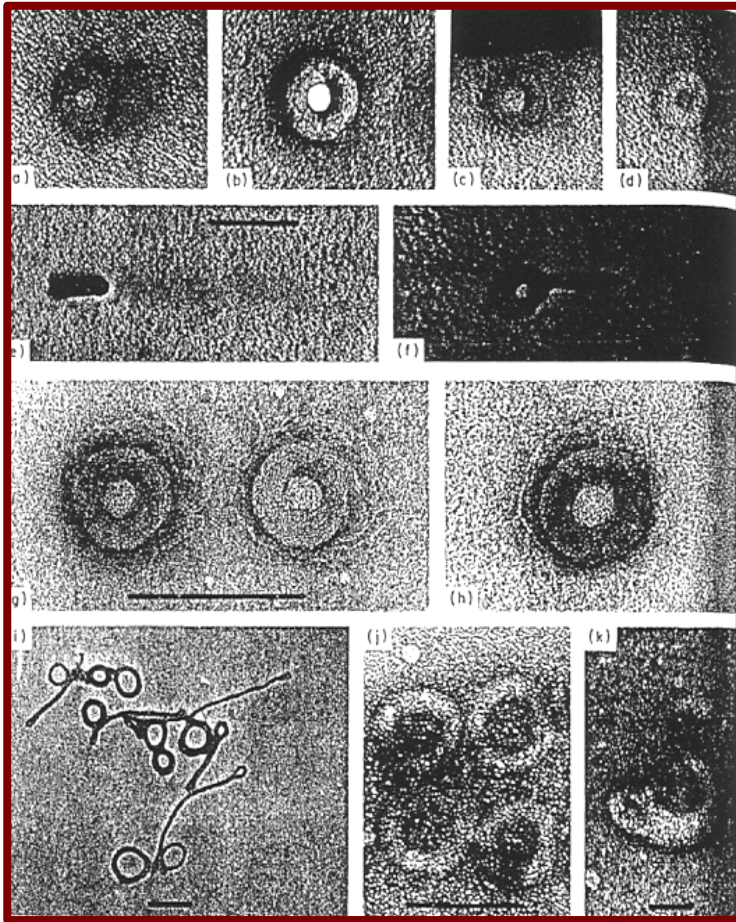
How can RTion regulate uncoating?

What is the state of vRNA and vDNA during RTion?

How can mutations in NC affect state of NA during RTion and capsid uncoating?

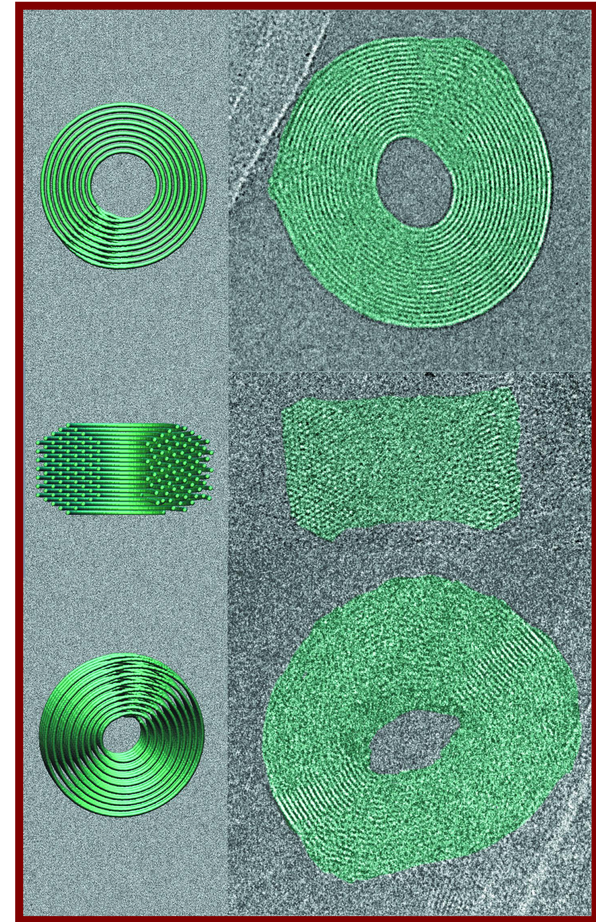
Multivalent cations with charge ≥3 condense polymeric dsDNA into tightly wound toroids

Chattoraj, Gosule, Schellman 1970



T7 phage DNA + spermidine^{4+} =~100nm diameter toroid

N.Hud et.al 2005

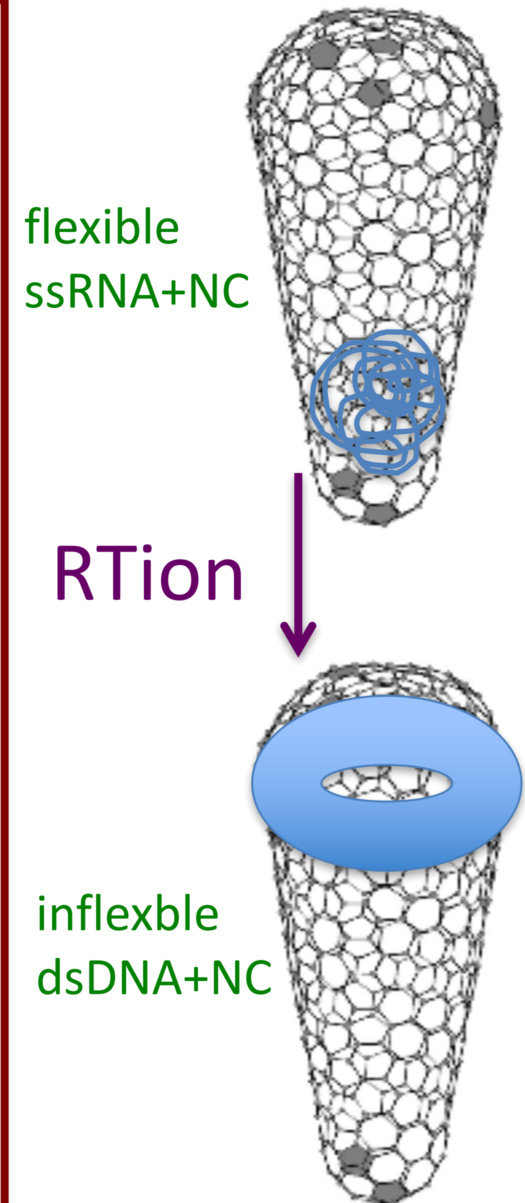


λ-phage DNA + Cobalt Hexamine^{3+}=~100nm diameter toroid

No reports of NC-induced dsDNA toroids yet

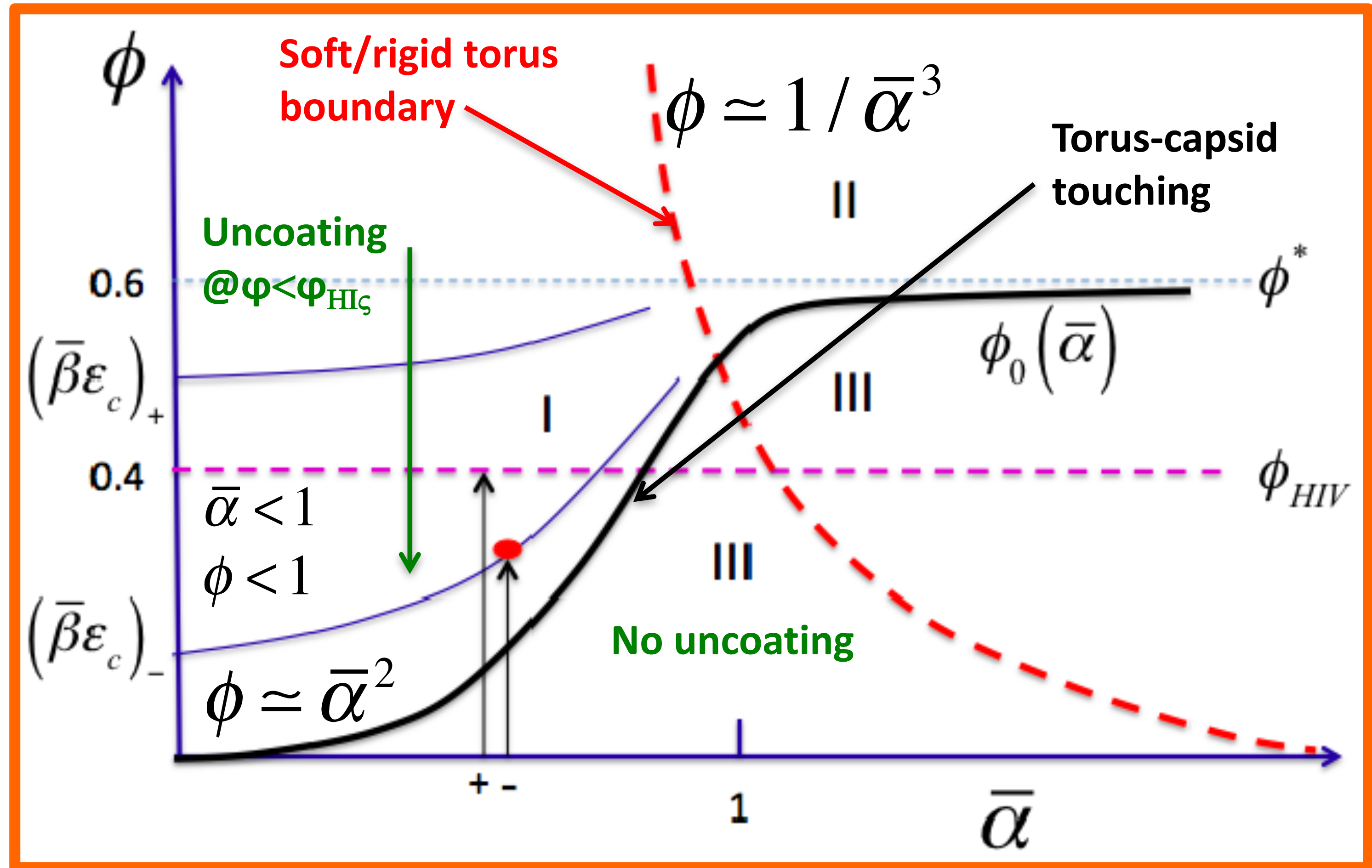
Possible scenario for NC-dependent RT-driven capsid uncoating

- Volume of mature capsid: V_{CA}=~$10^5 nm^3$;
- Self-volume of vRNA^{x}2 & v dsDNA =~$4x10^4 nm^3$ (fractional occupancy φ=40% V_{CA});
- Is it possible that such low φ of DNA will cause capsid uncoating? Yes, but only for low stability capsid with weakly condensed dsDNA.
- NC-condensed dsDNA is expected to form torus with size determined by dsDNA's length, persistence length and strength of NC-induced DNA self-attraction.
- dsDNA is rigid, and torus size can be large for small DNA length.
- Size of NC-condensed dsDNA toroid growing with RTion may lead to capsid uncoating.



"Uncoating" phase diagram

Fractional volume occupancy of dsDNA inside capsid

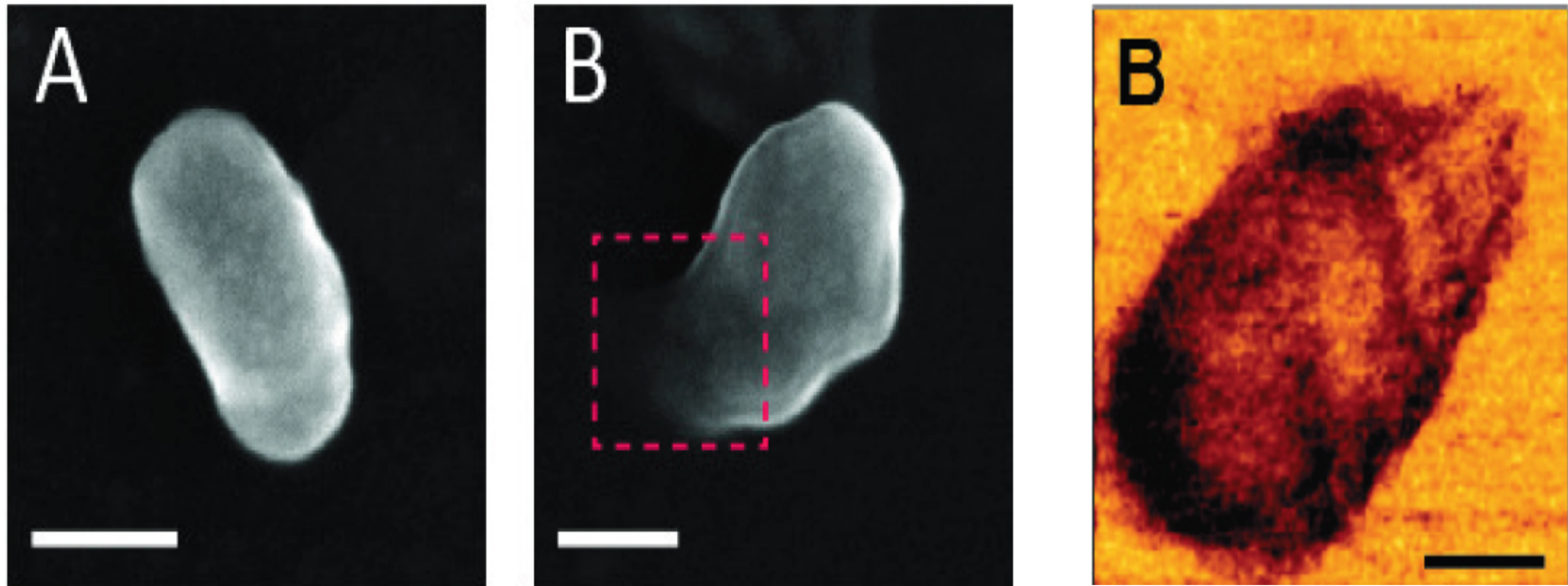


Strength of NC-induced dsDNA self-attraction

HIV capsid uncoating possible only for:

Marginally stable capsid: $\bar{\beta}\varepsilon_{cr} < \phi_{HIV}$ and weak DNA-DNA attraction: $\bar{\alpha} < \phi_{HIV}^{1/2}$

AFM imaging of RTion observes formation of rigid filament inside the mature core

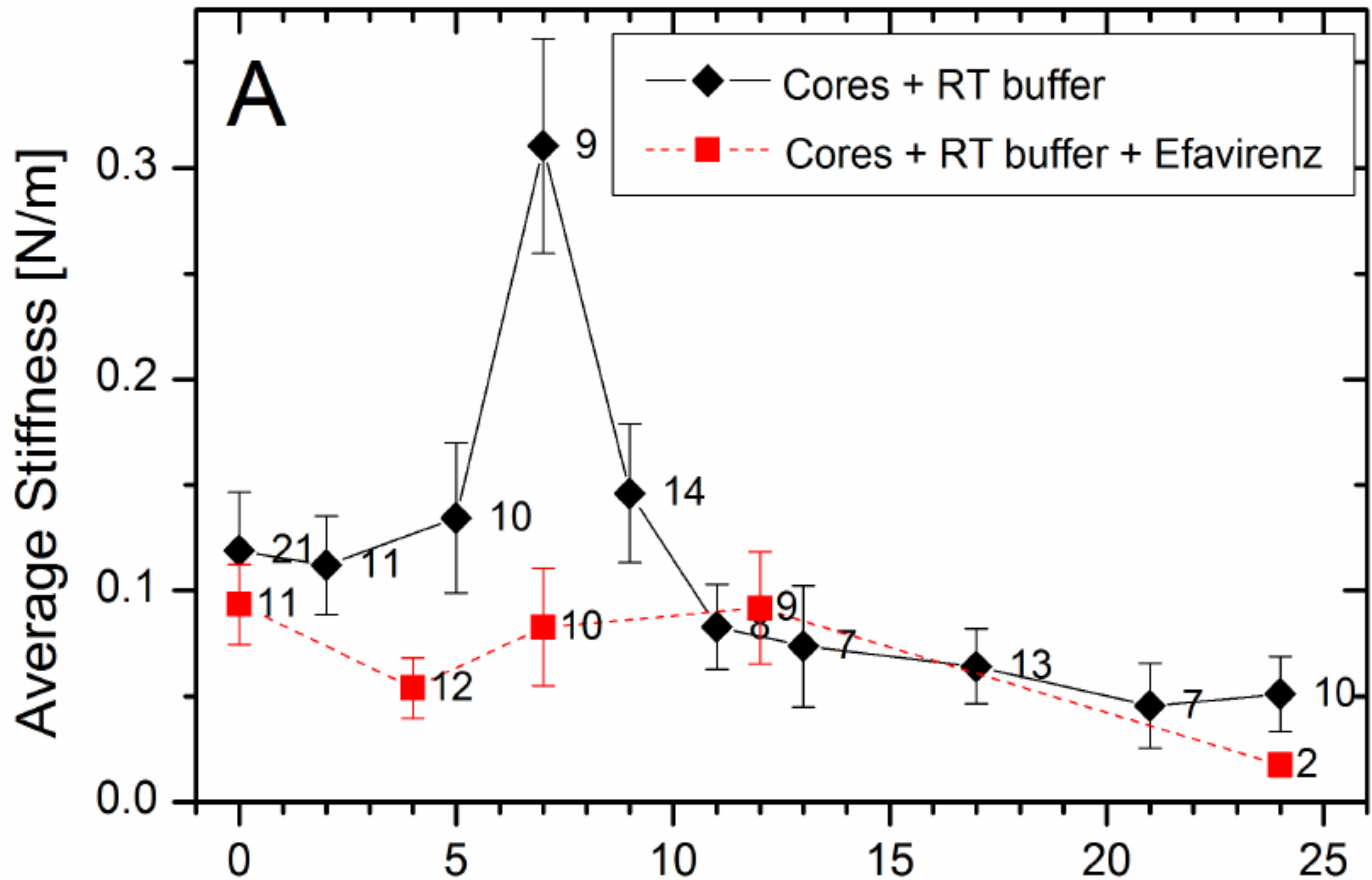


Rousso et.al. 2017

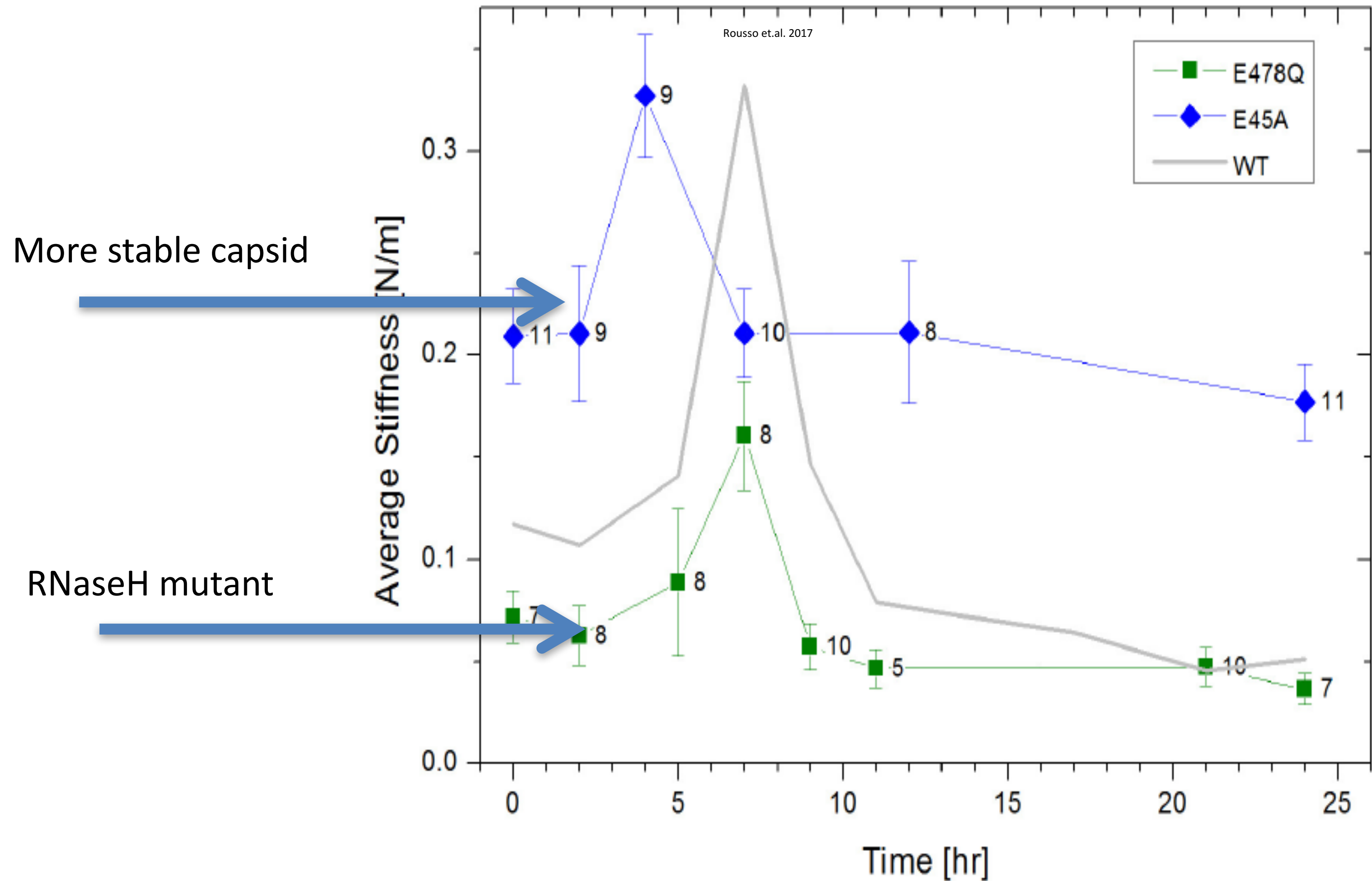
- Core pinching happens at the narrow capsid end and corresponds in time to burst in capsid rigidity.
- Capsid rigidity burst co-insides with formation of rigid filamentous coiled structure within the capsid, that disappears after the capsid burst.

Is uncoating driven by RTion?

Rousso et.al. JVI, 2017. RTion mechanically initiates HIV capsid disassembly



Stabilized CA mutant core does not break.



Conclusions

- RTion is expected to lead to condensed dsDNA+NC toroidal globule growing inside intact mature HIV capsid;
- Full length viral dsDNA would take up only ~20-40% of mature capsid volume. However, the size of NC-induced DNA torus can become larger then the capsid major radius, and can therefore push on the capsid and lead to its uncoating. This regime is only possible for weak NC-induced DNA self-attraction and weak capsid stability typical of HIV;
- We predict the uncoating DNA length (or fractional capsid volume occupancy by dsDNA) for any DNA self-attraction and capsid stability parameters. Weak capsid can be uncoated by weakly self-attracting DNA at low volume occupancy ≤1;

Conclusions - continued

- Mutations in NC causing changes in its DNA condensing ability are expected to lead to either early (weaker DNA attraction) or late (stronger DNA attraction) uncoating, both detrimental to HIV life cycle;
- Mutations in CA that make capsid more stable will take longer time and larger dsDNA length to uncoat;
- Small hole in the capsid (partial uncoating) will lead to the loss of the dsDNA-condensing NC and subsequently to complete uncoating.
- Mutations of Rnase H domain of RT precludes dsDNA synthesis and eliminates uncoating.
- Rtion rate may be modulated by solution conditions (dNTP cons, salt, pH) and presence or absence of mature core, as well as transparency of its pores to dNTPs. This may slow or facilitate Rtion, but the uncoating is expected to happen when the same length of dsDNA is synthesized.