Assembly Kinetics of Synthetic Capsids Made from DNA Origami

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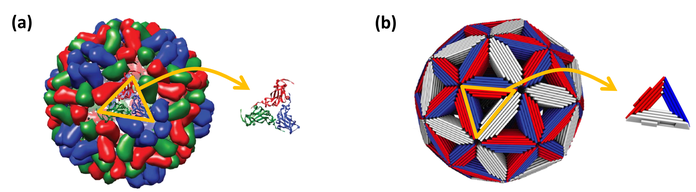
Introduction

The robust self-assembly of biological materials into large, but finite-size, superstructures is fundamental to life. One of the prototypical examples is a virus capsid, whose widely various geometries are built from either a single or a few species of repeating units. Inspired by this efficient paradigm, we previously developed a programmable engineering analog composed of user-prescribed DNA origami subunits [1].

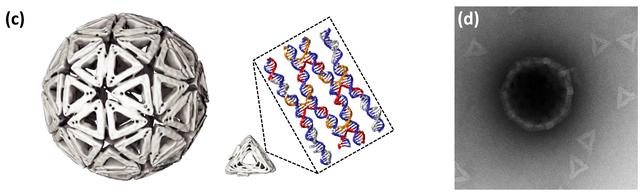
While the equilibrium structure of the synthetic capsid was determined, the dynamical transformation from a disorganized state of individual building blocks into an ordered state of a fully-closed capsid shell remains uncharacterized. To reveal the underlying mechanism, we firstly utilize static light scattering to quantify the association affinity between building blocks *in situ*. Specifically, the inter-block lock-and-key docking with base-stacking plus variable hybridizations enables precise control of binding strength.

We then non-invasively monitor the assembly kinetics and undertake a quantitative study of on- and off- rates of the monomer-dimer transition as a function of interaction strength. With the knowledge thus gained, we aim to realize assembly of various artificial capsids with optimized yield and reaction time, enabling scalable routes to new functional materials. The tool-kit thus far developed can then be applied to other systems in the IRG1 community, such as curvature-controlled tubule tiling and frustration-controlled curvamer assembly.

Synthetic Capsids with Icosahedral Symmetry



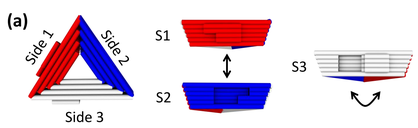
(a) Schematic represents an example of a natural virus capsid, cowpea chlorotic mottle virus (CCMV), with icosahedral symmetry (triangulation number T=3). 60 identical capsomers (or, 180 quasi-equivalent protein subunits) assemble into a capsid. Capsid diameter: 28 nm. **(b)** Schematic of an analogue synthetic capsid made from DNA origami. 60 identical triangular building blocks assemble into a T=3 capsid shell. Capsid diameter: ~170 nm.

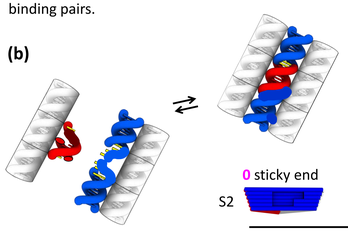


(c) Cryo-EM reconstructions of a fully assembled capsid (*left*) and a capsid subunit (*right*). Capsid diameter: ~170 nm. **(d)** Negative stain EM image shows a fully assembled capsid and several subunit monomers.

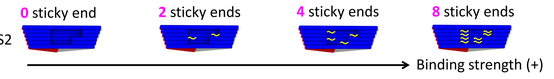
Precisely-controlled and Tunable Block-block Interactions

To ensure specific block-block interaction, **(a)** all three edges of the triangular building block are given unique shape-complementary protrusion (key) and/or recess (lock). The arrows indicate binding pairs.



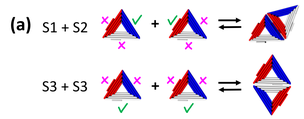


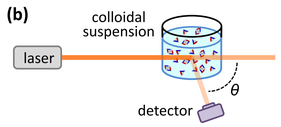
Individual docking site is further modified by **(b)** introducing single stranded sticky ends (3bp) (*left*). The thus achieved variable hybridizations (via different number of added sticky ends) enable precise control of binding strength (*bottom*).



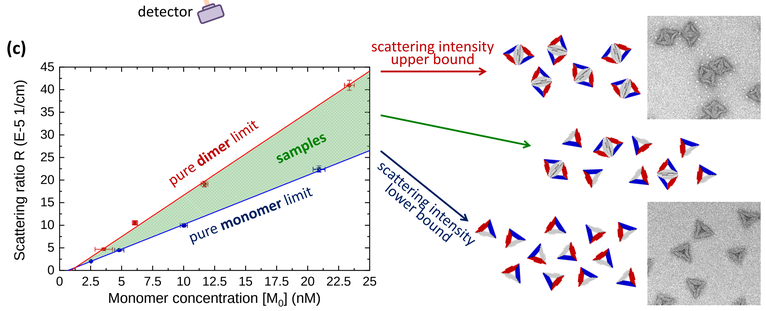
Light Scattering: *in situ* Assembly Monitor and "Gibbsometer"

To reveal the underlying mechanism of capsid assembly, we firstly undertake a quantitative study of the monomer-dimer transition. **(a)** Two specific block-block interactions are studied: side S1 binds with side S2 and side S3 binds with another side S3.



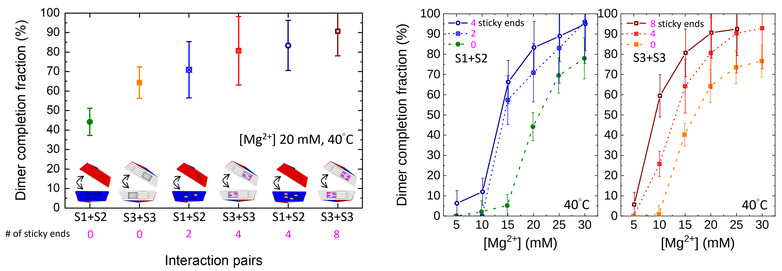


Static and dynamic light scattering (setup schematic in **(b)**) are then utilized to non-invasively quantify the association. The scattered light by targeted colloidal (monomer/dimer building blocks in this case) suspension is detected at a $\theta = 90^{\circ}$ scattering angle.

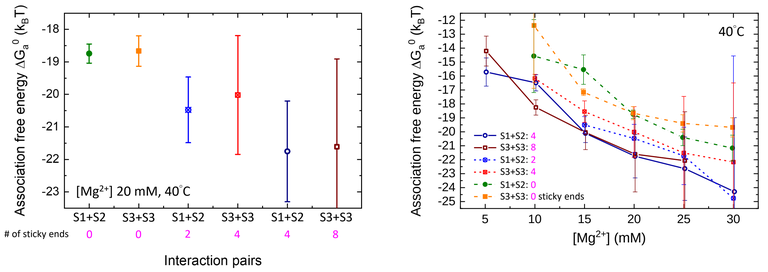


Any monomer-dimer assembly sample contains a mixture of monomers and dimers. **(c)** With the help of pure monomer standards (*lower right*) and pure dimer standards (*upper right*), the monomer/dimer population within each sample can be measured, followed by calculations of the standard Gibbs free energy of association.

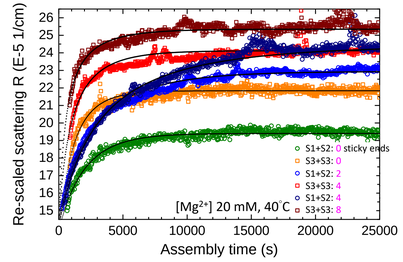
Dimer Completion Fraction of Various Block-block Interactions



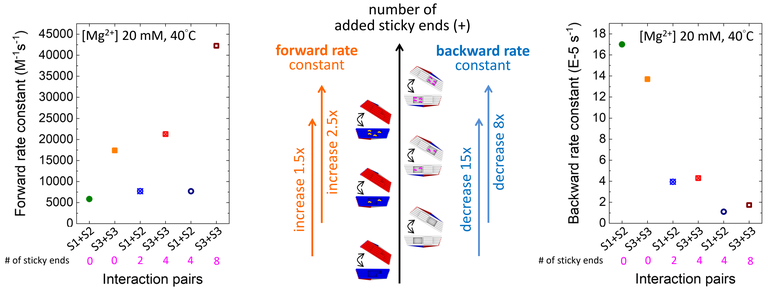
Standard Gibbs Free Energy of Association at Equilibrium



Monitor and Engineer Assembly Kinetics

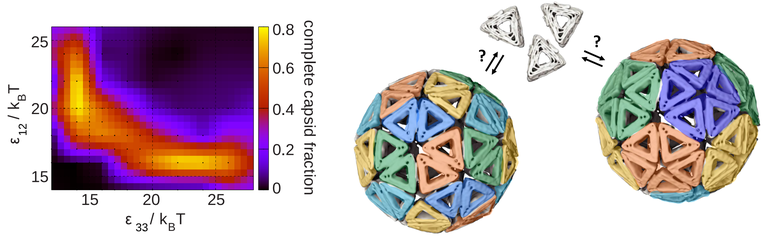


Light scattering also enables *in situ* monitor of the monomer-dimer assembly kinetics. At zero assembly time, only monomers exist in the solution, leading to the lowest scattering intensity. By adjusting buffer $[Mg^{2+}]$ (at t = 0 s), interactions between building blocks are turned on. Observed scattering intensity thus increases as monomers bind into dimers (and eventually reaches equilibrium).



On- and off- rate constants of the monomer-dimer transition are determined by fitting the experimental assembly curves. Preliminary results indicate that we can engineer both the system off-rate (*i.e.*, decrease significantly, *right*) and on-rate (*i.e.*, increase slightly, *left*) by adding more sticky ends to the block docking site.

Capsid Assembly: Pathways and Efficiency



Simulation shows that asymmetry interactions between building blocks are favorable (*left*), which hints that a dimer-bias pathway or a pentamer-bias pathway (*right*) can potentially lead to more efficient capsid assemblies. With the knowledge thus gained, we are currently working on realizing assembly of artificial capsids with optimized yield and reaction time.

References / Acknowledgements

[1] C. Sigl *et al.*, *Nature Materials* (2021)

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