Non-equilibrium Physics of Self-Assembly: from Viruses to Nano-containers

ICMS

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# Virus-like particles, packaging signals and assembly pathways

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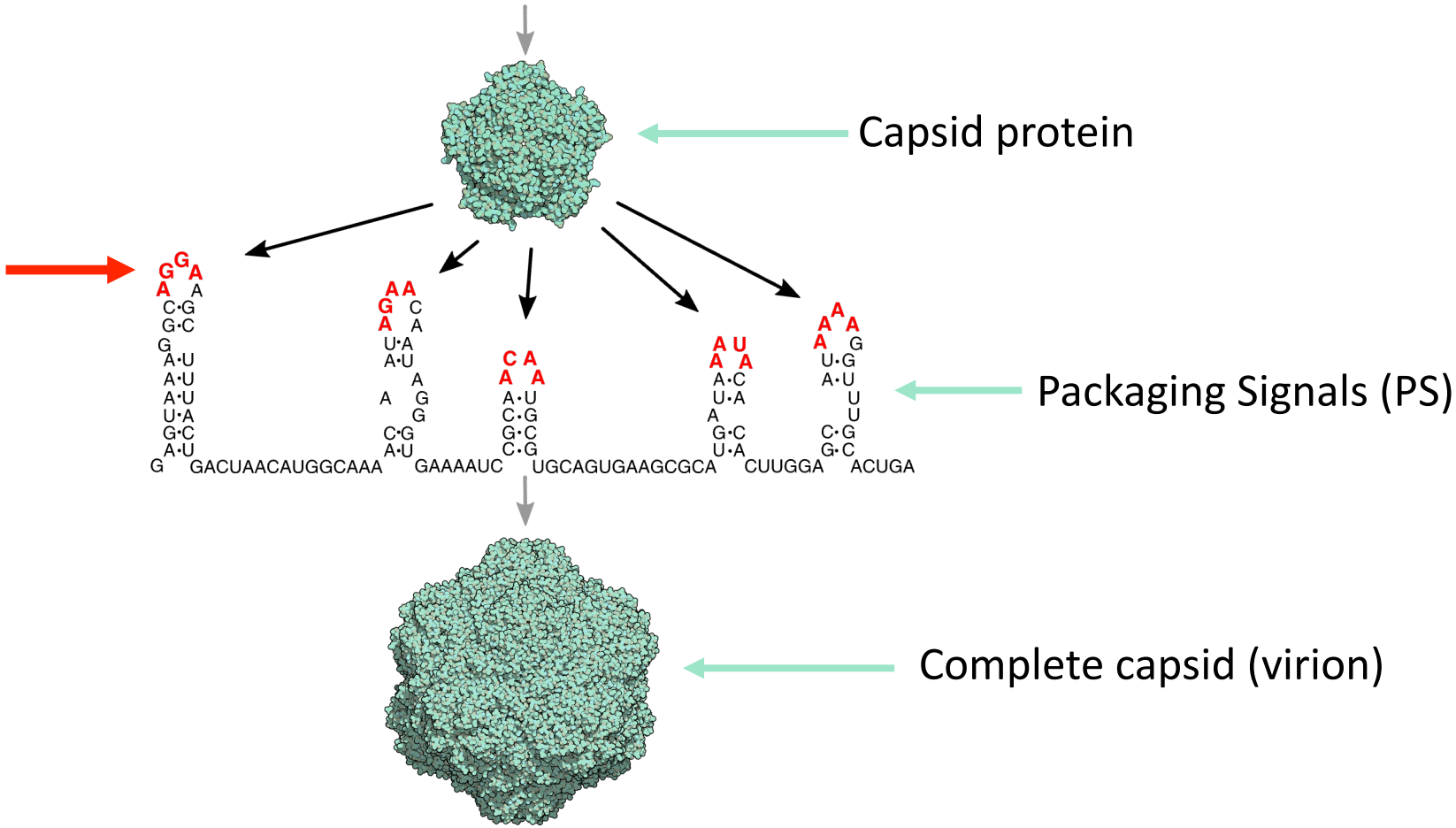
# Virus assembly instructions

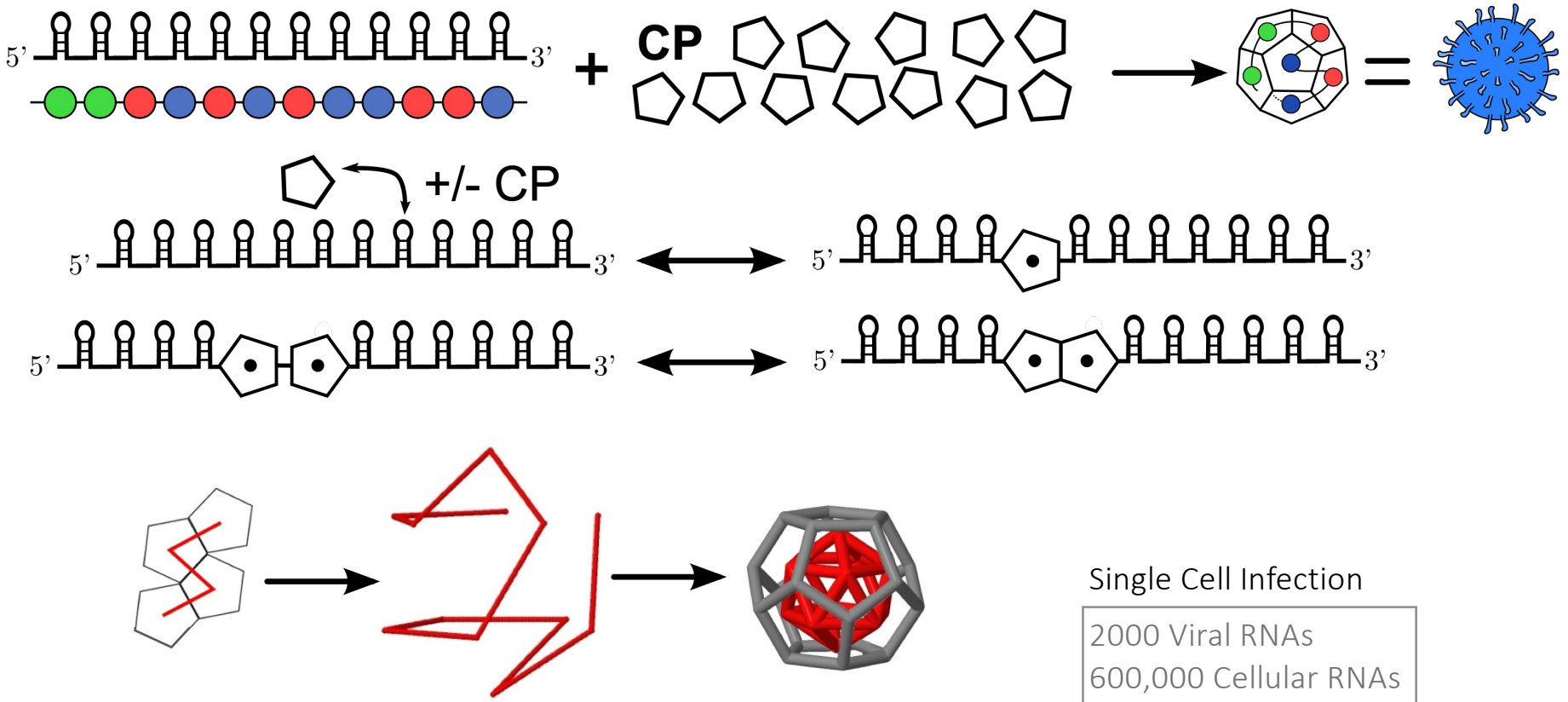
***A virus is a piece of bad news wrapped in protein.*** - Peter Medawar

Genomic material (RNA)

GGCAGGAAGCUUUACUGACUAACAUGGCAAAACAAUAGAACAAUAGGGUGAAAAUCCGCAACAAUGCGUGCAGUGAAGCGCAUGAUAAAUACACACUUGGAGCAUAAAAGGU



# *In Silico* (coarse-grained) viral assembly



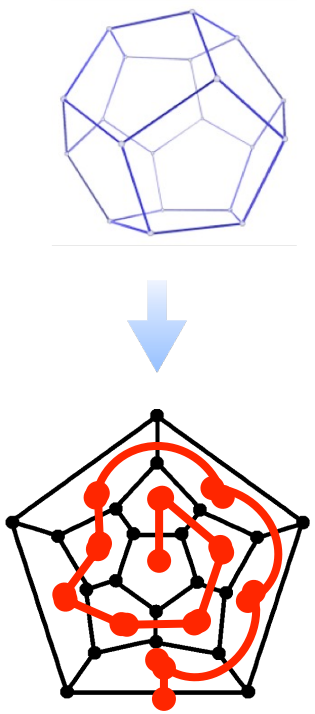

Single Cell Infection

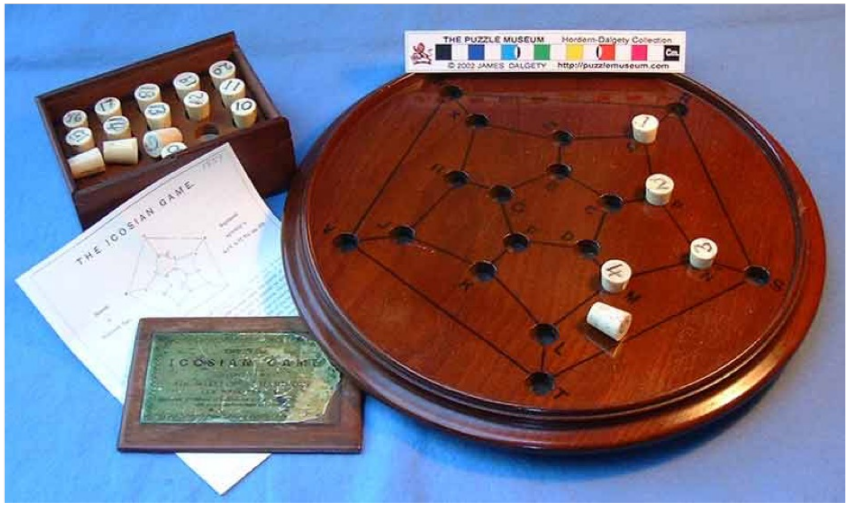
2000 Viral RNAs
600,000 Cellular RNAs
24000 Capsid subunits
Infection cycle : 1000s
Gillespie algorithm

Dykeman et al. *PNAS* **111** 5361-5366 (2014)

# Assembly Pathways

Assembly pathways are one-to-one with Hamiltonian paths



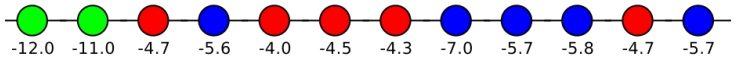



A board game designed by W.R. Hamilton based on the concept of Hamiltonian circuit (cycle)

**RNA Viruses play the Icosian game**

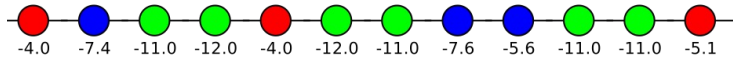
# What makes an efficient PS-assembling virus?

RNA 1

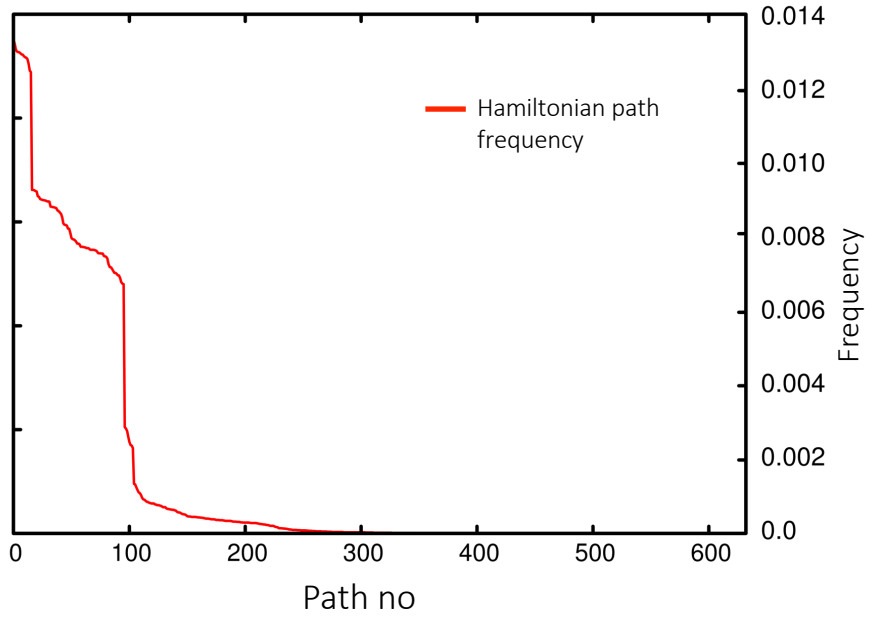


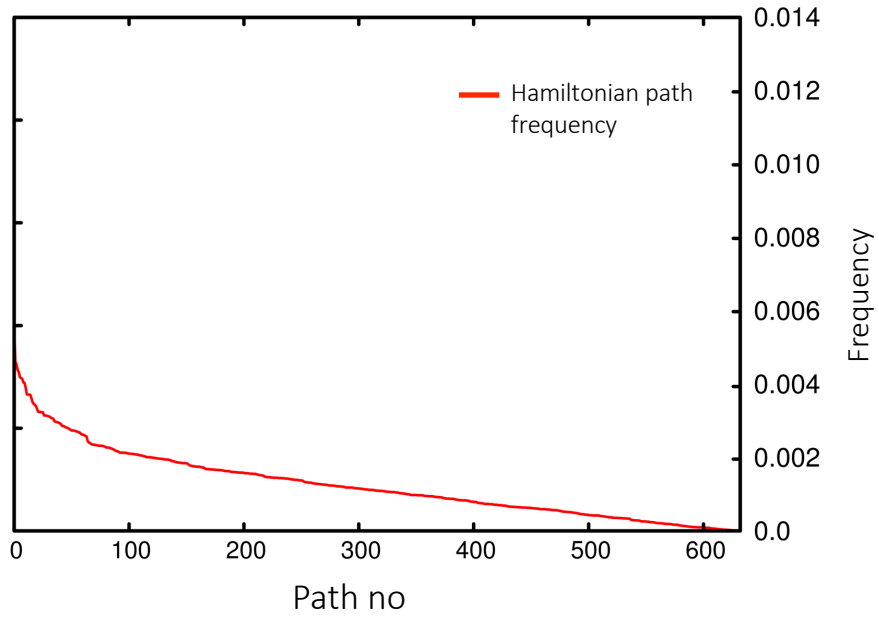

98.175 ± 0.044 % encapsulation

RNA 2



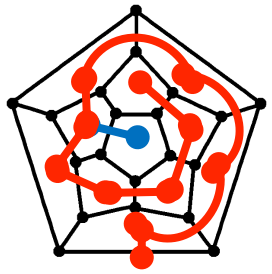

75.864 ± 0.110 % encapsulation



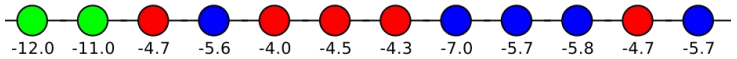




Efficient assembling vRNAs use a smaller subset of assembly pathways.

# What makes an efficient PS-assembling virus?



RNA 1

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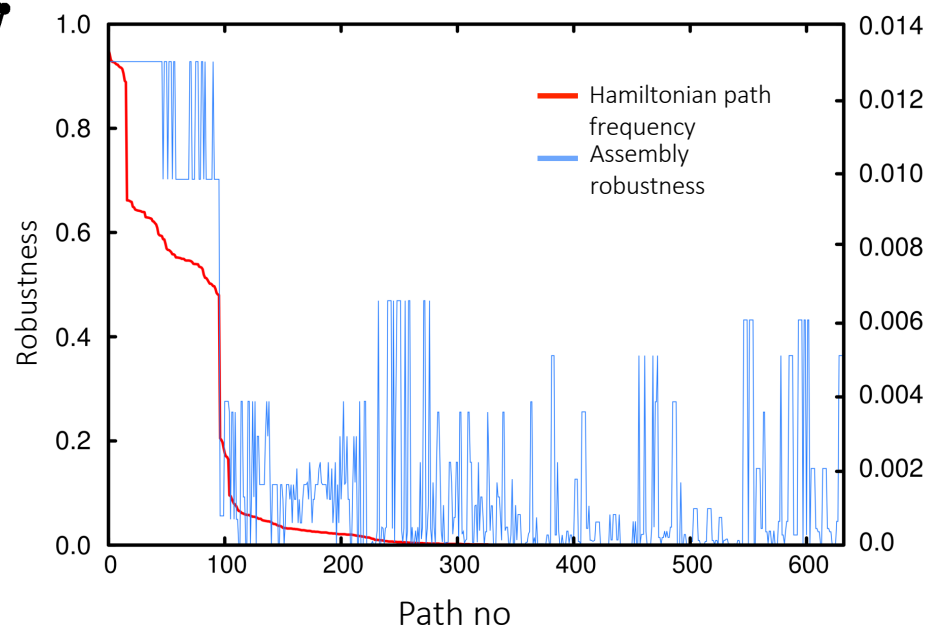
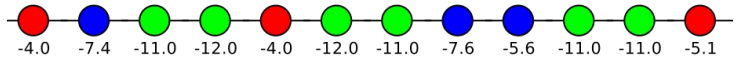



RNA 2

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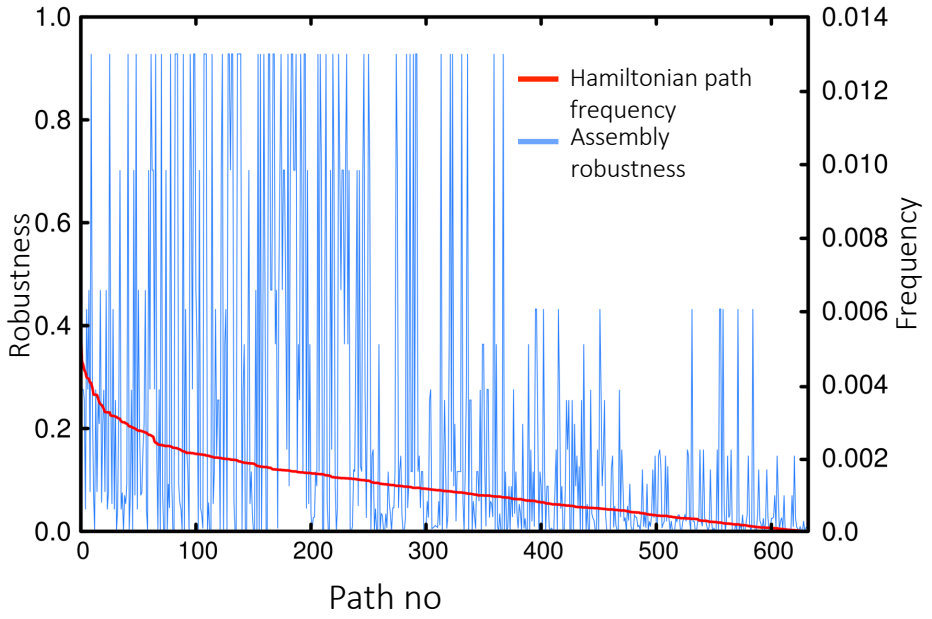
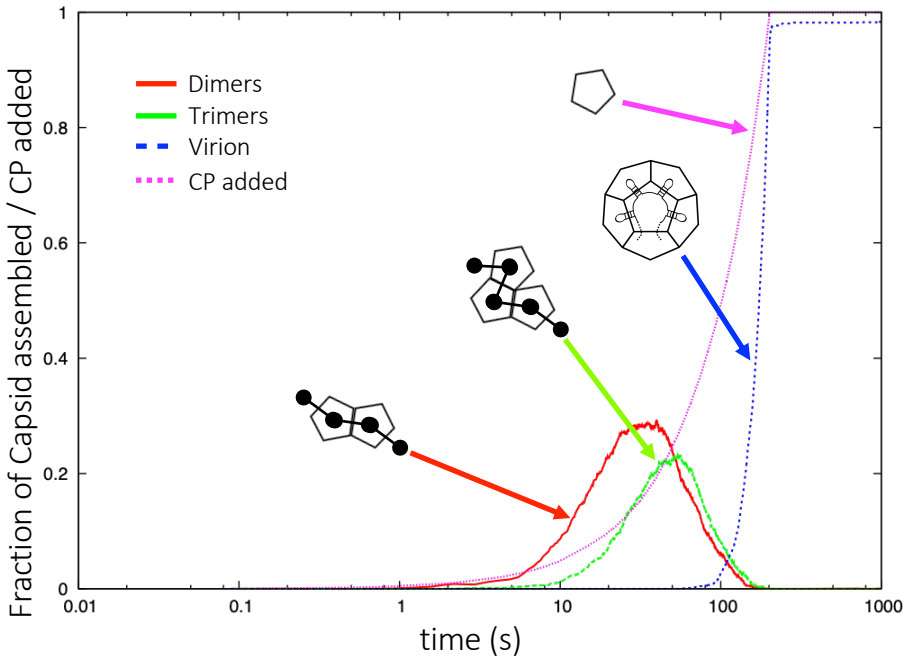
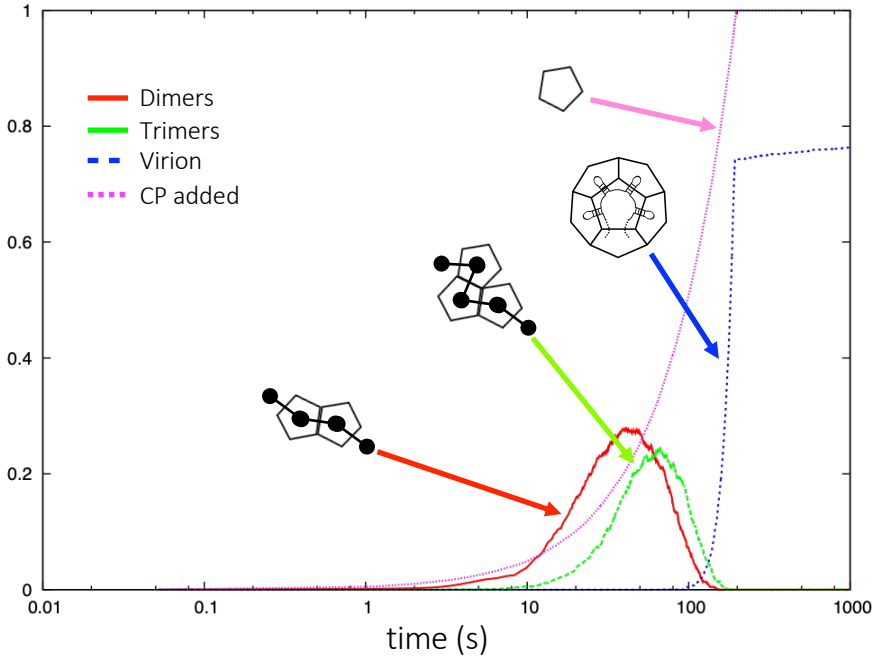


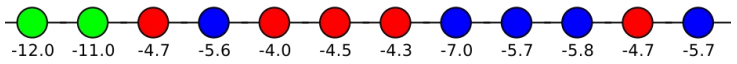

Efficient assembling vRNAs use assembly pathways that are less likely to get trapped

# Assembly efficiency as a measure of fitness




RNA 1

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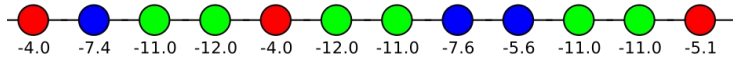
RNA 2

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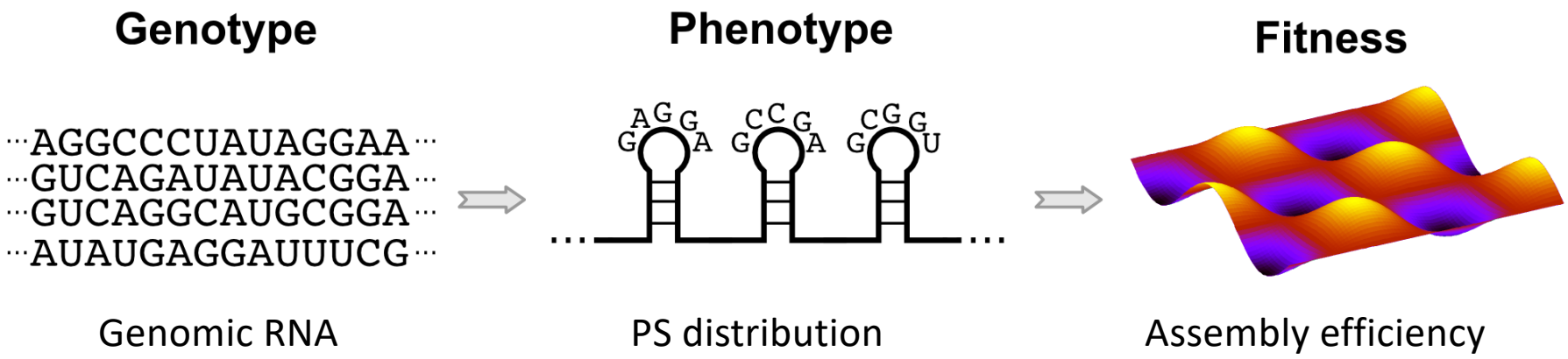
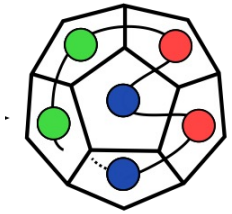
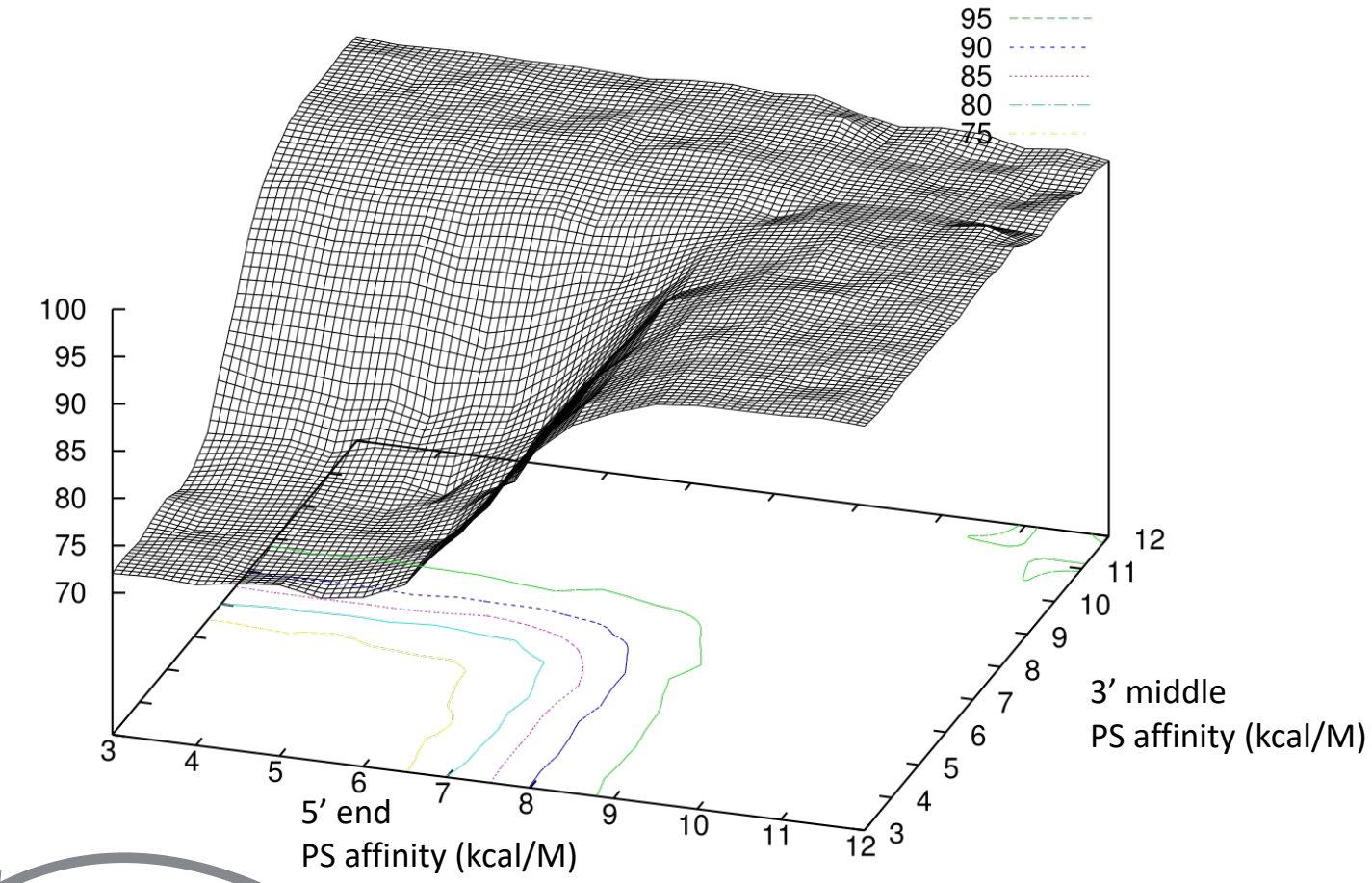
# Genotype-Phenotype-Fitness Maps



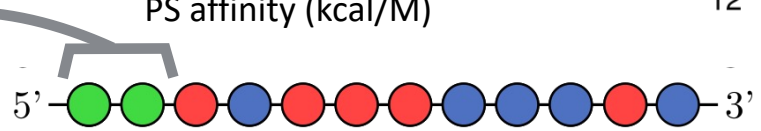
# Fitness landscapes



Fitness/
%Assembled

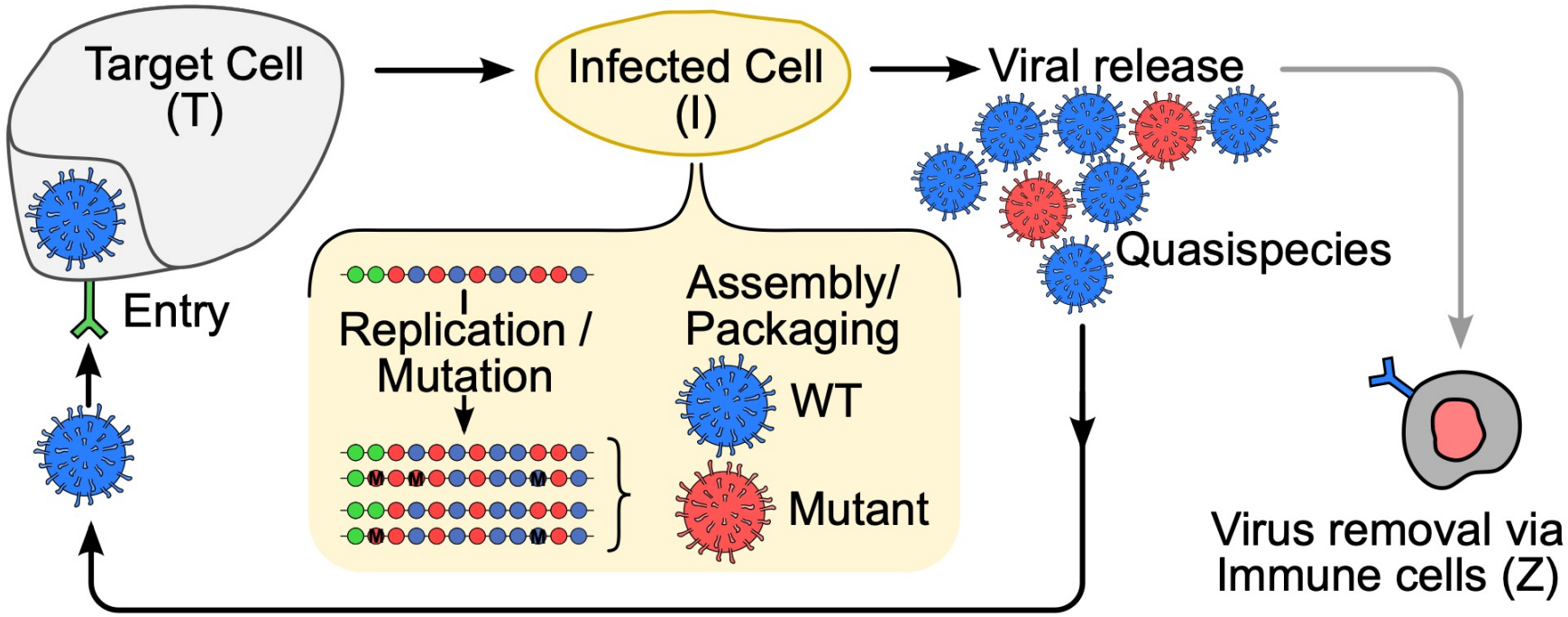



Important for
nucleation




Important for geometry
of capsid formation

# Infection model




Bingham *et al.* Viruses (2017)

# Infection model (via Nowak & May)

$$T \xrightarrow{\lambda} 2T \quad \text{(Target cell birth)}$$

$$T \xrightarrow{d_T} 0 \quad \text{(Target cell death)}$$

$$T + V_j \xrightarrow{\beta} I_j \quad \text{(Infection of target cell by phenotype j)}$$

$$I_j + Z \xrightarrow{p} Z \quad \text{(Infected cell removal by immune system)}$$

$$I_j \xrightarrow{a} \sum_l k_l V_l \quad \text{(Infected cell death/lysis)}$$

$$V_j + Z \xrightarrow{u} Z \quad \text{(Removal of virion by immune system)}$$

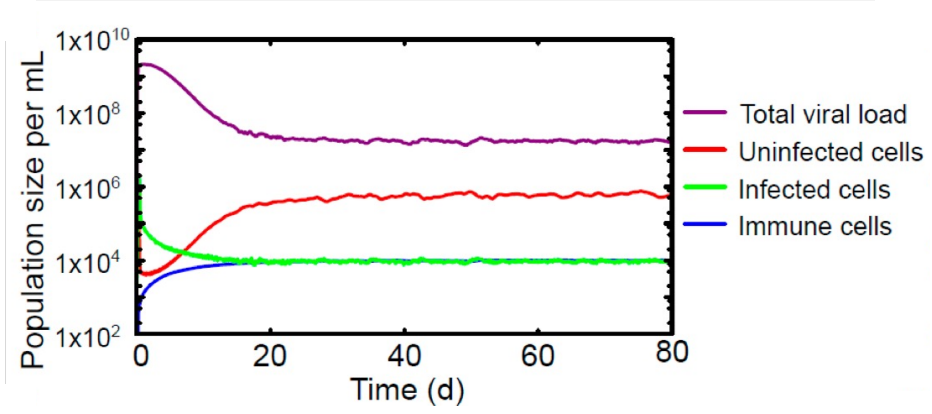
$$I + Z \xrightarrow{c} I + 2Z \quad \text{(Immune cell birth)}$$

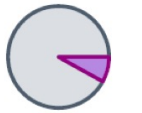
$$Z \xrightarrow{b} 0 \quad \text{(Immune cell death.)}$$

$10^6$ Target cells
$10^7$ Virions
$10^4$ Infected cells
Infection cycle :100d
Gillespie algorithm

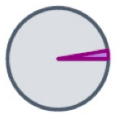
# Treating chronic infections (HCV)

**Infection dynamics**



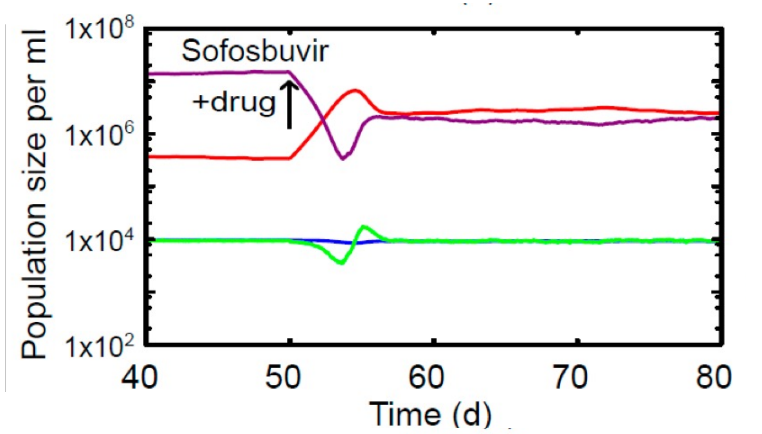



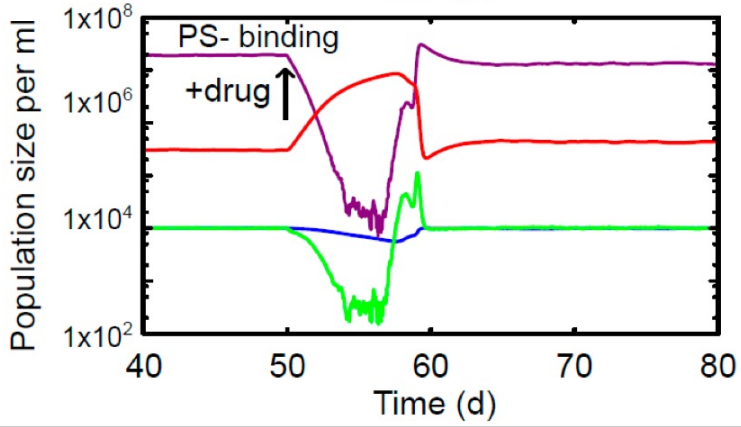
PSI-7977
(Sofosbuvir)
8% escape rate



PS- binding
2.6% escape rate

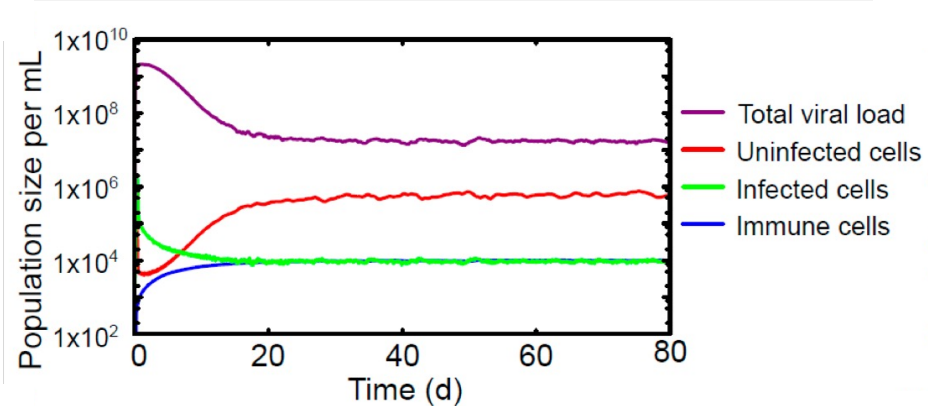
**Emergence of resistance**



# Treating chronic infections (HCV)

**Infection dynamics**

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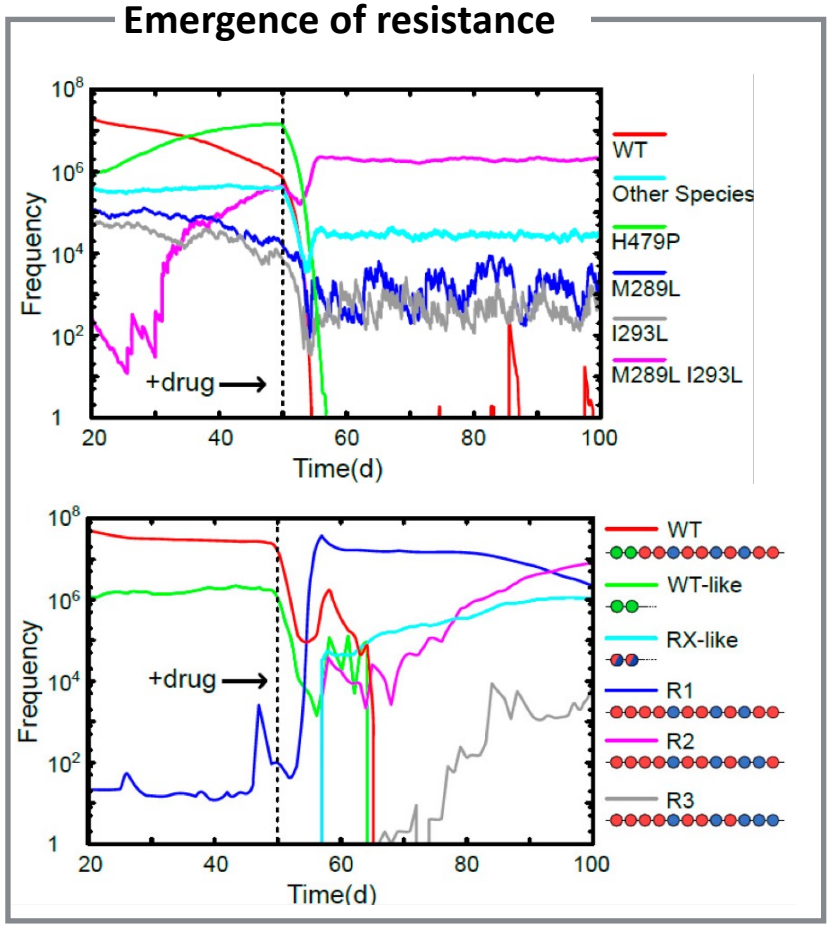
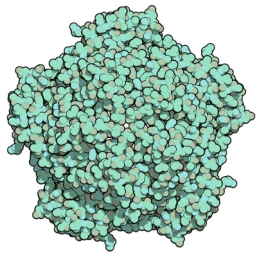
**Emergence of resistance**



# Finding Packaging Signals

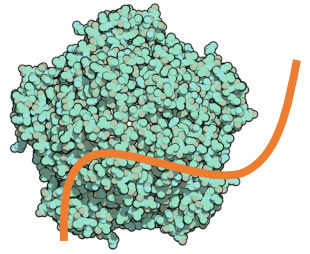
PS are degenerate, dispersed, sequence/structure motifs

Systematic Evolution of Ligands by EXponential enrichment

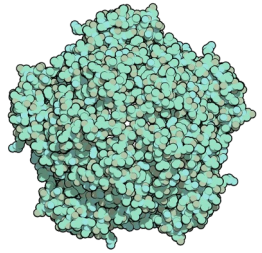


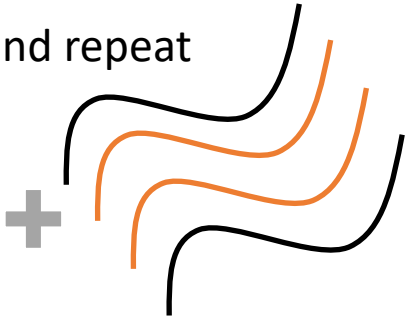
Viral Capsid Protein

Random RNA Aptamers

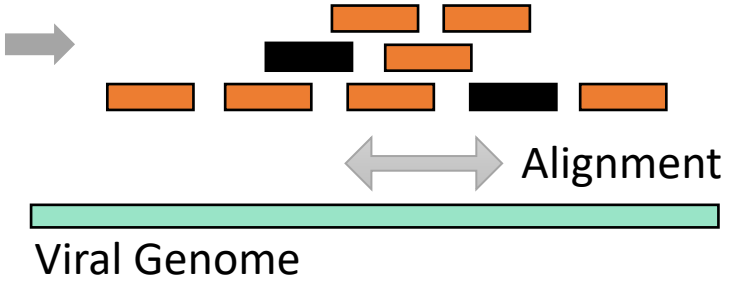


Protein+RNA binding





PCR and repeat



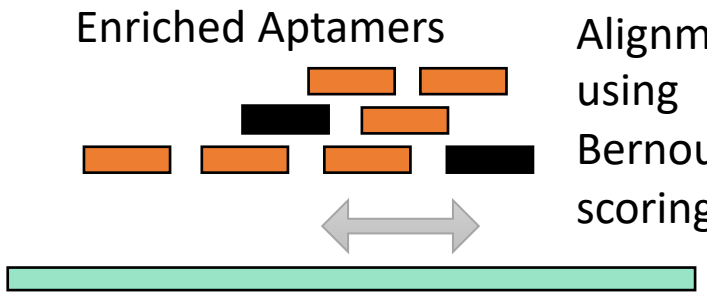

Enriched Aptamers

Viral Genome

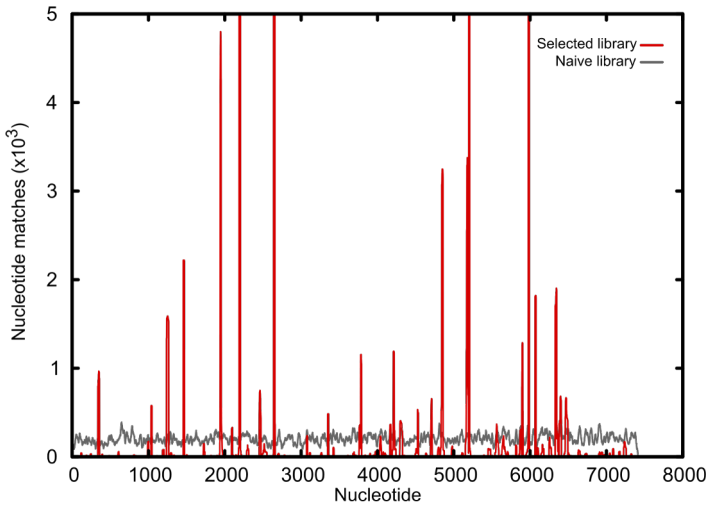
Alignment needs to take into account for a lot of mis-matches

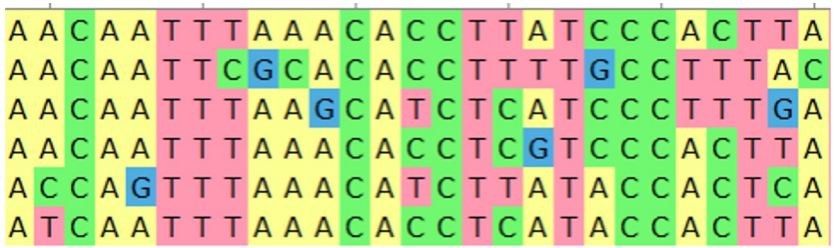
# Selex analysis

Systematic Evolution of Ligands by EXponential enrichment

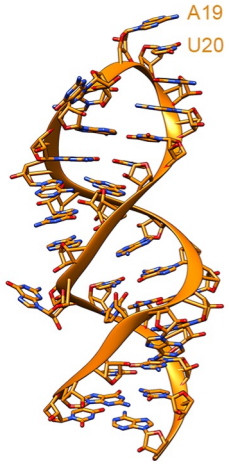


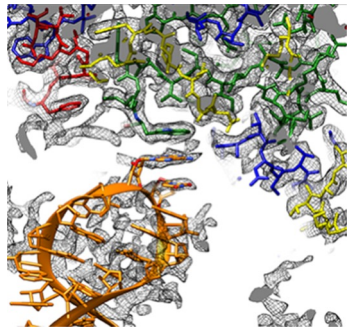

Bernoulli scoring




Conservation & Secondary Structure analysis

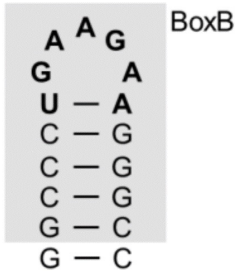


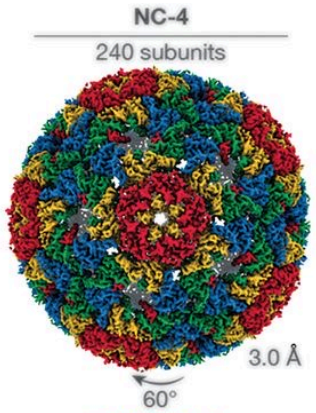
Cryo-EM validation

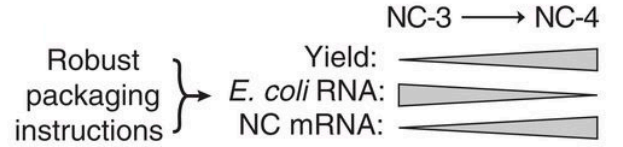
Packaging signals characterised in:
HBV (*Nat Microbiol* **2** *10*)
HCV(*Sci Rep* **6** *1*)
HPeV (*Nat Comms* **8** *5*)
EV-E *(PLoS Path* **16** *12*)
among others...

# Virus-like particles (VLPs)

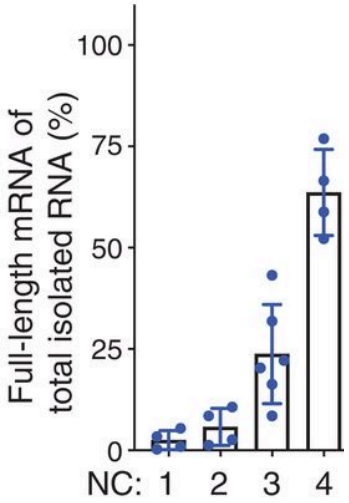




Directed Evolution

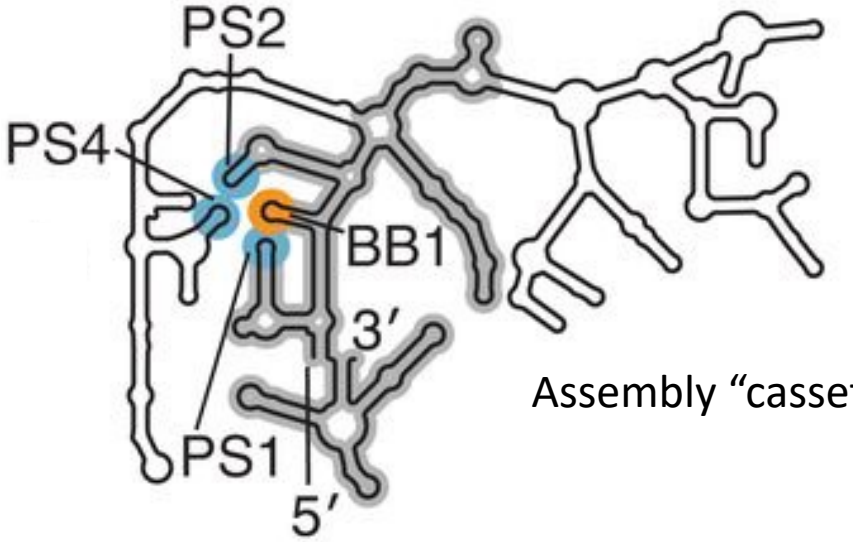



Increased efficiency & specificity




XRF analysis &
RNA Secondary structure sampling & prediction

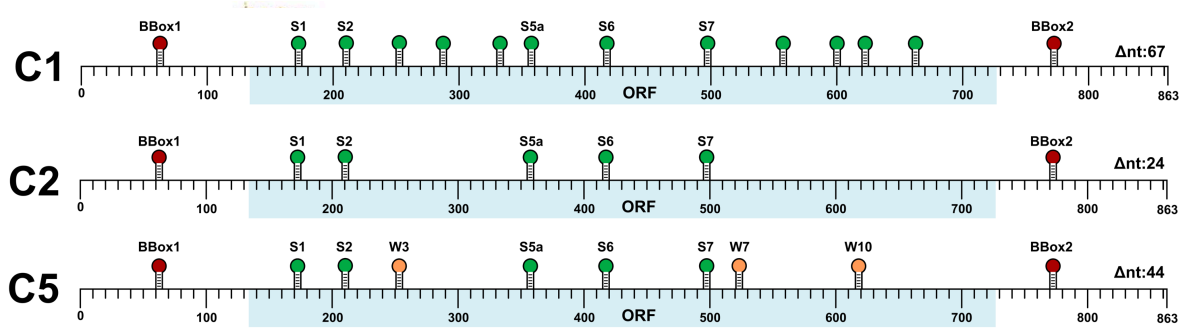


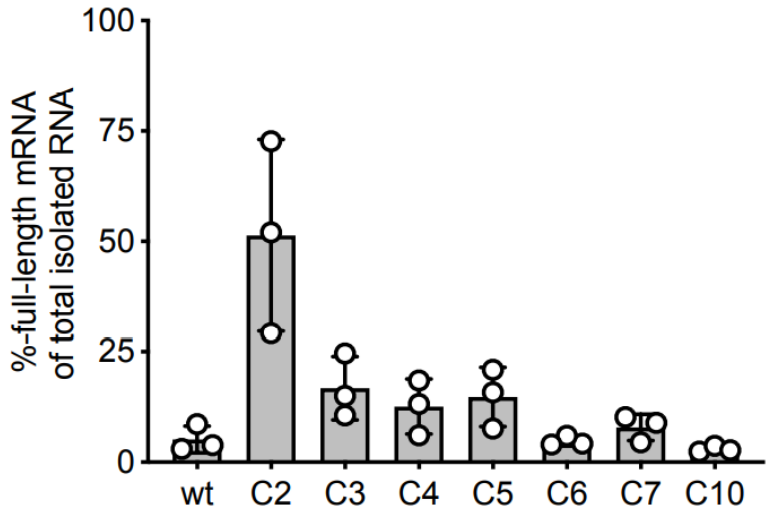

Assembly “cassette”

Tetter *et al.* Science **372**, *220* (2021)

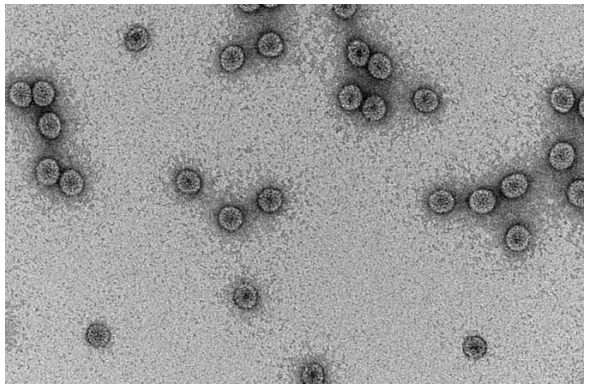
# Designing virus assembly instructions

Can we design VLPs rather than evolve them?

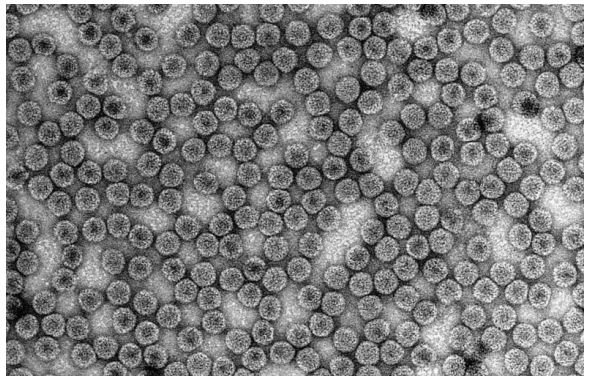



WT



C2



*In preparation.*

# Thanks

Reidun Twarock, Sam Clark, Farzad Fatehi, Eric Dykeman (York)

Peter Stockley & group (Leeds)

Don Hilvert & group (Zurich)