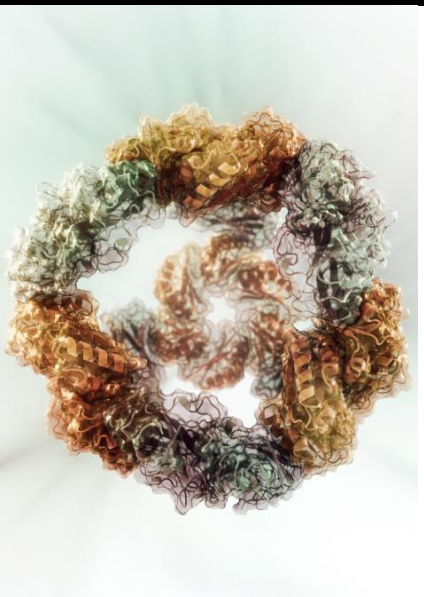


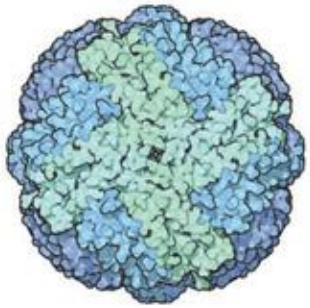
Design of self-assembling protein nanomaterials as next-generation vaccine scaffolds

INSTITUTE FOR Protein Design

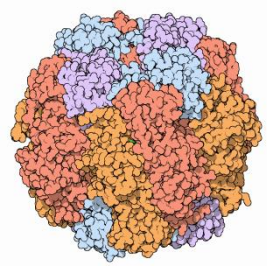
Neil King | March 15, 2016
UNIVERSITY of WASHINGTON

Protein self-assembly enables specialized functions; our goal is to design new self-assembling molecular machines

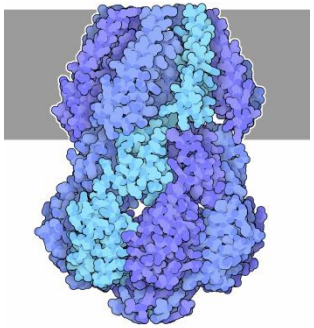
Storage containers



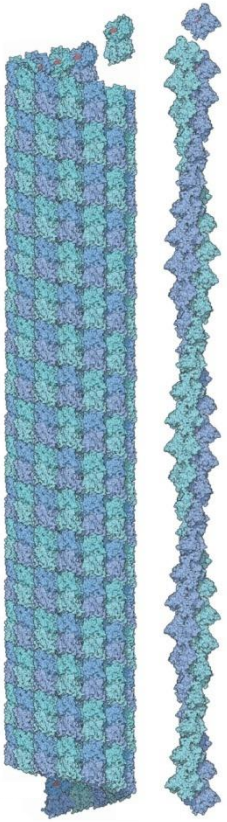
Catalysts



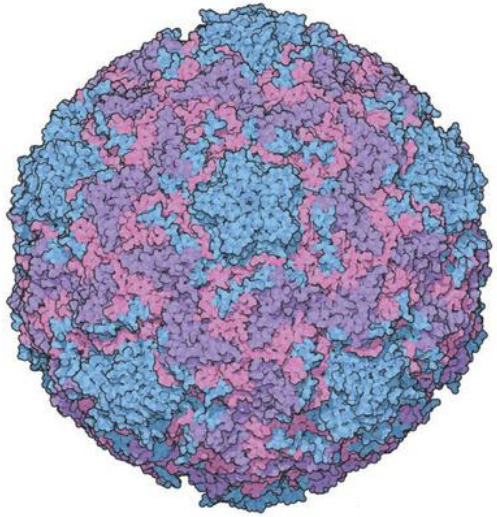
Signal Transducers



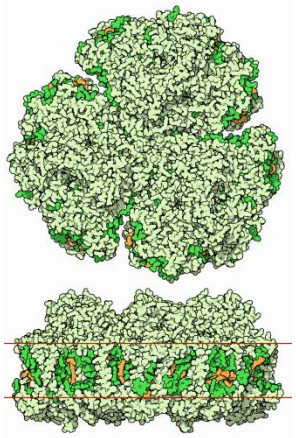
Cellular scaffolding, tracks for transport and motility



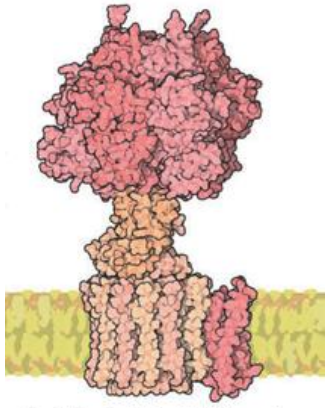
Delivery vehicles



Energy Converters



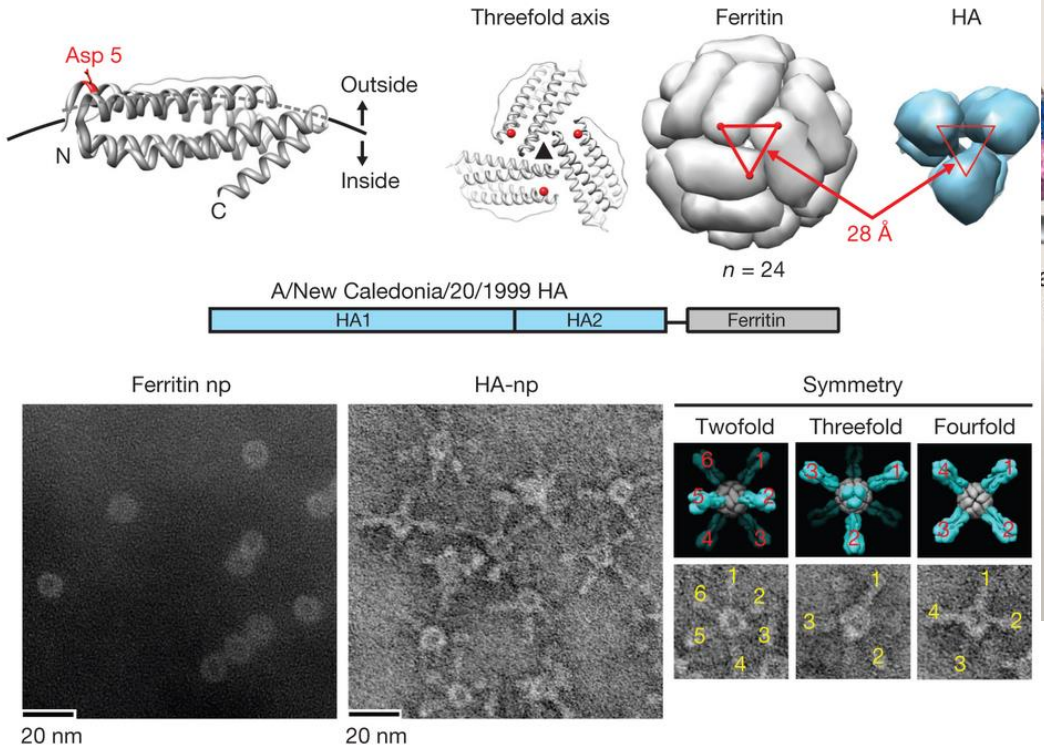
Molecular motors

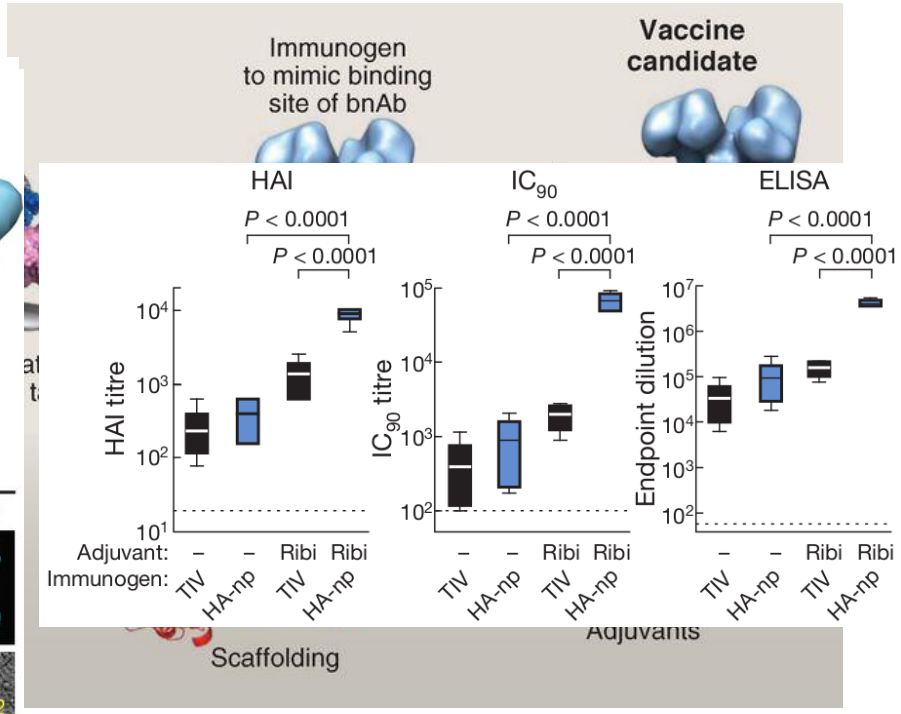


http://pdbbeta.rcsb.org/pdb/education_discussion/molecule_of_the_month/

Custom-designed self-assembling protein nanomaterials could facilitate new approaches to next-generation vaccine design

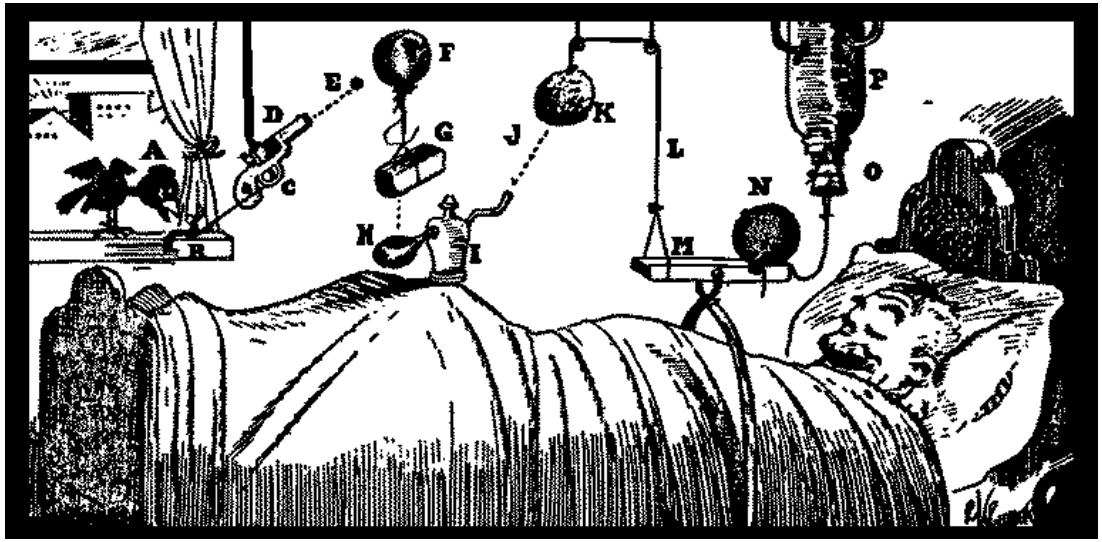
Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies





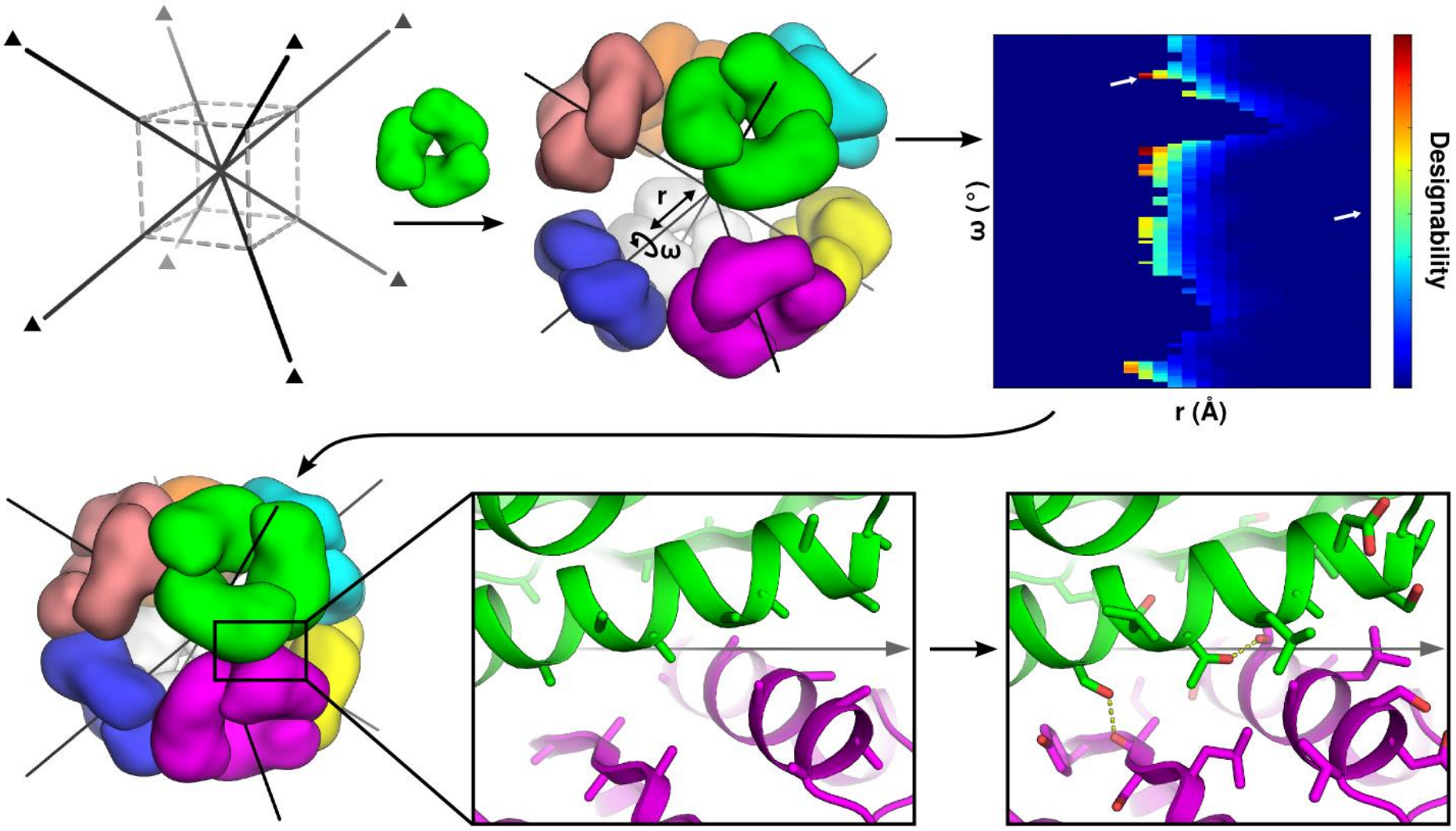
Koff WC, et al. (2013) *Science* **340**:1232910.
Kanekiyo M, et al. (2013) *Nature* **499**:102-6.

Design allows parts (proteins) to be built for a specific purpose



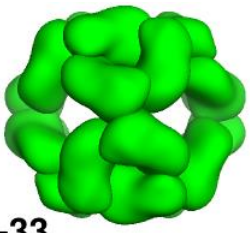
Images courtesy of Chris Negron

We have developed a general computational method for designing new self-assembling protein nanomaterials

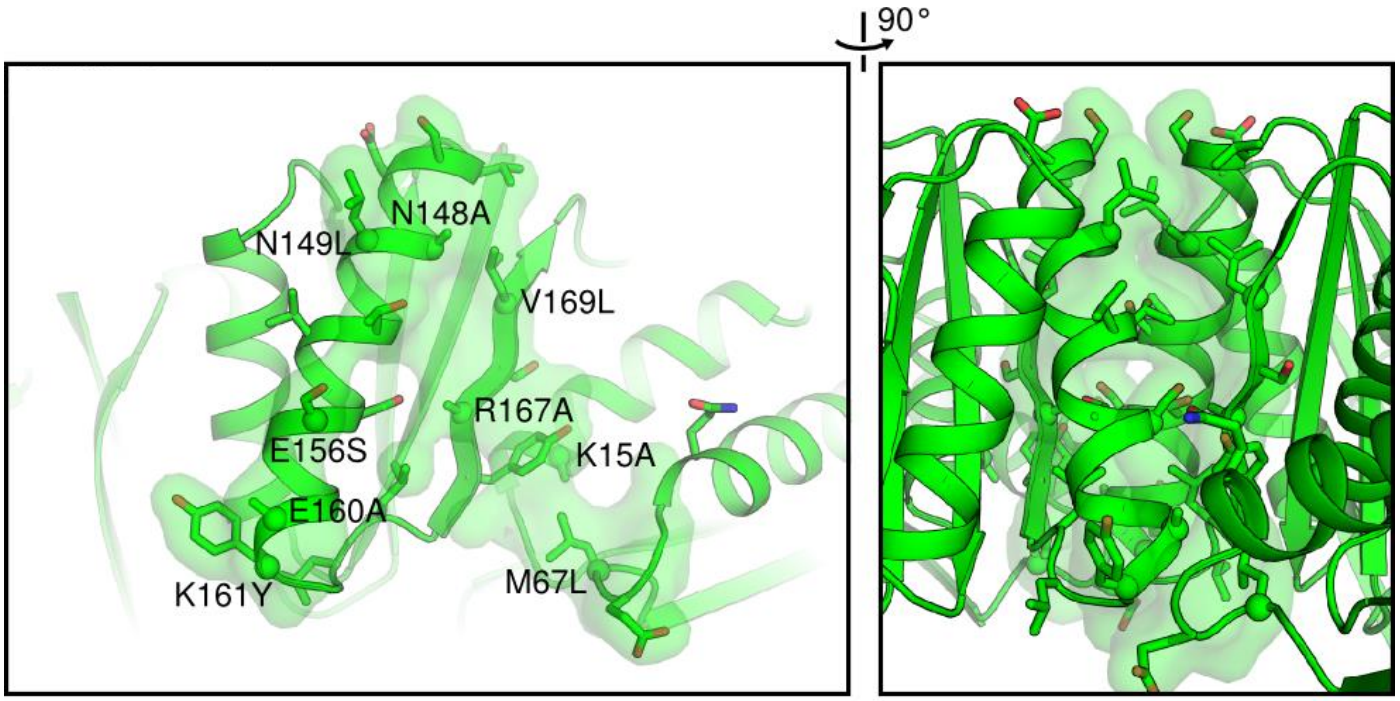


King NP, et al. (2012) *Science* **336**:1171-4.

The designed interfaces have features resembling natural protein-protein interfaces

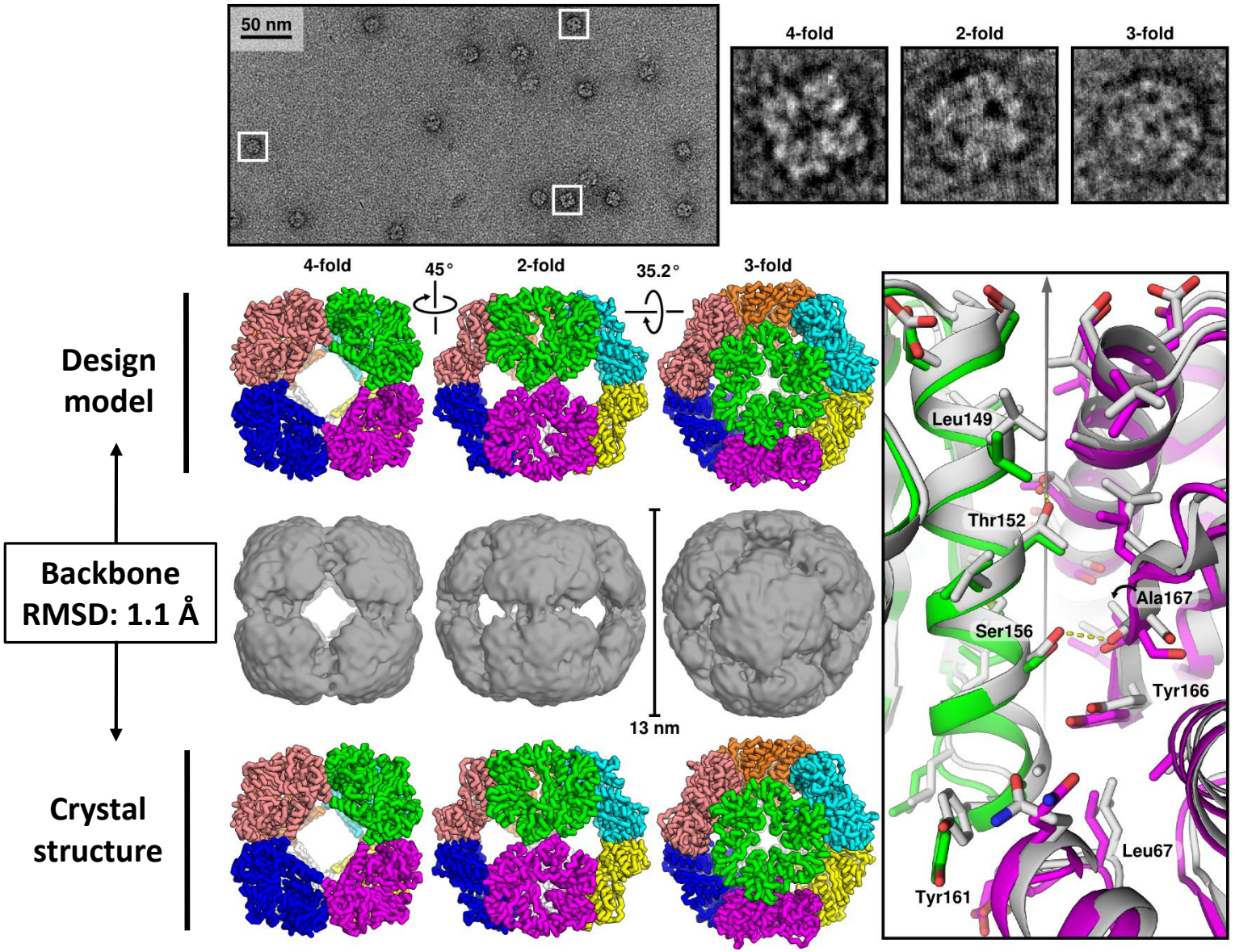


O3-33
Octahedral symmetry
8▲ (24 subunits), 480 kDa



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

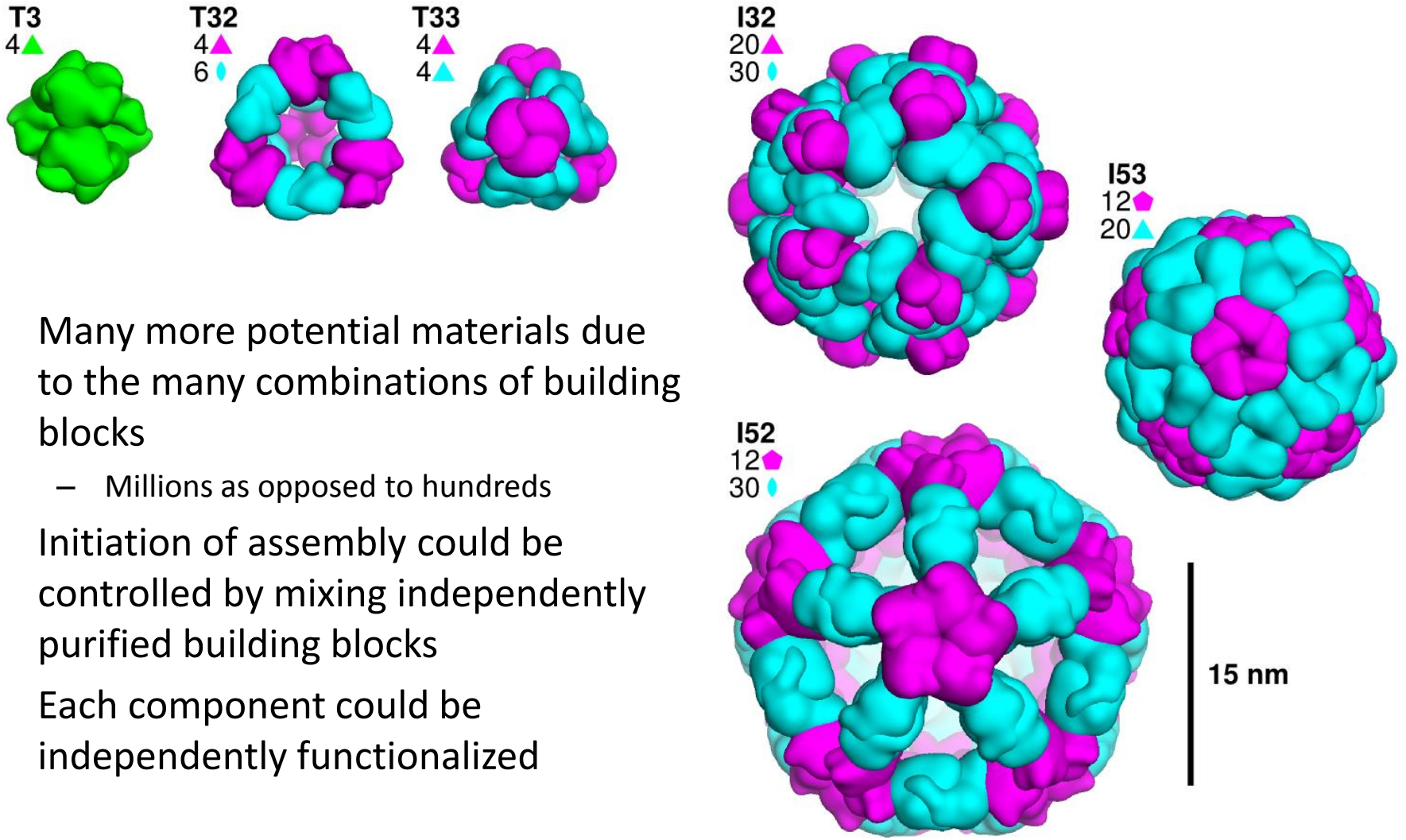
The method enables the design of novel protein nanomaterials with atomic-level accuracy



B. Vollmar, T. Gonen, M. Sawaya, T. Yeates, D. Baker

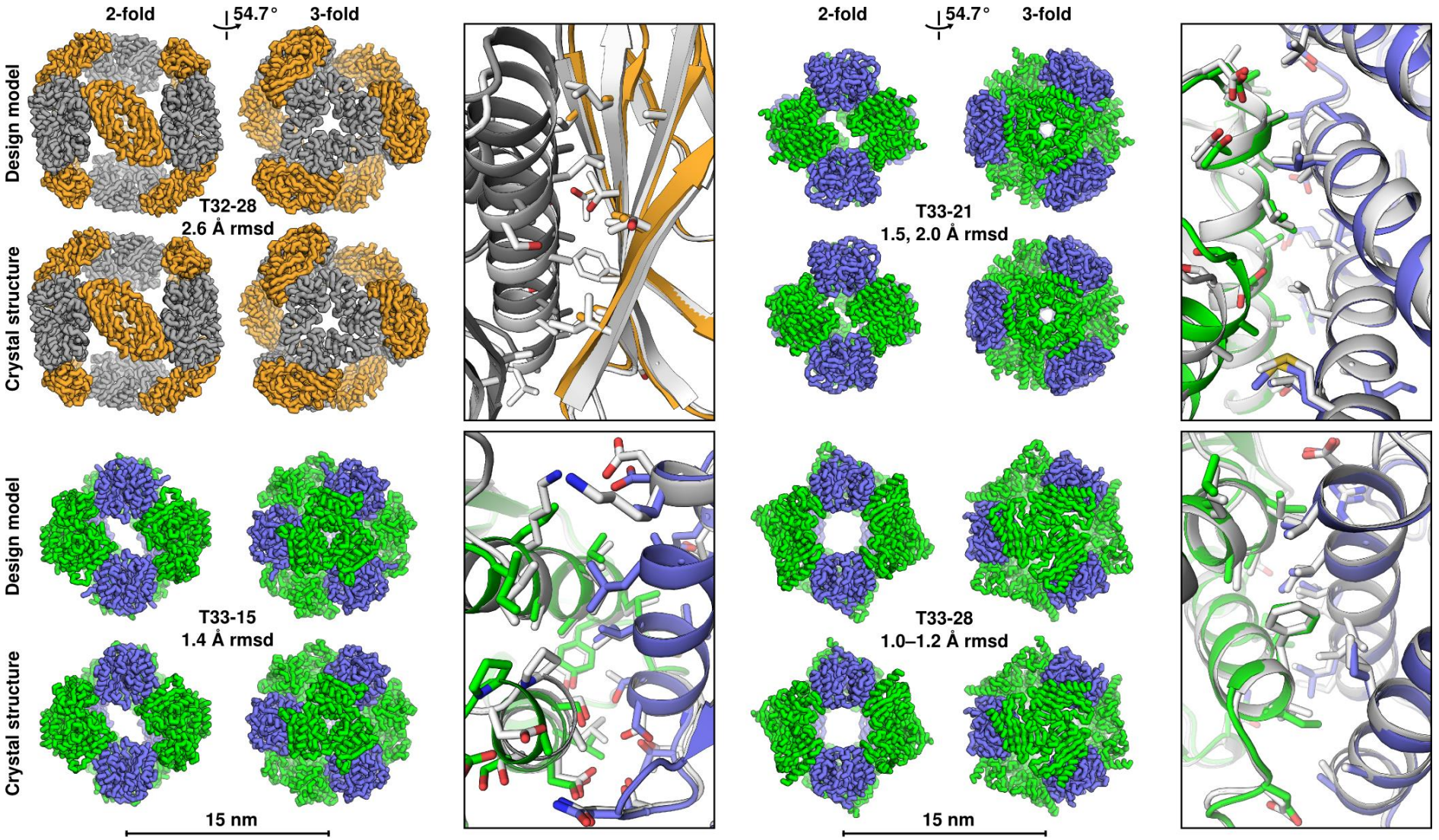
King NP, et al. (2012) *Science* **336**:1171-4.

“Two-component” materials should be much more versatile for various applications



- Many more potential materials due to the many combinations of building blocks
  - Millions as opposed to hundreds
- Initiation of assembly could be controlled by mixing independently purified building blocks
- Each component could be independently functionalized

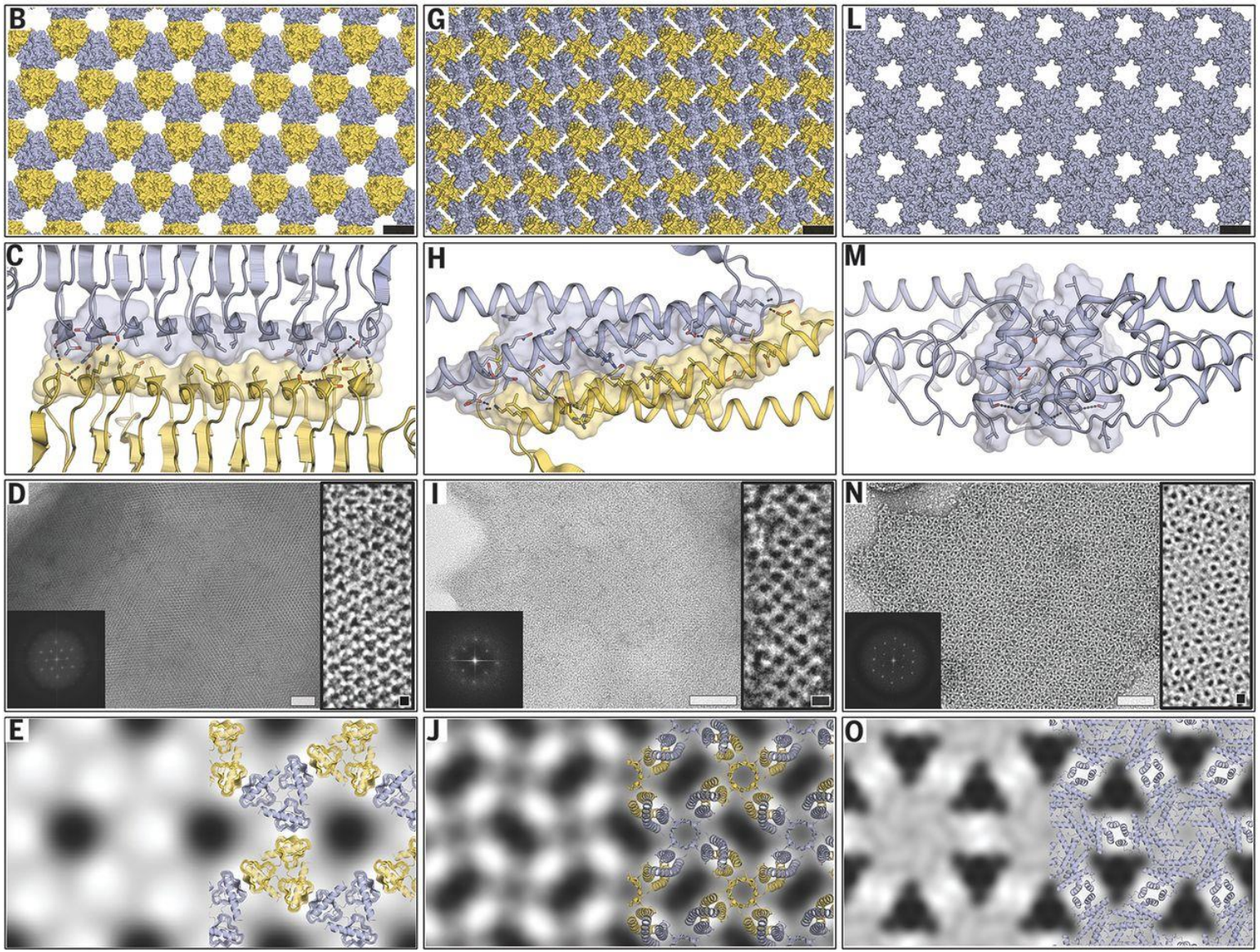
We extended the method to accurately design two-component co-assembling nanomaterials



J. Bale, W. Sheffler, D. McNamara, T. Yeates, D. Baker

King NP, et al. (2014) *Nature* **510**:103-8.

A wide variety of symmetric architectures can be designed using the approach (e.g., 2D layers)



S. Gonen, F. DiMaio, T. Gonen, D. Baker

Gonen S, et al. (2015) *Science* **348**: 1365-8.

Targeted drug delivery and nanoparticle vaccine design: twin applications for designed protein cages

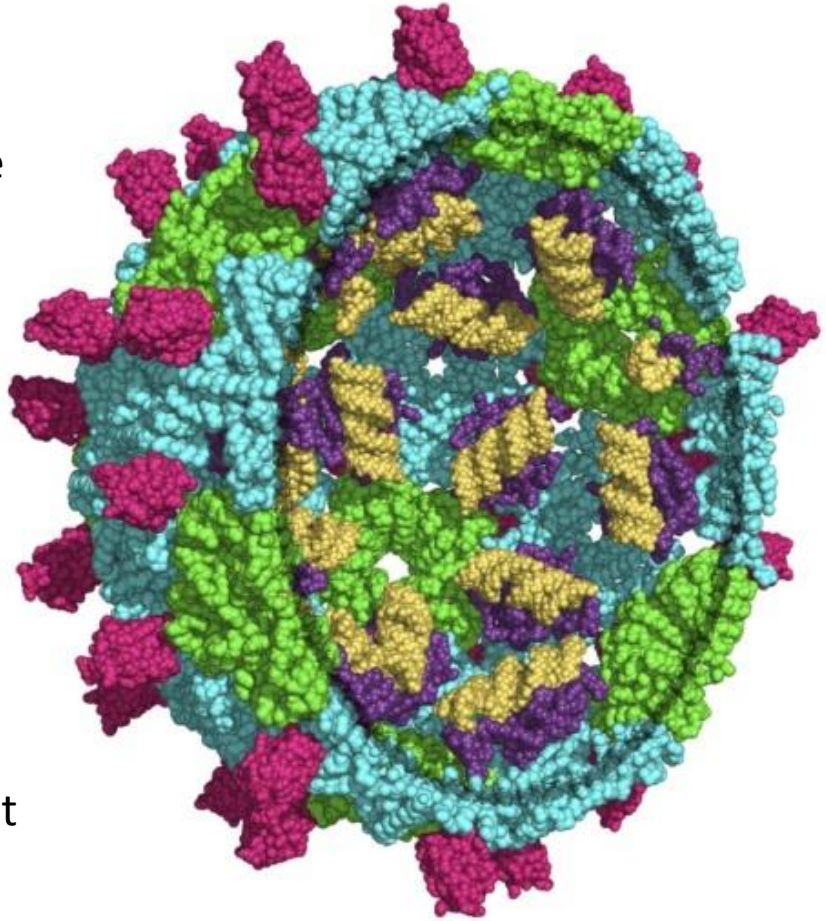
Features:

Two-component cage for encapsulation & multivalent display

Targeting domain or antigen/epitope

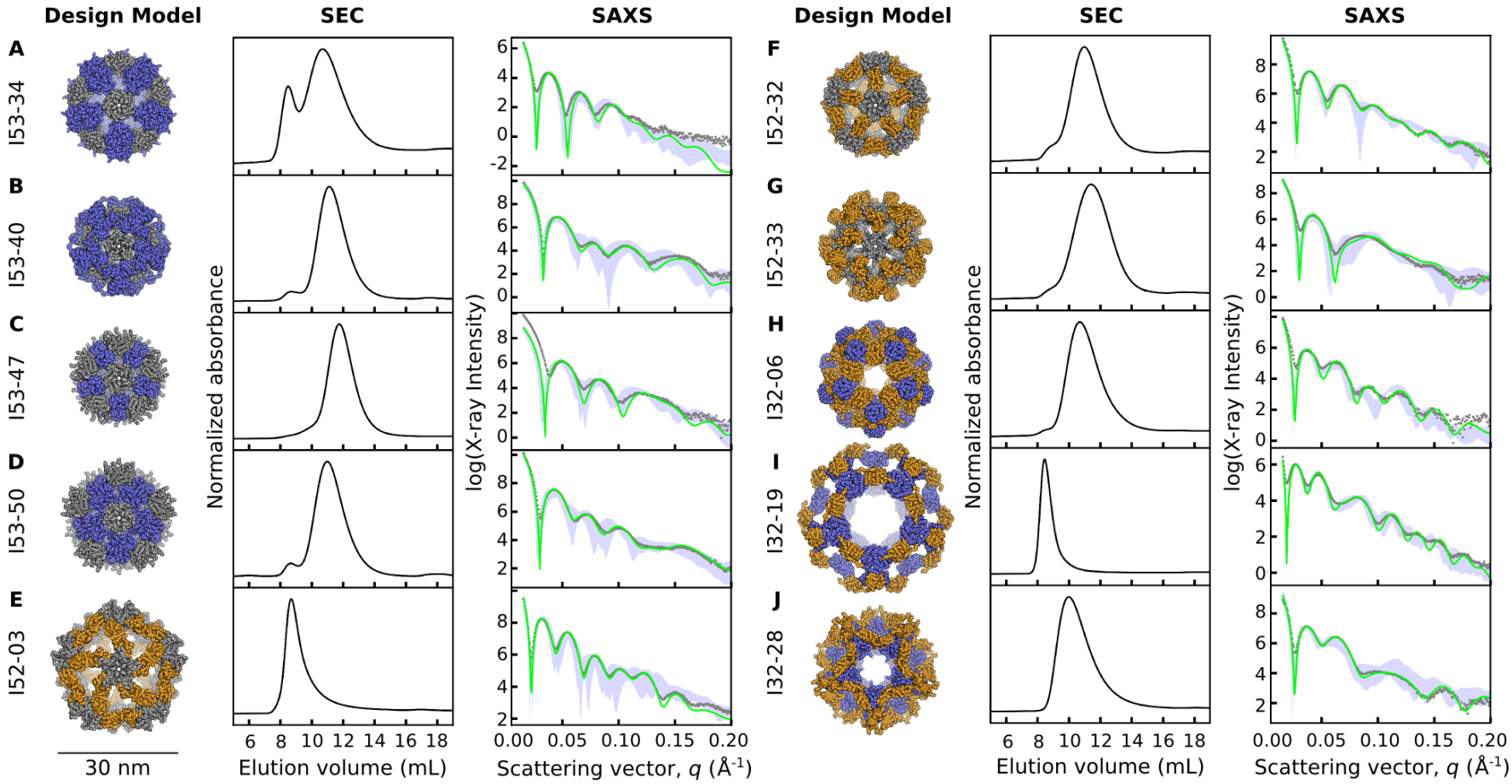
Drug- or adjuvant-loading domain/ residue/polymer

Small molecule/xNA/ protein drug/adjuvant



Additional potential features:
membranes, immune evasion/stimulation, environmental responsiveness, endosomal escape/subcellular localization, allostery, etc.

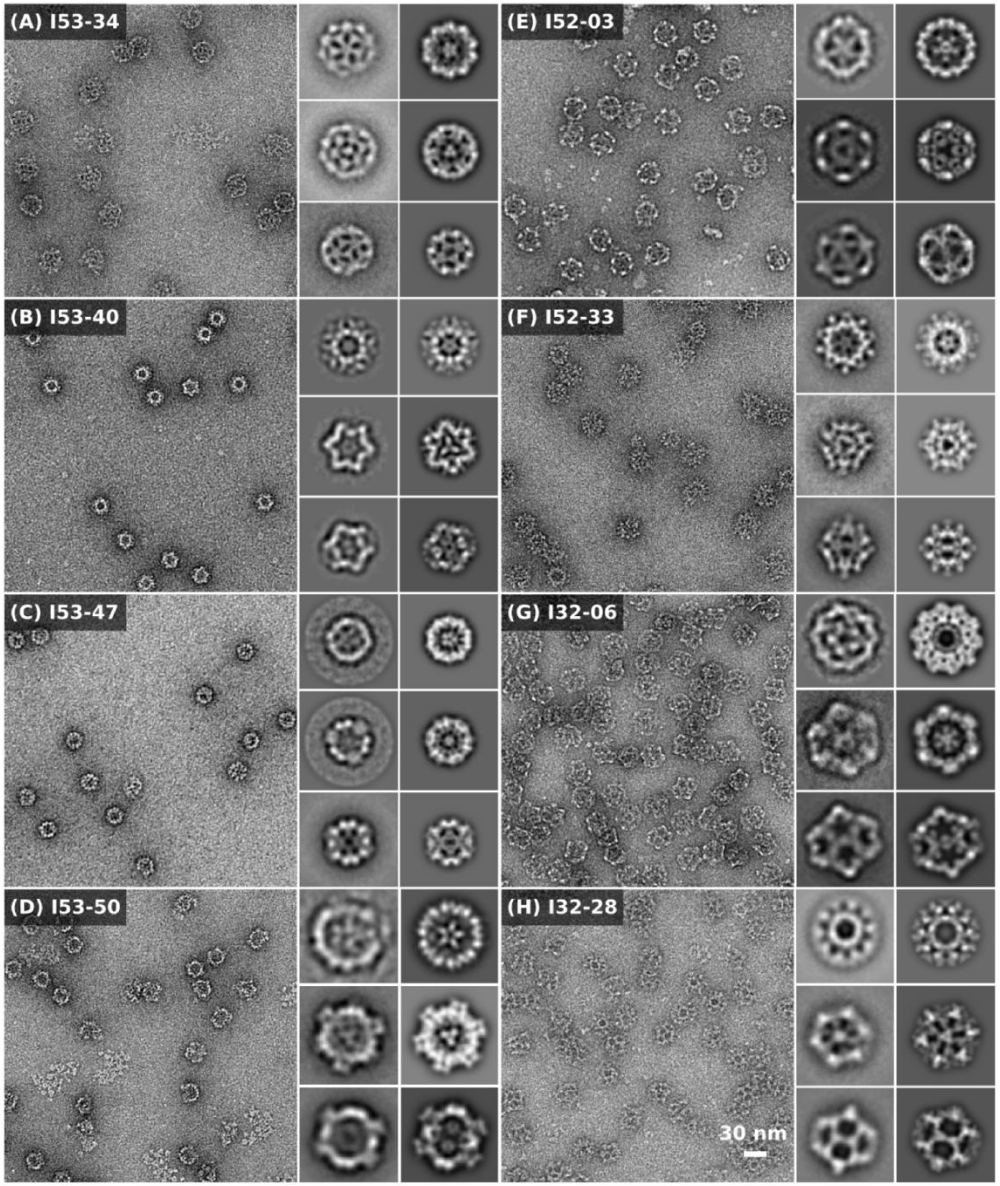
We have recently designed 12 two-component icosahedra, 10 of which are well-behaved, with large packaging capacities

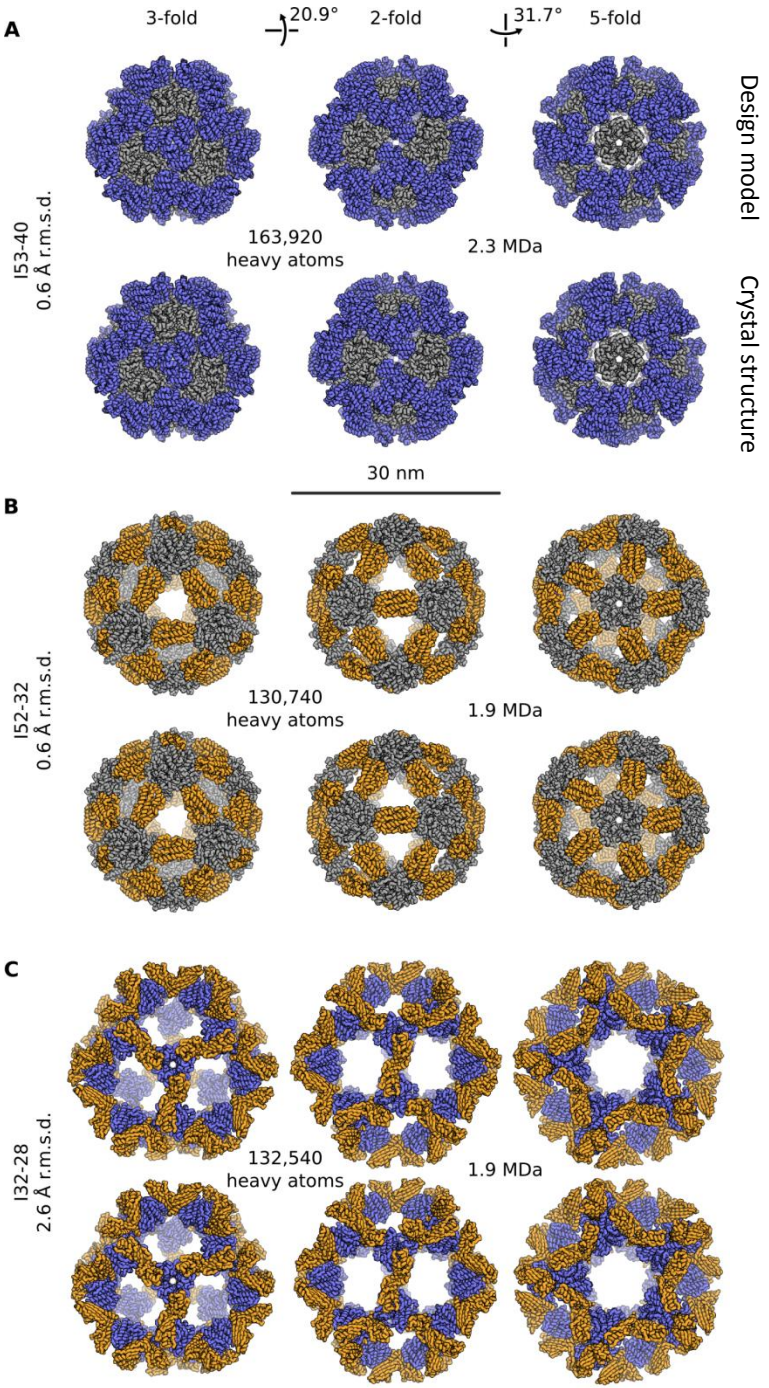


J. Bale, W. Sheffler, J. Tainer

Bale J et al., manuscript in preparation

The 120-subunit, megadalton-scale structures were designed with atomic-level accuracy



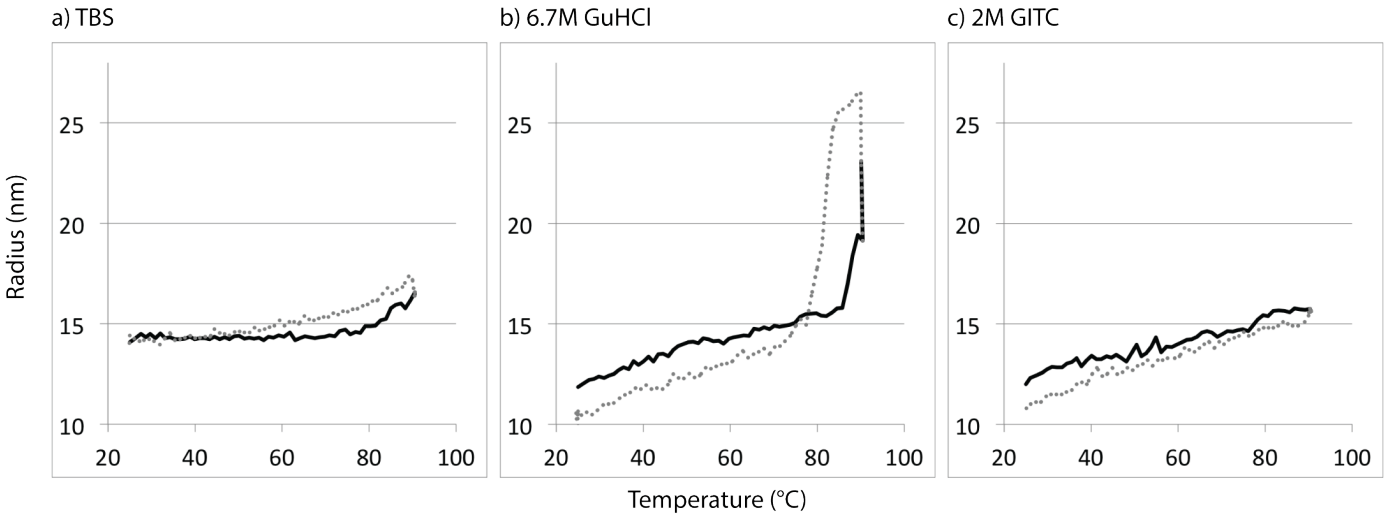


J. Bale, S. Gonen, W. Sheffler

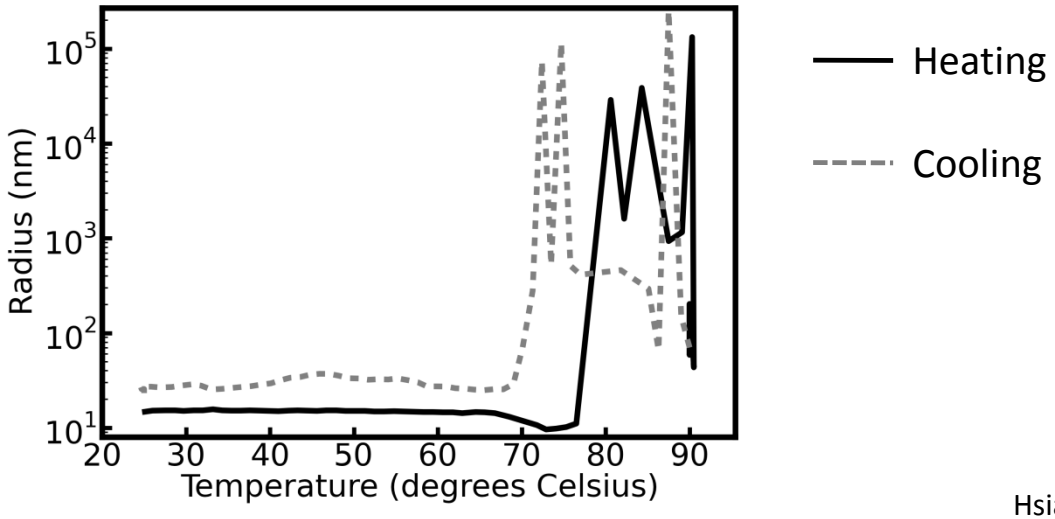
Bale J et al., manuscript in preparation

The nanoparticles are highly resistant to thermal stress

I3-01



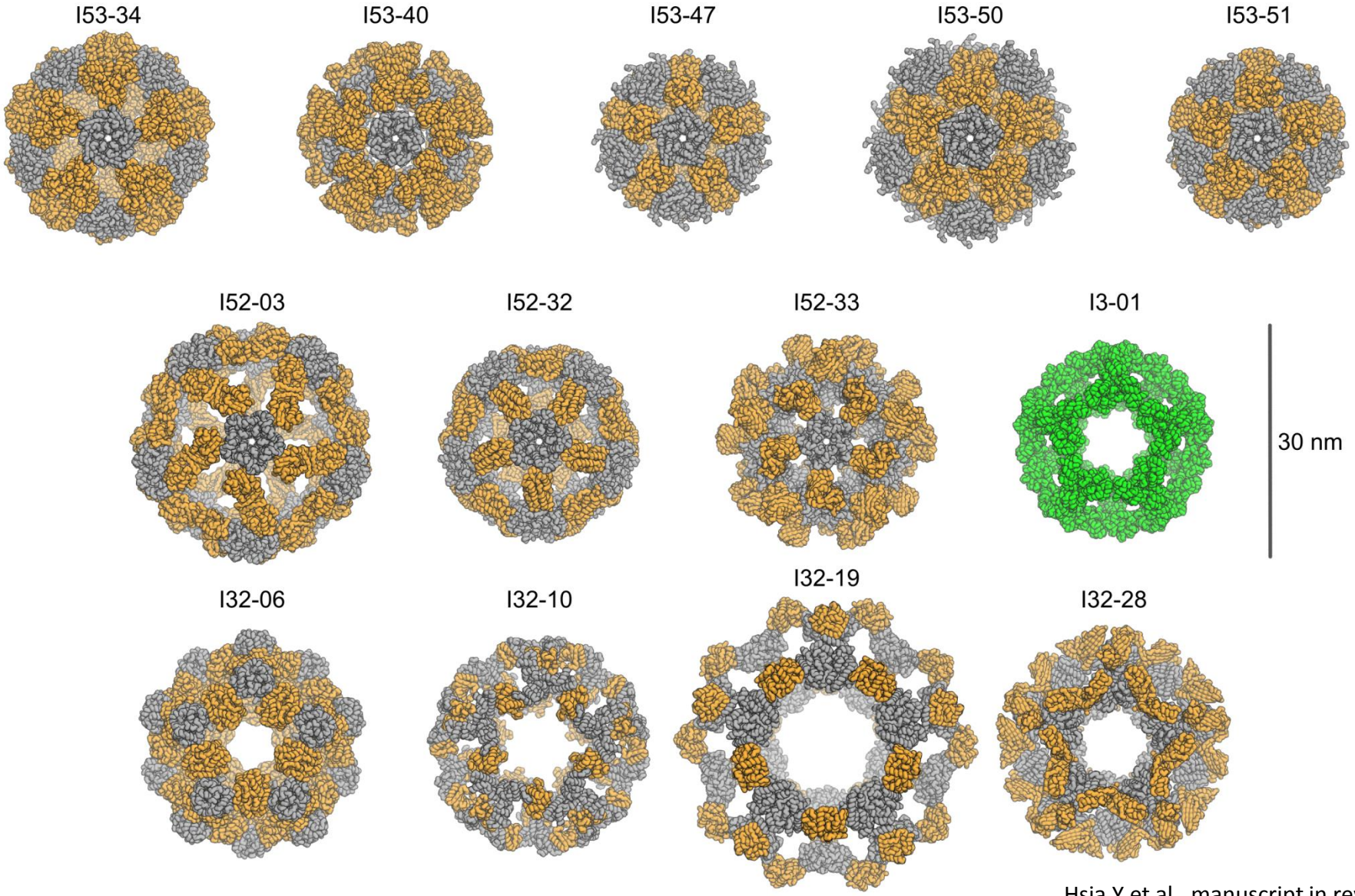
I53-50



Y. Hsia, J. Bale, D. Ellis

Hsia Y et al., manuscript in revision
Bale J et al., manuscript in preparation

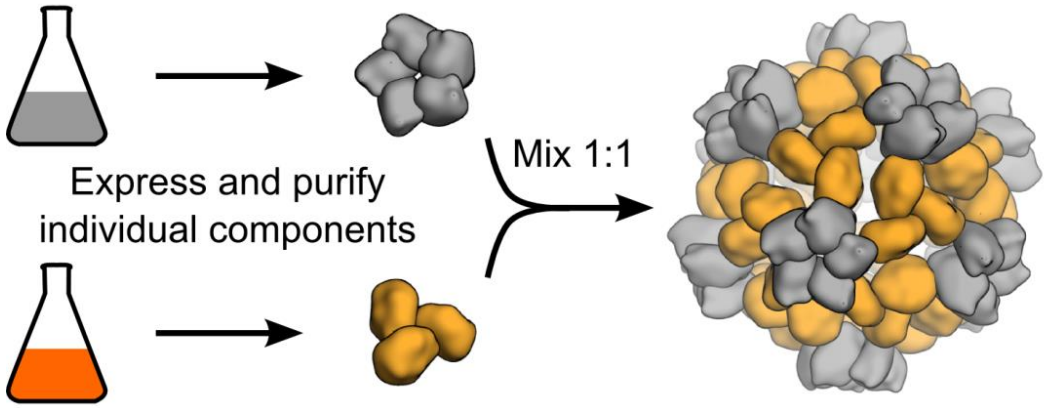
Our current crop of icosahedral nanoparticles

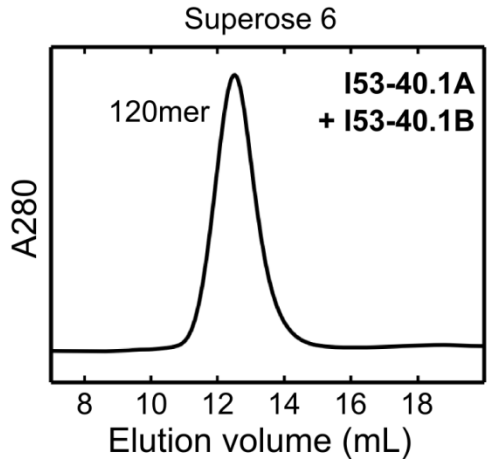


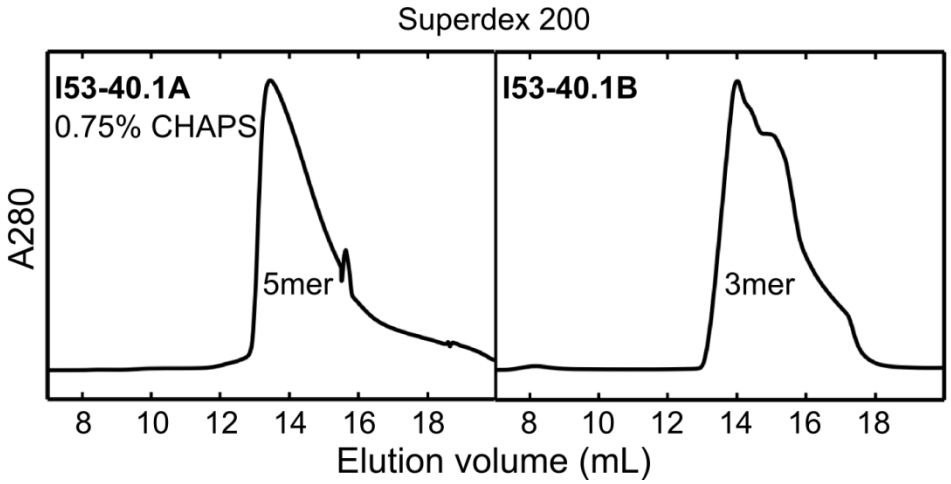
J. Bale, Y. Hsia, W. Sheffler

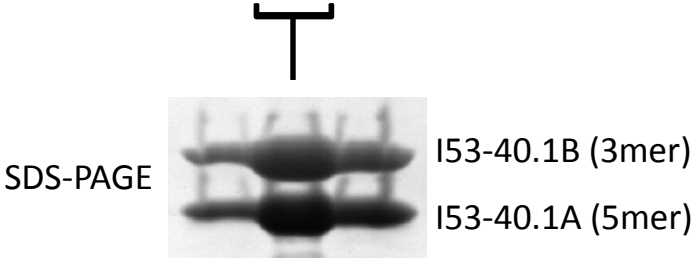
Hsia Y et al., manuscript in revision
Bale J et al., manuscript in preparation

Mixing independently purified components enables simple, efficient, and controlled *in vitro* assembly



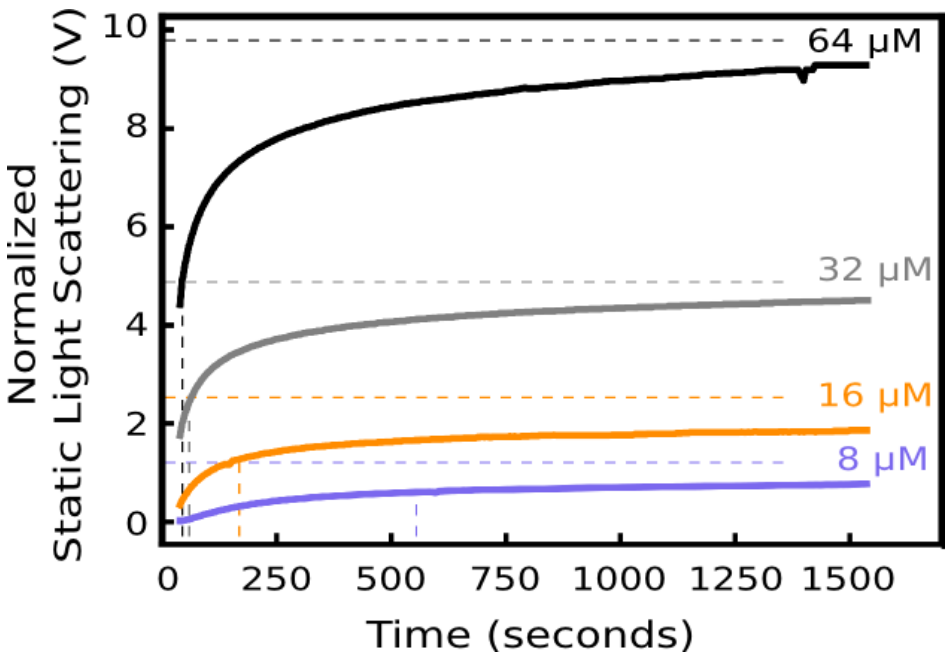






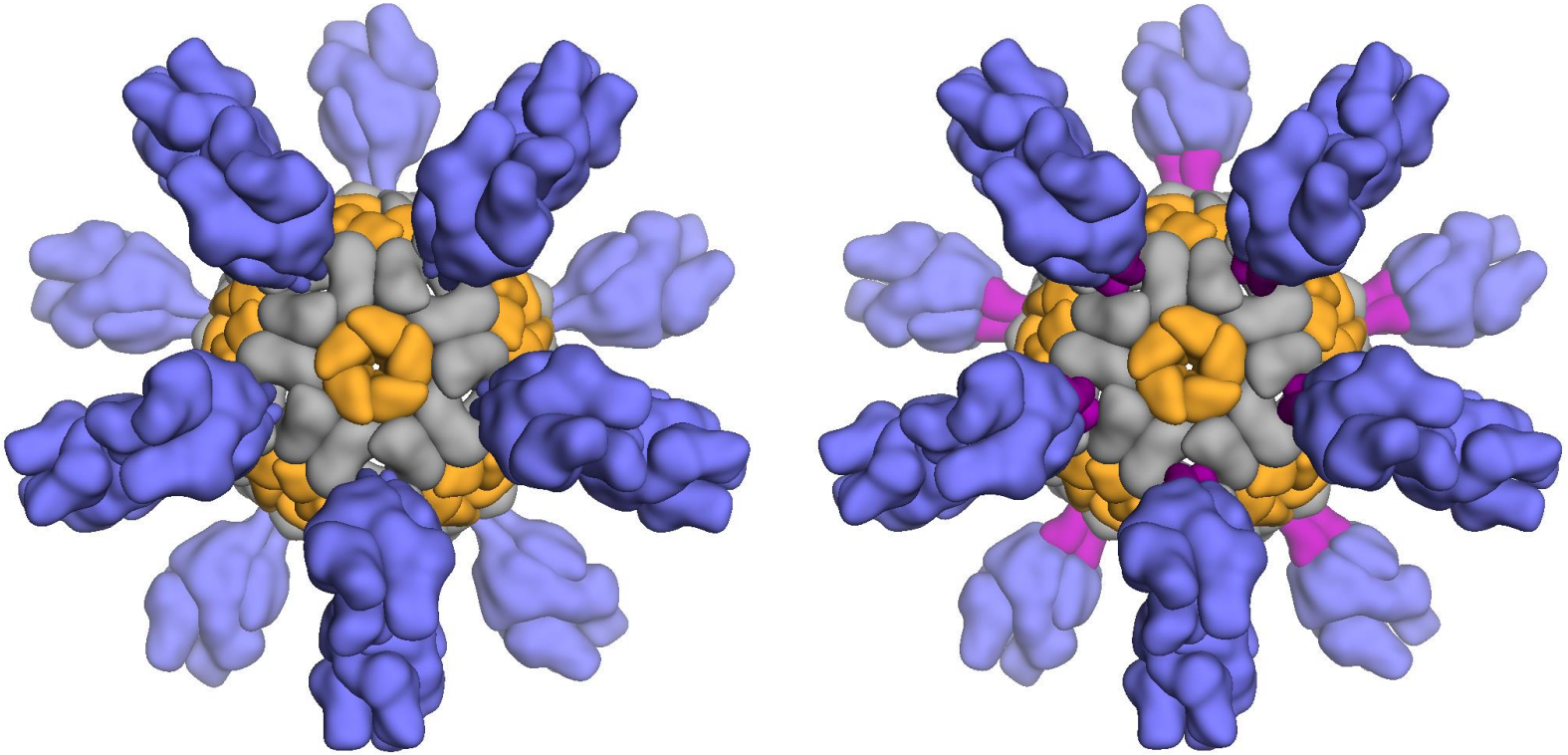
D. Ellis

Assembly occurs on the timescale of seconds to minutes



Two-component protein nanoparticles: a versatile platform for multivalent display

Component A Component B Functional domain Adaptor protein complex



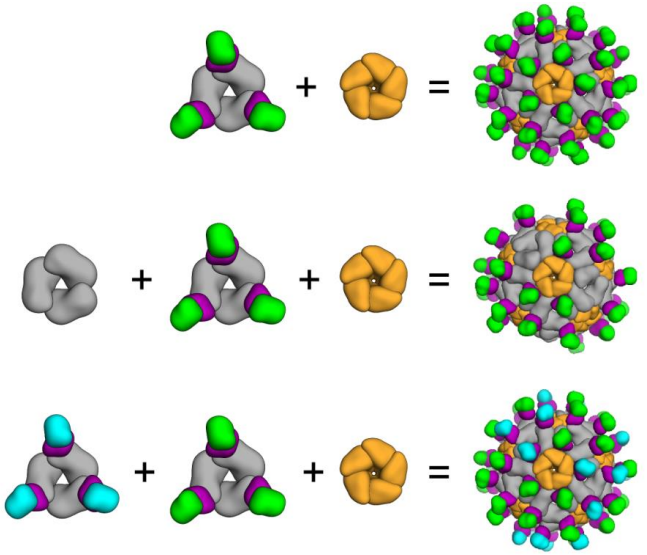
Functional domain expressed as a genetic fusion to cage component

Adaptor protein mediates attachment to nanoparticle components

Two-component protein nanoparticles: a versatile platform for multivalent display

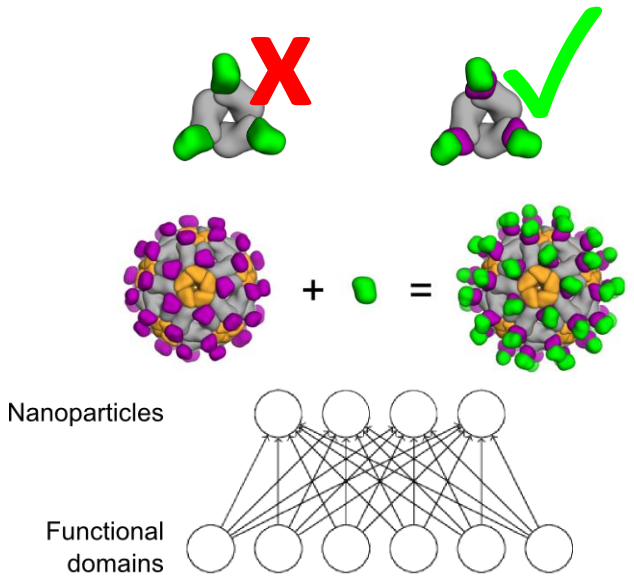
In vitro assembly

- Expression, purification, and quality control can be performed independently on distinct building blocks
- Antigen valency/copy number can be controlled by including unmodified components during *in vitro* assembly
- Distinct antigens/costimulatory proteins can be scaffolded in defined ratios



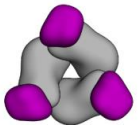
Adaptor-mediated labeling

- Complex antigens that fail as genetic fusions to nanoparticle subunits can be labeled with a small adaptor tag
- Post-assembly labeling
- Rapid prototyping of functional domain/nanoparticle combinations

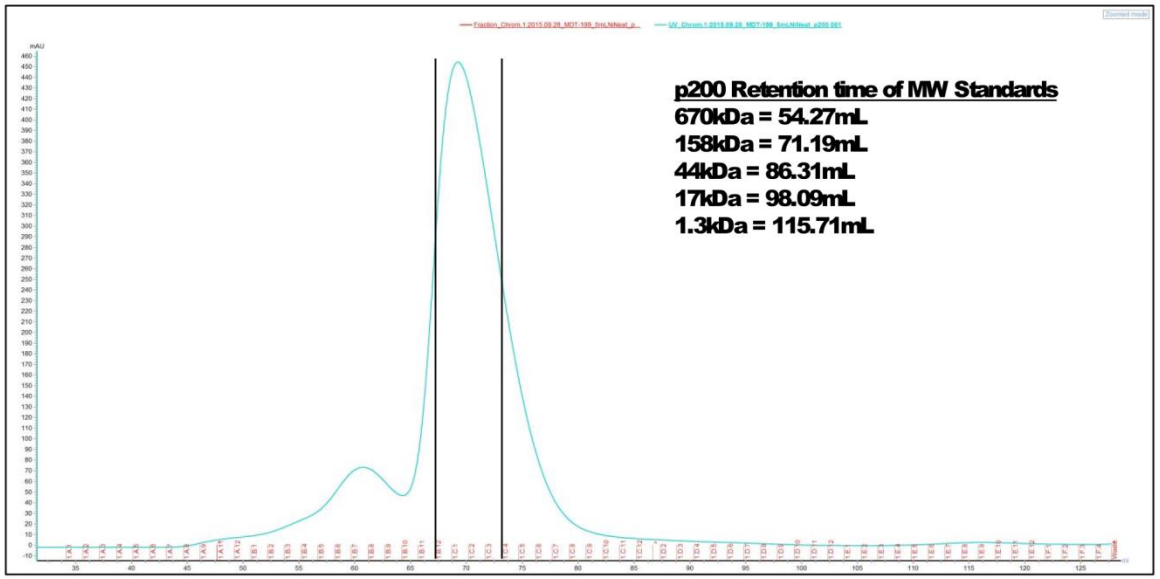


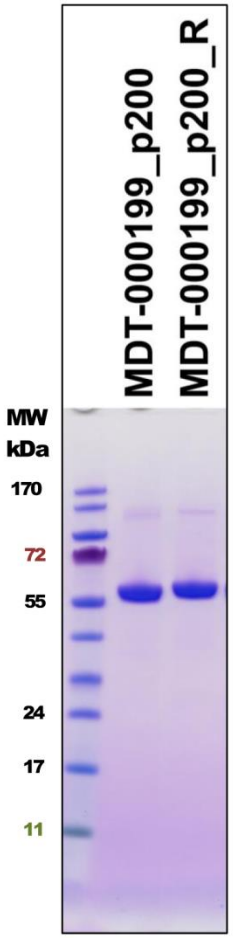
An anti-CD20 scFv–I53-50A fusion protein can be produced in good yield

scFv-trimer genetic fusion



Superdex 200 16/600 pg

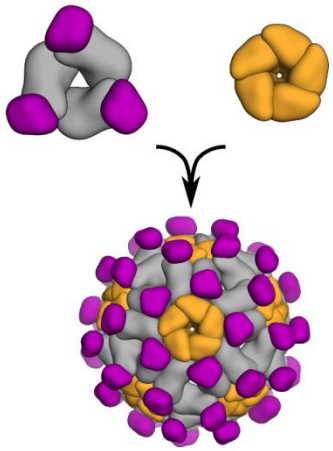




C. Correnti, W. van der Schueren

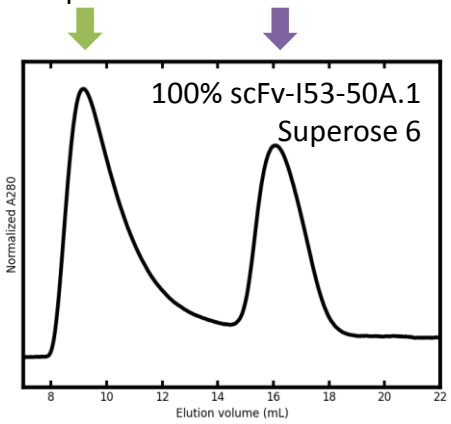
In vitro assembly allows control over scFv valency

100% scFv-trimer

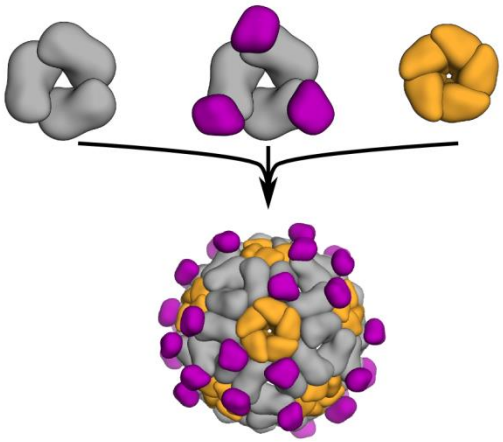


Full-valency nanoparticle

Nanoparticles Excess trimer

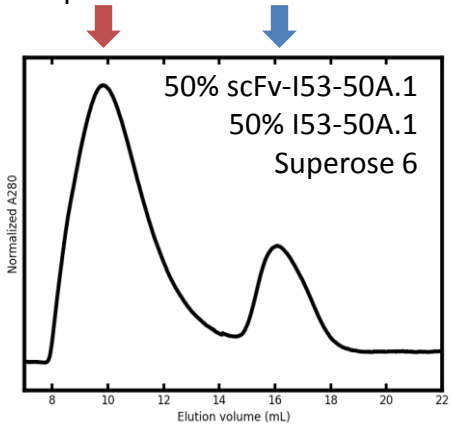


50% 50% scFv-trimer

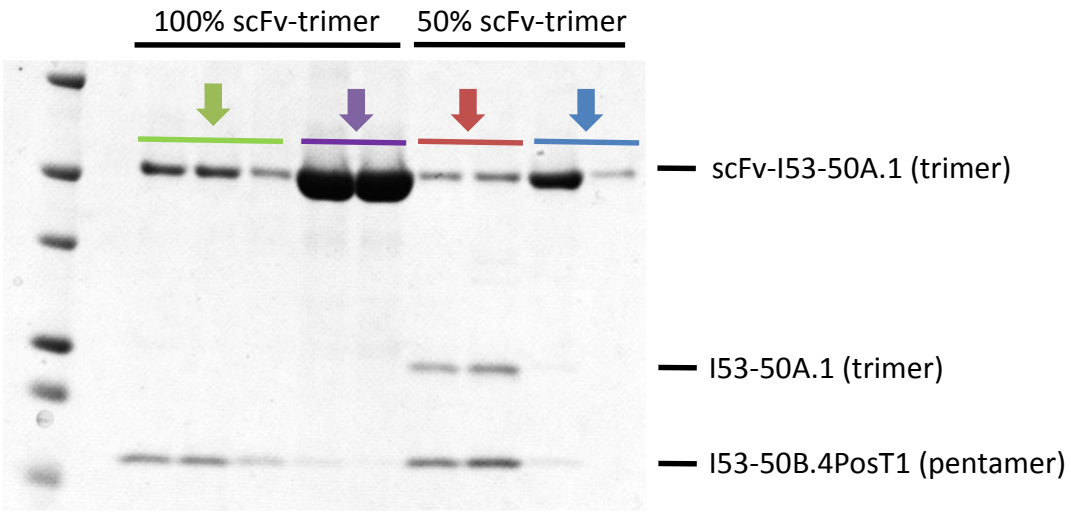


Half-valency nanoparticle

Nanoparticles Excess trimer

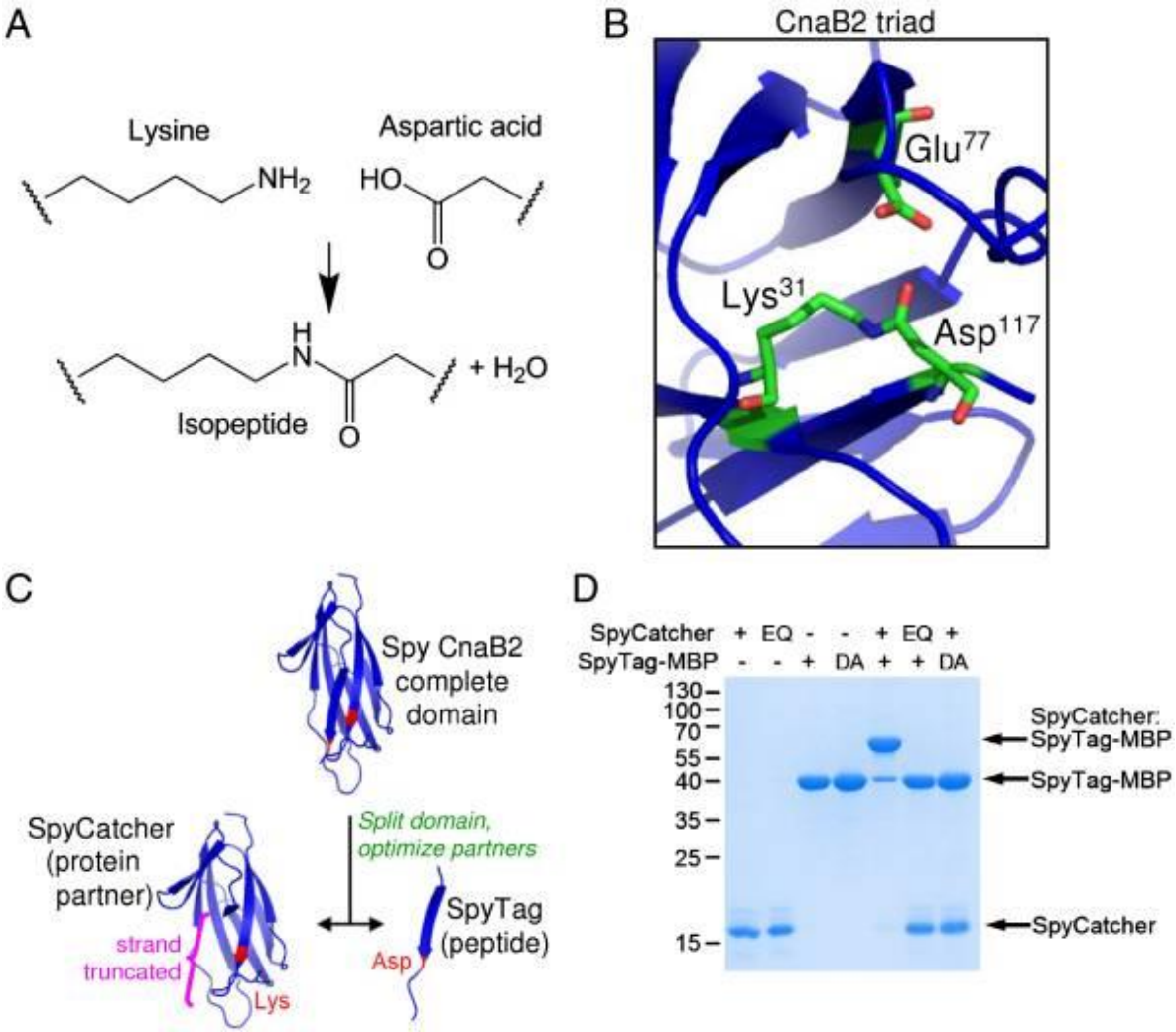


SDS-PAGE



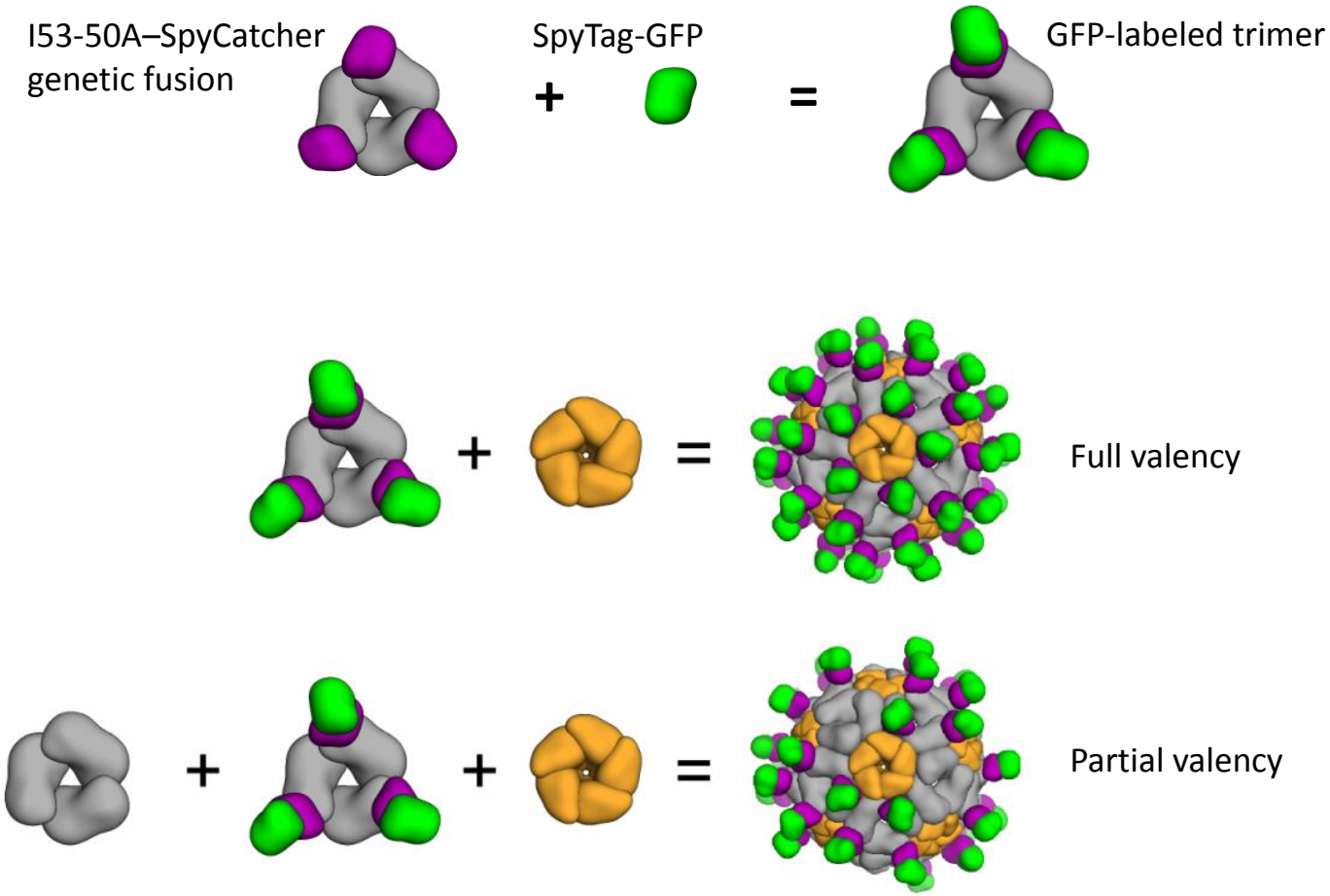
J. Burrows

SpyCatcher-SpyTag is a molecular adaptor capable of selective and stable labeling

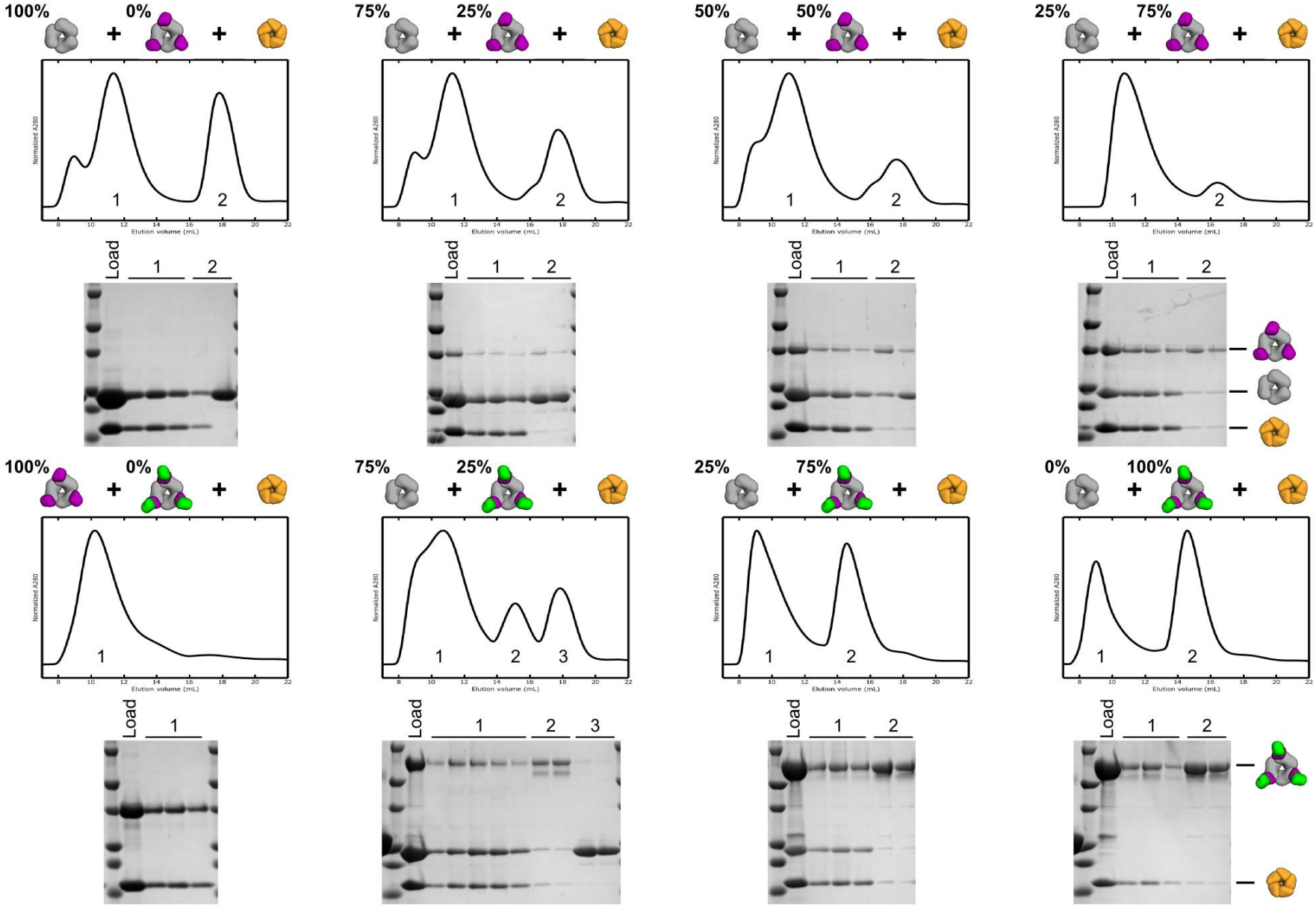


Zakeri B, *et al.* (2012) *Proc. Natl. Acad. Sci.* **109**:E690-7.

Conjugating SpyTag-GFP to purified SpyCatcher-I53-50A enables *in vitro* assembly of nanoparticles with variable GFP valencies

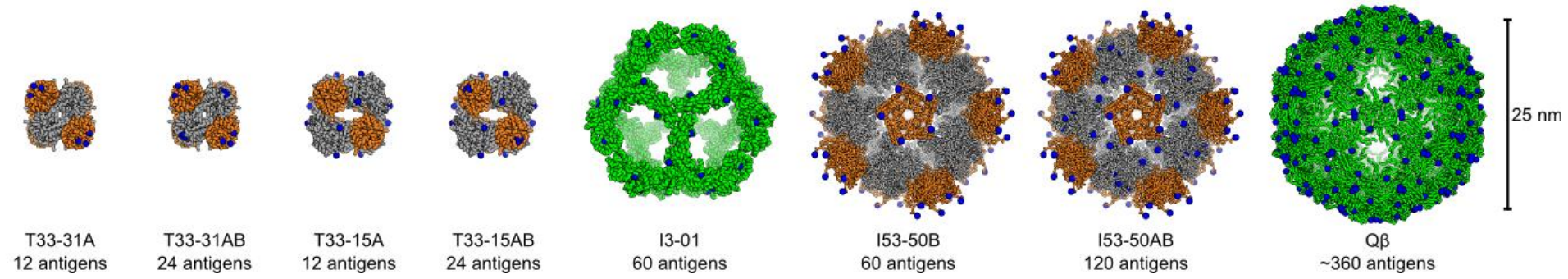


Conjugating SpyTag-GFP to purified SpyCatcher-I53-50A enables *in vitro* assembly of nanoparticles with variable GFP valencies



B. Nickerson

A pilot immunization study in mice revealed size- and valency-dependent immunogenicity

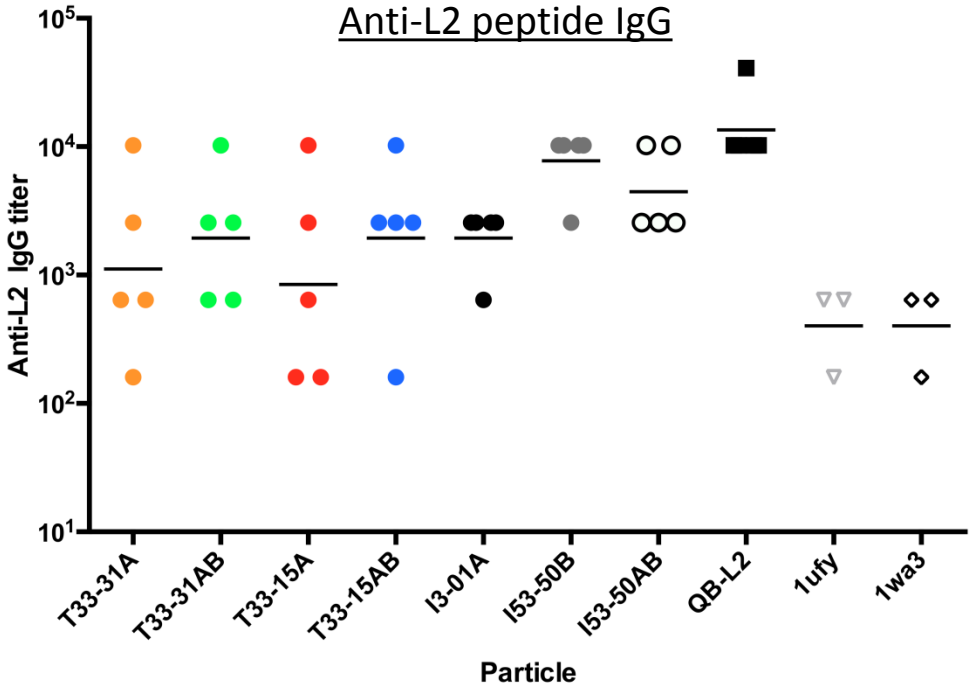


Antigen: consensus L2 peptide

Tyler M, et al. (2014) *Vaccine* **32**:4267-74.

HPV 16:	GTGGRTGYIPLGTR***PPT***ATDT
HPV 18:	GTGGRTGYIPLGGRSNT***VVDV***
Consensus:	GTGGRTGYVPLGTRPPTVVDV

2 immunizations, 5 µg protein each



B. Chackerian, J. Peabody (UNM)

Summary and future directions

Summary:

- We have developed a general computational approach to designing self-assembling protein nanomaterials with atomic-level accuracy
- We have recently designed and experimentally validated 120-subunit icosahedral nanoparticles with sizes and molecular weights comparable to small viruses
- We have demonstrated the multivalent display of complex proteins (e.g., scFvs, viral envelope glycoproteins) on the nanoparticles using both direct genetic fusion and molecular adaptors
- The designed nanoparticles boost the immunogenicity of a multivalently displayed peptide antigen comparably to RNA-containing bacteriophage particles in mice

Future directions:

- Need to obtain additional immunogenicity data on nanoparticles bearing antigens of interest
- Further modify antigen-bearing nanoparticles to co-package adjuvants to increase/tailor immune response
- Explore possibilities afforded by two-component nanoparticles to display multiple antigens or combinations of antigens and costimulatory proteins

Acknowledgements

UW
Julia Burrows
Kate DaPron
Quinton Dowling
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Sue Yi

David Baker
Lance Stewart
Jacob Bale
Gabe Butterfield
Steve Haushcka
Yang Hsia
Marc Lajoie
Una Natterman
George Ueda

Betsy Gray
Dan Stetson

FHCRC
Colin Correnti
Chen Fang
Ollie Press

UNM
Julie Peabody
Bryce Chackerian

Utah
Joerg Votteler
Wes Sundquist

AMC
Ilja Bontjer
Philip Brouwer
Rogier Sanders

Scripps
Chris Cottrell
Andrew Ward

IRB
Davide Corti
Antonio Lanzavecchia
Laurent Perez