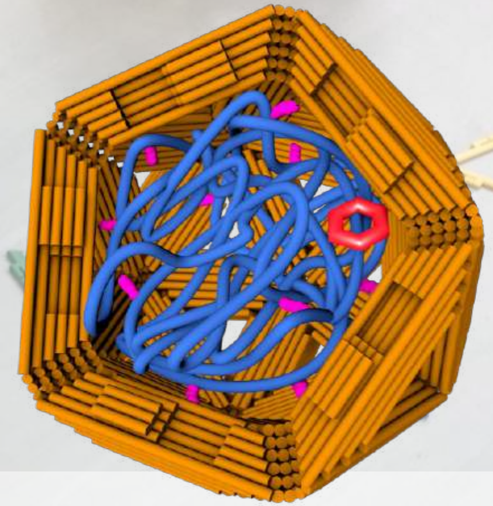
Brandeis bioinspired MRSEC



Hierarchical assembly of capsids is robust

S. Fraden

The Team


Gregory Grason


Douglas Hall


Hendrik Dietz


Chris Sigl


Fabian Kohler


Alba Monferrer


Daichi Hayakawa


Botond Tyukodi


Wei-Shao Wei


Rupam Saha


Anthony Trubiano


Thomas Videbæk

Brandeis bioinspired MRSEC


Seth Fraden


Mike Hagan


Ben Rogers


Tijana Ivanovic

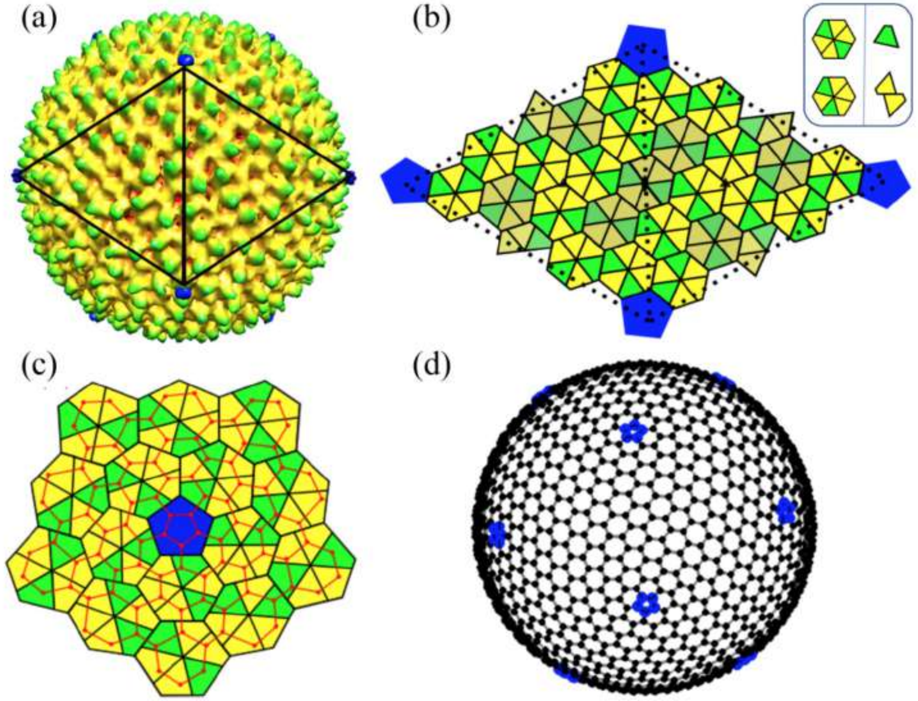
EMAIL US FIND US Visiting Fellows Partnerships About Support ICMS People Coming To ICMS Gallery

What's On Mathematics For Humanity Funding Knowledge Exchange Public Engagement

Caspar-Klug

Reidun Twarock

Geometrical tiling



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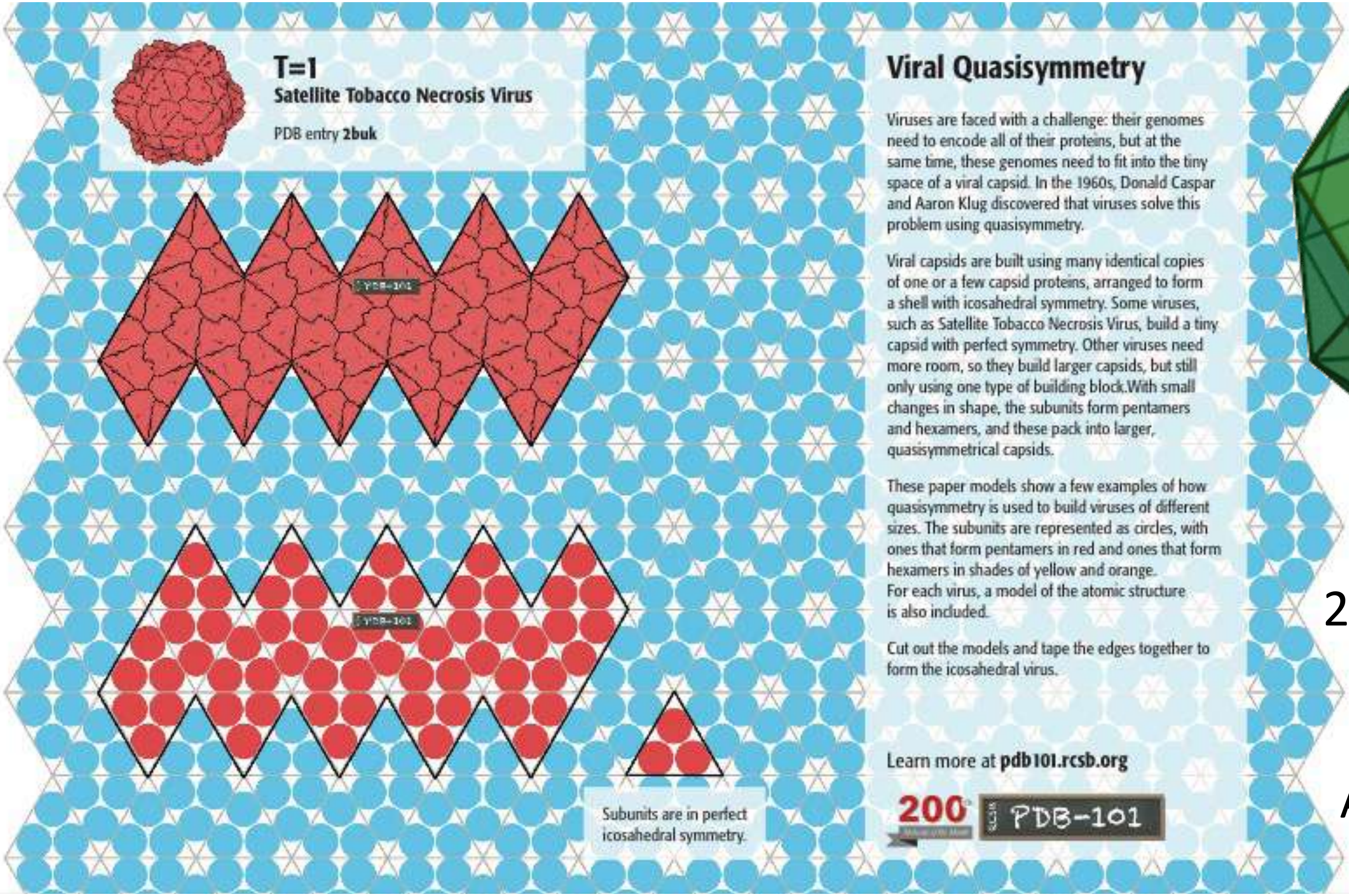
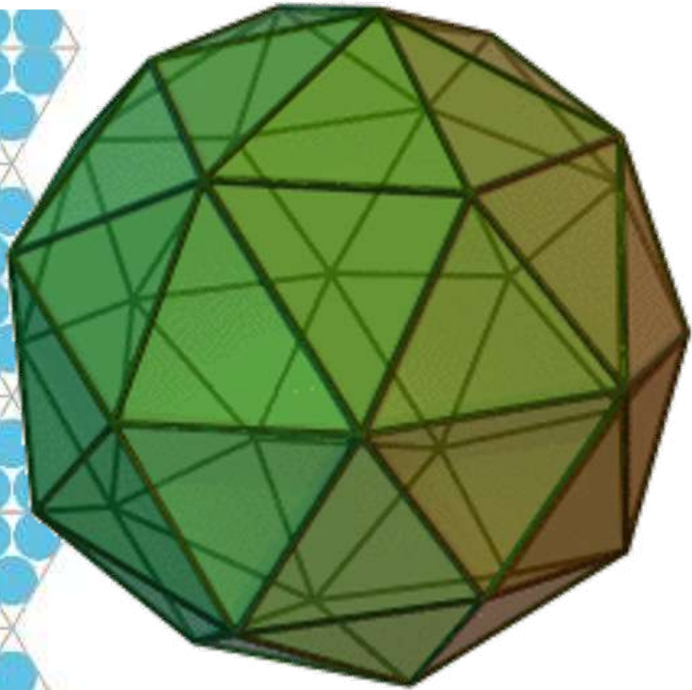
Caspar & Klug, Cold Spring Harbor Symp. Quant. Biol. (1962)



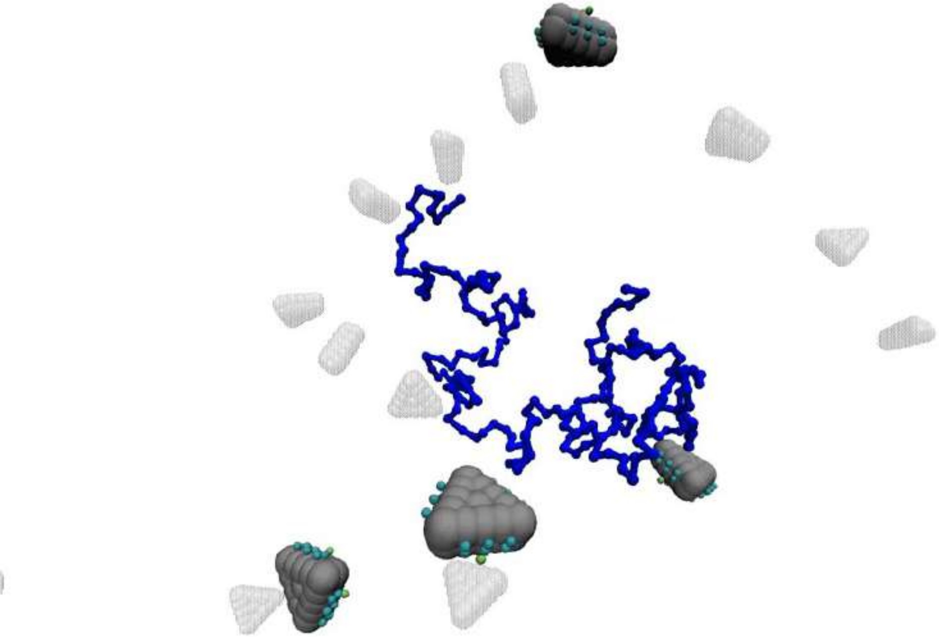


Icosahedra
20 identical triangles

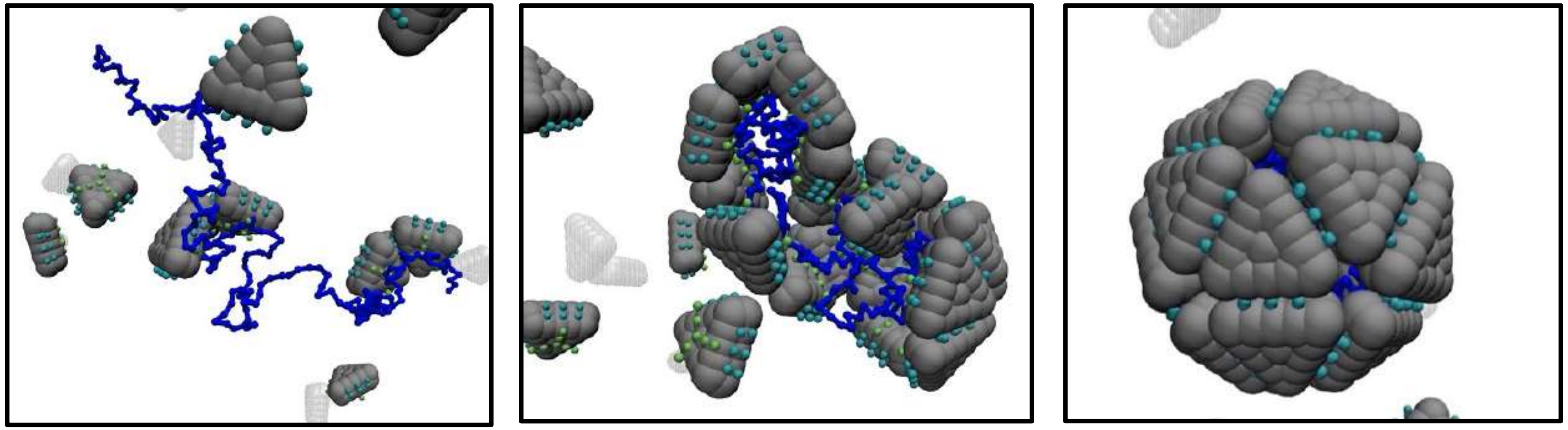
T=1
All sides equivalent

Icosahedron: largest possible structure built out of **identical** subunits

Icosahedral capsid assembly around a flexible polymer

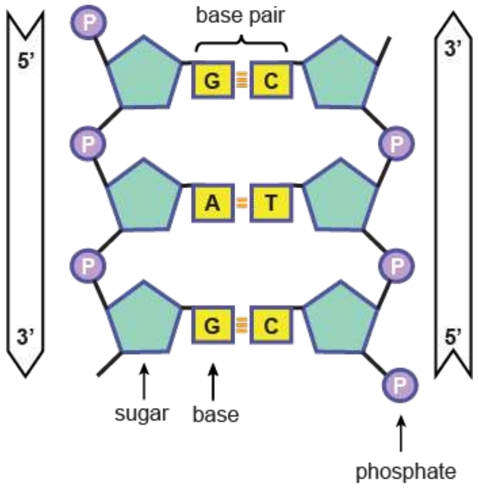


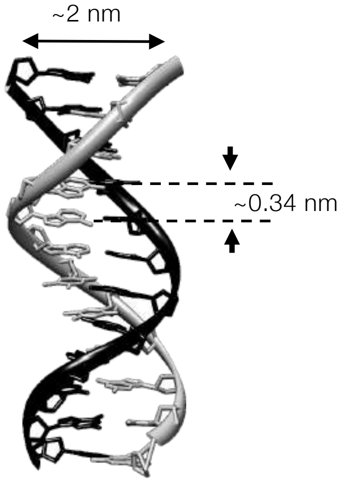
- Colloidal triangles
- Control rigidity
- Control dihedral angle
- Control valency
- Control specificity

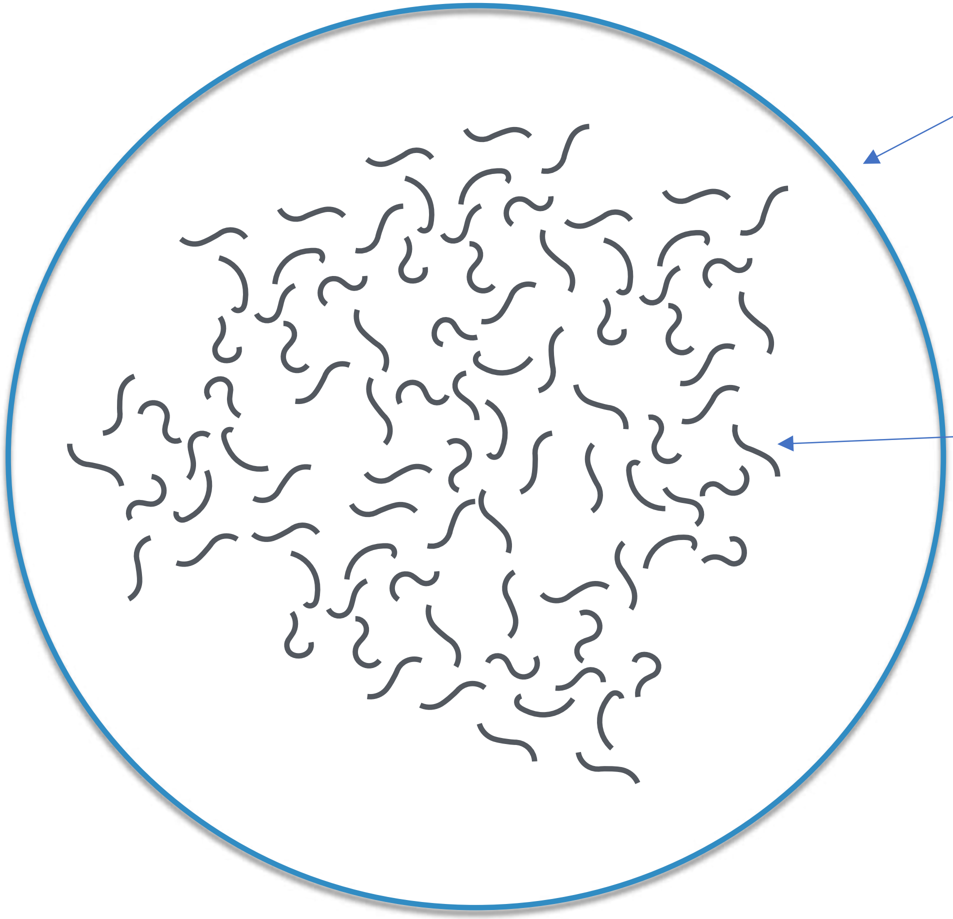


Elrad O.M., & Hagan M.F.
Physical biology (2010)

DNA origami







Scaffold

- M13Mp18
- ~8000 bases
- 1 circular

Staples

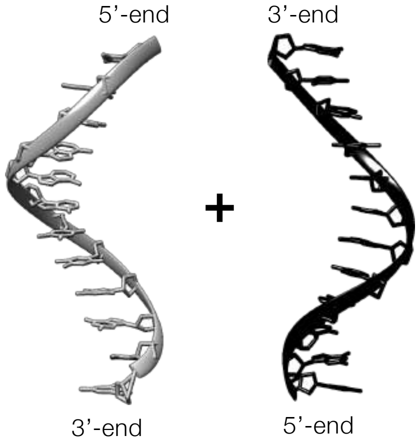
- chemical synthesis
- ~50 bases
- ~200 linear

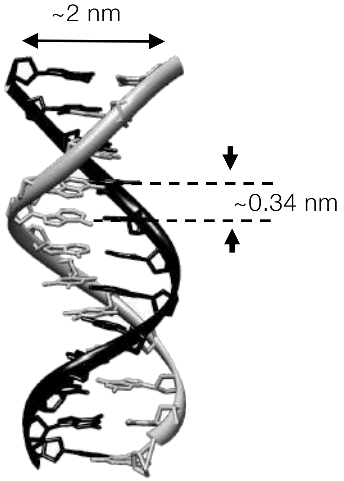
Hendrik Dietz

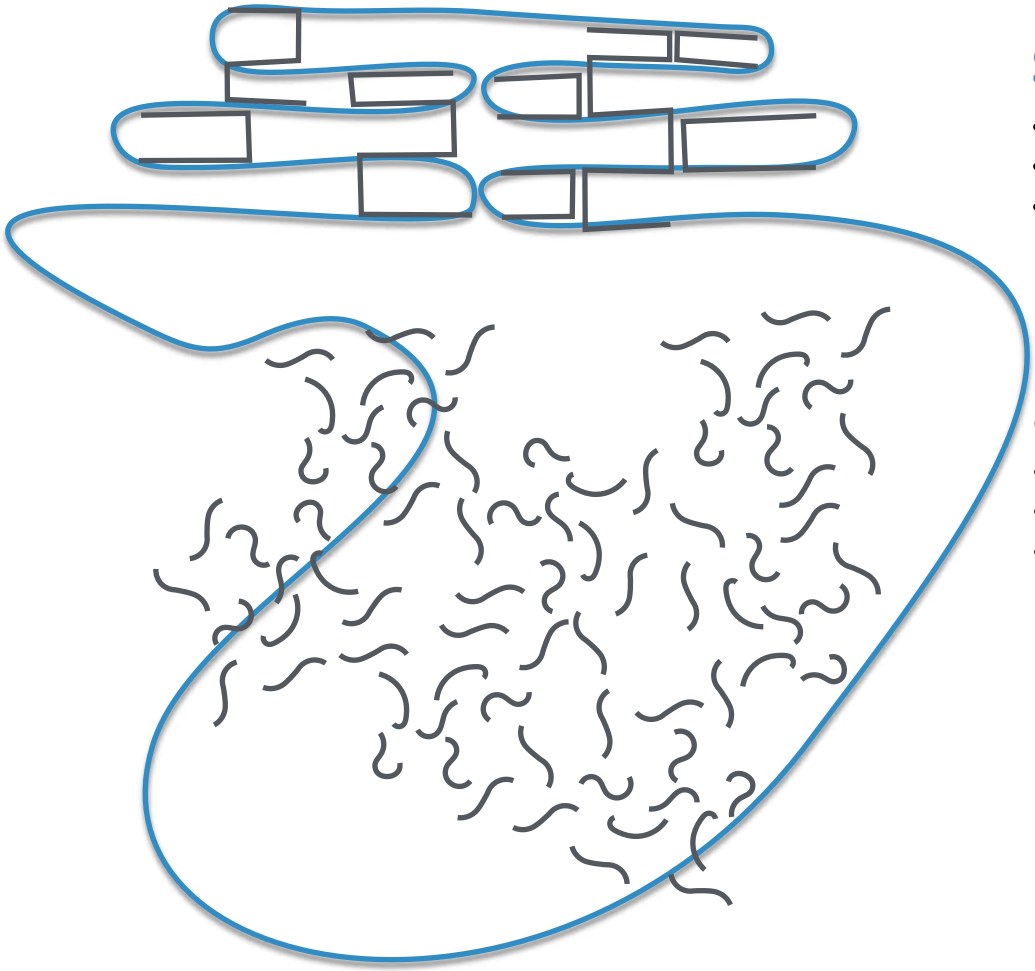
Chris Sigl

Paul W. K. Rothemund, Nature, 2006

DNA origami







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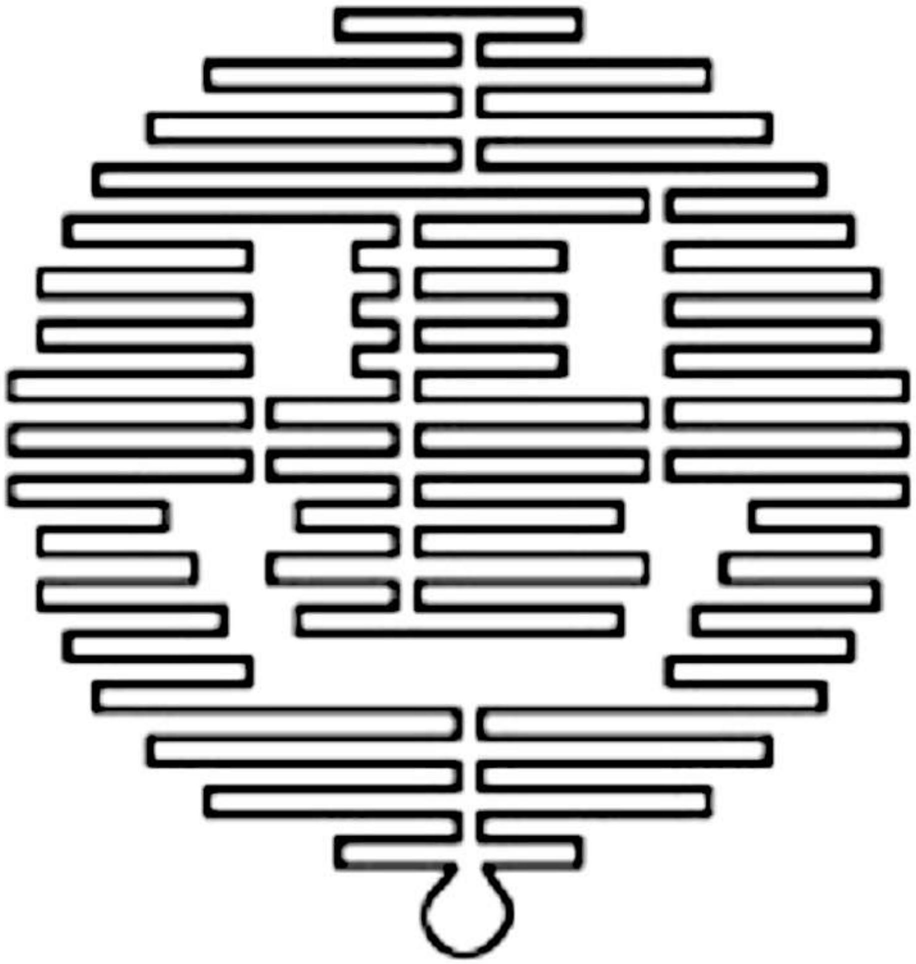

Paul W. K. Rothemund, Nature, 2006

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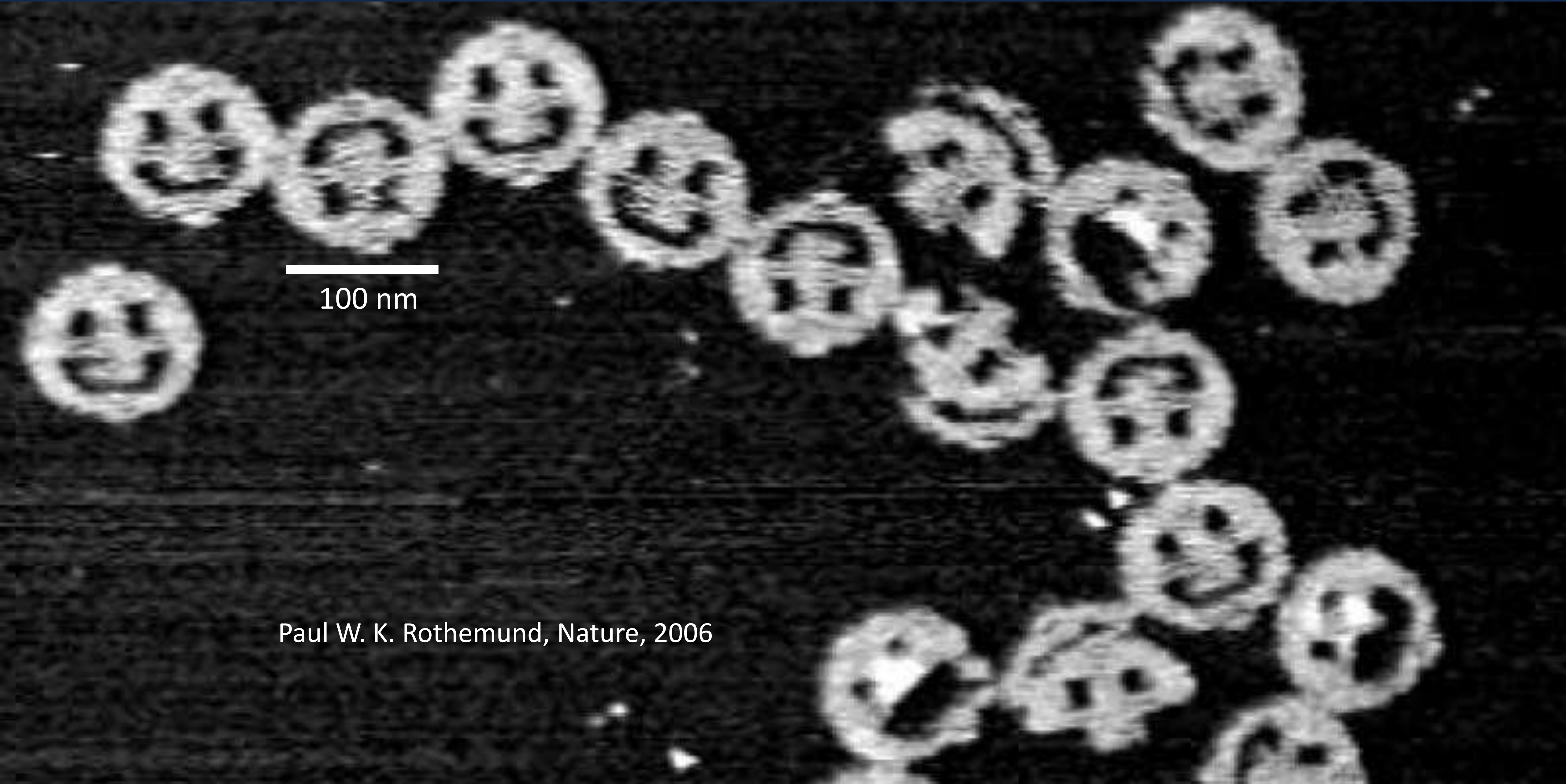
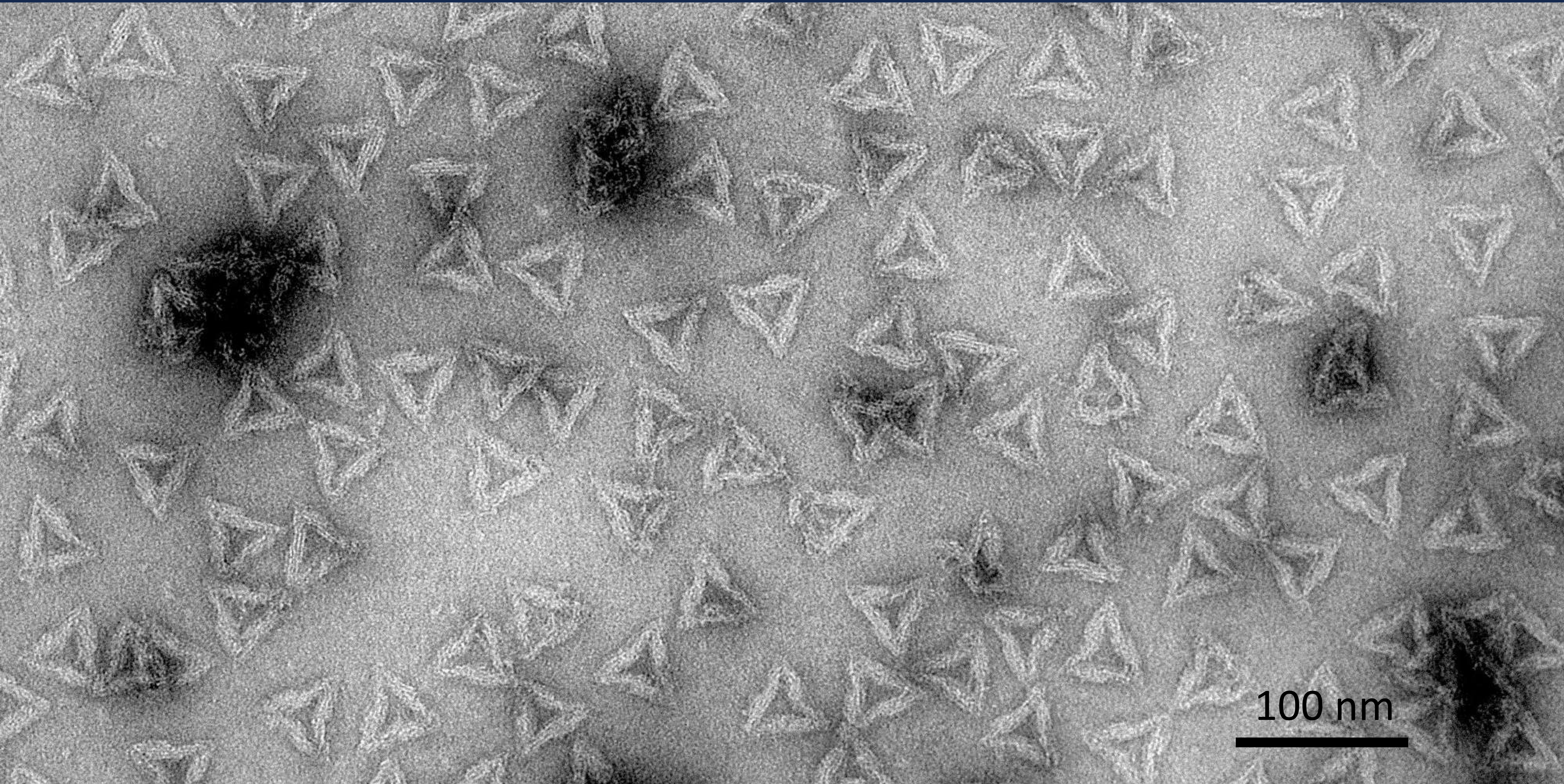


Paul W. K. Rothemund, Nature, 2006

Origami triangles as building blocks



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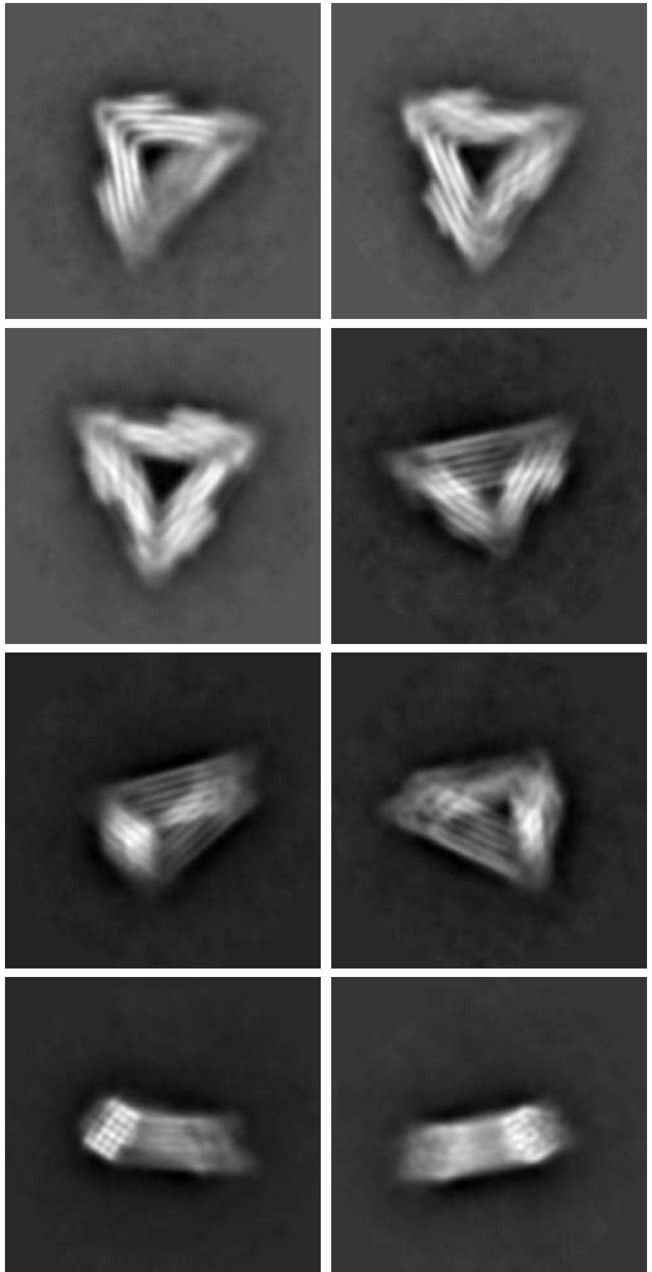
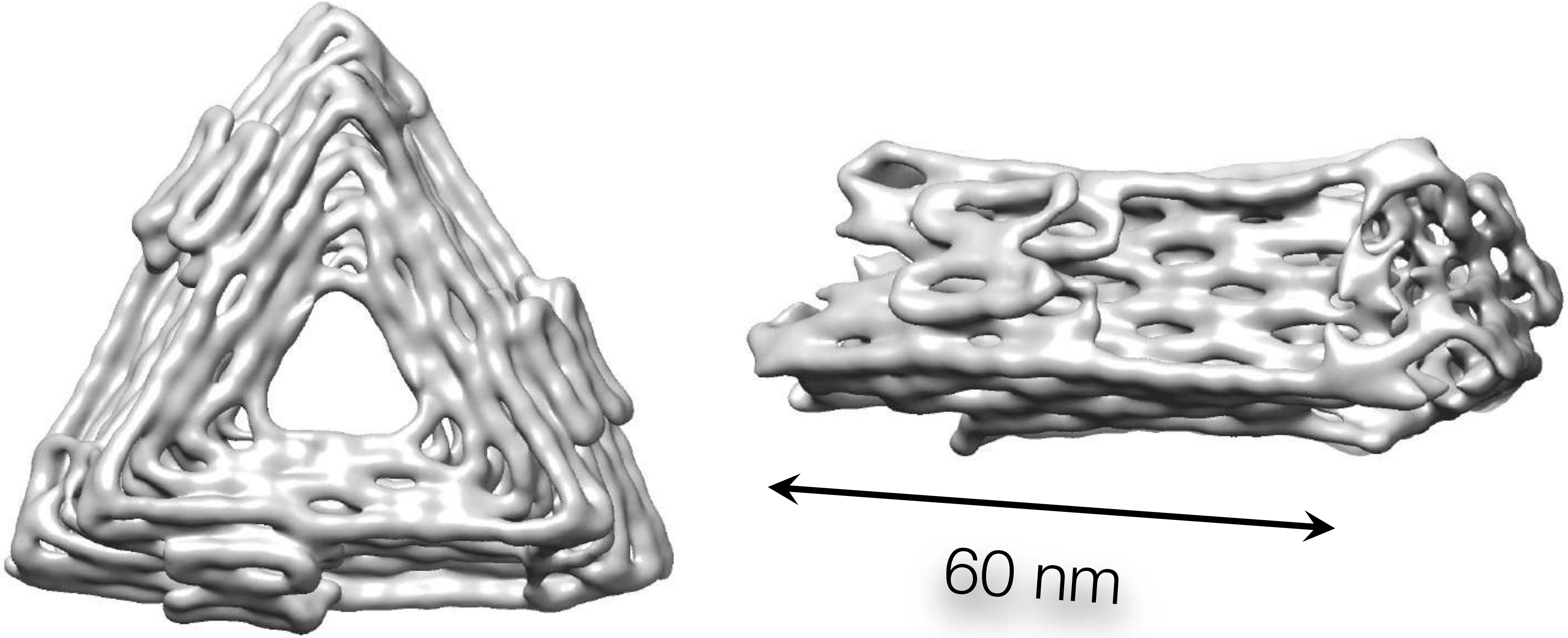




Cryo electron microscopy, 2 nm resolution

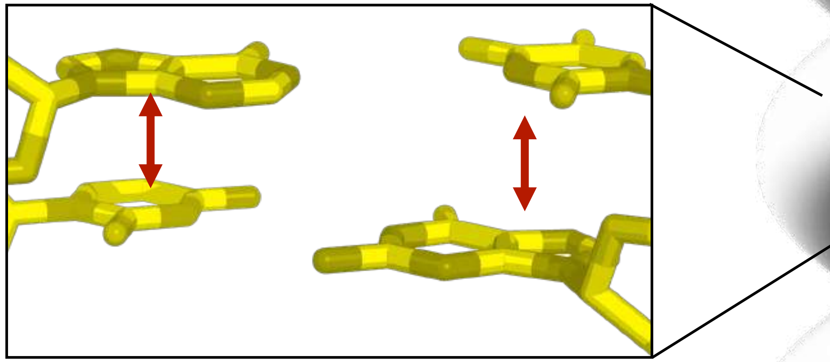
Octahedral capsids from origami

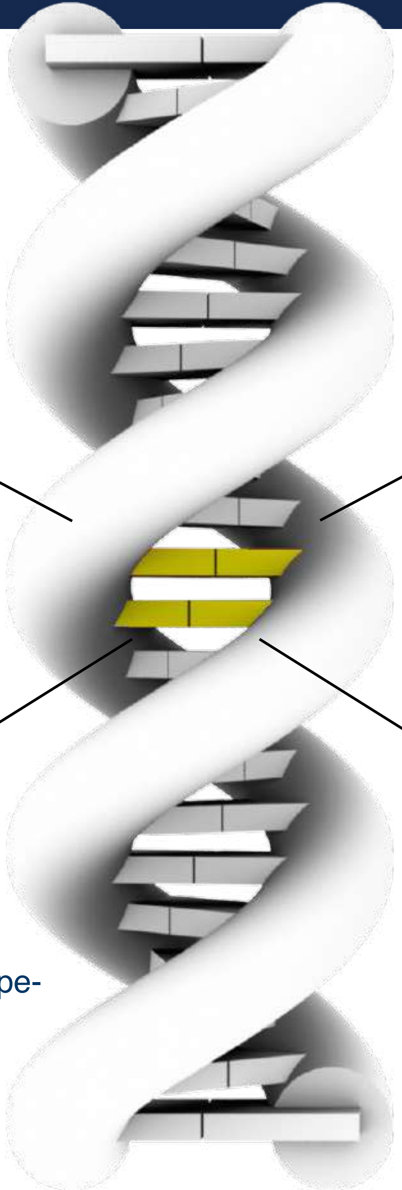
Base Stacking vs Hybridization

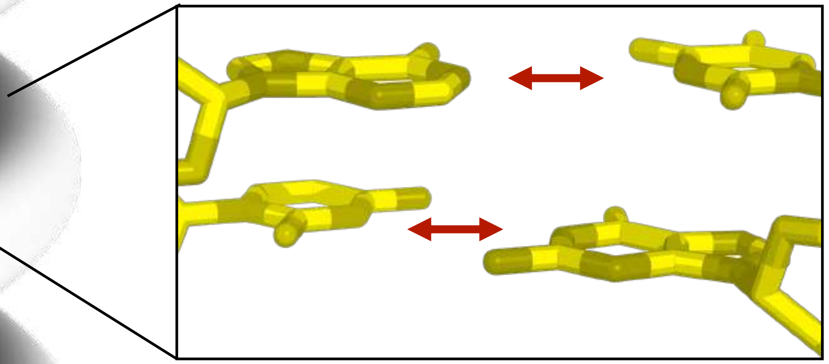
Base-Stacking

van der Waals

Non-specific







Base-Pairing

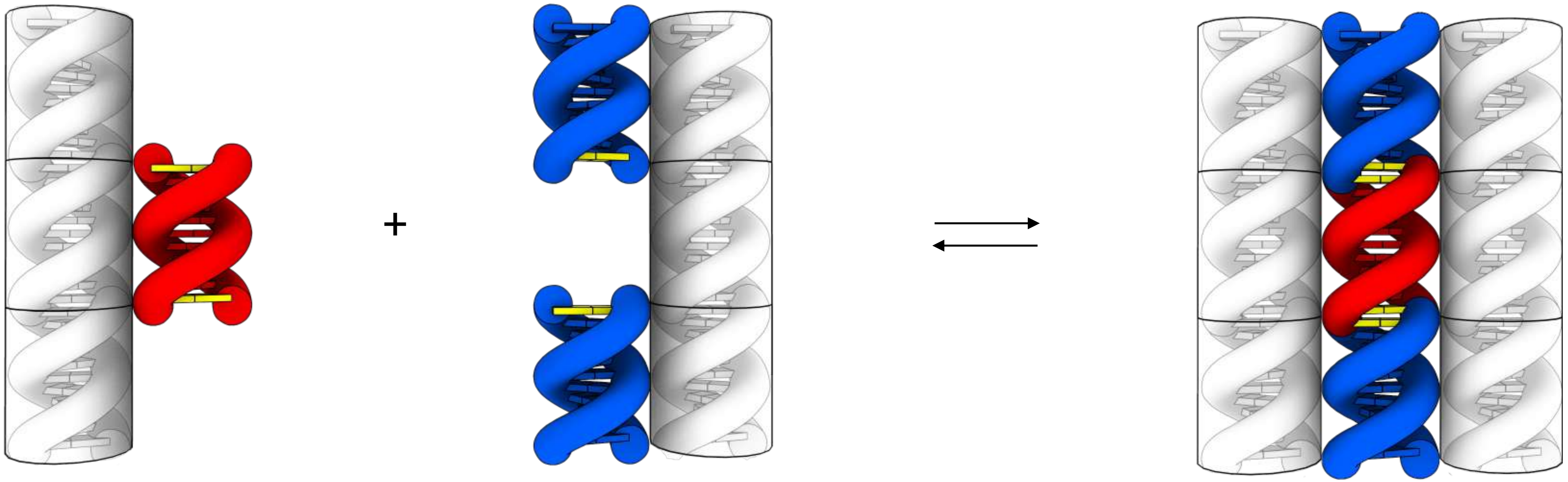
Hydrogen bonds

Specific: AT, GC

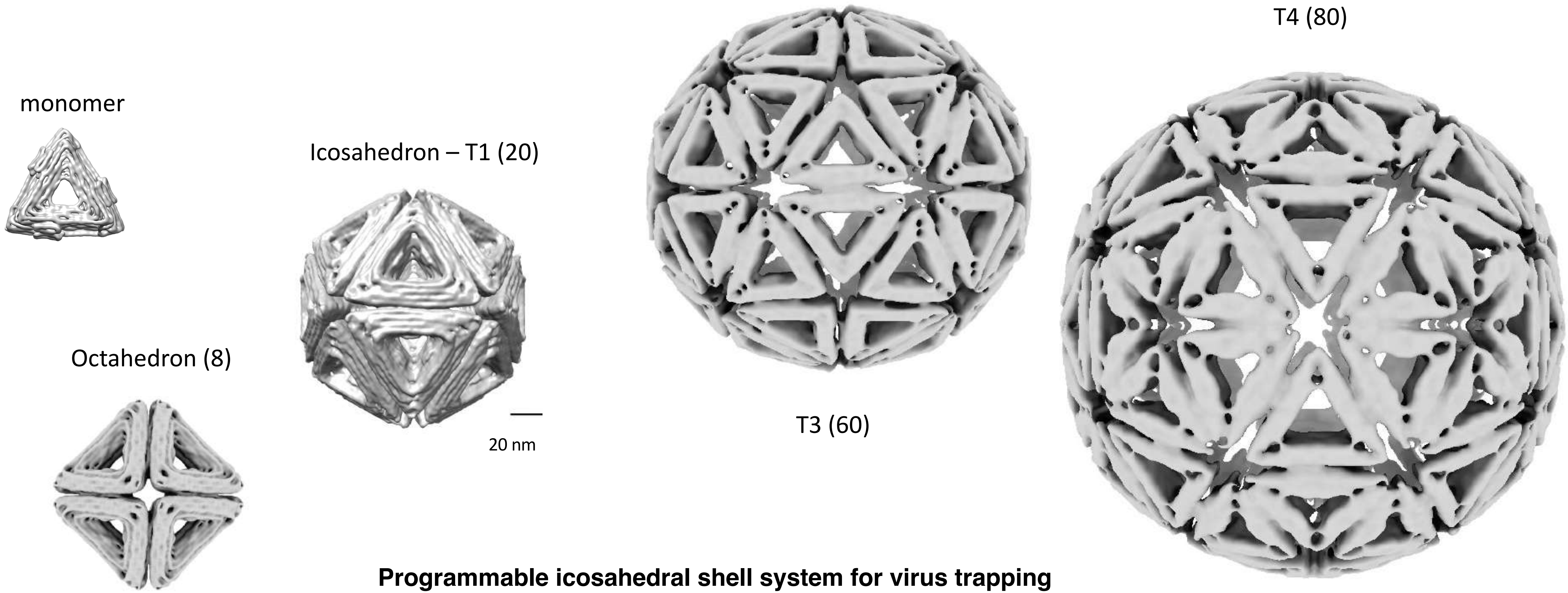
T. Gerling, K. Wagenbauer, A. Neuner, and H. Dietz
'Dynamic DNA devices and assemblies formed by shape-complementary, non-basepairing 3D components'
Science, vol 347 (2015), p1446-1452

Shape-Complementarity & Base-Stacking

Shape complementarity (lock and key)
restores specificity



Origami capsids



Programmable icosahedral shell system for virus trapping
Sigl, …, Hagan, Fraden and Dietz, ***Nature Materials*** (2021)
DOI: 10.1038/s41563-021-01020-4

Geometrically programmed self-limited assembly of tubules using DNA origami colloids
Hayakawa, Videbæk, Hall, Fang, Sigl, Feigl, Dietz, Fraden, Hagan, Grason, Rogers, ***PNAS*** (2022)
https://doi.org/10.1073/pnas.2207902119

encapsulation
scaffolding

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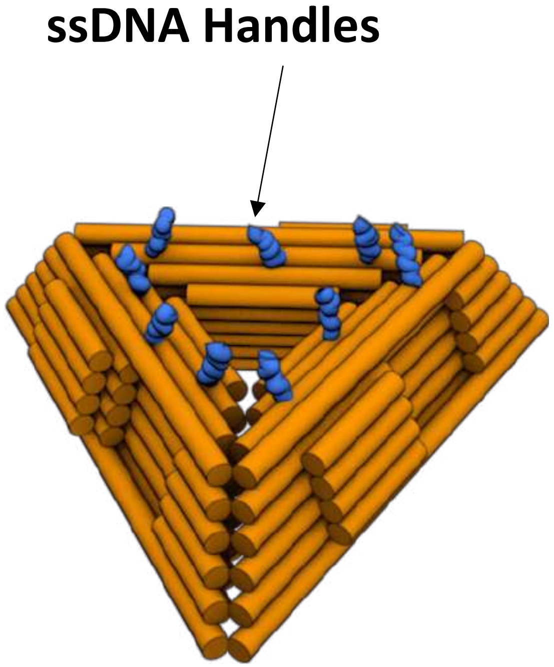
Cargo

Encapsulating a scaffold as cargo in a T1 icosahedron



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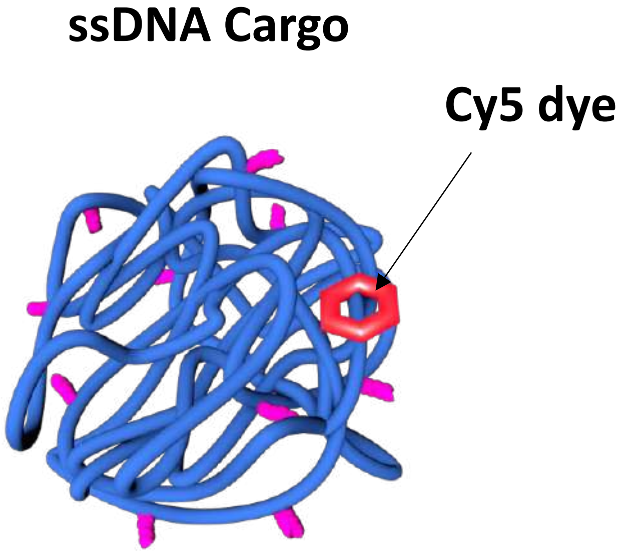

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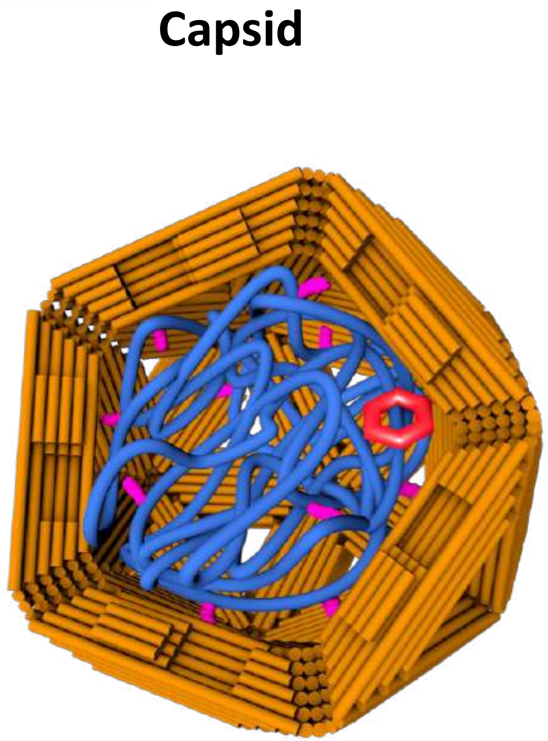


Ali Aghvami

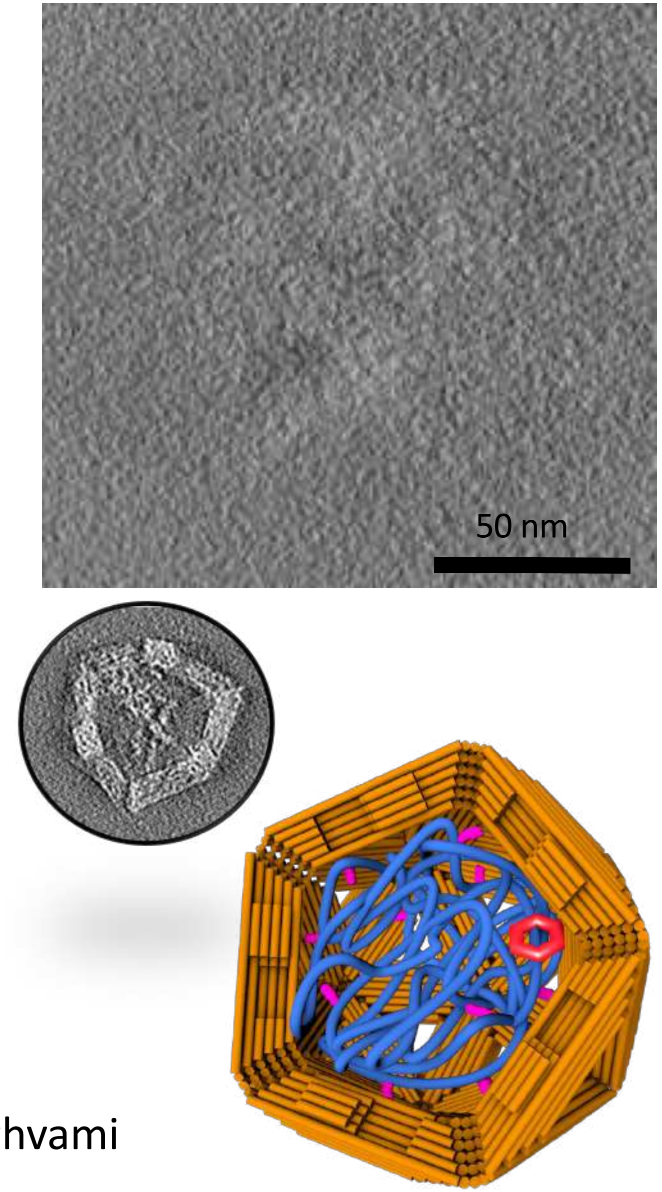
8064 bp scaffold with 10 handles

Complementary handles on scaffold and monomers

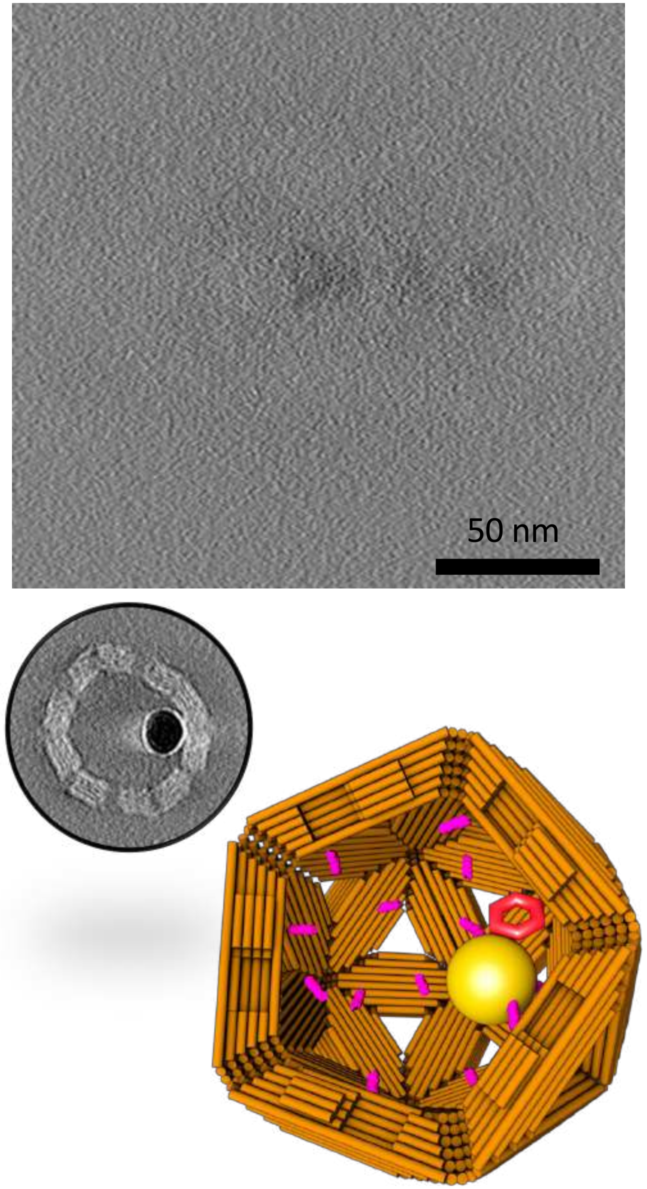
Elrad O.M., & Hagan M.F.
Physical biology (2010)

Cargo: TEM tomograms of encapsulated cargo in T1 capsids

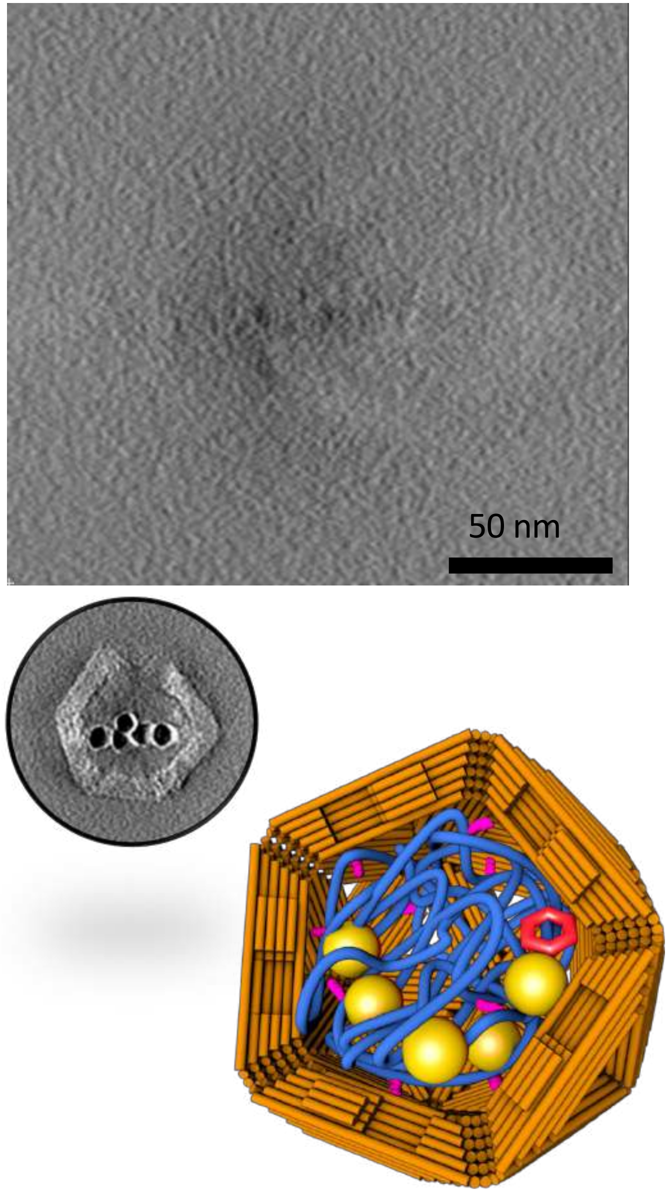
Scaffold



Gold Nano-particles



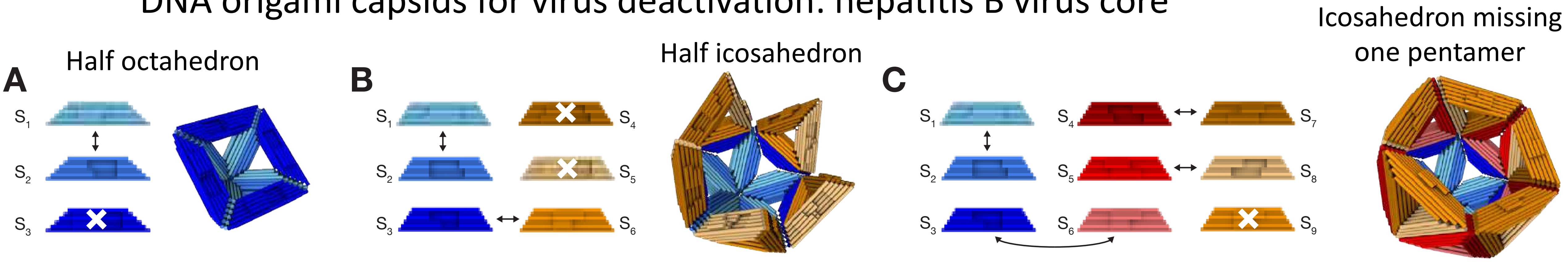
Scaffold & Gold n.p.



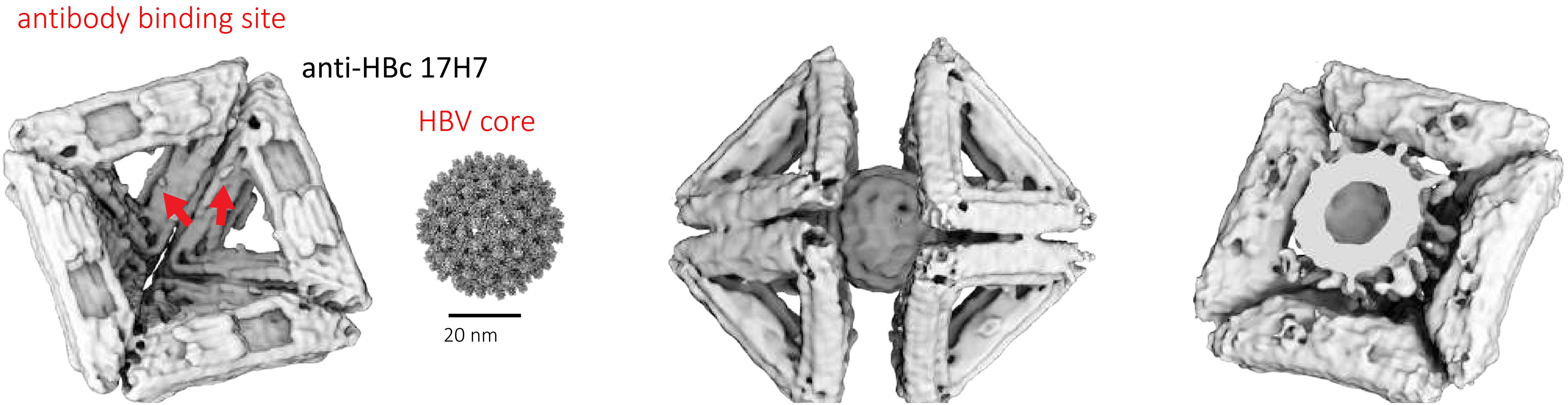
Ali Aghvami

Virus deactivation

DNA origami capsids for virus deactivation: hepatitis B virus core

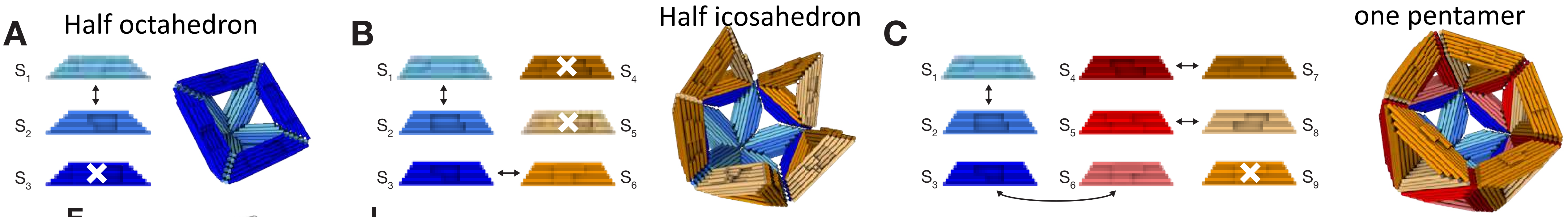


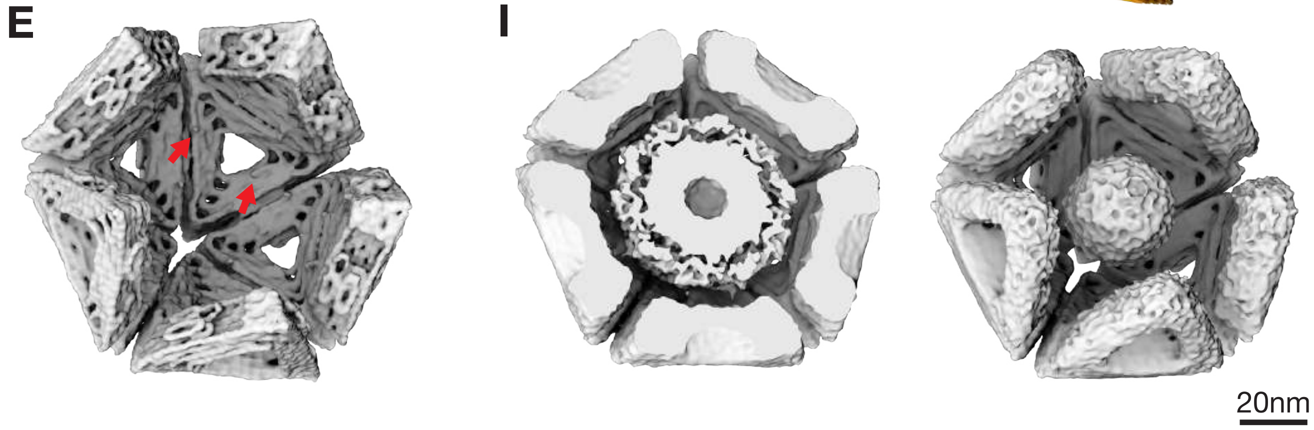
DNA origami capsids capture hepatitis B virus core



Exploit viral assembly principles to deactivate virus

DNA origami capsids capture hepatitis B virus core





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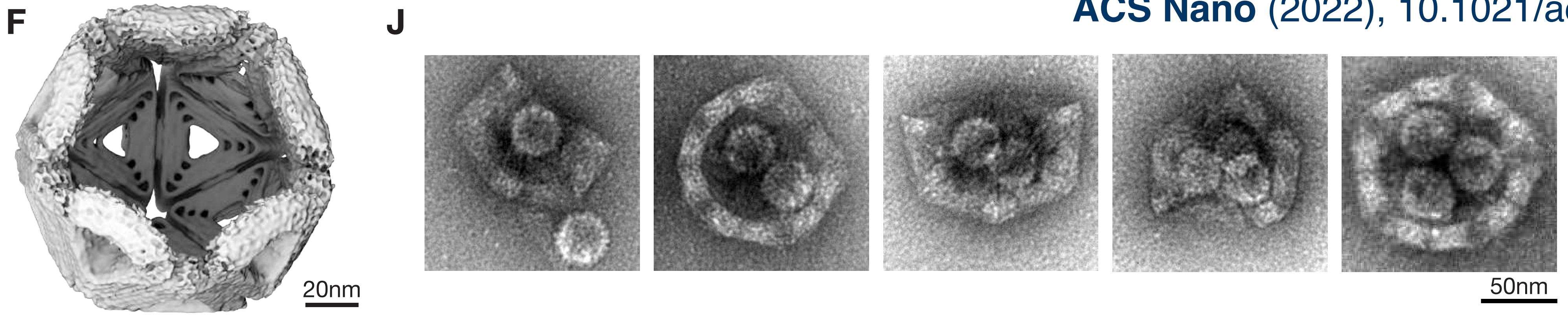
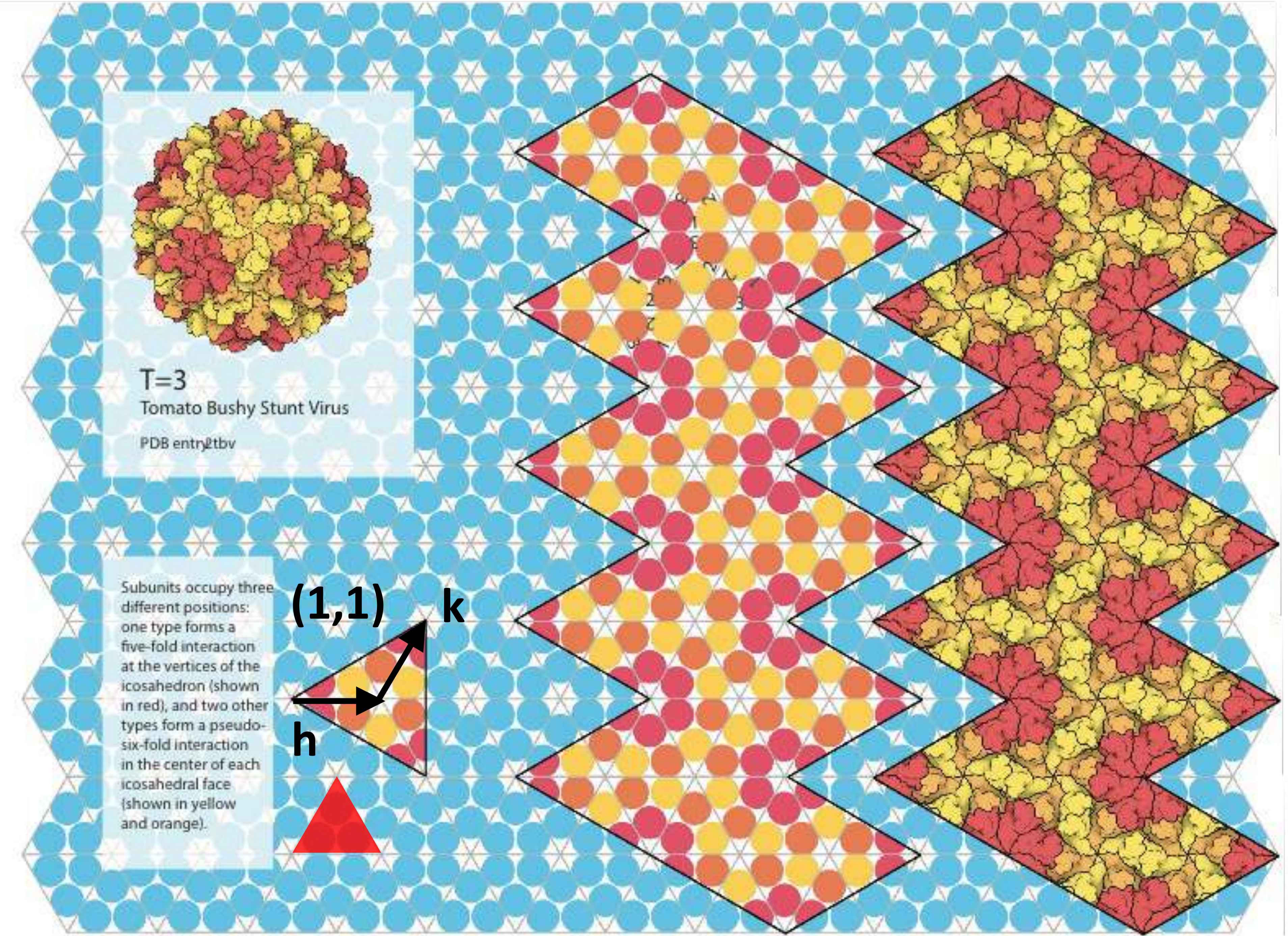
Hendrik Dietz
virofight.eu

ACS Nano (2022), 10.1021/acsnano.1c11328

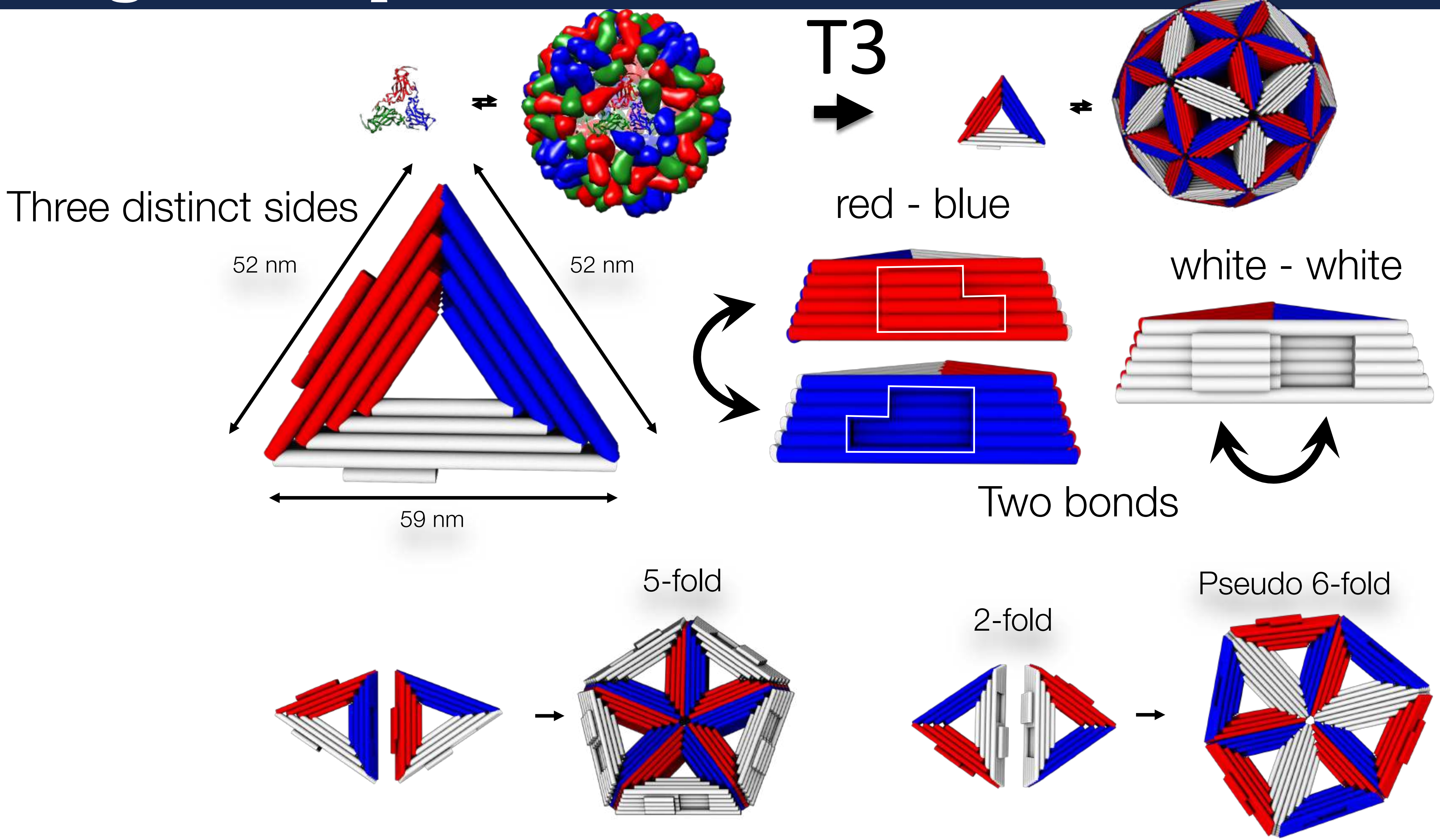


T=3

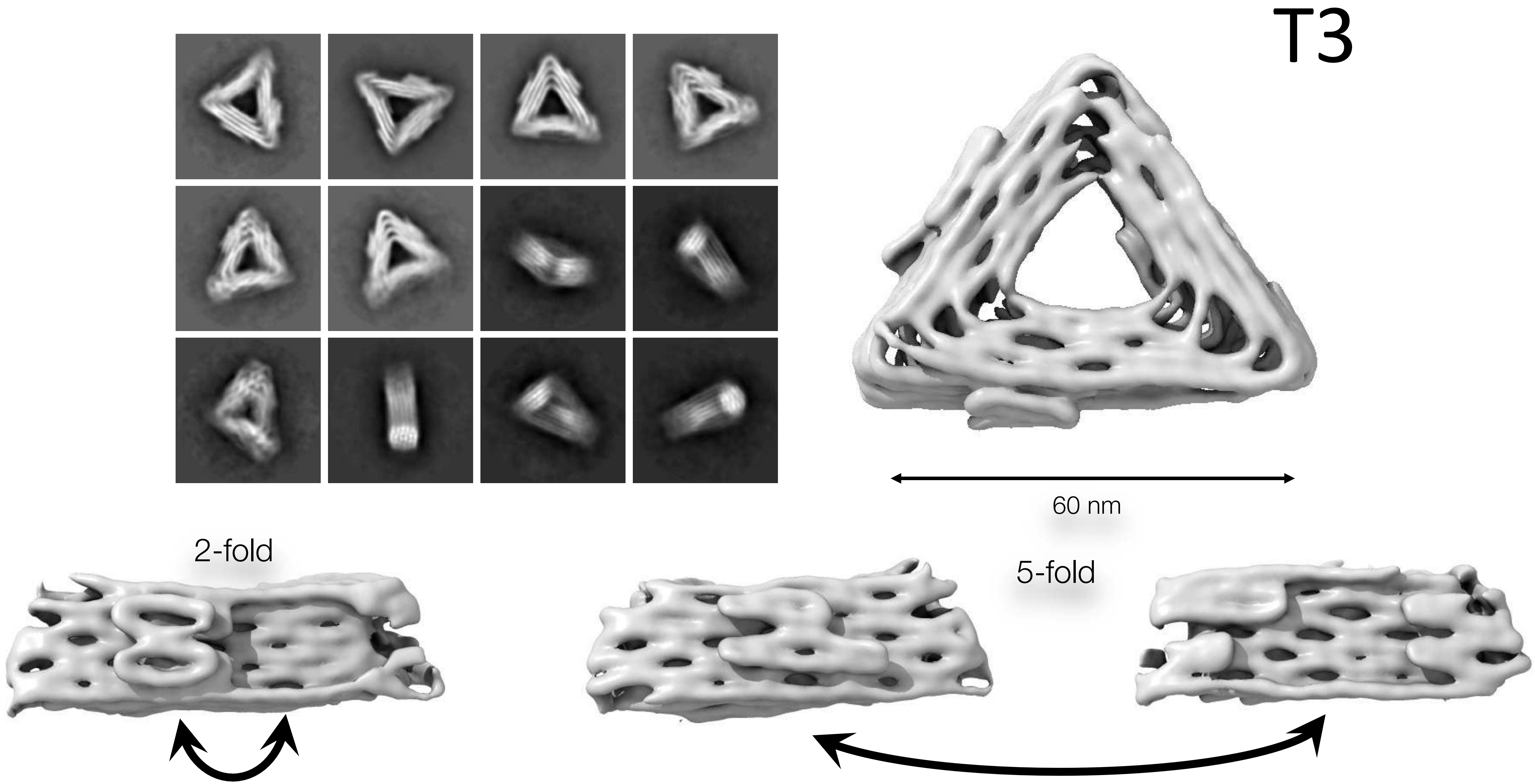


T=3
Tomato Bushy Stunt Virus
PDB entry 2tbv
Subunits occupy three different positions: one type forms a five-fold interaction at the vertices of the icosahedron (shown in red), and two other types form a pseudo-six-fold interaction in the center of each icosahedral face (shown in yellow and orange).
(1,1)
k
h

Origami capsids

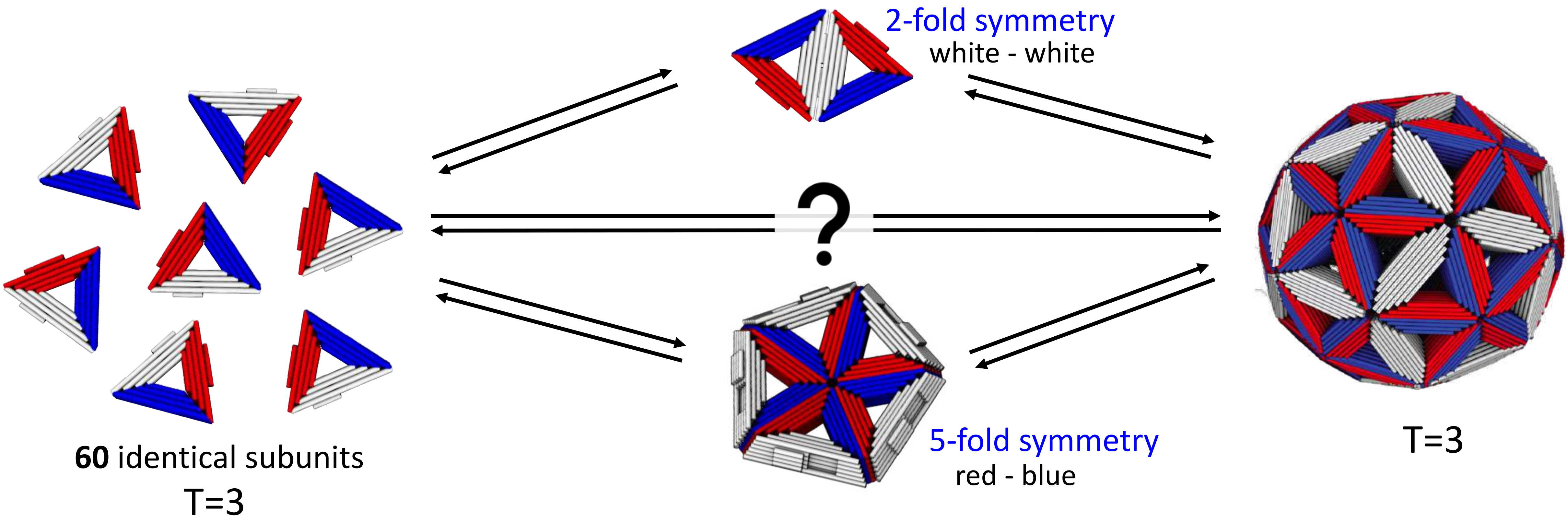


Origami capsids



What are the T=3 assembly pathways?

Symmetry is only one piece of the puzzle.



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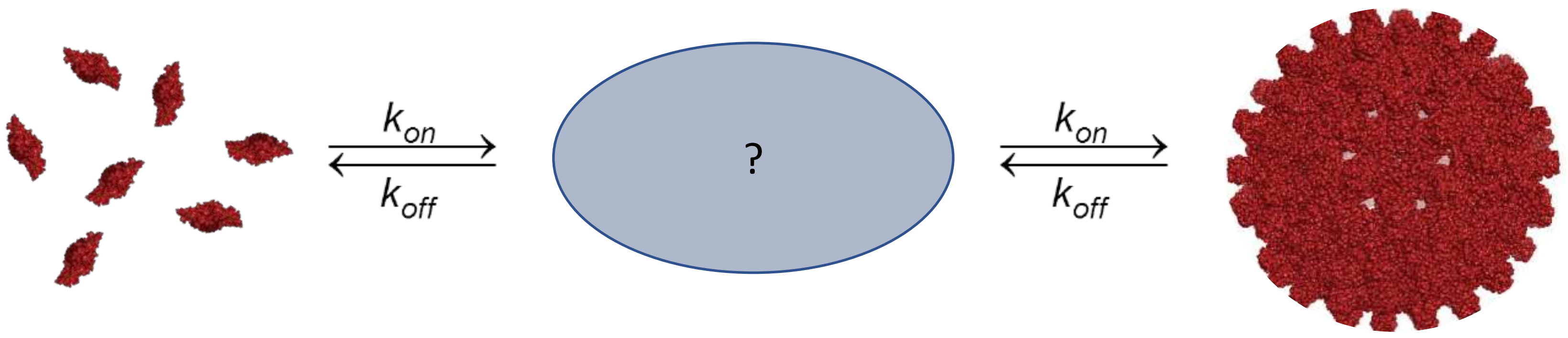
Assembly pathways?

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

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



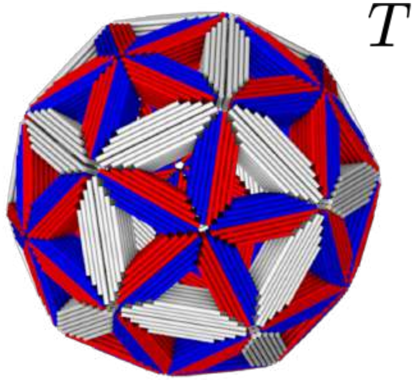
~10^{30} possible intermediates

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

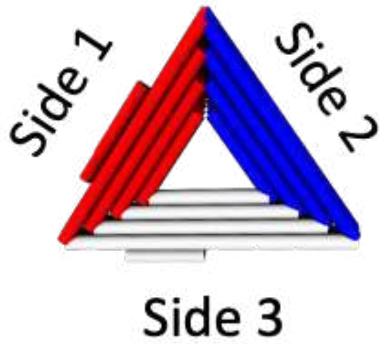
Symmetry is only one piece of the puzzle

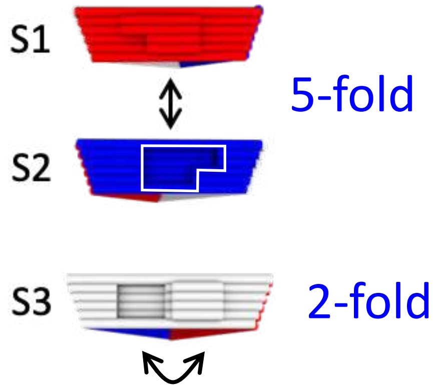
Question: What are the optimal kinetic pathways?

$T = 3$

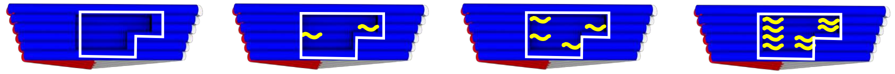


From symmetry





But what about the relative strength of the two interactions?



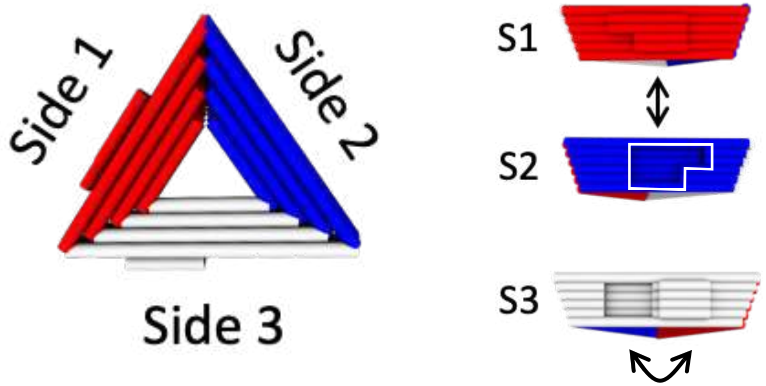
Increase # complementary ssDNA

Binding strength ↑

Symmetry is only one piece of the puzzle

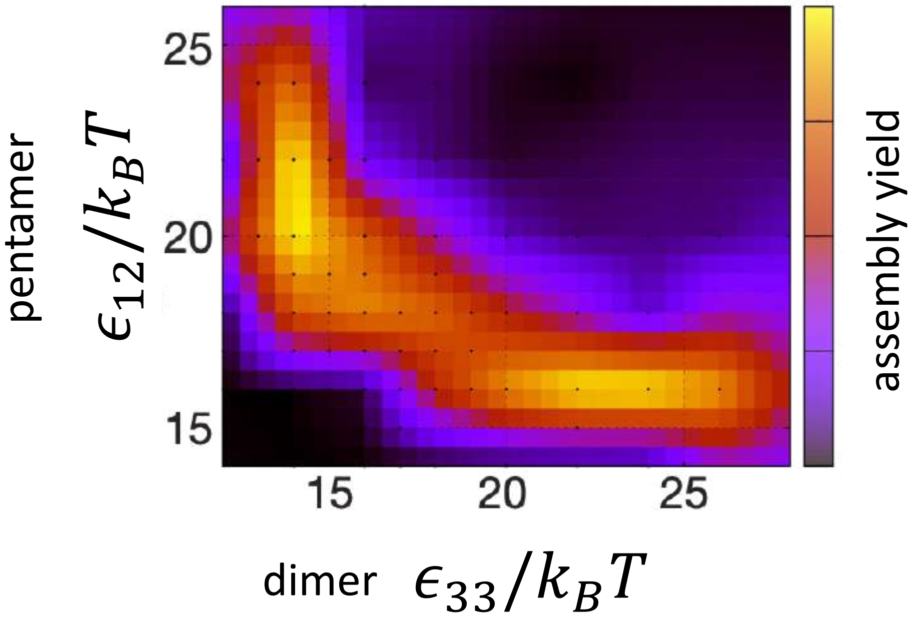
Question: What are the optimal kinetic pathways?

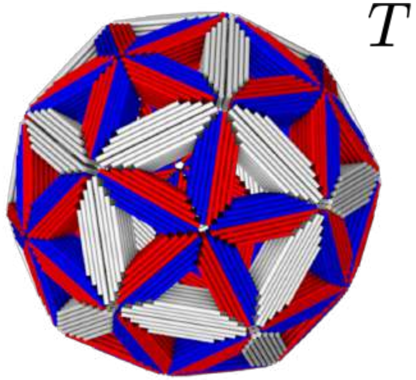
From symmetry



But what about the relative strength of the two interactions?

From kinetic simulation





Wei, Trubiano, Sigl, Paquay, Dietz, Hagan, Fraden, *under review* (2023)

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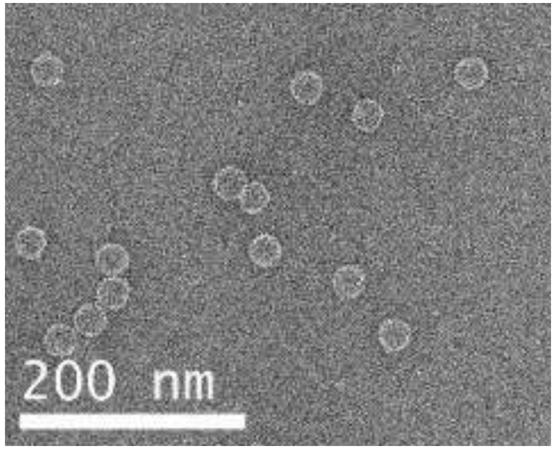
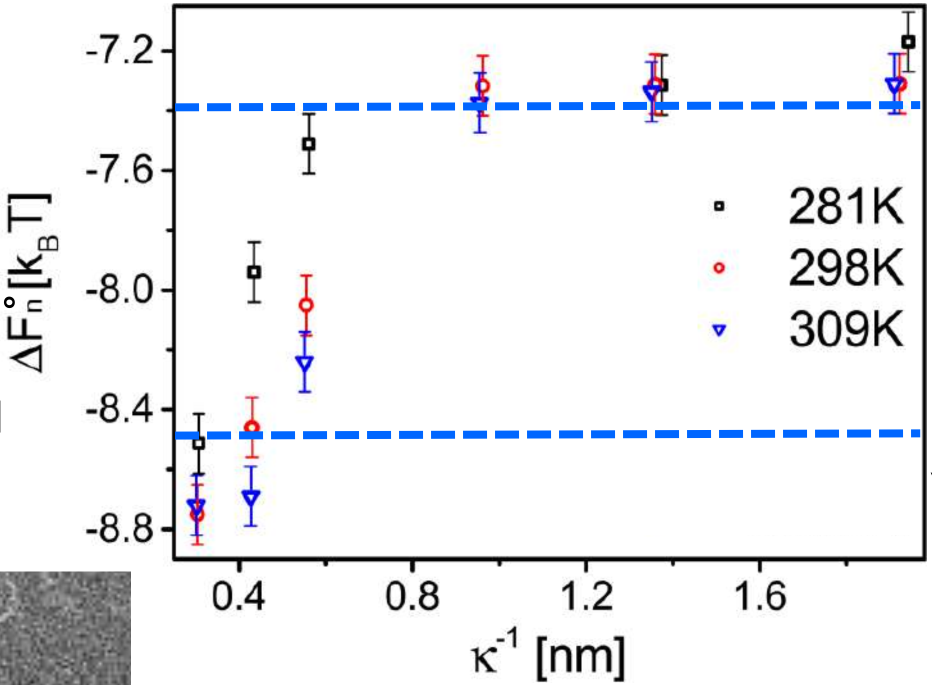
Uri Raviv

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)



No assembly

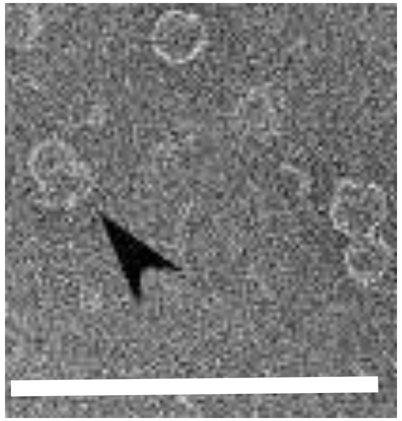


Successful assembly

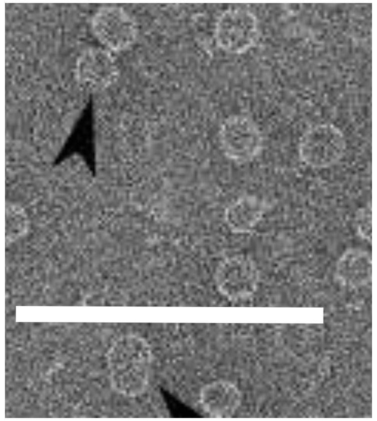
Malformed particles

Debye screening length

overgrown capsids



aggregated capsid fragments

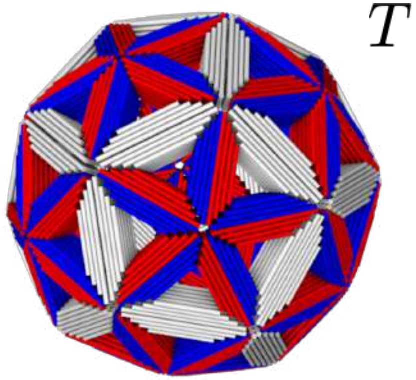


~ $1k_BT$ (2.4 kcal/mole) difference in ΔF°_n leads to the formation of malformed capsids with aberrant geometry

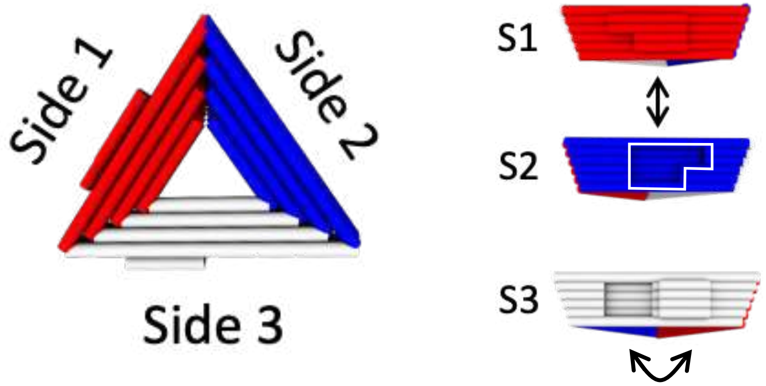
Symmetry is only one piece of the puzzle

Question: What are the optimal kinetic pathways?

$T = 3$

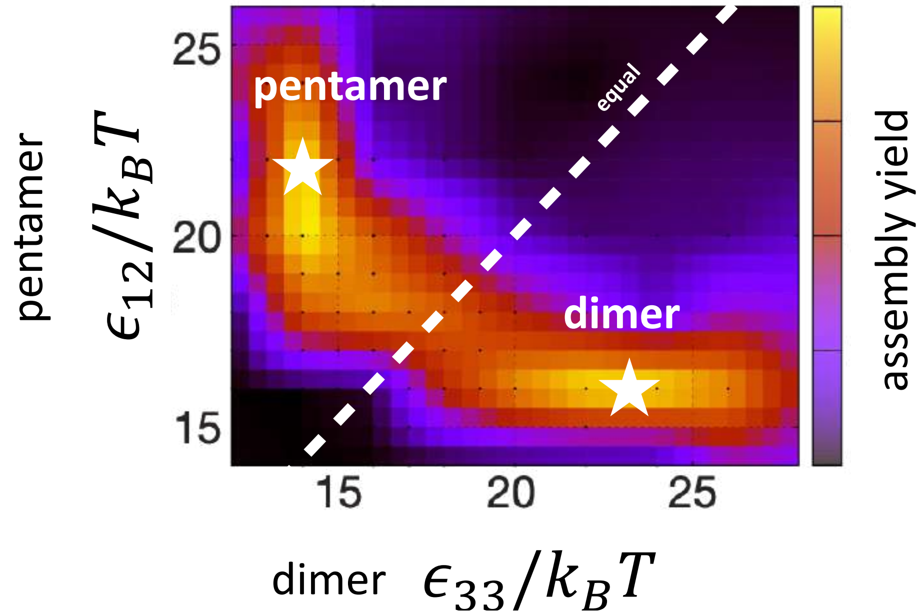


From symmetry



But what about the relative strength of the two interactions?

From kinetic simulation



Takeaway: Hierarchical pathways predicted* to improve assembly yield

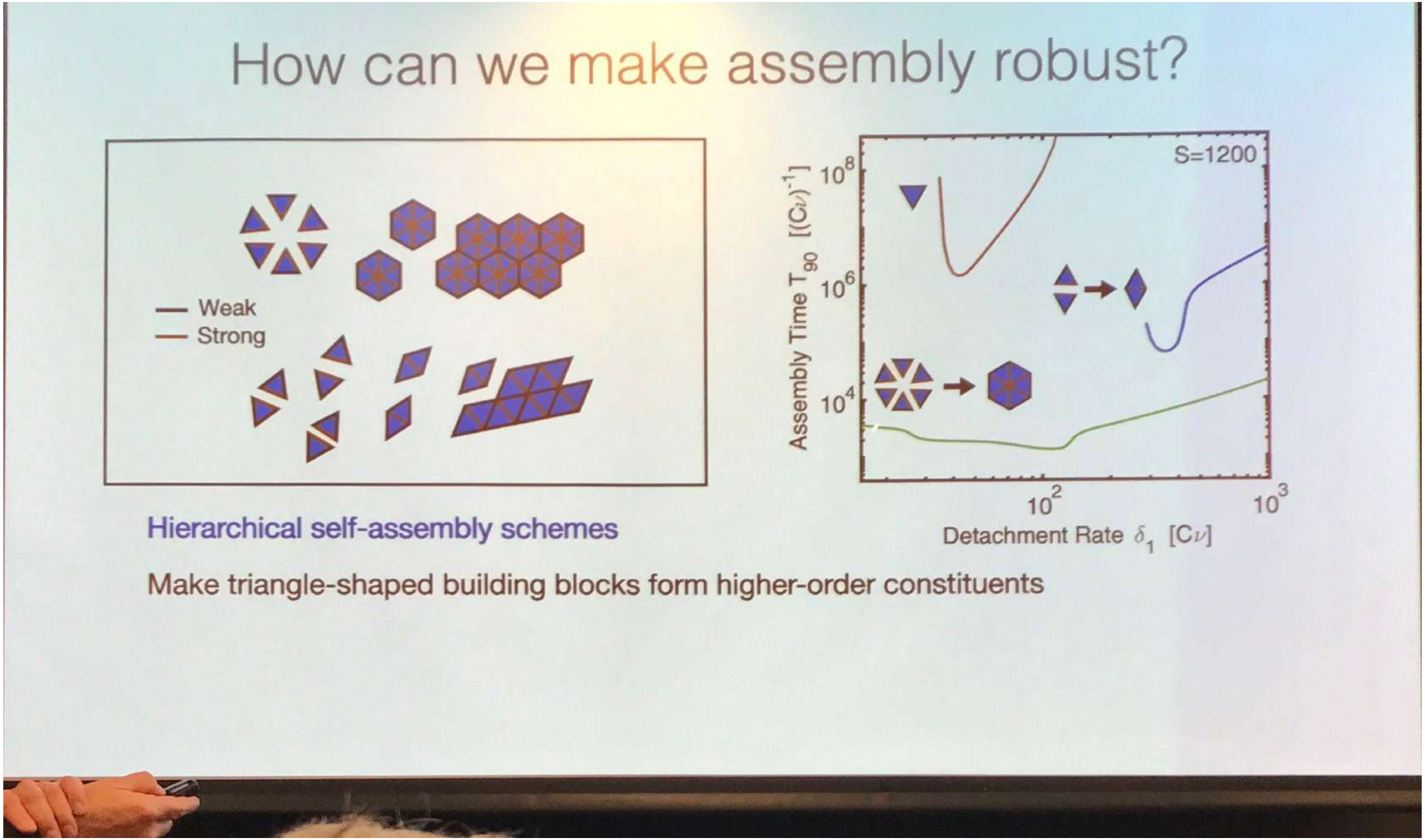
predicted*: Before experiment!

Wei, Trubiano, Sigl, Paquay, Dietz, Hagan, Fraden, *under review* (2023)

MONDAY 13 NOVEMBER 2023

09.00 - 09.50	**Registration and Refreshments**		
09.50 – 10.00	**Welcome and Housekeeping**		
10.00 - 10.30	**William Gelbart**, UCLA	IN CELLULO versus IN VITRO RECONSTITUTION OF RNA-SPECIFIC VIRUS-LIKE PARTICLES	Abstract
10.30 - 11.00	**Dek Woolfson**, University of Bristol	Rationally and computational design of functional protein barrels	Abstract
11.00 - 11.30	**Farzad Fatehi**, University of York	Geometry as a key to the virosphere – from viral infections to virus nanotechnology	Abstract
11.30 - 12.00	**Refreshments**		
12.00 - 12.30	**Roya Zandi**, University of CA, Riverside	The role of flexibility of capsid proteins in the symmetry of viral shells	Abstract
12.30 - 13.00	**Danielle Tullman-Ercek**, Northwestern University	Designing with nanoscale building blocks: engineering self-assembling protein superstructures for applications in vaccines, drug delivery and biochemical production	Abstract
13.00 - 14.30	**Lunch**		
14.30 - 15.00	**David Bhella**, MRC - University of Glasgow Centre for Virus Research	Capsid stability and receptor mediated priming in calicivirus entry	
15.00- 15.30	**Alexander Borodavka**, University of Cambridge	How do Viruses Use RNA Granules and RNA Chaperones to Assemble Their Segmented Genomes?	Abstract
15.30 - 16.00	**Refreshments**		
16.00 - 16.30	**Uri Raviv**, The Hebrew University of Jerusalem	Mechanism of Virus Capsid Assembly	Abstract
16.30- 17.00	**Erwin Frey**, Ludwig-Maximilians-Universitaet Muenchen	The time complexity of self-assembly	Abstract

Erwin Frey

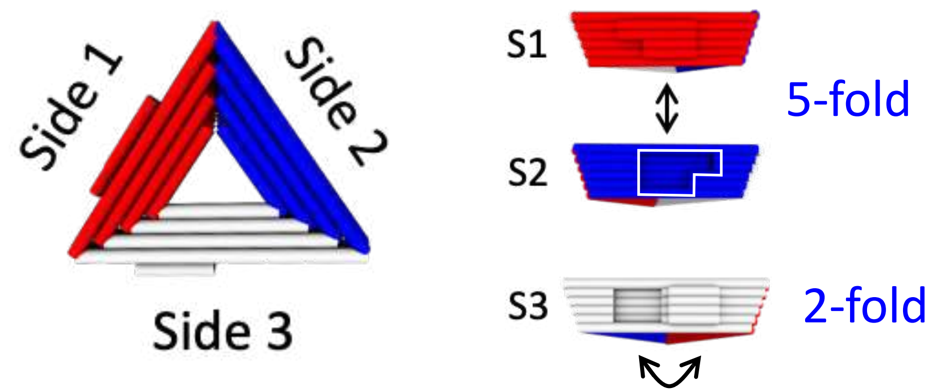


How can we make assembly robust?
Weak
Strong
S=1200
10^8
10^6
10^4
Assembly Time T_{90} $[(C\nu)^{-1}]$
10^2
10^3
Detachment Rate δ_1 $[C\nu]$
Hierarchical self-assembly schemes
Make triangle-shaped building blocks form higher-order constituents

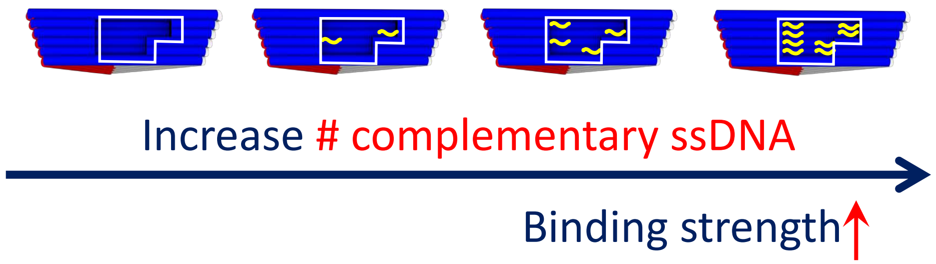
Tuned block-block interactions

What are the optimal kinetic pathways?

From symmetry

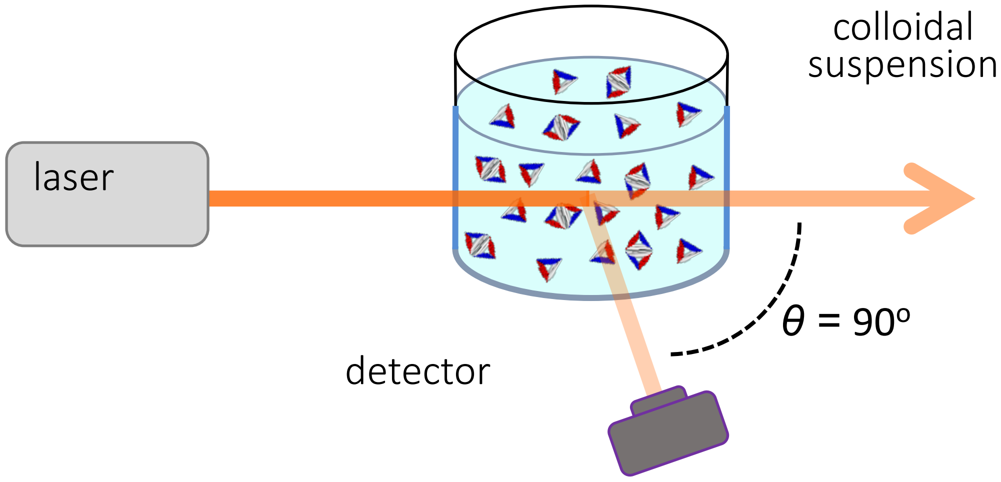


But what about the relative strength of the two interactions?



Free energy measurement

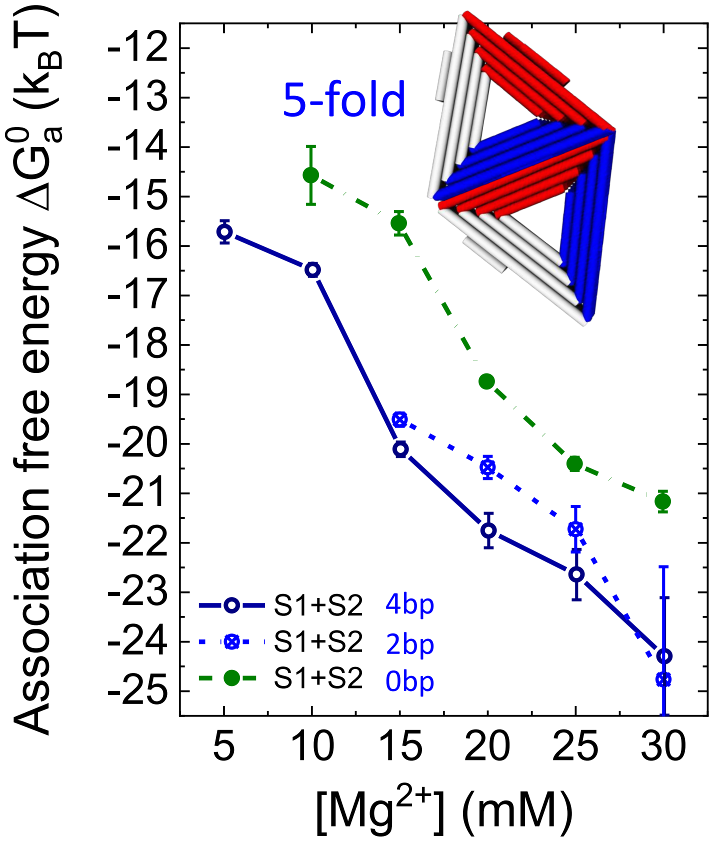
Static light scattering - Gibbsometer & real time assembly monitor

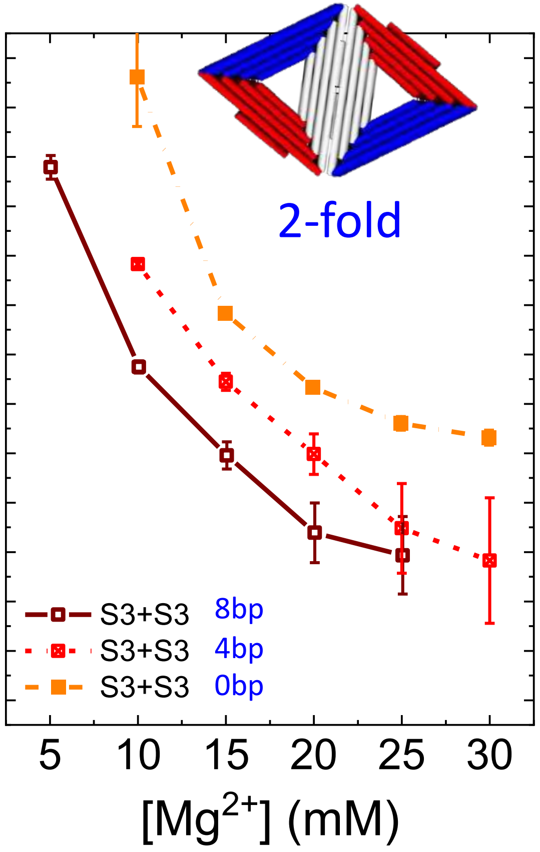


Wei, Trubiano, Sigl, Dietz, Hagan, Fraden, *under review* (2023)

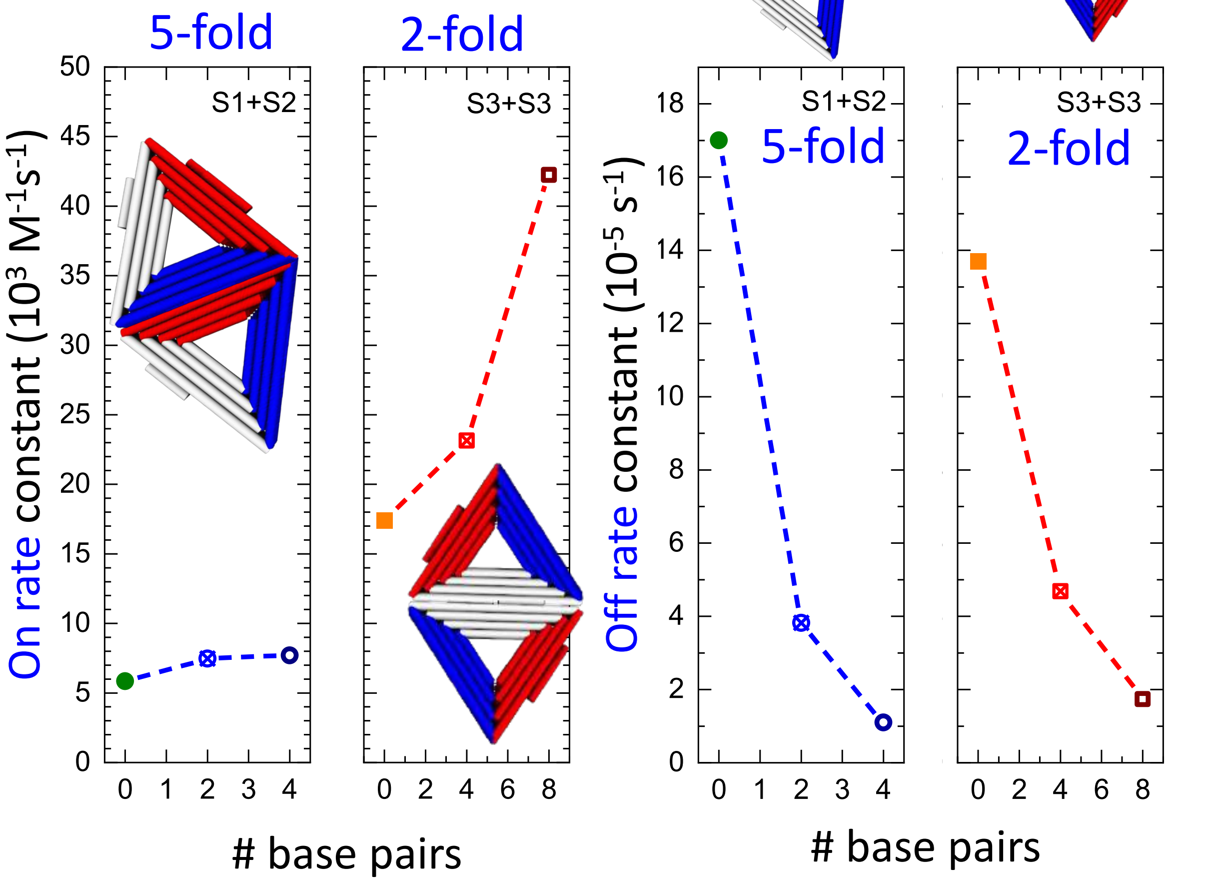
Monomer – dimer free energy

(1) Bond strength increases with the number of added base pairs & the magnesium ion concentration.
(2) Off rate constant strongly decreases with number of added base pairs.
(3) On rate constant shows a weak dependence with number of added base pairs.
(4) On rate 5 orders of magnitude slower than diffusion limited rate.





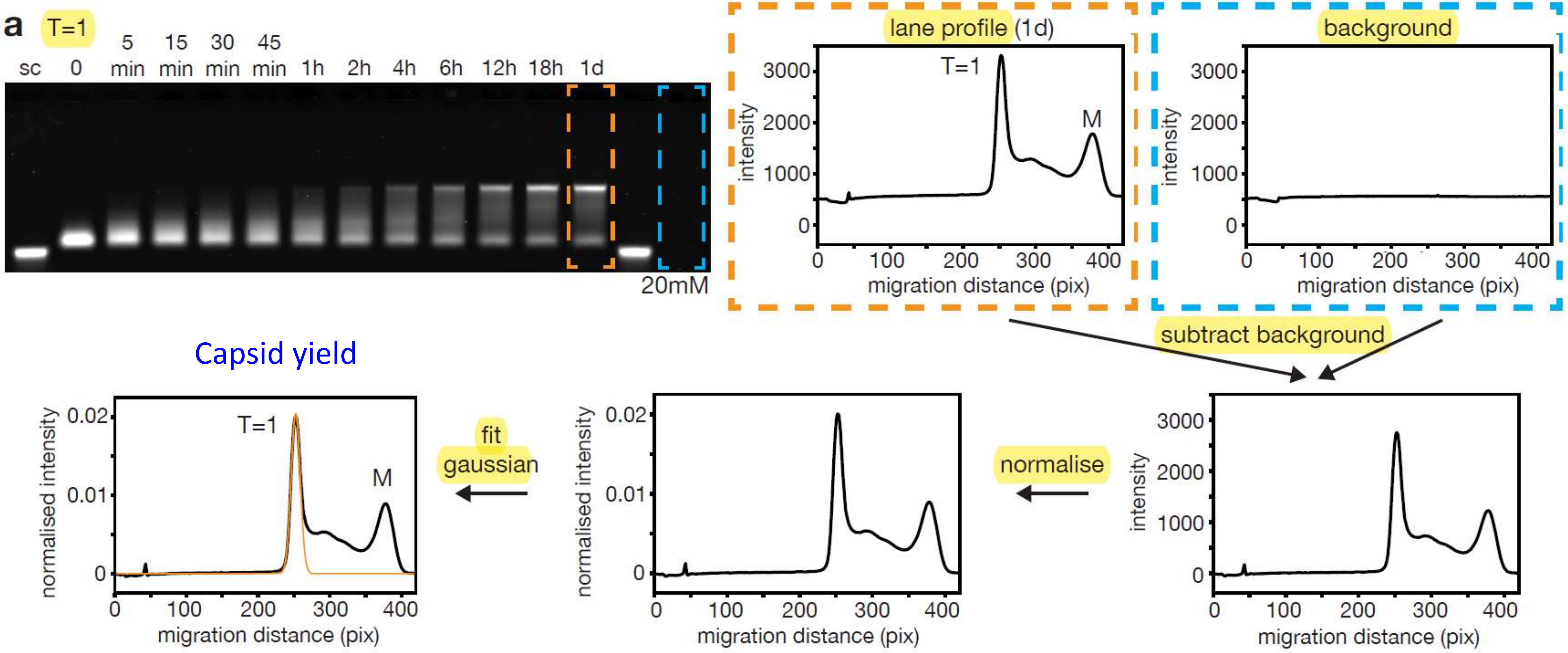
Gibbs free energy vs Mg^{2+} concentration



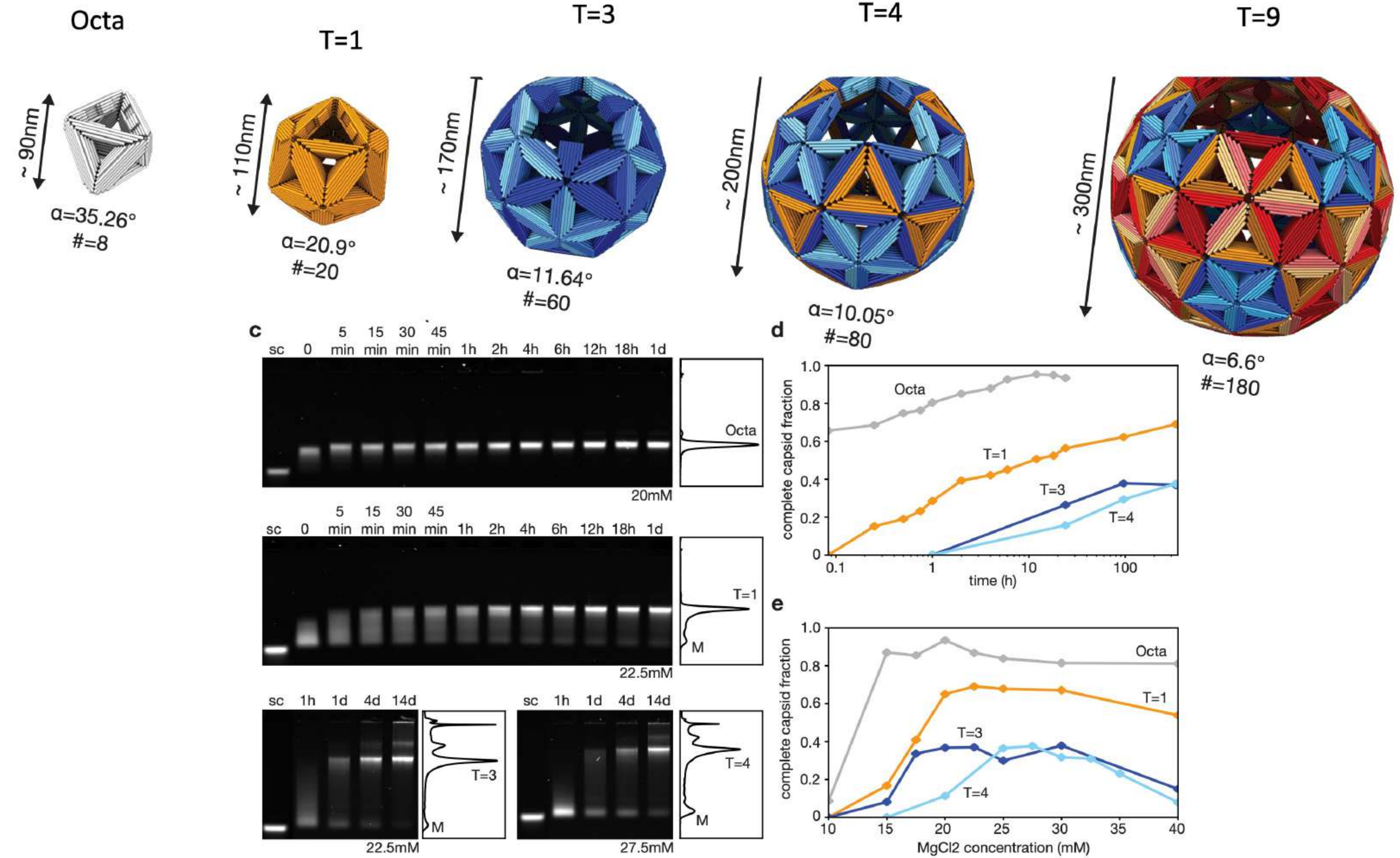
On and off rate constants vs. # base pairs (20 mM Mg^{2+})

Kinetics: Experiment

Gel Electrophoresis



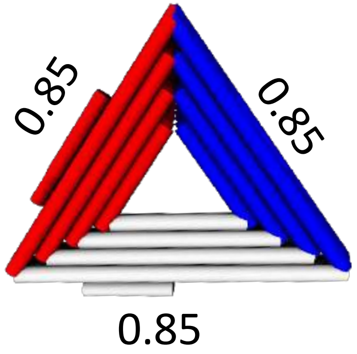
Kinetics: Experiment

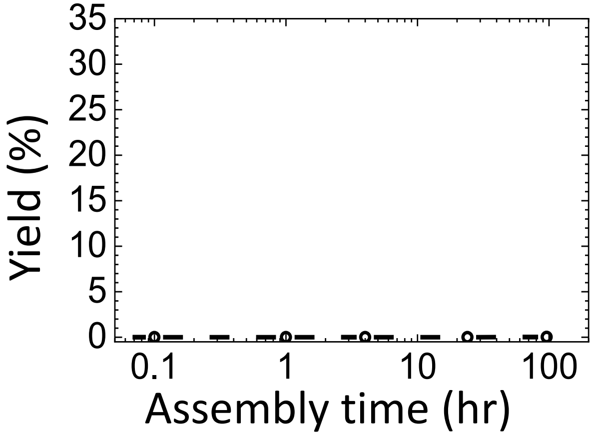


Equalitarian assembly (T=3)

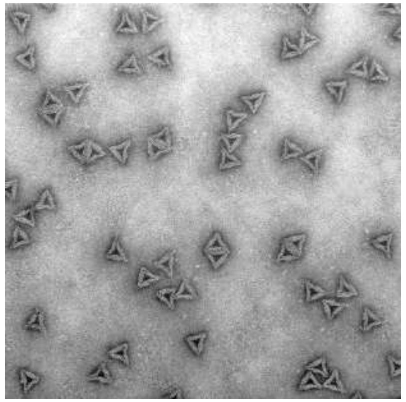
Weak binding – no assembly

Equal-binding
(-15.0 k_BT)

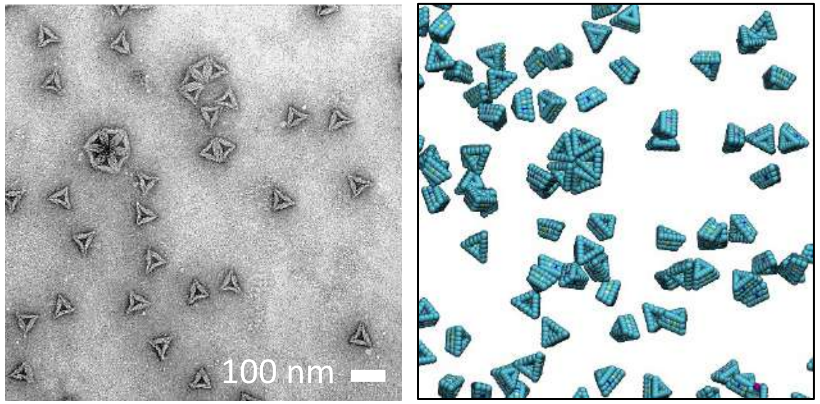


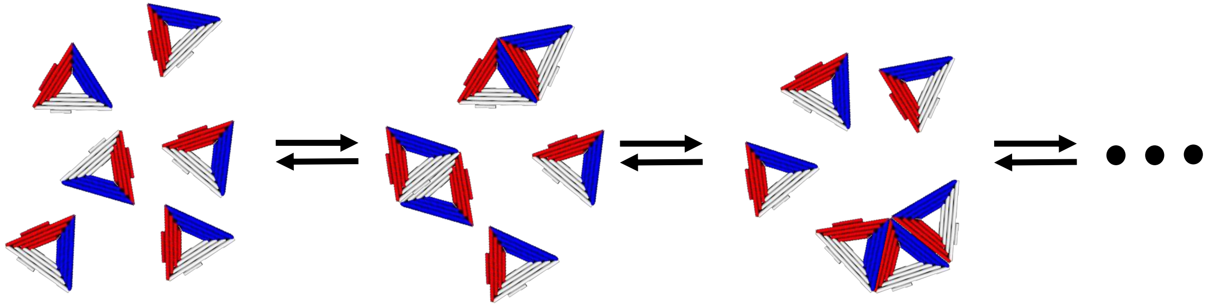


early assembly stage



final assembly stage



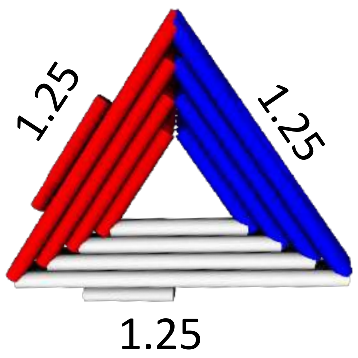


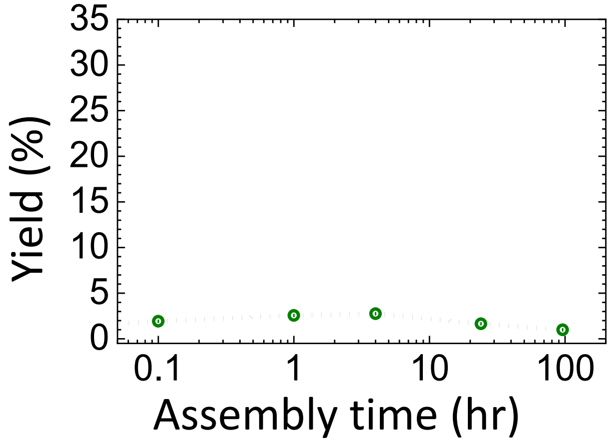
Bonds are too weak.
Nuclei don’t form.
Majority subunits remain as monomers.

Equalitarian assembly (T=3)

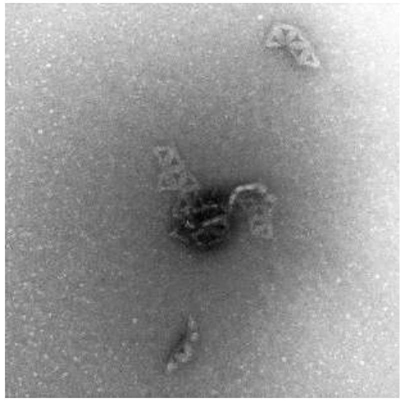
Strong binding – kinetic traps

Equal-binding
(-21.7 k_BT)

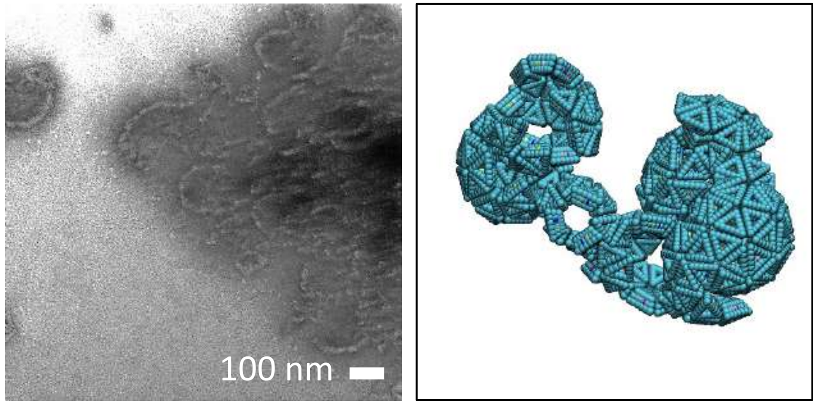




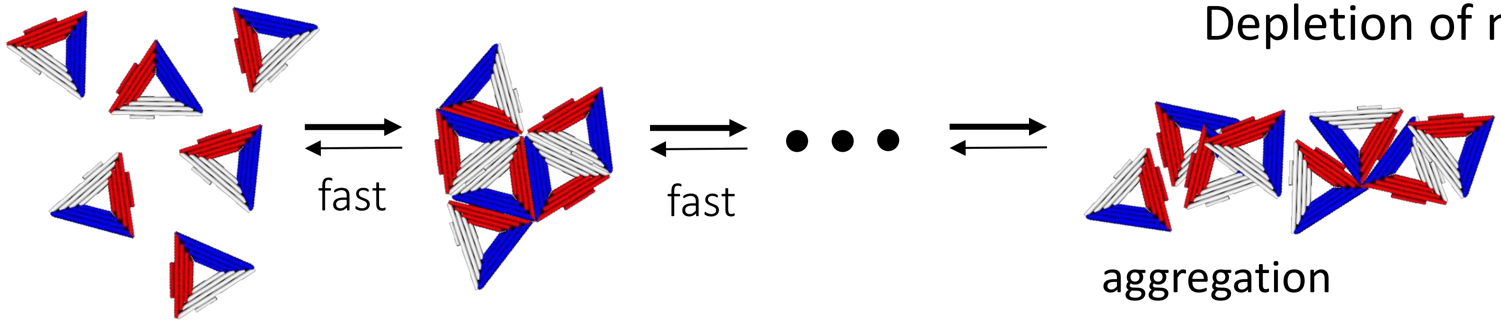
early assembly stage



final assembly stage



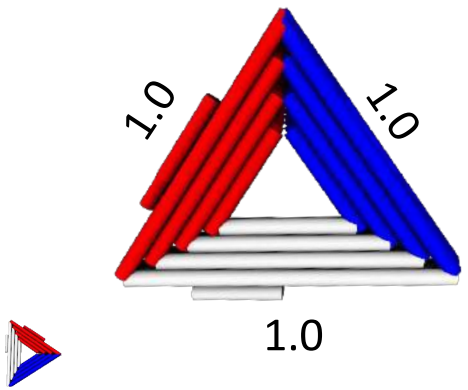
Bonds are too strong.
Subunits rapidly assemble into intermediate species.
Depletion of monomers and low off-rate forms kinetic traps.

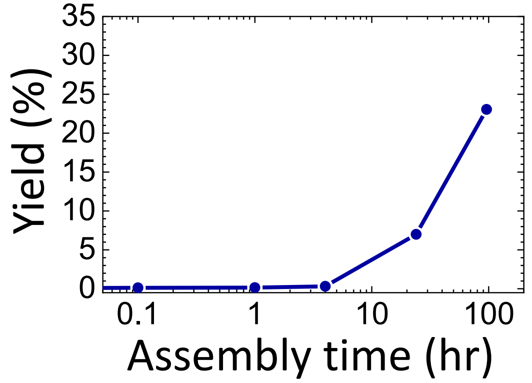


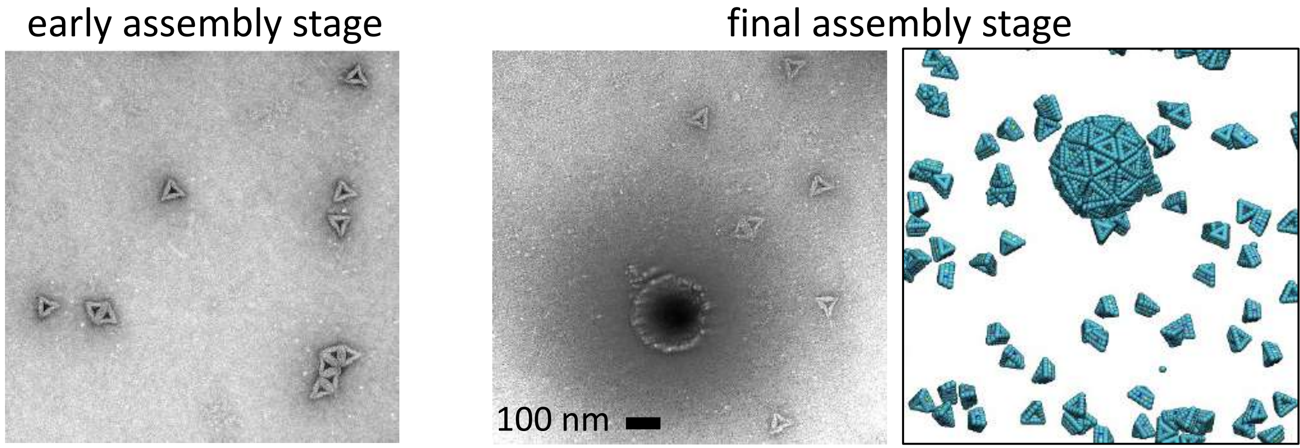
Equalitarian assembly (T=3)

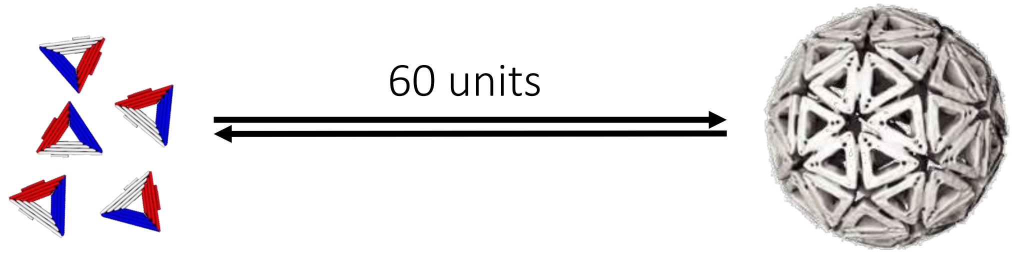
Intermediate binding

Equal-binding
($-17.4\ k_BT$)







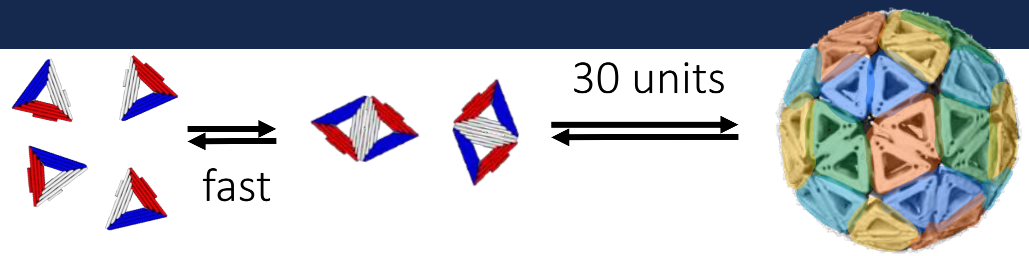


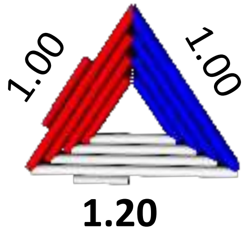
Intermediate species form after a-few-hour-long lag phase.
Quick consumption of free subunits.
Monomer – capsid equilibrium is consistent with a nucleation and growth mechanism.

Goldilocks!

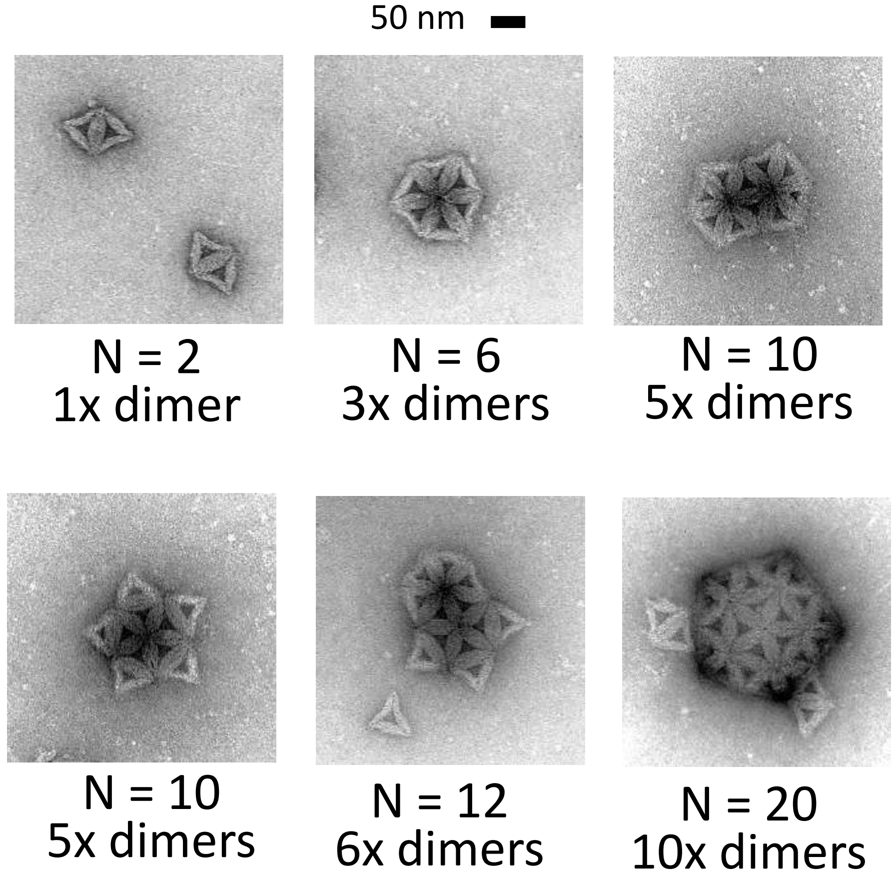
Dimer-bias (T=3)

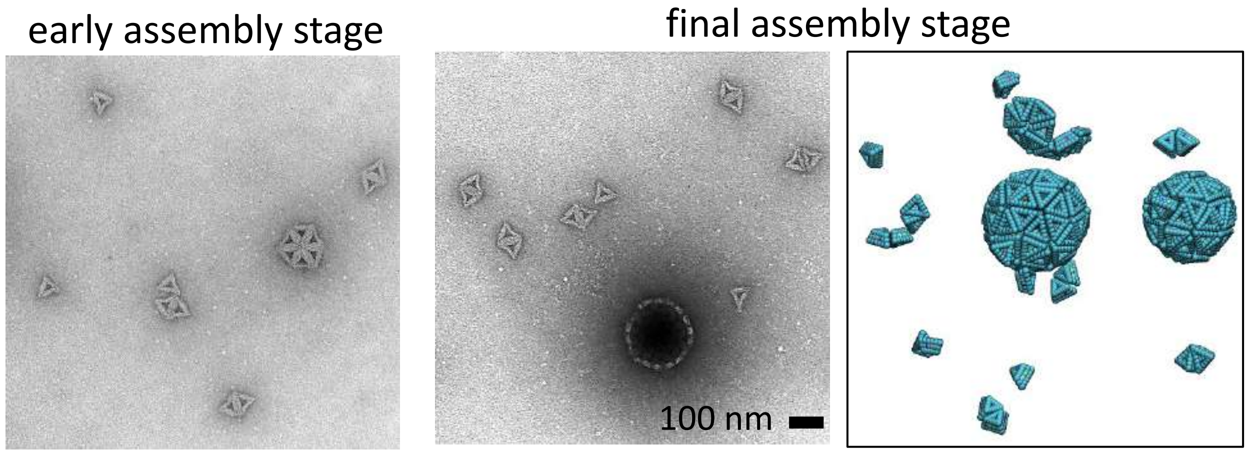
Hierarchical assembly pathways

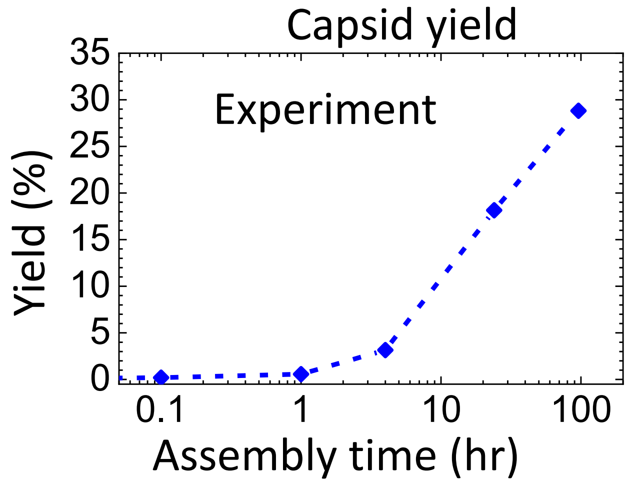


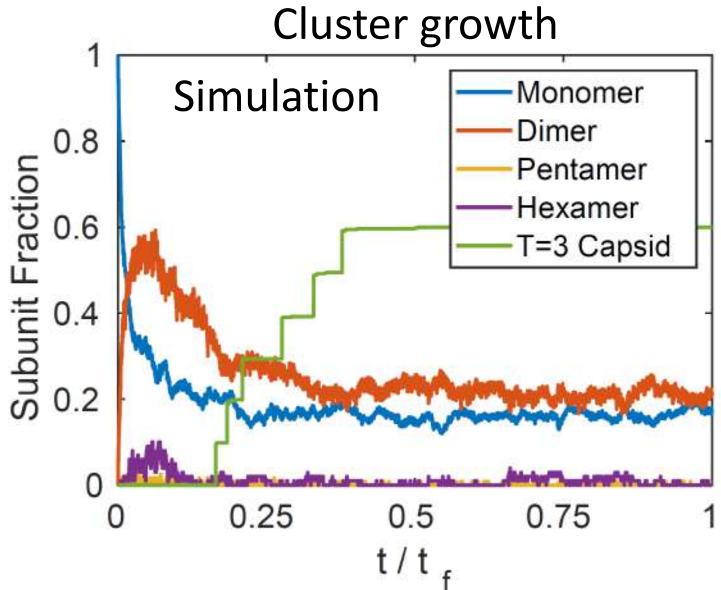


Dimer-bias
(by 3.7 k_BT)



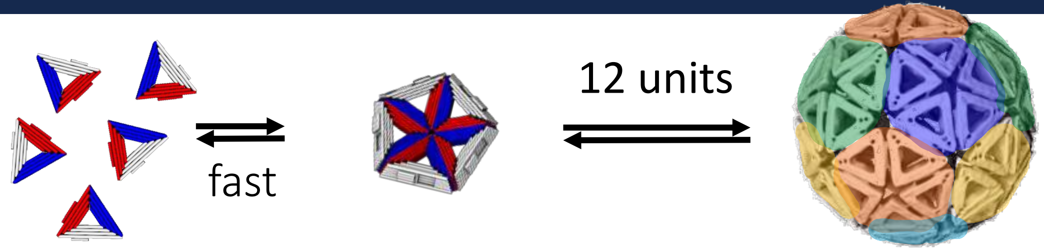


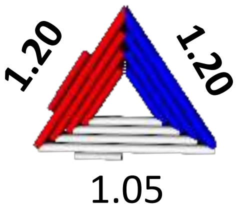




Pentamer bias T=3 assembly

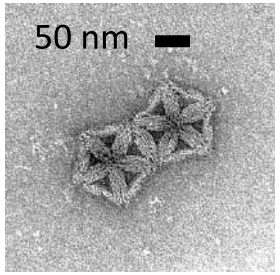
Hierarchical assembly pathways



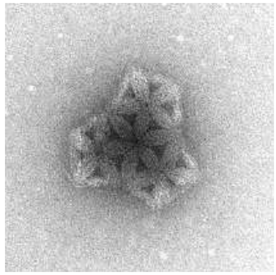


Pentamer bias
(by 3.0 k_BT)

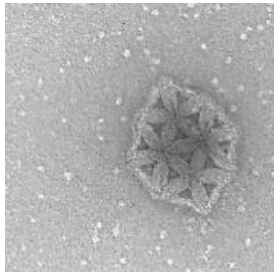
N = 5
1x pentamer



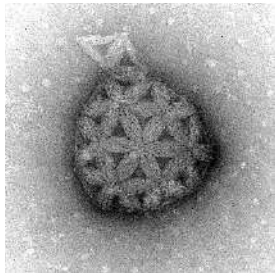
N = 10
2x pentamers



N = 15
3x pentamers

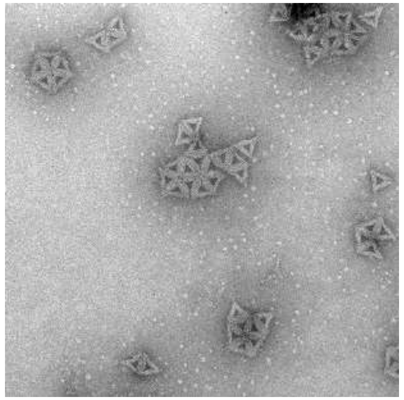


N = 14
2x P + 2x D

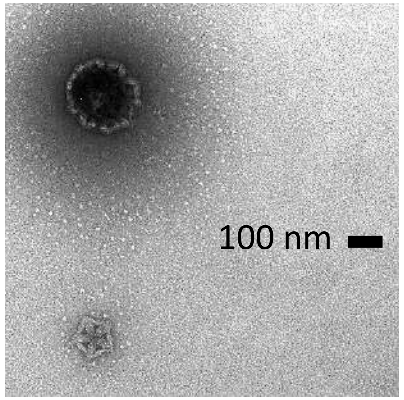


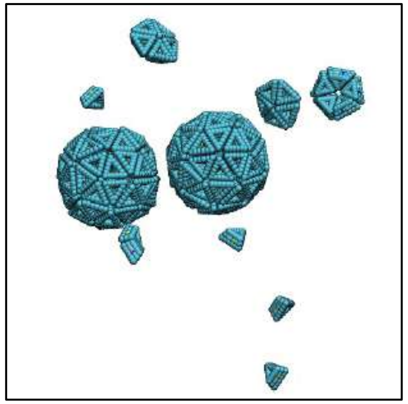
N = 21
3x P + 3x D

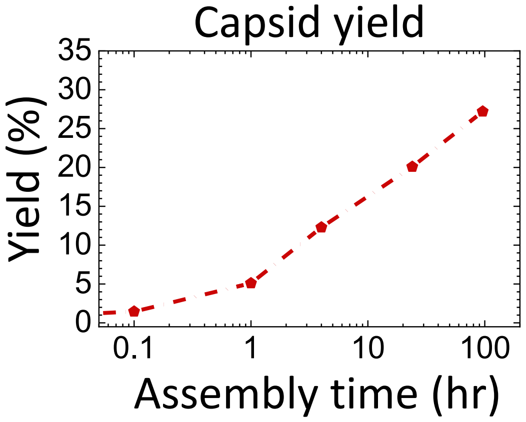
early assembly stage

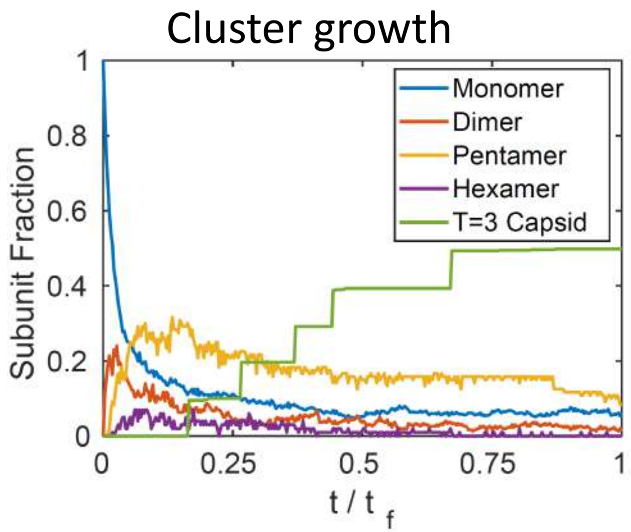


final assembly stage





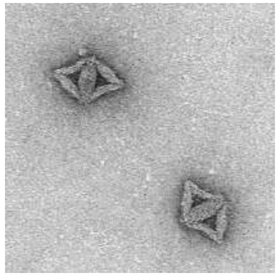




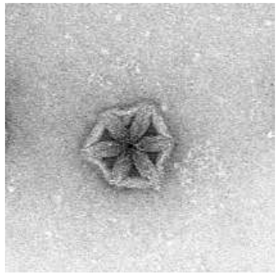
Funneling free energy landscape

Experiment

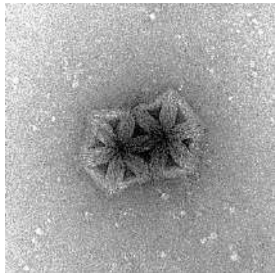
Dimer-bias (by 3.7 k_BT)



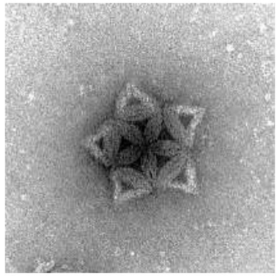
N = 2
1x dimer



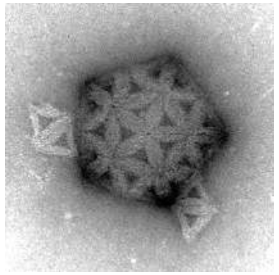
N = 6
3x dimers



N = 10
5x dimers



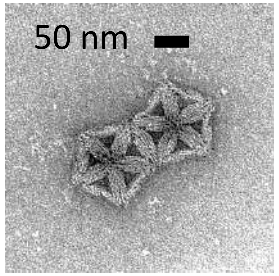
N = 10
5x dimers



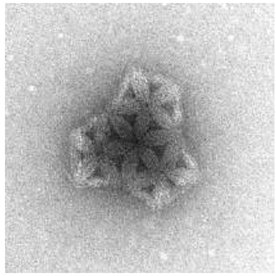
N = 20
10x dimers

Pentamer-bias (by 3.0 k_BT)

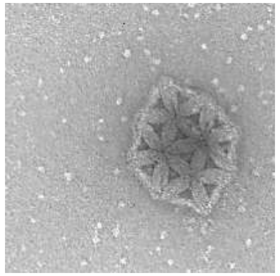
N = 5
1x pentamer



N = 10
2x pentamers



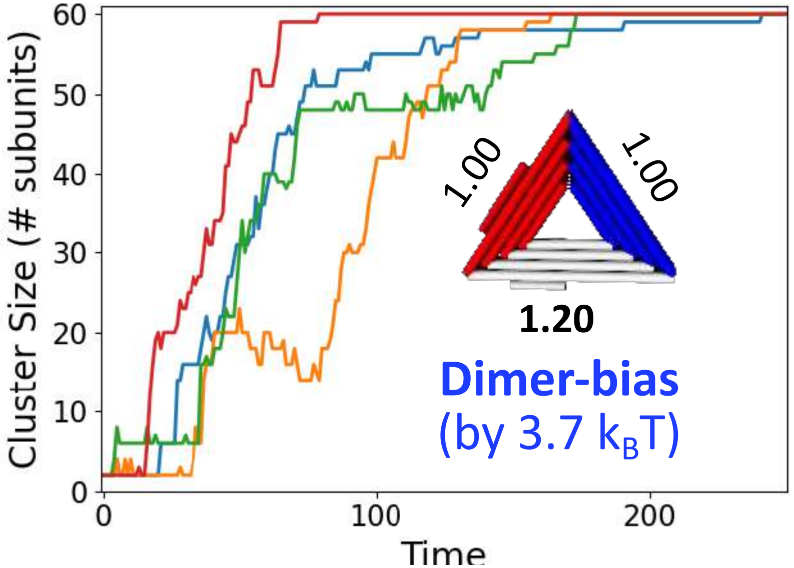
N = 15
3x pentamers

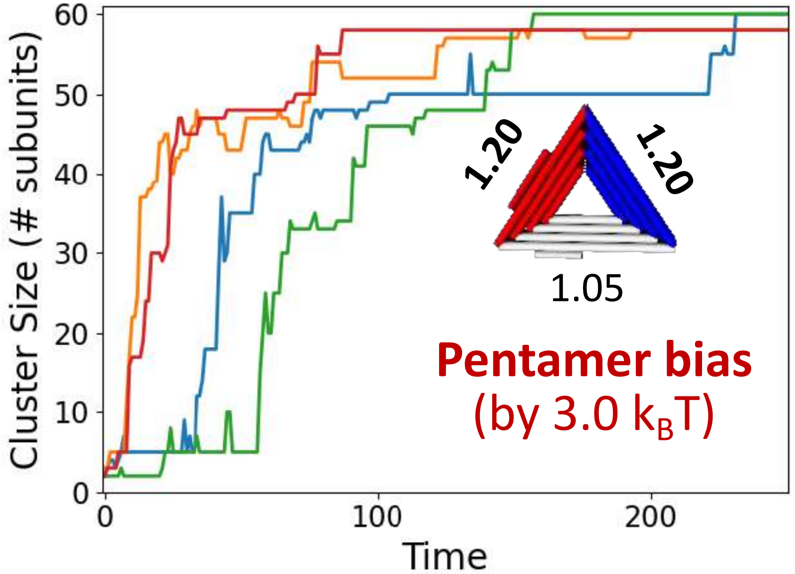


N = 14
2x P + 2x D

N = 21
3x P + 3x D

Simulation: Individual trajectories

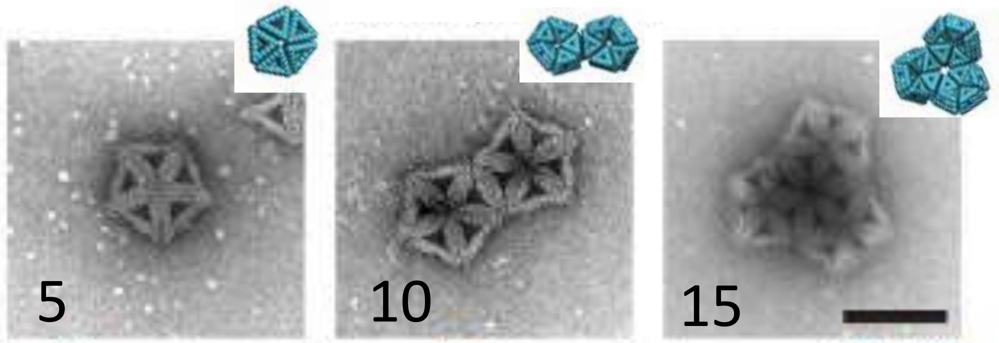




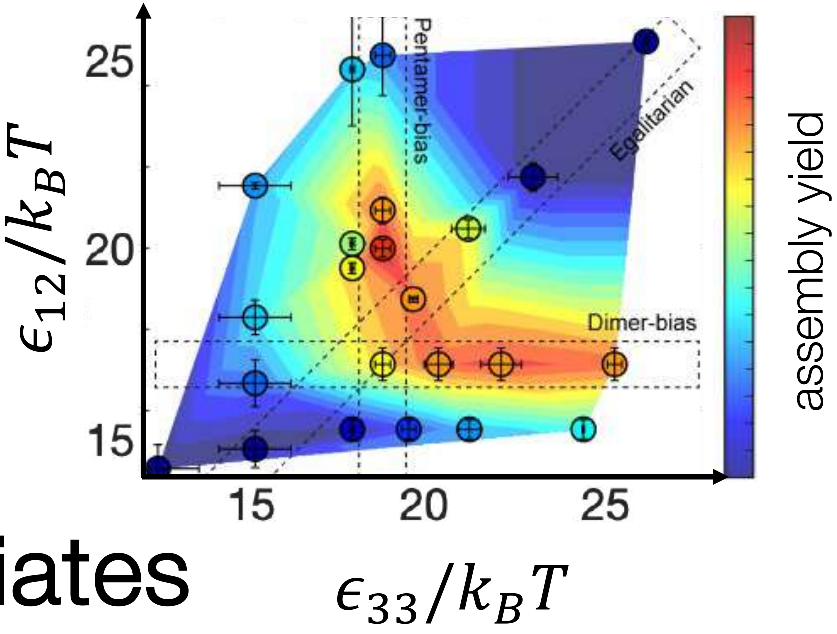
Hierarchical assembly is more robust than egalitarian assembly

Experiments confirm predictions

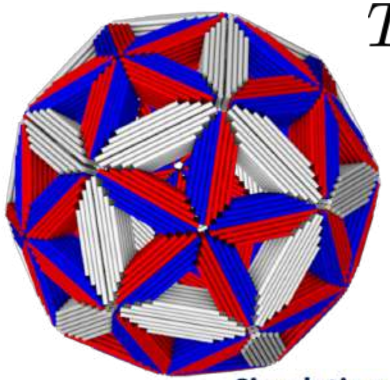
pentamer pathway intermediates



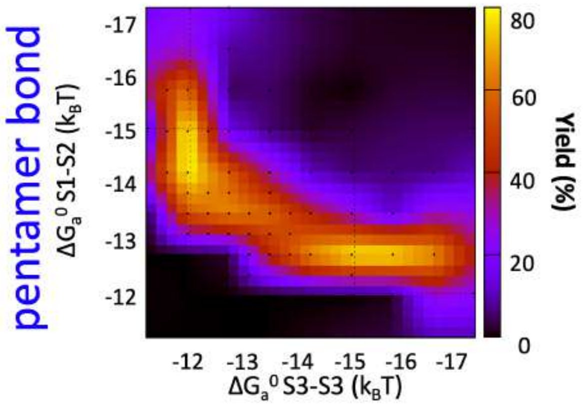
Experiment



$T = 3$

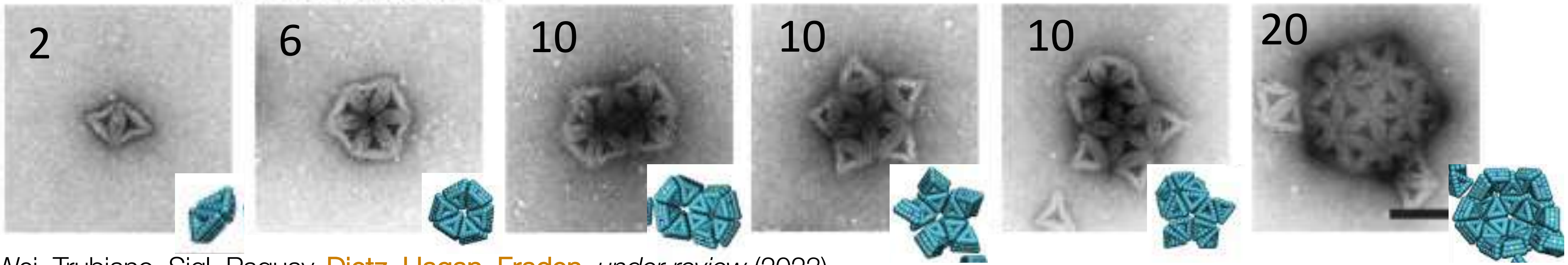


Simulation



dimer bond energy

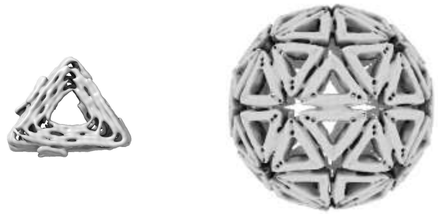
dimer pathway intermediates



Wei, Trubiano, Sigl, Paquay, Dietz, Hagan, Fraden, *under review* (2023)

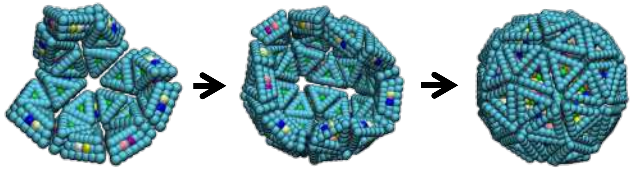
Summary

- **You can do anything with DNA origami. Build subunits with programmable bond energy, direction, valency and addressability, for studying self-assembly pathways, kinetics, and yield.**

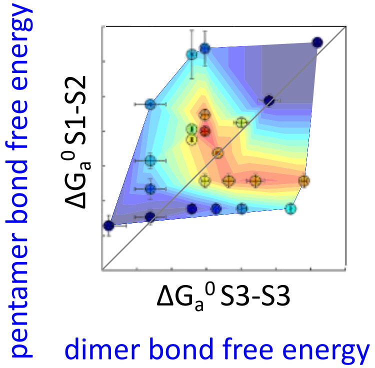


- Construct building blocks with designed structures in 3D to sub-nm accuracy and bond strengths controlled to k_BT precision, quantified *in situ* by cryo-EM and static light scattering.

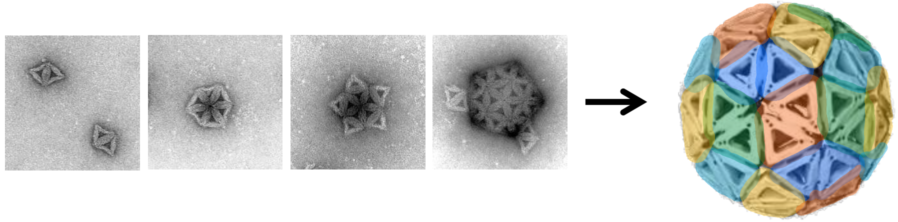
- Coarse-grained representation of subunits, short-ranged potentials, and Langevin dynamics.

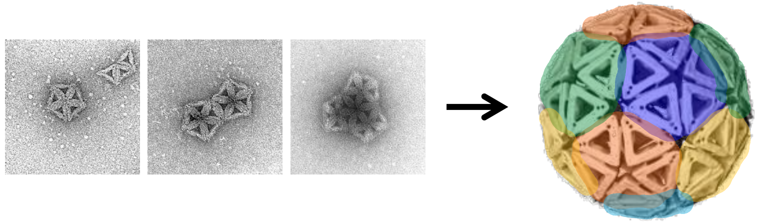


- **Hierarchical assembly is robust and has high yield.**



- Avoids kinetic traps by funneling intermediate assemblies towards the designed final target by reducing number of intermediates and excluding off-path structures.





Hierarchical assembly is more robust than egalitarian assembly in synthetic capsids
https://arxiv.org/abs/2310.18790

Economical routes to size-specific assembly of self-closing structures
https://arxiv.org/abs/2311.01383