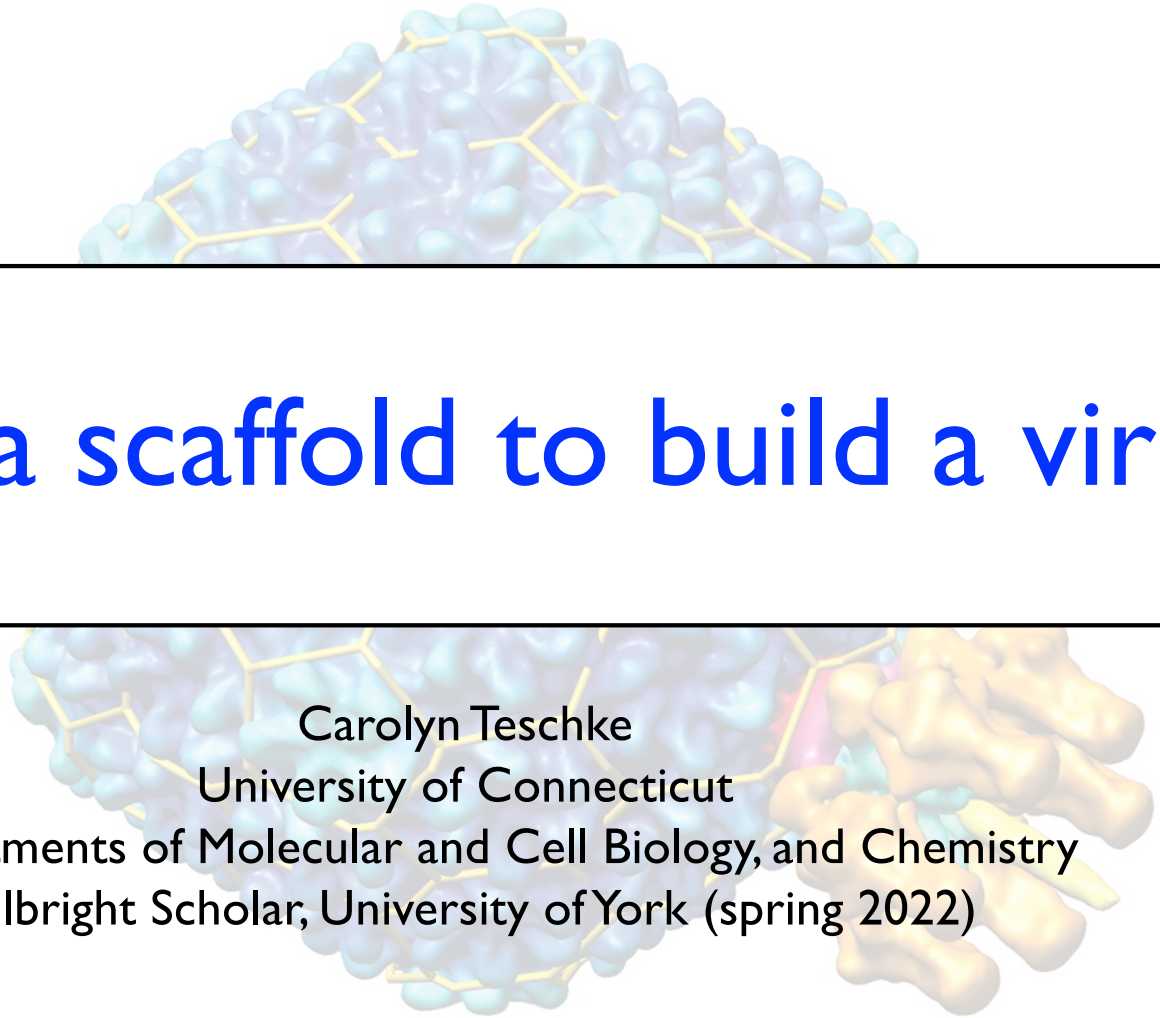

Using a scaffold to build a virus

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Acknowledgements

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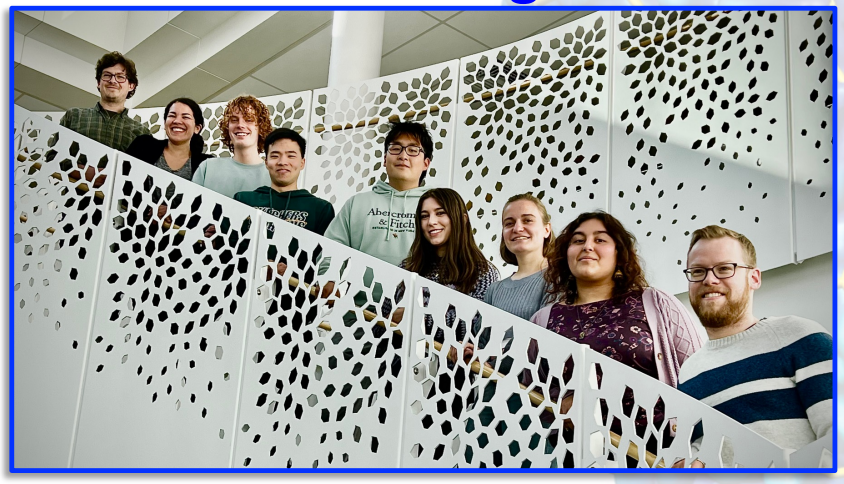
Team Phage



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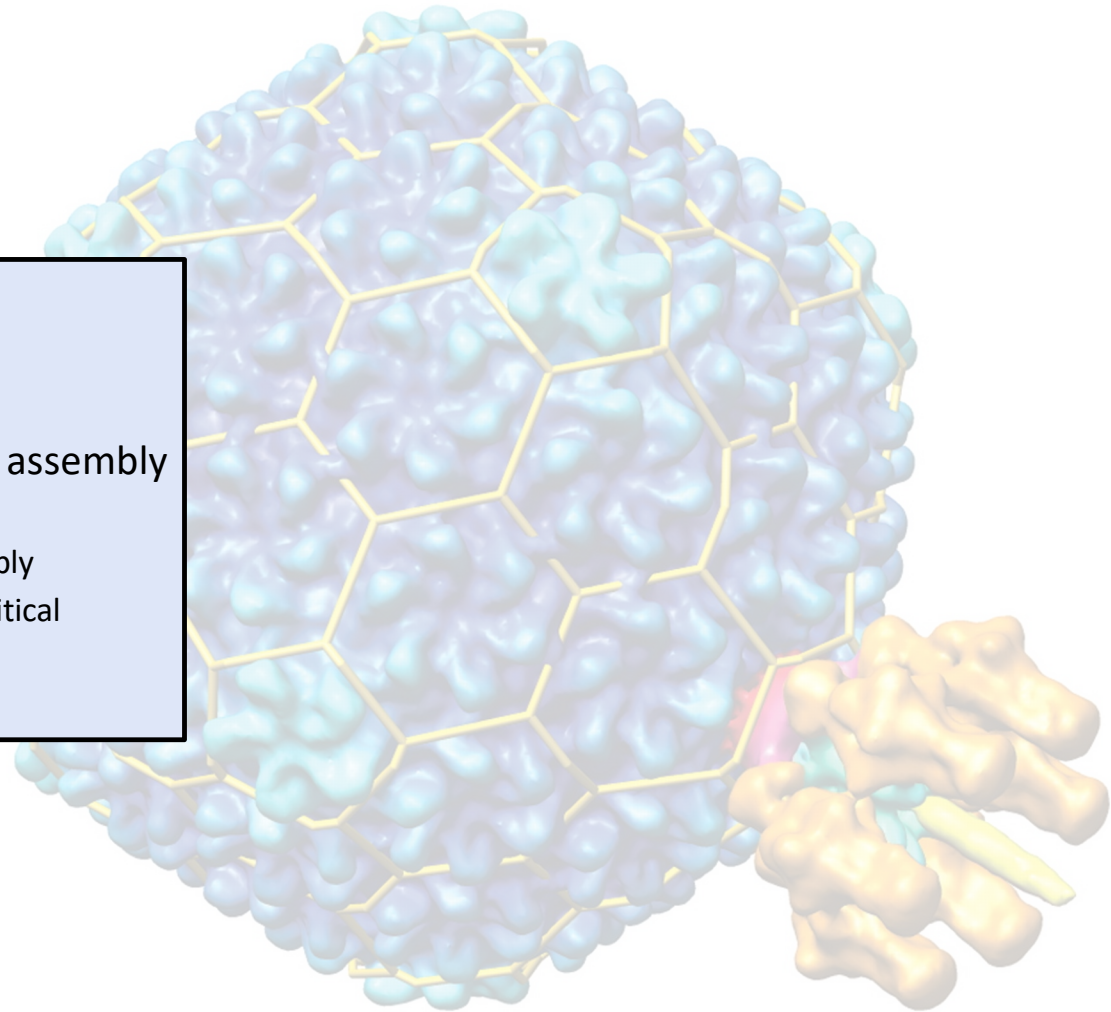
BASIC DISCOVERIES FOR BETTER HEALTH

NIH National Institute of Allergy and Infectious Diseases

Overview

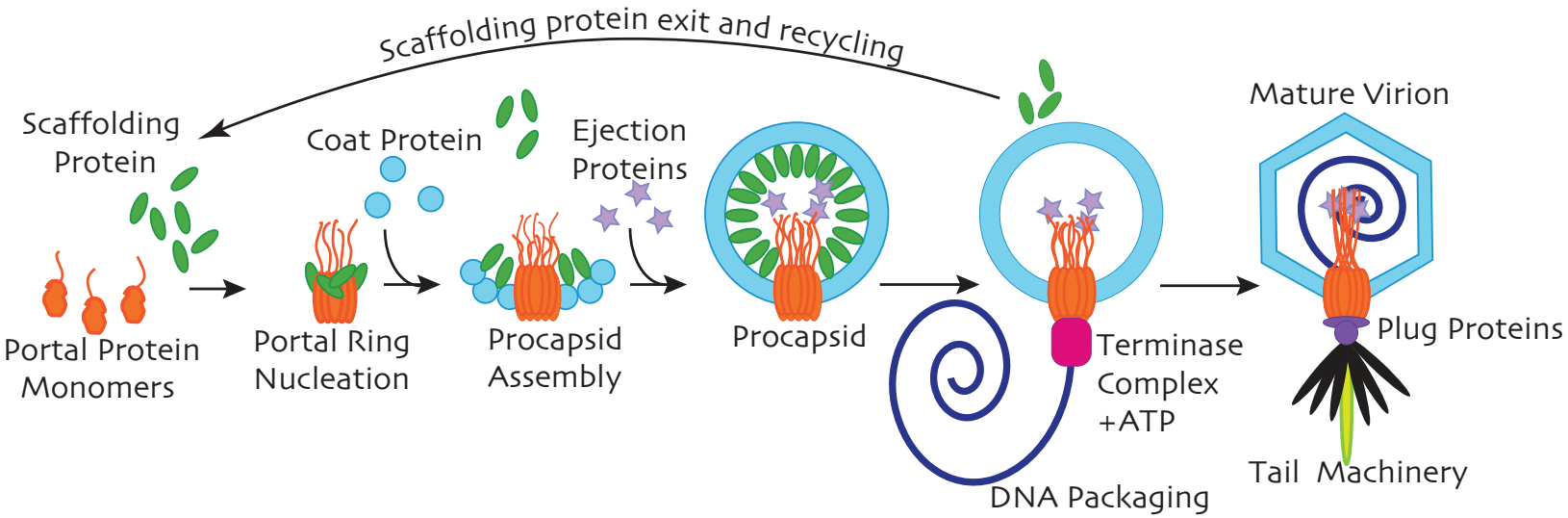
- dsDNA virus design principles
- A bit about phage P22
- Roles of P22 scaffolding protein in capsid assembly
  - Control of SP concentration is critical
  - SP monomer:dimer ratio affect proper assembly
  - Interaction affinity with coat protein is also critical
- Mathematical model of assembly



Lander et al. (2006) Science

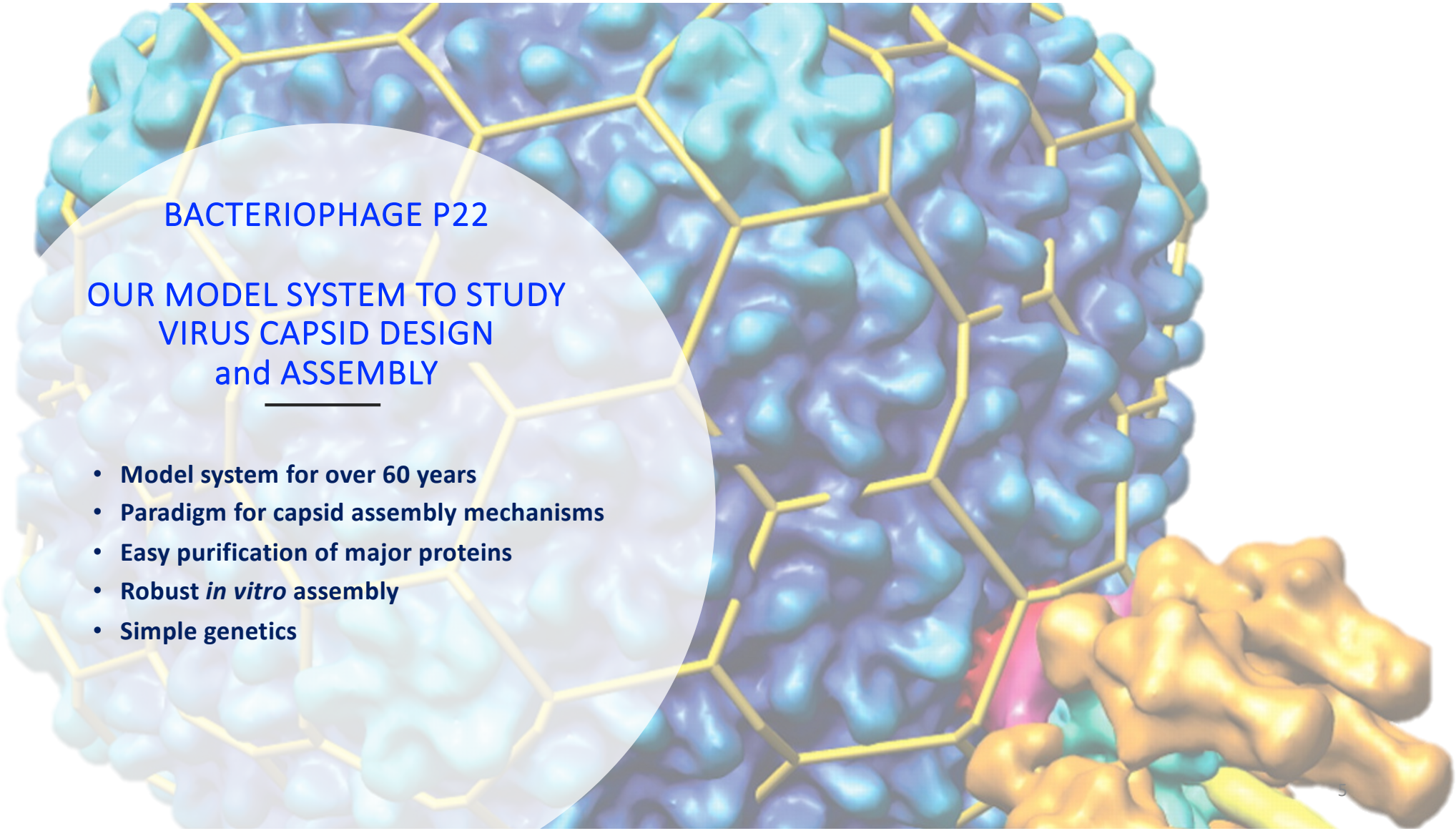
dsDNA virus design principles: assembling the head

Tailed phages
Herpes viruses



Key general features:

- Self-assembly of precursor capsid catalyzed by **essential scaffolding protein**
- Scaffolding protein leaves the procapsid
- DNA actively packaged through the portal protein
- Coat protein rearrangements during assembly and maturation



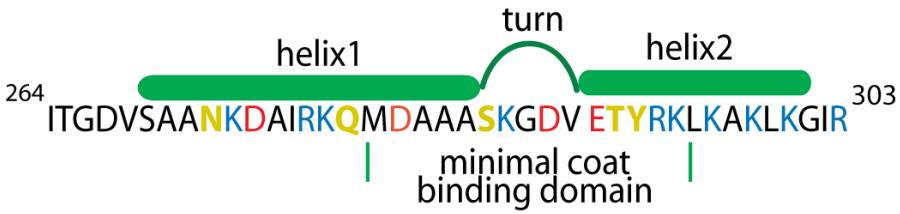
BACTERIOPHAGE P22

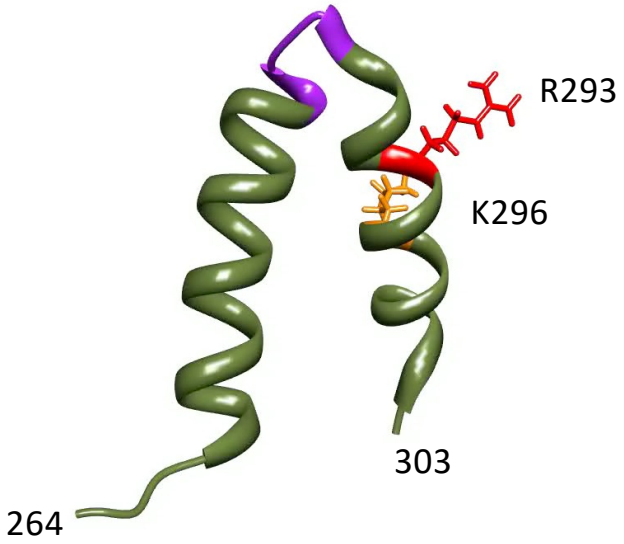
OUR MODEL SYSTEM TO STUDY VIRUS CAPSID DESIGN and ASSEMBLY

- **Model system for over 60 years**
- **Paradigm for capsid assembly mechanisms**
- **Easy purification of major proteins**
- **Robust *in vitro* assembly**
- **Simple genetics**

P22 scaffolding protein: Fun facts and figures

- 303 amino acids
- Monomer:dimer:tetramer association
- Dimensions-- 22 Å diameter x 247 Å length
- Intrinsically disordered
- Minimal coat binding fragment:
  - C-terminal amino acids 280-294
  - NMR structure of amino acids 264-303 (PDB 2gp8)
- About the coat binding domain:
  - **R293 and K296 essential for coat protein interaction**
  - **Binds coat protein N-arm residue D14**
  - The interface stabilizes the HTH structure
  - The turn-- for proper configuration of the helices

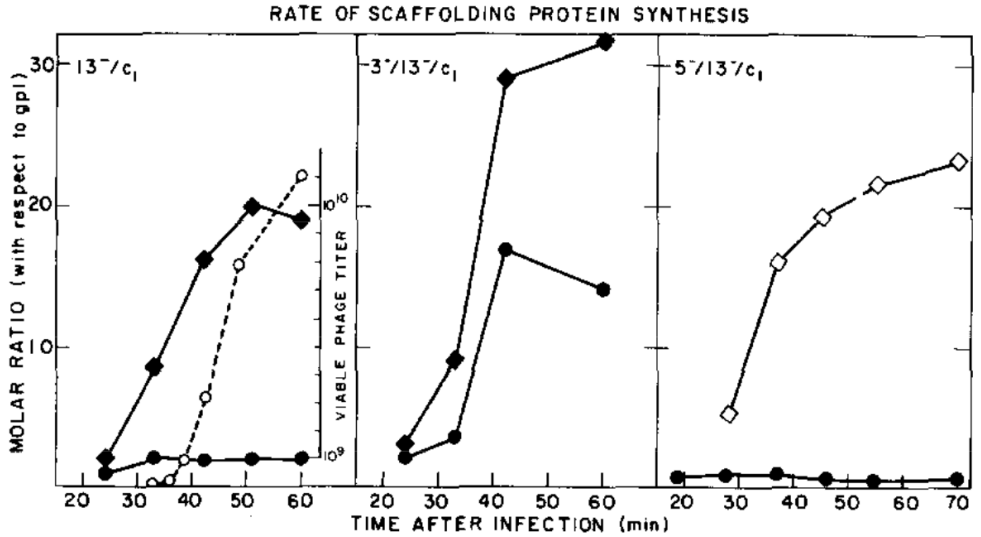




Cortines et al., 2011; Padilla-Meier et al., 2012; Parker et al., 1997; 1998; Weigele et al., 2005; Tuma *et al.*, 1998; 1996; Sun *et al.*, 2000

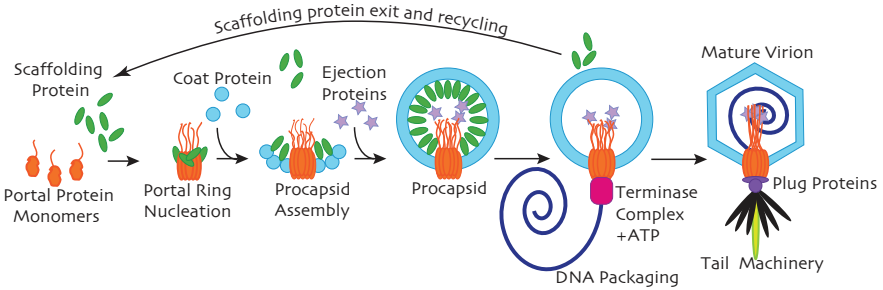
Scaffolding protein expression is autoregulated

Infection making: phages procapsids coat fragment



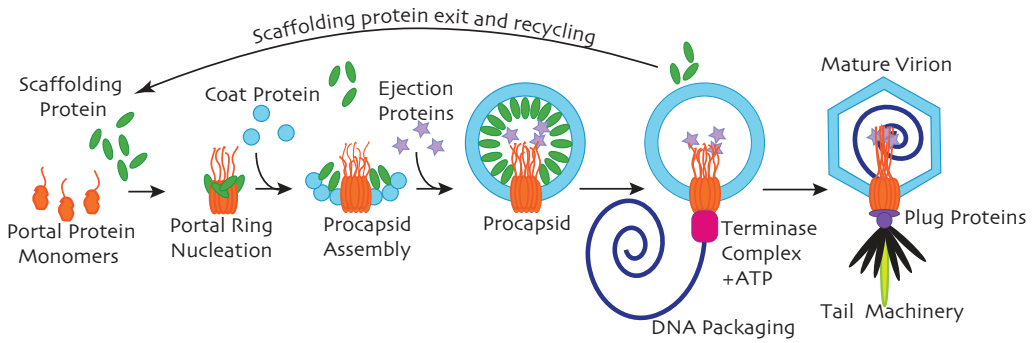
Wyckoff and Casjens (1985) showed that scaffolding protein regulates its own translation by binding to its RNA.

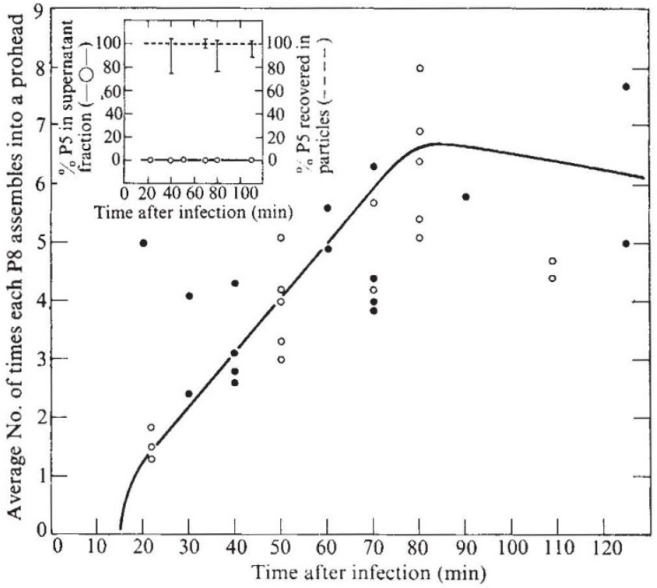
We have UV X-linked scaffolding protein to its RNA and are characterizing the binding site on each.



King et al., Cell, 1978

Scaffolding protein is recycled during infections





Synthesis of scaffold is low when phages are made but is higher when the infection is halted at procapsids.

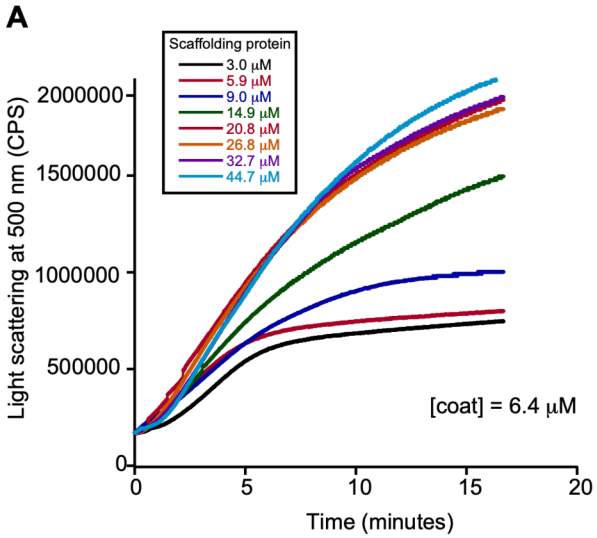
Recycled up to 6x during an infection

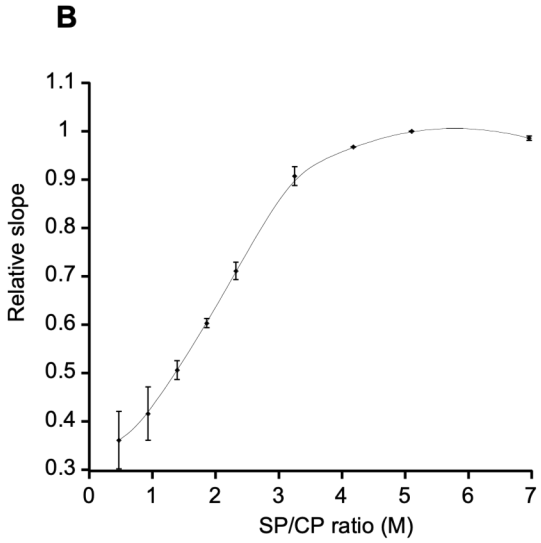
Scaffolding protein exits PCs during DNA packaging.

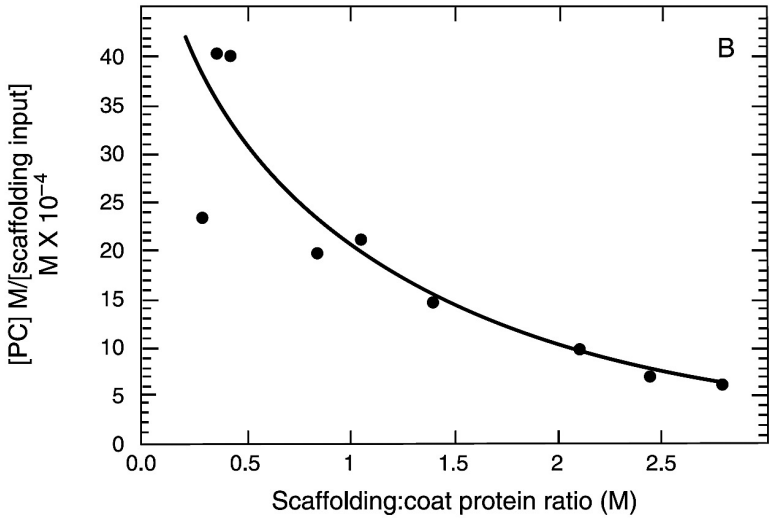
Casjens et al., JVi, 1985
King and Casjens, Nature 1974

Why is [scaffolding protein] so regulated?

Too much scaffolding protein slows assembly kinetics and decreases yield *in vitro*





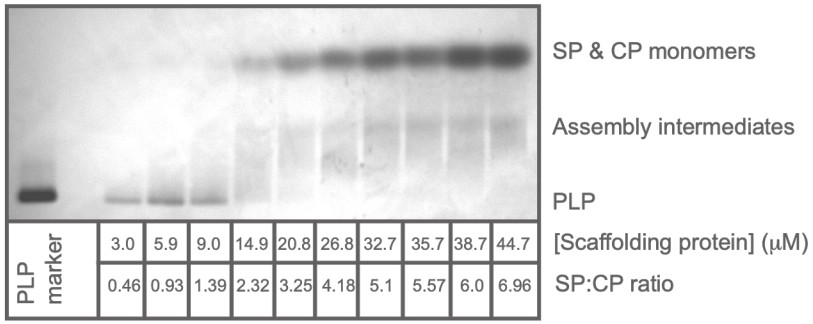


Motwani and Teschke, JVi, 2019; Parent et al., JMB, 2006

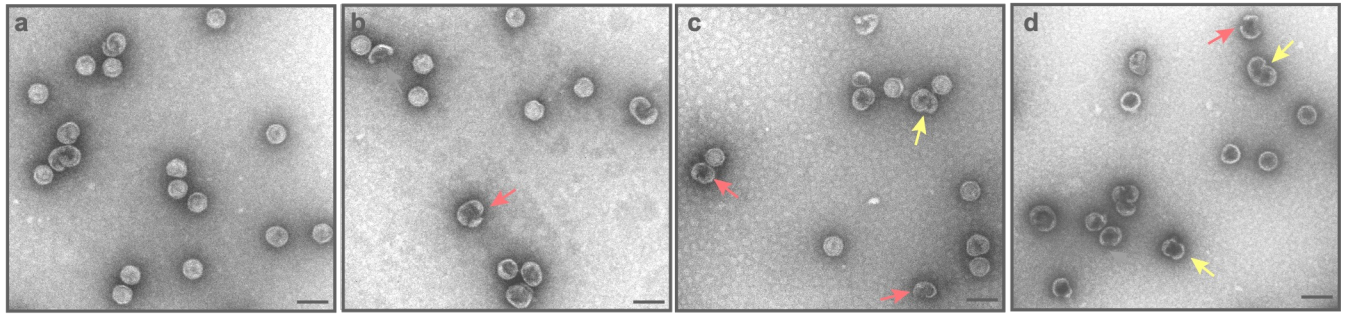
Too much scaffolding protein leads to mis-assembly

C

Agarose gel



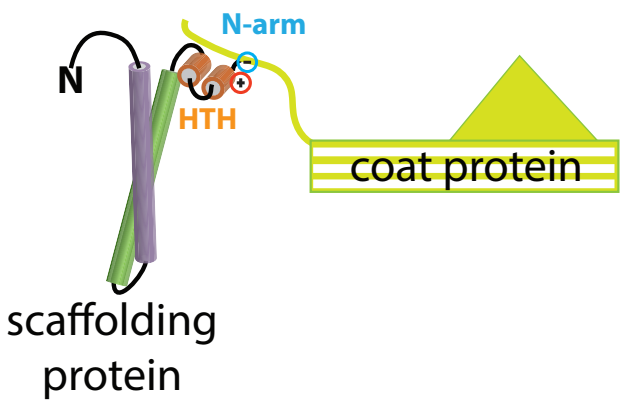
D



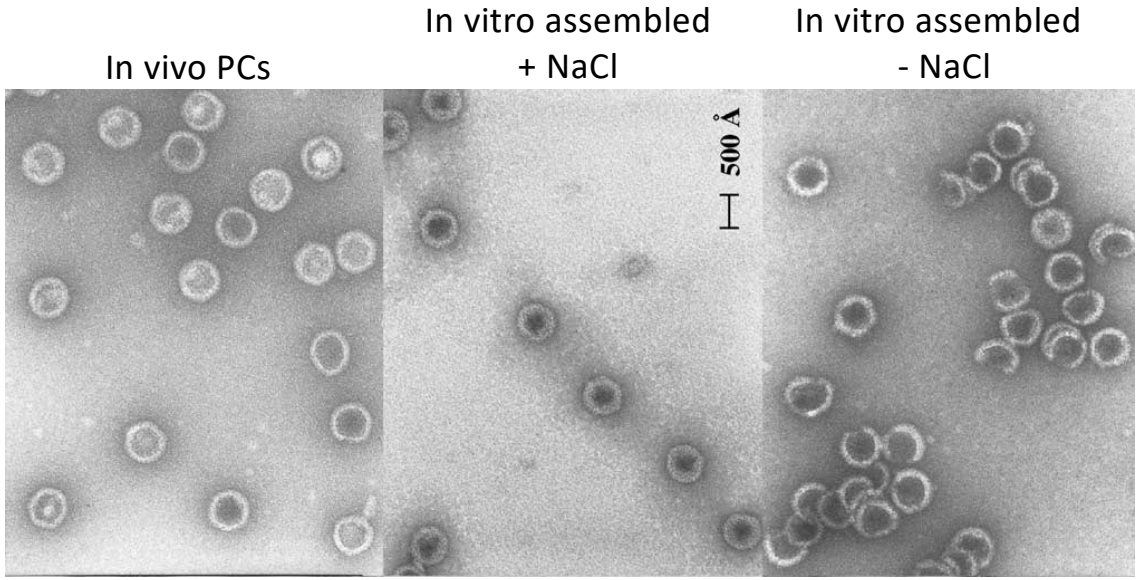
Scale bar = 100 nm, red arrow: halves, yellow arrow: two halves fused

Parent et al., Virology, 2005

Coat:scaffold affinity also affects assembly products



* SP:CP is an ionic interaction.
* Changing the [salt] affects affinity



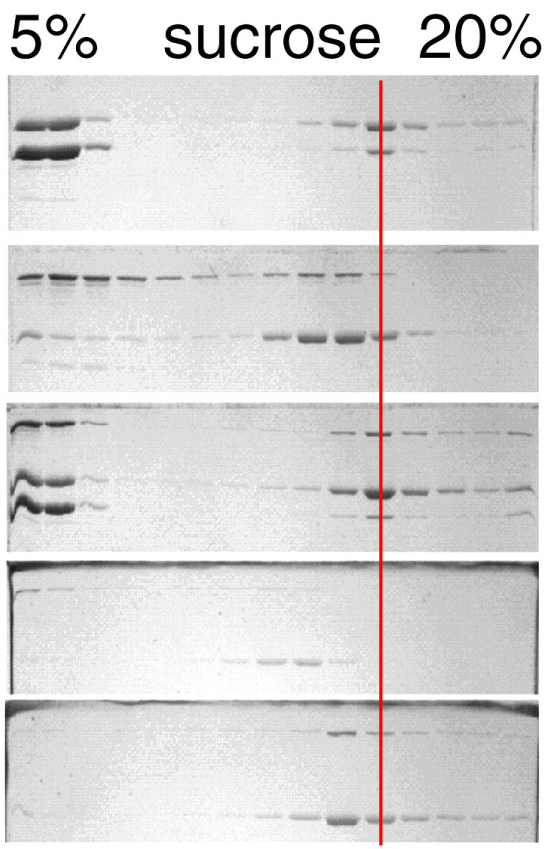
Need some free coat protein to complete PCs

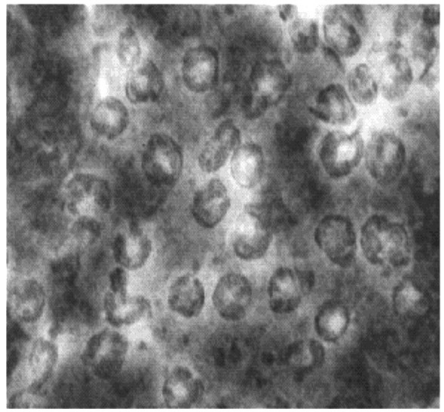
Cortines et al., Virology, 2011; Cortines et al., JVi, 2014; Parent et al., Virology, 2005

Monomeric scaffolding protein required for proper assembly

COAT	WT gp8	DIMER (μM)
10.6	14.7	0.0
	~5 μM dimer	
10.6	0.0	7.4
23.4	7.4	3.7
7.7	0.0	4.4
23.4	0.0	4.4

Covalent SP dimer

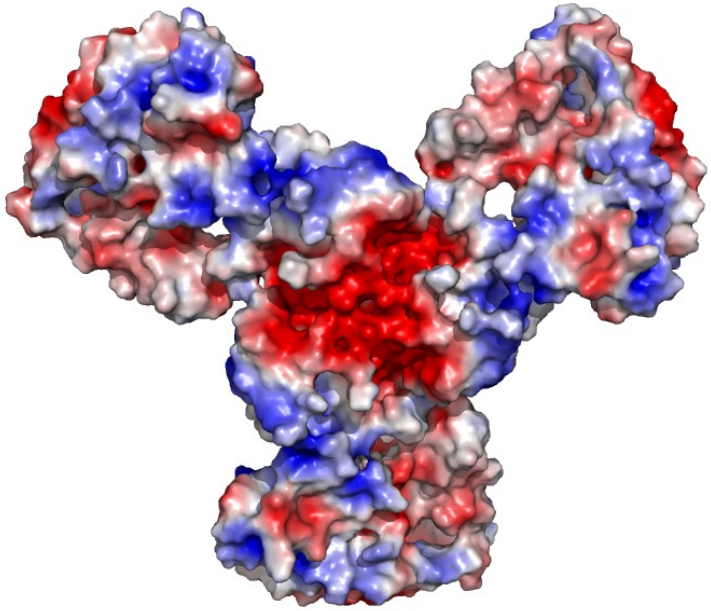




Tuma et al., JMB 2008

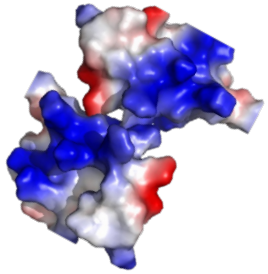
How does scaffolding protein interact with coat protein to catalyze assembly?

3-fold axis of symmetry from inside of PC



PDB 2XYY

Scaffolding HTH 'dimer'



PDB 2gp8

- charge
+ charge

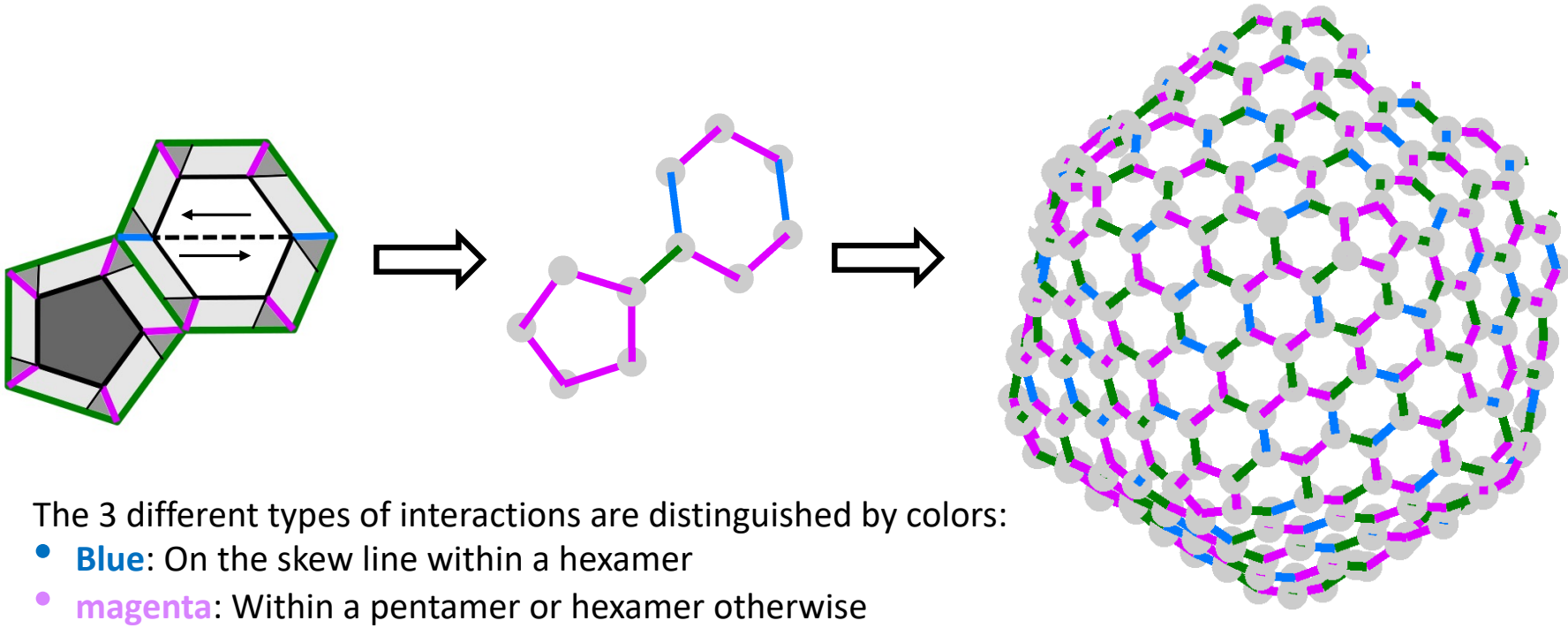
- Icosahedral three-fold axes of symmetry suggested to be the sites of interaction with scaffolding protein.
- Coat protein N-arm and P-loop move during maturation based on structures.
- Possible trimer interactiom suggested by Huet at al., Sci Adv, 2023

Parent et al., 2010; Cortines et al., 2014
Chen et al., 2011; Thuman-Commike et al., 1998; Zhang et al, 2000

Can we model scaffolding driven assembly?

A mathematical model of the interaction network

Each capsid protein (CP) is replaced by a vertex, and edges denote interaction between them:



The 3 different types of interactions are distinguished by colors:

- **Blue**: On the skew line within a hexamer
- magenta: Within a pentamer or hexamer otherwise
- **Green**: Between pentamer/hexamer and a hexamer

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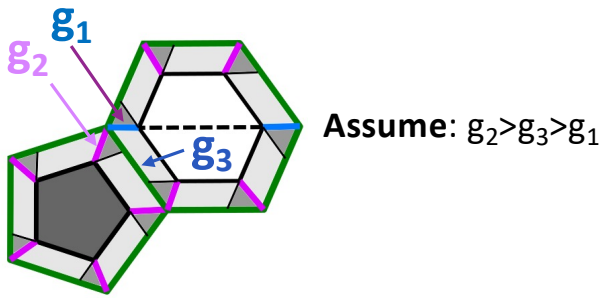
Modeling assembly

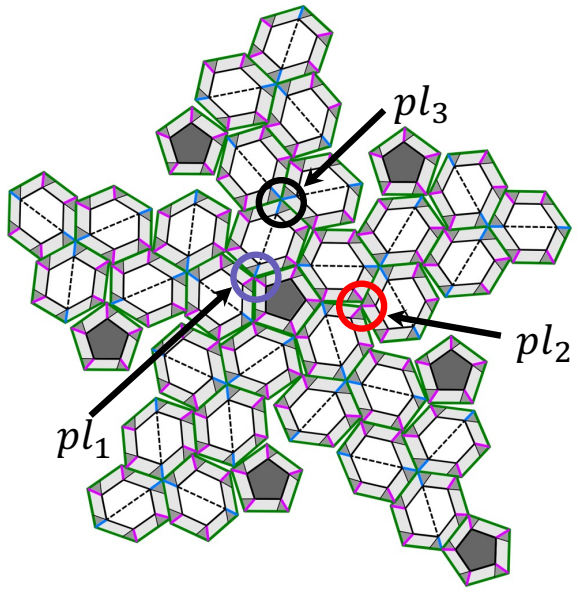
Model assumption:

- Assembly initiates around a five-fold axis, which could contain the portal, and follows the template of the tiling/interaction network.
- Adding CS or C at each site



Model depends on:

- The level of CS (CP:SP complex) and CP.
- The relative strengths of the 3 types of bonds.
- The probability of acquiring of CSs in a 3-fold axis. The first CS at probability pl_i (lower half) and pu_i (upper half), the second and third CS at a reduced level by factors of p_d and p_t, respectively.



Implementation:

- One pentamer/portal forms completely and nucleates assembly.
- Dependent on the relative bond strengths, the most favorable binding sites are identified, and all sites filled by either CP or CS.
- This is repeated until the capsid is formed.

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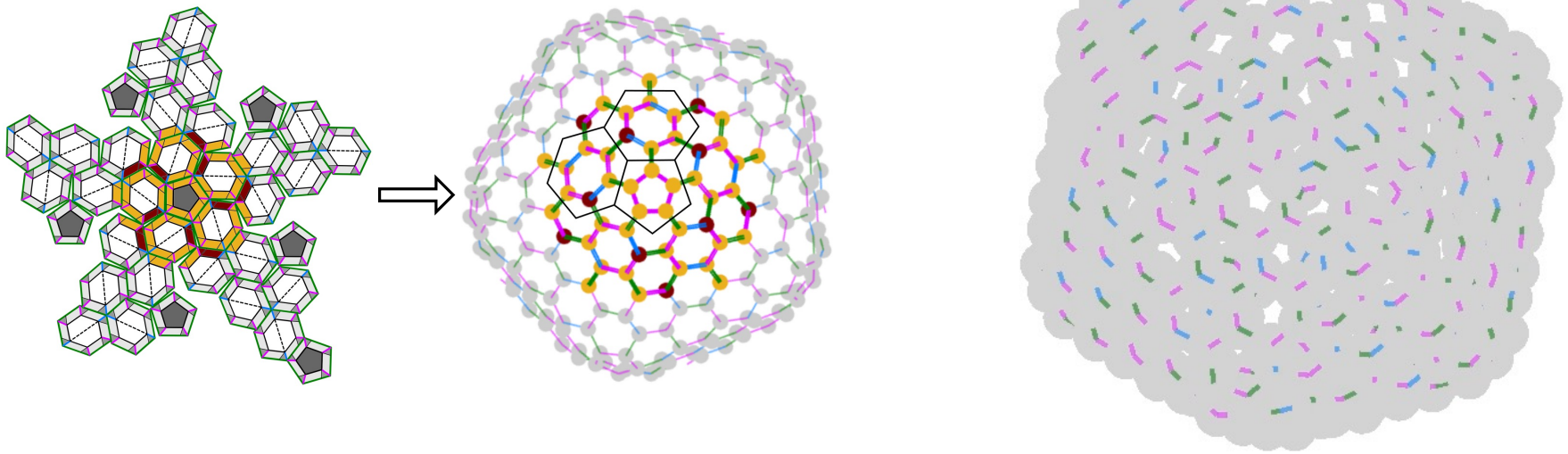
Simulation of the model

GOLD if a CP is associated with an SP (CS), and in **MAROON** otherwise.



Simulations are performed under the assumption that CP and CSs (CP:SP complexes) are always available.

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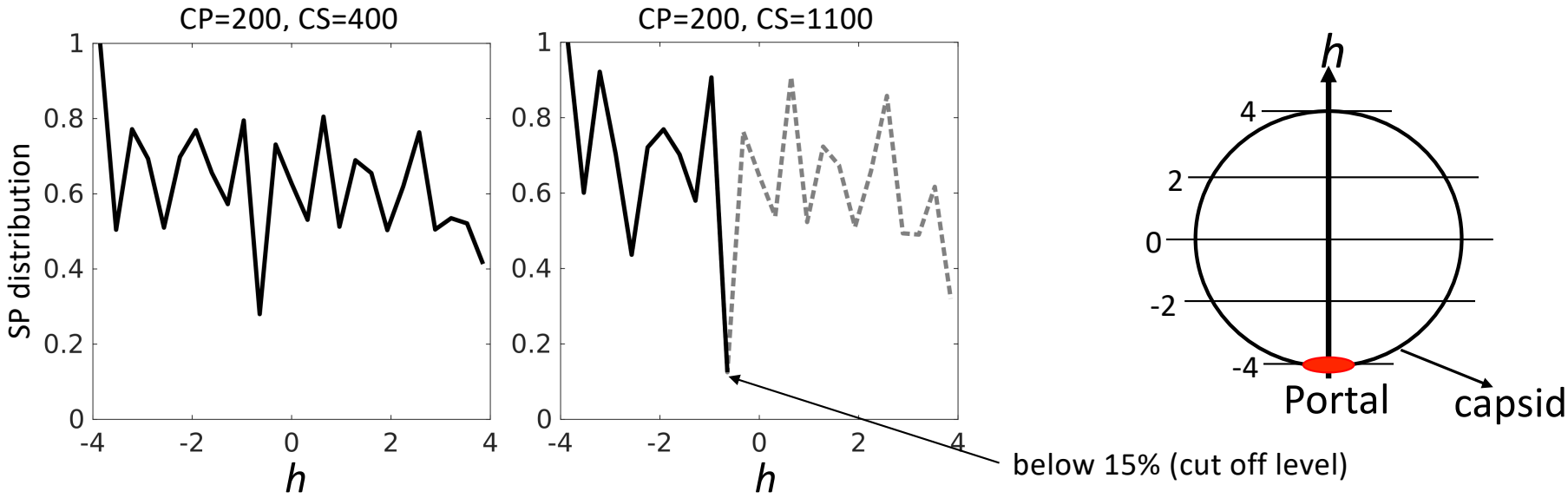
Distribution cut-off level



- We assume that assembly stalls when the SP distribution drops below 15% (cut-off level).
- If $p_t = 0$ increasing CS, i.e., increasing SP concentration, causes a dip in the SP distribution around the equator of the particle. **This explains why we are seeing half capsids for high SP concentrations.**
- For positive values of p_t, or small values of p_d, the distribution does not fall below the cut-off value by increasing CS concentration. Thus, SPs form dimers with a high probability and are less likely to form trimers.
- These results are independent of choice of parameters.

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Conclusions

The model shows that:

- For high SP concentrations, assembly stalls around the equator. Recapitulates experimental conditions.
- There is an ideal range of SP concentration for assembly, this necessitating control of translation *in vivo*.
- SPs mainly form monomer and dimers and are less likely to form trimers.