MECHANISM OF VIRUS CAPSID ASSEMBLY

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

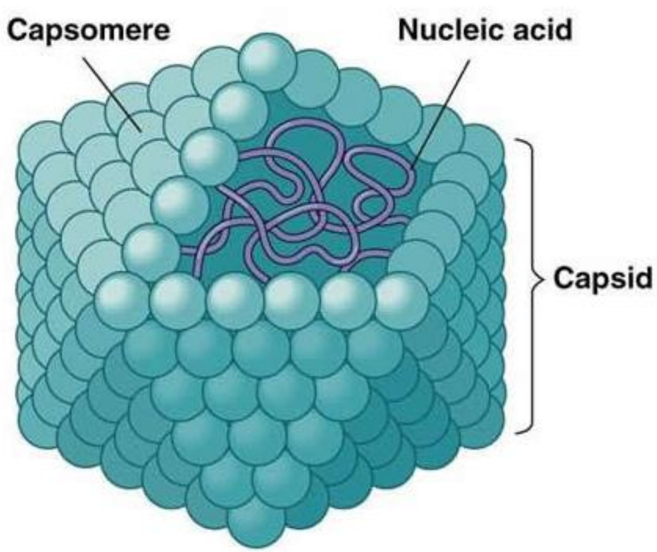
Icosahedral viruses

Half of known virus families

Regular icosahedron → 60 identical subunits

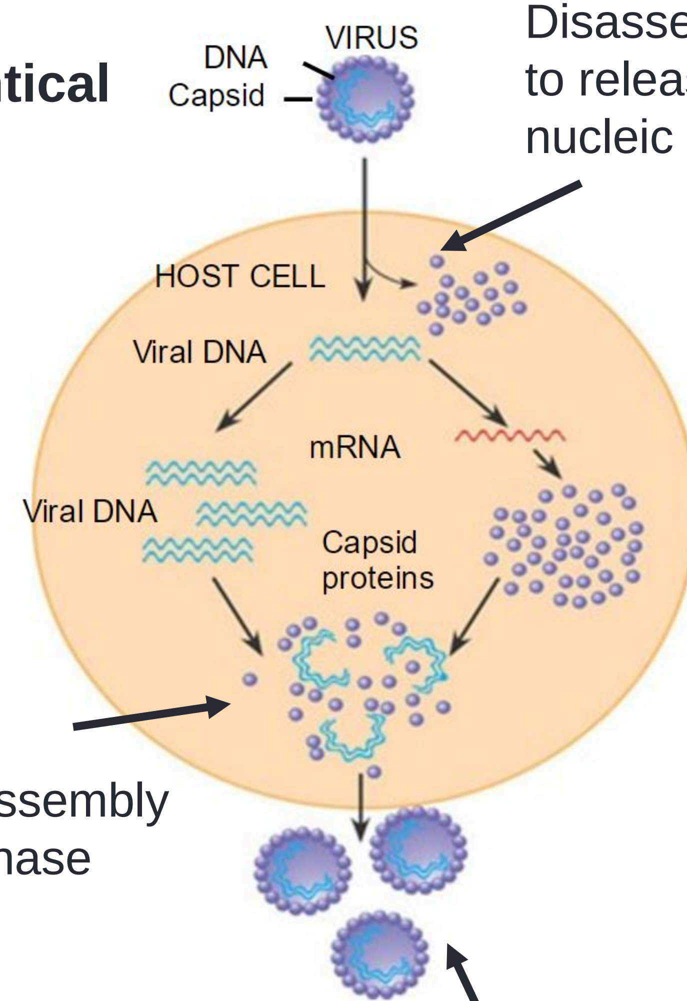
Larger viruses → 60T subunits
T = 1,3,4,7 …

Caspar and Klug (1962)



capsid - many copies of a single protein or few different proteins

nucleic acid –double or single stranded RNA/DNA



Disassemble to release its nucleic acid

Assembly phase

Product must be very stable

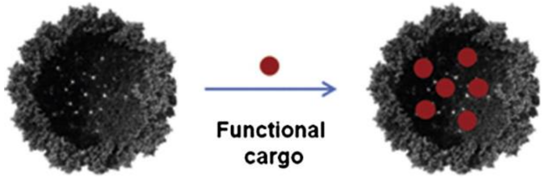
HBV Assembly

- Chronic infection 250M → ~1M deaths/year
- Capsids may package nucleic acid during assembly or remain empty (90% of the particles *in vivo* during infection)
  - storage form
  - immature particles for subsequent nucleic acid packaging

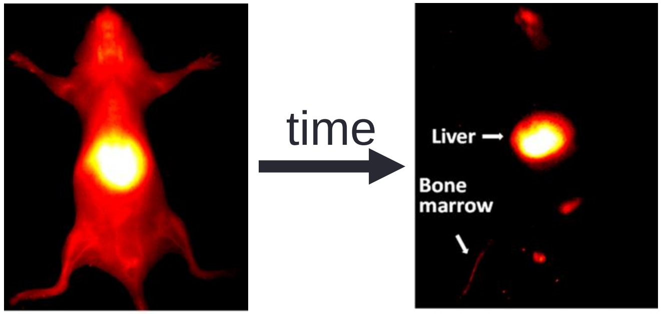
a promising target for antiviral agents

Virus like particles as materials

Protein nanocapsules



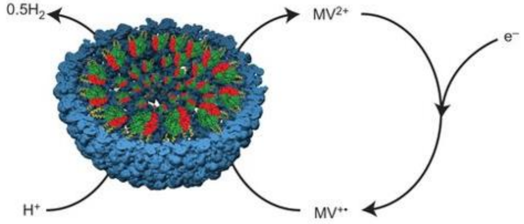
Ma et. al. Adv. Drug Deliv. Rev. (2012)



Li et. Al. Nano Lett. (2019)

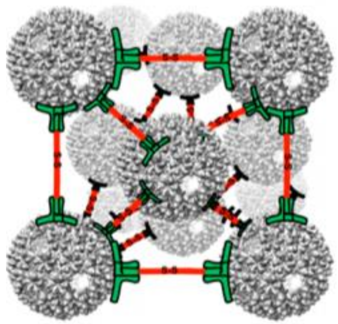
Delivery systems

Scaffolds for displaying antigens for vaccine applications



Nanoreactors for catalysis

Jordan et. al. Nat. Chem. (2016)

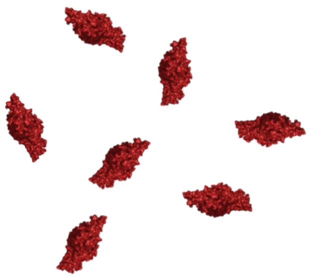


3D functional materials

K. McCoy et. al. ACS Nano (2018)

Structure **Stability** **Dynamics of assembly**

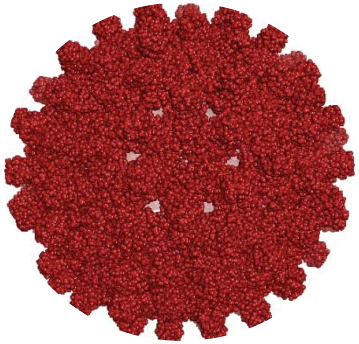
Assembly of empty capsids of Hepatitis B can be recapitulated with purified capsid protein



Dimer subunits
at pH 7.5 and at 4°C

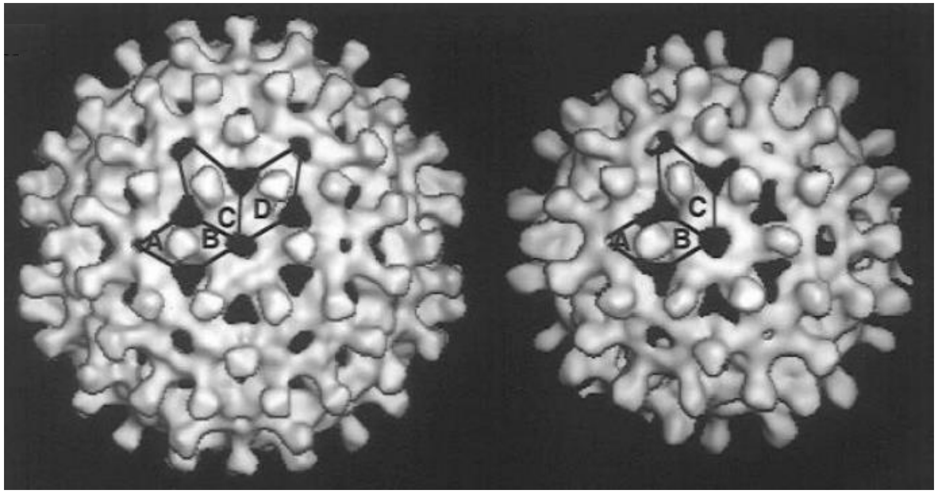
Increase temperature

Increase the ionic strength
(screen repulsive interactions)



Associate by burial of
hydrophobic surfaces

T = 4
120 dimers
~33nm



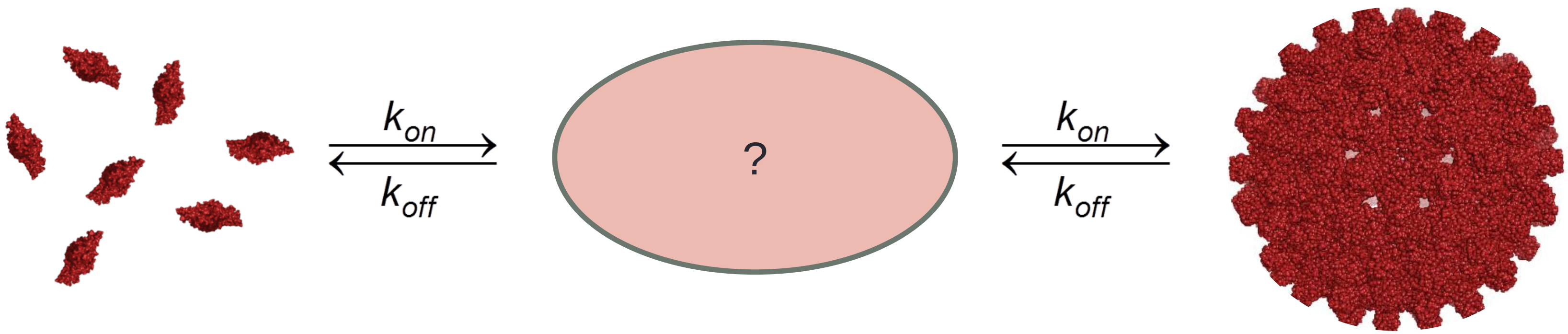
T = 3
90 dimers
~28nm

Zlotnick et al. Biochemistry 1996

Assembly pathways?

- alleviate errors
- follow the most efficient path
- avoid kinetic traps

→ Weak protein-protein interactions, slow nucleation, reversible association

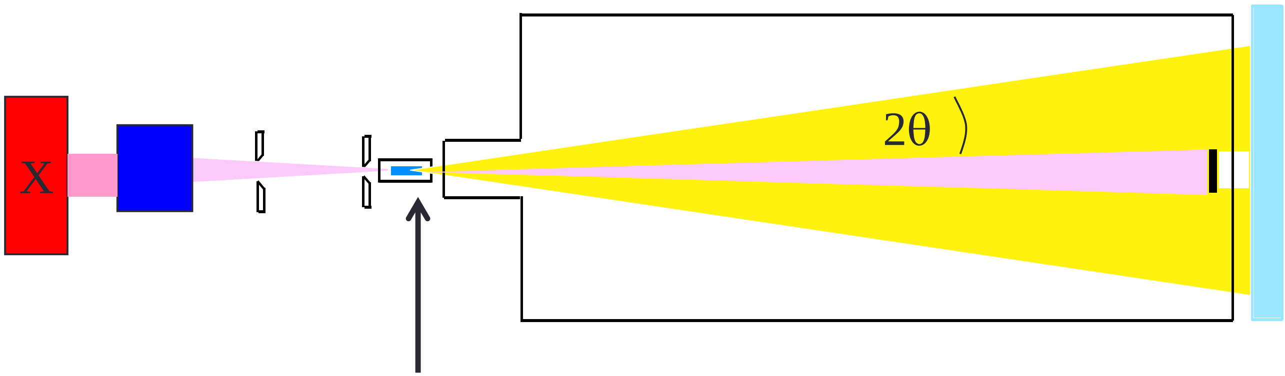


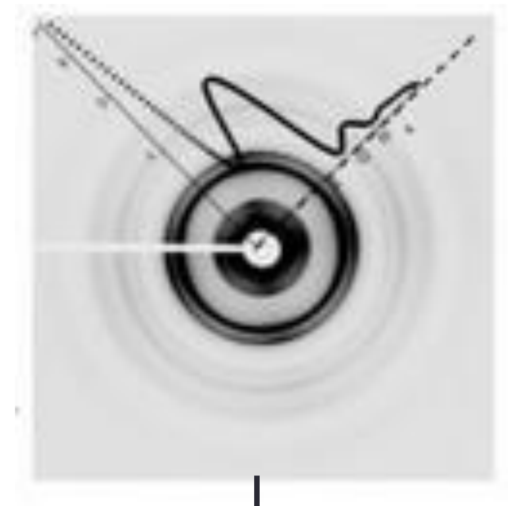
~10^{30} possible intermediates

Prevelige et al. Biophys. J 1993, Zlotnick A. Virology 2003, Rapaport D. PRL 2008

solution X-ray scattering

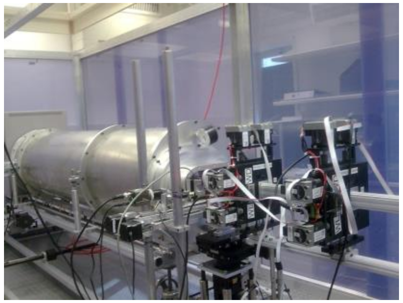
Detector





Biochemistry

Data Analysis



N. Nadler, A. Steiner, T. Dvir, O. Szekely, P. Szekely, A. Ginsburg, R. Asor, R. Resh, C. Tamburu, M. Peres, U. Raviv, Soft Matter, 7, 1512, 2011

Advantages of solution X-ray scattering

Simultaneously:

- in solution
- label-free
- high spatial resolution: 1 – 1000 Å
- high temporal resolution: 20 msec and up
- highly sensitive bulk method

Data analysis is challenging

Scattering Amplitude =

Fourier transform of the electron density contrast

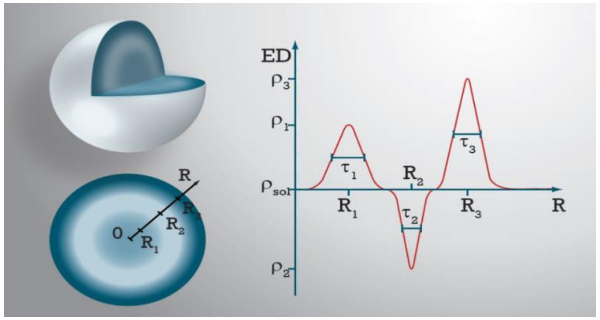
$\Rightarrow$ Phase problem

Orientation Averaging

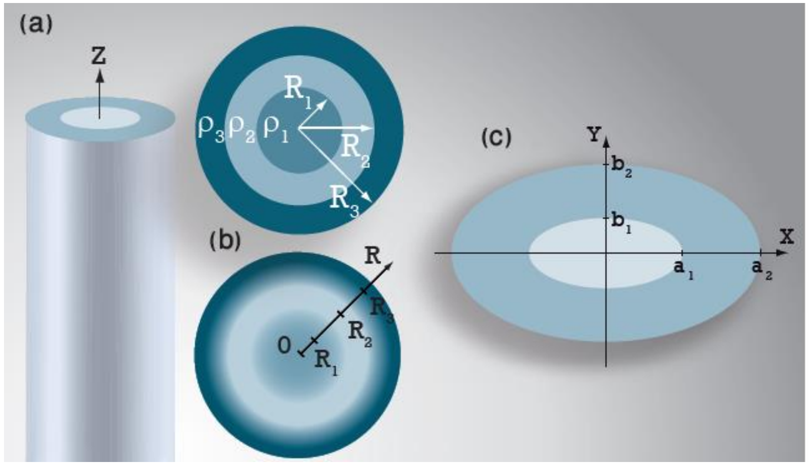
1D data $\Rightarrow$ 3D structures

X+: *Multilayered* - single-geometry and lattices

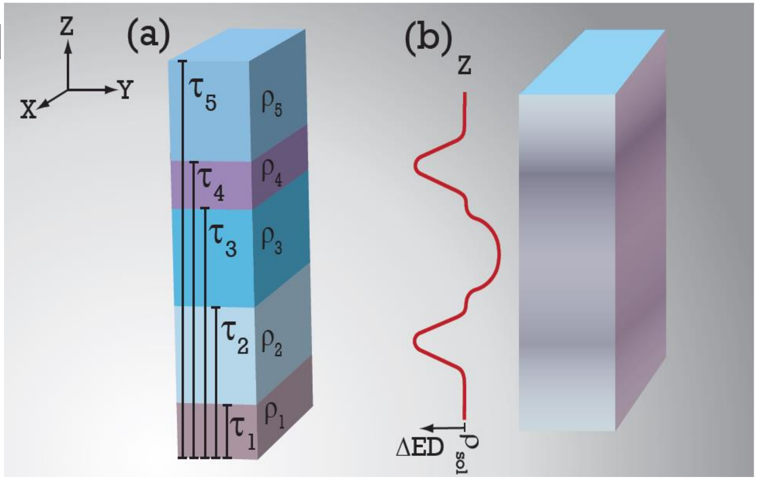
Spherical



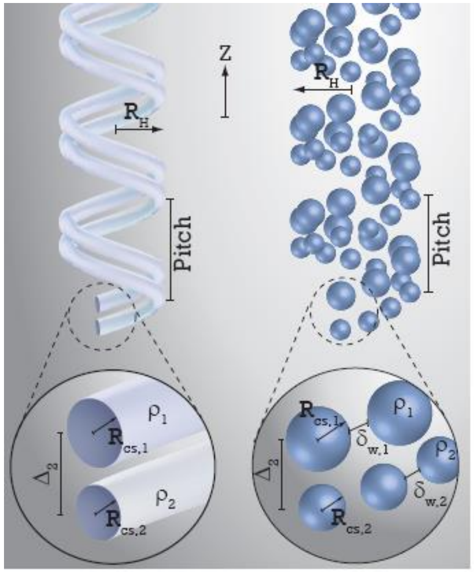
Cylindrical



Layered



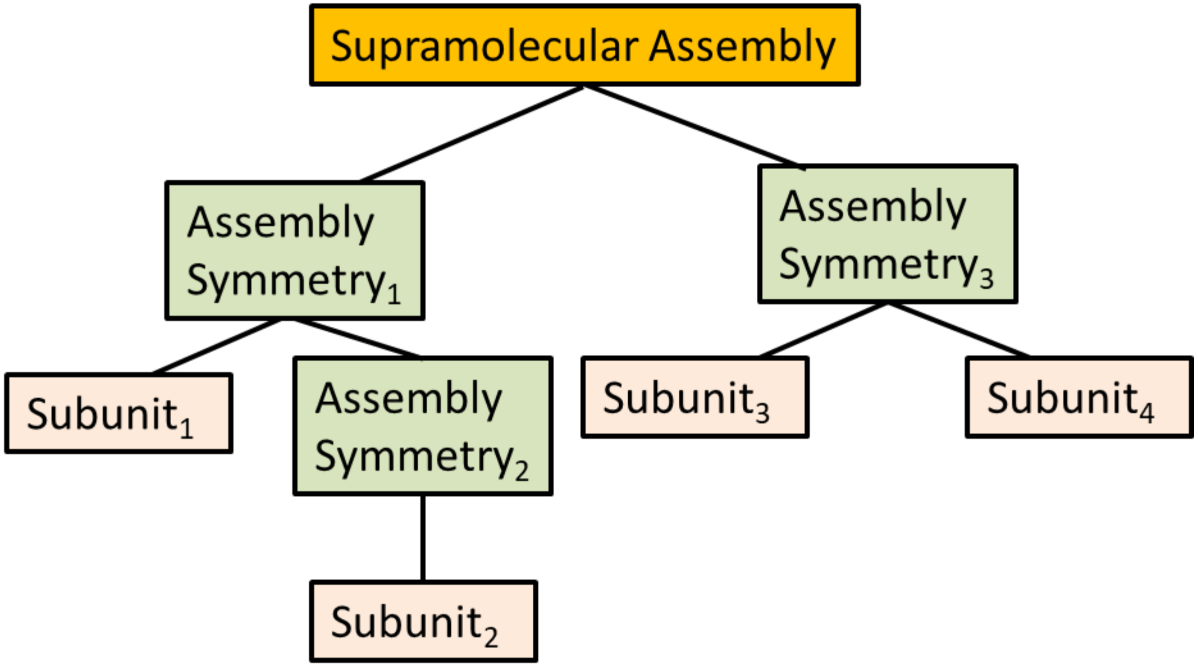
Helical



P. Szekely, A. Ginsburg, T. Ben-Nun, U. Raviv, Langmuir, 2010
T. Ben-Nun, A. Ginsburg, P. Szekely, U. Raviv, J. Appl. Cryst., 2010
T. Ben-Nun, R. Asor, A. Ginsburg, U Raviv, Isr. J. Chem., 2015

https://scholars.huji.ac.il/uriraviv/software/x

D+: Hierarchical docking of geometric and atomic models

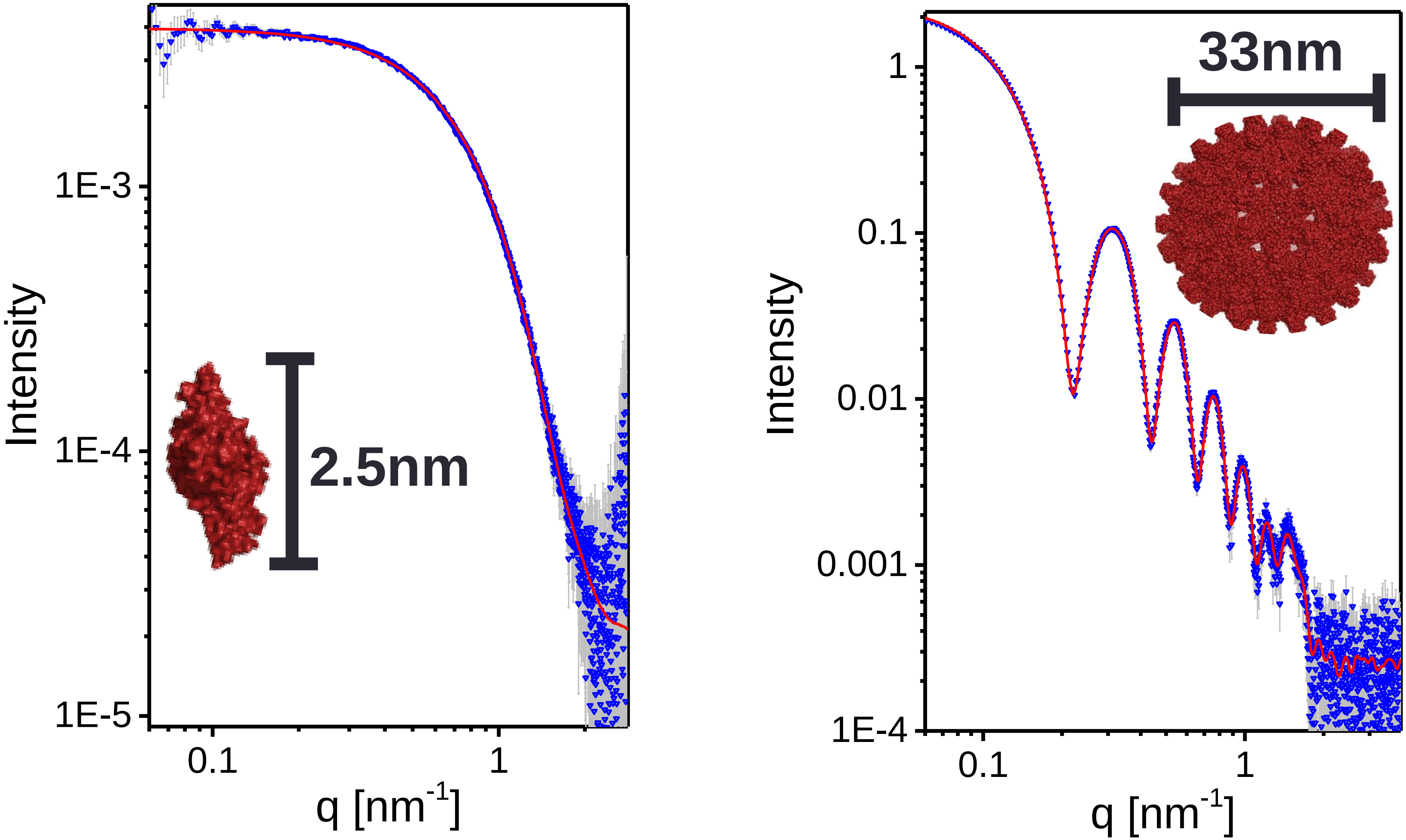


https://scholars.huji.ac.il/uriraviv/software/d-software

A. Ginsburg, T. Ben-Nun, R. Asor, A. Shemesh, I. Ringel, U. Raviv, *J. Chem. Information and Modeling*, 2016
A. Ginsburg, T. Ben-Nun, R. Asor, A. Shemesh, L. Fink, R. Tekoah, Y. Levartovsky, D. Khaykelson, R. Dharan, A. Fellig, U. Raviv, *J. Appl. Cryst., 2019*.

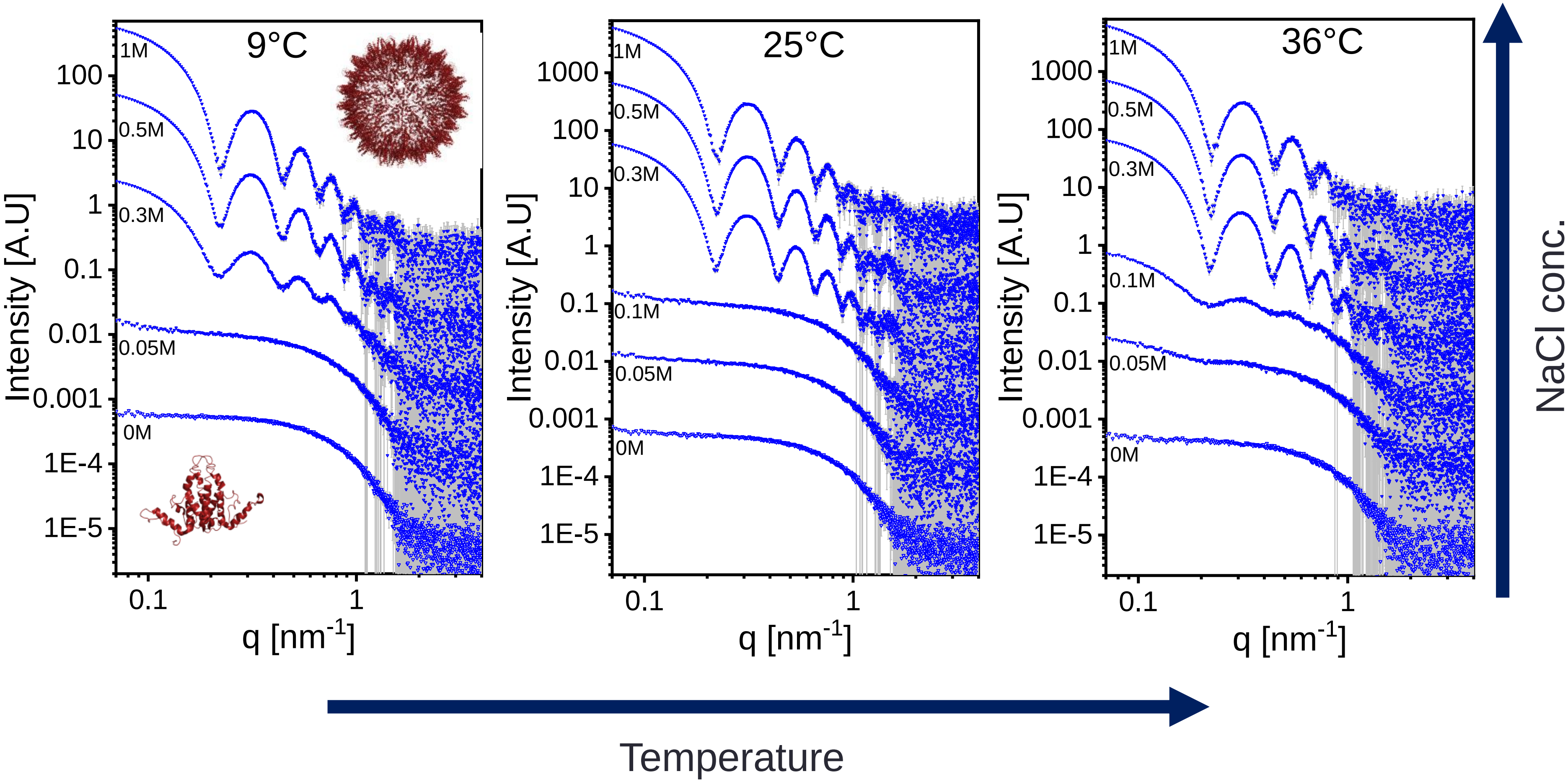
E. Balken, I. Ben-Nun, A. Fellig, D. Khaykelson, U. Raviv, J. Appl. Cryst. 2023.

Scattering intensities from atomic models



Ginsburg A., Ben-Nun T., Asor R., Shemesh A., Ringel I. and Raviv U. *J. Chem. Inf. Model.*, 2016

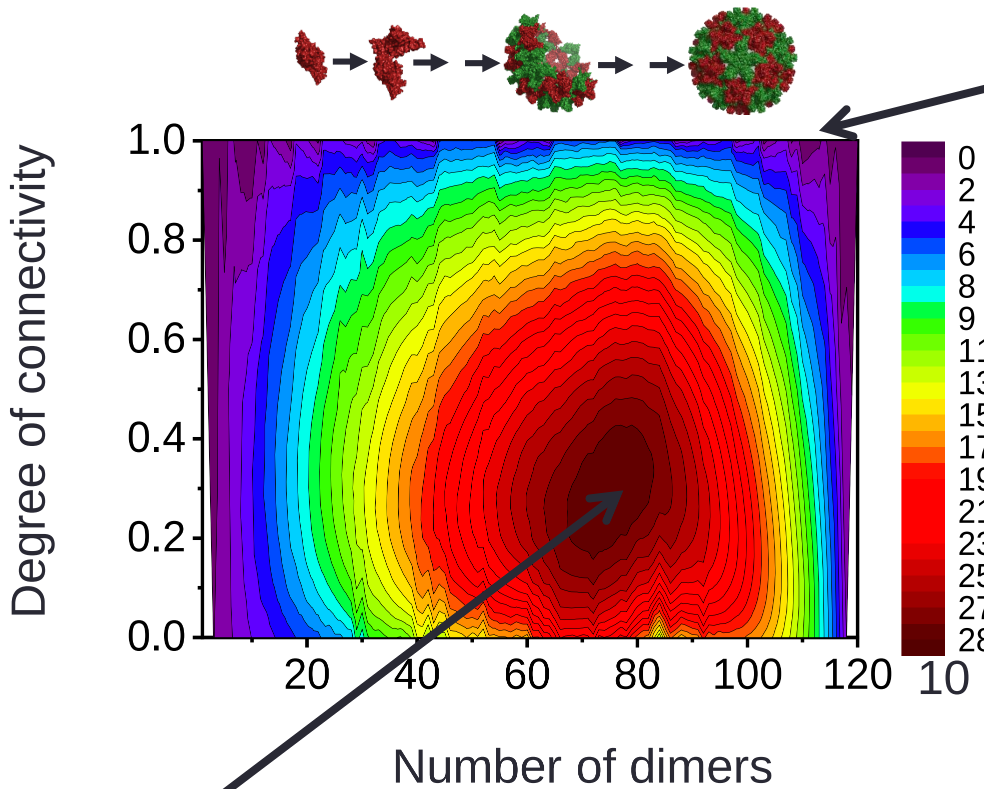
Capsid assembly conditions



R. Asor, L. Selzer, C.J. Schlicksup, Z. Zhao, A. Zlotnick , U. Raviv, ACS Nano 2019

Density map of ~10^{30} distinguished capsid intermediates

Compact/Stable



Umbrella sampling of MC Simulation:

-Graph representation
-Add/Remove a subunit in each step
-Verify detailed balance and uniqueness

→ a comprehensive library of distinct intermediates

Configurational entropy favors holey capsid structures

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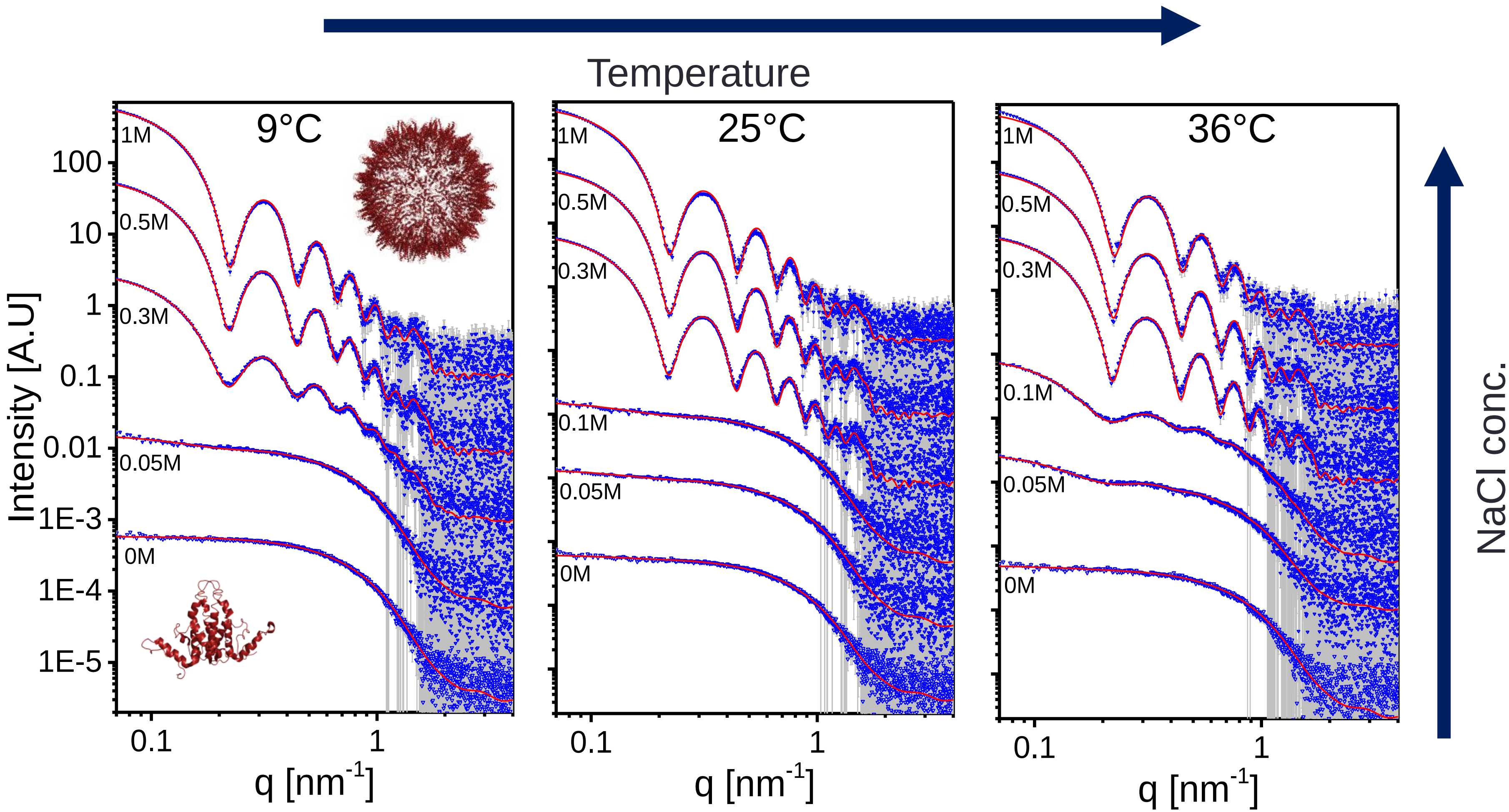
Fitting to a thermodynamically weighted sum of basis spectra

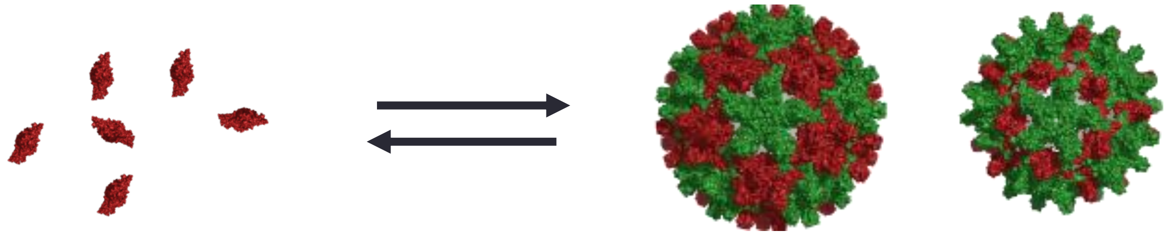


$$X_{n,c,s} = s \times \exp\left(-\frac{c\Delta F_n^\circ - k_B T \ln\Omega_{n,s,c} - s \cdot k_B T \ln X_1}{k_B T}\right)$$

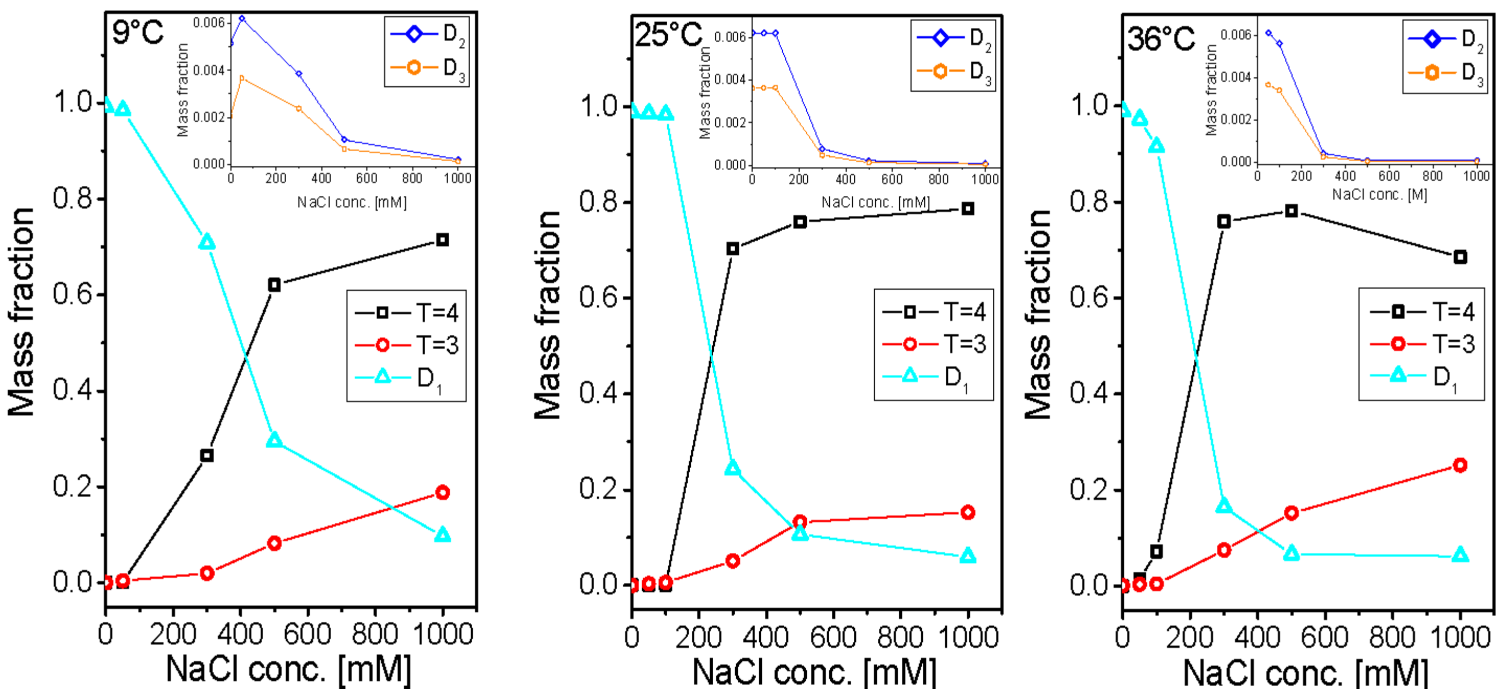
Asor *et. al.* ACS Nano 2019

Thermodynamic filtering of assembly products:

less stable complexes dissipate in favor of more stable species



99% of the mass is found in the dimer and full capsids forms (T=4 and T=3)



±2%

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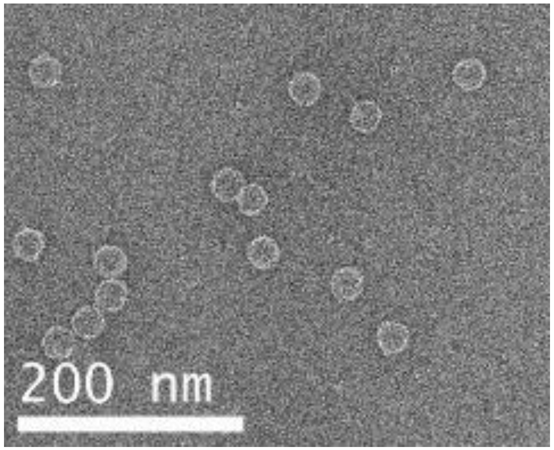
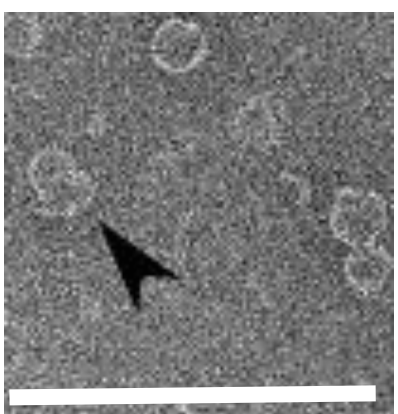
Thermodynamic analysis of assembly products

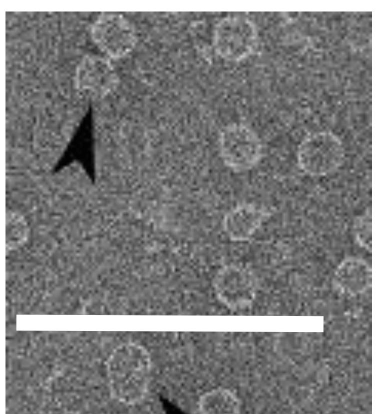
At 20μM protein successful assembly can be realized within a narrow range of association free energies (7.4 – 8.5kʙT)

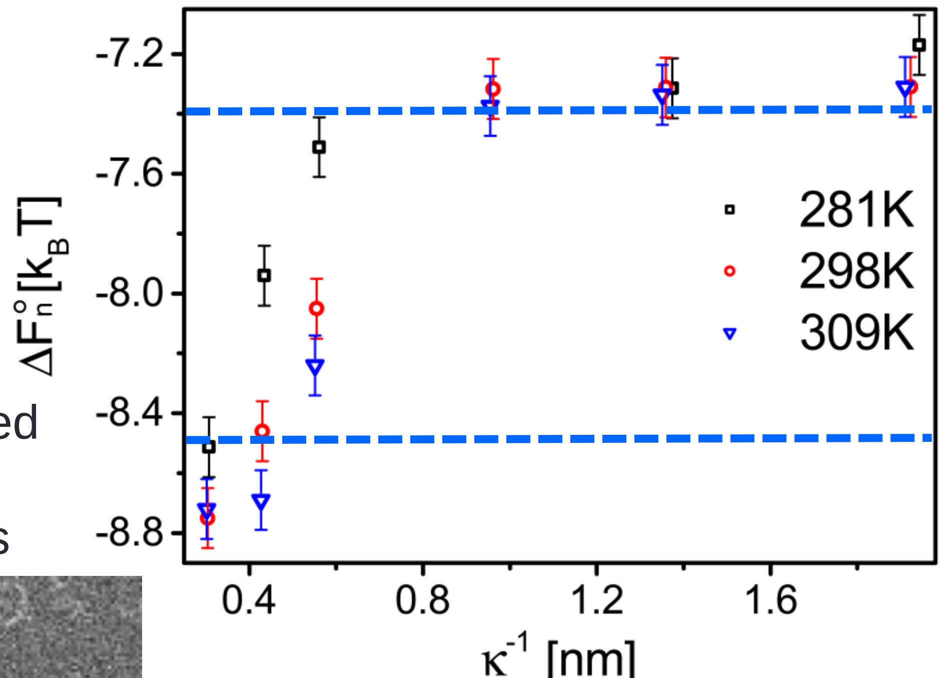


overgrown capsids



aggregated capsid fragments





No assembly

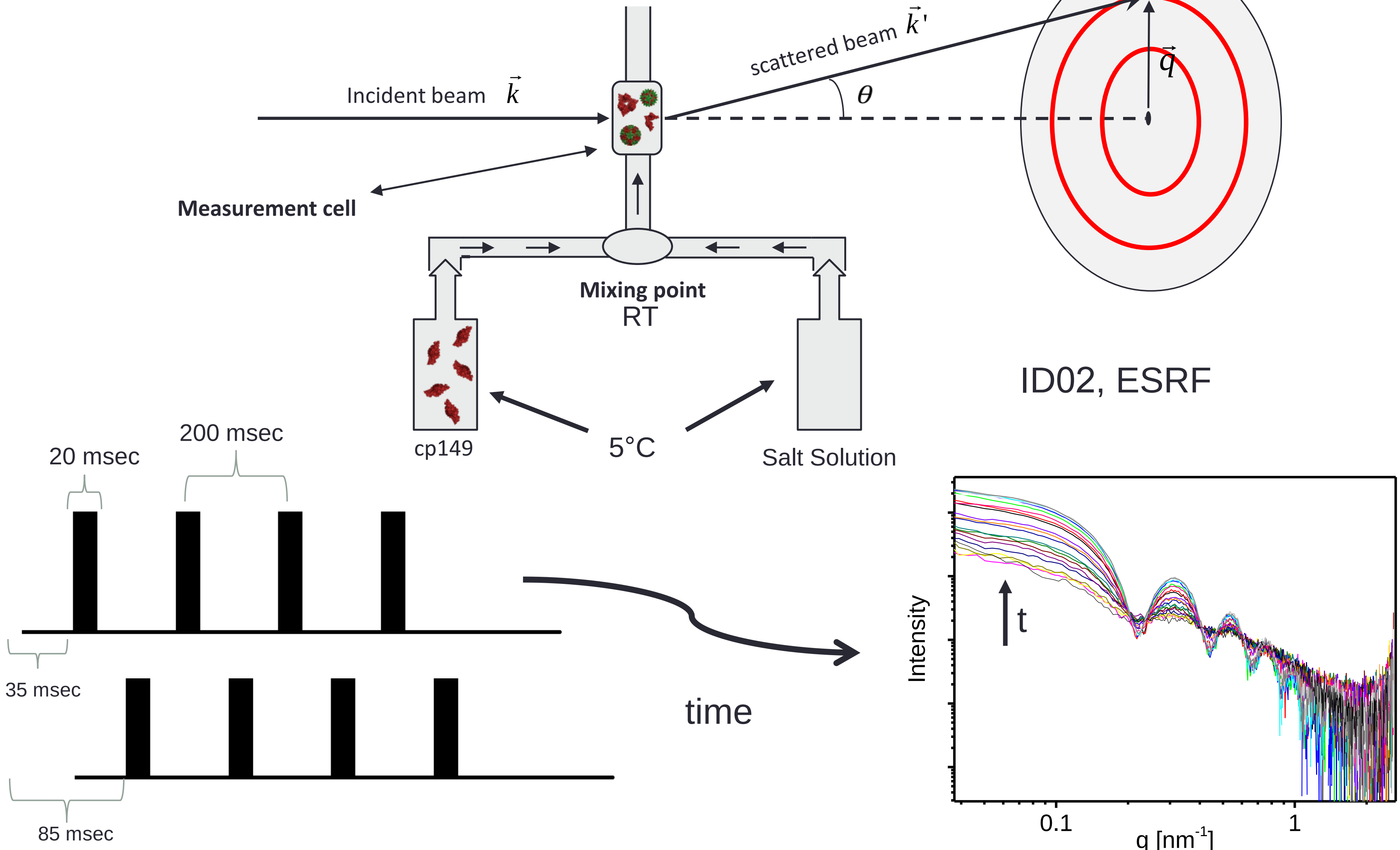
Successful assembly

Malformed particles

Debye screening length

~ $1k_BT$ (2.4 kcal/mole) difference in $\Delta F°_n$ leads to the formation of malformed capsids with aberrant geometry

Time-resolved SAXS – Stopped flow experiments



Maximum Entropy Analysis

Estimate the intermediate probability distribution that maximizes the informational entropy (minimum bias) and consistent with the experimental data and the available information

Maximize $$-\sum_i p_i \cdot \ln p_i$$

subject to: $$\sum_i p_i = 1$$

$$p_i \geq 0$$

$$-\sigma_q \leq \sum_i p_i I_i^{mod}(q) - \langle I(q) \rangle \leq \sigma_q$$

Shannon, C. E. Bell System Tech. (1948); Jaynes, E. T. Phys. Rev. (1957); Levine, R. D. J. Phys. A (1980)

$$p_i = \frac{p^\circ}{Z} \exp\left(-\sum_q \lambda_q \cdot I_i^{mod}(q)\right)$$

The number of parameters is proportional to the number of active data constrains

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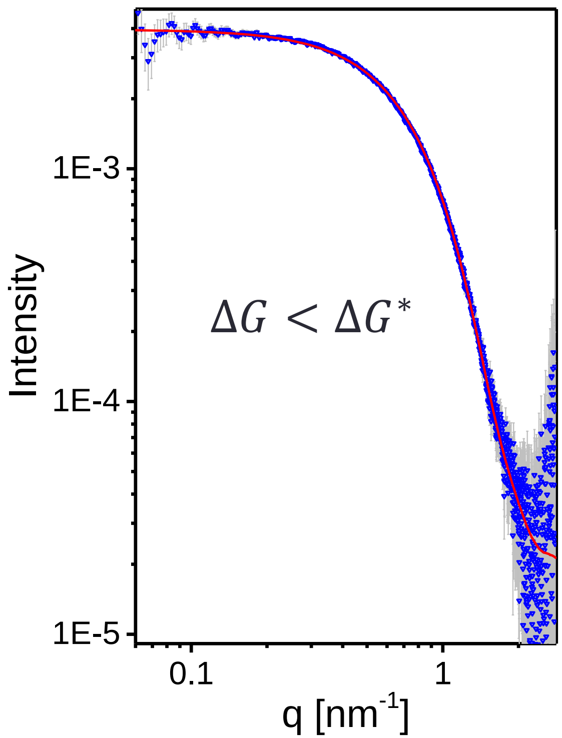
Stability bias (filter) as a prior knowledge

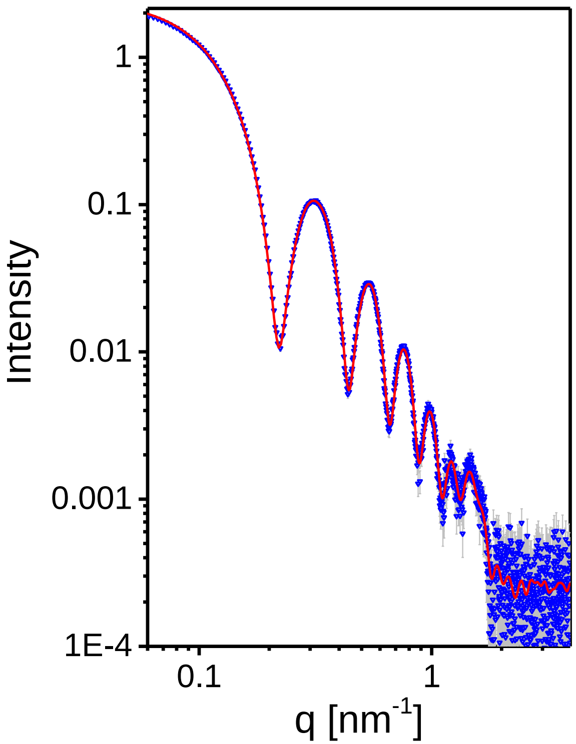
Confining the configurational space ($\sim 10^{30}$ states) into a more physically accessible space by adding stability bias to the initial distribution of states.

Reactant-like



Cold dimer before mixing

Product-like

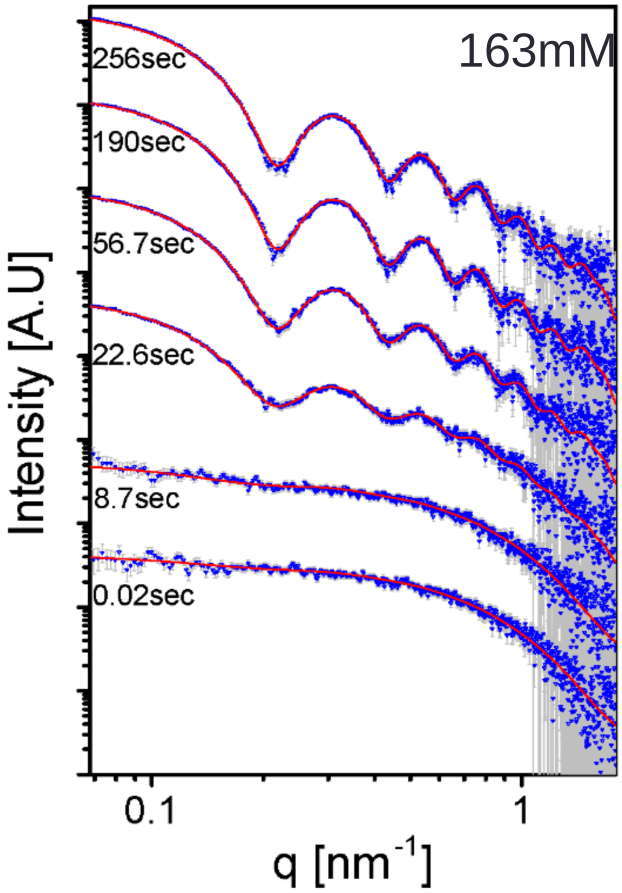


At equilibrium

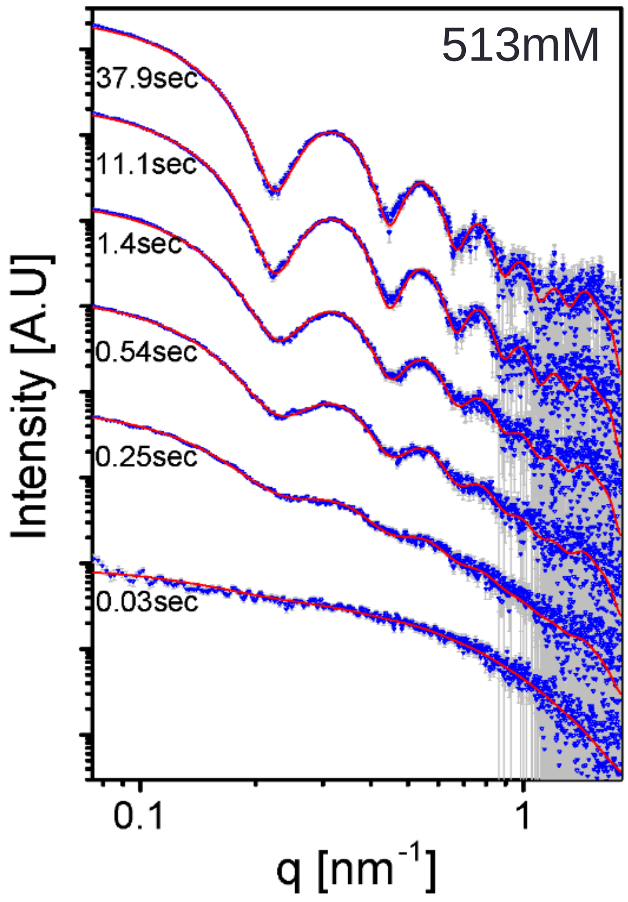
Other prior distributions were based on the fitted distributions of earlier and successive time points

Time resolved analysis results using maximum entropy

Mild conditions

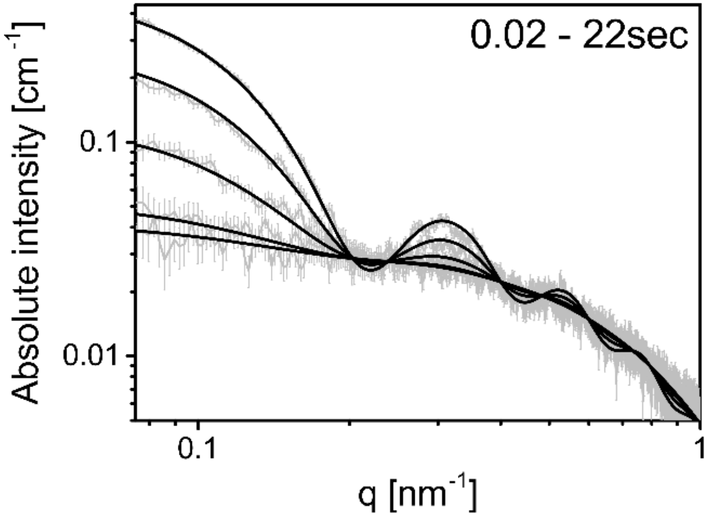


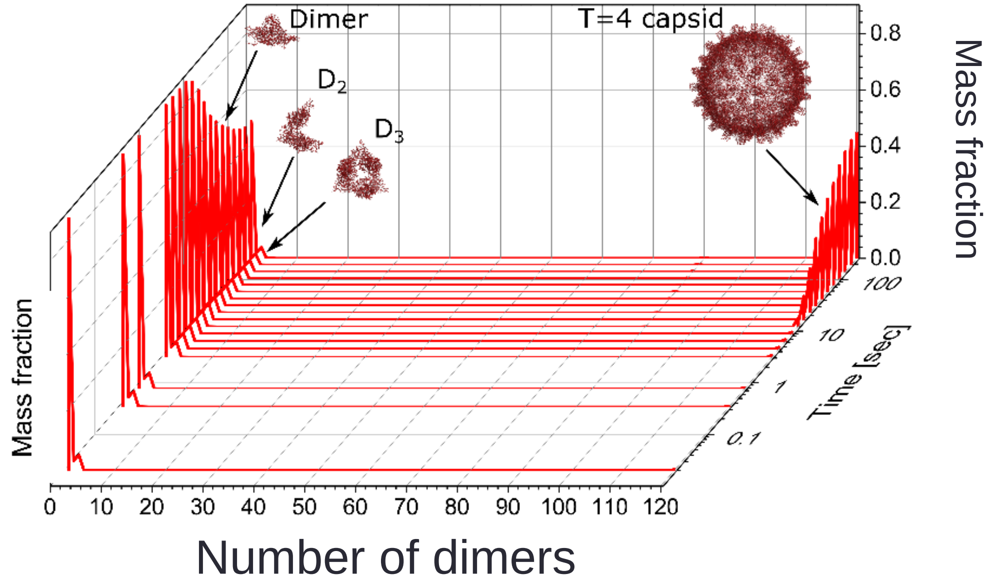
Aggressive conditions

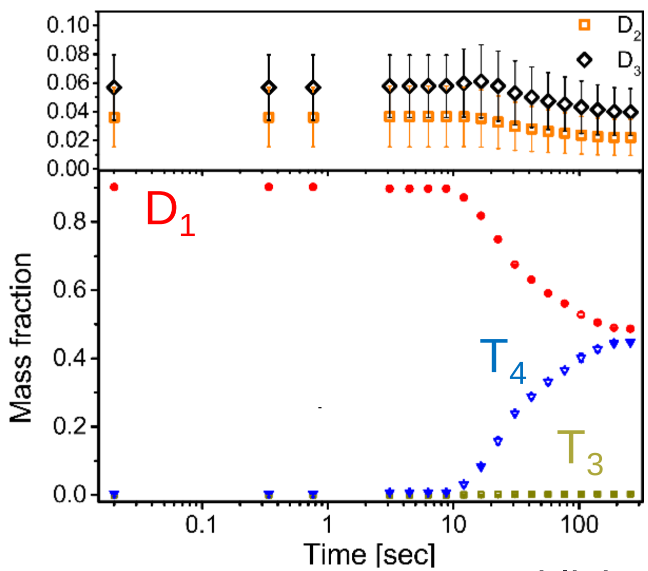


Time

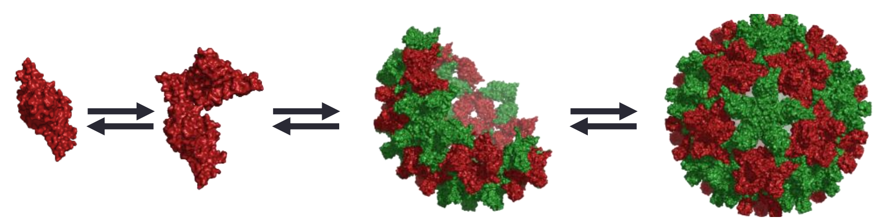
Reaction dynamics – Mild Conditions







No (±2%) intermediates accumulated throughout the assembly reaction



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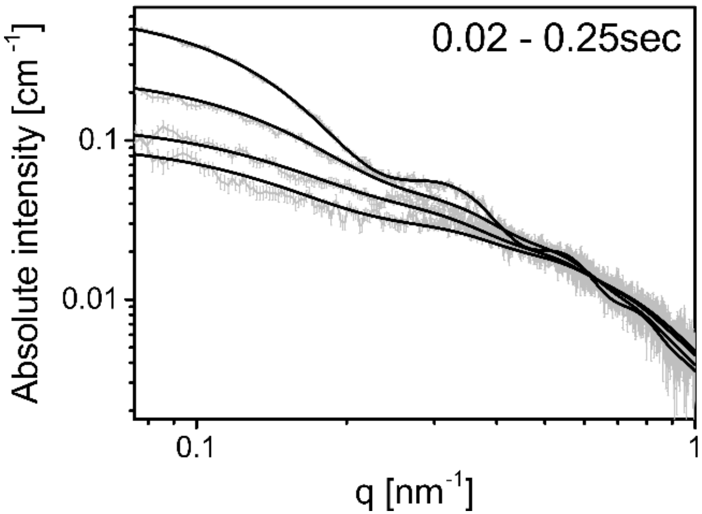
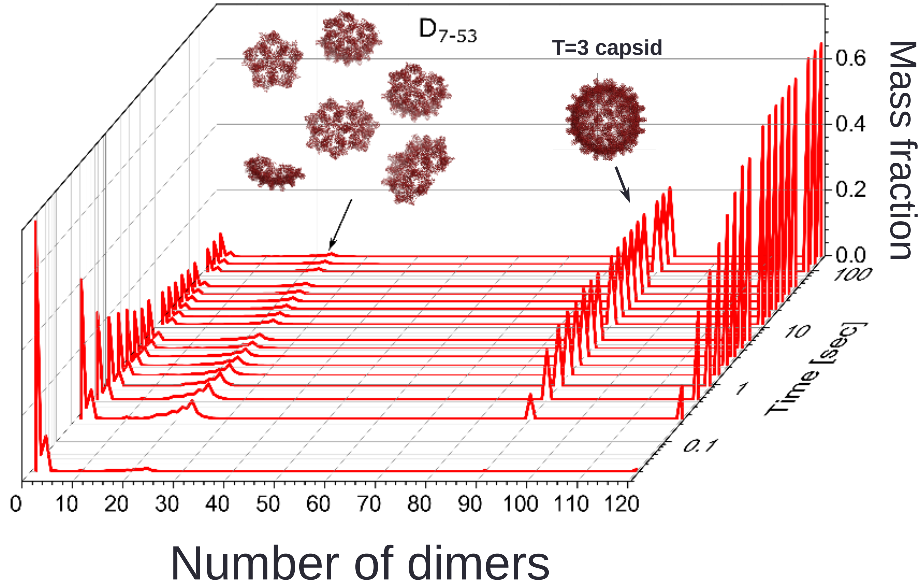
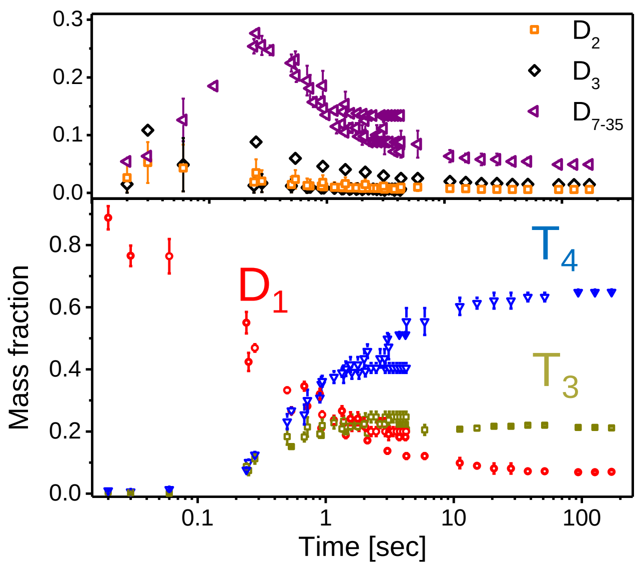
Reaction dynamics – Aggressive Conditions







Fast depletion in the concentration of free subunits

Small intermediates accumulated within the first 300 msec

The first second of the reaction dictated the steady state product composition

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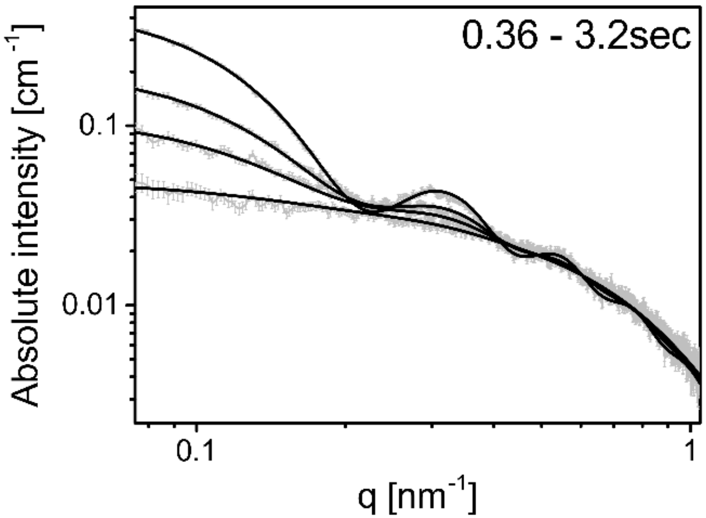
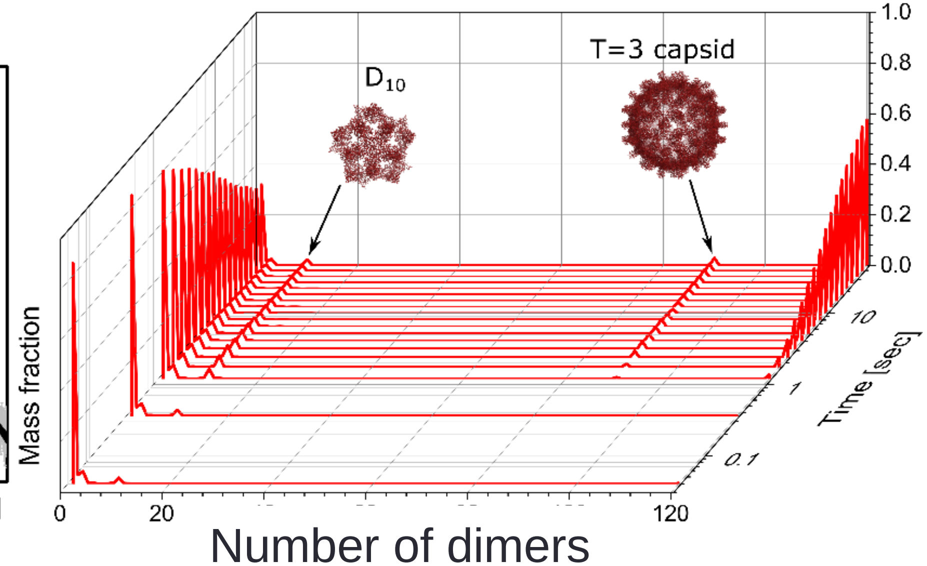
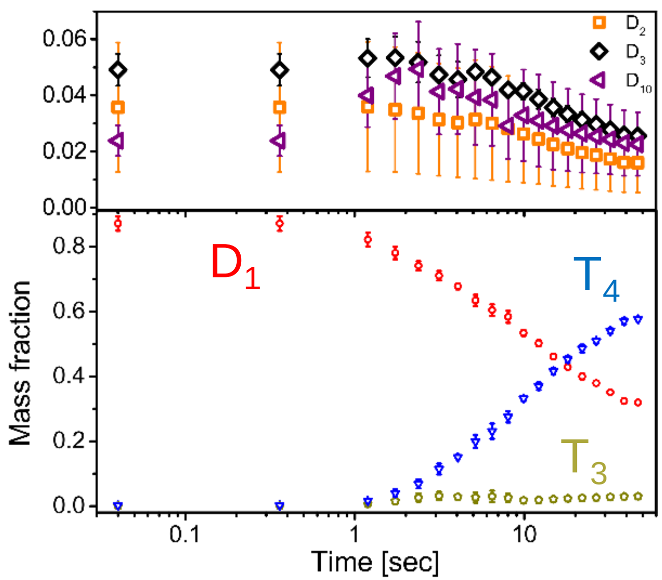
Reaction dynamics – intermediate ionic strength

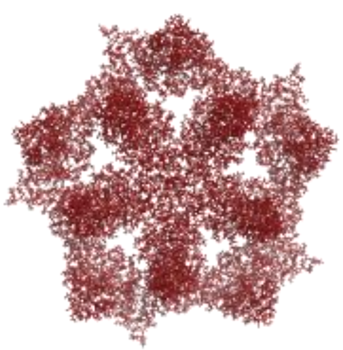


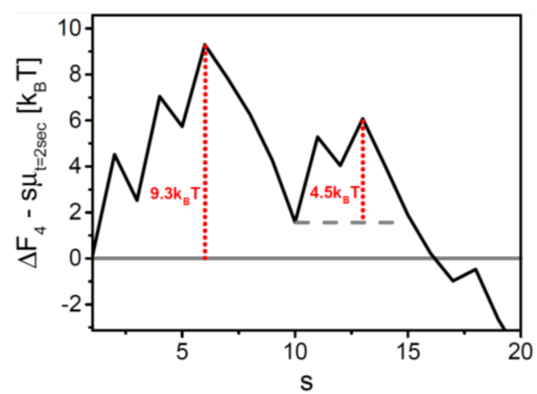




Short (0.4 sec) lag phase

Accumulation of compact 10-mer intermediate





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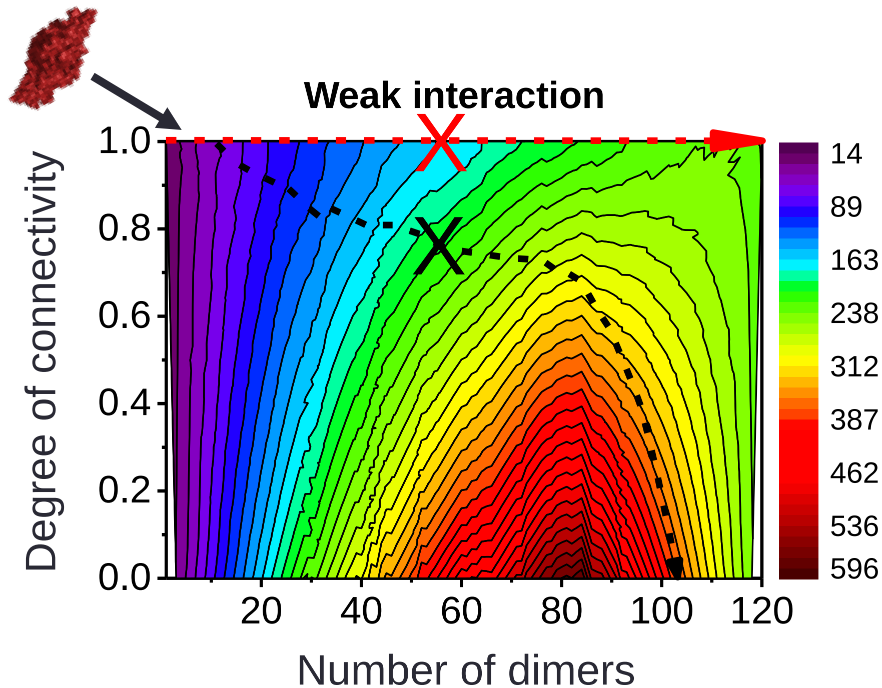
Free energy landscape at the onset of assembly reaction – weak interaction



$$\Delta\Omega_G\left(\Delta F_n^{\circ}, \mu_{1,t=0}\right) = c \cdot \Delta F_n^{\circ} - k_B T ln\Omega_{s,c} - s \cdot k_B T \ln \mathrm{X}_{1,\mathrm{t=0}}$$

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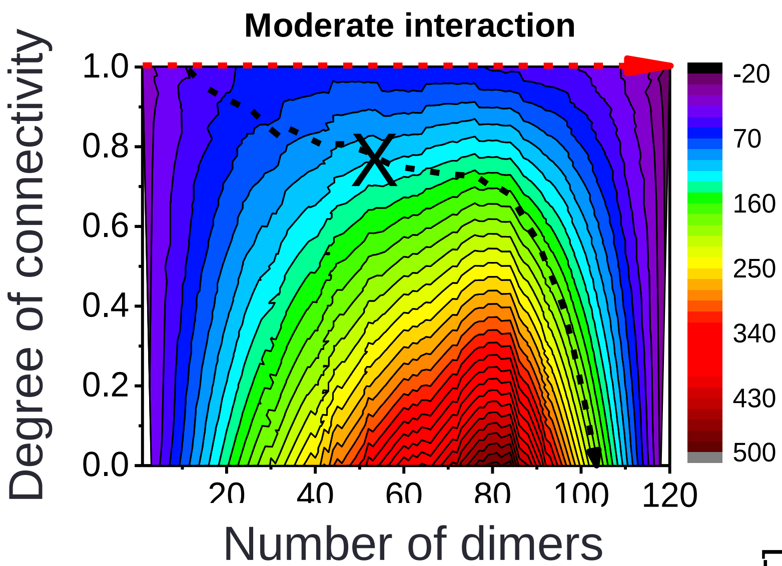
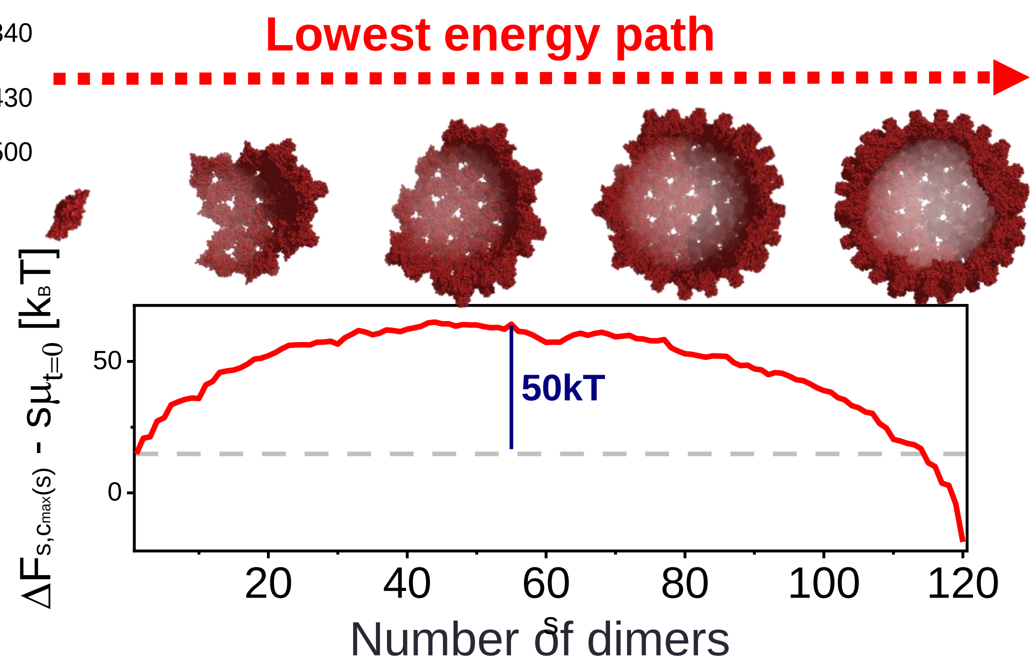
Narrow assembly path: intermediates either pass the barrier or completely disassemble



Broad assembly barrier
→ **Weak and reversible** binding
→ Nonoptimal contacts can be corrected



Beyond the barrier:
Rapid elongation, limiting accumulation of intermediates

Nucleation and growth mechanism

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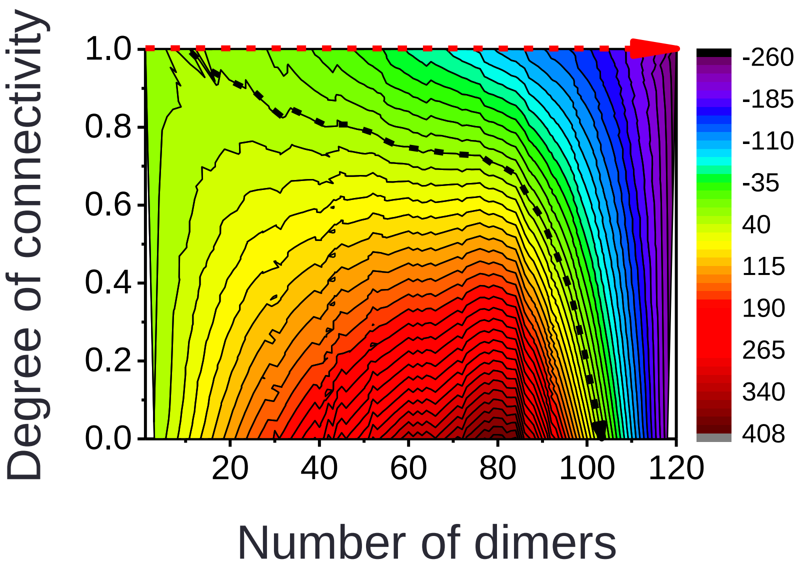
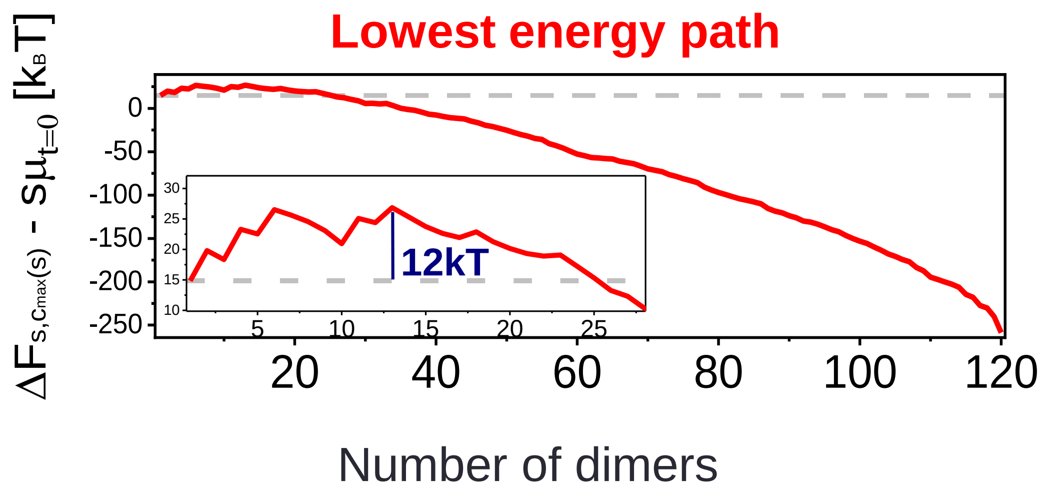
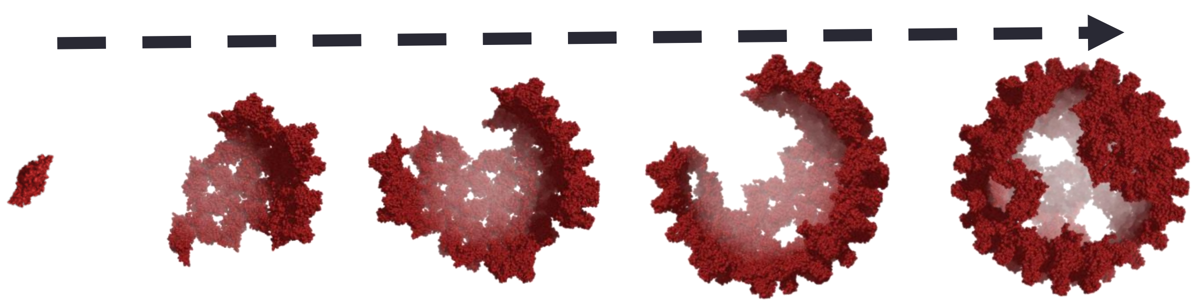
Strong interaction: Low assembly barrier leads to rapid accumulation of intermediates







Fast nucleation
High disassembly barrier

Low barrier for sampling less compact states
& high barrier for disassembly

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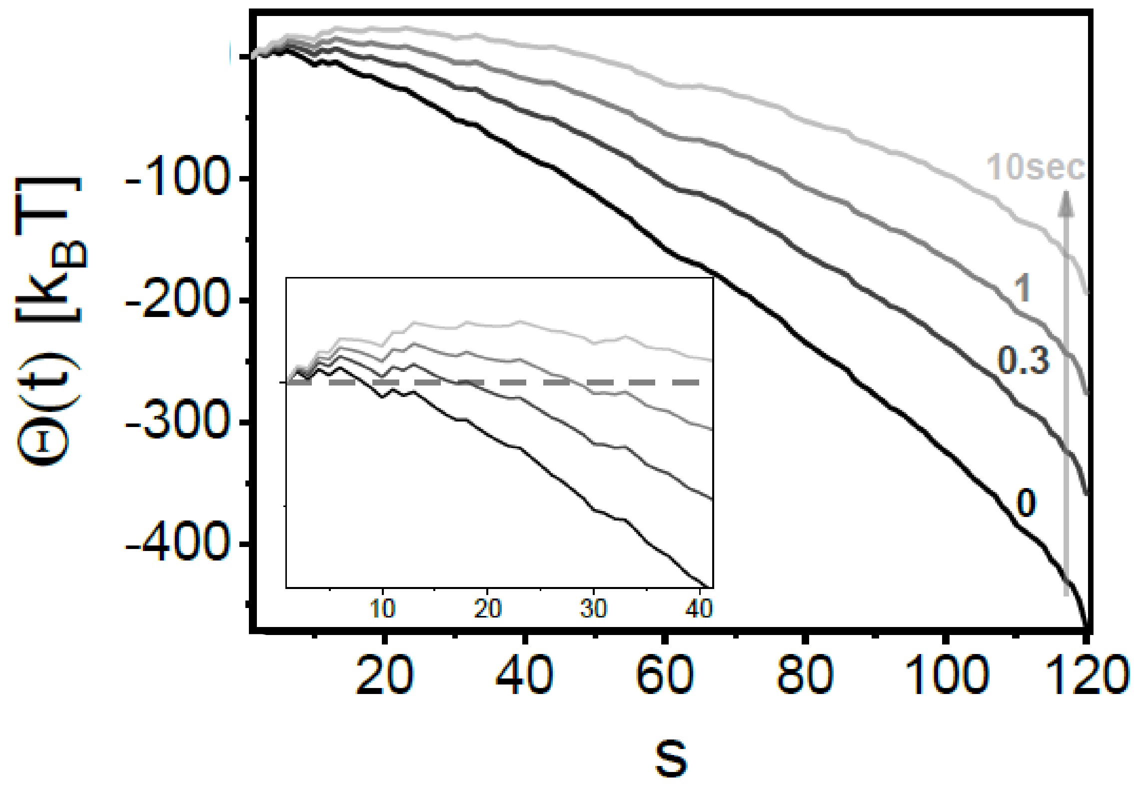
Minimum grand canonical free energy (along the stable path) as a function of time under aggressive conditions

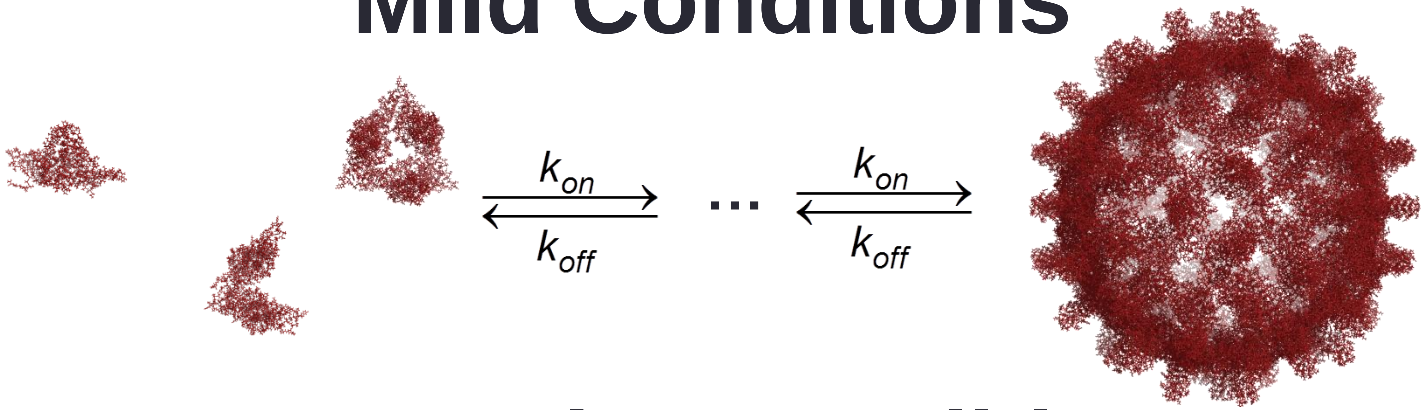


New capsid assembly lines following the nucleation and elongation mechanism

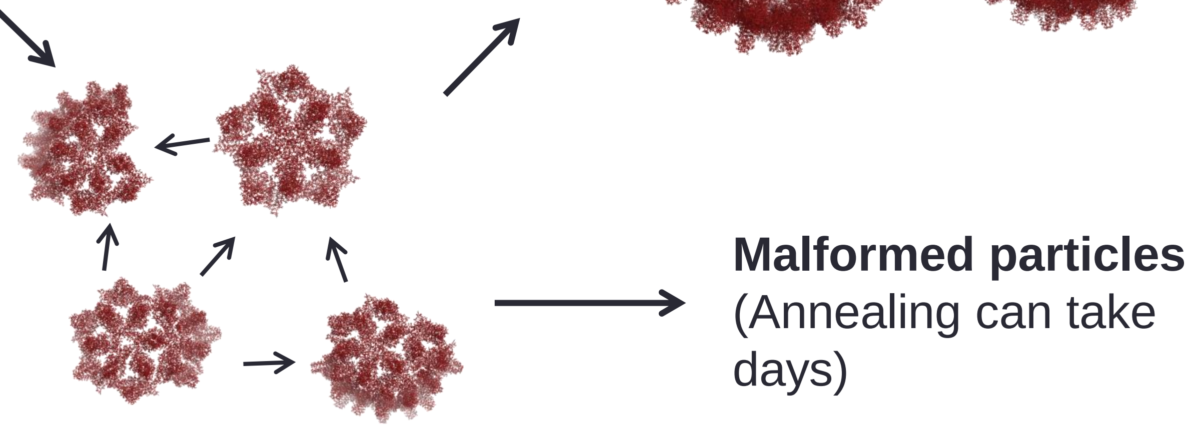
Slow elongation of medium size intermediates

Reversibility is crucial for correct capsid assembly

Mild Conditions



Aggressive Conditions



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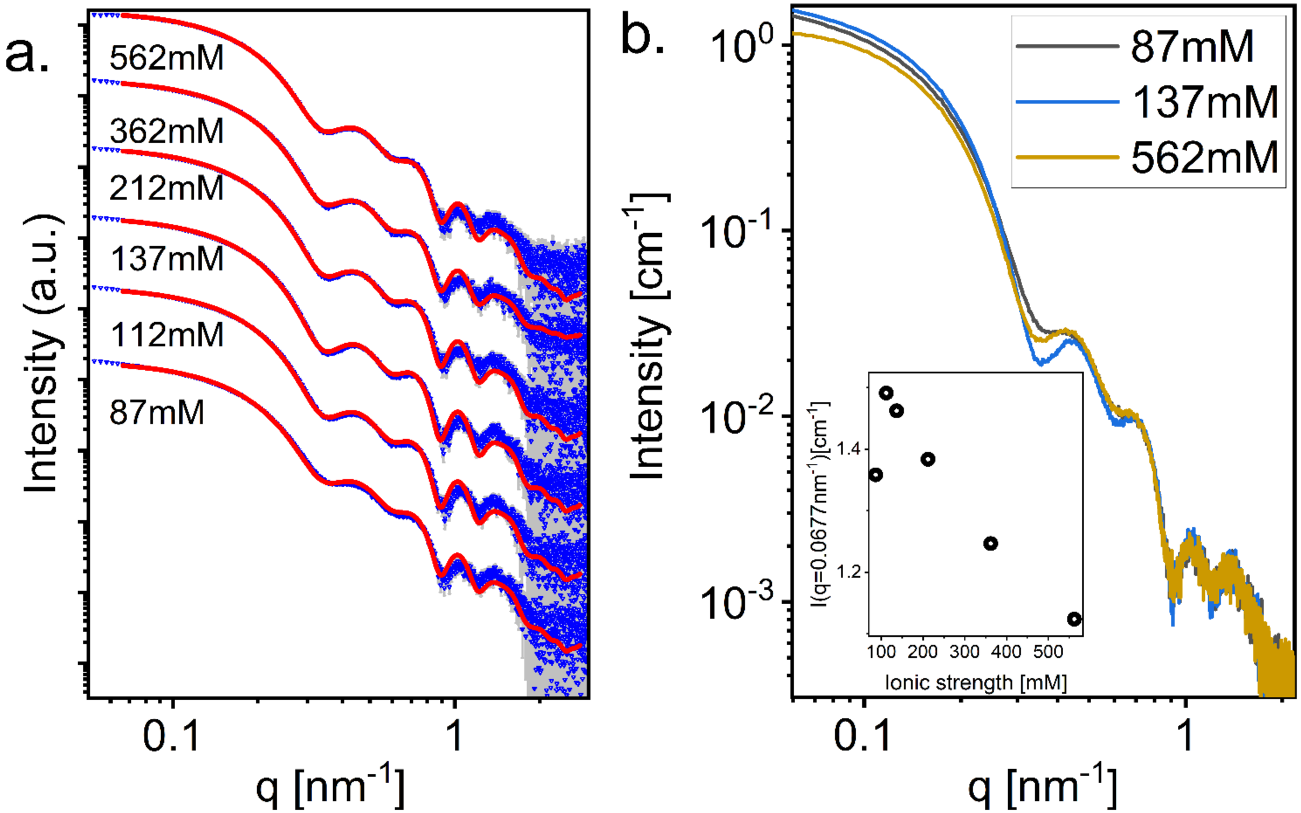
R. Asor, C.J. Schlicksup, Z. Zhao, A. Zlotnick , U. Raviv, JACS 2020

Effect of ionic strength on T=1 VLPs from PSS and SV40 VP1 pentamers



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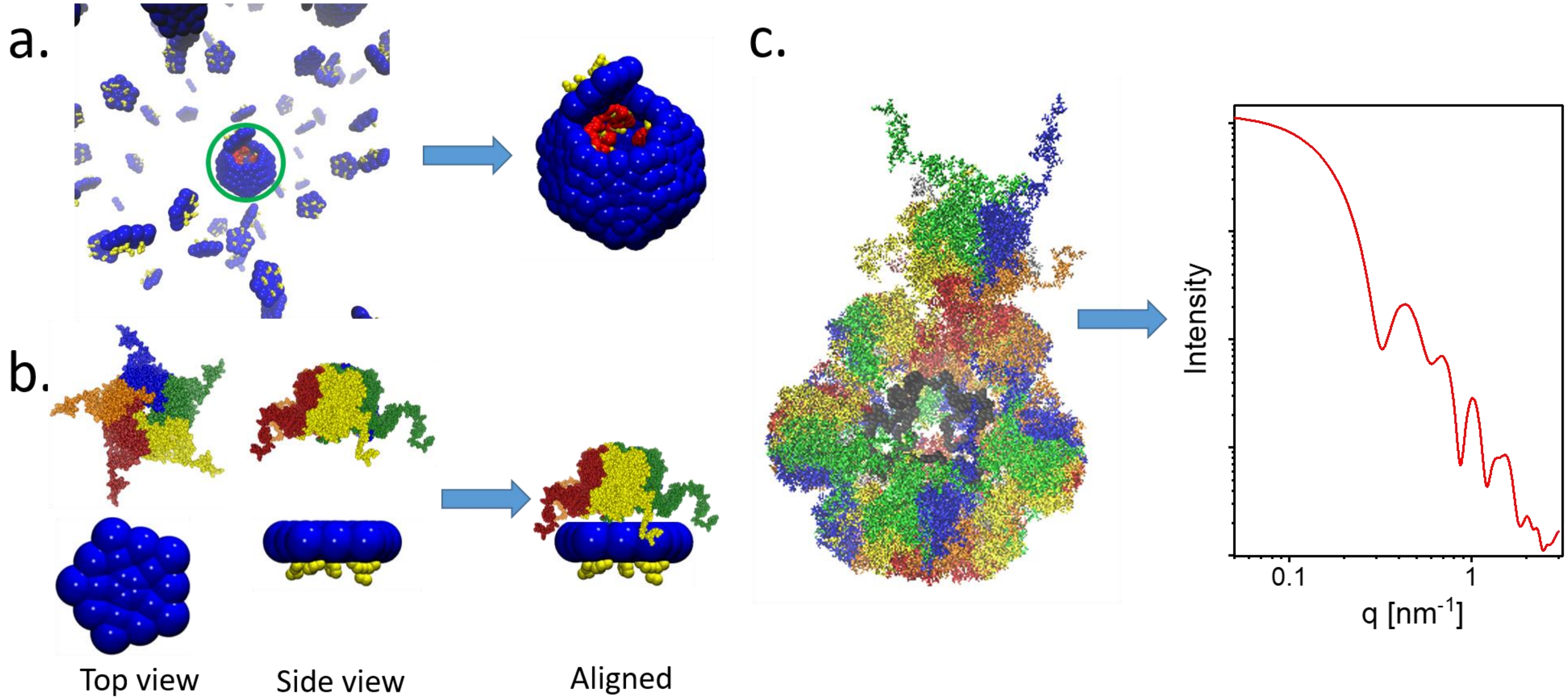
Simulated coarse-grained models to atomic models and SAXS curves



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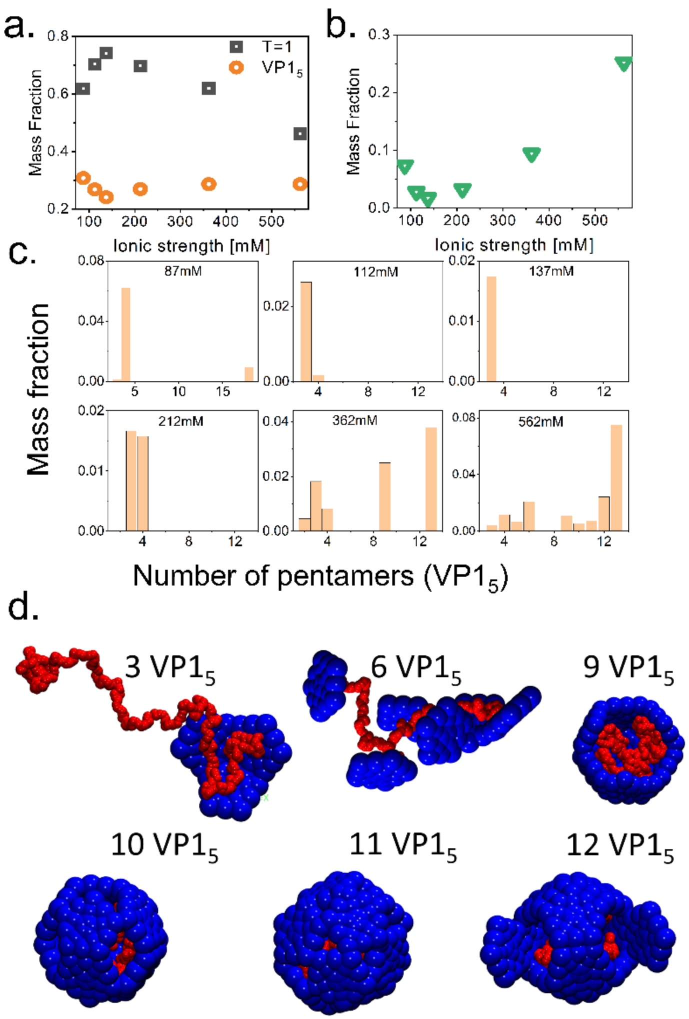
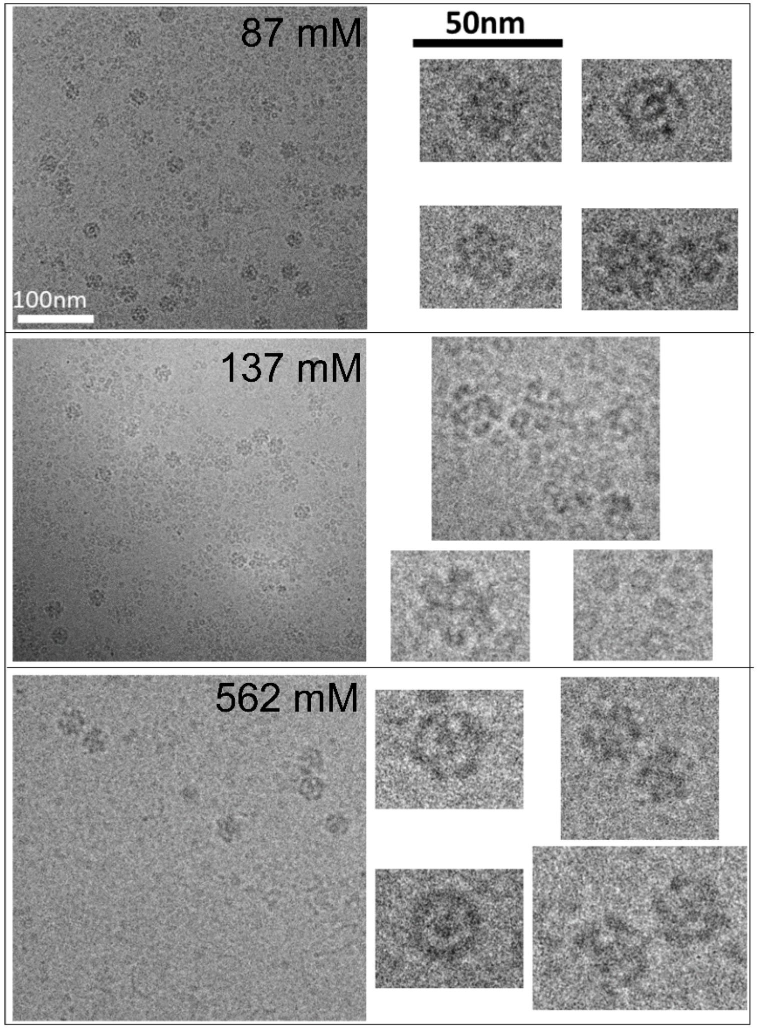
Dominant assembly states





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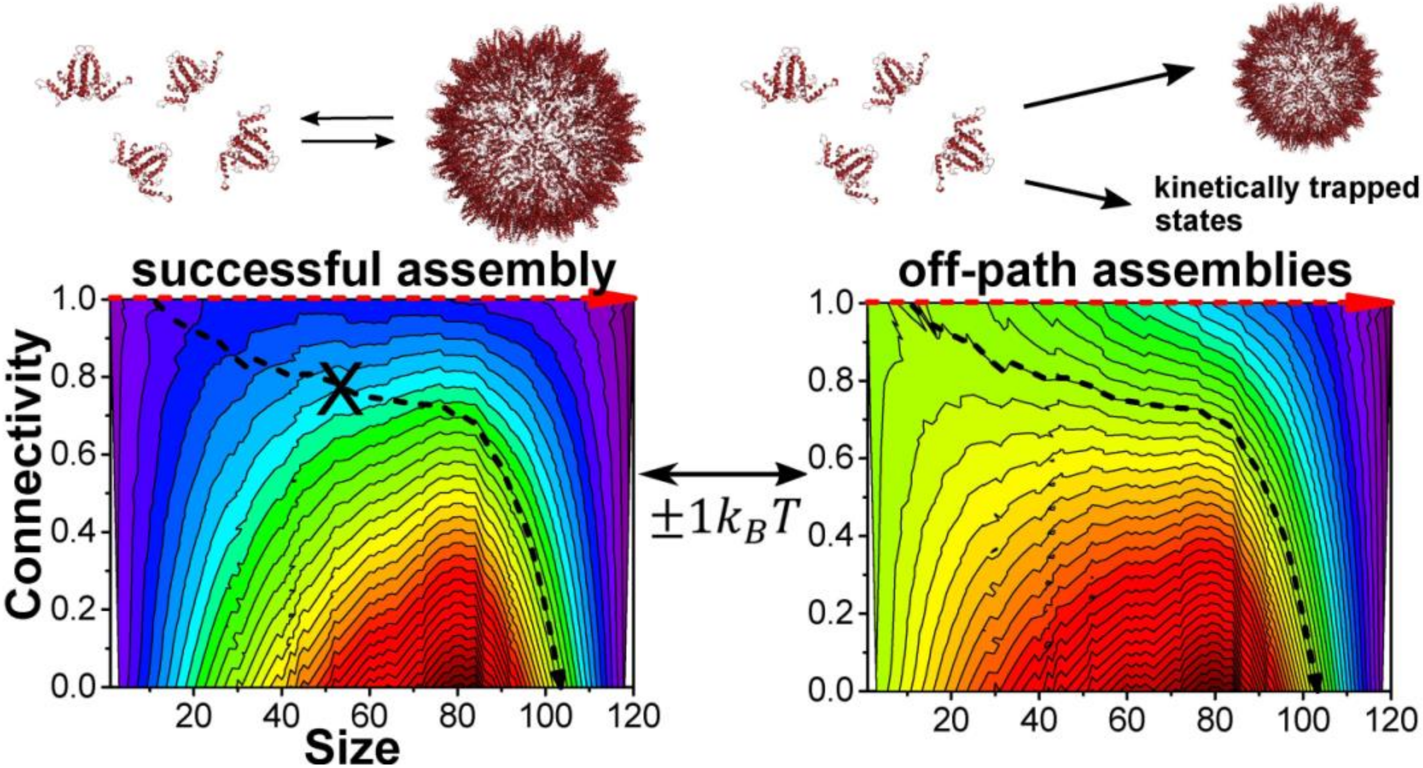
Summary

SAXS detects structure, interactions, and dynamics of HBV assembly in native conditions



Geometry describes the possible intermediates

Energy is tunable and makes the actual selection

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Hamburg (P12) & Soleil (SWING) **– Steady state data**

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