Controlling capsid assembly with antivirals and liquid-liquid phase separation

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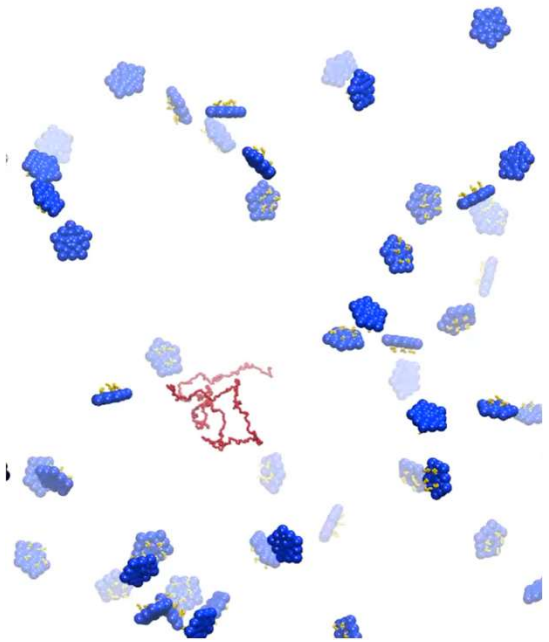
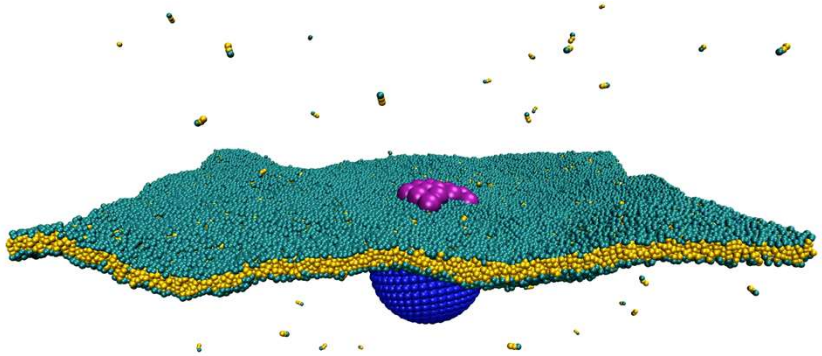
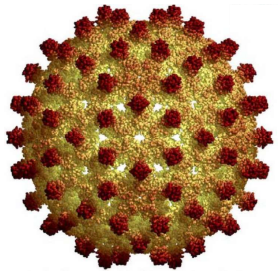




Understanding and controlling capsid self-assembly

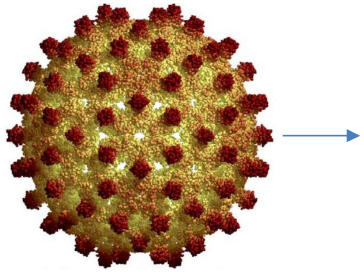
1. What factors control assembly pathways and outcomes

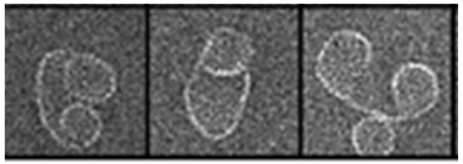
Example: HBV capsids form T=3 and T=4 shells



2. Can we design molecules to redirect assembly to different sizes or morphologies?

Example: HBV antivirals

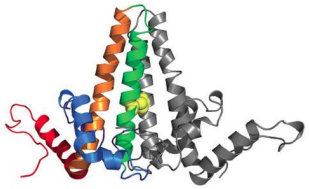


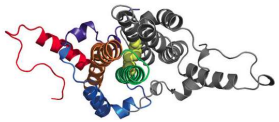


3. Coupling to liquid-liquid phase separation (biomolecular condensates) can change assembly rates by orders of magnitude and make it more robust

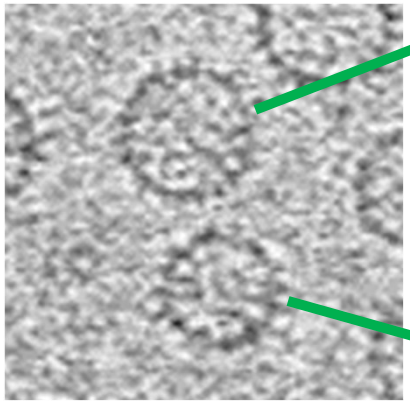
HBV capsid (core) comes in 2 sizes

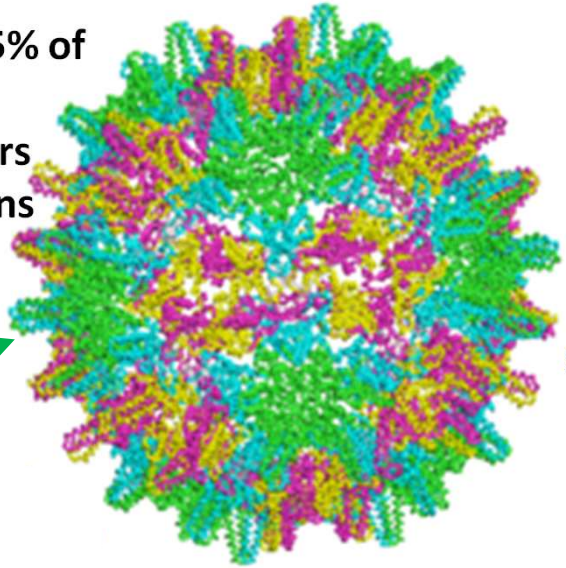
HBV core protein dimer

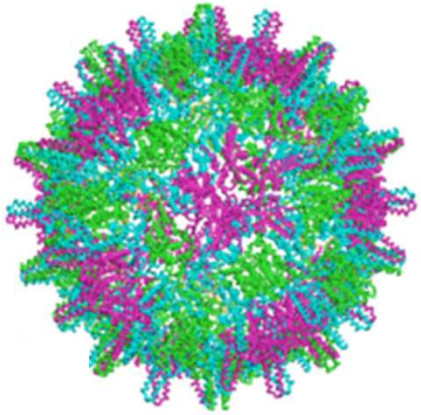




T=4 Capsid (infectious, 95% of assemblies)
120 core protein dimers
4 protein conformations







T=3 Capsid (5%)
90 core protein dimers
3 protein conformations

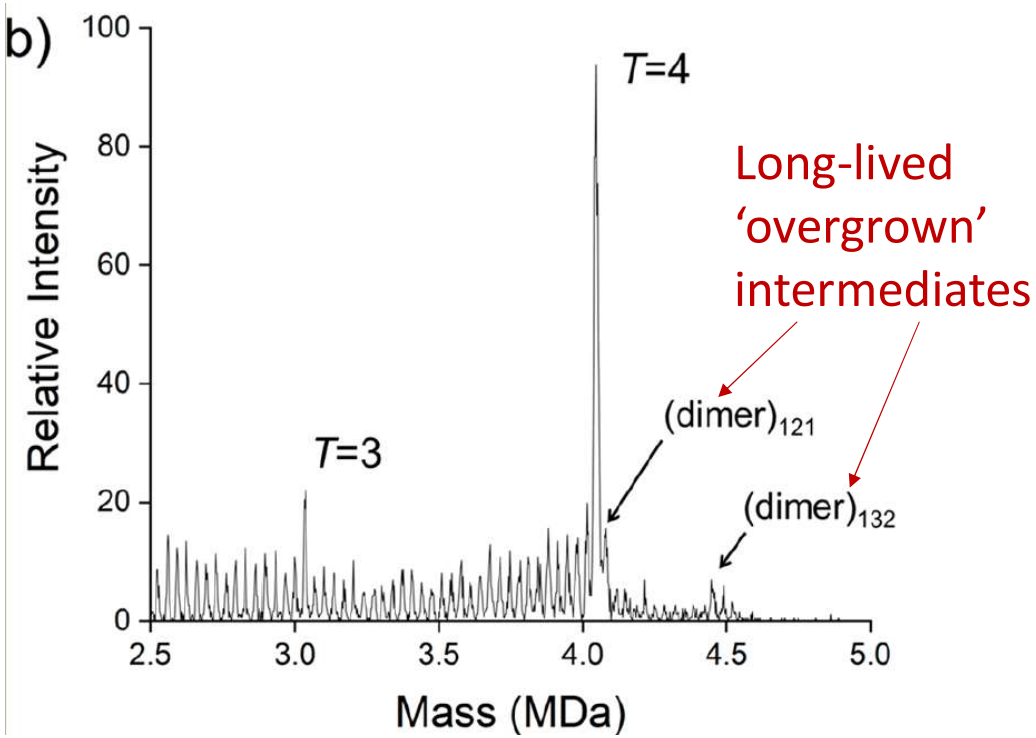
Different colors indicate different protein conformations

see Caspar & Klug (1962), Cold Spring Harb Symp Quant Biol 1962;27:1-24. doi: 10.1101/sqb.1962.027.001.005)

Experiments on HBV assembly

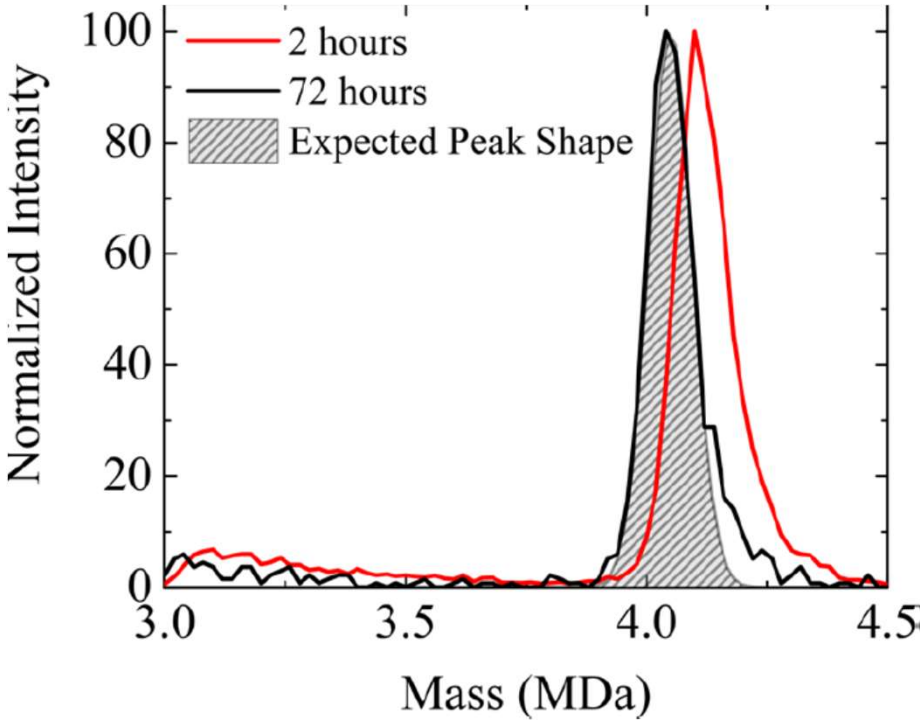
Charge Detection Mass Spec (CDMS) measures capsid sizes with single-dimer resolution

CDMS of human HBV



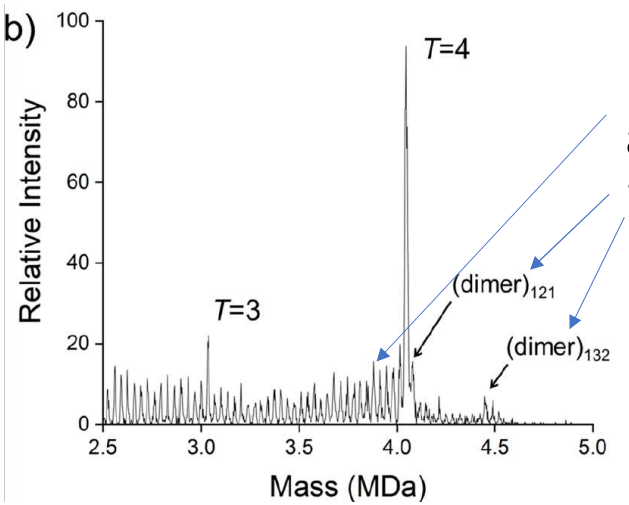
Todd, Barnes, Young, Zlotnick, Jarrold, Anal. Chem. **92**, 11357 (2020)

Capsids assemble overgrown intermediates before forming T=4 capsid



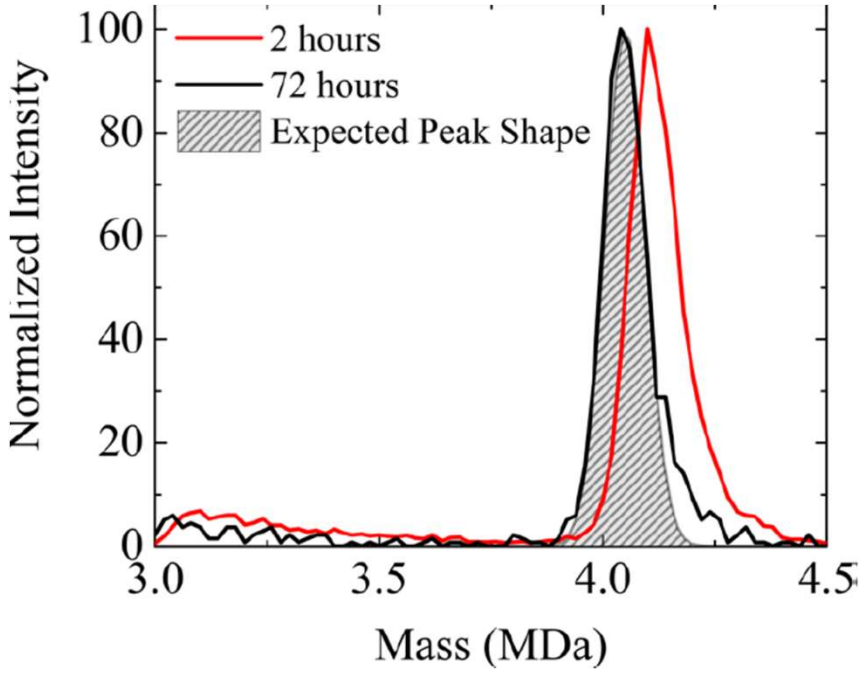
Lutomski, Lyktey, Zhao, Pierson, Zlotnick, Jarrold, JACS, **139**, 16932 (2017)

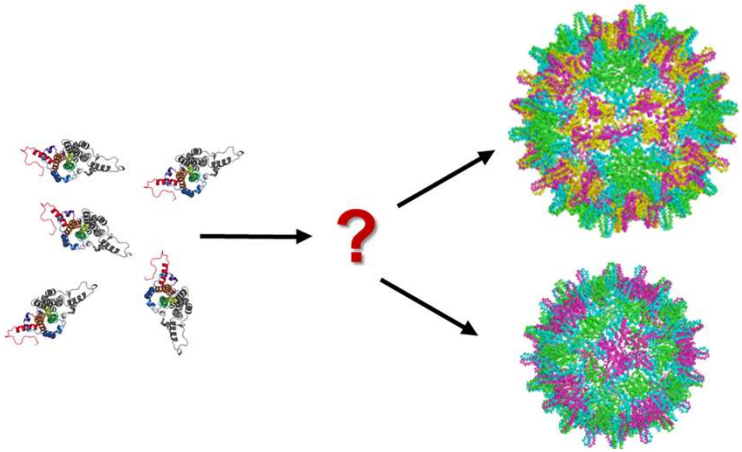
Experiments on HBV assembly



Long-lived assembly intermediates

Todd, Barnes, Young, Zlotnick, Jarrold, Anal. Chem. **92**, 11357 (2020)



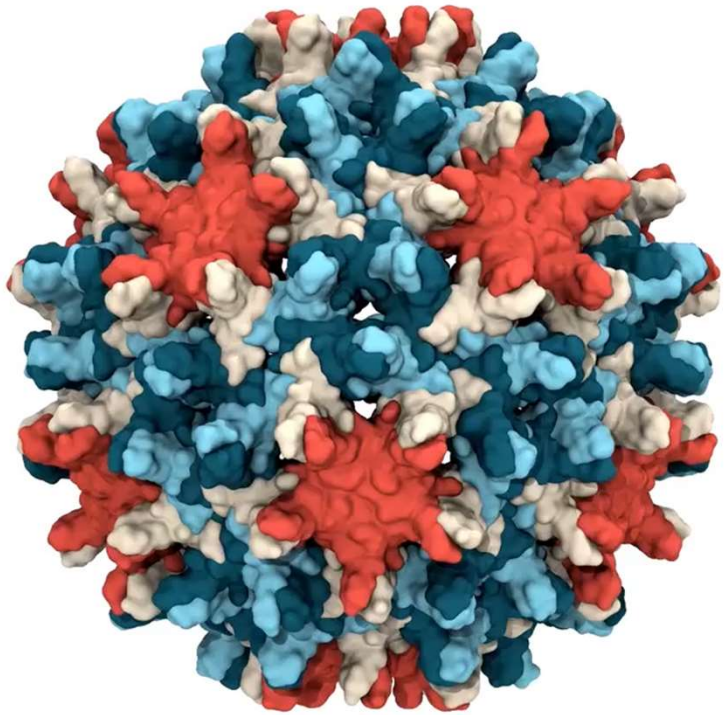


Experiments can't resolve structures of intermediates along assembly pathways or overgrown intermediates

All-Atom simulations of HBV

All-atom simulation of complete (but empty) HBV capsid, ~1 μs

Jodi Hadden et al., eLife (2018)



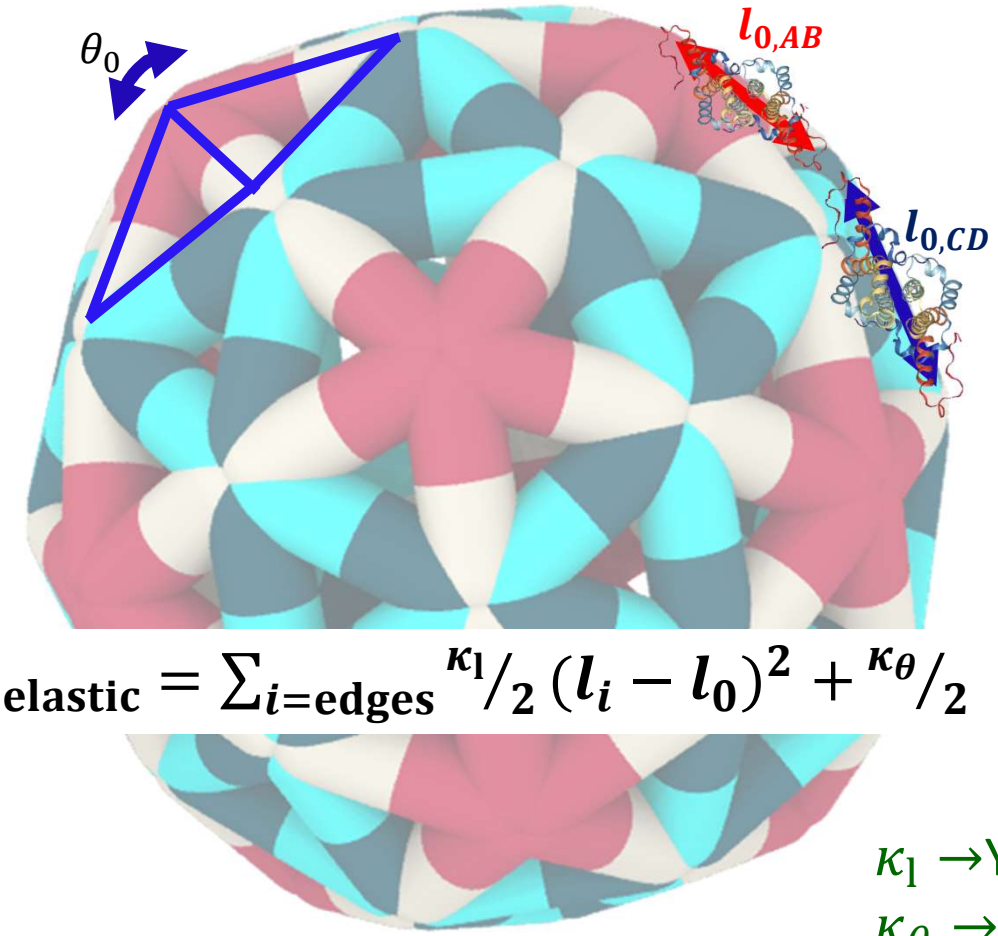
Not yet tractable to simulate **assembly** with all-atom simulations

We need a model that can link all-atom simulations and experimental data with assembly dynamics

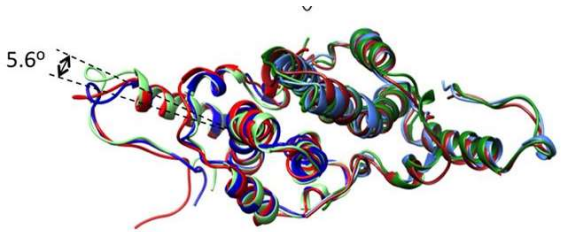
Computational model

capsid = elastic network with edges corresponding to protein dimers

Farri Mohajerani



dimers have two conformations: AB and CD



Mohajerani et al. ACS Nano (2022)

adapted from:
-GM Rotskoff, PL Geissler, PNAS (2018)
-Panahandeh, Li, Marichal, Rubim, Tresset, Zandi, ACS Nano (2020)
-Tyukodi, Mohajerani, Hall, Grason, Hagan, ACS Nano (2021)

$$G_{\text{elastic}} = \sum_{i=\text{edges}} {}^{\kappa_l}/_2 \, (l_i - l_0)^2 + {}^{\kappa_\theta}/_2 \, (\theta_i - \theta_0)^2 + \cdots$$

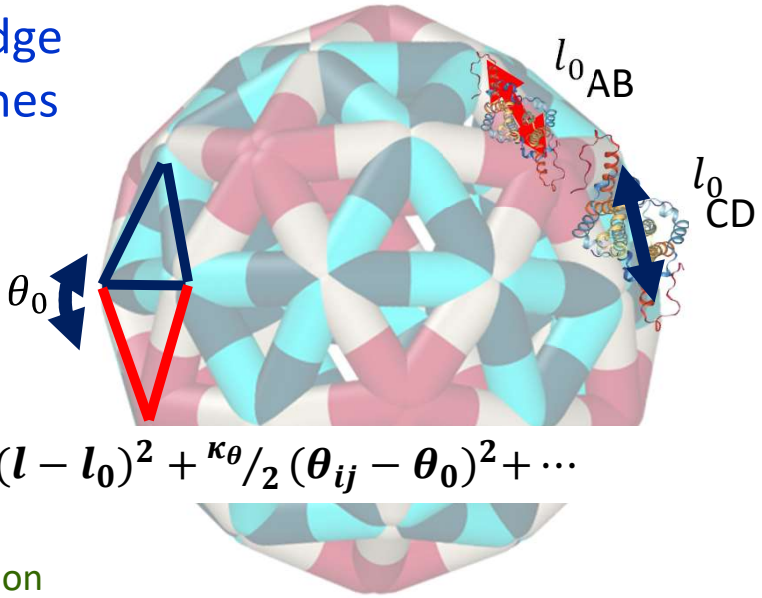
simulate assembly with dynamical Monte Carlo

κ_l →Young's modulus (stretching)
κ_θ → bending modulus

Estimating model parameters from all-atom simulations

Optimize model parameters so that distributions of edge lengths and angles in coarse-grained simulation matches all-atom

fit parameters: $\kappa_{\mathrm{l}}, \kappa_\theta, \kappa_\phi, l_{0,\mathrm{AB}}, l_{0,\mathrm{CD}}, \theta_{0,\mathrm{AB}}, \theta_{0,\mathrm{CD}}, \ldots$



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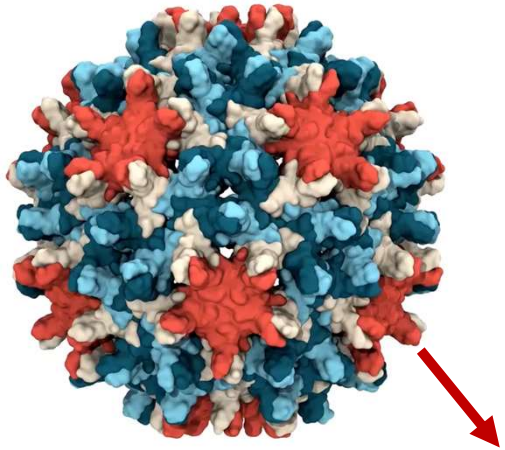
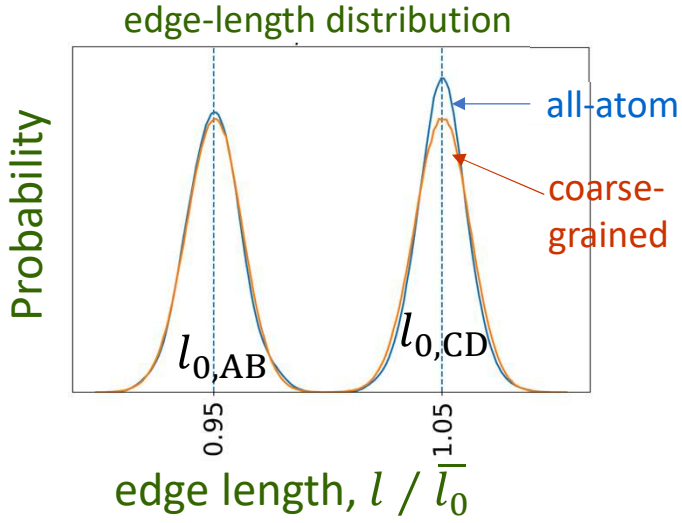


Jodi Hadden et. al., eLife (2018)

$$\kappa_{\mathrm{l}} = 4200\frac{k_{\mathrm{B}}T}{\bar{l}_0}, \kappa_\theta = 40\ k_{\mathrm{B}}T$$

Föppl-von Kármán # = 500



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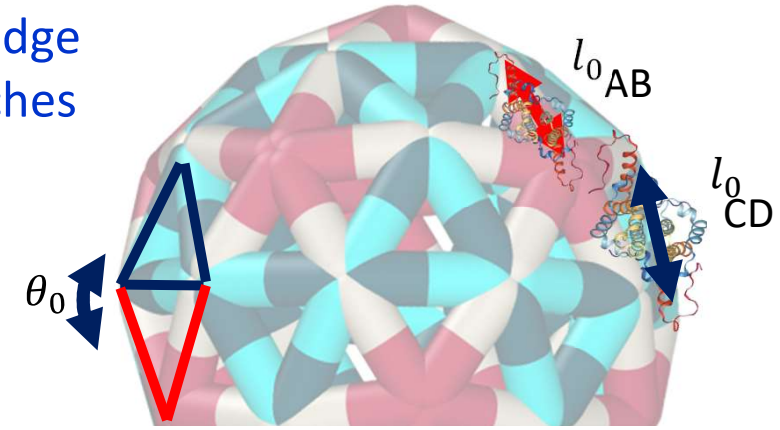

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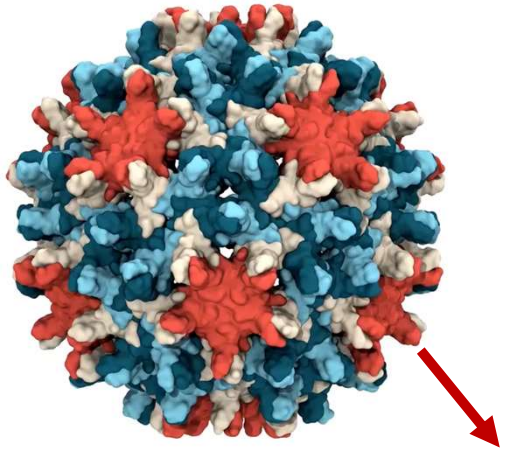

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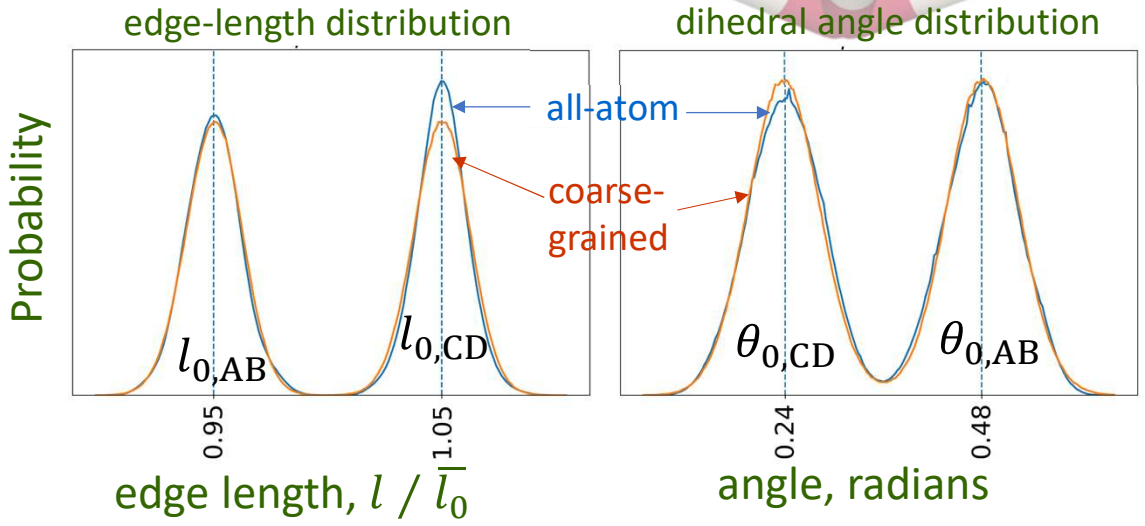

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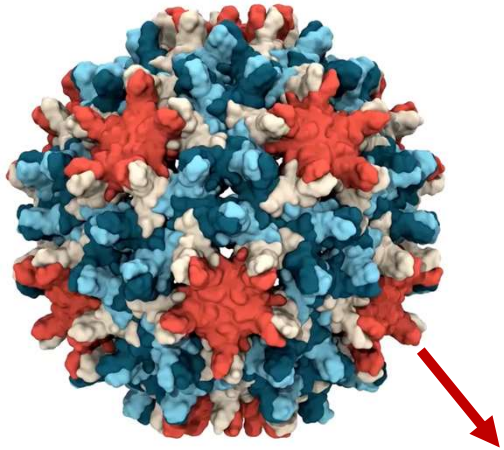

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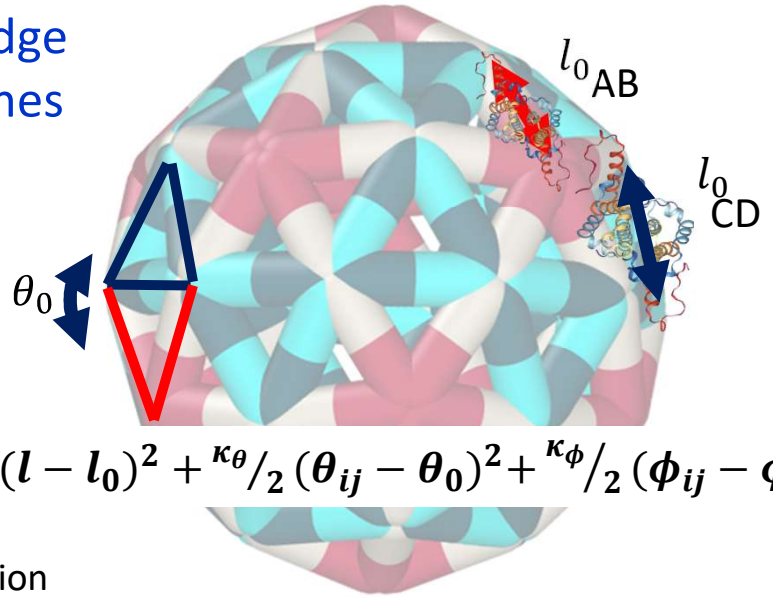

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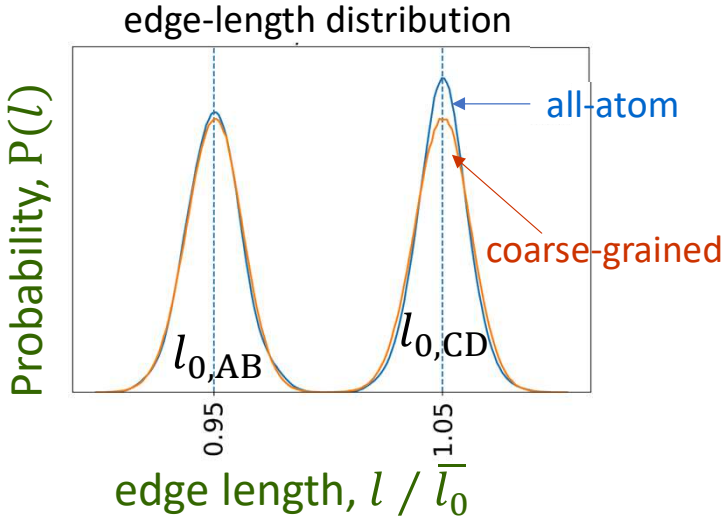


previous models have only 1 conformation, cannot fit all-atom data

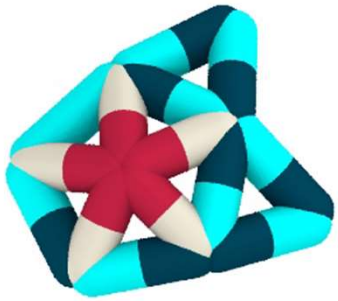
need 2 subunit types ('ultra-coarse graining'):

Elrad&MFH, Nanolett (2008); Grime, ..., Voth Nat. Comm. (2016); Dama, Jin, Voth JCTC (2017)

Subunit association/dissociation/relaxation

Addition of one edge
Removal of one edge

Addition of two edges
Removal of two edges



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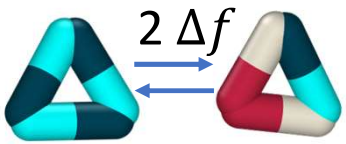
Panahandeh, Li, Marichal, Rubim, Tresset, Zandi, ACS Nano (2020)
Tyukodi, Mohajerani, Hall, Grason, Hagan, ACS Nano (2021)

Relaxation of the shell
between addition/removal moves

- Monte-Carlo moves are reversible → a well-defined equilibrium distribution
- Monte-Carlo moves mimic real dynamics (hopefully)
- dimer-dimer binding affinities for different conformations estimated from buried surface area

2 control parameters (depend on [salt], pH, temperature)

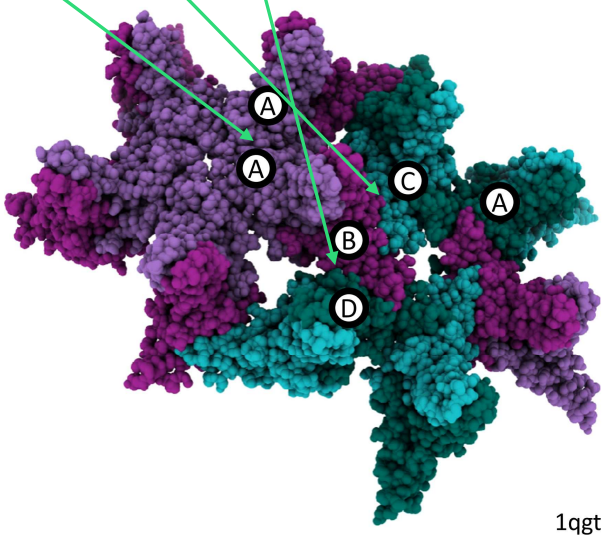
- mean subunit-subunit binding affinity, g_{b}
- Equilibrium ratio AB/CD dimer conformations, $K_{\mathrm{AB}} = \frac{[\mathrm{AB}]}{[\mathrm{CD}]} = \exp\left[{}^{-\Delta f}/_{k_{\mathrm{B}}T}\right]$



Subunit conformations

AA/CD	BC/CD	DB/DC
1.3	1.1	0.9

Estimate relative dimer/dimer binding affinities from buried surface area (PDBePISA)



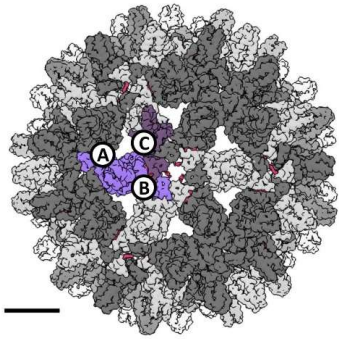
1qgt

- Monte-Carlo moves are reversible → a well-defined equilibrium distribution
- Monte-Carlo moves mimic real dynamics (hopefully)
- dimer-dimer binding affinities for different conformations estimated from buried surface area

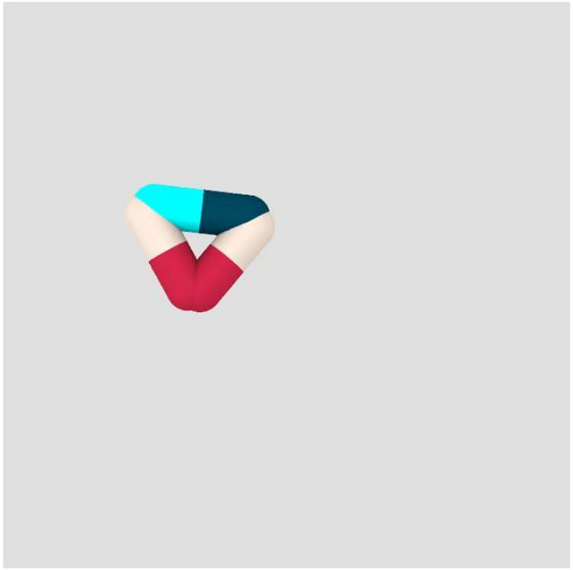
2 unknown parameters (can't estimate from atomistic simulations or structures):

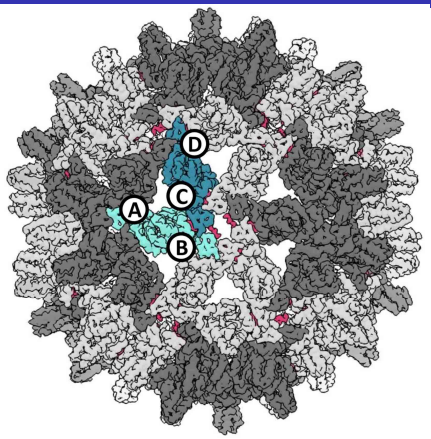
- mean subunit-subunit binding affinity, g_{b} (depends on [salt], temperature, pH)
- Equilibrium ratio AB/CD dimer conformations, $\frac{[\mathrm{AB}]}{[\mathrm{CD}]} = \exp\left[{}^{-\Delta f}/_{k_{\mathrm{B}}T}\right]$

Example simulation trajectories

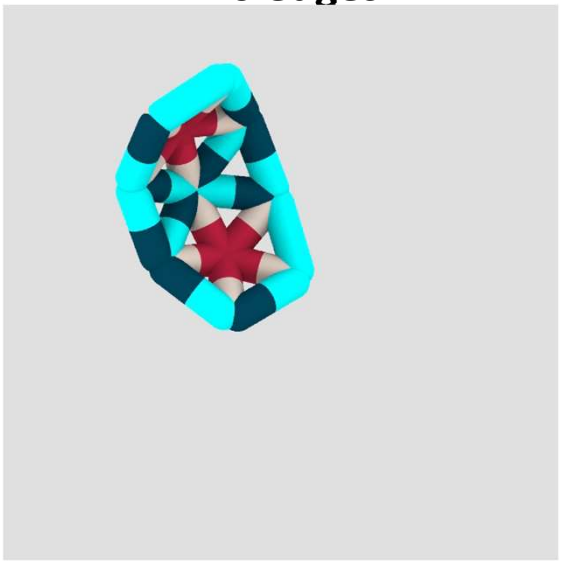


90 edges (dimers)





120 edges

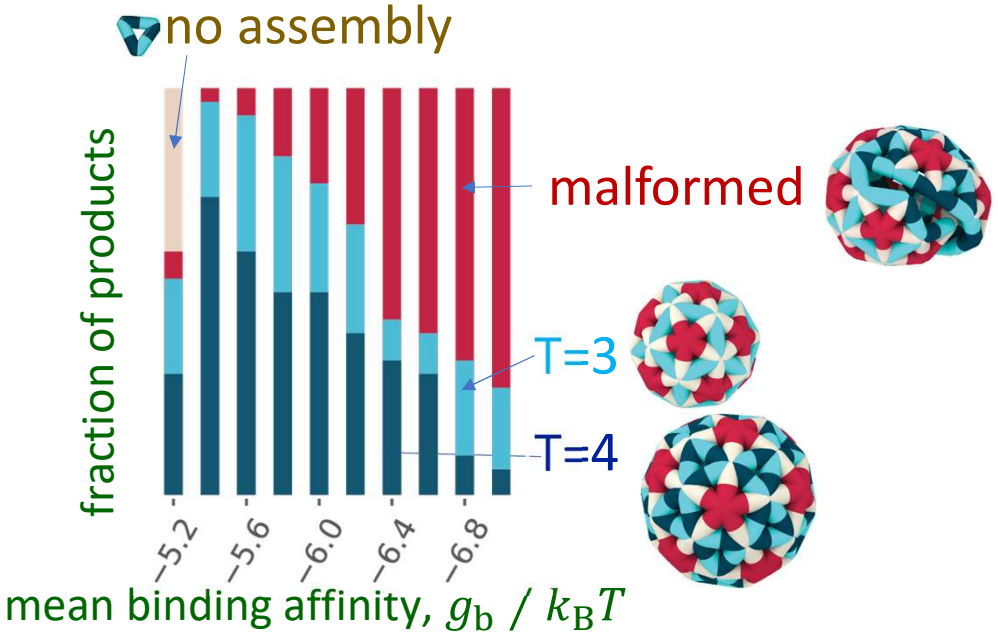


need two dimer types to get T=4 capsids

Mohajerani et al. ACS Nano (2022)

$g_{\mathrm{b}} = 6.5\ k_{\mathrm{B}}T$
$\Delta f = 3.5\ k_{\mathrm{B}}T$
[dimer]$= 10\ \mu$M

Parameters that control assembly morphologies



- malformed structures assemble when binding affinity too strong compared to k_BT (1 k_BT = 0.6 kcal/mol)

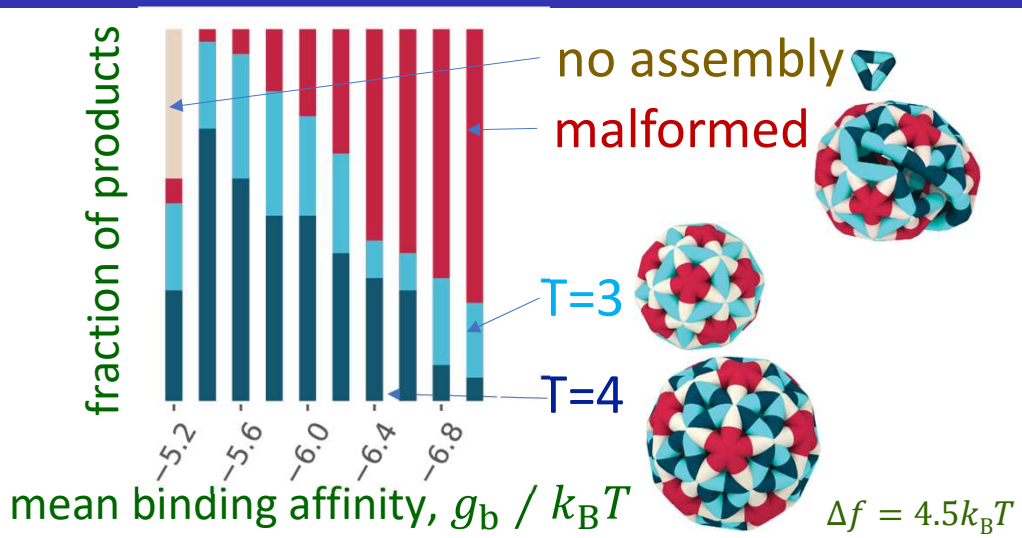
  consistent with CDMS, lightscattering experiments and previous simulations

- [T=4]/[T=3] ratio not sensitive to mean dimer-dimer binding affinity, g_b

strong interactions lead to kinetic traps:
Ceres & Zlotnick 2002, Hagan & Chandler 2006

$\Delta f = 3.6 k_B T$

Parameters that control assembly morphologies



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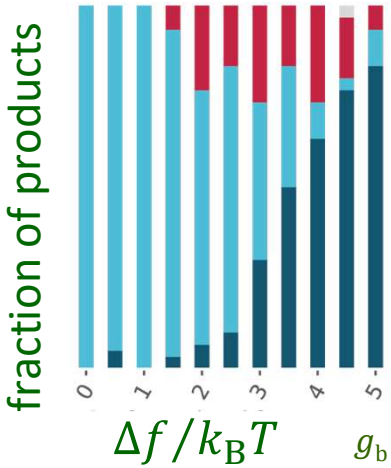


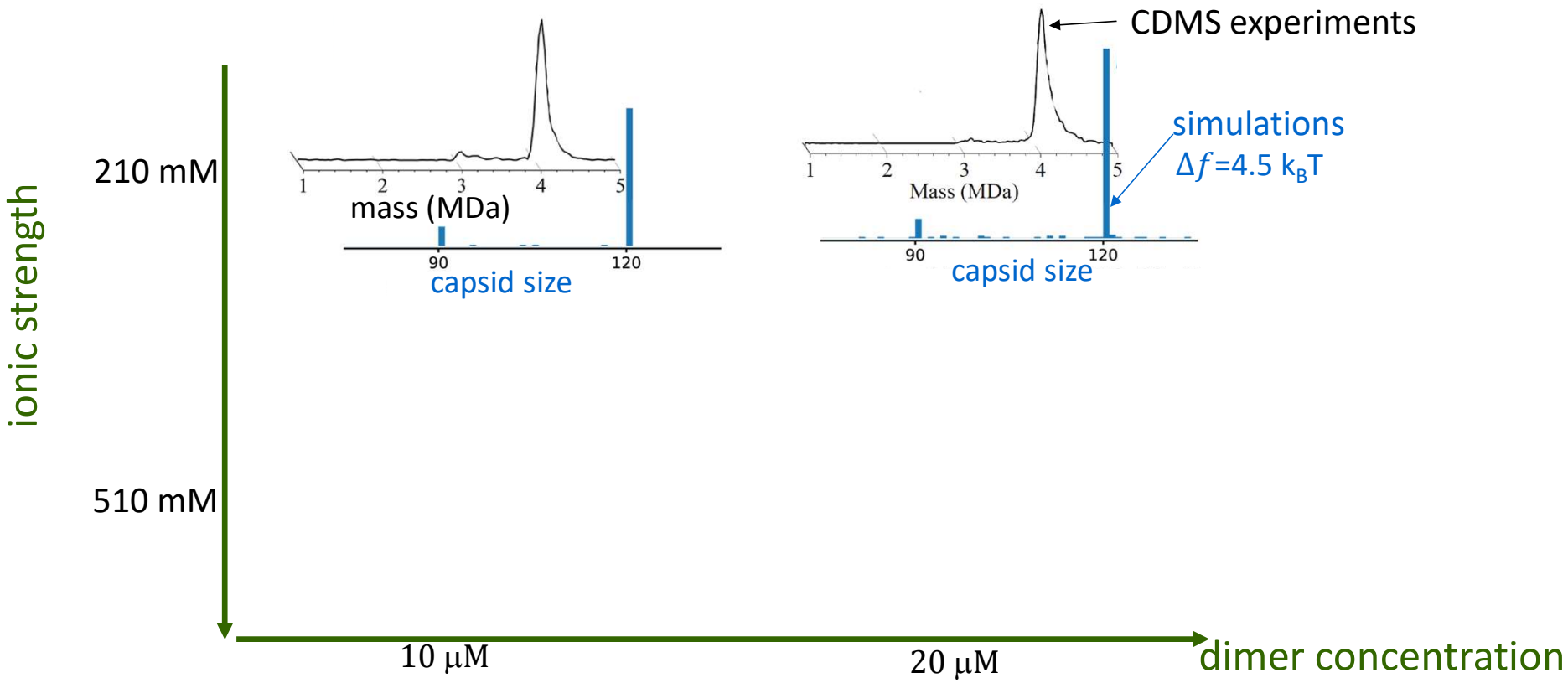
- [T=4]/[T=3] ratio depends on conformational free energy landscape

Δf =free energy difference between AB and CD dimers:

$$K_{AB/CD} = \frac{[AB]}{[CD]} = \exp\left[-\Delta f / k_BT\right]$$

Mohajerani et al. ACS Nano (2022)

Qualitative comparison with experiments



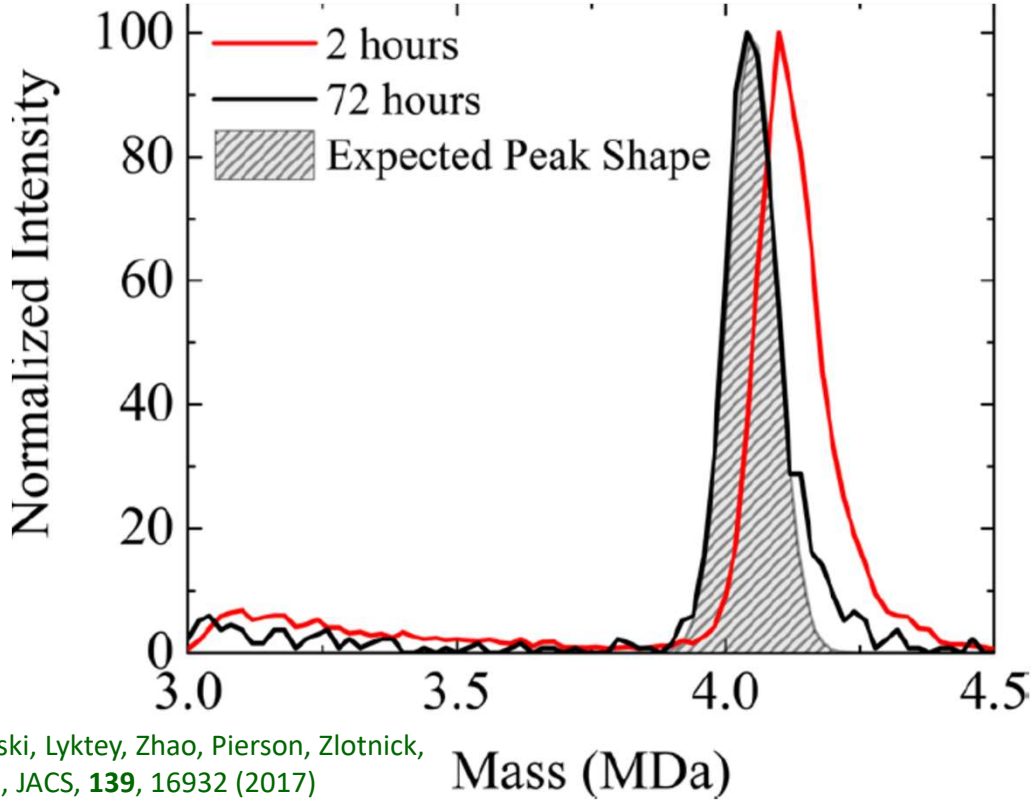
-Experiments: T=3/T=4 increases with [salt] but is independent of [dimer]

-Computational results match if Δf decreases ($K_{\mathrm{AB/CD}}$ increases) with increasing [salt]

(consistent with Ceres and Zlotnick, Biochemistry (2002))

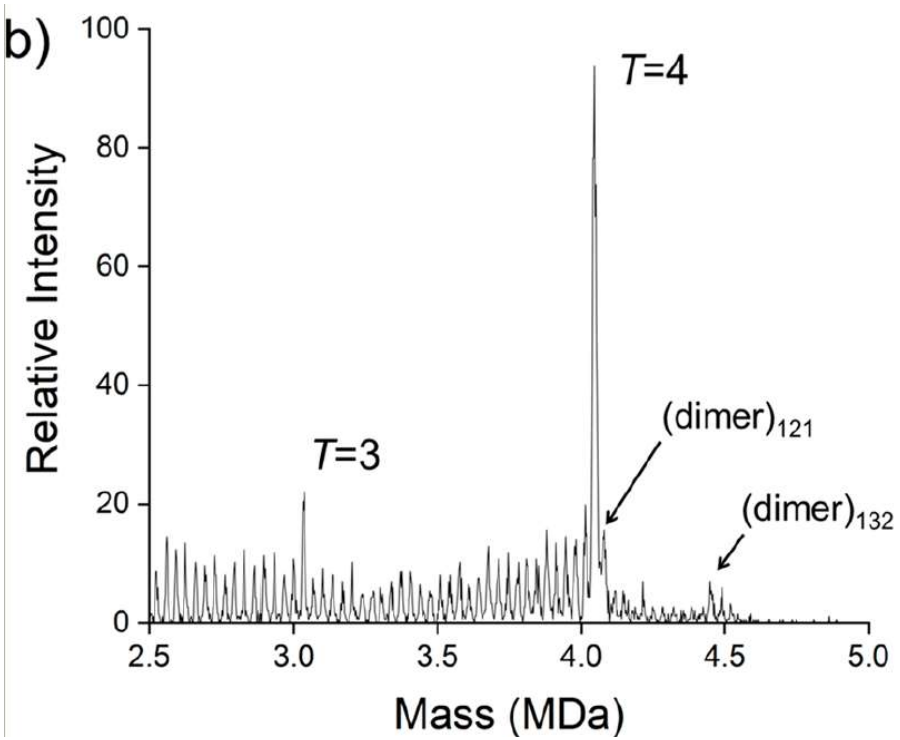
Error correction during HBV assembly (overgrown intermediates)

Charge Detection Mass Spec (CDMS) shows that HBV capsids assemble 'over-grown' intermediates before forming T=4 capsid



Lutomski, Lyktey, Zhao, Pierson, Zlotnick, Jarrold, JACS, **139**, 16932 (2017)

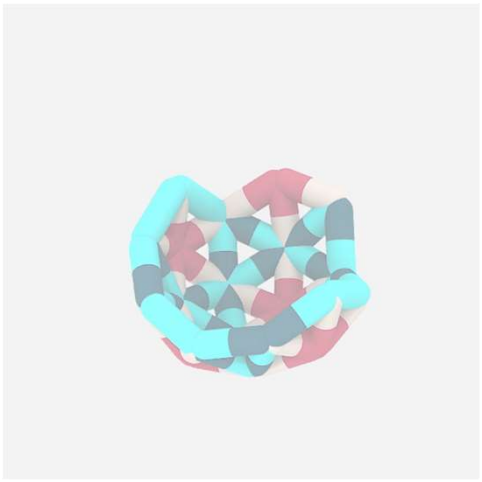
Long-lived 121-mer and 132-mer



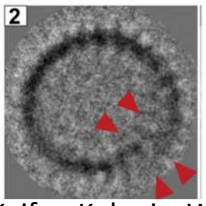
Todd, Barnes, Young, Zlotnick, Jarrold, Anal. Chem. **92**, 11357 (2020)

Error correction during assembly

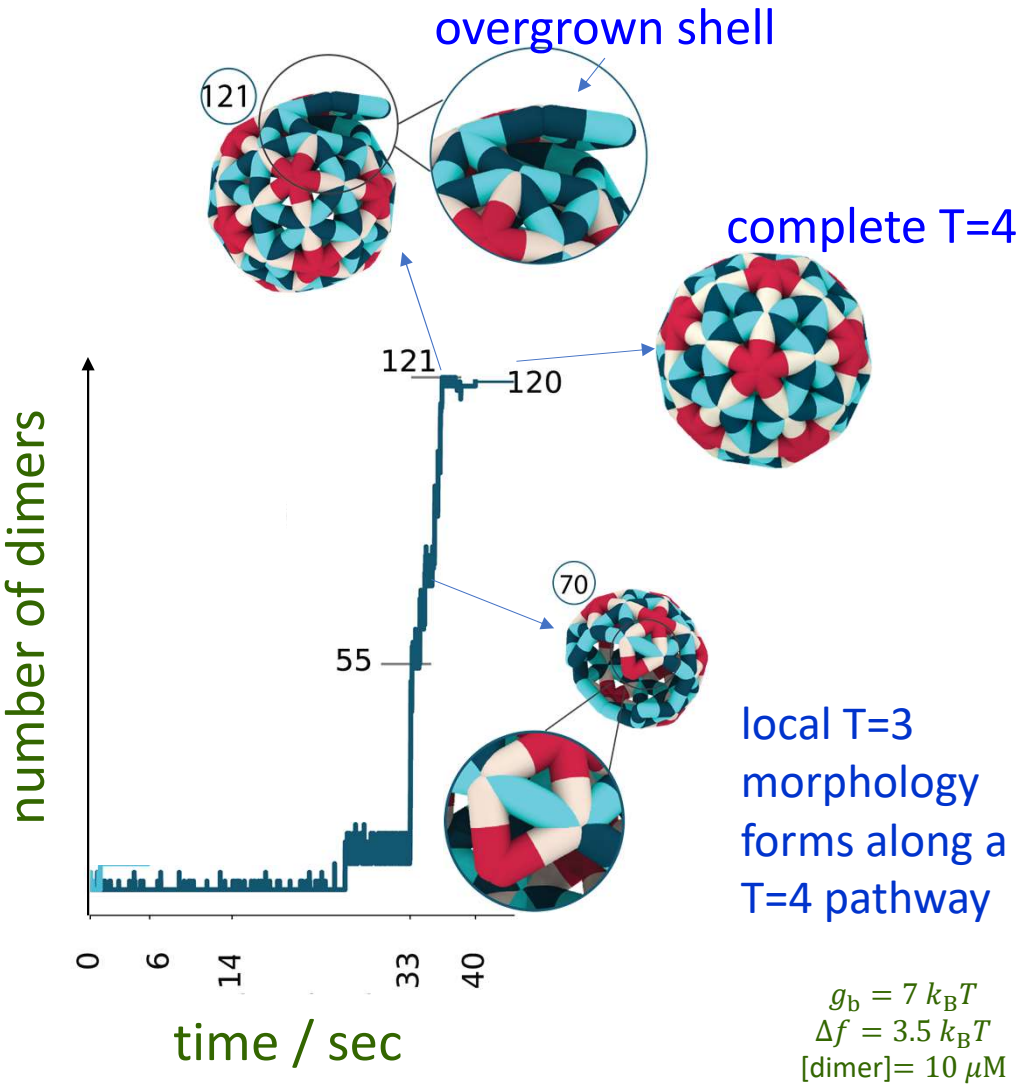
Capsid overgrows and then sheds excess subunits (as seen in experiments)



Overgrown woodchuck HBV capsids

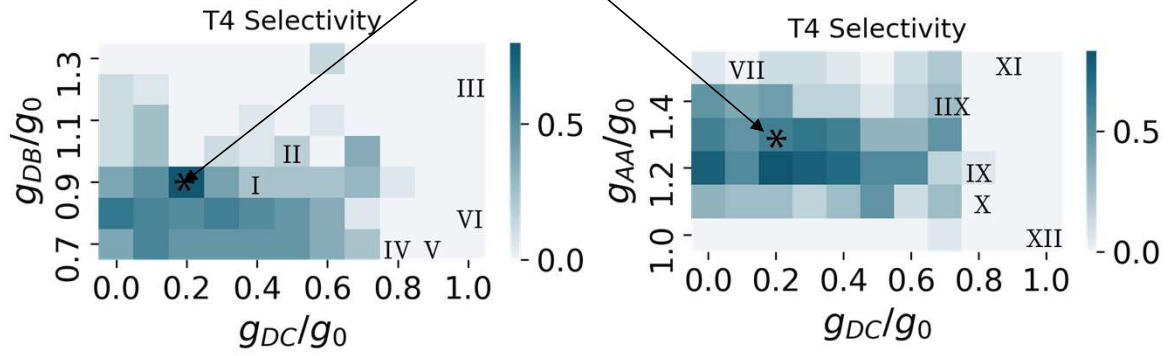


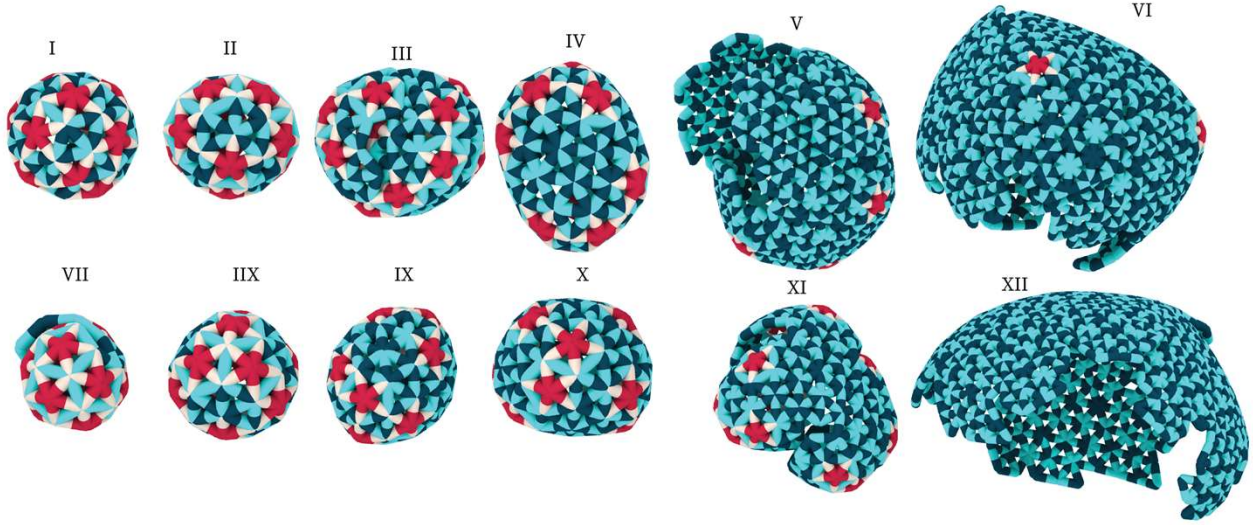
Pierson, Keifer, Kukreja, Wang, Zlotnick, Jarrold, J Mol Biol **428**, 292–300 (2016)



Importance of conformational specificity

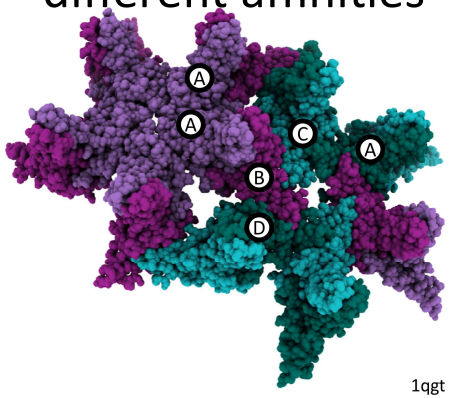
buried surface area estimate for wild-type interface affinities





Mohajerani et al. ACS Nano (2022)

different interfaces have different affinities

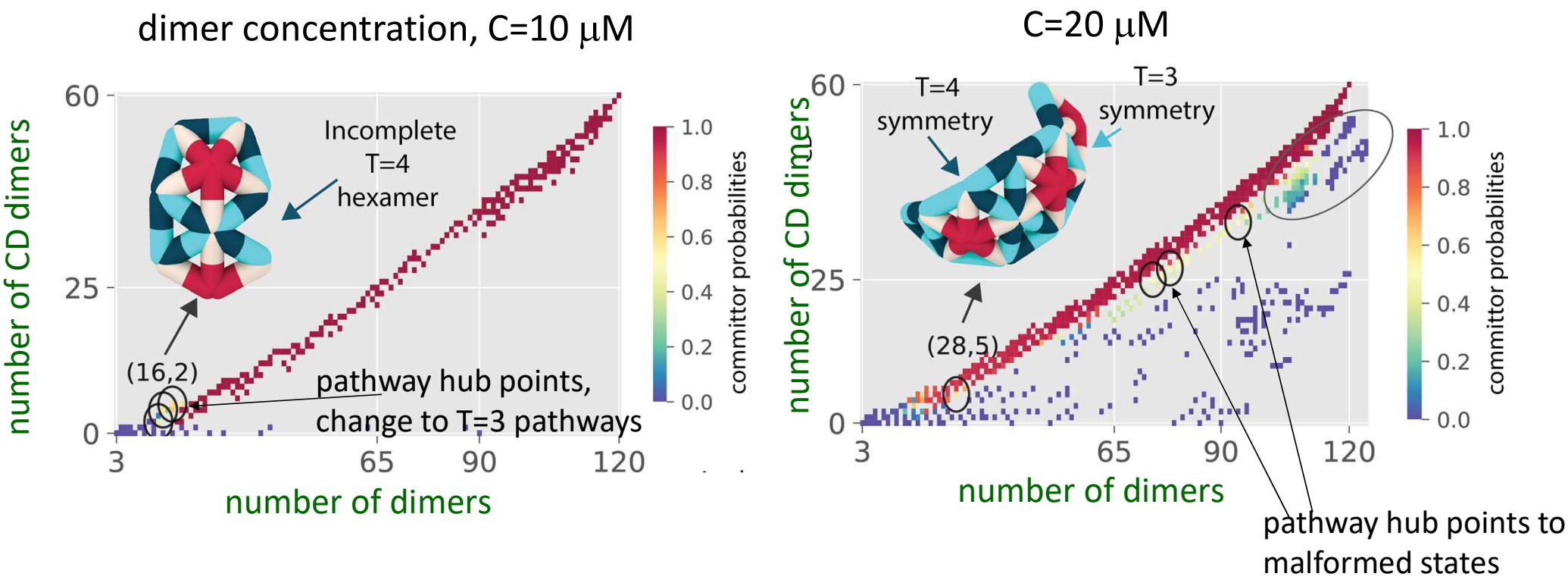


species-specific binding important design tool for synthetic programmable assemblies

Bale et al. Science (2016) https://doi.org/10.1126/science.aaf8818; Sigl et al Nat. Mater. 2021; Videbæk et al, arXiv:2111.04717 (2021)

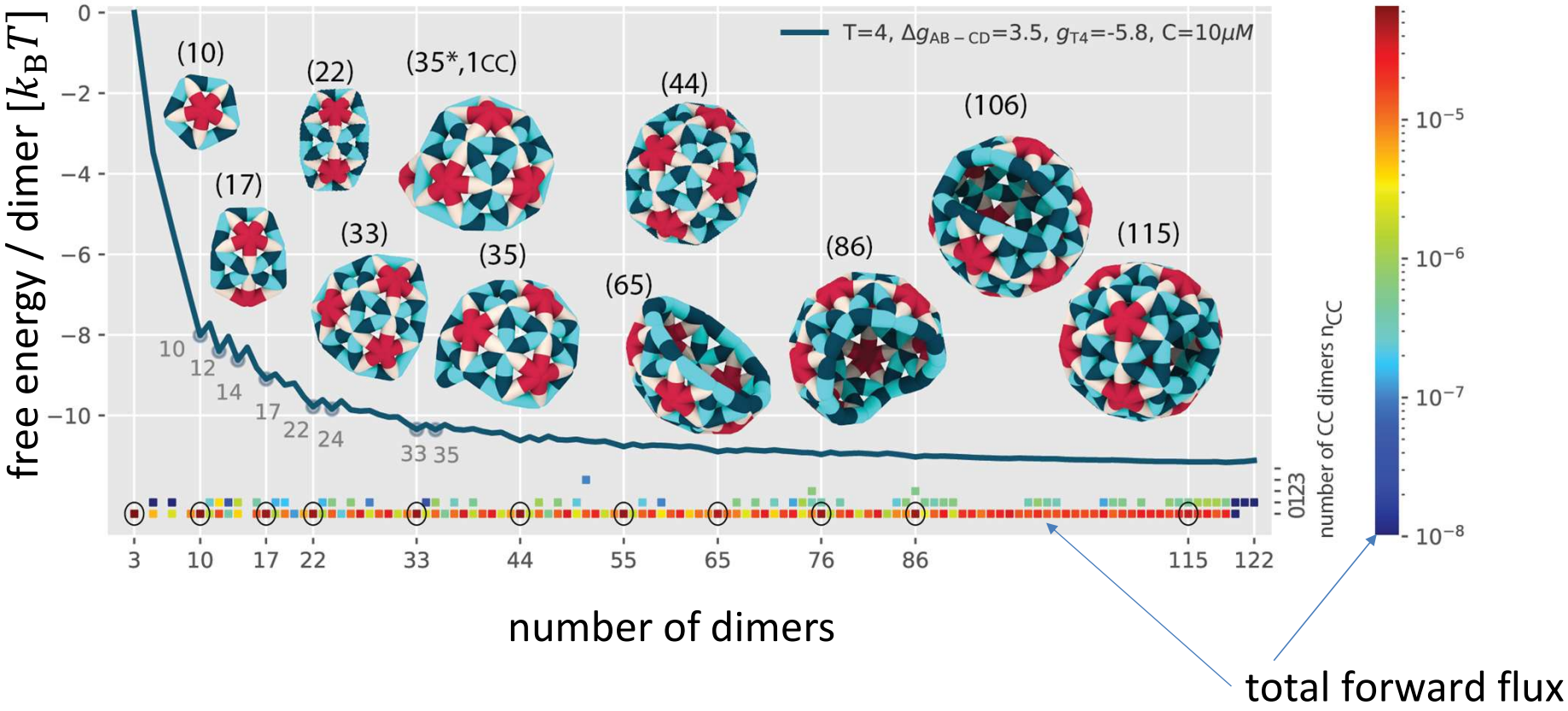
Pathway analysis

'commitor probability' = conditional probability that a structure will end up in a T=4 capsid

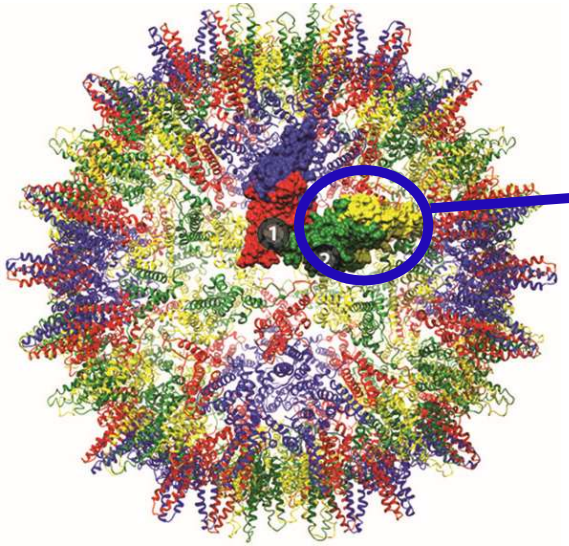


pathways can diverge to malformed pathways at large sizes with higher concentration

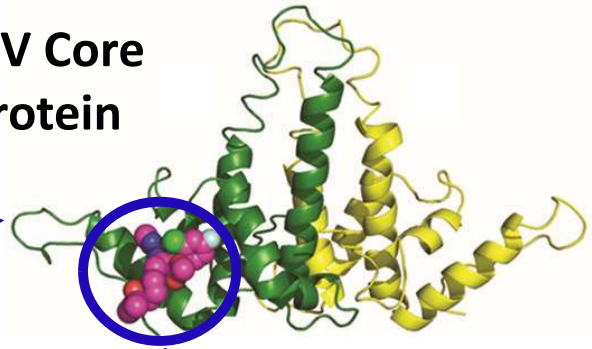
Prevalent intermediates



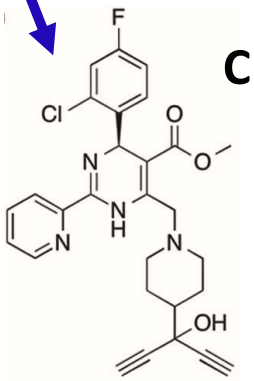
Antiviral Agents: Core protein Allosteric Modulators (CpAMs)



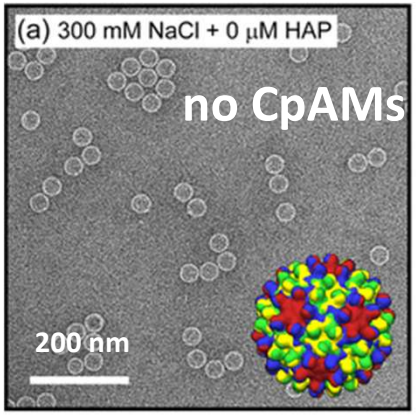
HBV T=4
Capsid



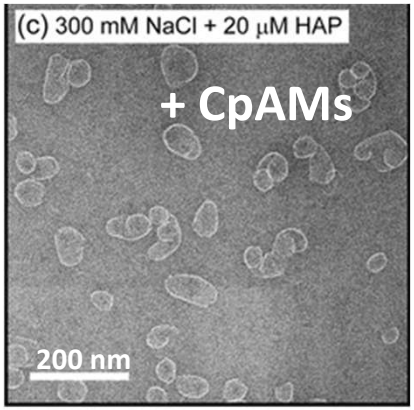
HBV Core protein



CpAM



Adam Zlotnick

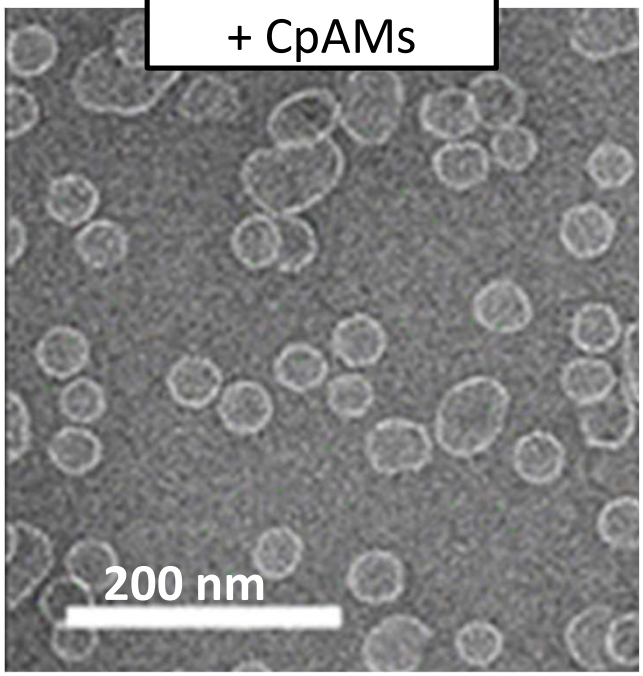


CpAMs bind to core proteins during assembly, resulting in aberrant structures.

Schlicksup, C. J. , et al. Elife 7 (2018)
Kondylis, P., et al. JACS (2018)

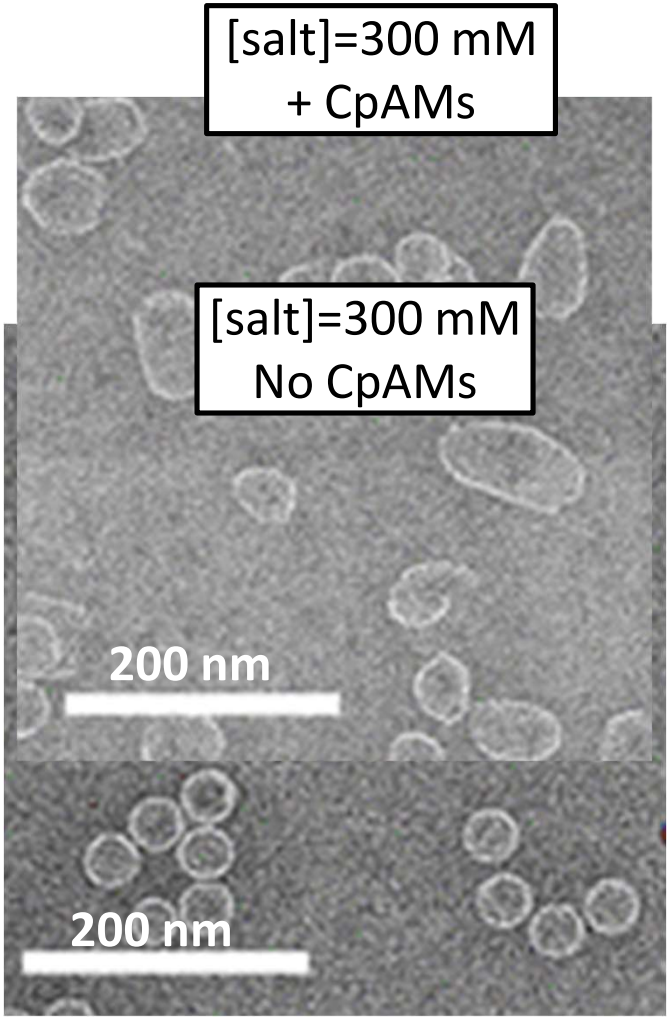
In Vitro Assembly with CpAMs

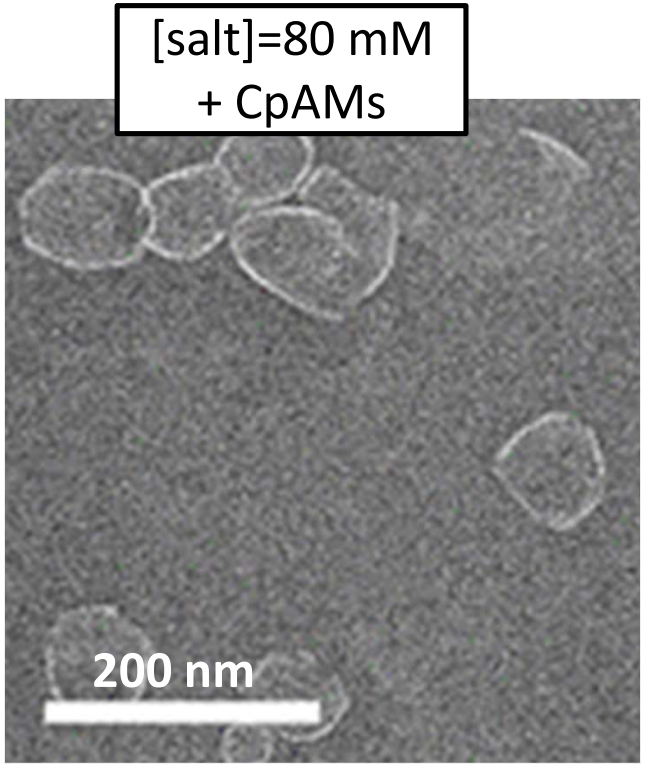
Kondylis, P. , et al. *JACS* (2019)



Strong protein-protein interactions:

- Higher curvature
- mostly T=4 (like native)



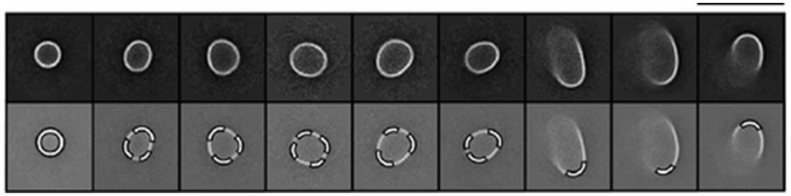


Weak protein-protein interactions:

- lower curvature
- Larger, more aberrant products

Adding CpAMs to the model

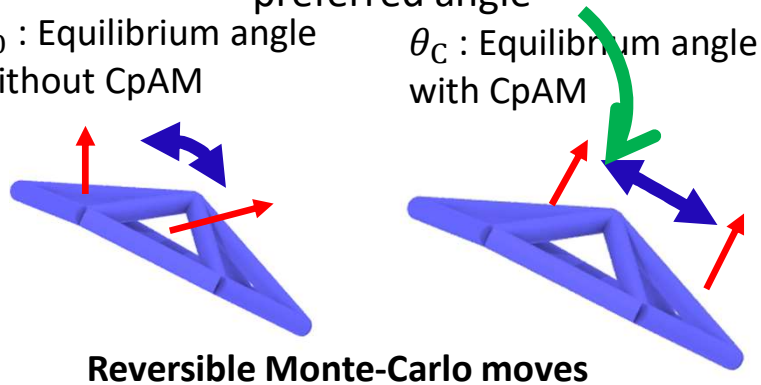
TEM of capsids assembled with CpAMs



CpAM binding changes the preferred angle

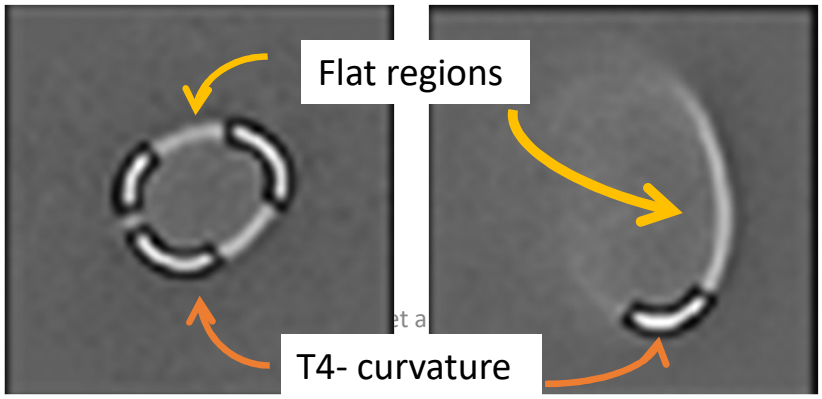
θ_0 : Equilibrium angle without CpAM

θ_C : Equilibrium angle with CpAM



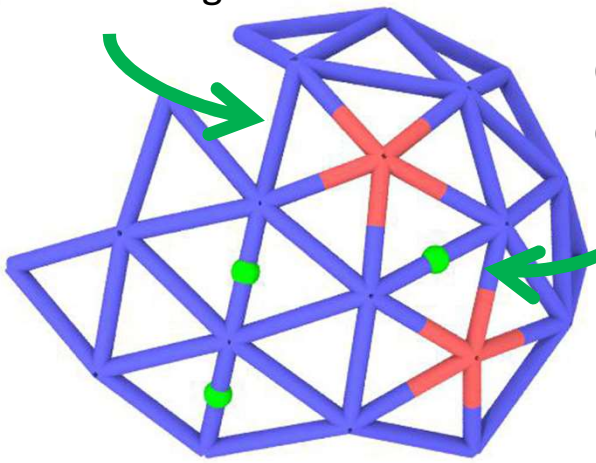
Reversible Monte-Carlo moves

CpAMs flatten binding angles



CpAM binding

CpAM dissociation



Simulations with CpAMs

dimer-dimer affinity:

$g_{\mathrm{b}} = 6.5\ k_{\mathrm{B}}T$

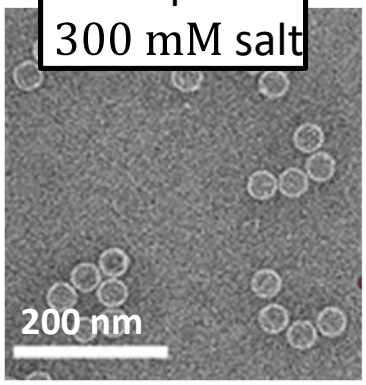
(moderate salt)

g_{b} =16 $k_{\mathrm{B}}T$

(high salt)

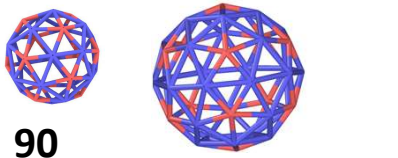
$k_{\mathrm{B}}T$ = 0.6 kcal/mol

Comparison with Experiments



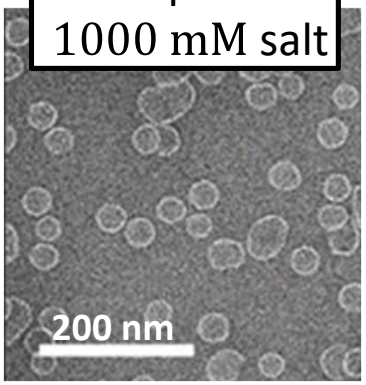
No CpAM

G_{bind} = 6.5 $k_{\mathrm{B}}T$



90

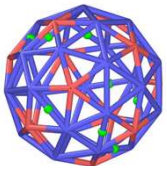
120 dimers

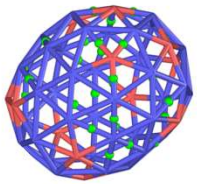


+ CpAM

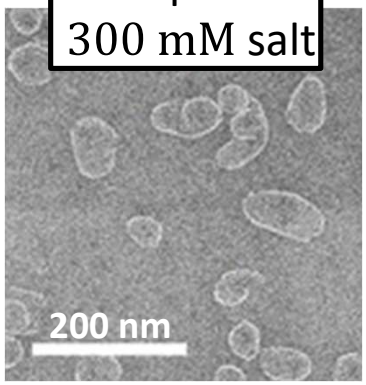
$G_{\mathrm{bind}} = 16.5\ k_{\mathrm{B}}T$

120





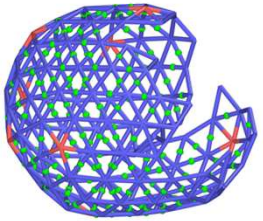
129



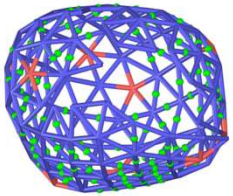
+ CpAM

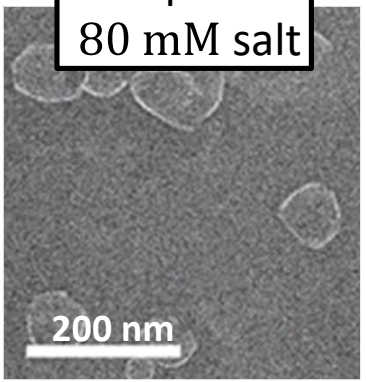
G_{bind} =6.5 $k_{\mathrm{B}}T$

357



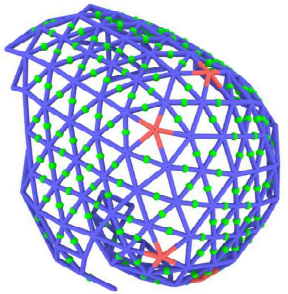
315





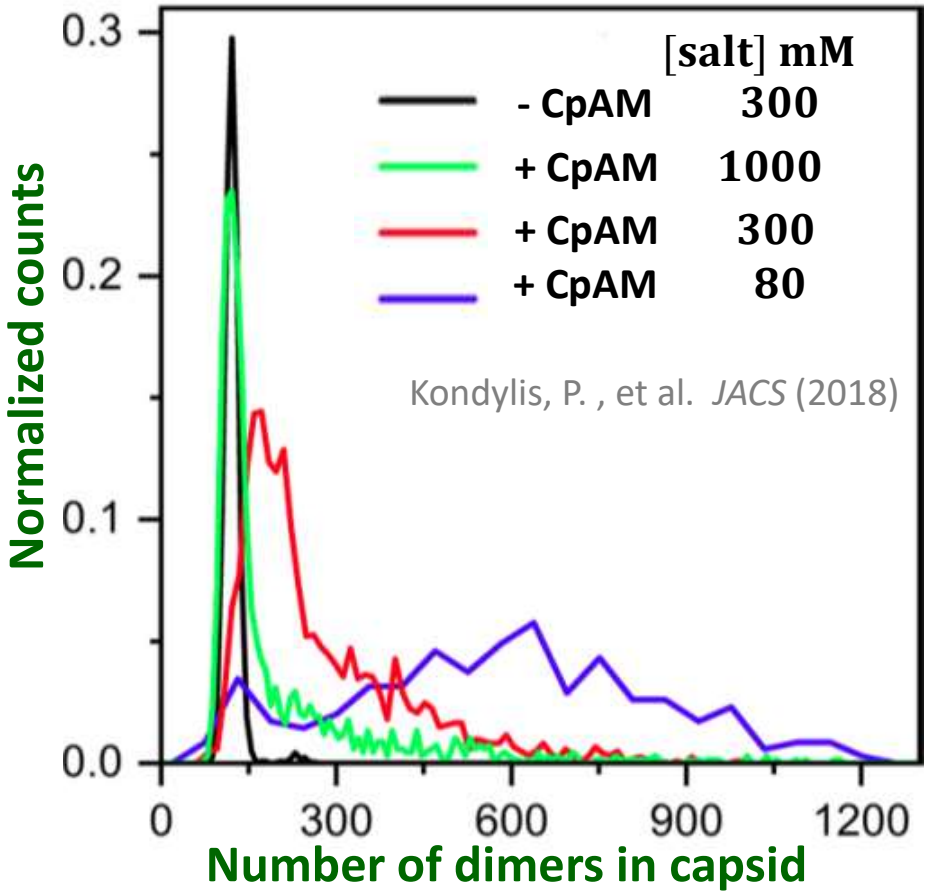
+ CpAM

G_{bind} =4.5 $k_{\mathrm{B}}T$

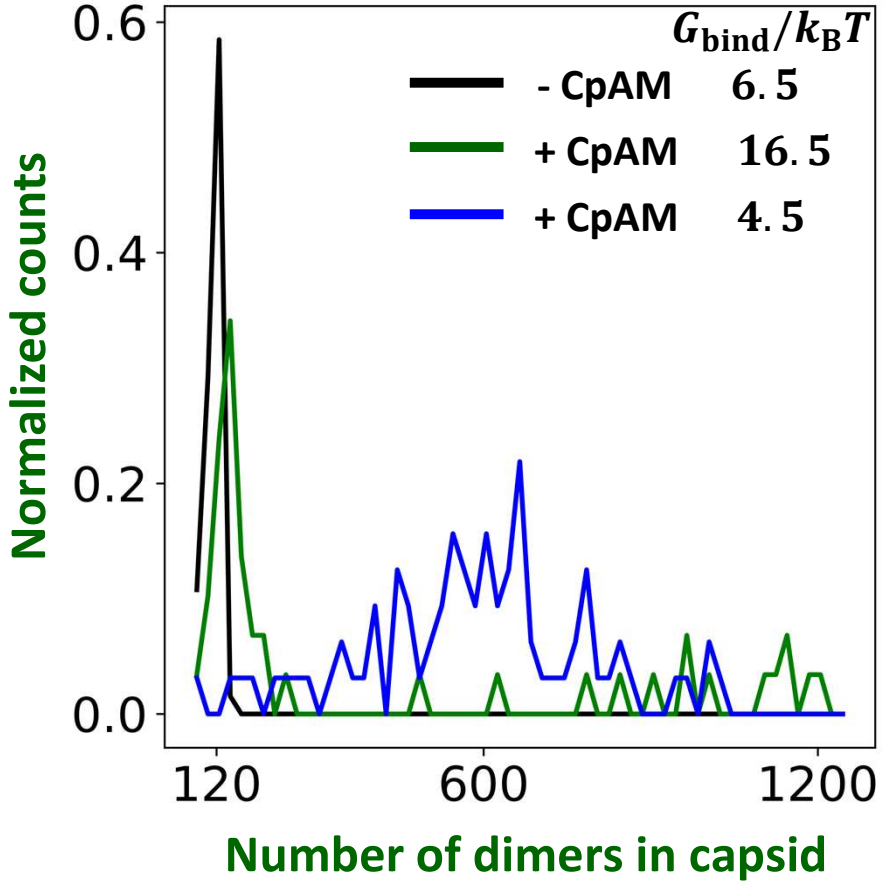


Distribution of capsid sizes

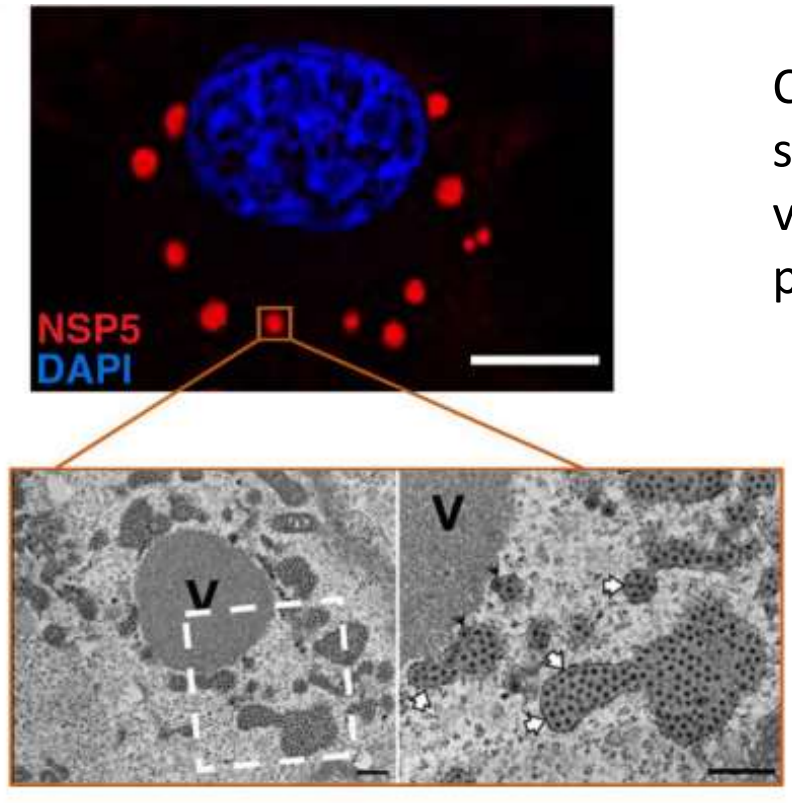
Experiments



Simulations



Viruses exploit liquid-liquid phase separation (LLPS)



Cells infected by rotaviruses form phase separated compartments called viroplasms (V) within which new viral particles assemble

Viroplasms: Assembly and Functions of Rotavirus Replication Factories

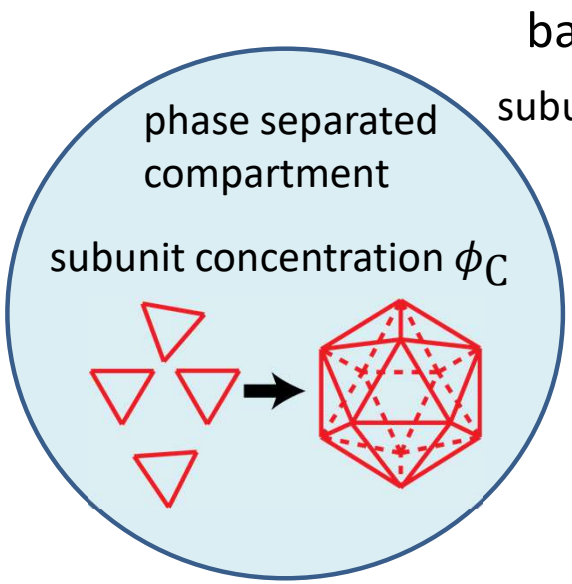
Guido Papa [1], Alexander Borodavka [2], Ulrich Desselberger [3]

Viruses. 2021 Jul; 13(7): 1349.

doi: 10.3390/v13071349

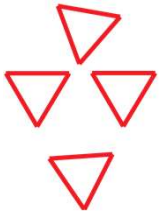
See also: Etibor et al., *Viruses* **2021**, *13*, 366

Self-Assembly Coupled to Liquid-Liquid Phase Separation (LLPS)



background

subunit concentration ϕ_B



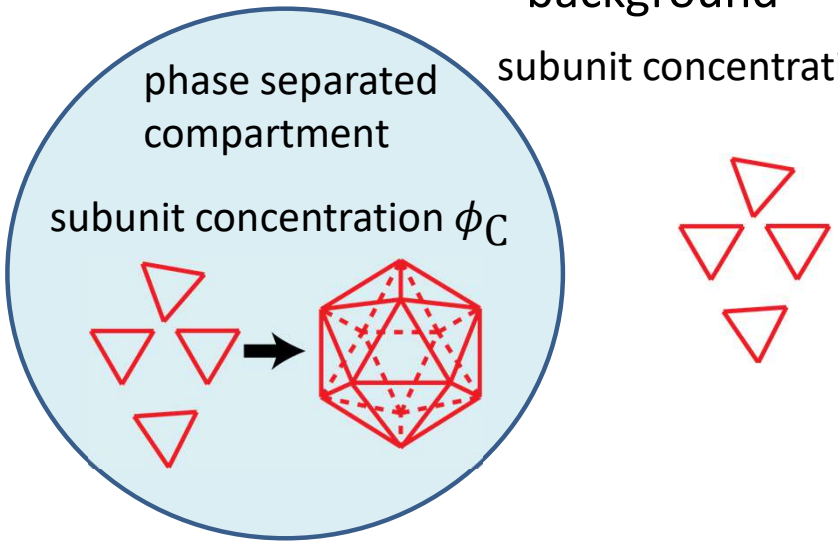
$$\phi_C = K_C \phi_B$$

K_C = partition coefficient = $\dfrac{[\text{subunit in droplet}]}{[\text{subunit in background}]}$

$K_C > 1$ so subunits preferentially partition into domain

Hagan & Mohajerani, Plos Comp. Biol. (2023) https://doi.org/10.1371/journal.pcbi.1010652
see also, Weber et al. eLife 2019;8:e42315 for similar model for irreversible filament assembly

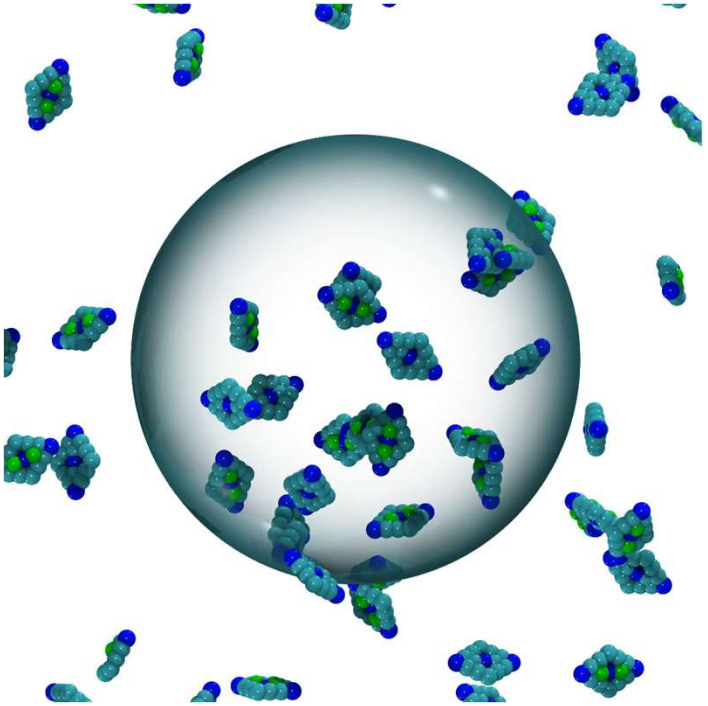
Self-Assembly Coupled to Liquid-Liquid Phase Separation (LLPS)



background

subunit concentration ϕ_B

$$\phi_C = K_C \phi_B$$



K_C = partition coefficient = $\frac{[\text{subunit in droplet}]}{[\text{subunit in background}]}$

$K_C > 1$ so subunits preferentially partition into domain

Naren Sundararajan

Hagan & Mohajerani, Plos Comp. Biol. (2023) https://doi.org/10.1371/journal.pcbi.1010652

Rate equation model for assembly coupled to LLPS

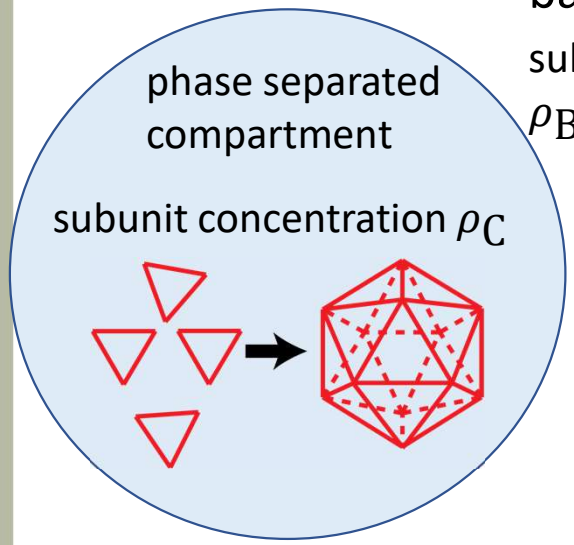
$$\frac{d\rho_1^\alpha}{dt} = -2f_1(\rho_1^\alpha)^2 + b_2\rho_2^\alpha + \left(\sum_{n=2}^{N-1} -f_n\rho_n^\alpha\rho_1^\alpha + b_n\rho_n^\alpha\right) + b_N\rho_N^\alpha + \mathcal{D}_1^\alpha$$

$$\frac{d\rho_n^\alpha}{dt} = f_{n-1}\rho_1^\alpha\rho_{n-1}^\alpha - (f_n\rho_1^\alpha + b_n)\rho_n^\alpha + b_{n+1}\rho_{n+1}^\alpha + \mathcal{D}_n^\alpha \quad \text{for } n = 2\ldots N-1$$

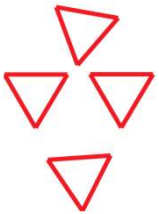
$$\frac{d\rho_N^\alpha}{dt} = f_{N-1}\rho_1^\alpha\rho_{N-1}^\alpha - b_N\rho_N^\alpha + \mathcal{D}_N^\alpha$$

$$\mathcal{D}_n^{\mathrm{c}} = \frac{1}{V_{\mathrm{c}}}k_{\mathrm{DL}}(n)\left(\rho_n^{\mathrm{bg}} - \rho_n^{\mathrm{c}}/K_{\mathrm{c}}^n\right)$$

$$\mathcal{D}_n^{\mathrm{bg}} = -V_{\mathrm{r}}\mathcal{D}_n^{\mathrm{c}}$$



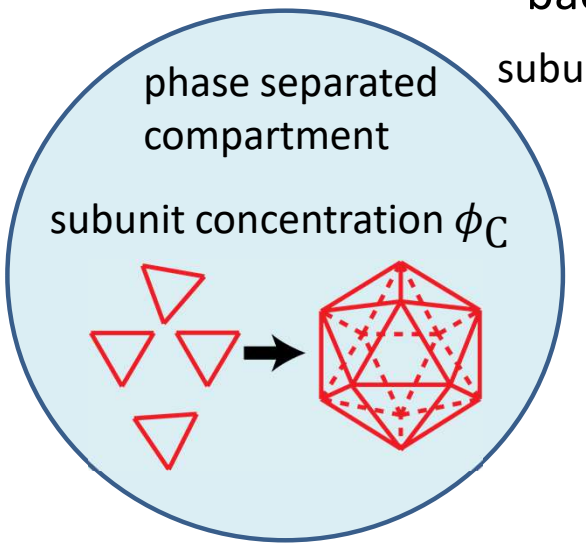
background subunit concentration ρ_{B}



$$\rho_{\mathrm{C}} = K_{\mathrm{C}}\rho_{\mathrm{B}}$$

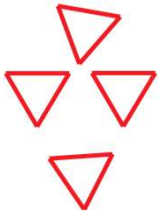
Hagan & Mohajerani, Plos Comp. Biol. (2023)

Self-Assembly Coupled to Liquid-Liquid Phase Separation (LLPS)



background

subunit concentration ϕ_B

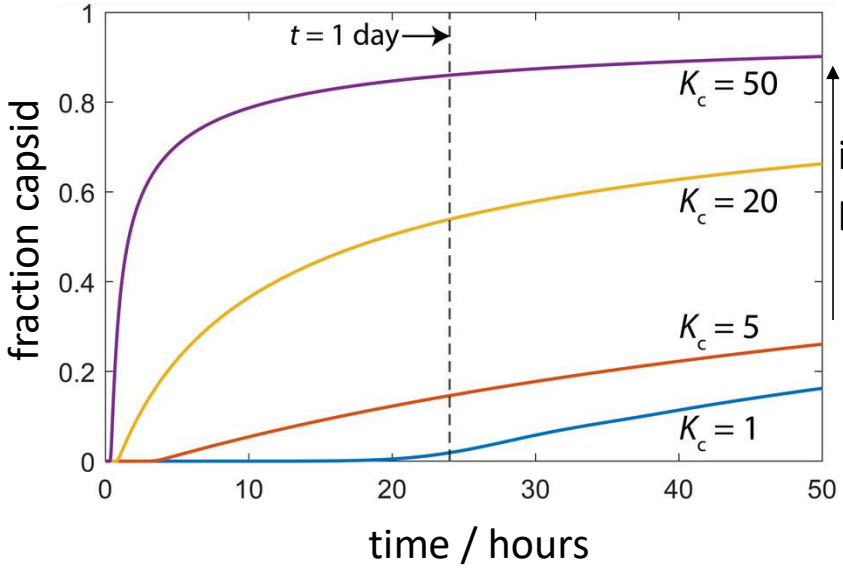


$$\phi_C = K_C \phi_B$$

K_C= partition coefficient

$K_C > 1$ so subunits preferentially partition into domain

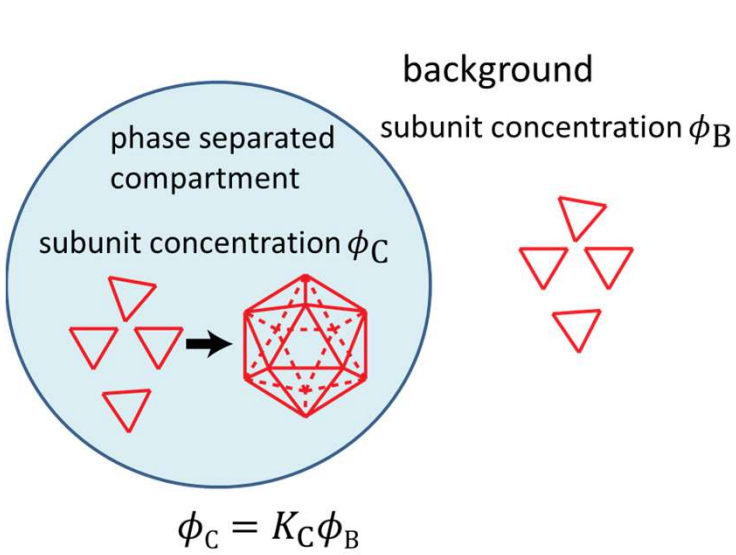
results from rate equation model

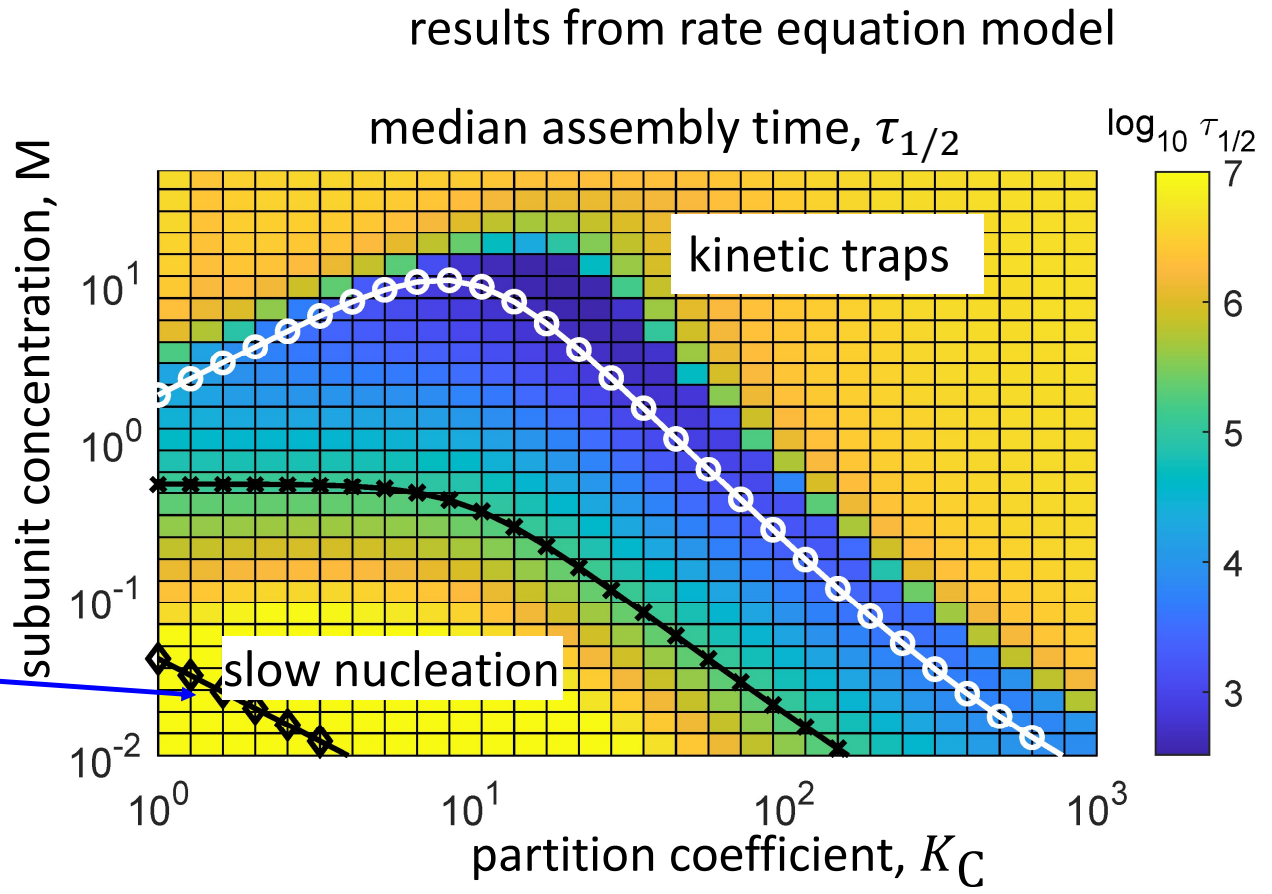


LLPS can increase assembly rates by orders of magnitudes

Hagan & Mohajerani, Plos Comp. Biol. (2023) https://doi.org/10.1371/journal.pcbi.1010652

Self-Assembly Coupled to Liquid-Liquid Phase Separation (LLPS)

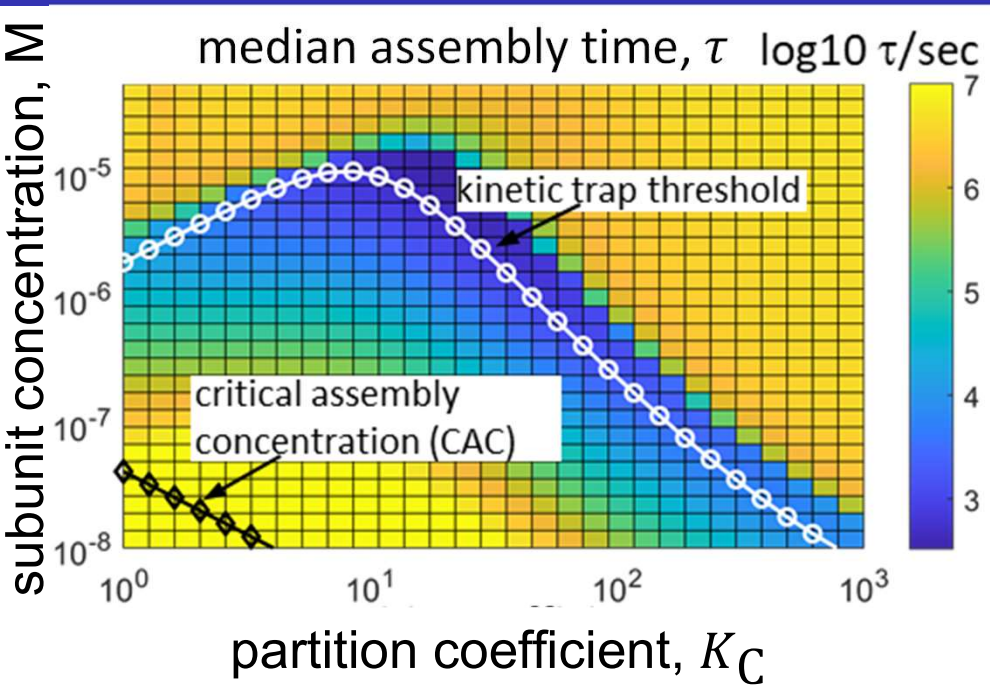


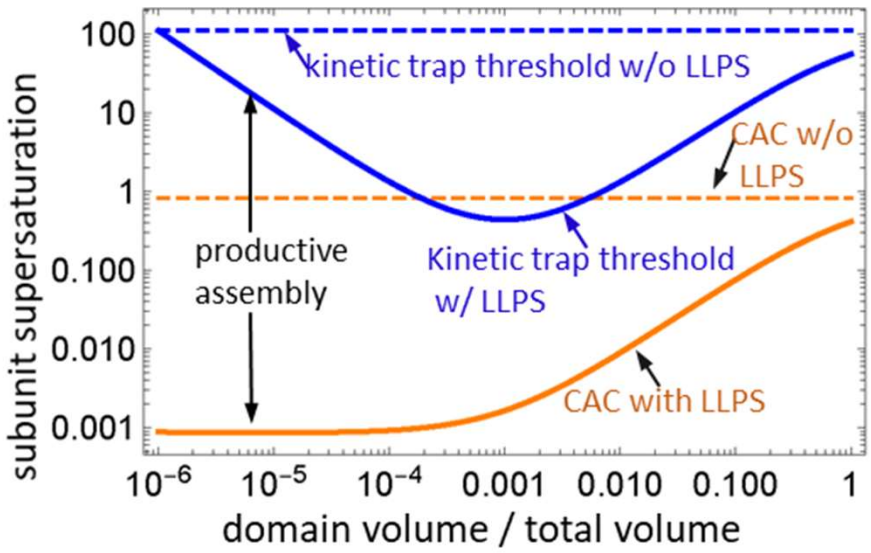


LLPS increases assembly rates and **robustness to parameter variation**

Hagan & Mohajerani, Plos Comp. Biol. (2023) https://doi.org/10.1371/journal.pcbi.1010652

Self-Assembly Coupled to Liquid-Liquid Phase Separation





partition coefficient $= K_C = \frac{[\text{subunit in droplet}]}{[\text{subunit in background}]}$

LLPS can:

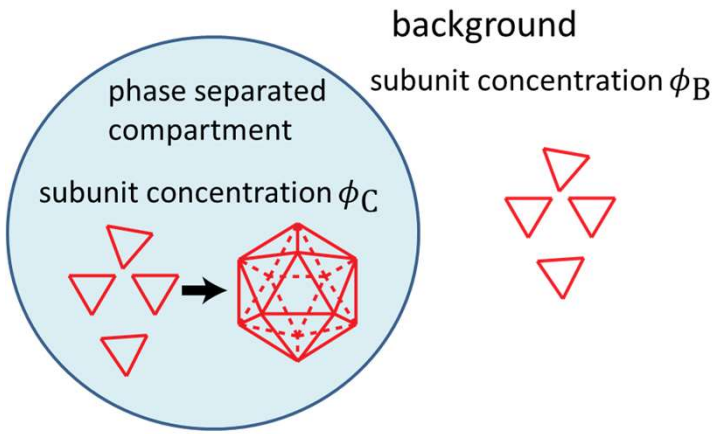
-dramatically accerelate assembly rates

-make assembly robust, by expanding range of concentrations and binding affinities that lead to good assembly, by avoiding kinetic traps

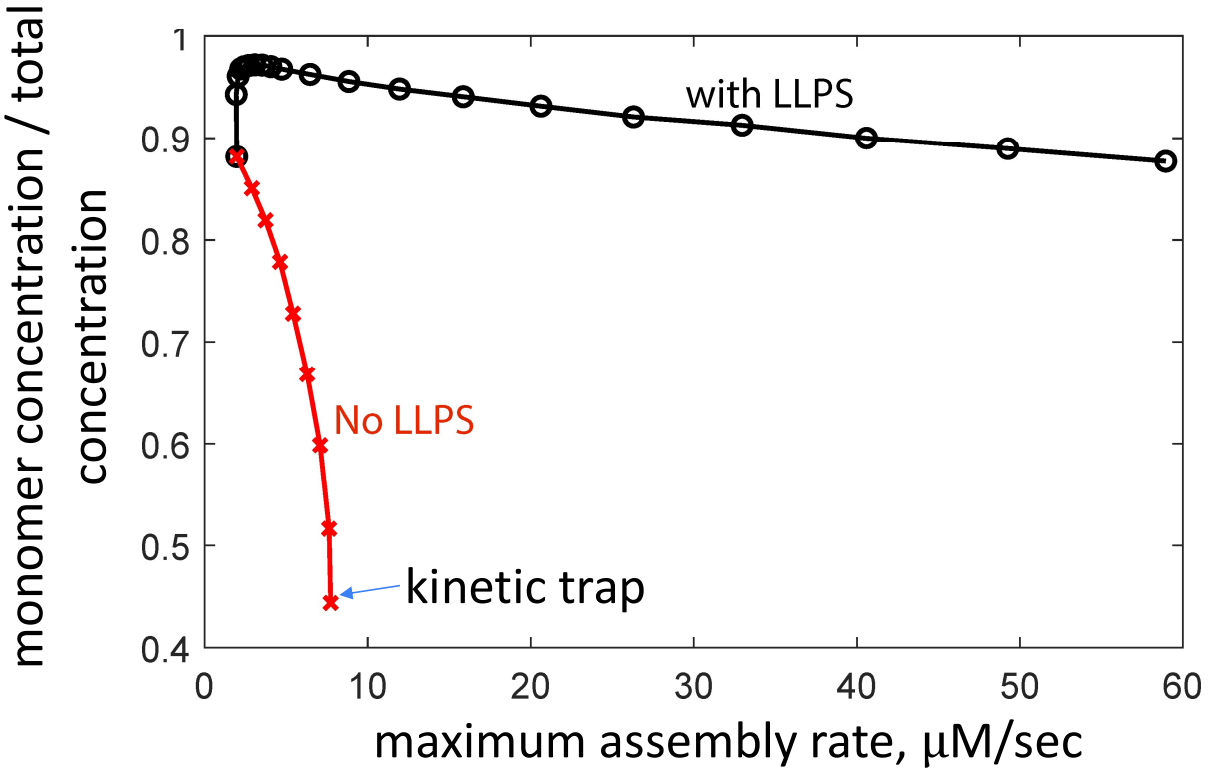
Hagan & Mohajerani, Plos Comp. Biol. (2023) https://doi.org/10.1371/journal.pcbi.1010652

see also, Weber et al. eLife 2019;8:e42315 for similar model for irreversible filament assembly

Bulk solution acts as a buffer of free subunits



$$\phi_C = K_C \phi_B$$

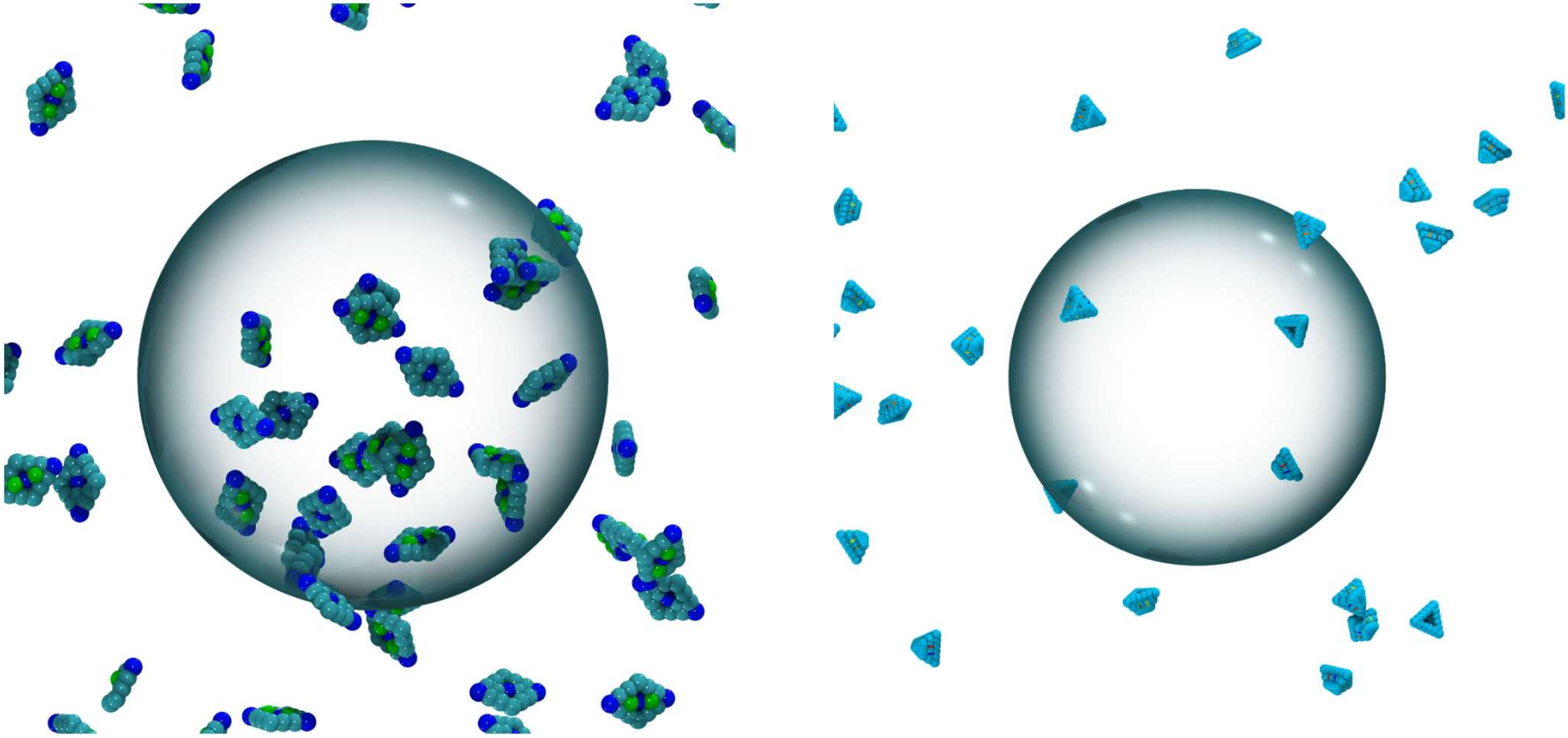


fast assembly without depleting subunits because assembly is localized to compartment

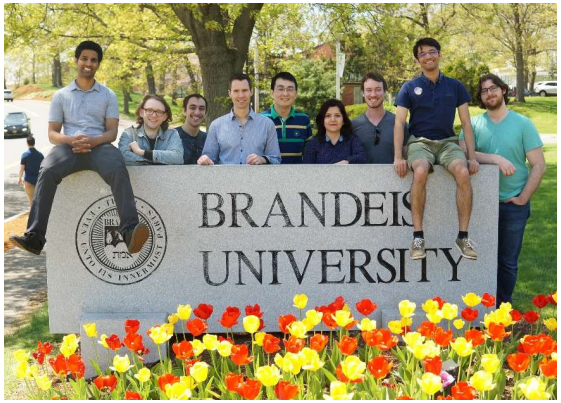
increasing concentration or interaction strength

Hagan & Mohajerani, Plos Comp. Biol. (2023)

Brownian dynamics simulation of self-assembly with LLPS

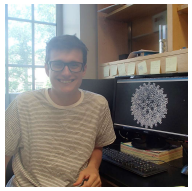


Naren Sundararajan



Farri Mohajerani

Naren Sundararajan



Chris Schlicksup

Adam Zlotnick

Jodi Hadden-Perilla

Botond Tyukodi, Stefan Paquay, Seth Fraden,Ben Rogers, Wei-Shao Wei

NIH (R01GM108021)
$$: DOE: Machine learning approaches to understanding and controlling 3D active matter
NSF (CMMT DMR-1855914, **Brandeis MRSEC**)
Brandeis Provosts Research Grant Computation: NSF XSEDE, Brandeis HPCC

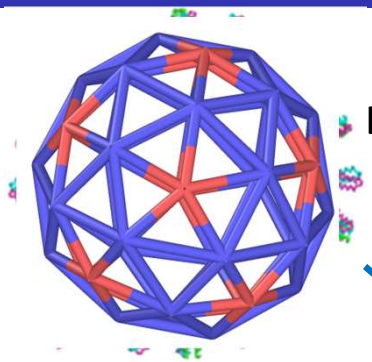
Hagan Group: Layne Frechette, Fernando Caballero, Anthony Trubiano, Phu Tran, Chris Amey, Yingyou Ma, Saptorshi Ghosh, Sarvesh Uplap, Naren Sundararajan, Smriti Pradhan

Brandeis MRSEC, AMC Crawford Notch, New Hampshire, 2023

Postdoc position on active matter available

Capsid symmetry

Model capsids with 120 dimers do not have icosahedral symmetry

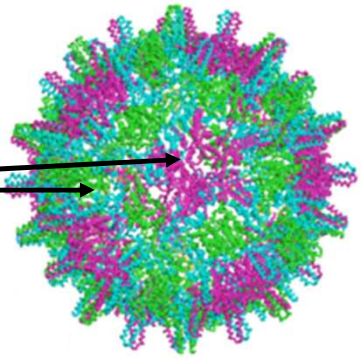


HBV capsid with 120 dimers
T=4 Icosahedral symmetry

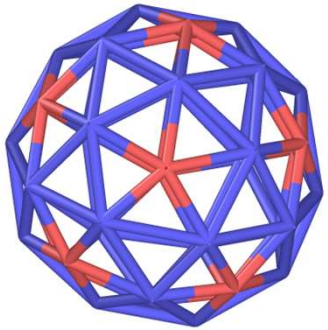
View from all sides

preliminary results: 2 dimer conformations are required for T=4 symmetry

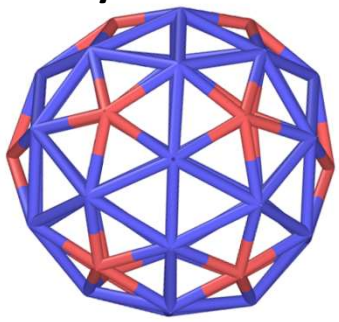
2 dimer conformations in T=4 HBV capsids



Model capsid with 120 edges
D5h symmetry



Top view



Side view

Other works showing D5h is favored: Wagner & Zandi, Biophys. J. (2015); Sanaz, Li, Zandi 2018; Lorente, Hernandez-Rojas, Breton, Soft Matter (2018)