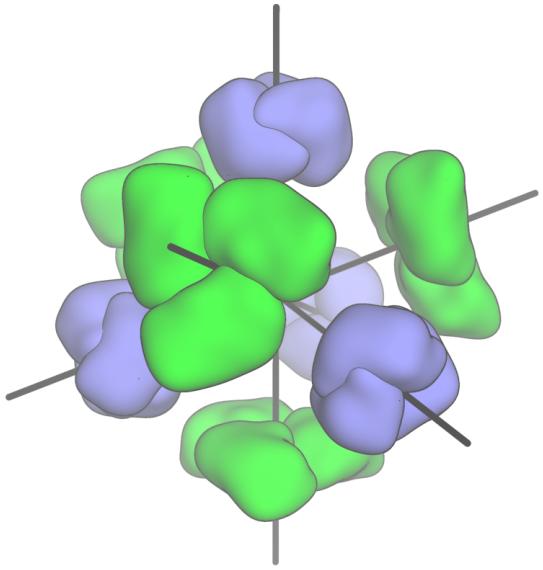
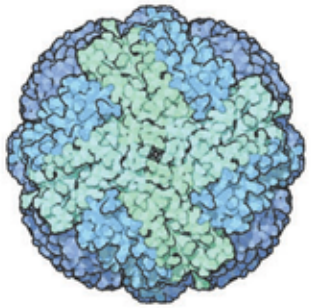
Design of self-assembling two-component protein nanomaterials



Jacob Bale
Baker Lab, UW
RosettaCon
July 29th, 2013

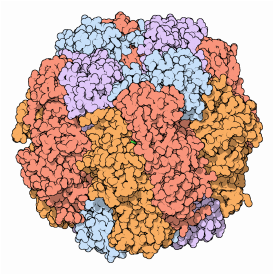
Self-assembling proteins have evolved to perform many extraordinary functions

Storage containers

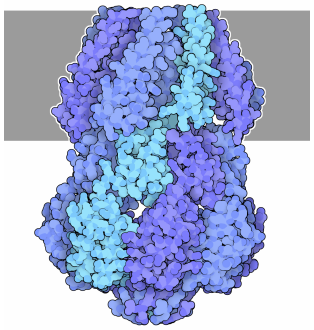


1hrs Ferritin

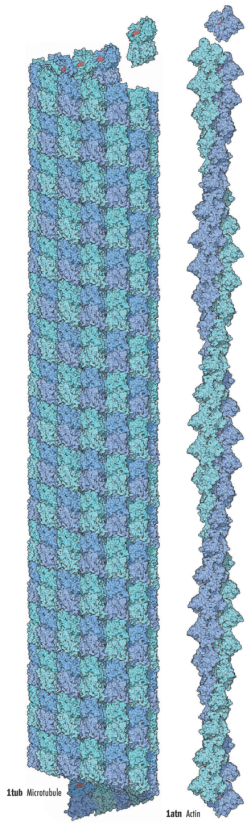
Enzymes



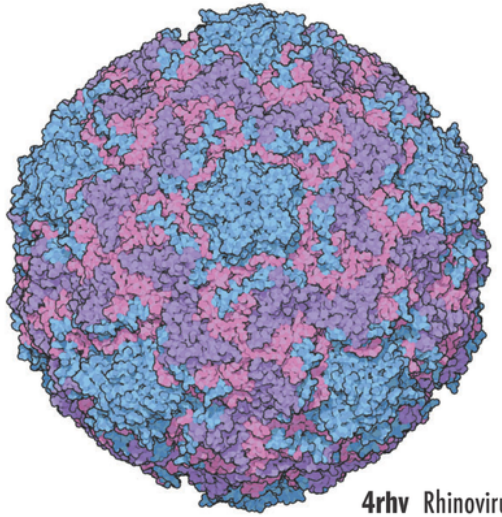
Signal Transducers



Cellular scaffolding, tracks for transport and motility

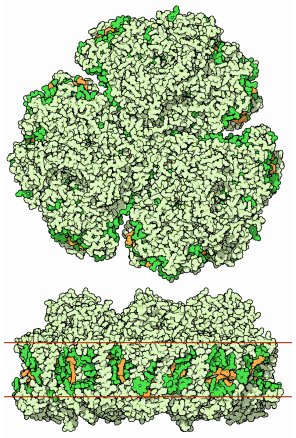


Delivery vehicles

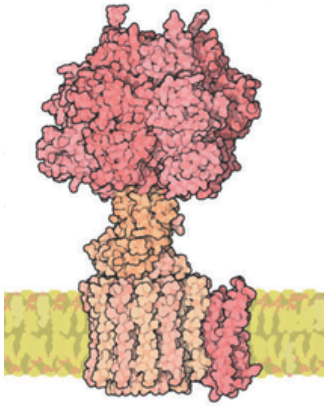


4rhv Rhinovirus

Energy Converters



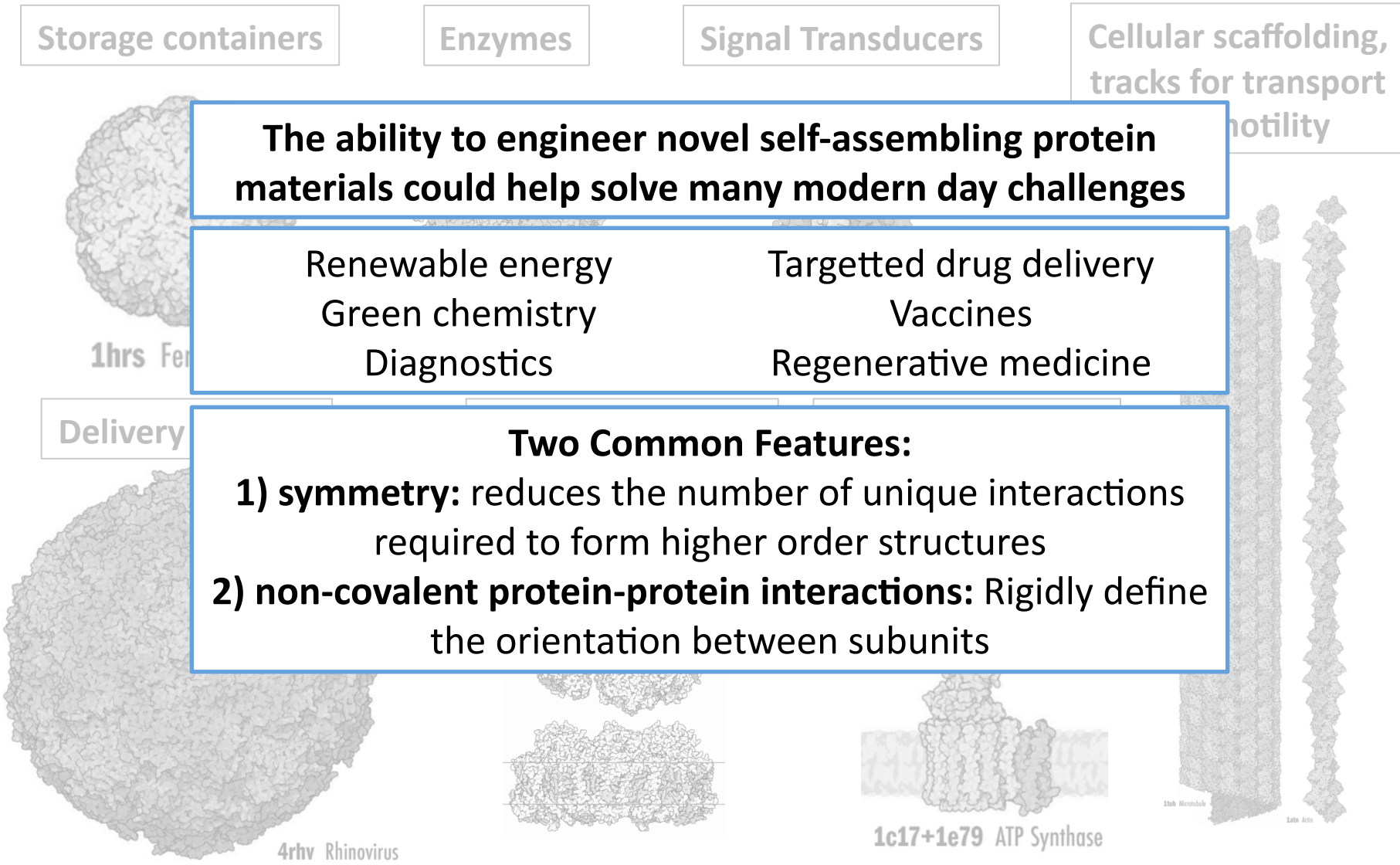
Molecular motors



1c17+1e79 ATP Synthase

http://pdbbeta.rcsb.org/pdb/education_discussion/molecule_of_the_month/poster_quickref.pdf

Self-assembling proteins have evolved to perform many extraordinary functions



The ability to engineer novel self-assembling protein materials could help solve many modern day challenges

Renewable energy	Targetted drug delivery
Green chemistry	Vaccines
Diagnostics	Regenerative medicine

Two Common Features:

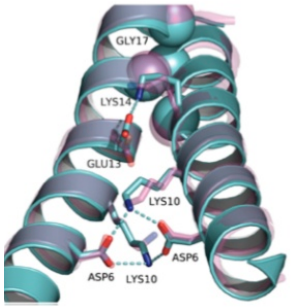
1) symmetry: reduces the number of unique interactions required to form higher order structures

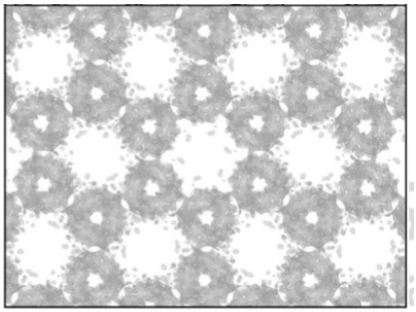
2) non-covalent protein-protein interactions: Rigidly define the orientation between subunits

http://pdbbeta.rcsb.org/pdb/education_discussion/molecule_of_the_month/poster_quickref.pdf

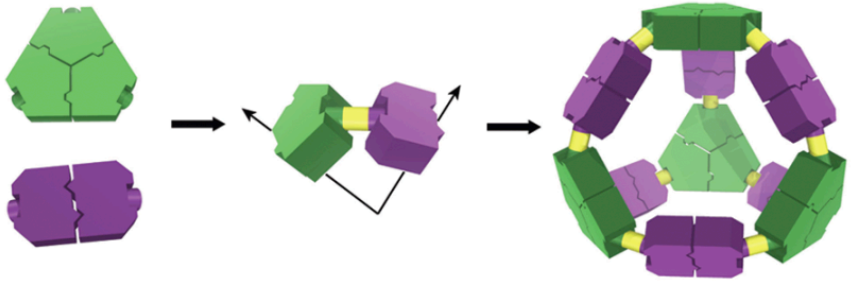
Many approaches have been taken to engineer novel self-assembling protein structures

Coiled-coil assembly

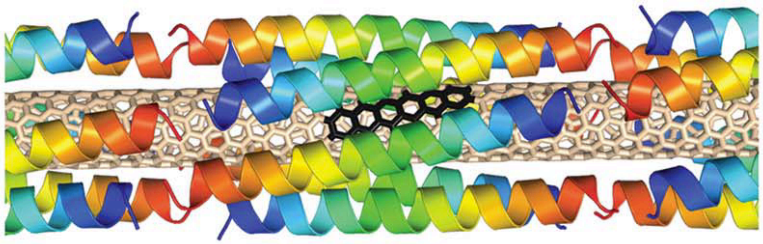




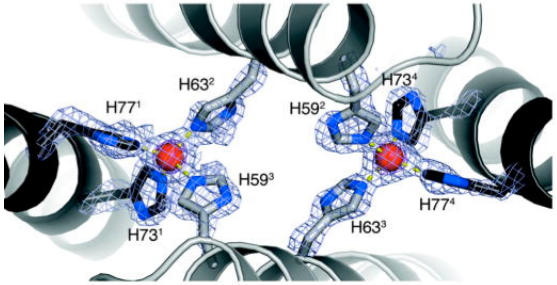
Fusion of naturally self-assembling domains

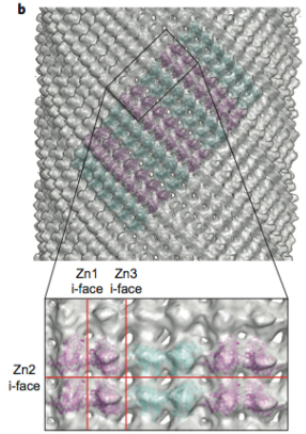


Nanomaterial-templated assembly



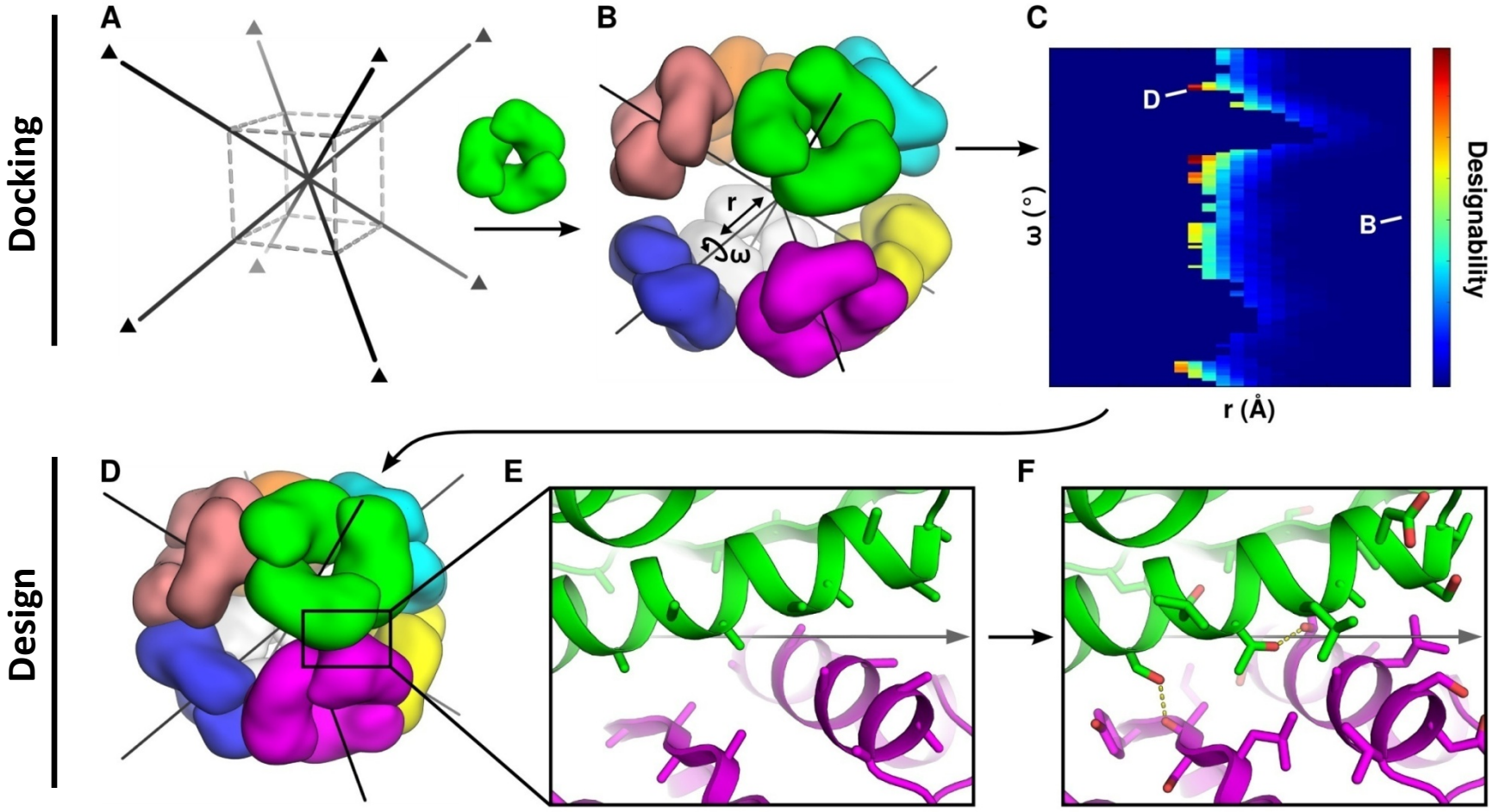
Metal-Directed Assembly





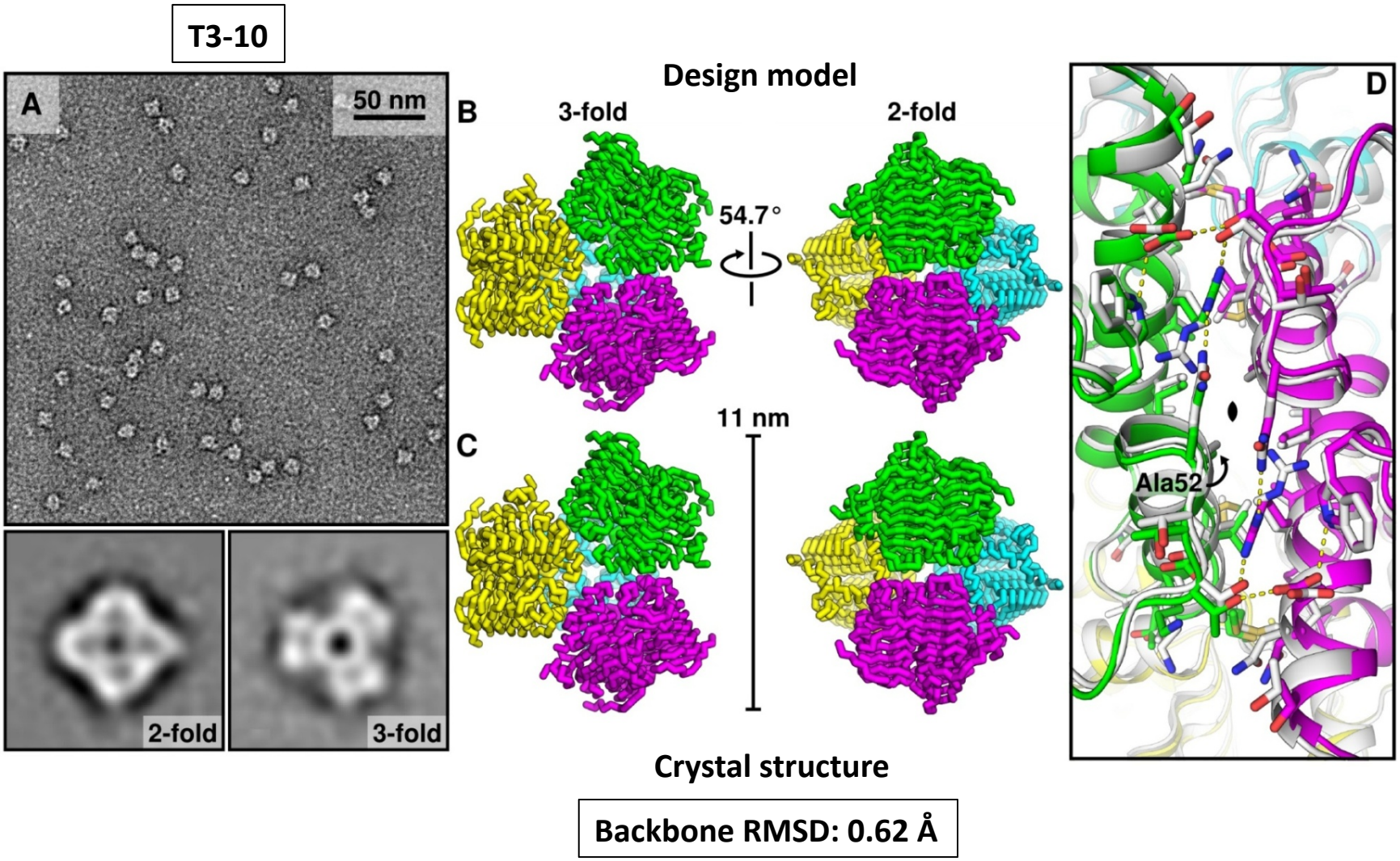
Lanci CJ, et al. (2012) *PNAS* **109**:7304-9.
Lai YT, et al. (2012) *Science* **336**:1129.
Grigoryan G, et al. (2011) *Science* **332**:1071-6.
Brodin JD, et al. (2012) *Nat. Chem.* **4**:375-82.

We developed a general method for designing assemblies composed of multiple copies of a single protein building block



King NP, et al. (2012) *Science* **336**:1171-4.

Our method was validated by designing two novel protein cages with atomic-level accuracy



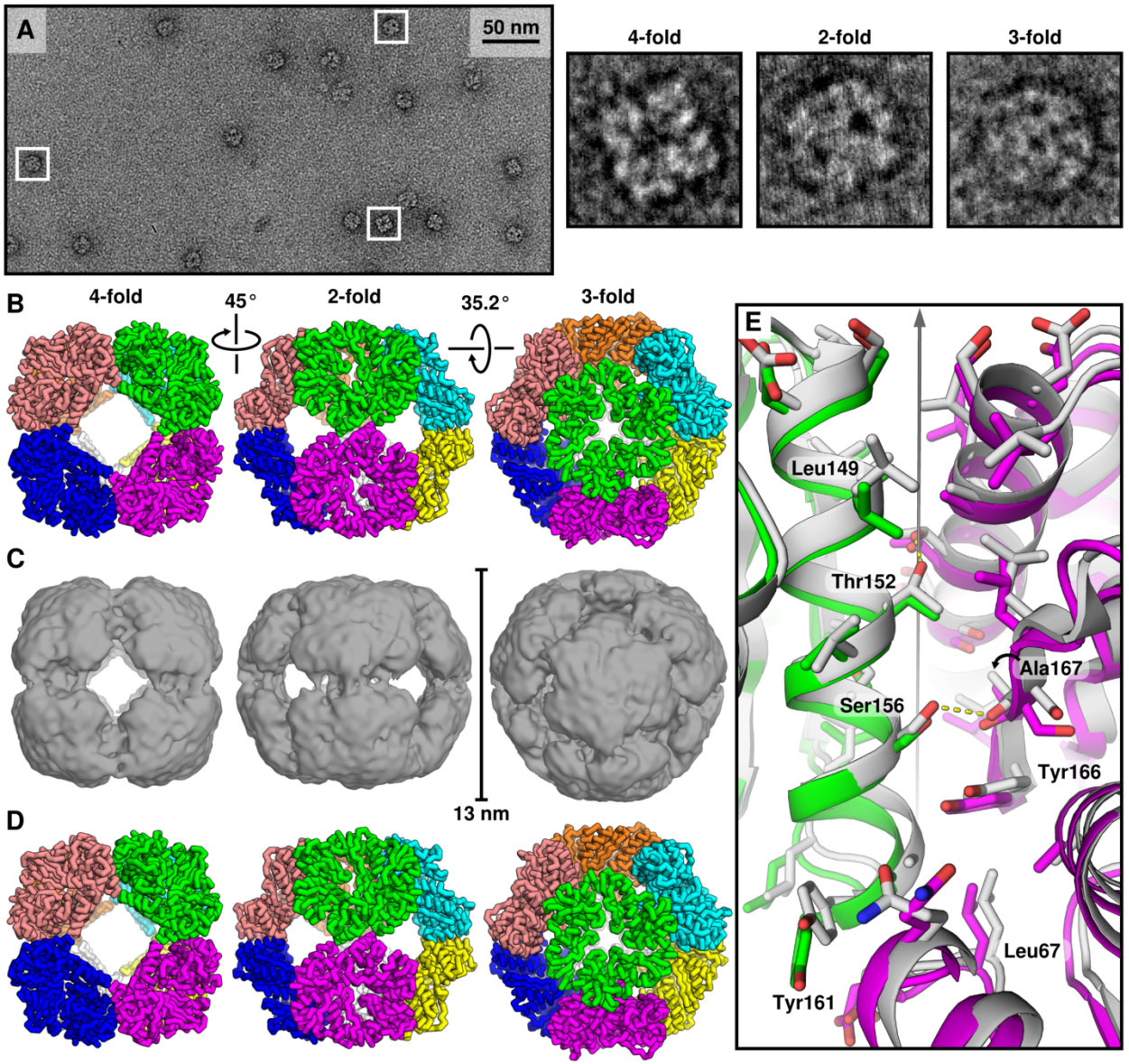
Backbone RMSD: 0.62 Å

EM: Iadanza/Gonen (Janelia)
Xtal: Sawaya/Yeates (UCLA)

King NP, et al. (2012) *Science* **336**:1171-4.

Our method was validated by designing two novel protein cages with atomic-level accuracy

O3-33



Design model

Backbone RMSD: 1.07 Å

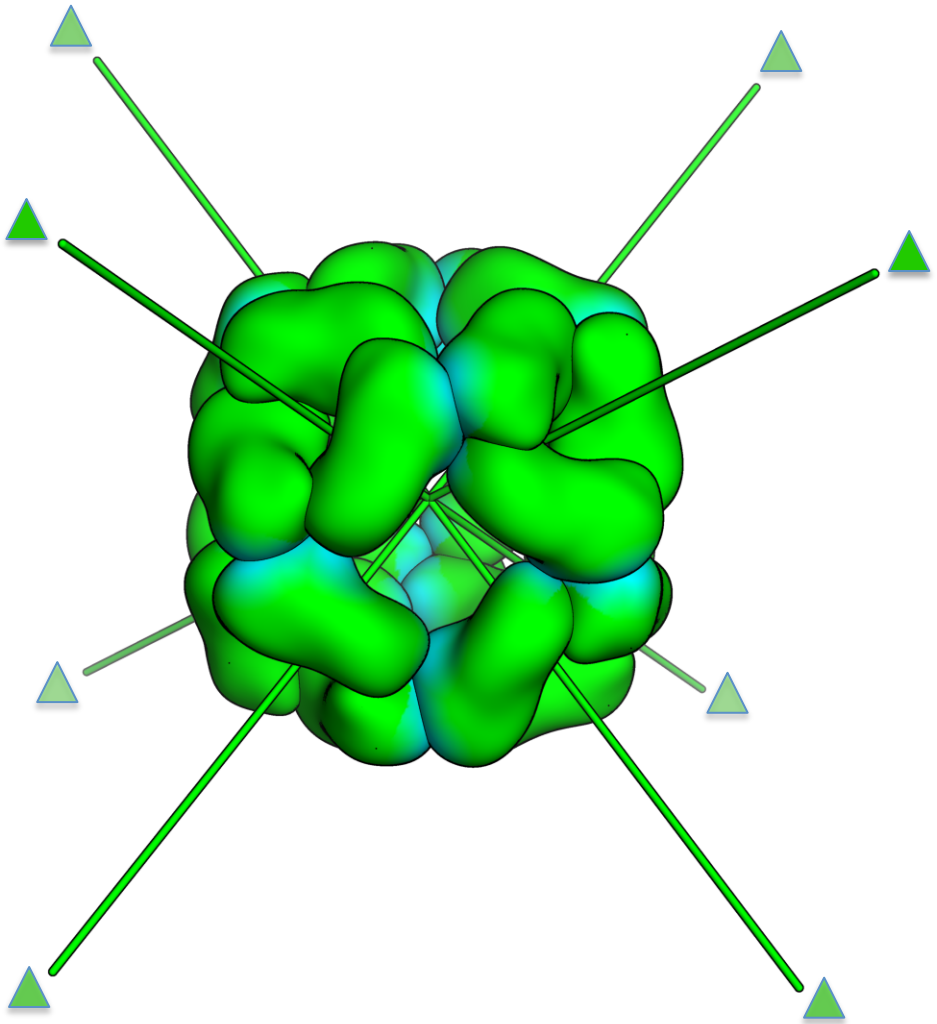
Crystal structure

EM & Xtal:
Vollmar/Gonen (Janelia)
Sawaya/Yeates (UCLA)

King NP, et al. (2012) *Science* **336**:1171-4.

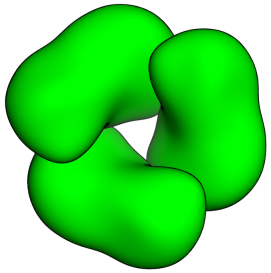
Our approach could be extended to incorporate multiple distinct protein building blocks

<u>One-Component Octahedron</u>



- One scaffold
  - C3 homotrimer

8 x



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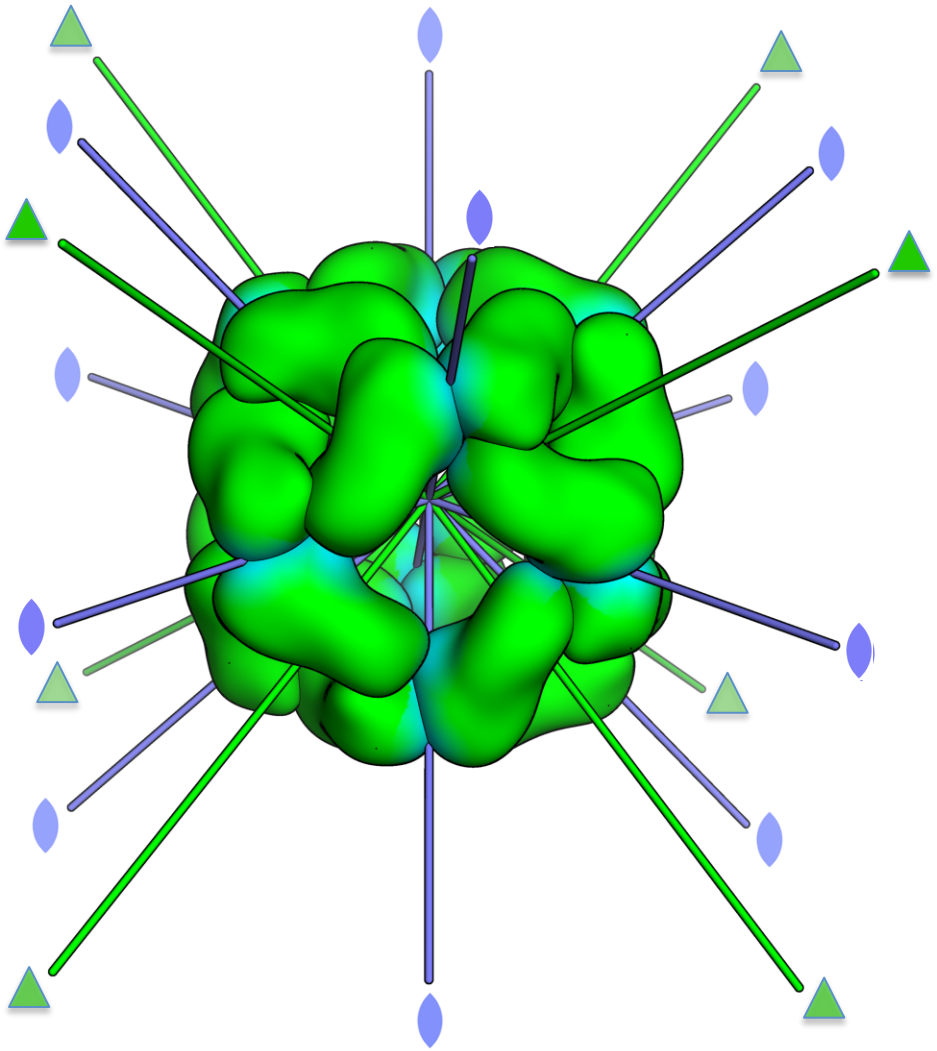
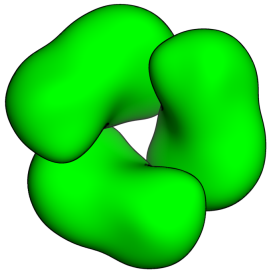
One-Component Octahedron



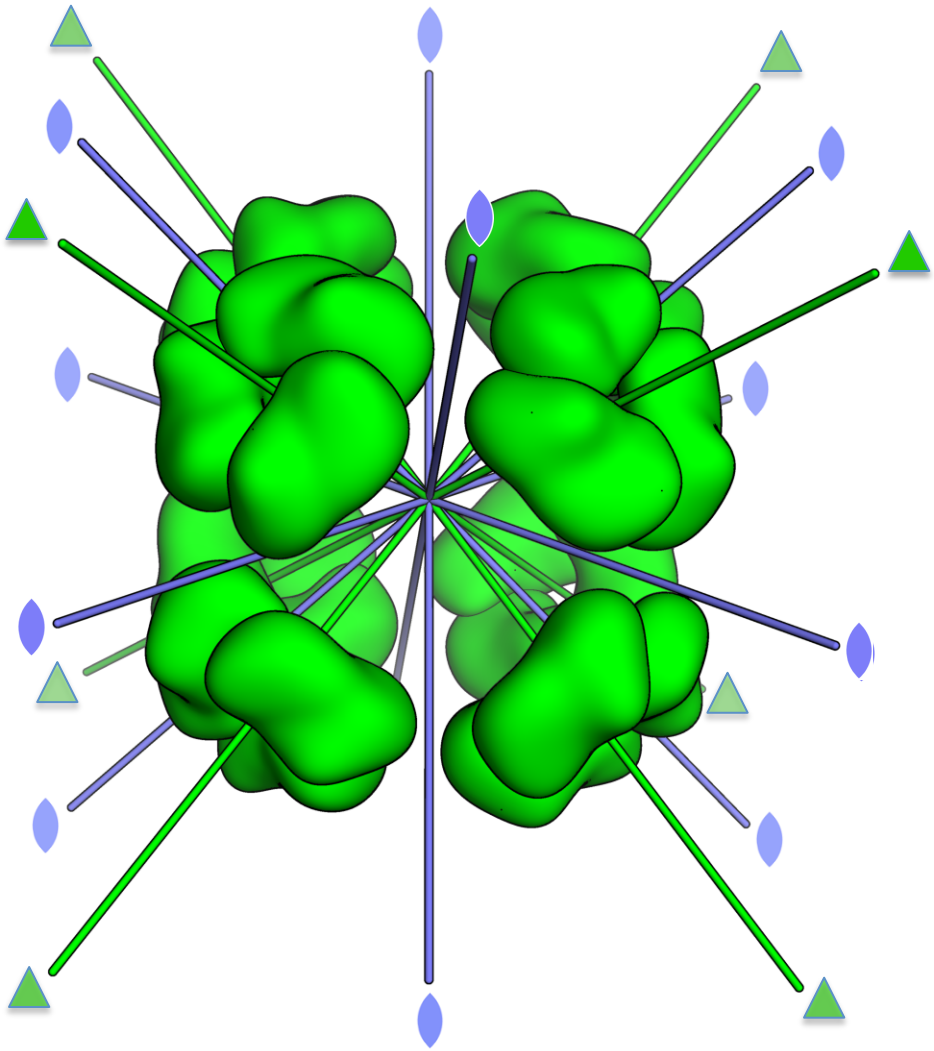
- One scaffold
  - C3 homotrimer

8 x 

- One designed interface
  - 2-fold symmetric

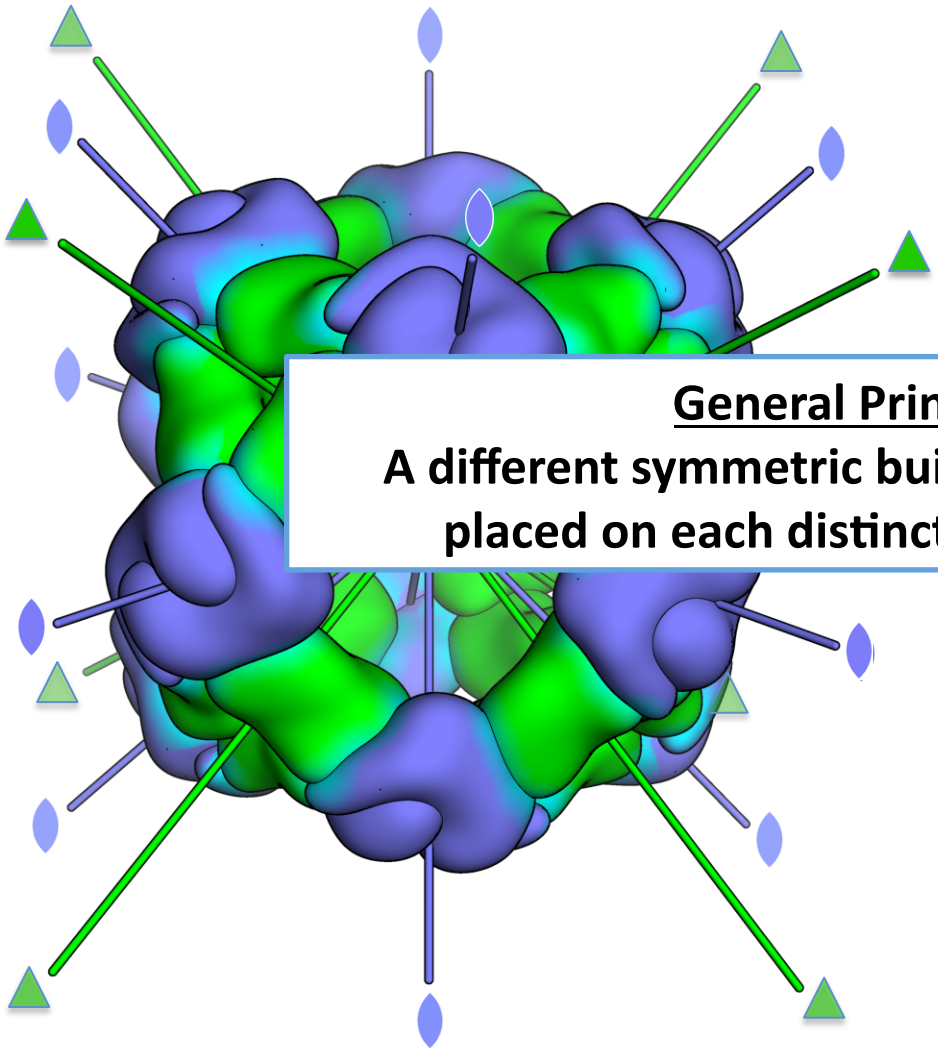
Our approach could be extended to incorporate multiple distinct protein building blocks

One-Component Octahedron

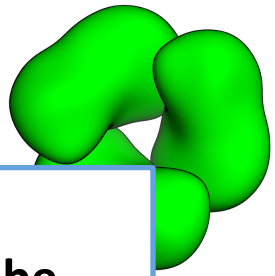


Our approach could be extended to incorporate multiple distinct protein building blocks

Two-Component Octahedron

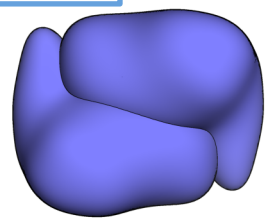


- Two scaffolds
  - C3 homotrimer



8 x

dimer



12 x

- One designed interface
  - Heterologous

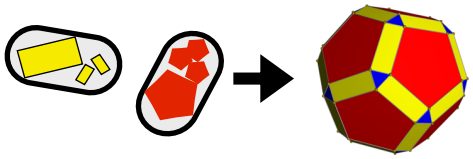
General Principle

A different symmetric building block can be placed on each distinct symmetry axis

Two-component nanomaterials offer many advantages over one-component systems

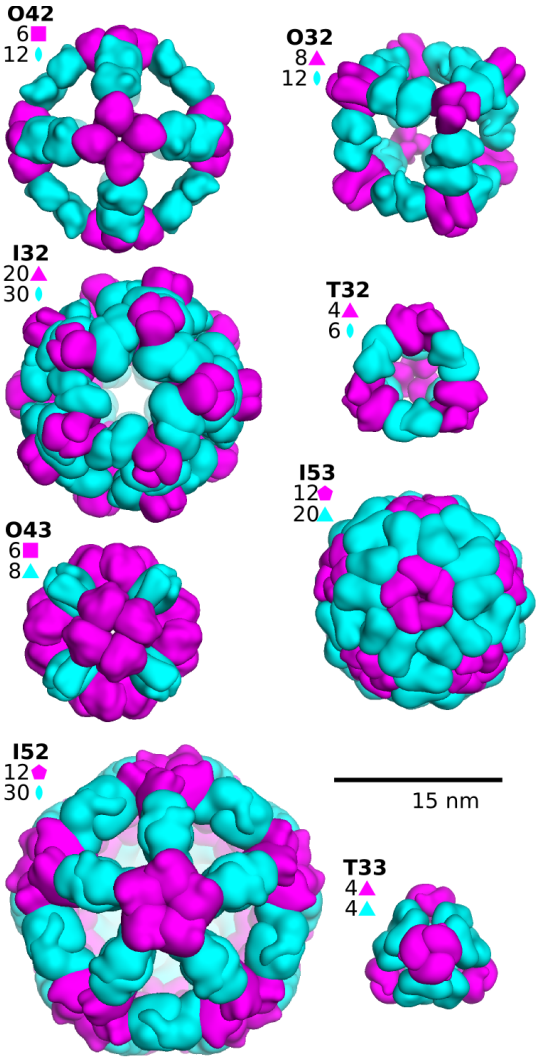
- Many more potential materials due to the many combinations of building blocks
  - 542 one-component cages docked
  - 992,824 two-component cages docked
- Each component could be independently functionalized and characterized
- Initiation of assembly could be controlled by mixing independently purified building blocks

Controlled Assembly

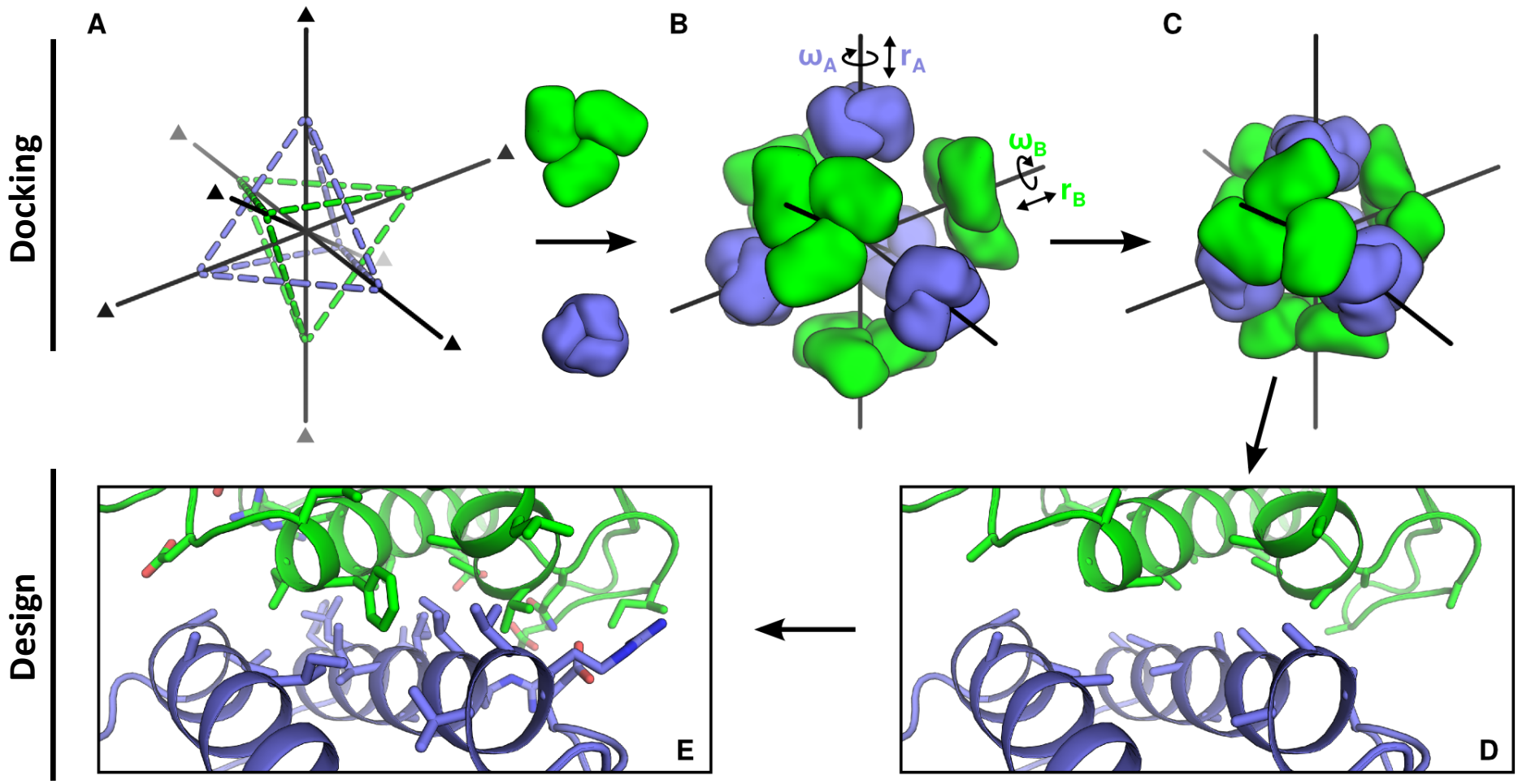


Combinatorial Functionalities

Structural			Structural
Catalytic			Catalytic
Recognition	x		Recognition
Photoactive			Photoactive
Electronic			Electronic

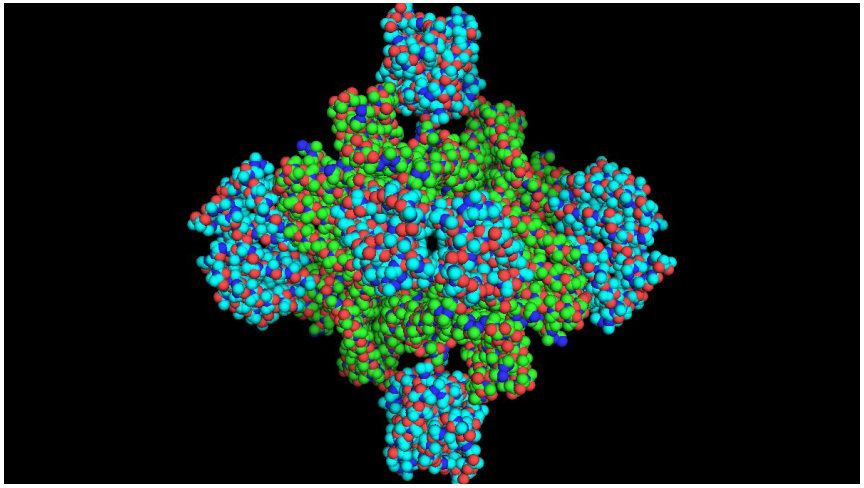


We have now developed a general method for the design of two-component symmetric protein assemblies



New docking code developed to sample additonal degrees of freedom and provide improved scoring metrics

- Enumeratively sample rigid body DOFs: rotation of each component about its symmetry axis and radial displacement along its symmetry axis
  - Search space reduced by sliding components into contact



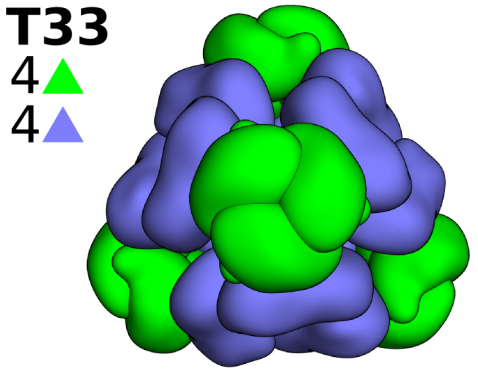
- Score each configuration for “designability”:
  - Sum of Cβ-Cβ contacts within 10 Å, weighted by secondary structure and average degree
  - Normalized by number of residues at interface

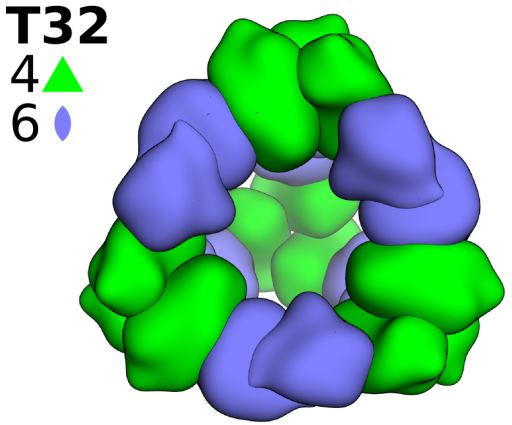
RosettaScripts movers, filters, and task operations developed/modified for two-component design and optimization

- Filter top-docked configurations by number of residues at interface
- Design (nstruct=50-100):
  - Randomly perturb 4 RB DOFs
  - Clash check filter
  - Fixbb soft rep design (reduced AA set + natives), hard min (chi + rb)
  - SASA filter
  - Fixxbb hard rep design (reduced AA set + natives), hard min (chi + rb)
  - Filter by shape complementarity, ddG, interface size, number of mutations, and number of buried unsatisfied polars
- Optimize shape complementarity using the GreedyOptMover
- Perform automated reversion to native using the GreedyOptMover
- Manually inspect and guide resfile based redesign

We chose T32 and T33 as our first targets and selected 60 designs for experimental characterization

- 252,100 pairs of building blocks were docked
- 1000 docked configurations for each architecture were sent through design
- Designs were filtered on ddG, sc, interface size, buried polars, etc.
- Greedy optimization of sc and governed aggressive reversion improved designs and reduced manual refinement

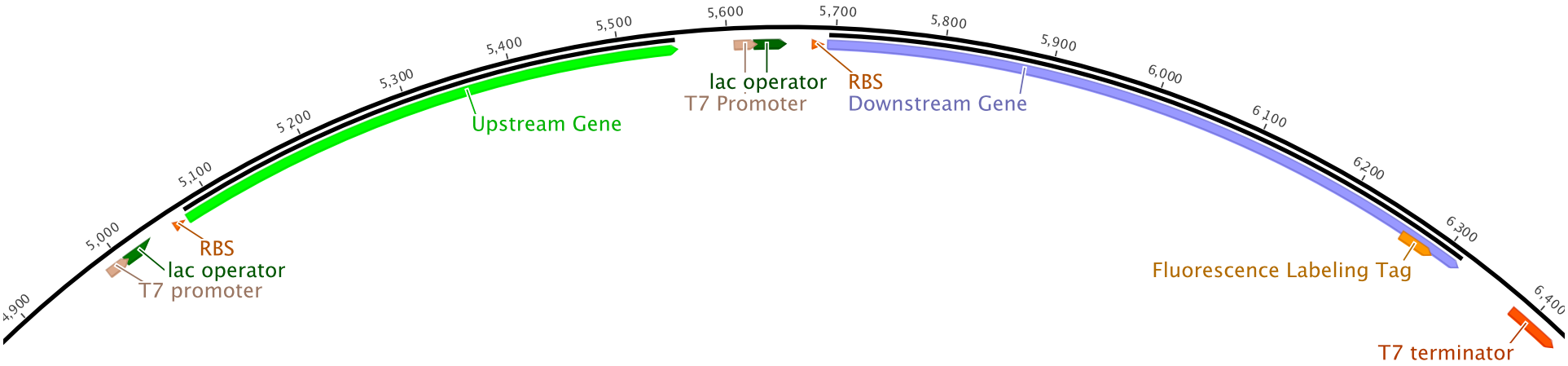




T32: Results from optimization

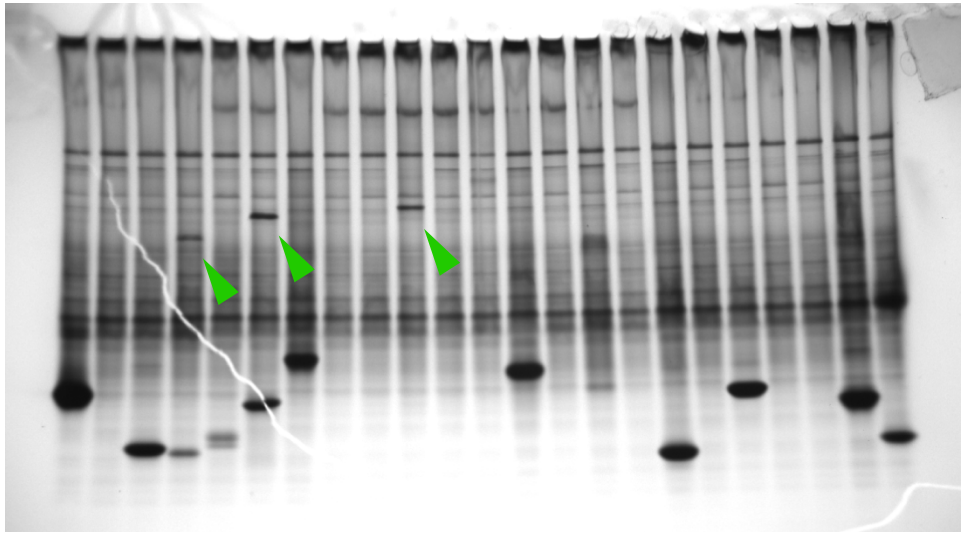
	Δ ddG	Δ uhb	Δ sc	Δ mutations
sc opt.	-1.326	-0.401	0.047	-2.75
Reversion	1.197	-0.005	-0.005	-7.7382

Designs were co-expressed in *E. coli* and analyzed for self-assembly to the intended architectures

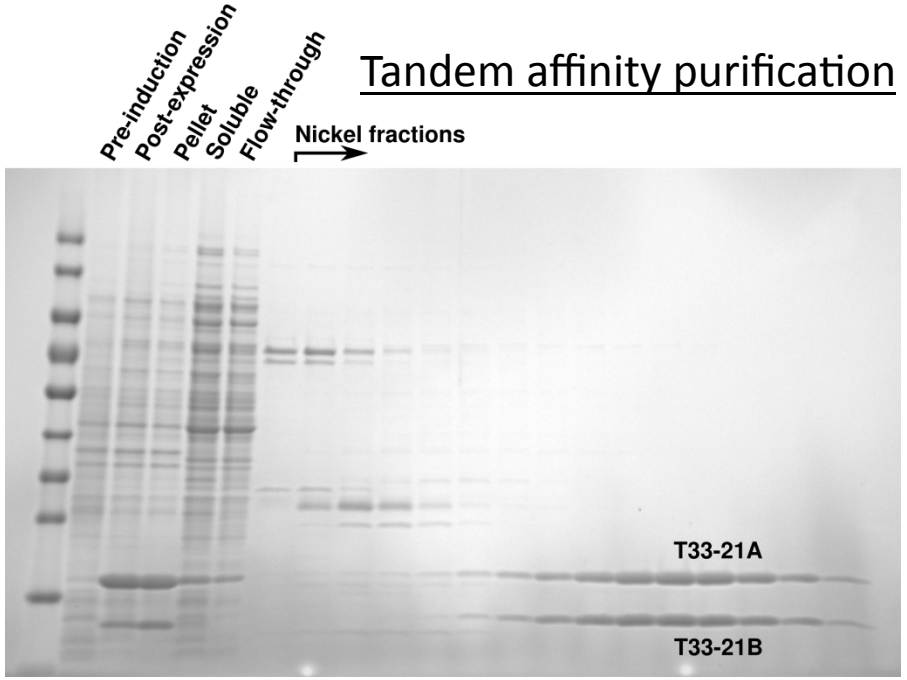


Designs were co-expressed in *E. coli* and analyzed for self-assembly to the intended architectures

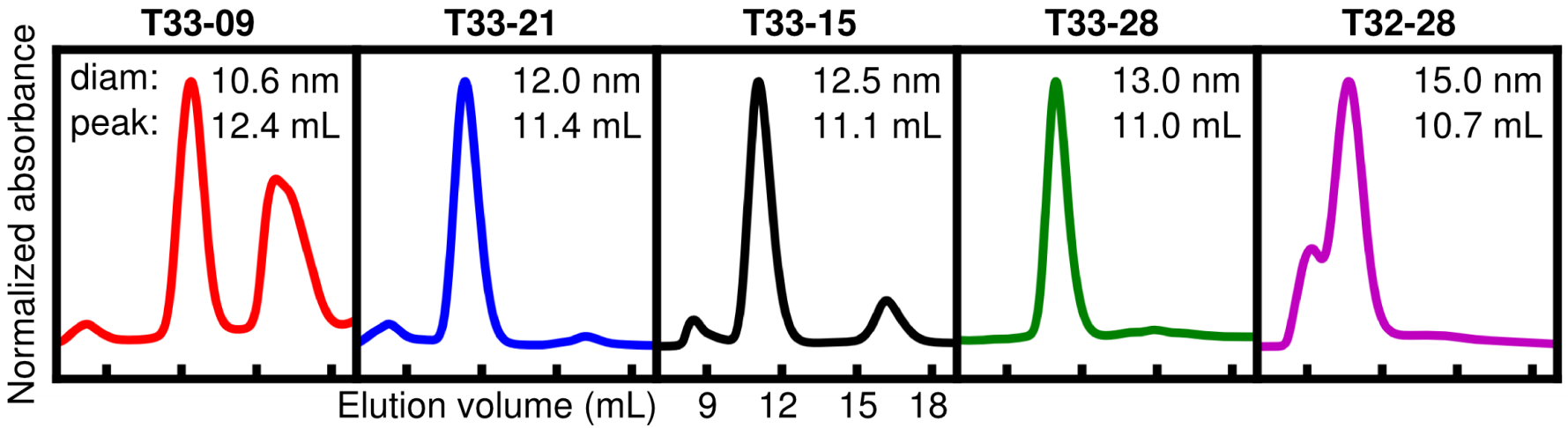
Native PAGE of cell lysates



Tandem affinity purification

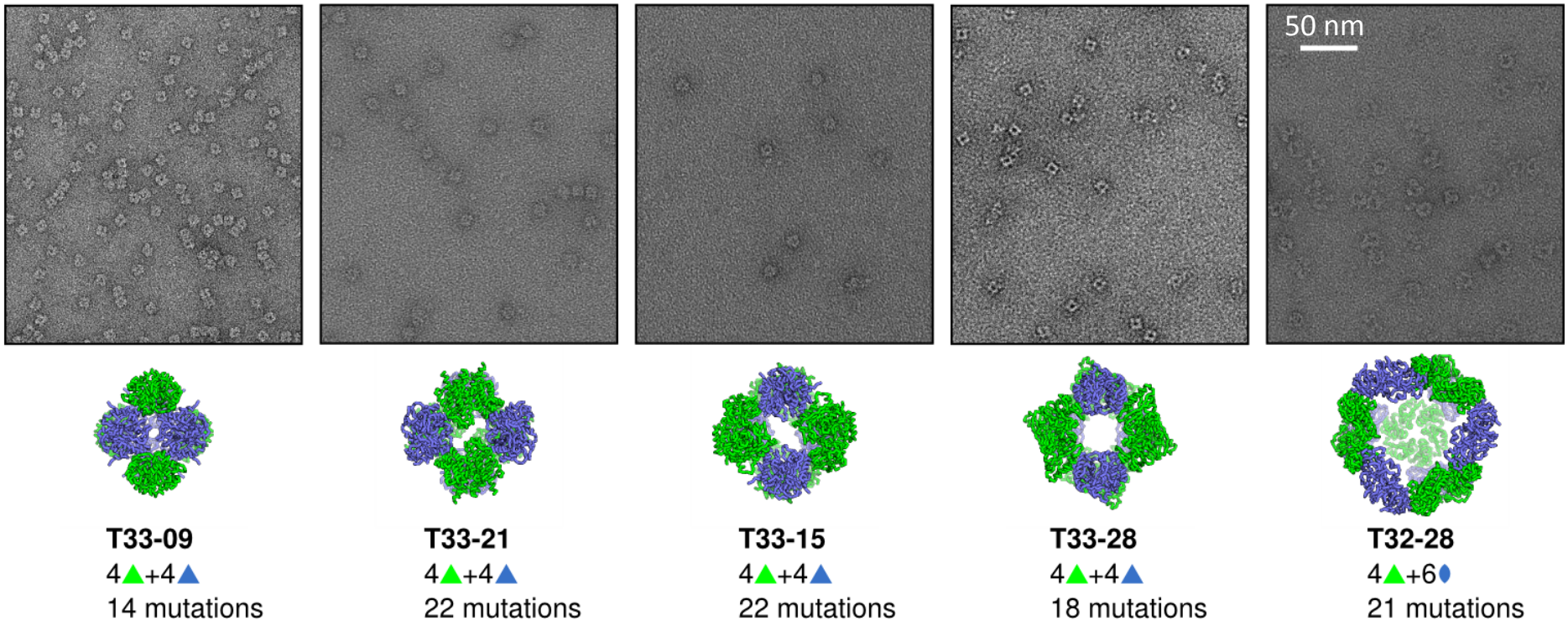


Size-exclusion Chromatrography



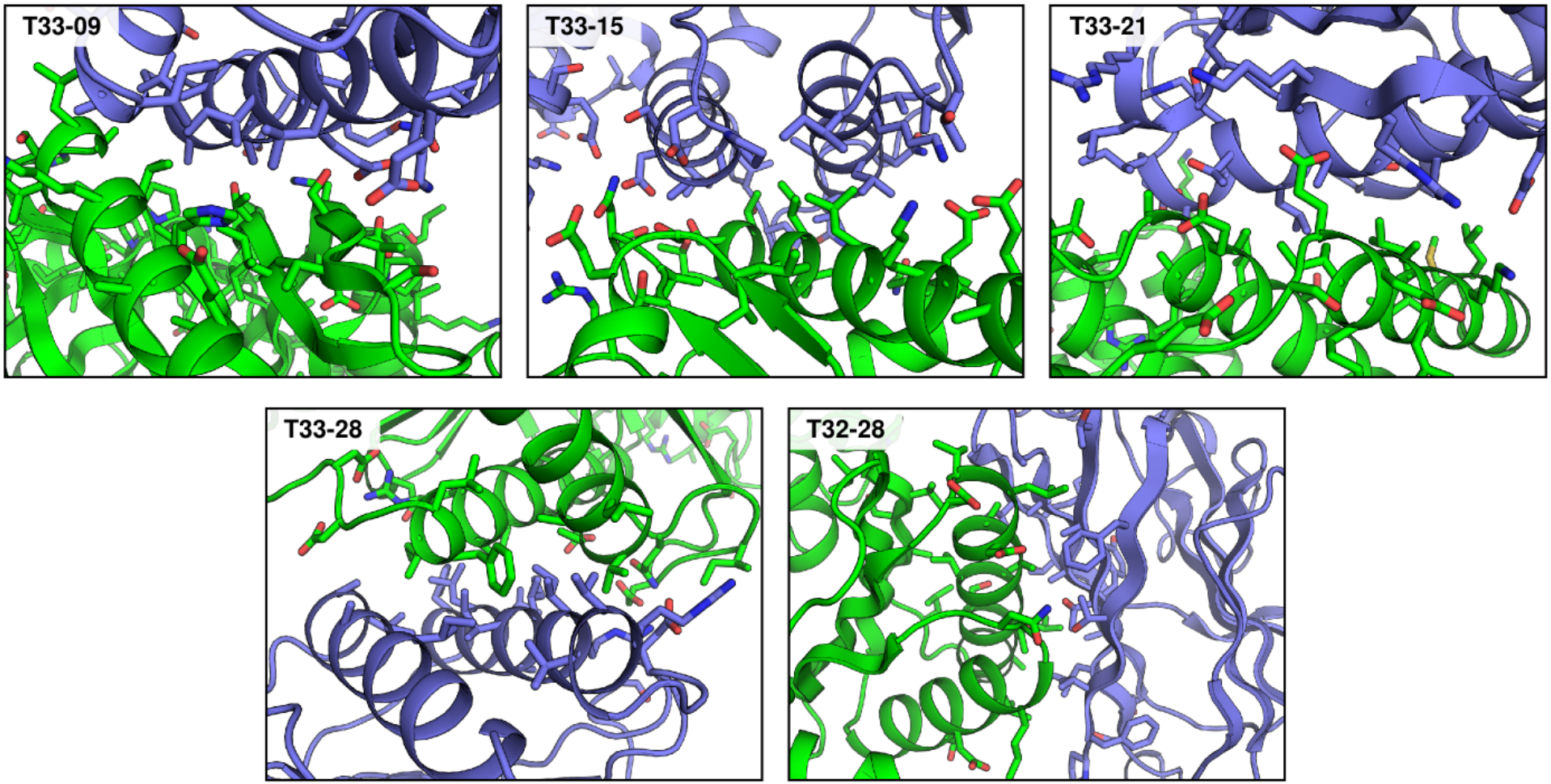
Five out of 57 designs assemble to the target architecture

<u>Negative Stain TEM</u>

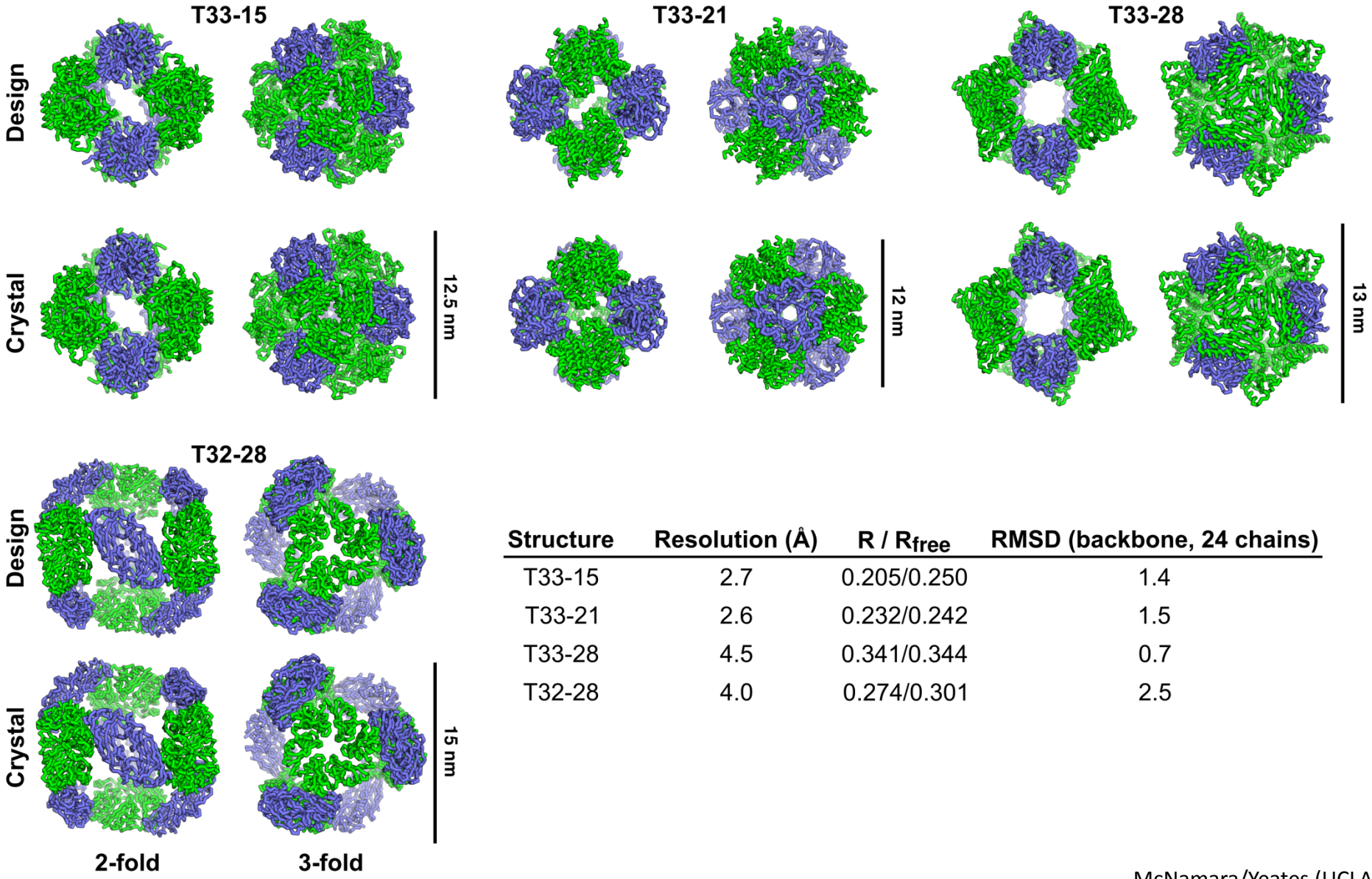


Gonen/Gonen (Janelia)

The designed interfaces resemble natural interfaces and reside mostly on elements of secondary structure



Preliminary crystal structures suggest the materials were designed with high accuracy



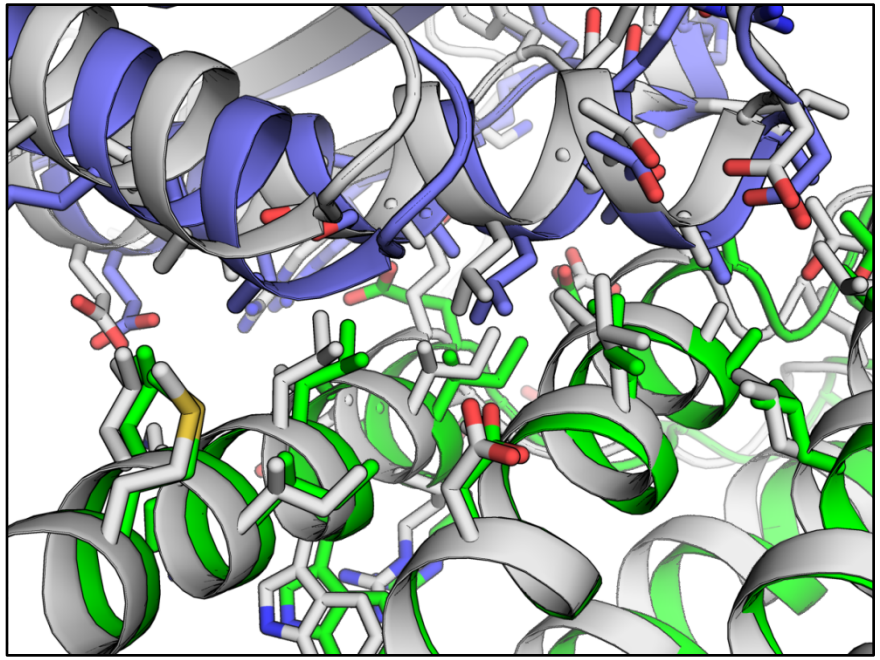
Structure	Resolution (Å)	R / R_{free}	RMSD (backbone, 24 chains)
T33-15	2.7	0.205/0.250	1.4
T33-21	2.6	0.232/0.242	1.5
T33-28	4.5	0.341/0.344	0.7
T32-28	4.0	0.274/0.301	2.5

McNamara/Yeates (UCLA)

Preliminary crystal structures suggest the materials were designed with high accuracy

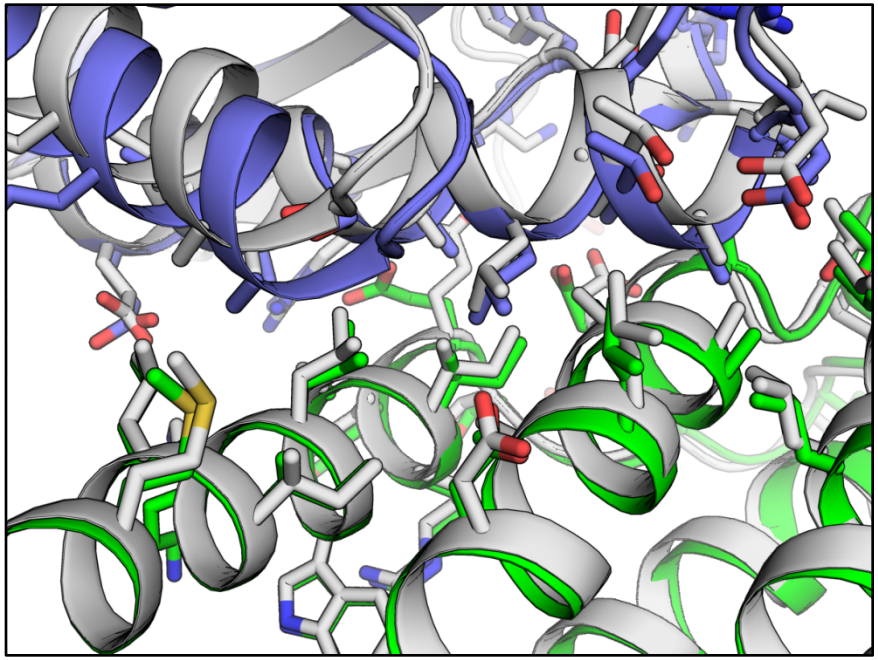
T33-21

Global backbone alignment



Backbone RMSD: 1.50 Å

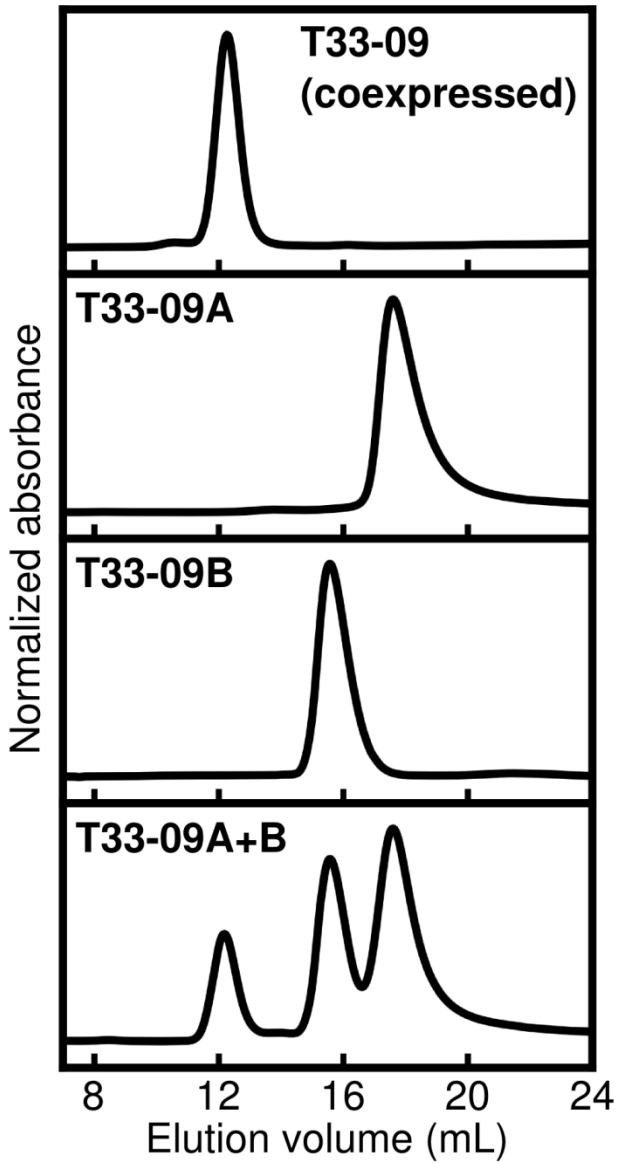
Chains A+B backbone alignment



Backbone RMSD: 0.74 Å

McNamara/Yeates (UCLA)

Preliminary experiments suggest efficient *in vitro* assembly may be relatively straightforward



Conclusions and next steps

- Protein-protein interface design makes highly accurate nanomaterials design possible
- Two-component nanocage design may be a good system for testing new protein-protein interface design methods
  - Relatively high success rate with relatively simple design approach
  - One of few successful examples of simultaneous two-sided interface design
  - Designs are relatively simple to screen and highly crystallizable
- The method could represent a platform technology
- Next steps:
  - Design other two-component architectures: icosahedra, layers, etc.
  - Continue to refine methods to improve success rates
  - Develop general methods for designing regulatory mechanisms into the materials
  - Develop experimental methods for step-wise assembly
  - Design materials custom-tailored to particular applications

Acknowledgements

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David Baker

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Dan McNamara

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Janelia Farm

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Shane Gonen

Breanna Vollmar

Matt Iadanza

Brent Nannenga

Support

HHMI

IAVI

NSF GRFP