

Background

- Human Immunodeficiency Virus type 1 (HIV-1) is responsible for the development of Acquired Immunodeficiency Syndrome (AIDS).
- Approximately 37 million people are currently infected by the HIV-1 worldwide (WHO).

World Map of HIV-1 Prevalence

Global HIV Prevalence = 0.8%

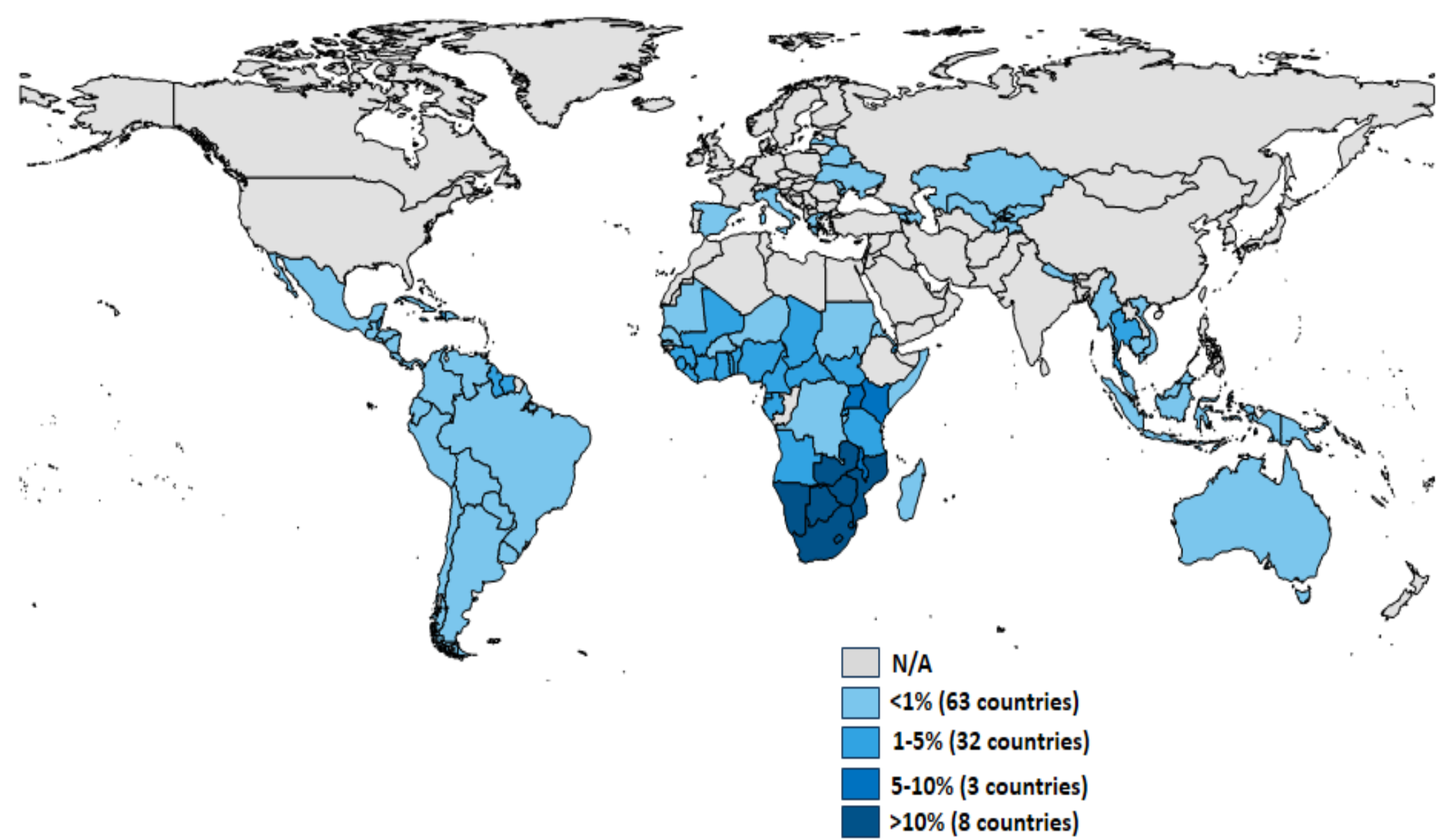


Figure from The Kaiser Family Foundation (2016)

HIV-1 Life Cycle

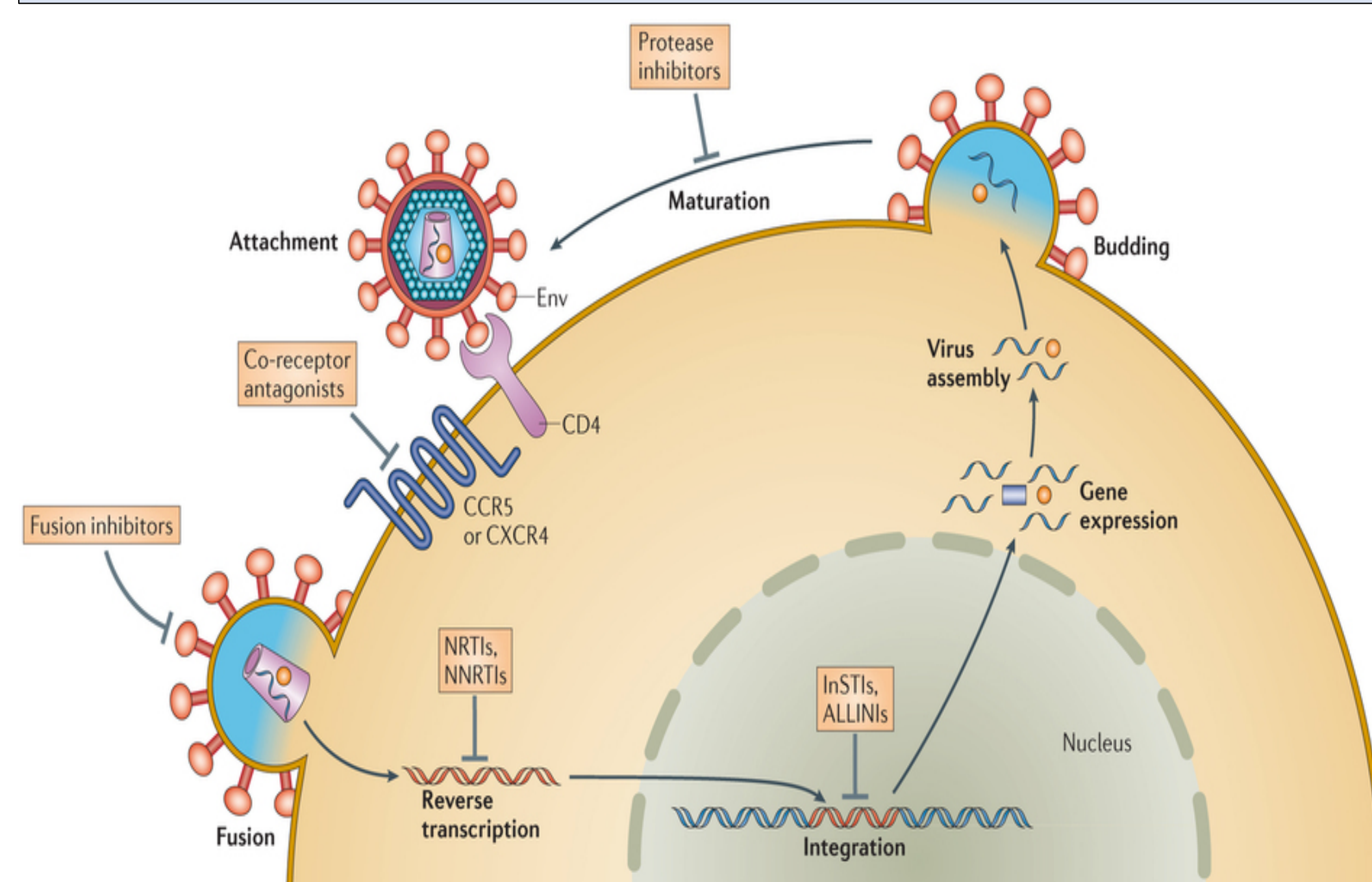


Figure from Laskey, S. B., & Siliciano, R.F. (2014)

Specific Interactions between Gag & Psi Facilitate Genomic RNA Packaging

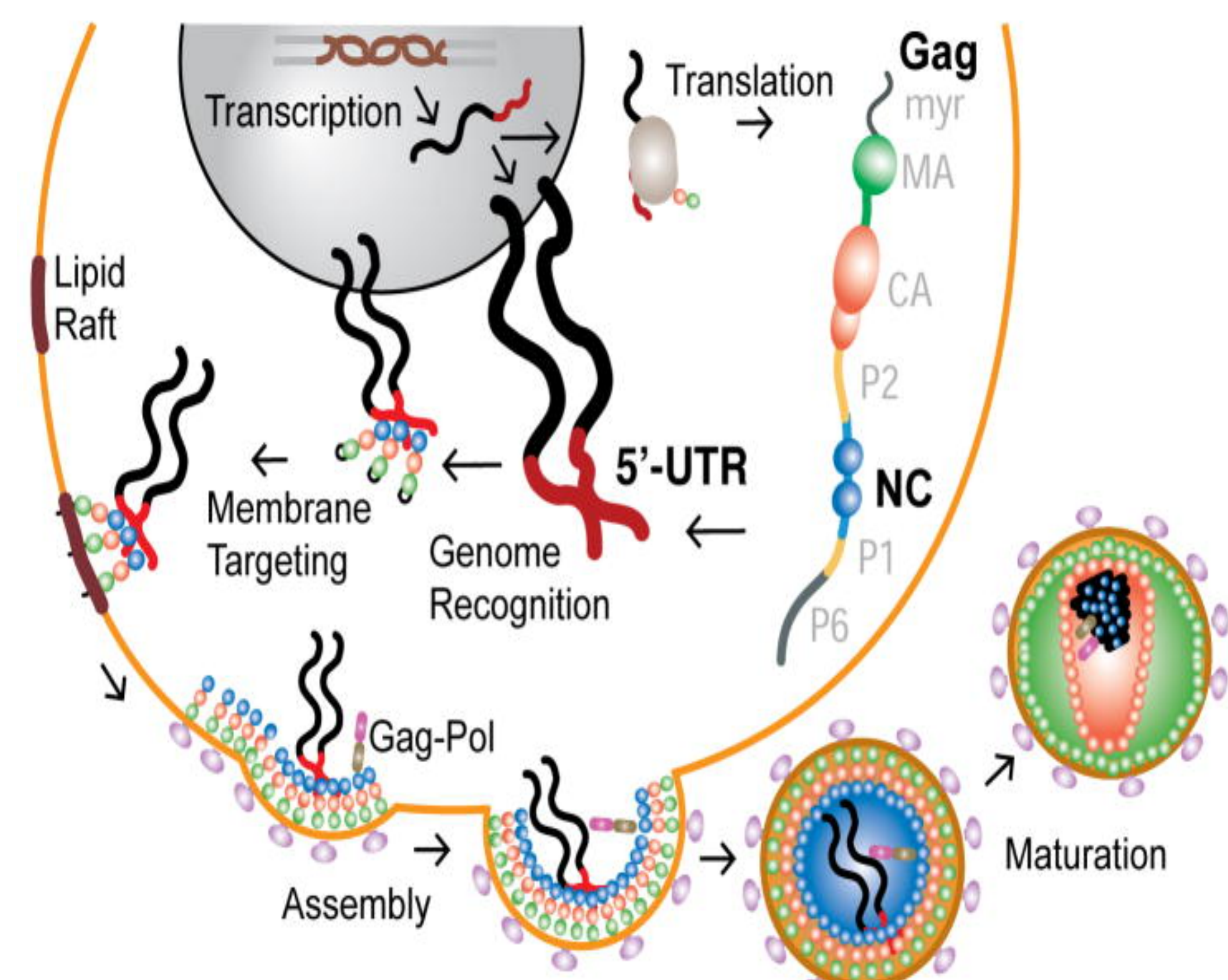


Figure from Lu, K. et. al. (2011)

Model: Psi RNA Alters Gag Binding Mode

Non-Psi RNA binding mode: NC & MA
Psi RNA binding mode: NC-only

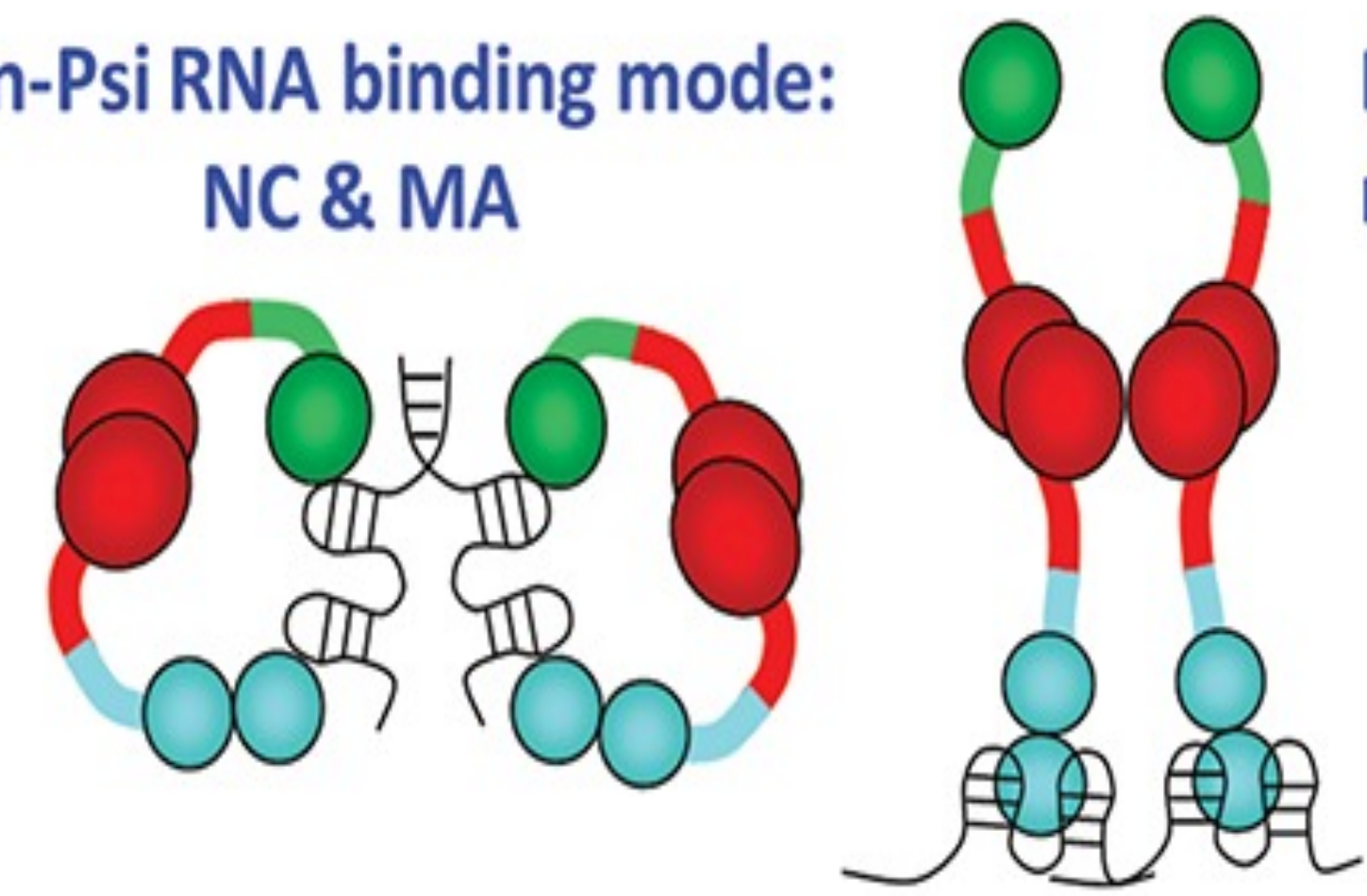


Figure from Webb, J. A. et. al. (2013)

Hypothesis: Positive and Negative Regulatory Elements Flank Psi in the 5'UTR

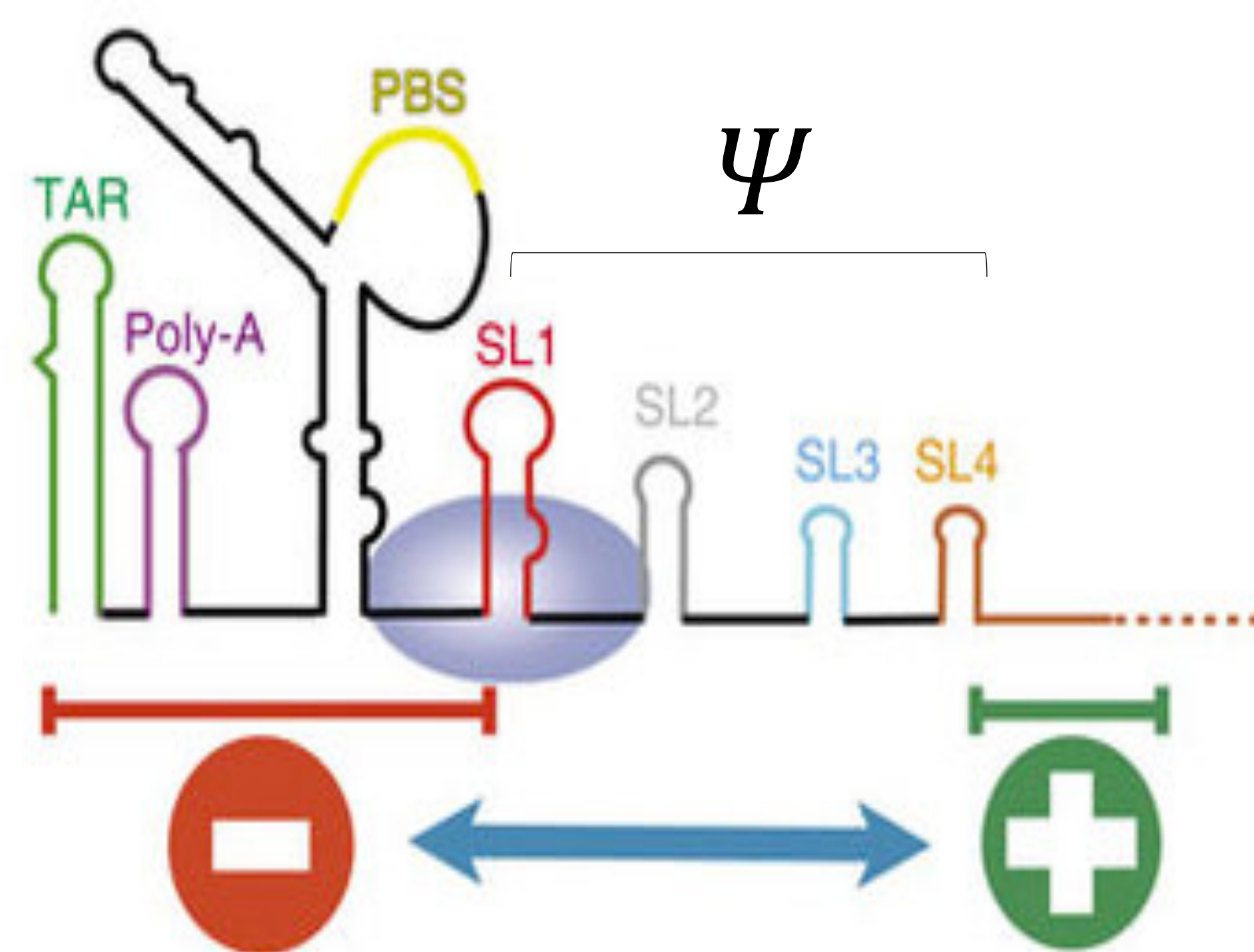


Figure from El-Wahab, E. W. et. al. (2014)

- Recent studies from the Marquet Lab suggest that regions upstream of Psi in the 5'UTR inhibit high affinity Gag binding, allowing for preferential packaging of genomic RNA over viral spliced RNAs.

Experimental 5'UTR Constructs

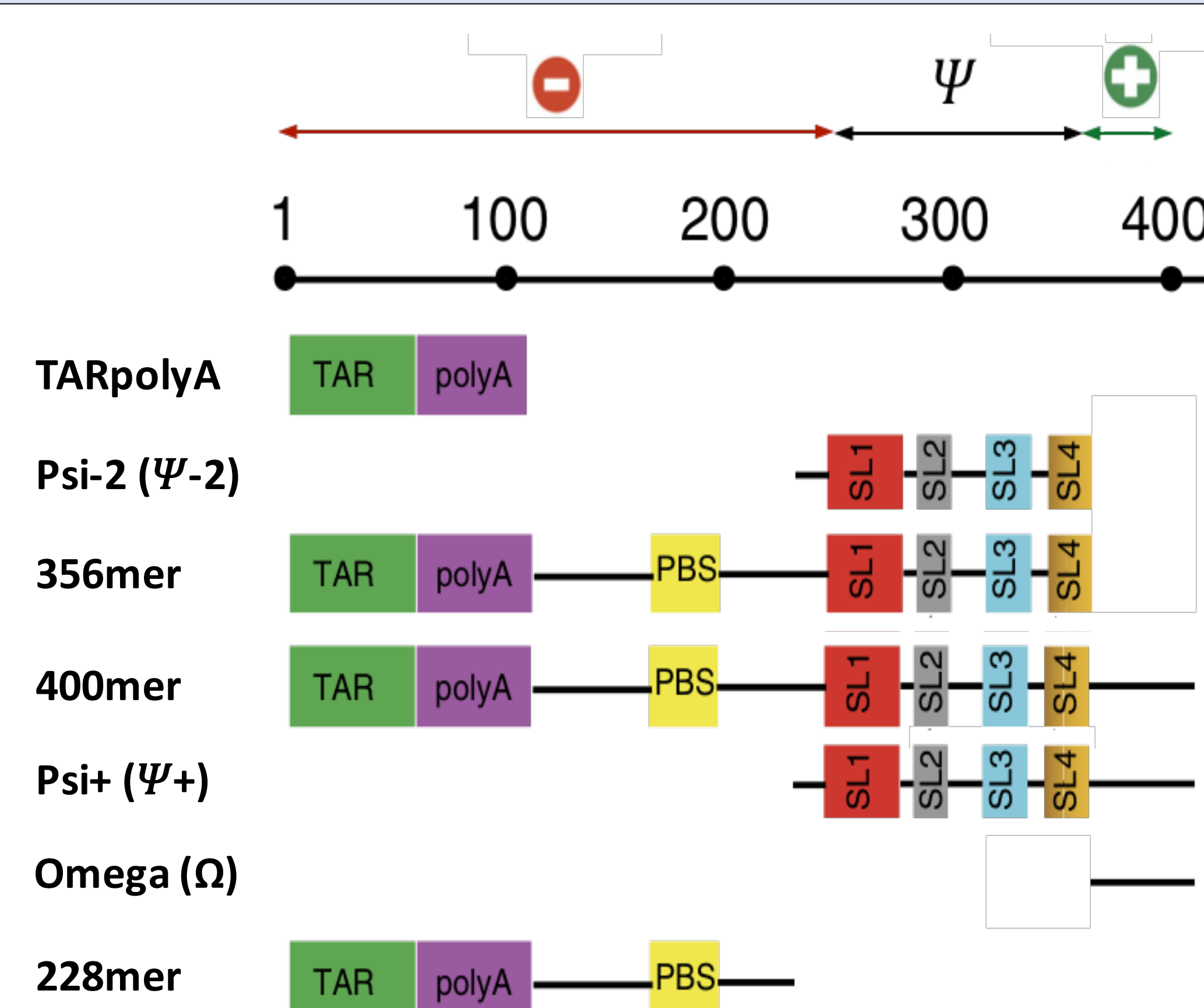
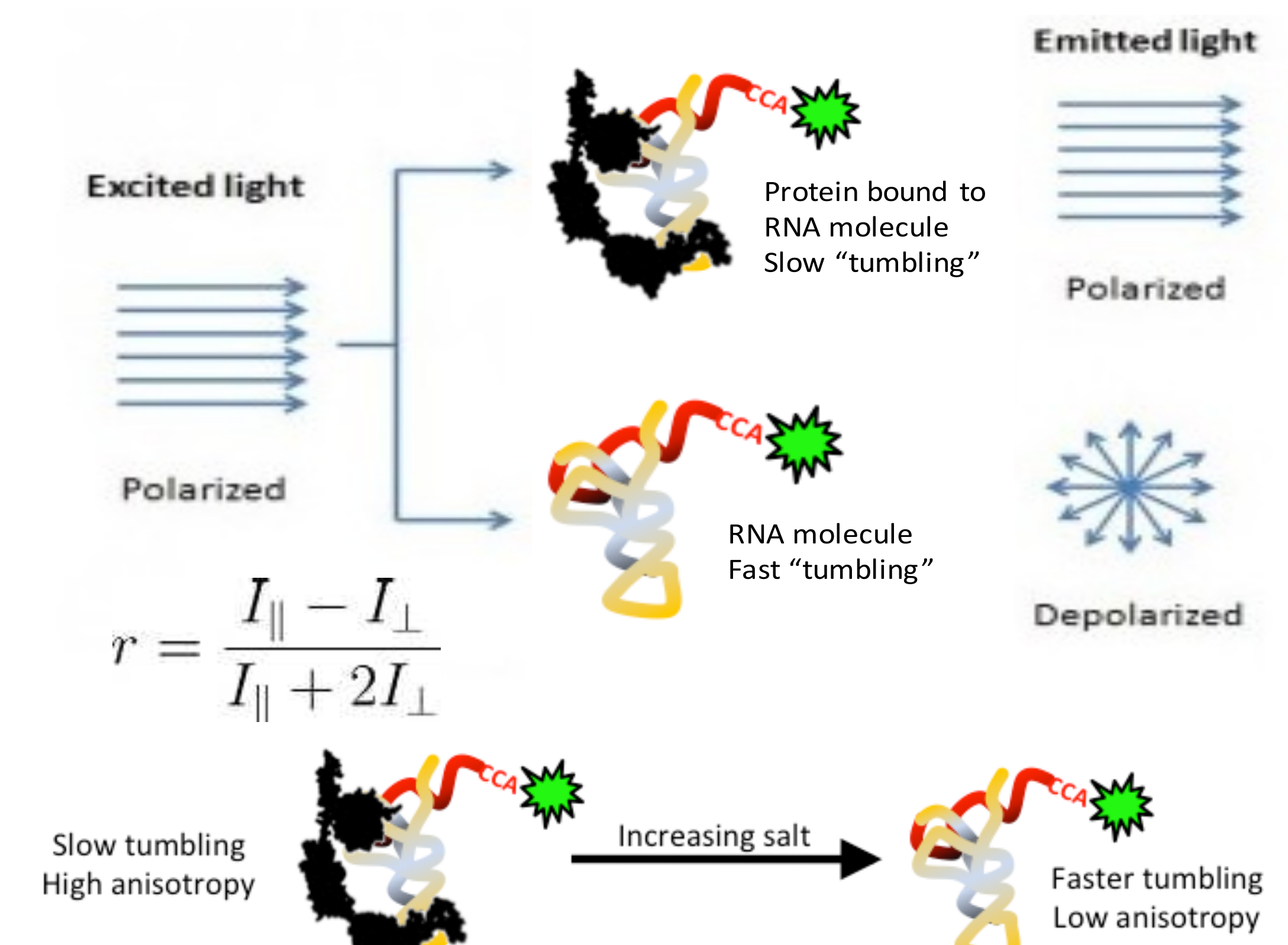
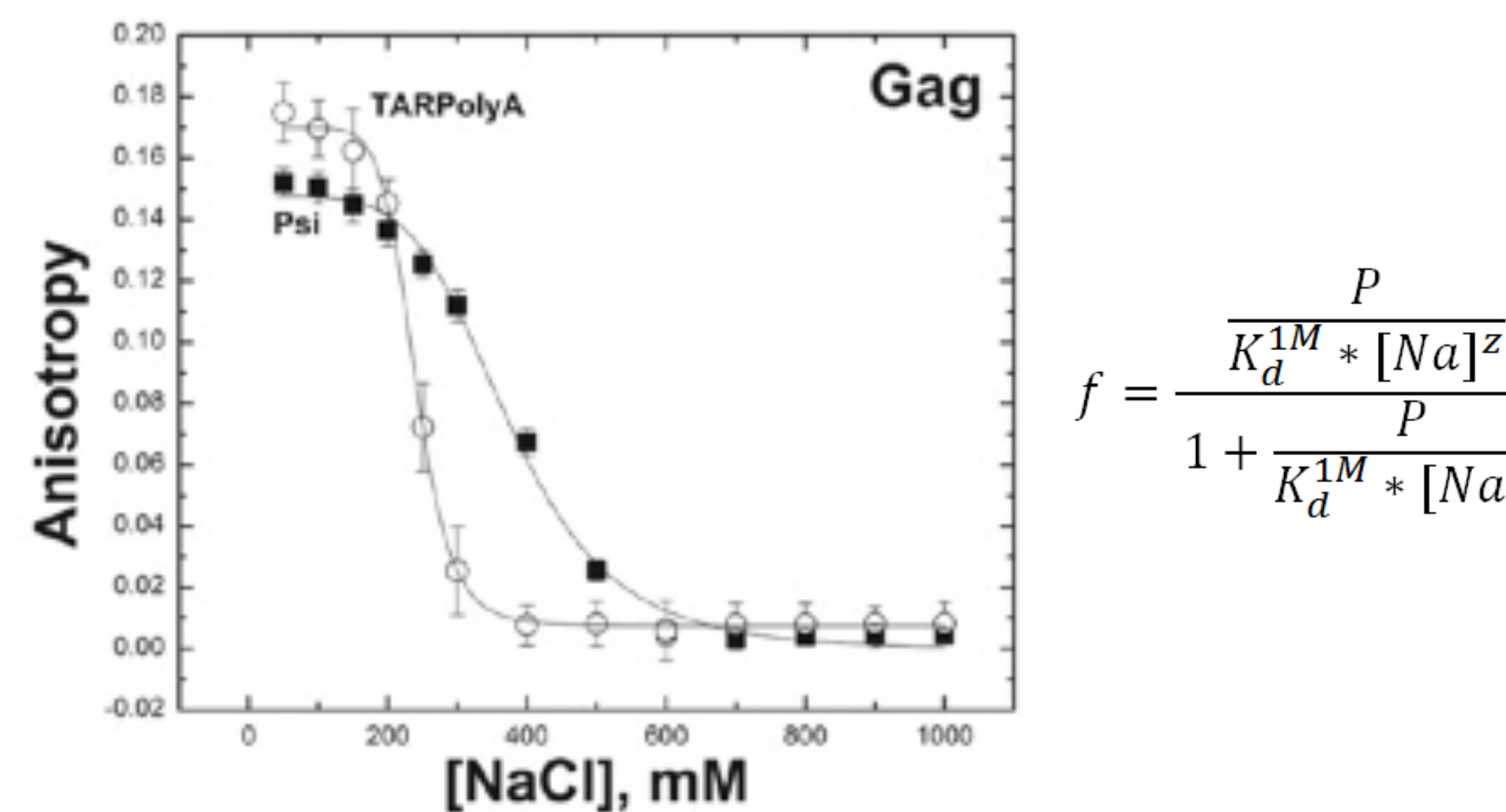


Figure adapted from El-Wahab, E. W. et. al. (2014)

Fluorescence Anisotropy-Based Salt Titration Assays



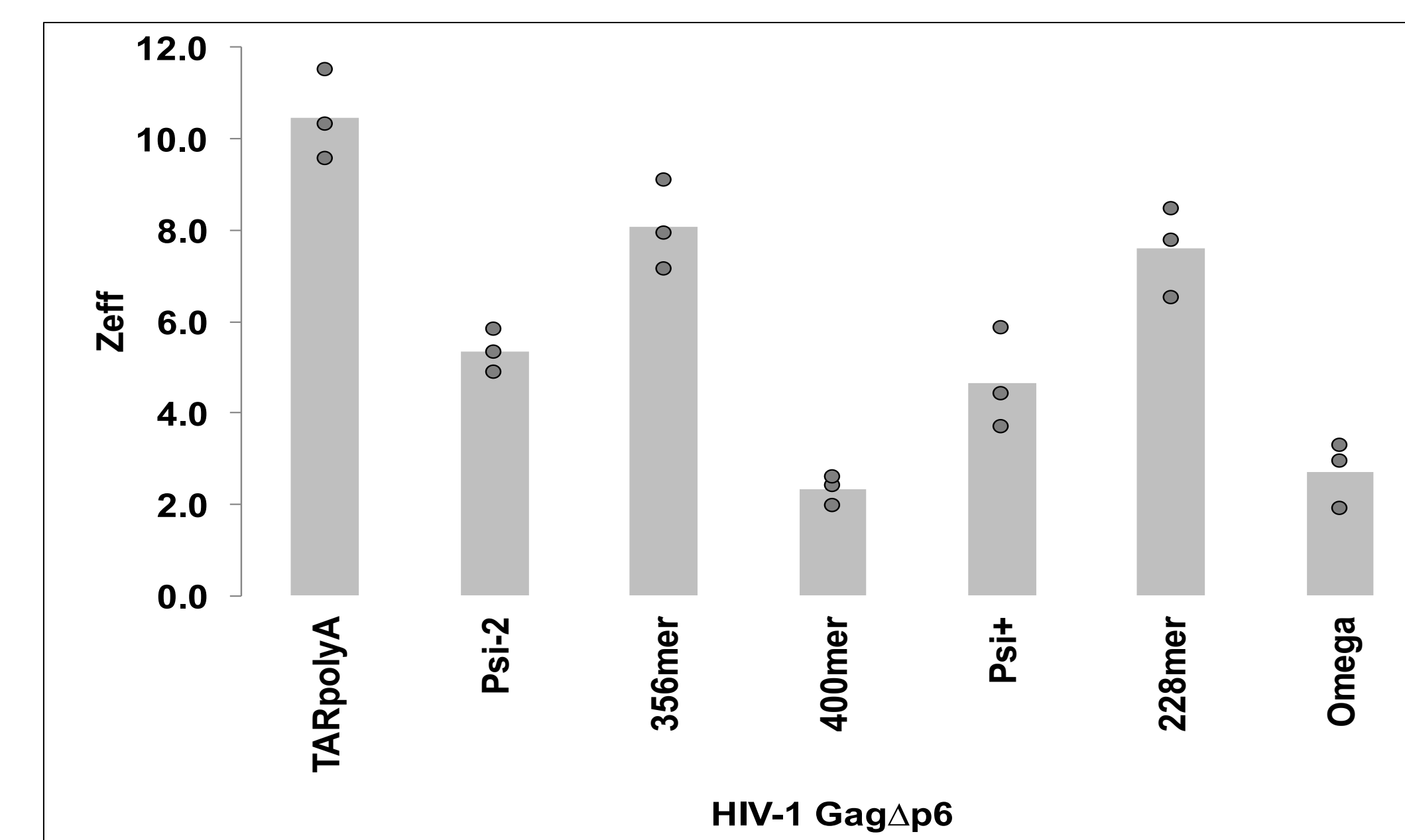
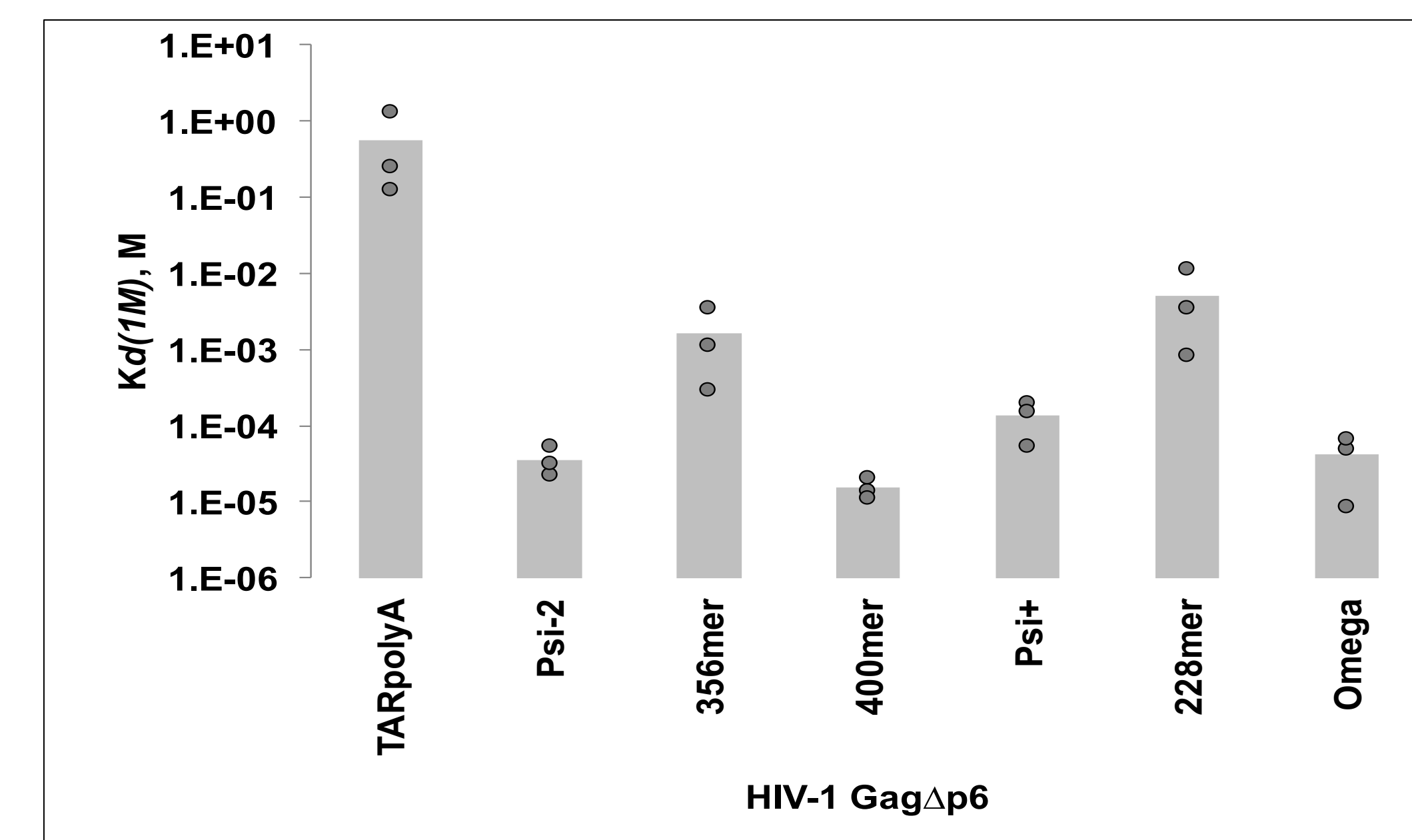
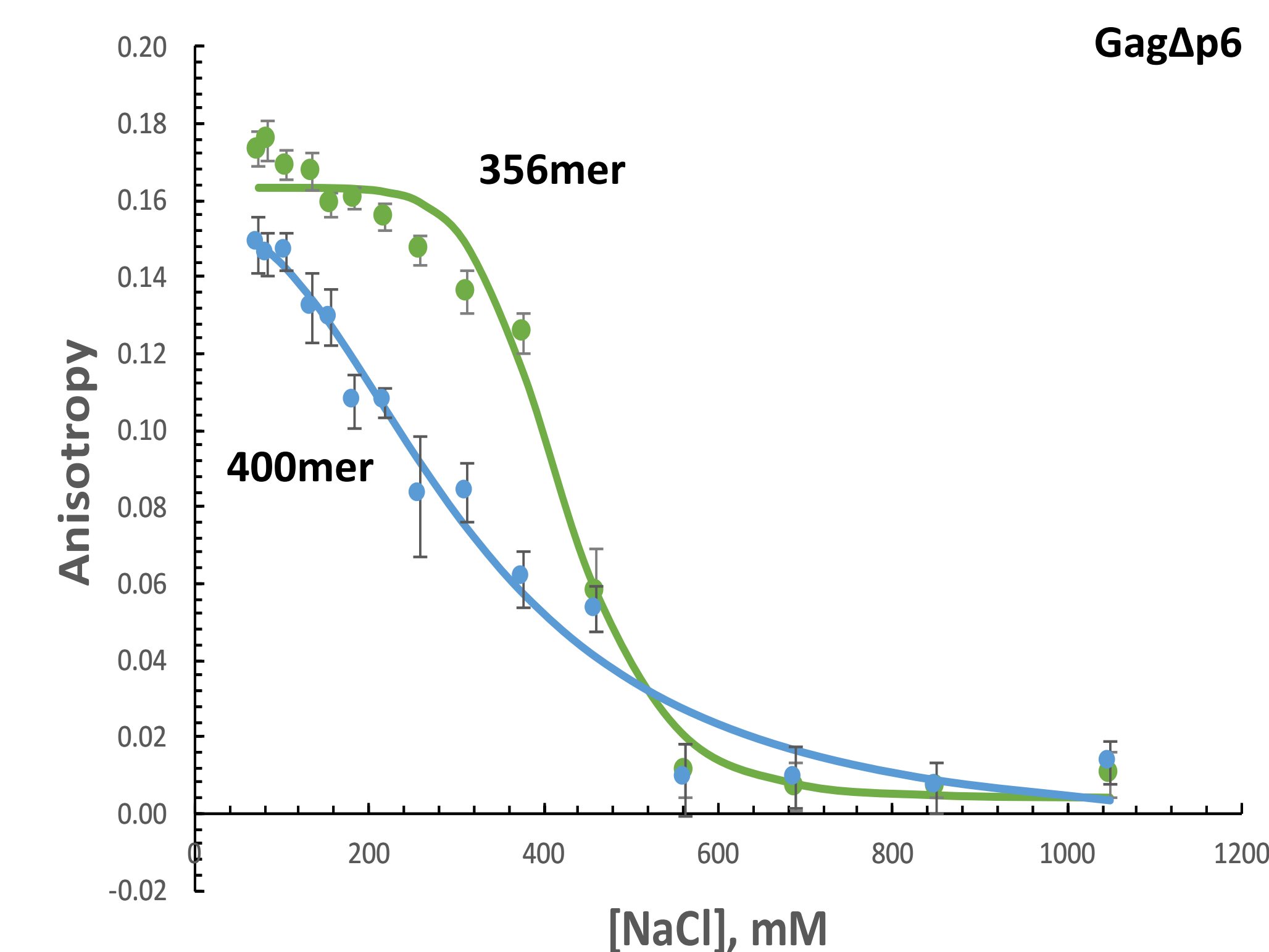
$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$



Figures from Webb, J. A. et. al. (2013)

- $K_{d(1M)}$: Describes the nonelectrostatic (specific) component of binding.
- Z_{eff} : Reflects the number of charges mediating the protein-RNA interaction.

Positive Regulatory Element Required for Specific Gag Binding

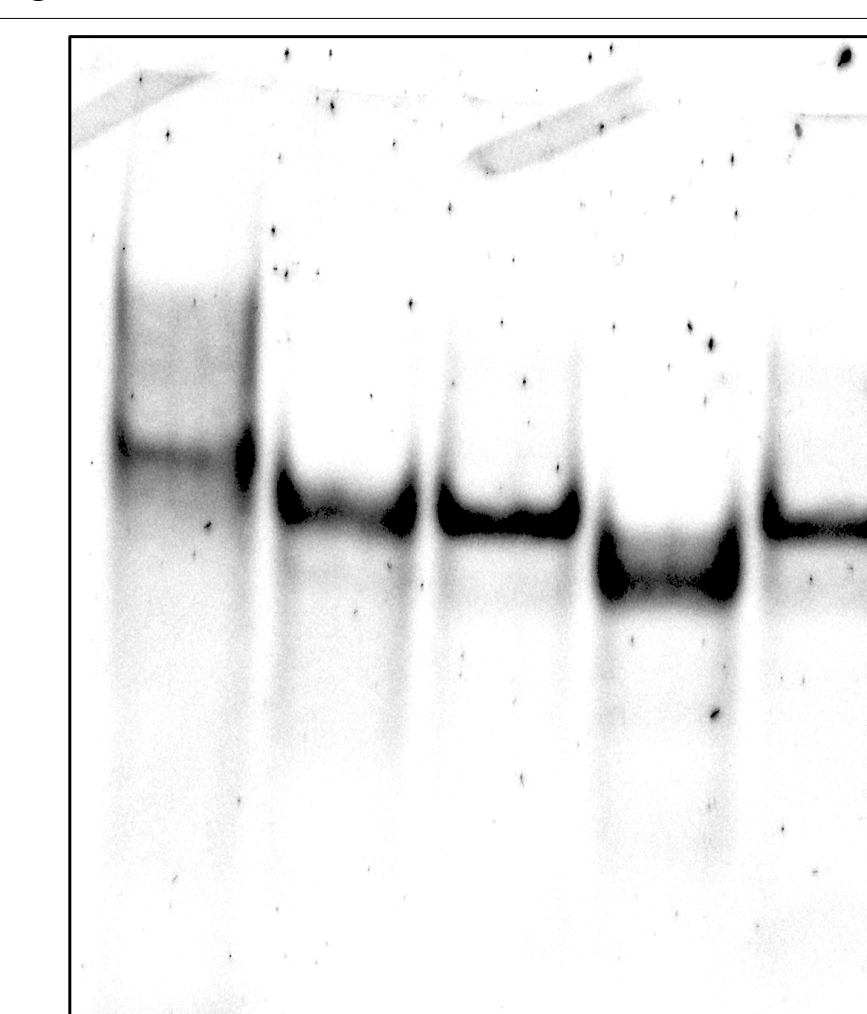


GagAp6 RNA Variant (20.5 nM)	$K_{d(1M)}$	Z_{eff}
TARpolyA	$5.60 (\pm 6.45) \times 10^{-1}$	$10 (\pm 1.0)$
Psi-2	$3.59 (\pm 1.56) \times 10^{-5}$	$5.4 (\pm 0.5)$
356mer	$1.63 (\pm 1.64) \times 10^{-3}$	$8.1 (\pm 1.0)$
400mer	$1.51 (\pm 0.49) \times 10^{-5}$	$2.3 (\pm 0.3)$
Psi+	$1.34 (\pm 0.74) \times 10^{-4}$	$4.7 (\pm 1.1)$
228mer	$5.15 (\pm 5.32) \times 10^{-3}$	$7.6 (\pm 1.0)$
Omega	$4.14 (\pm 3.01) \times 10^{-5}$	$2.7 (\pm 0.7)$

- The negative region present in the HIV 356mer diminishes Psi-like binding. The positive region in addition to the negative region in the HIV 400mer restores Psi-like binding.
- Psi+ interacts similar to Psi-2 suggesting that the positive region does not increase binding specificity by itself.

Native-PAGE Assesses RNA Conformation

Native-PAGE				
RNA	400	400	356	356
Mutant	wt	Δ DIS	wt	Δ DIS
Omega	-	-	-	+



Future Work

- Optimize native gel conditions for HIV 400mer and 356mer.
- Run native gels on all of the experimental constructs to study how RNA conformation changes in the presence and absence of Omega.
- Probe the secondary structure of the HIV 400mer using selective 2'-hydroxyl acylation and primer extension (SHAPE).