



CENTRO DE INVESTIGACIÓN EN
**CIENCIAS DE LA SALUD
Y BIOMEDICINA**



National Institutes
of Health



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Selective Packaging of the HIV-1 genomic RNA

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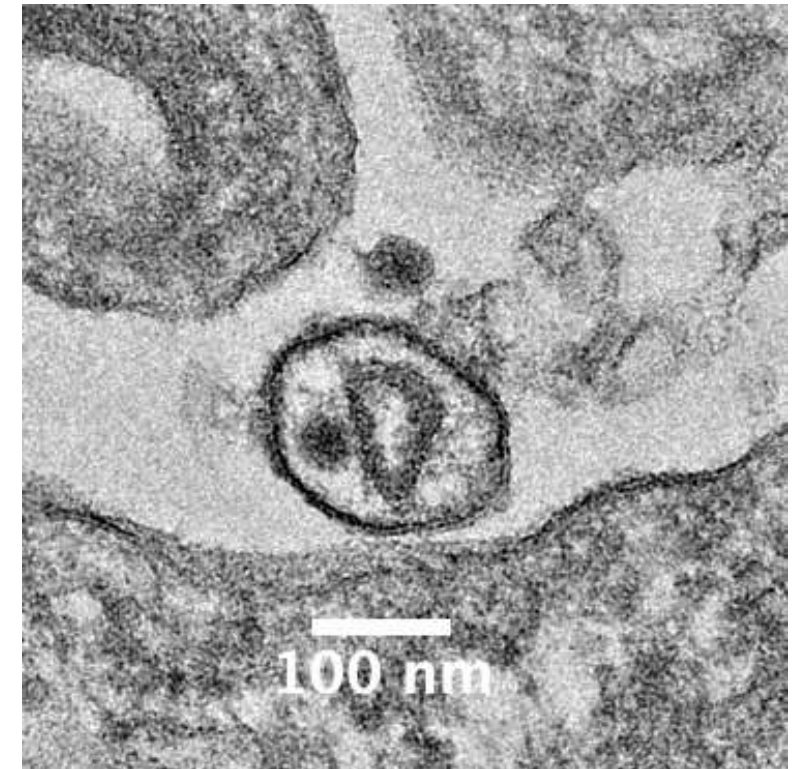
- HIV-1 is the etiological agent of AIDS.
- Since the beginning of this AIDS pandemics between 64 and 113 millions of people have been infected.
- Between 33.6 and 48.6 millions have died.
- In 2021, 38.4 million people KNEW they had HIV-1.
- In 2021 650,000 people dies from HIV-related complications.
- There are regions in Africa were 1 in 25 adults have HIV-1; this population represents 2/3 of the world-wide infected population.

Summary of the global HIV epidemic, 2021

	People living with HIV in 2021	People acquiring HIV in 2021	People dying from HIV-related causes in 2021
Total	38.4 million [33.9–43.8 million]	1.5 million [1.1–2.0 million]	650 000 [510 000–860 000]
Adults (15+ years)	36.7 million [32.3–41.9 million]	1.3 million [990 000–1.8 million]	560 000 [430 000–740 000]
Women (15+ years)	19.7 million [17.6–22.4 million]	640 000 [480 000–870 000]	240 000 [180 000–320 000]
Men (15+ years)	16.9 million [14.6–19.7 million]	680 000 [500 000–920 000]	320 000 [250 000–430 000]
Children (<15 years)	1.7 million [1.3–2.1 million]	160 000 [110 000–230 000]	98 000 [67 000–140 000]

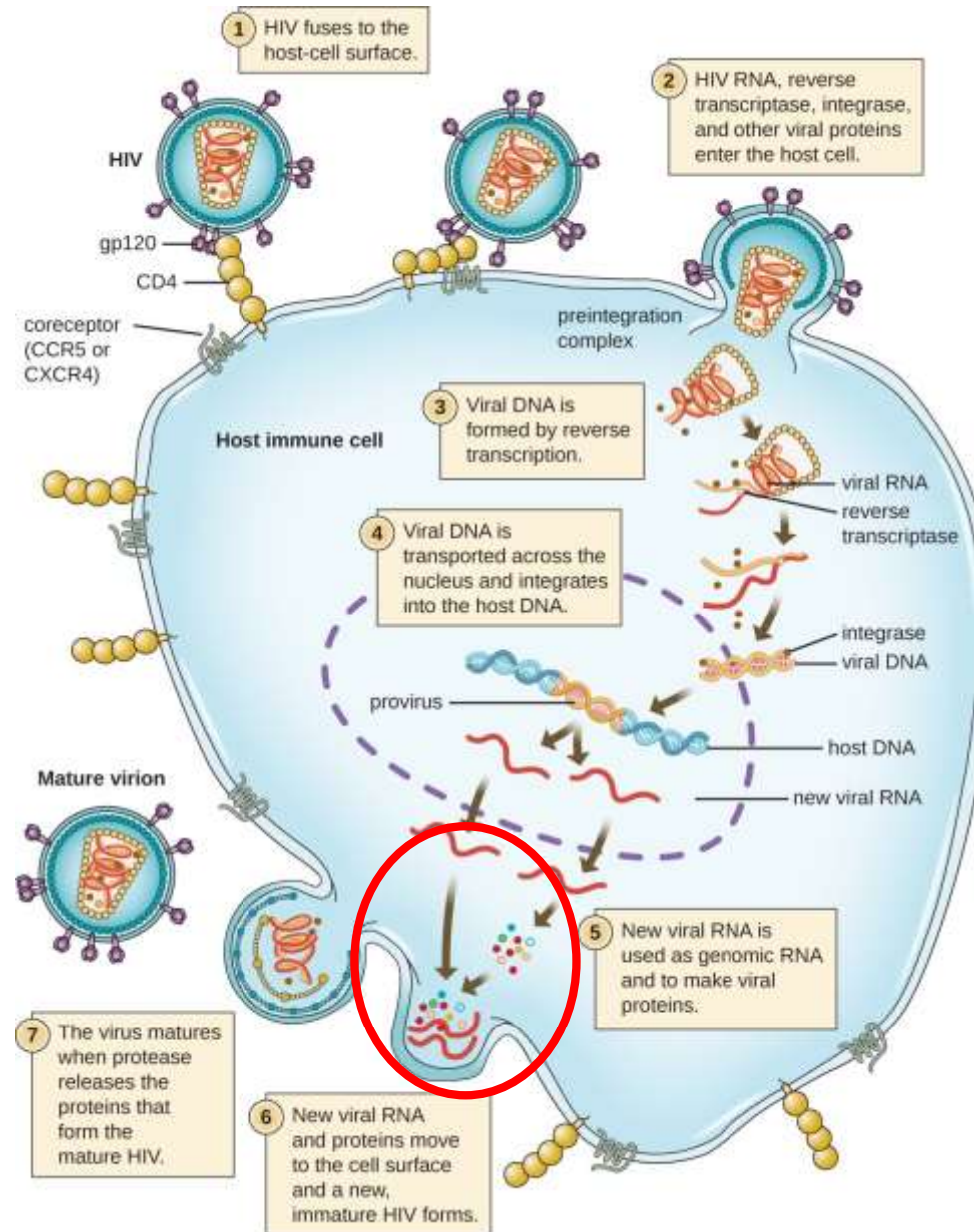
Source: UNAIDS/WHO estimates

Updated: July 2022

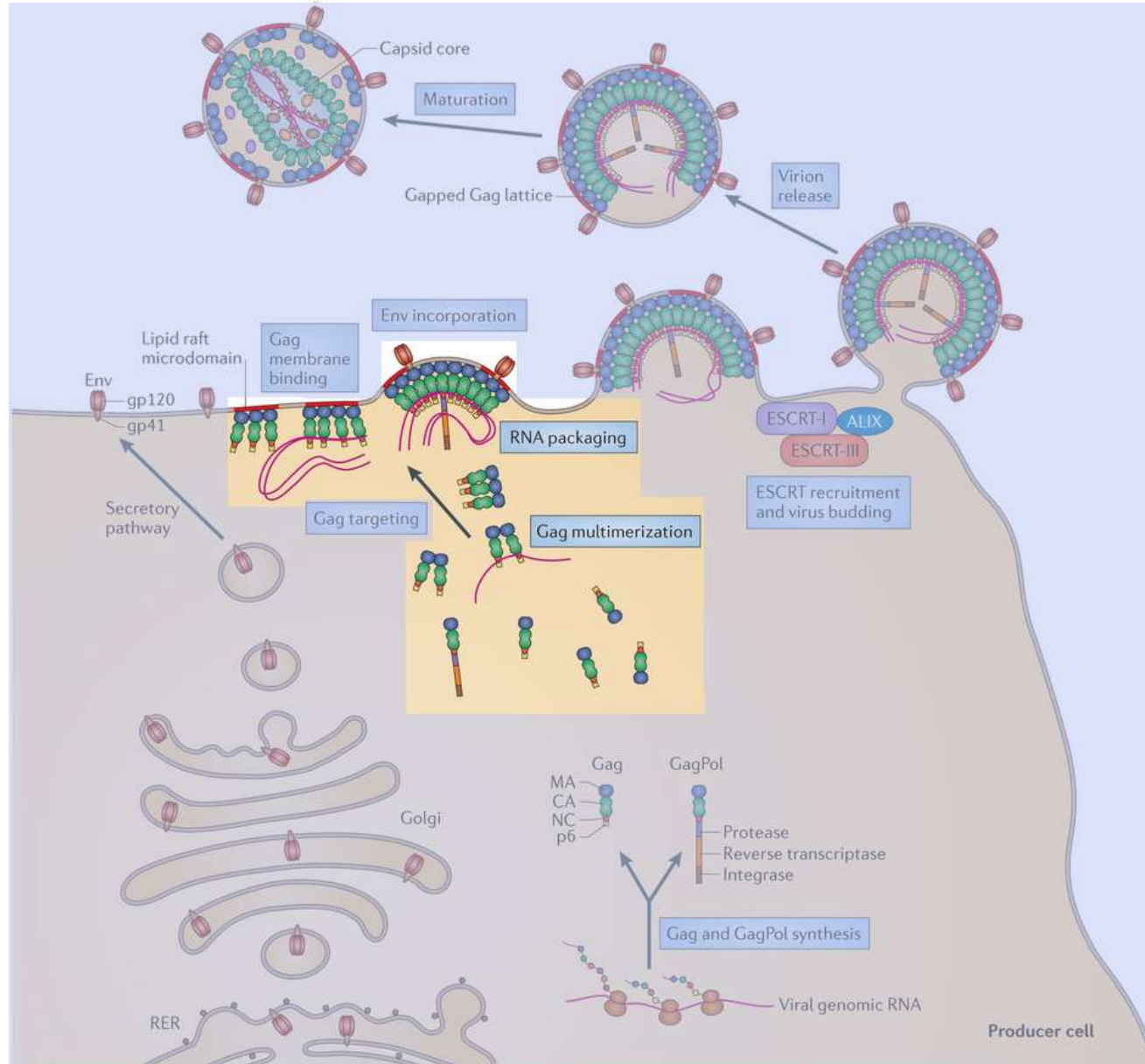


**Kidney biopsy (< 15 years old kid)
from our lab**

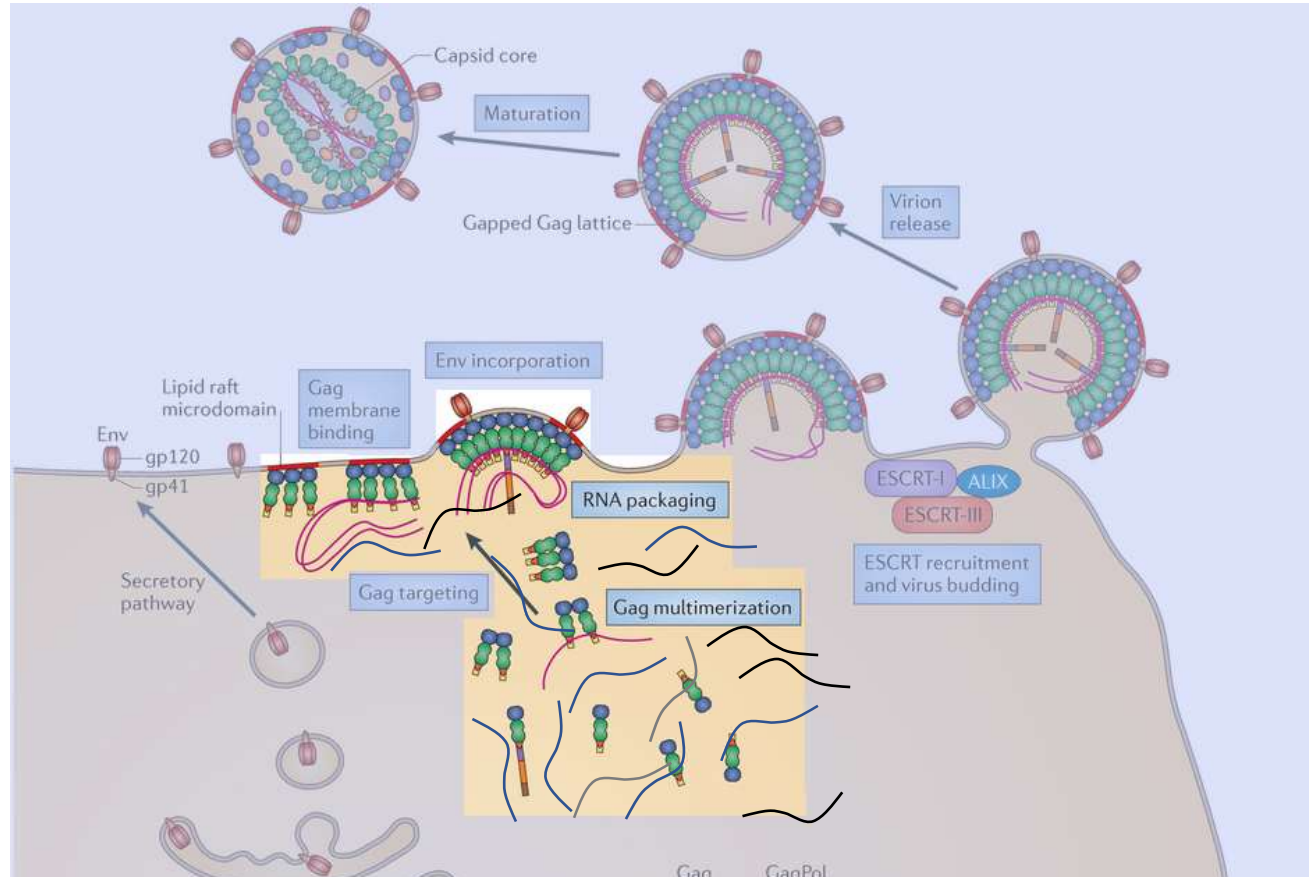
The infectious cycle



Virion assembly



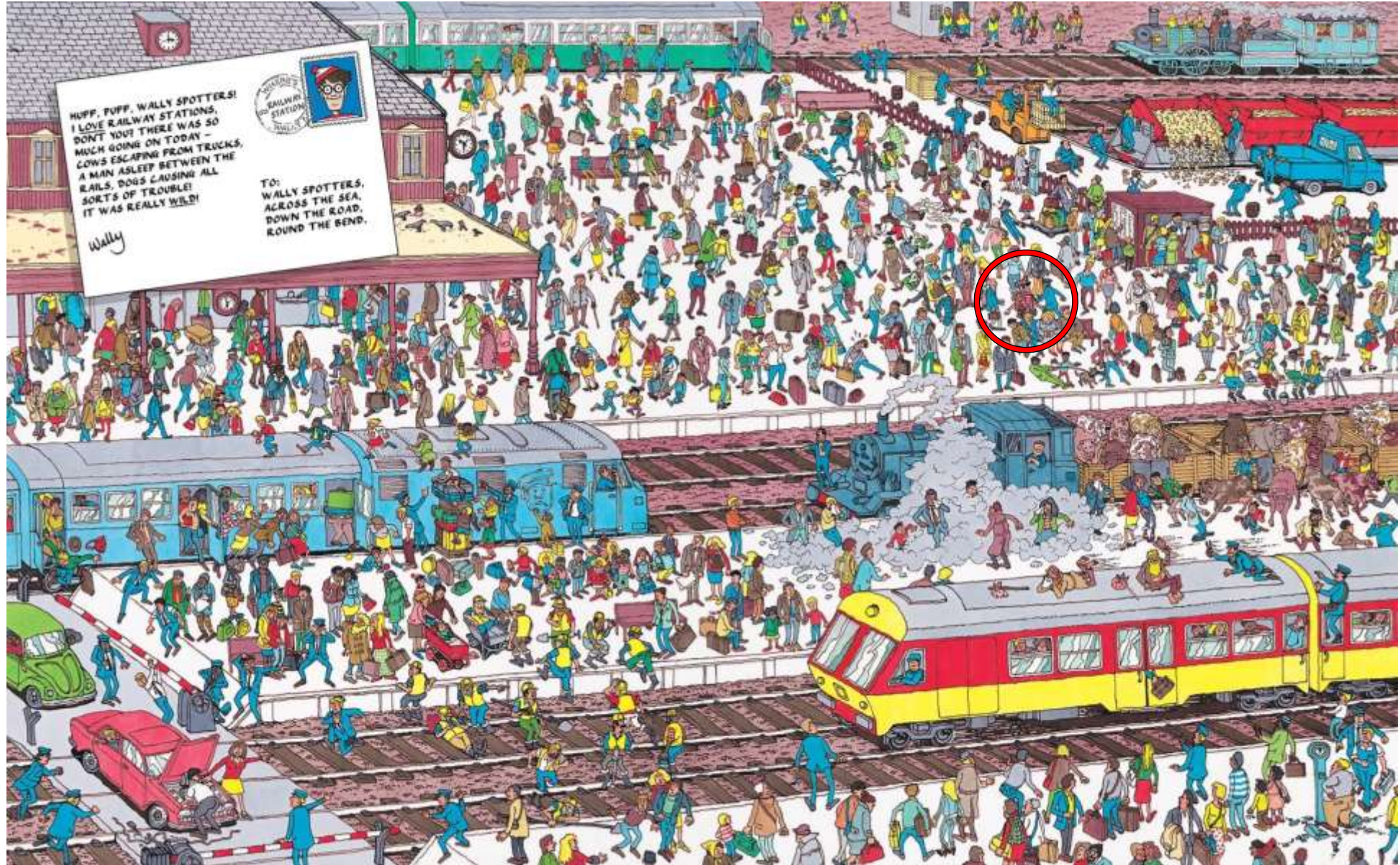
Virion assembly



!The HIV-1 genomic RNA is in constant competition for Gag binding against a large excess of viral and cellular mRNAs!



Where is Waldo (Wally)?





Packaging signal or Ψ

- Many (+)ssRNA (& retroviruses) use Ψ to selectively package their genome.
- There is no one "type" or class of Ψ .
- There is no single "selective" packaging mechanism.
- This mechanism depends on the viral species.

- Ψ is a “sequence” and/or “structure” of the genomic RNA that is responsible for selective packaging of the full-length viral RNA. We thought to be a short stem-loop region at the 5′ UTR.

Lever et al. & Sodroski JVI 1989 & Aldovini and Young JVI 1990.

- However, in the absence of Ψ , Gag interacts non-specifically with cellular RNAs, thus assembles into virus-like-particles. *Nonetheless, under these conditions Gag prefers RNAs with extremely long UTRs.*

Muriaux et al. & Rein PNAS 2001, Rulli et al. & Rein JVI 2007, Comas-Garcia, M. et al. & Rein Viruses 2016.



- Gag- Ψ interactions are **needed** for **specific packaging** but are **dispensable** for virion assembly.

Muriaux et al. & Rein PNAS 2001, Rulli et al. & Rein JVI 2007, Comas-Garcia, M. et al. & Rein Viruses 2016, Comas-Garcia, M. et al. & Rein. eLife 2017, Comas-Garcia, M. et al. & Rein. eLife 2018,

- One hypothesis is that selective packaging is a consequence of a high binding affinity between Gag and Ψ .

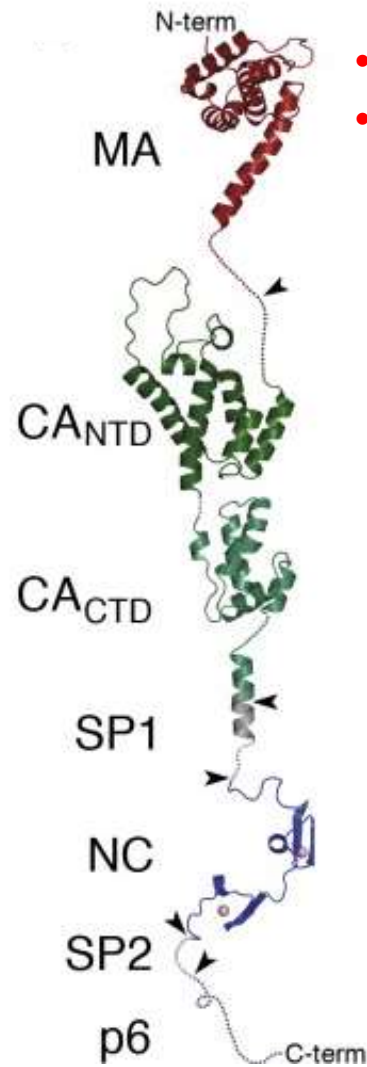
Webb et al. & Musier-Forsyth RNA 2013, Abd El-Wahab et al. & Marquet Nat Comm. 2014, Bernacchi et al. & Palliard RNA Biol. 2017.

- However, there is also evidence that mechanism by which selective packaging occurs is far more complicated than a high-affinity binding scenario.

Nikolaitchik et al. & Hu PLOS Path 2013, Comas-Garcia, M. et al. & Rein Viruses 2016, Dilley et al. & Hu JVI 2017, Liu et al. & Hu J. Mol. Bio. 2017, Comas-Garcia et al. & Rein eLife 2017. Comas-Garcia et al. & Rein eLife 2018.

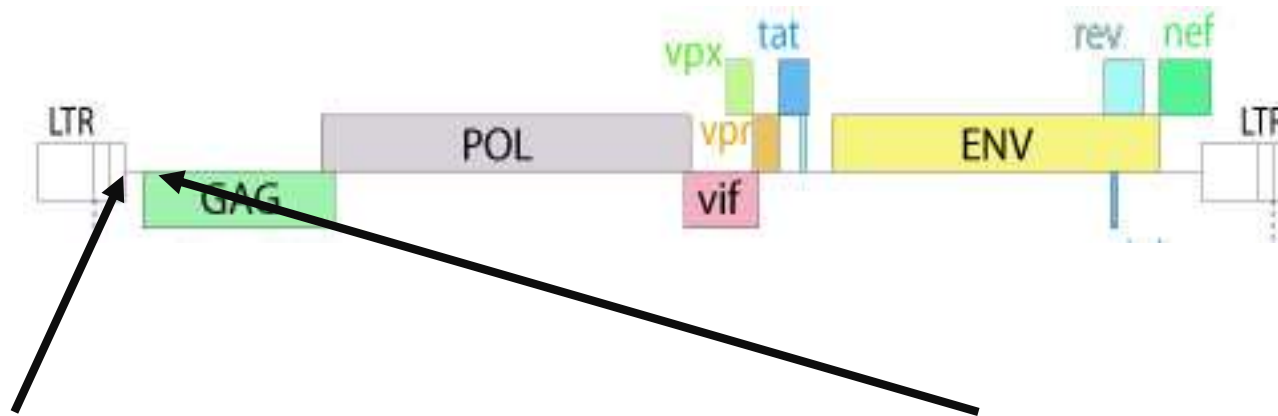


Gag: a Swiss army knife

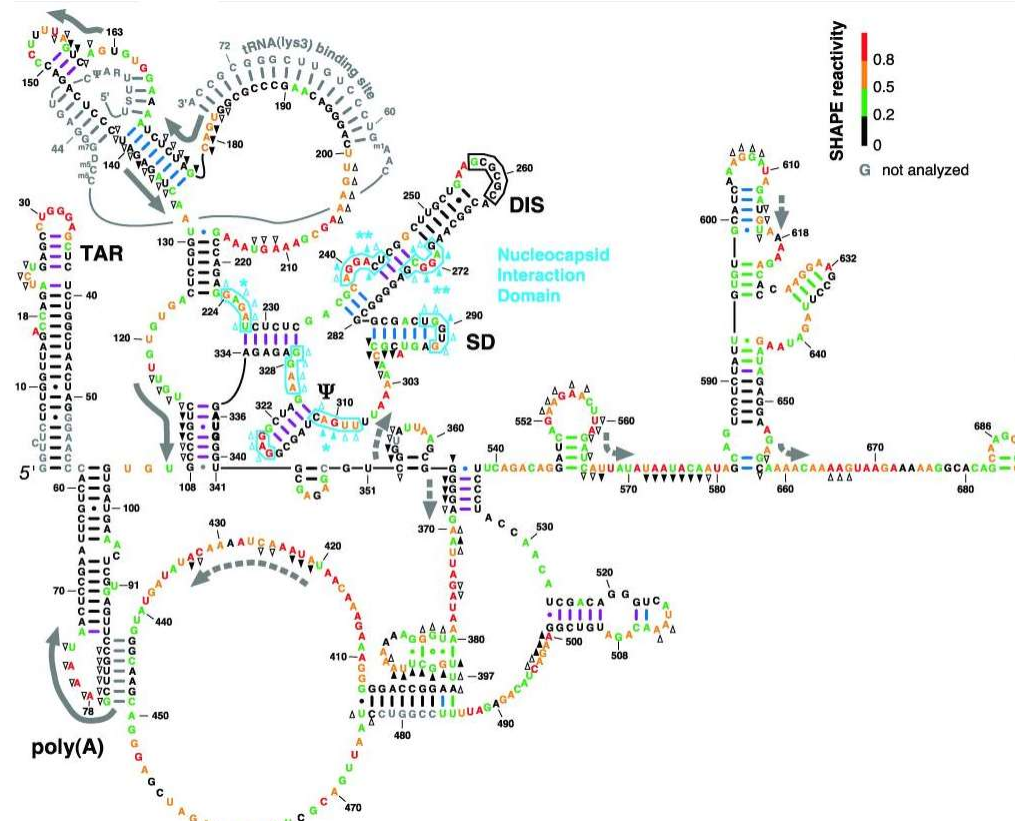


- **Basic (positively charged)**
- **Binds to nucleic acids < tRNAs < plasmatic membrane**
- **Gag-Gag interactions**
- **Dimerization.**
- **Multimerization**
- **Virion assembly**
- **Basic (positively charged)**
- **Zinc fingers (specific RNA-Gag ingeractions)**
- **Gag-RNA interactions**
- **Chaperone (RT)**

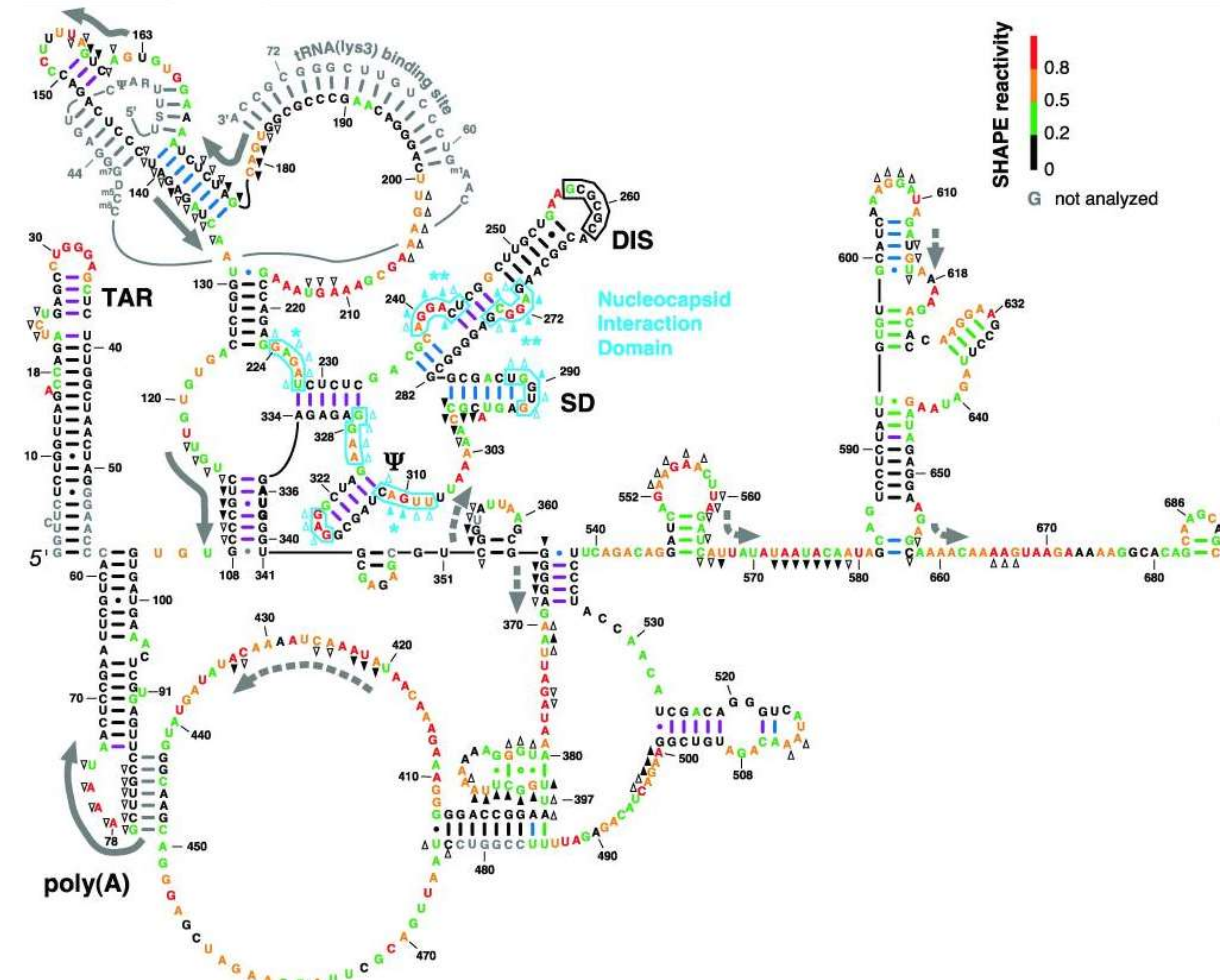
The genomic RNA



HIV-1 genome (DNA)



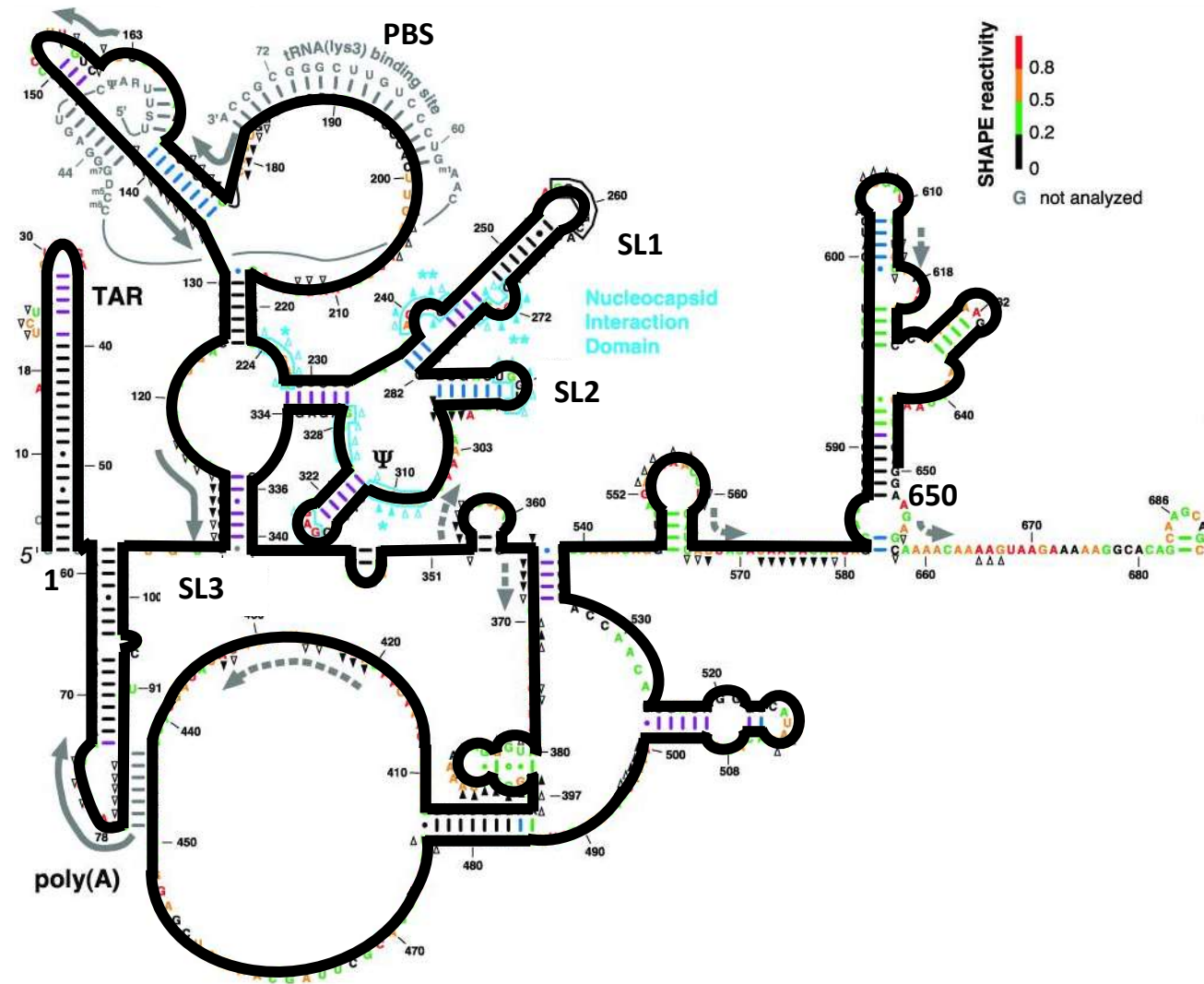
5' UTR: also Swiss army knife



This region controls:

- Transcription.
- Translation.
- Splicing.
- Dimerization.
- Selective packaging.
- Primer binding (retrotranscription).

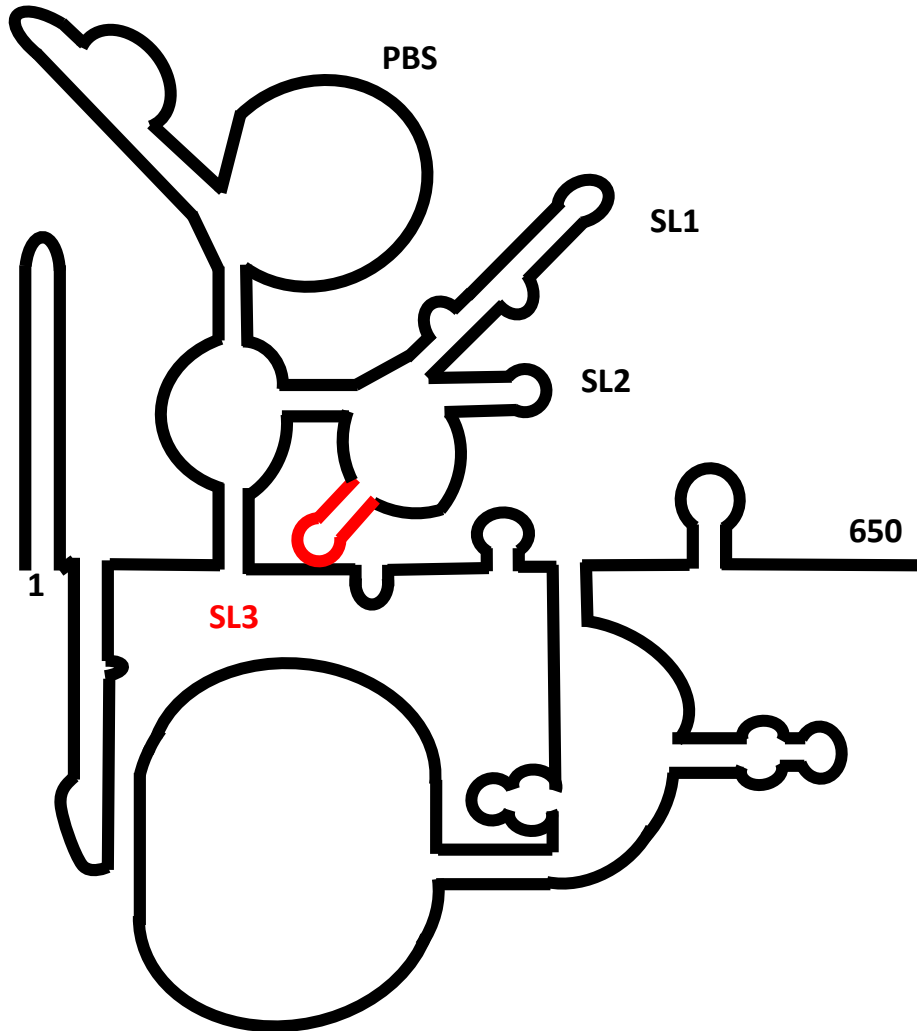
Let's take a look at it



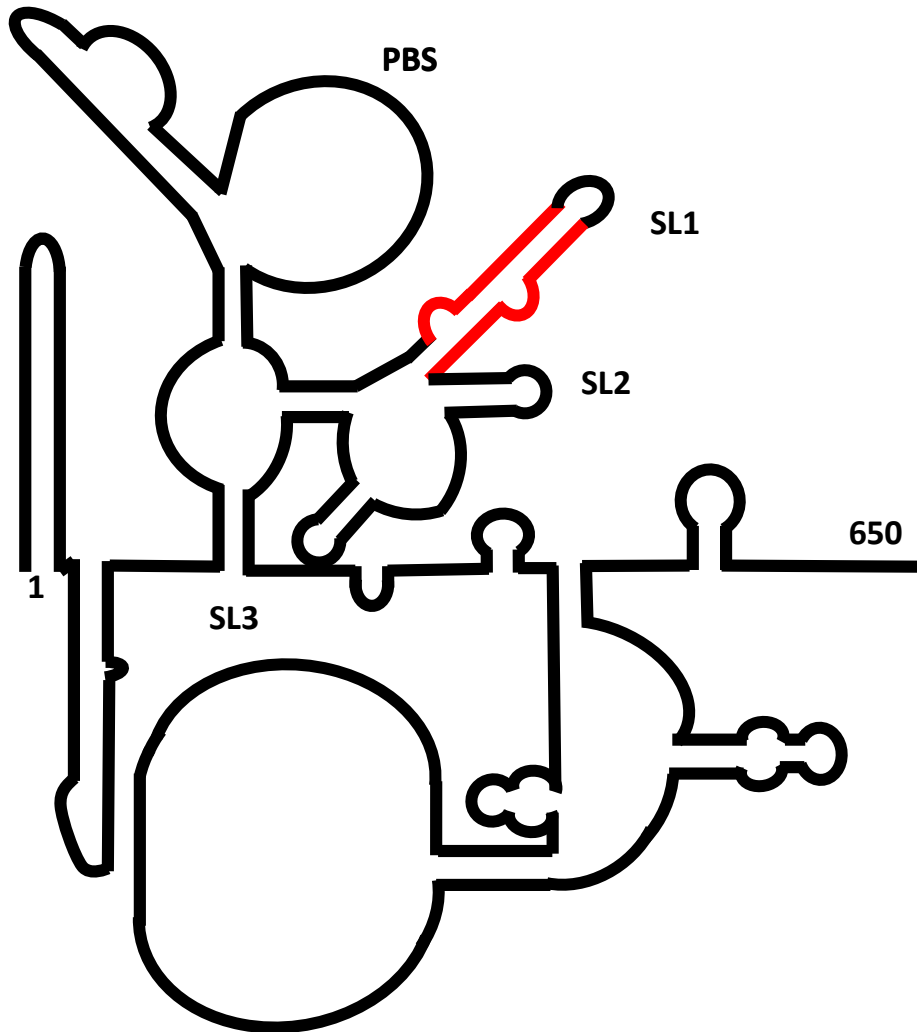
Let's take a look at it



- SL3 was the original Ψ .

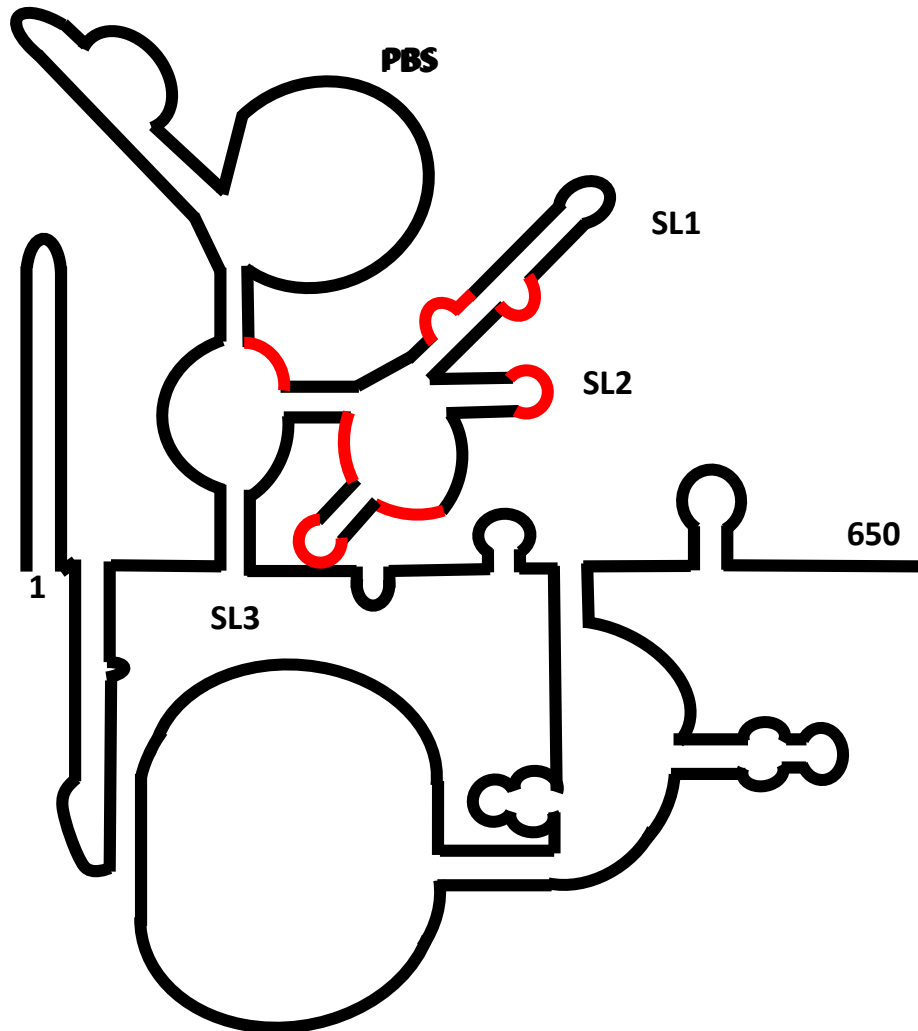


Let's take a look at it



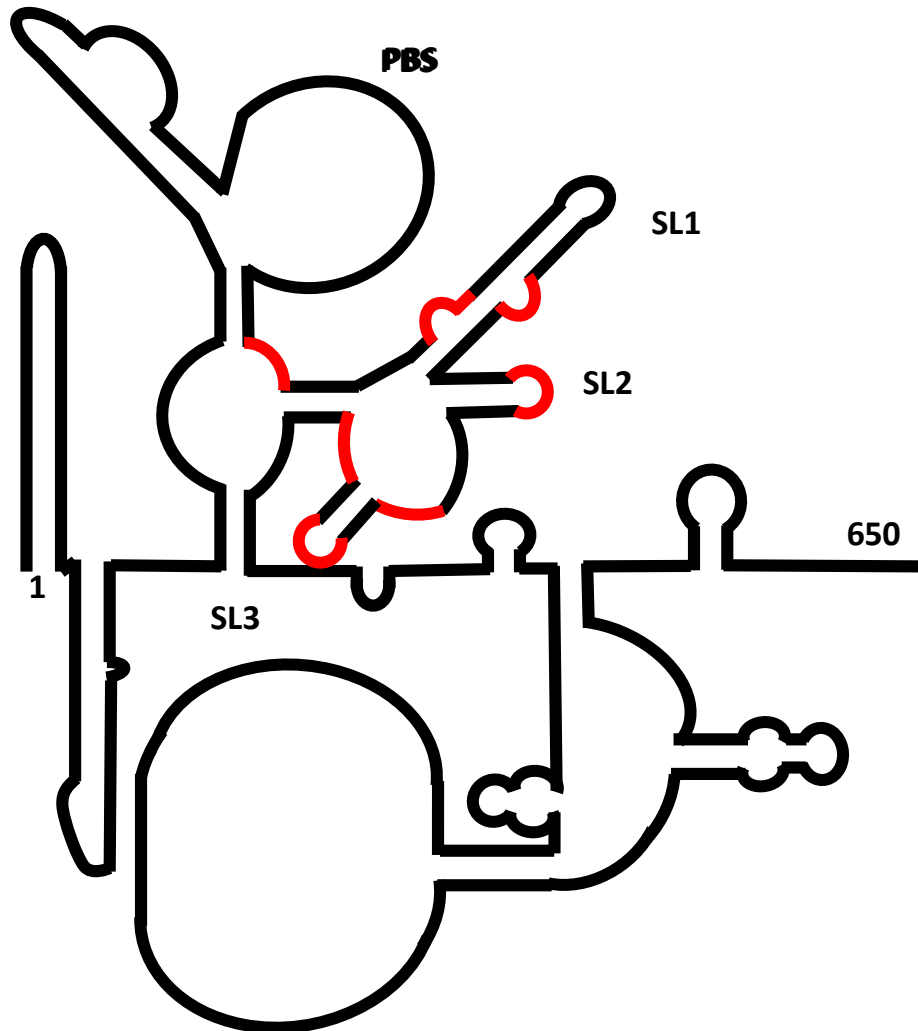
- SL3 was the original Ψ .
- Then SL1 was proposed to be the real Ψ (high-affinity binding).

Let's take a look at it



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- There was the observation that the unpaired guanosines within these regions strongly interact with the Gag.

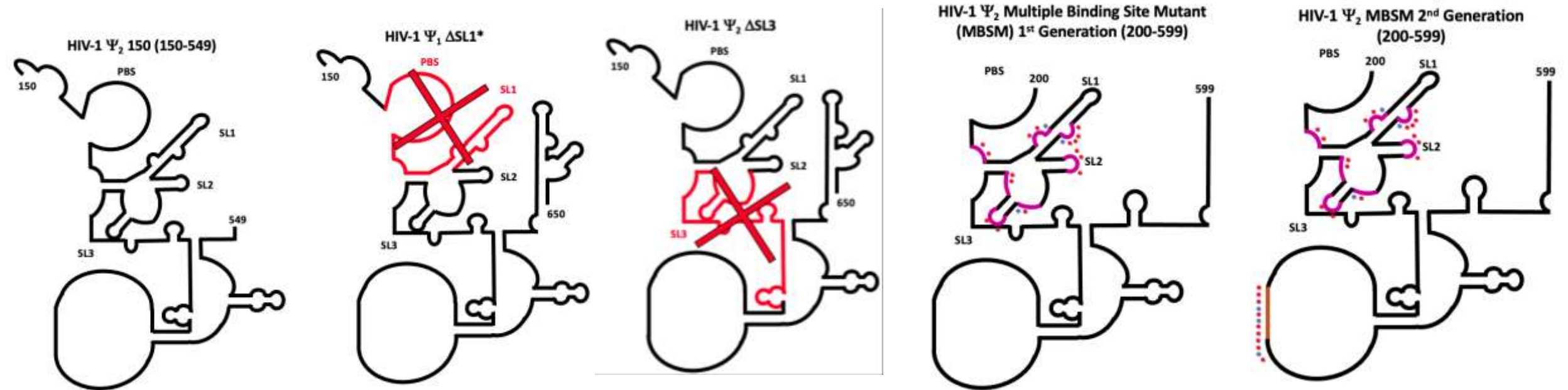
Let's take a look at it



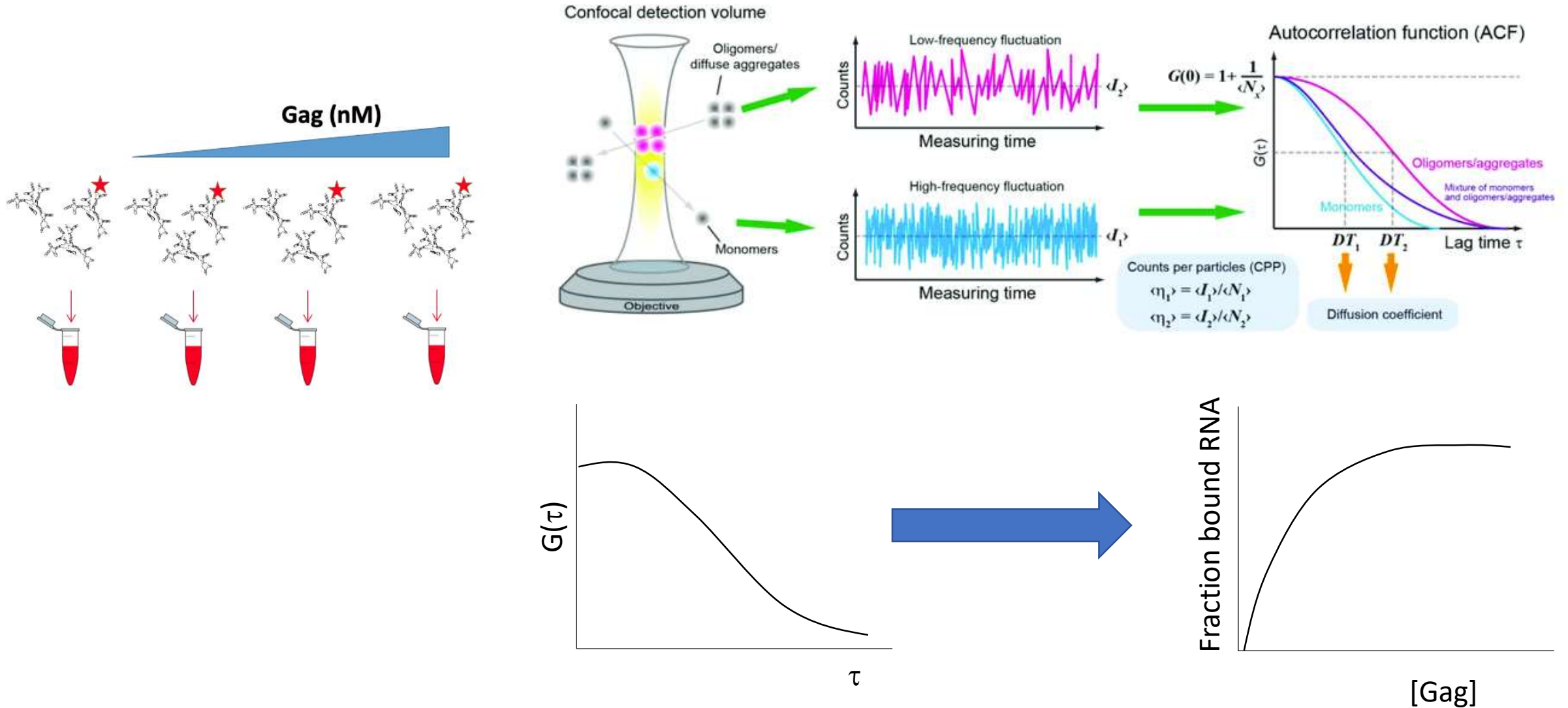
- SL3 was the original Ψ .
- Then SL1 was proposed to be the real Ψ (high-affinity binding).
- There was the observation that the unpaired guanosines within these regions strongly interact with the Gag.
- Mutation of some of these guanosines to adenosines decreases *in vitro* binding to NC and impacts packaging in cell lines.

What is going on?

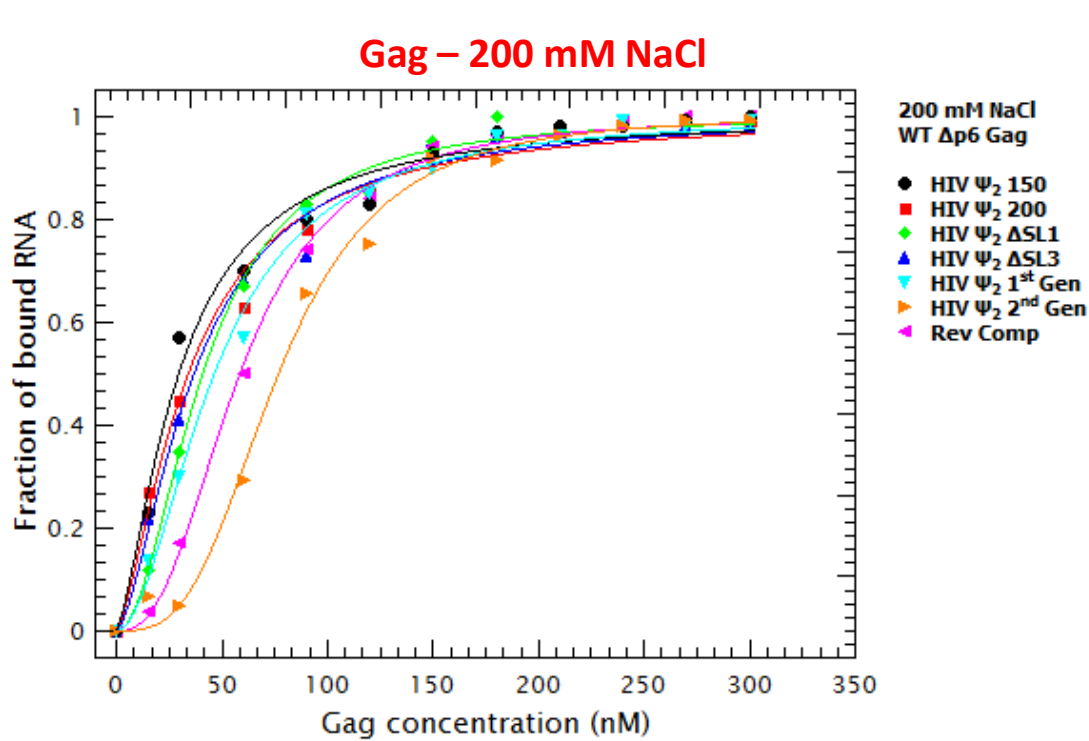
Making mutants



Measuring RNA-Gag binding



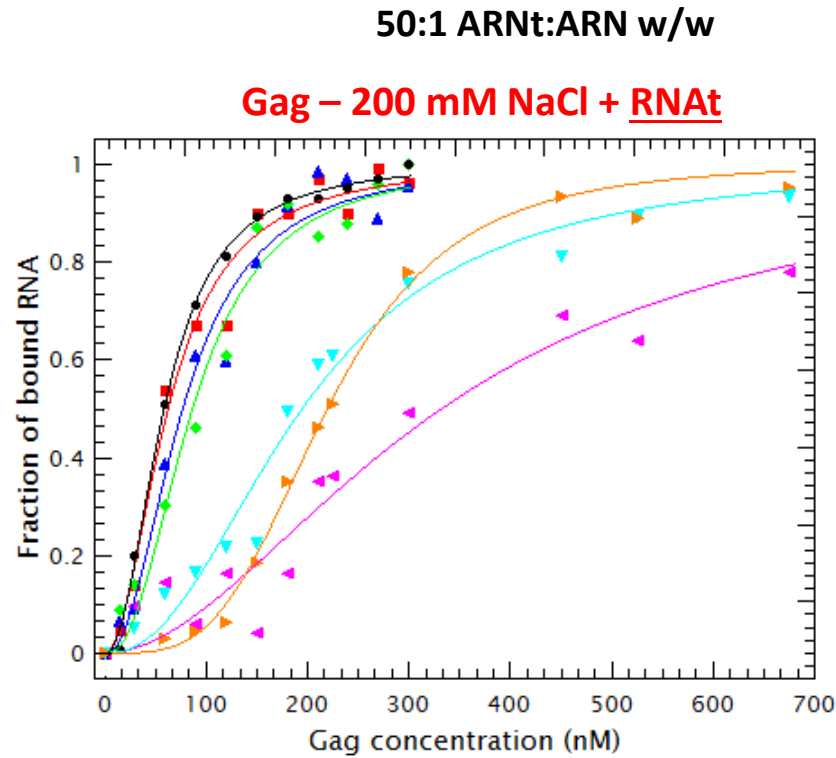
Measuring RNA-Gag binding



RNA	K_D (nM)	n_H
HIV Ψ ₂ 150	31	1.6
HIV Ψ ₂ 200	32	1.5
HIV Ψ ₁ ΔSL1	47	2.5
HIV Ψ ₂ ΔSL3	35	1.6
HIV Ψ ₂ MBSM 1 st Gen	48	1.8
HIV Ψ ₂ MBSM 2 nd Gen	77	3.1
Rev Comp	58	2.5

Under solution condition similar to those of the cytoplasm there is no real difference in binding affinities.

Measuring RNA-Gag binding



200 mM NaCl
WT Δp6 Gag + tRNAs

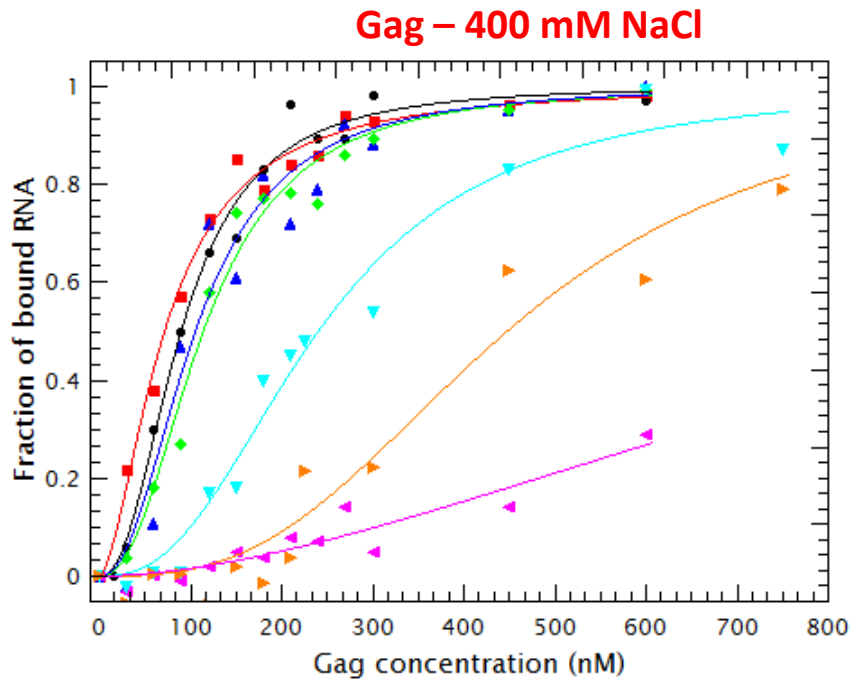
- HIV Ψ₂ SD
- HIV Ψ₂ UD
- ◆ HIV Ψ₂ ΔSL1
- ▲ HIV Ψ₂ ΔSL3
- ▼ HIV Ψ₂ 1st Gen
- ▲ HIV Ψ₂ 2nd Gen
- ◆ Rev Comp

200 mM NaCl

RNA	K _D (nM)	K _D (nM)
HIV Ψ ₂ 150	31	59
HIV Ψ ₂ 200	32	63
HIV Ψ ₁ ΔSL1	47	87
HIV Ψ ₂ ΔSL3	35	78
HIV Ψ ₂ MBSM 1 st Gen	48	195
HIV Ψ ₂ MBSM 2 nd Gen	77	222
Rev Comp	58	333

Addition of a competitor RNA for the MA domain reveals specific binding

Measuring RNA-Gag binding

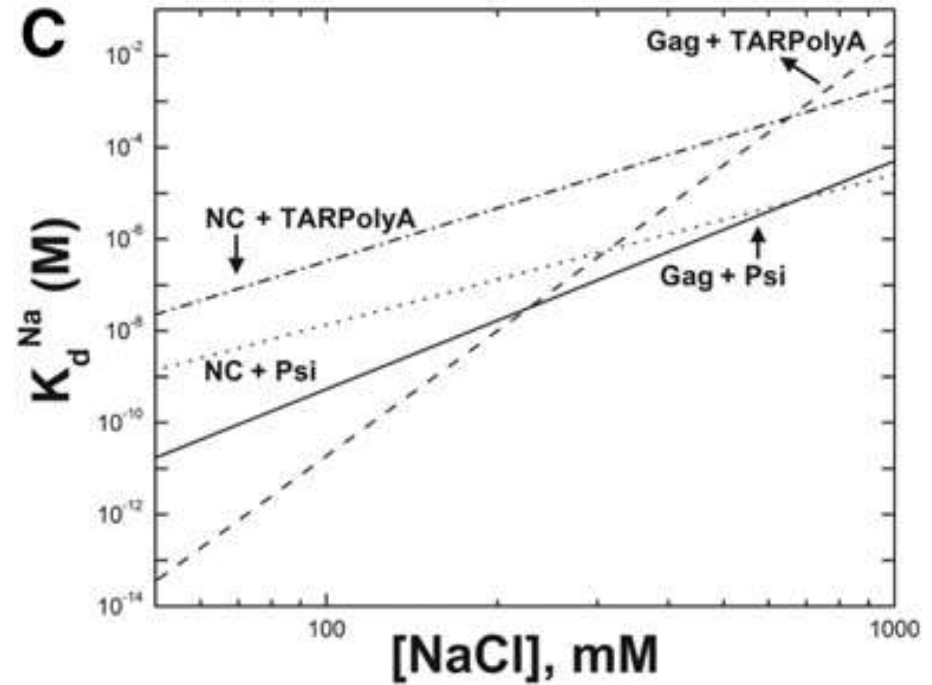
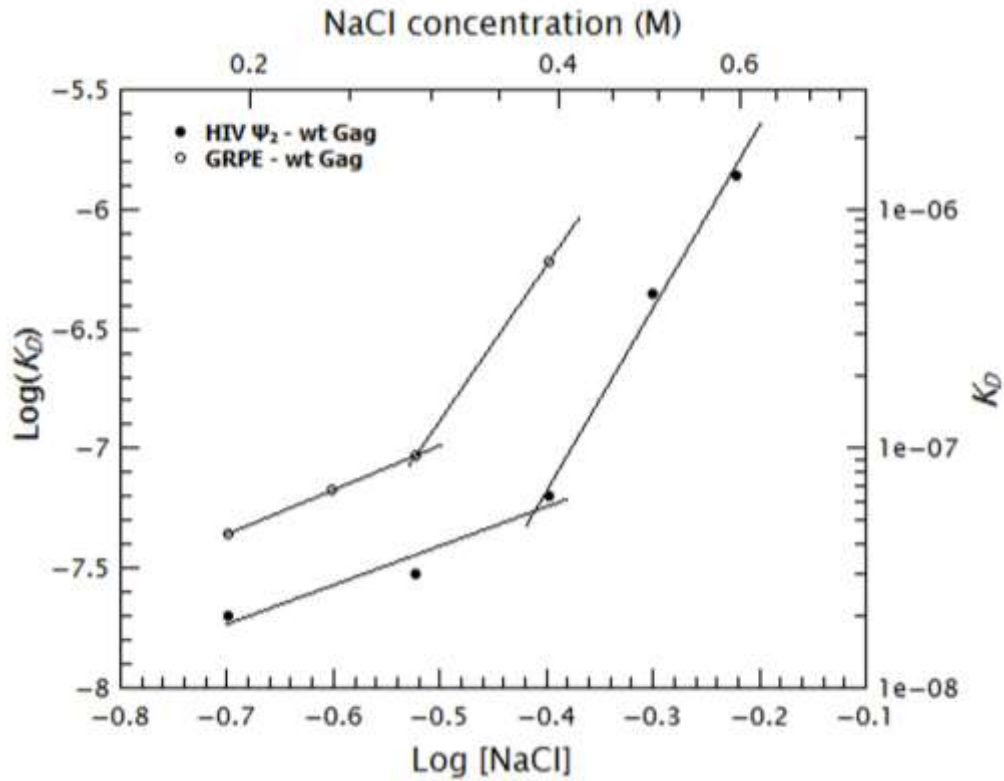


- 400 mM NaCl
WT Δp6 Gag
- HIV Ψ₂ 150
 - HIV Ψ₂ 200
 - ◆ HIV Ψ₂ ΔSL1
 - ▲ HIV Ψ₂ ΔSL3
 - ▼ HIV Ψ₂ 1st Gen
 - ▶ HIV Ψ₂ 2nd Gen
 - ◆ Rev Comp

RNA	200 mM NaCl K _D (nM)	400 mM NaCl K _D (nM)
HIV Ψ ₂ 150	31	93
HIV Ψ ₂ 200	32	65
HIV Ψ ₁ ΔSL1	47	102
HIV Ψ ₂ ΔSL3	35	103
HIV Ψ ₂ MBSM 1 st Gen	48	→ 204
HIV Ψ ₂ MBSM 2 nd Gen	77	→ 402
Rev Comp	58	→ 1,202

Non-specific binding is mostly electrostatic

Measuring RNA-Gag binding

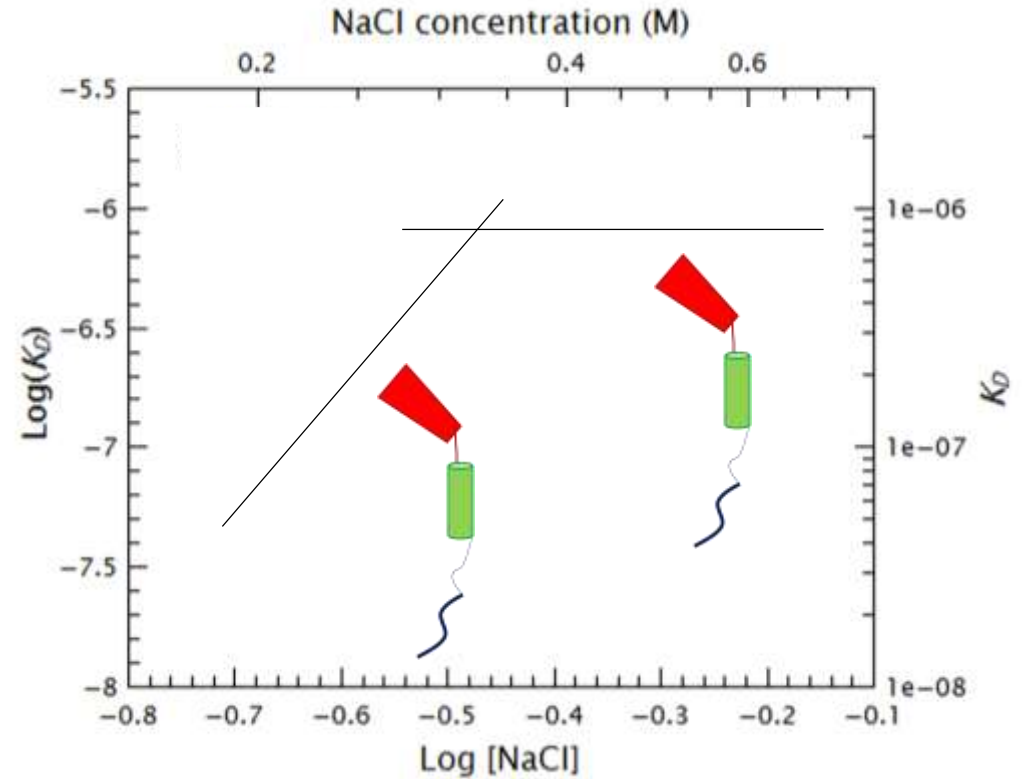
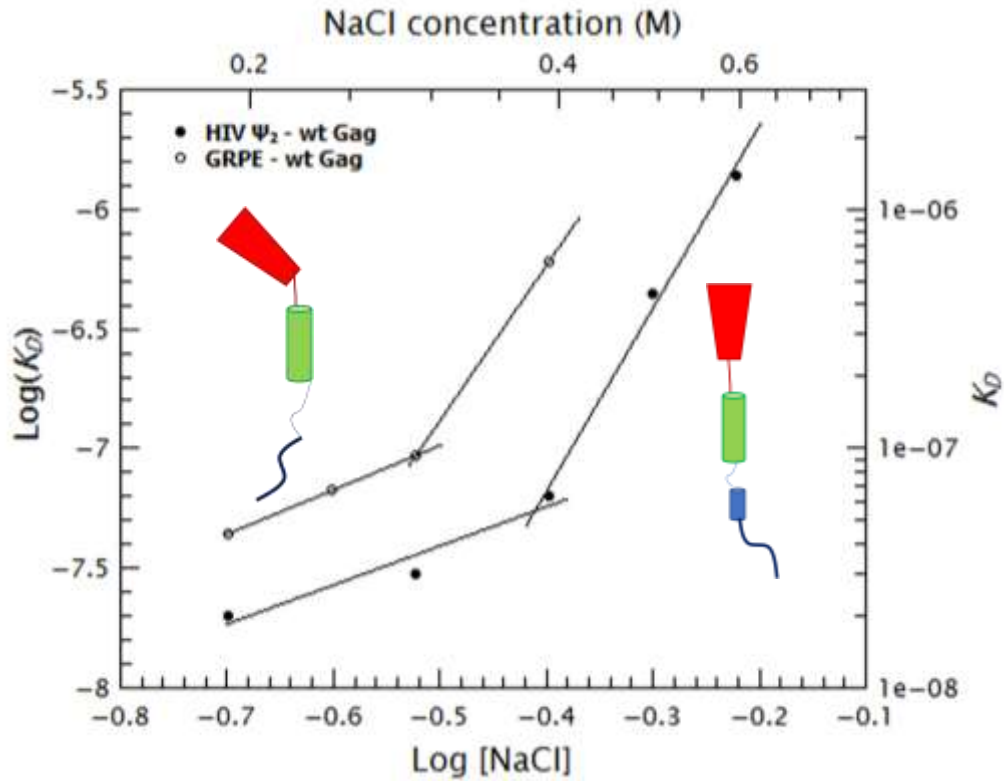


Non-specific binding is mostly electrostatic

Measuring RNA-Gag binding

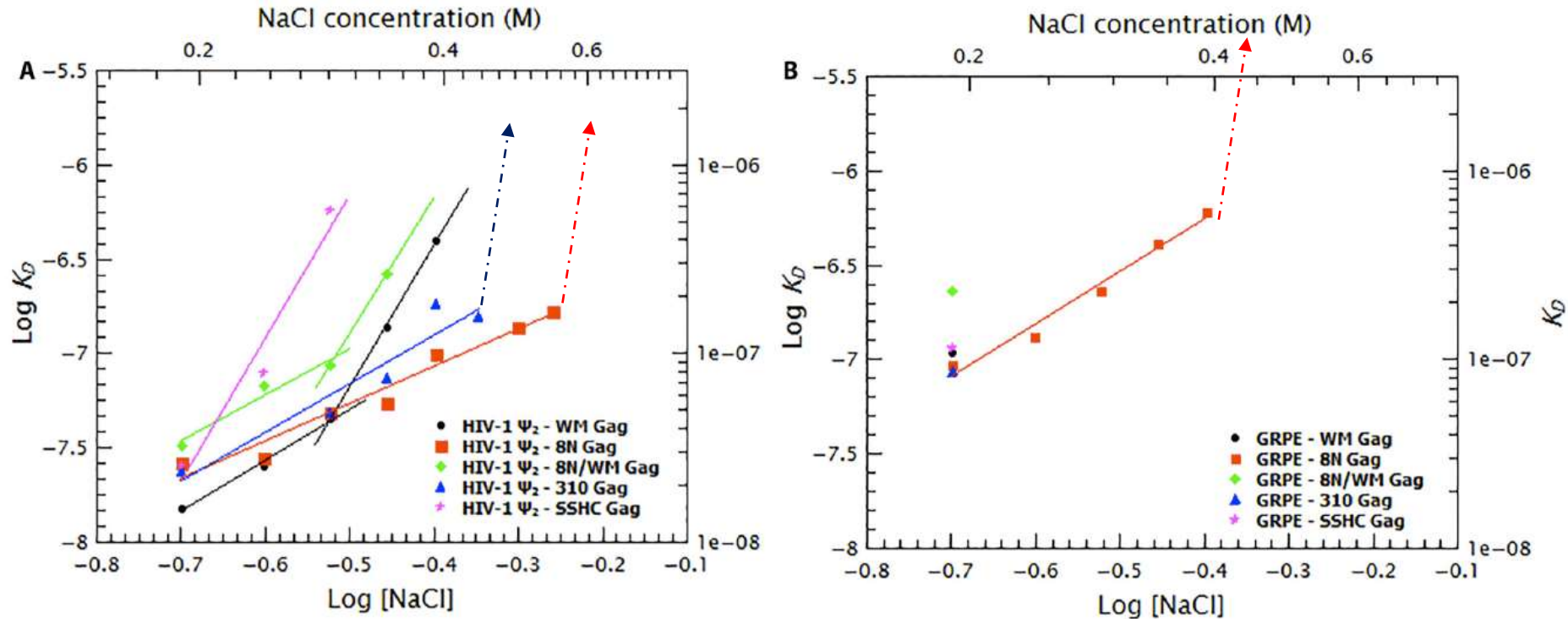


What we were expecting



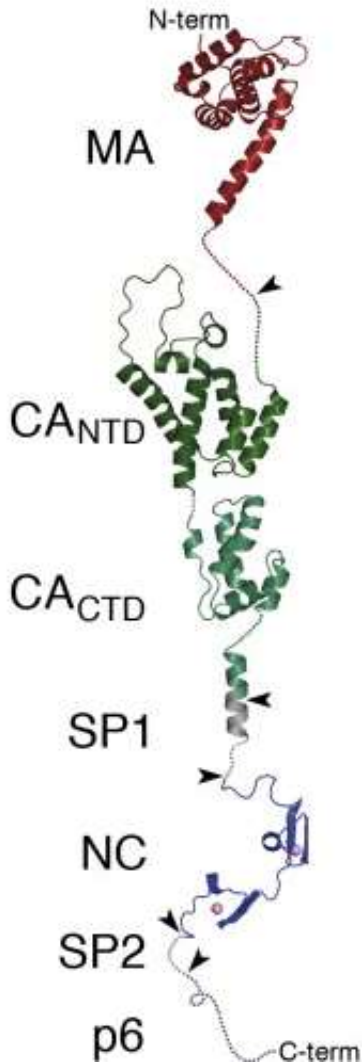
What is going on?

Specific binding is complicated



- WM Gag – Weak Gag-Gag interactions
- 8N Gag - No MA-RNA interactions
- WM/8N Gag – Weak Gag-Gag interaction & No MA-RNA interaction
- 310 Gag - Weak NC-RNA non-specific electrostatic interactions
- SSHC – Weak NC-RNA specific non-electrostatic interactions

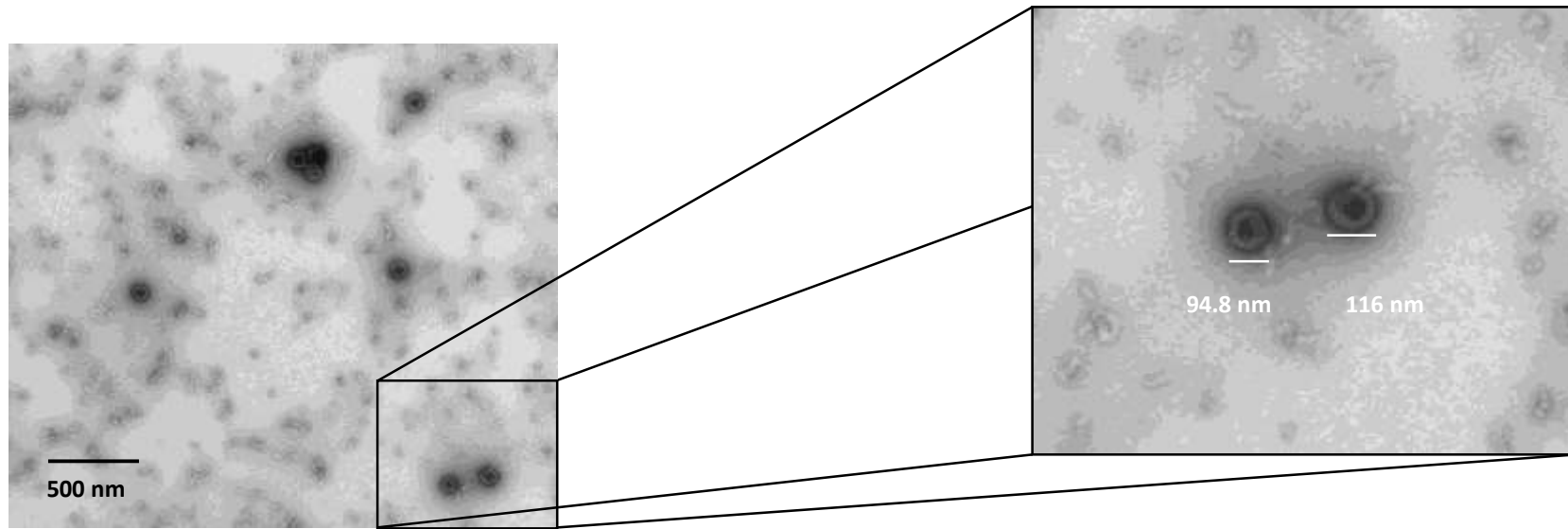
Ψ specific binding can be revealed



- Mutations that neutralize this basic domains results in selective binding to Ψ.
- Mutations that decrease the strength of Gag-Gag interactions results in binding only to Ψ.
- Mutations that decrease the non-specific electrostatic RNA-Gag interactions results in binding only to Ψ.
- Mutations that decrease the specific non-electrostatic interactions results in the inhibition of binding to any RNA and severely decreases binding to Ψ.

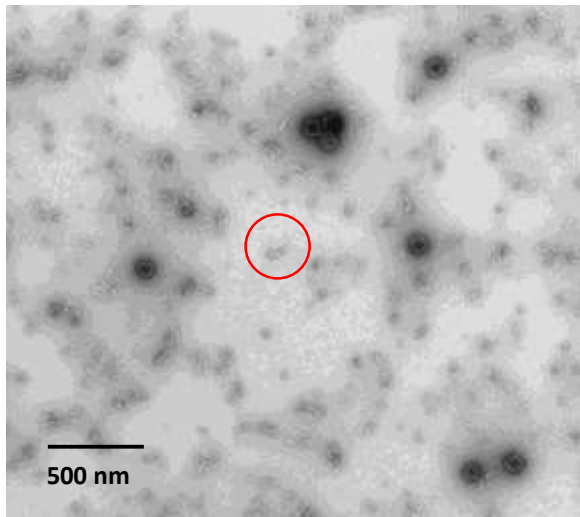
How is this achieved inside the cell?

In vitro assembly

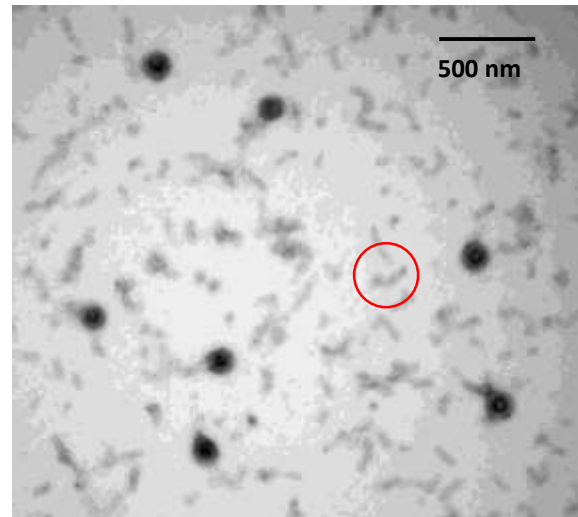


**61 nM HIV-1 Ψ ARN
2 μ M Δ MA Δ p6 Gag**

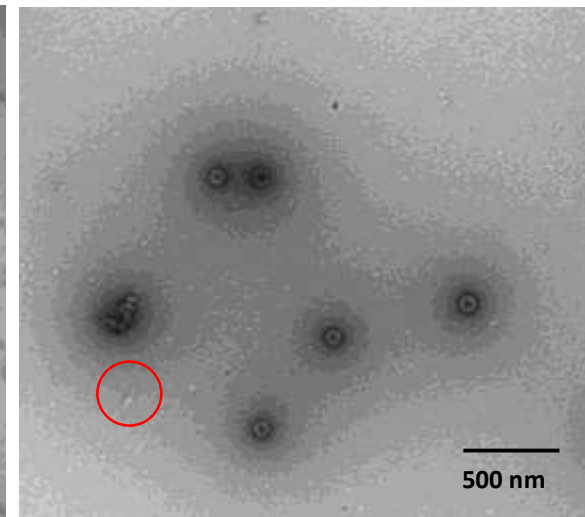
In vitro assembly



**61 nM HIV-1 Ψ ARN
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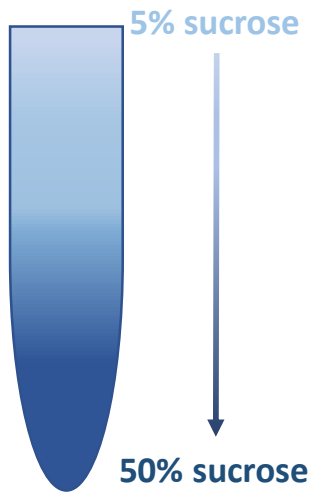
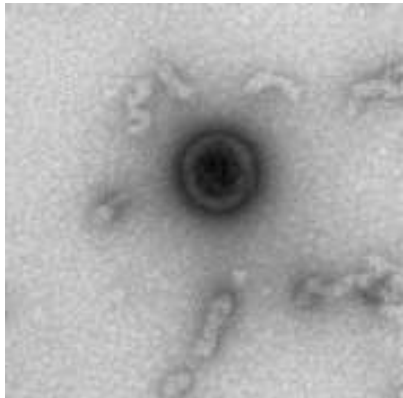


**61 nM MBSM 2nd Gen ARN
2 μ M Δ MA Δ p6 Gag**

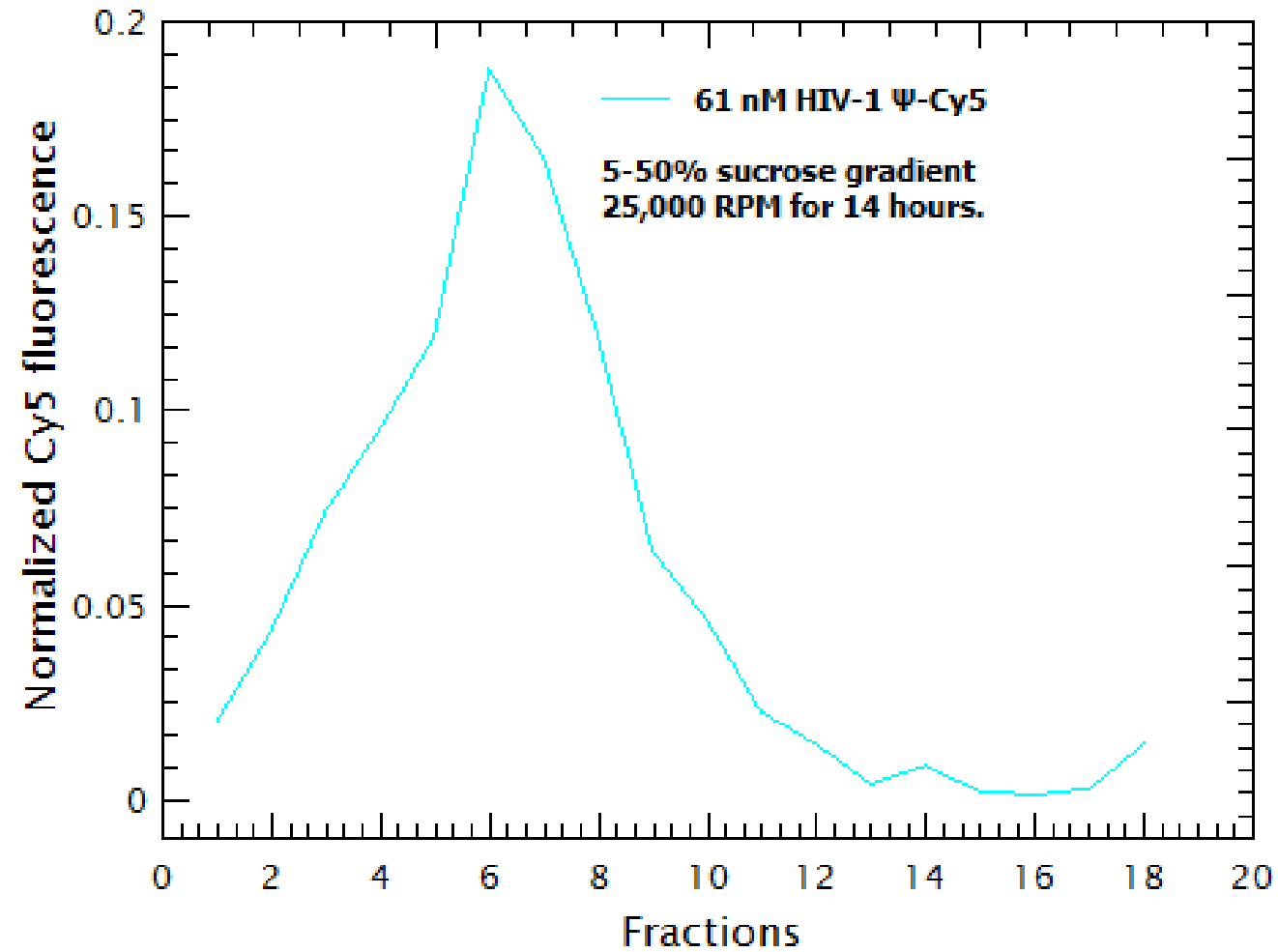


**61 nM Rev Comp ARN
2 μ M Δ MA Δ p6 Gag**

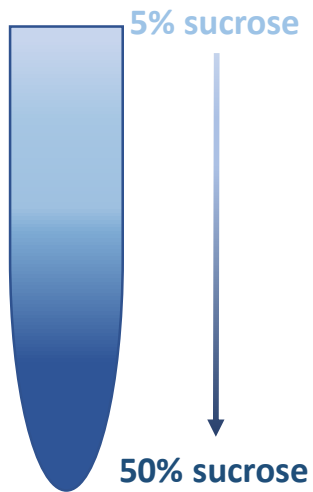
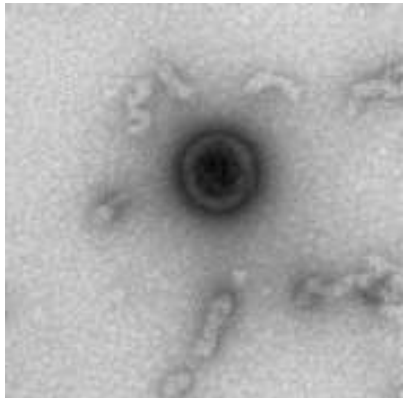
In vitro assembly efficiency



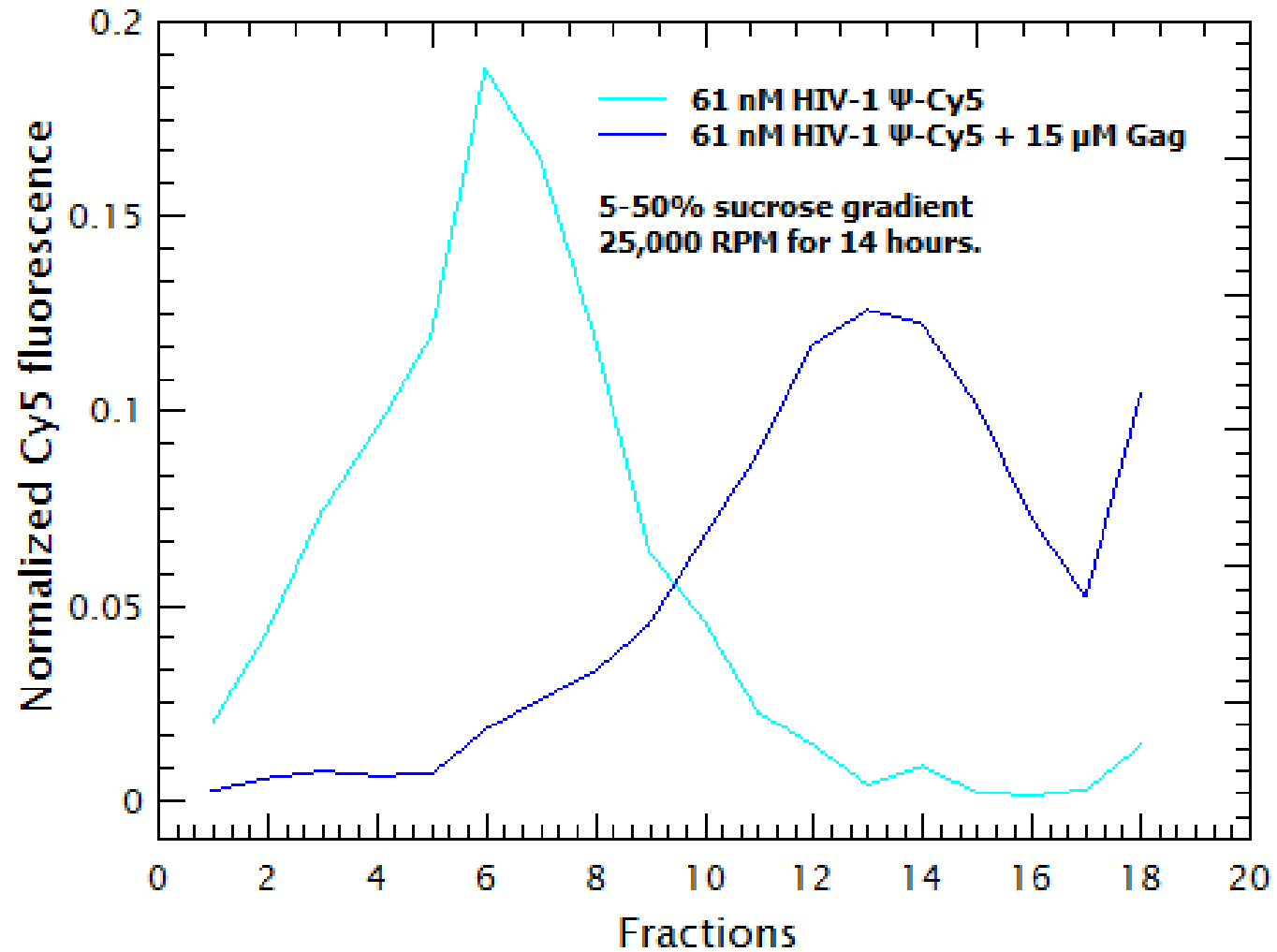
ULTRACENTRIFUGATION



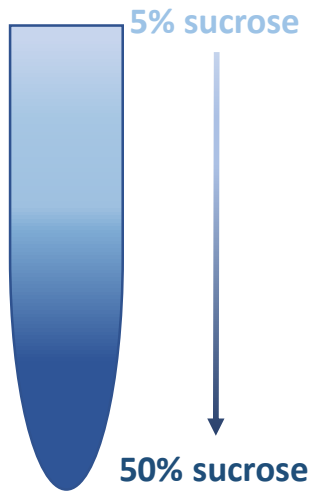
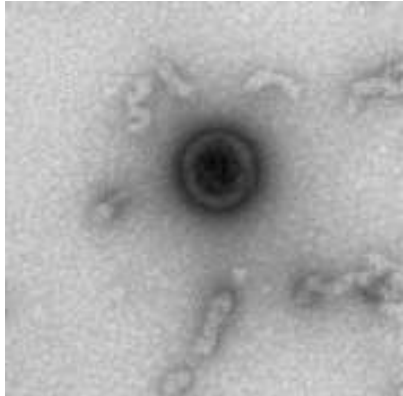
In vitro assembly efficiency



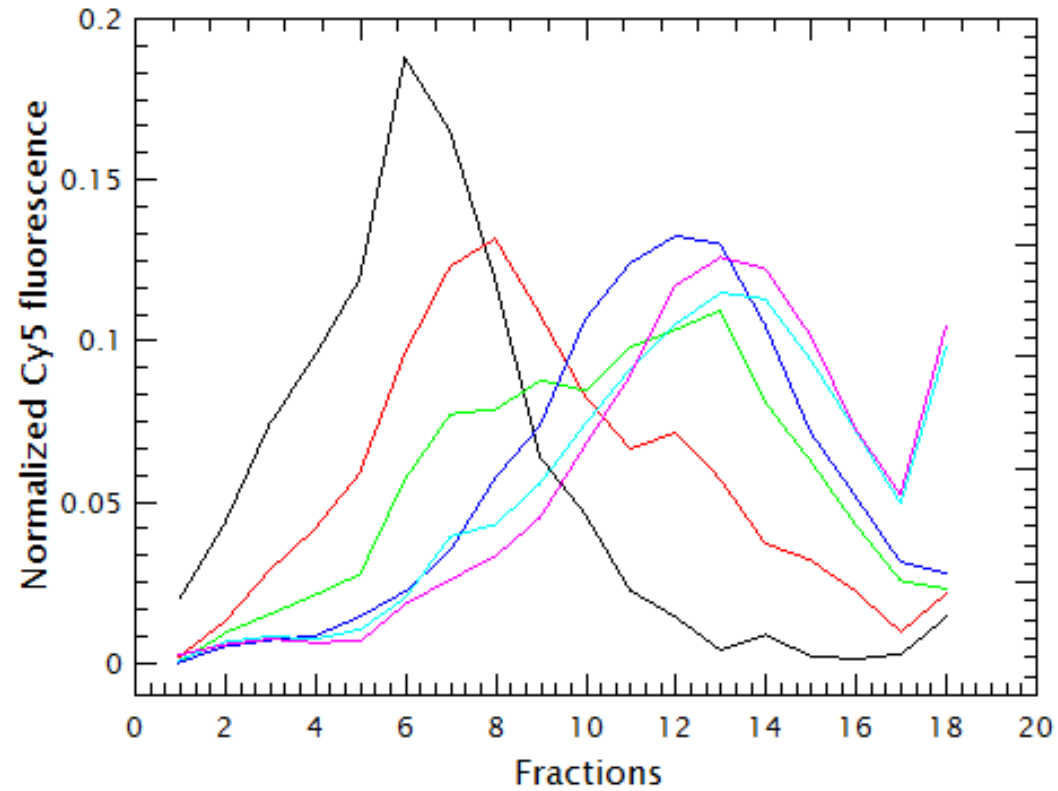
ULTRACENTRIFUGATION



In vitro assembly efficiency



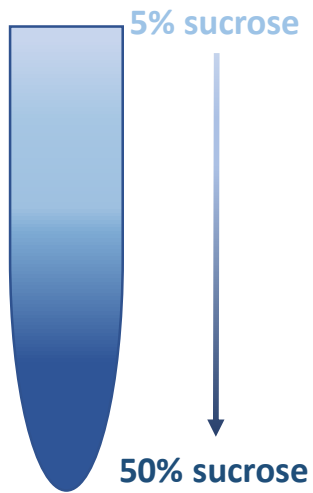
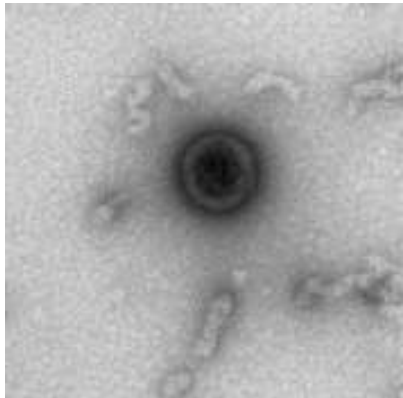
ULTRACENTRIFUGATION



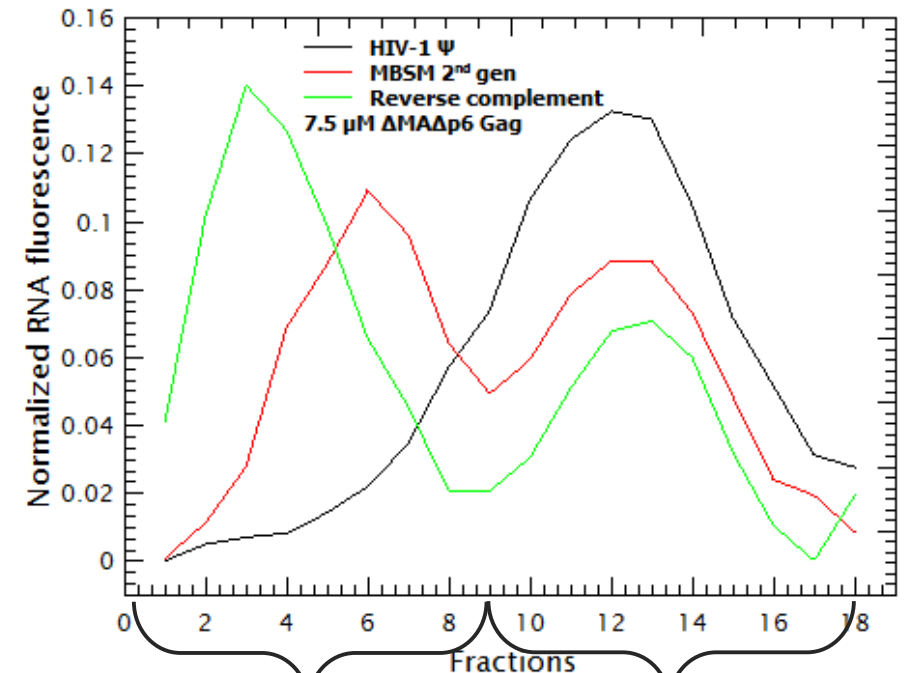
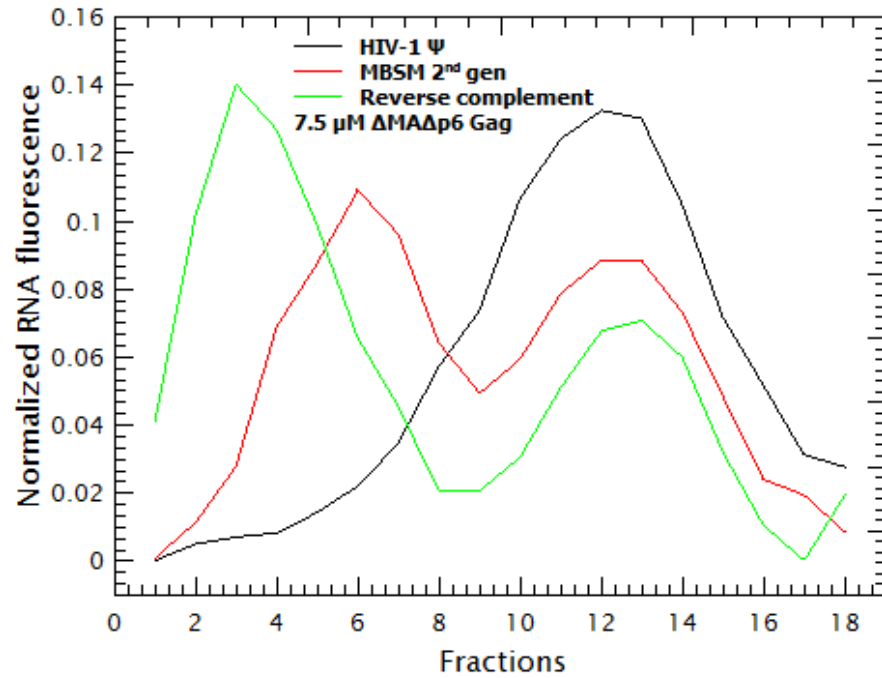
- 61 nM HIV-1 Ψ-Cy5
- 61 nM HIV-1 Ψ-Cy5 + 2 μM ΔMAΔp6 Gag
- 61 nM HIV-1 Ψ-Cy5 + 5 μM ΔMAΔp6 Gag
- 61 nM HIV-1 Ψ-Cy5 + 7.5 μM ΔMAΔp6 Gag
- 61 nM HIV-1 Ψ-Cy5 + 10 μM ΔMAΔp6 Gag
- 61 nM HIV-1 Ψ-Cy5 + 15 μM ΔMAΔp6 Gag

5-50% sucrose gradient
25,000 RPM for 14 hours.

In vitro assembly efficiency



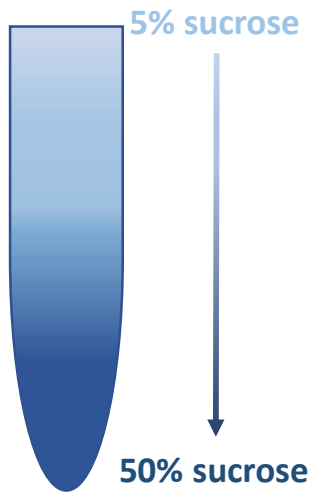
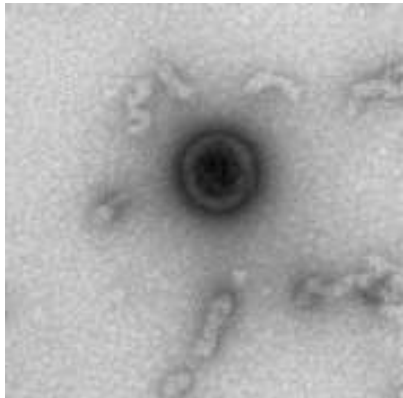
ULTRACENTRIFUGATION



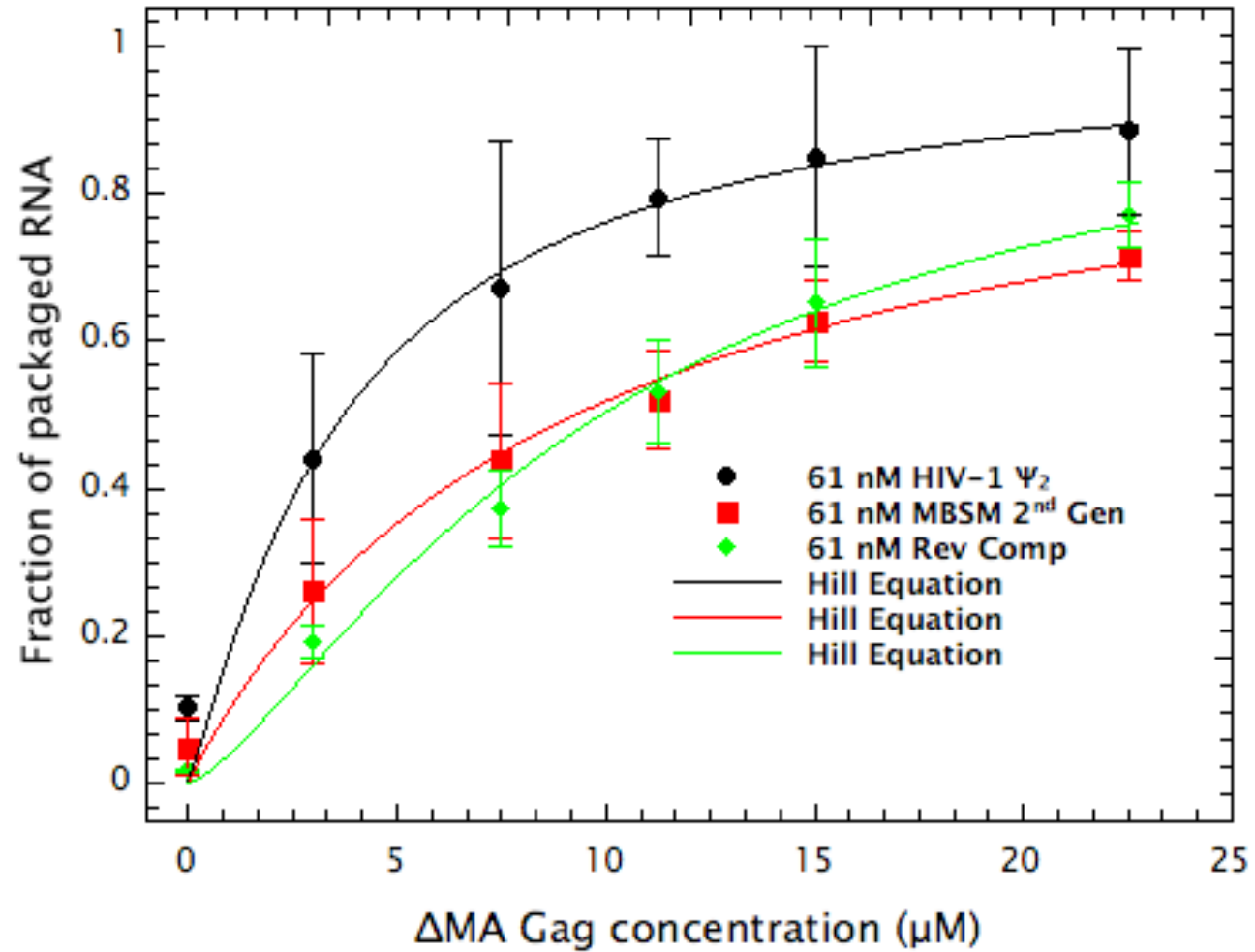
“Low MW”

“Packaged RNA”

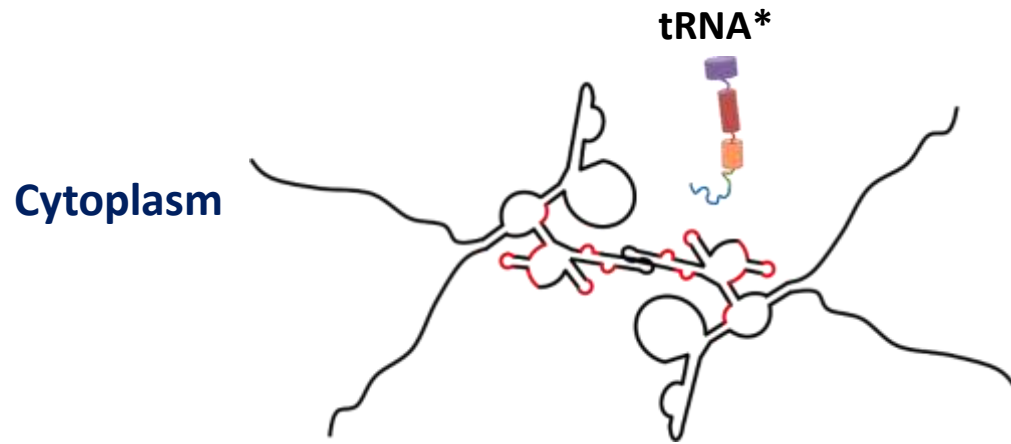
In vitro assembly efficiency



ULTRACENTRIFUGATION

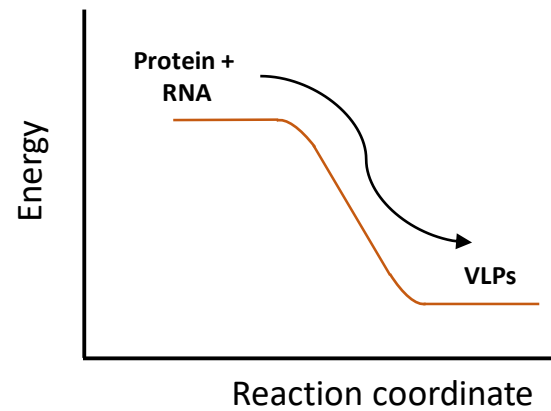
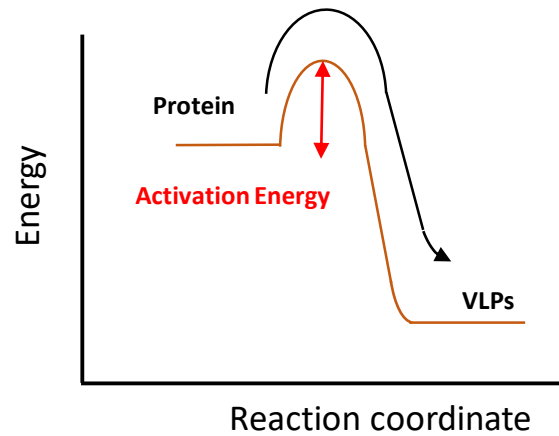


Our model



Without Ψ

With Ψ



- Gag binds to almost any nucleic acid with nM affinity.
- It is only in the presence of “stringent” conditions that specific binding can be revealed (*i.e.*, high-salt, competitor RNAs, inhibiting Gag-Gag interactions).
- Specific binding under “stringent” conditions depends on a series of unpaired guanosines distributed in 6 clusters and not of a particular SL.
- There could be other guanosines outside this cluster that plays a role in assembly and binding.
- The *in vitro* assembly experiments indicate that Ψ lowers the activation energy for assembly.
- Lowering the activation energy requires at least 3 clusters of unpaired guanosines.

**HIV Dynamics and Replication Program (NIH-NCI)
Retroviral Assembly Section (Rein Lab)**

Alan Rein (The boss)

Siddhartha Datta (The underboss)

Laura Baker

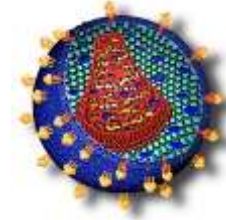
Tomáš Kroupa

Demetria Harvin

Rajat Varma (XENCOR Inc)

Prabhakar Gudla (Leidos Biomedical Inc-NIH)

Stephen Lockett (Leidos Biomedical Inc-NIH)



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Intramural AIDS Targeted Antiviral Program