mRNA-launched nanoparticle vaccines
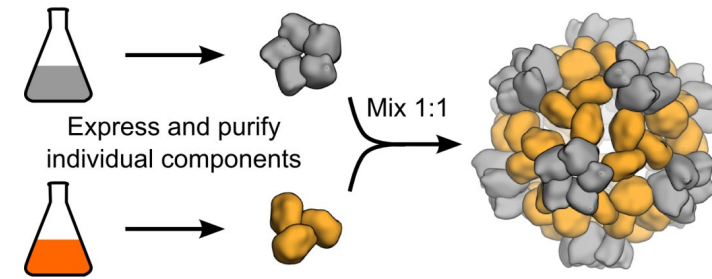
Neil King, Grace Hendricks, Naveen Jasti, Cyrus Haas, Isaac Lutz, Shunzhi Wang, Chris Norn, John Wang, Susan Kleinfelter, Elias Kinfu

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UNIVERSITY *of* WASHINGTON

Novel nanoparticles have been designed and validated
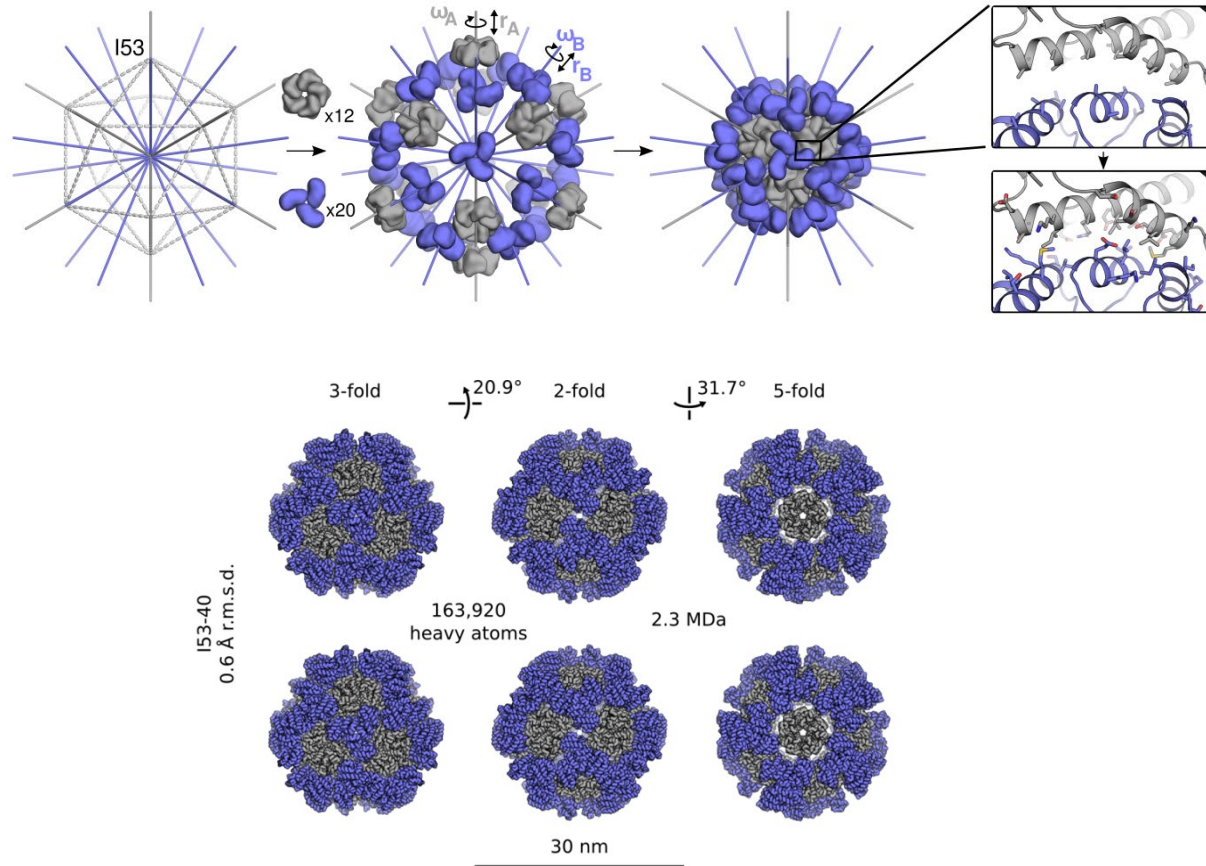
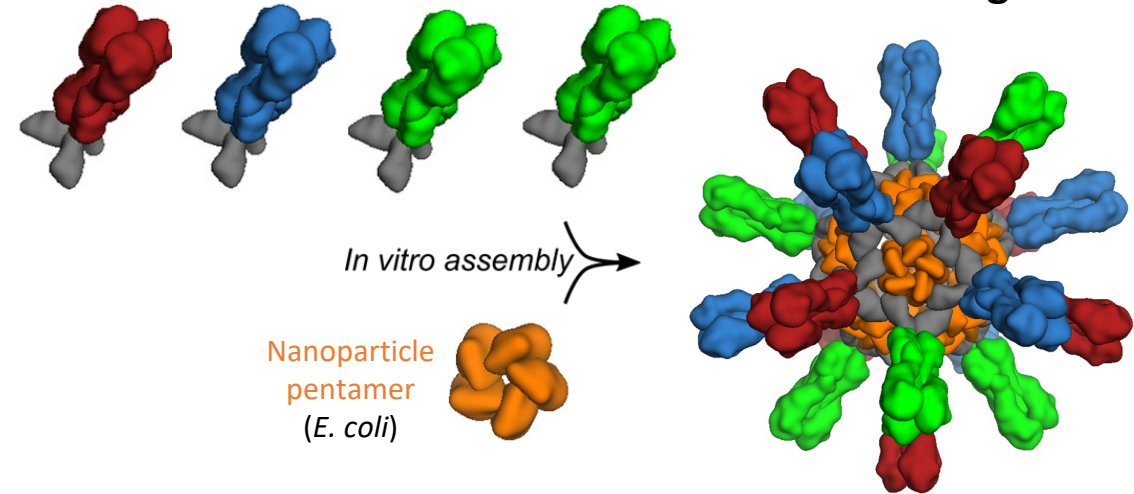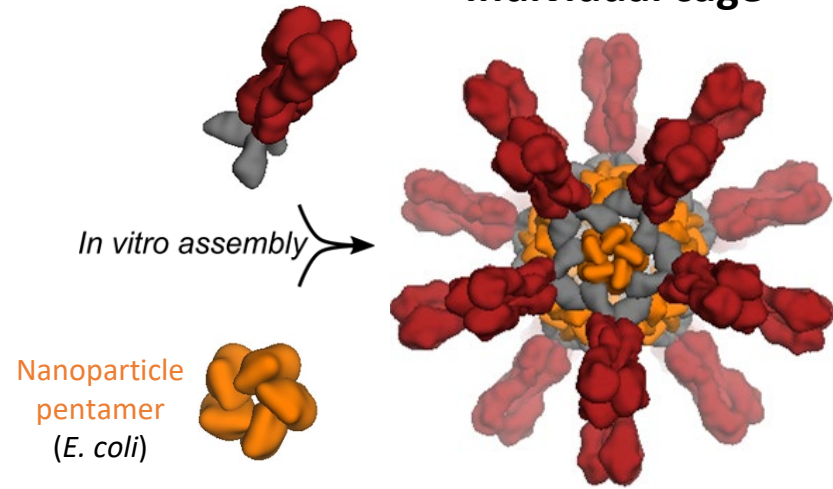**Two-component architecture allows for *in vitro* assembly**

Bale J. et al, 2016
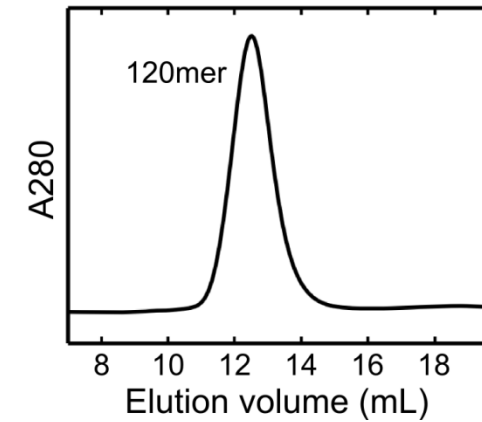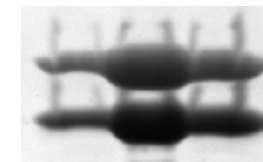
Two component nanoparticles
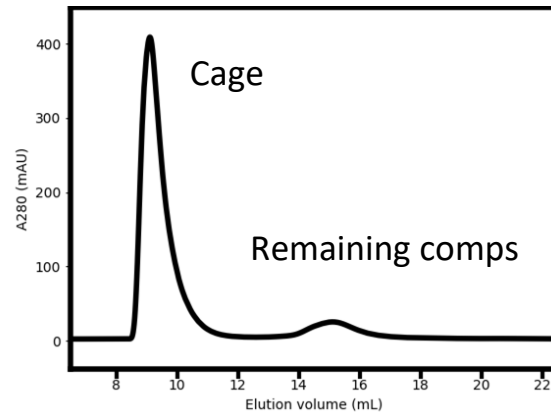

Antigen-nanoparticle trimer fusion (293F)

Individual cage

In vitro assembly

Nanoparticle pentamer (*E. coli*)

Mix of antigen-nanoparticle trimer fusions (293F)

Mosaic cage

In vitro assembly

Nanoparticle pentamer (*E. coli*)

A few examples of what we consider to be successful assemblies

Superose 6 Increase 10/300 GL

Cage

Remaining comps

Cage

Remaining comps

Cage

Remaining comps

B   A
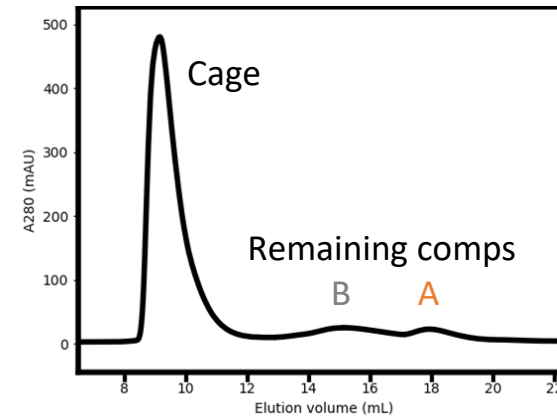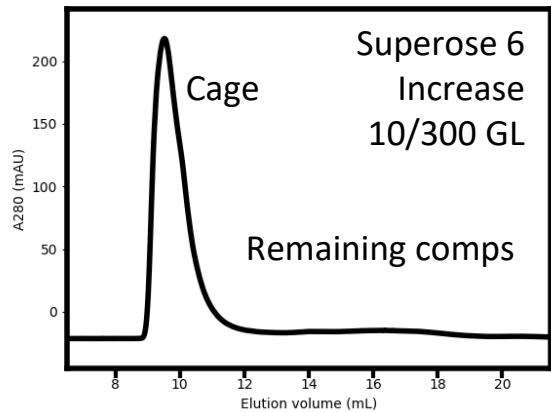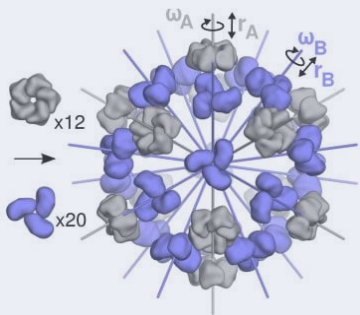
PD question:
Could we get away with not purifying the assembled particles based on size?

Nanoparticle vaccine platform

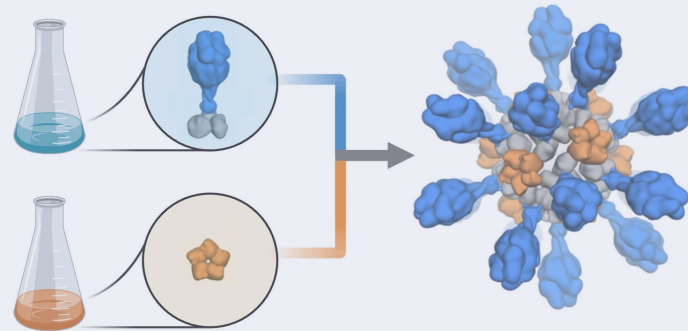| METHODS DEVELOPMENT	TECHNOLOGY PLATFORM	REAL-WORLD IMPACT

**Design of
protein self-assembly**

King et al. *Science*, 2012; King et al. *Nature*, 2014; Bale et al. *Science*, 2015; Bale et al. *Science*, 2016; Hsia et al. *Science*, 2016; etc.

**Self-assembling
nanoparticle immunogens**

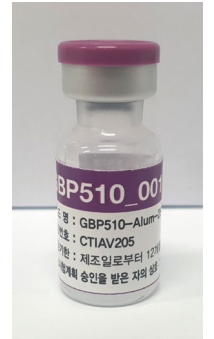Marcandalli et al. *Cell*, 2019; Brouwer et al. *Nature Commun*, 2019; Walls et al. *Science*, 2020; Antanasijevic et al, *PloS Pathology*; Walls et al, *Cell* 2020

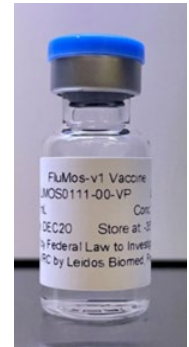**GBP510** **SARS-CoV-2 vaccine**
– *Phase 3 (SK bioscience) and Phase 1 (Icosavax)*
– *$173M in follow-on funding from CEPI*
– *Planned distribution through COVAX*

FluMos-v1 **Supraseasonal flu vaccine**
– *Phase 1 (NIAID)*

IVX-121 **RSV vaccine**
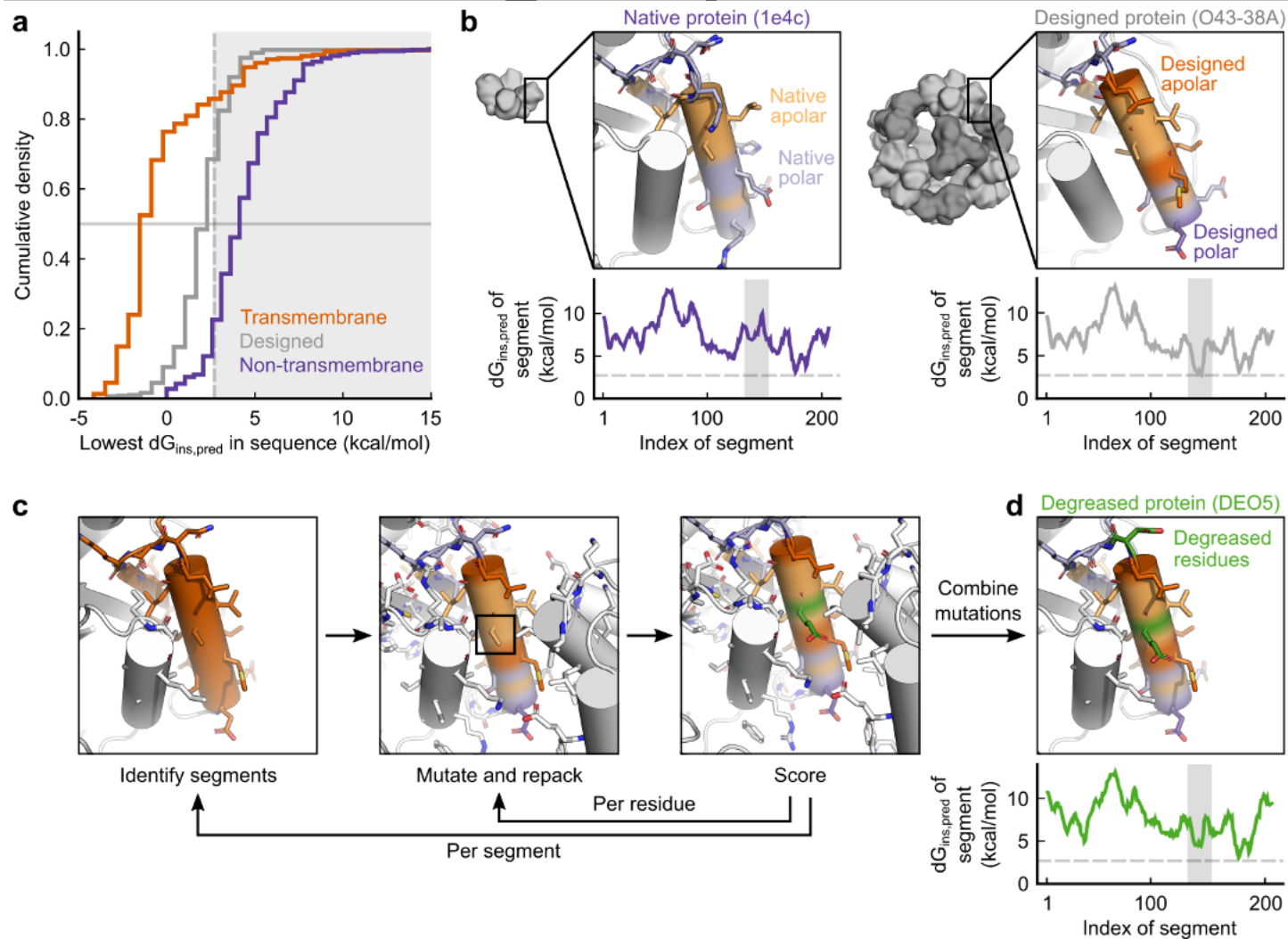– *Phase 1 (Icosavax)*

2012 – 2016   2016 – 2020   2021

nature
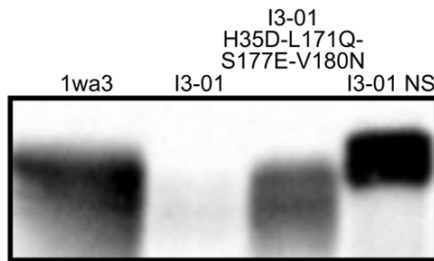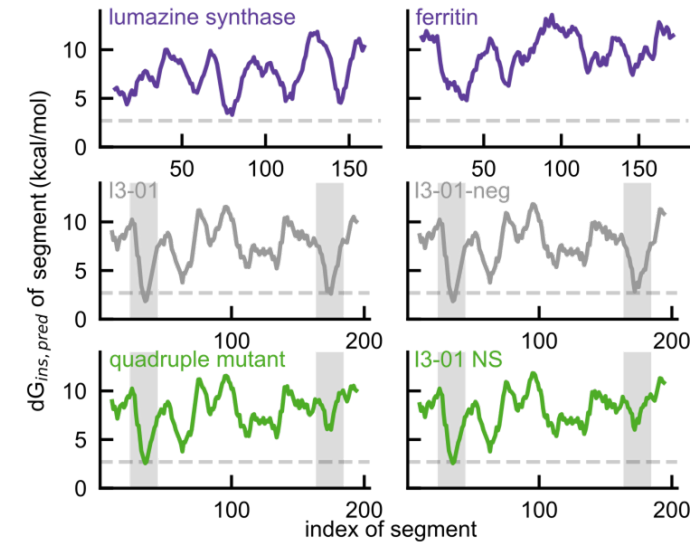*"Tiny particles could make a powerful COVID vaccine"* Sep. 23 2021

First-generation mRNA-launched nanoparticles

Cryptic transmembrane domains reduce secretion of designed proteins

Retroactive "Degreasing" of a designed protein nanoparticle improves secretion yield
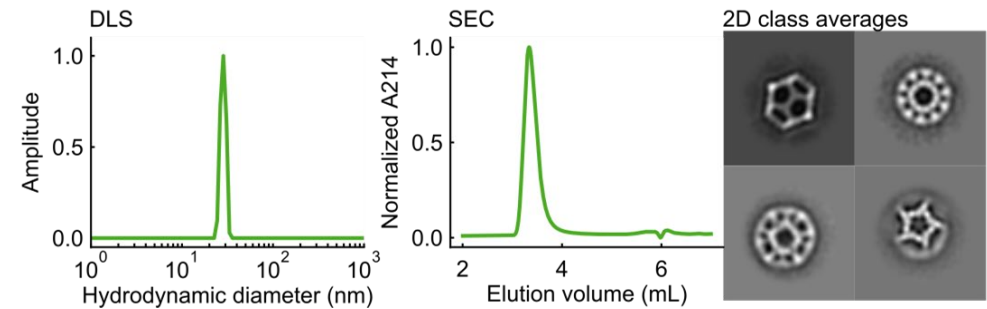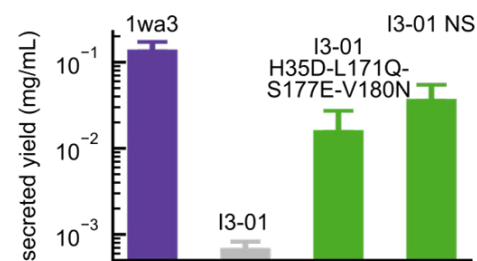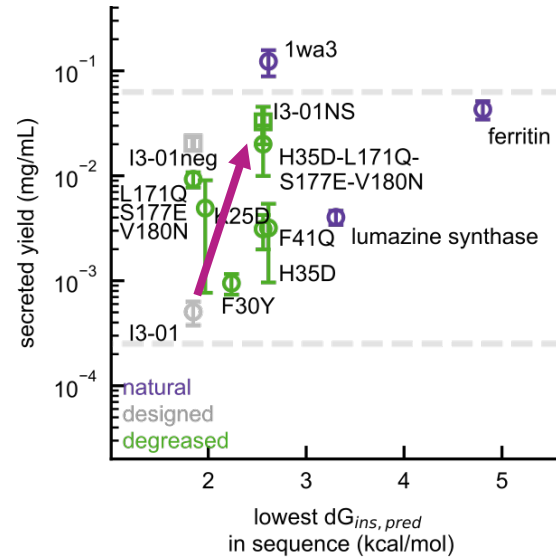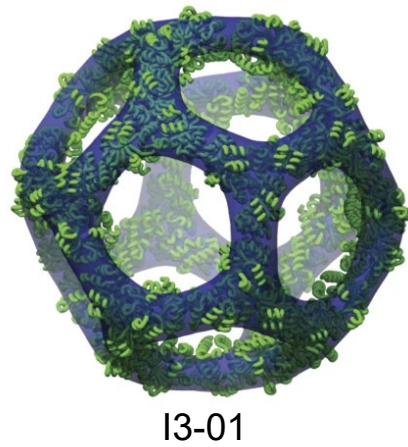
We are now designing new mRNA-launched nanoparticle scaffolds that are tailored to display specific antigens

De novo nanoparticle scaffold design by Monte Carlo tree search with reinforcement learning

monomer:

assembly:

J. Wang, S. Kleinfelter, E. Kinfu

We compared mRNA-launched nanoparticle vaccines to membrane-anchored spike and secreted RBD trimer

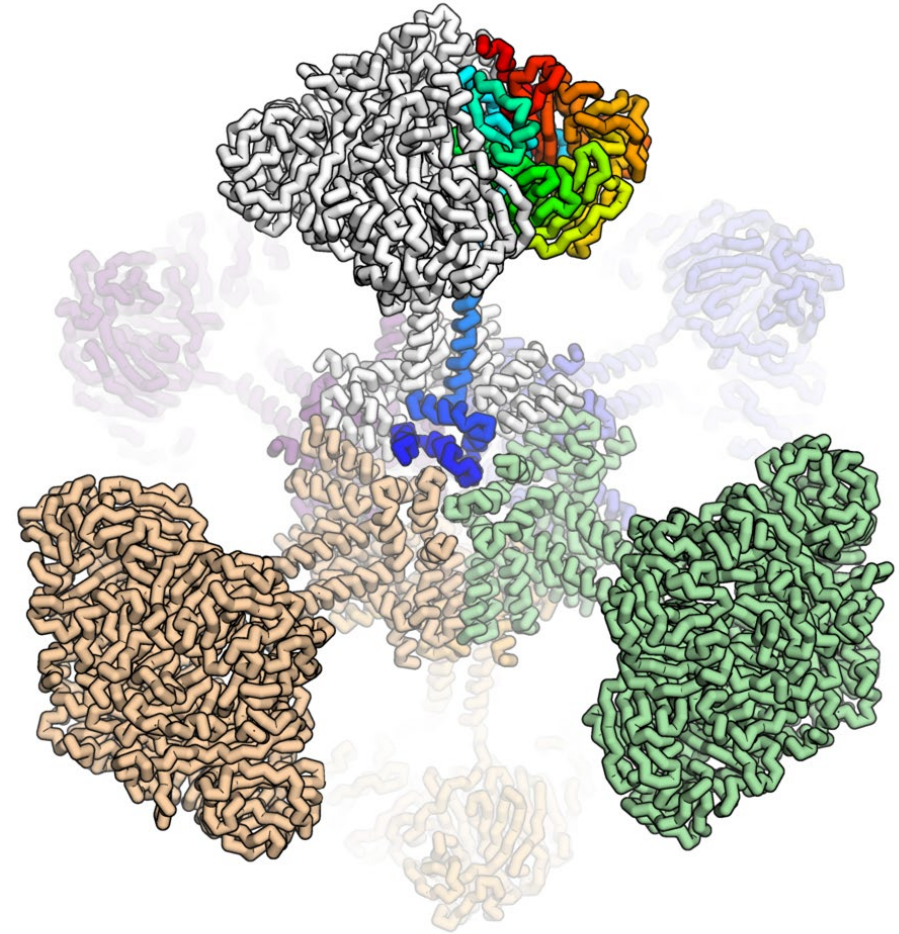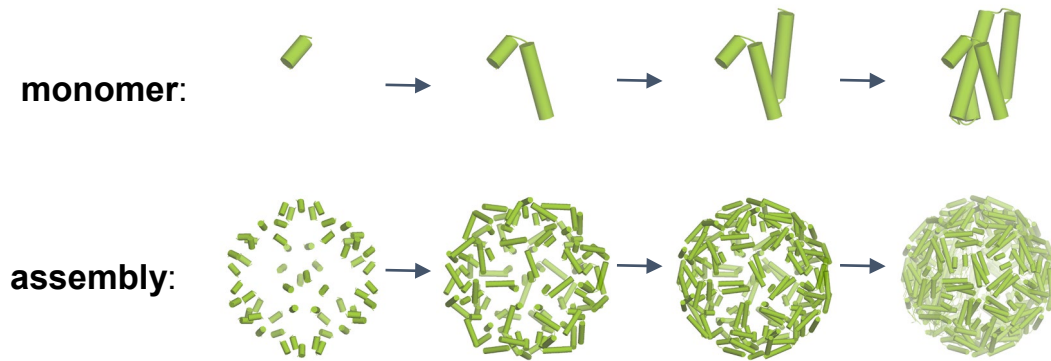| Protein	Format	Valency
Rpk9-I3-01	Nanoparticle	60
Rpk9-I53-50A	Soluble trimeric RBD	3
S-2P	Soluble spike	3
HexaPro	Soluble spike	3
Rpk9-I53-50	Nanoparticle	60
I53-50	Neg. Control	0

mRNA-LNP	Format	Valency
Rpk9-I3-01	Nanoparticle	60
Rpk9-I53-50A	Soluble trimeric RBD	3
S-2P trimer	Membrane-anchored spike	Surface
Empty LNP	N/A	N/A

Weeks: 0 — 2 — 3 — 5

D. Ellis, Y. Tam, C. Treichel, E. Soberg
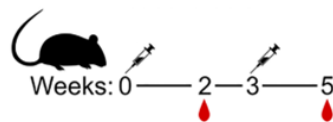
Secreted RBD-I3-01 nanoparticles are several-fold more potent than membrane-anchored spike and secreted RBD trimer

D. Ellis, Y. Tam, C. Treichel, E. Soberg, L. Walls, M.J. Navarro, D. Veesler
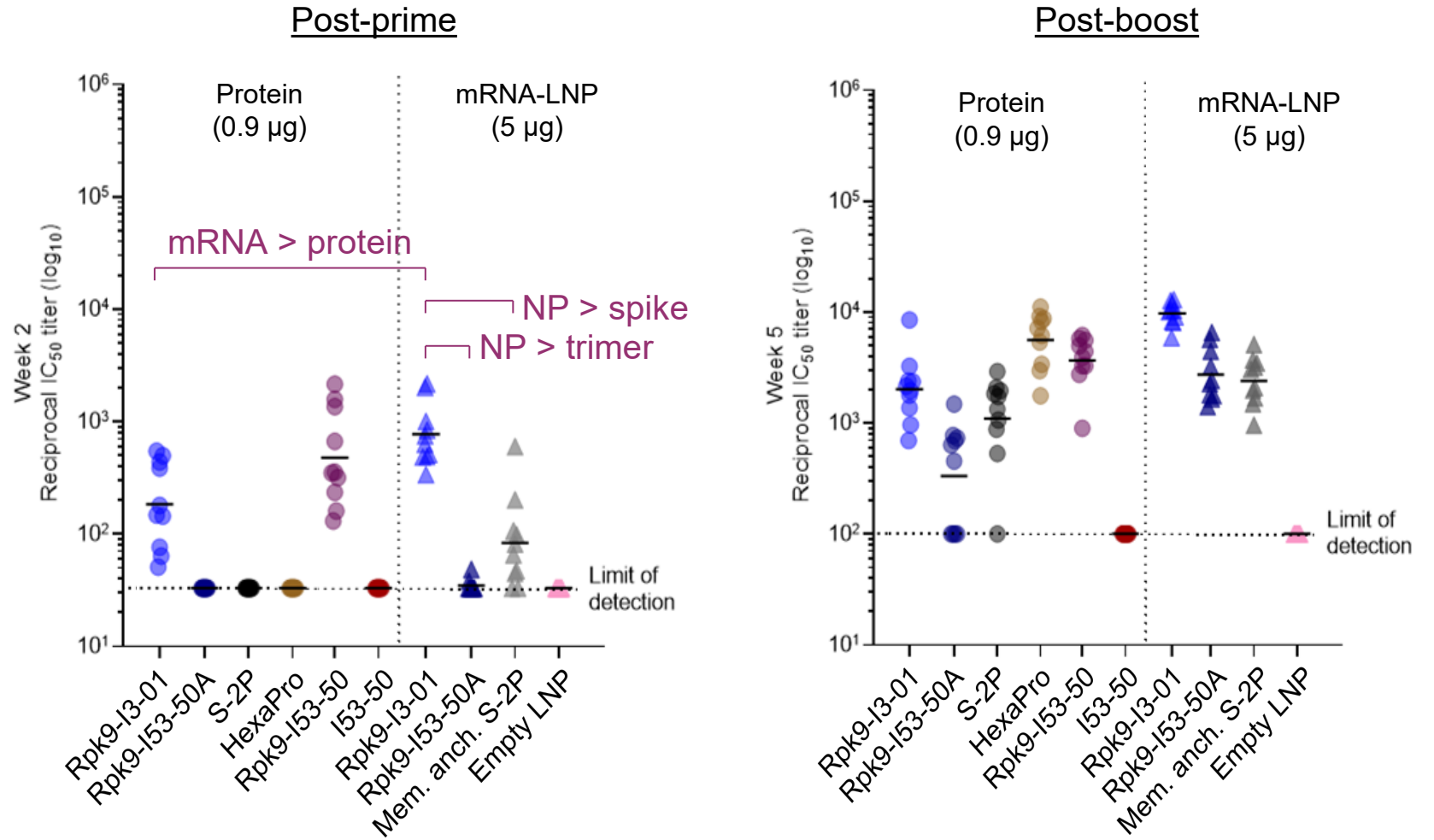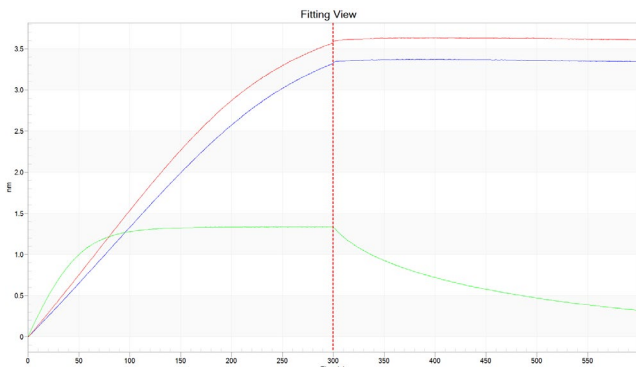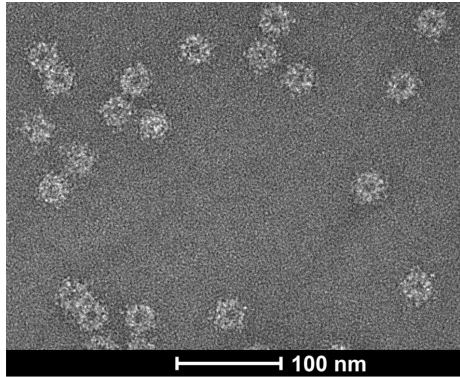
Methods development for the next generation of mRNA-launched nanoparticles
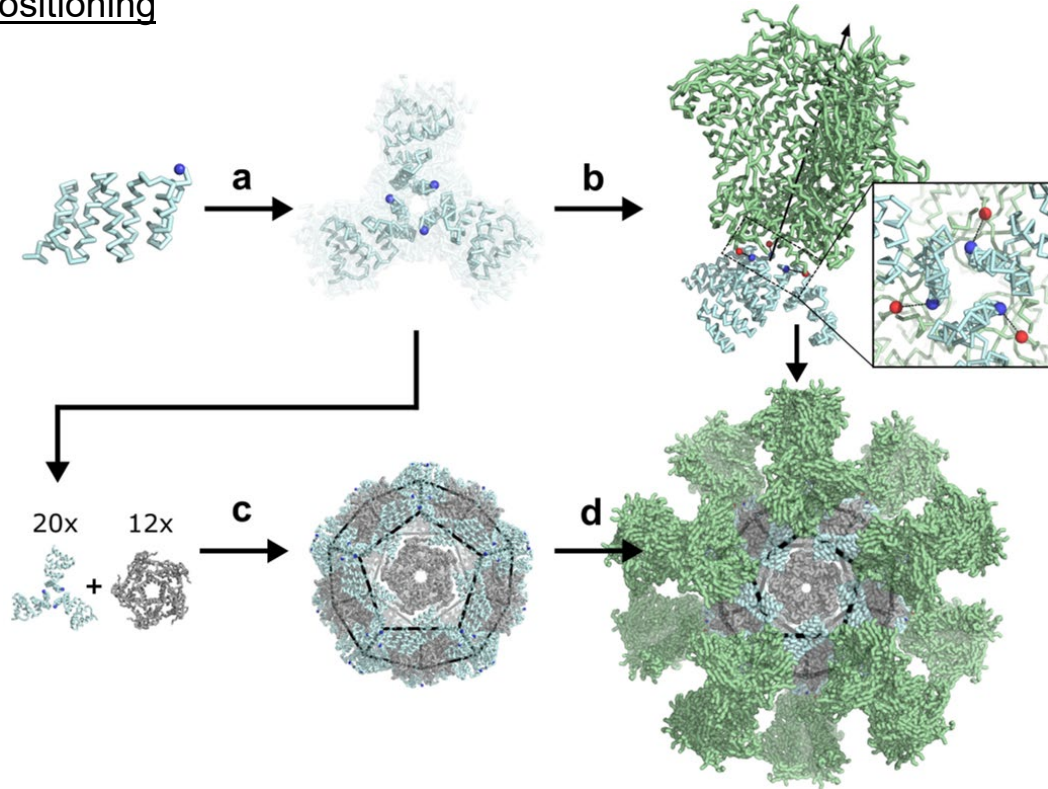
Goal: Develop methods that enable the rapid generation of mRNA-launchable nanoparticles tailored to specific antigens

e.g., design of trimeric building blocks with optimal terminus positioning

Problem:
- few one-component nanoparticles
- even fewer with optimal stability, solubility, and secretability
- only one with termini appropriate for class I fusion protein display
- limited design space for one-component nanoparticles
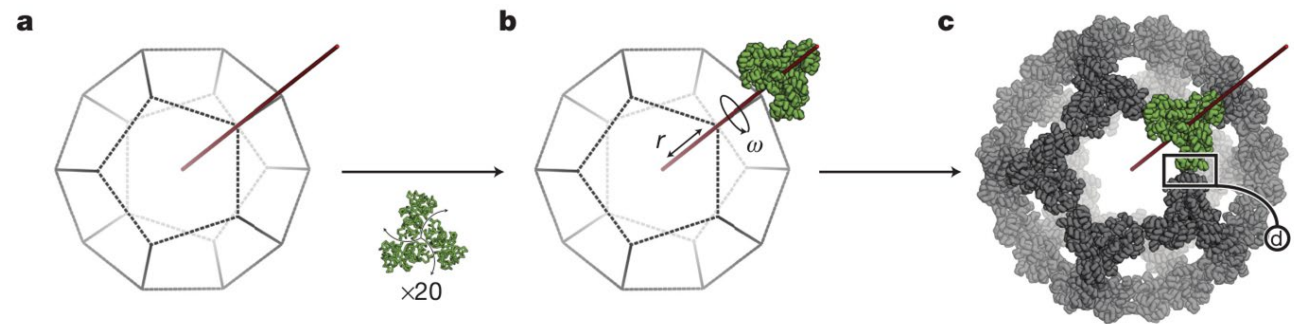
Solutions:
- Dramatically expand the oligomeric scaffold set
- De novo design

Ueda et al. (2020) *eLife*
Hsia et al. (2016) *Nature*

Approach 1: dock & design using AF2-predicted oligomers from thermophilic organisms

① Search for C3s from the PDB
Filters: resolution, helical structure, species of origin, no membrane proteins → **PDB Seeds**

② Search for all sequences with >50% identity to the seed
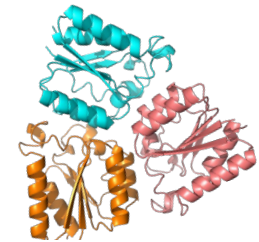**Filters:** thermophiles (>55ºC optimal growth) → **Thermophilic C3 Sequences**
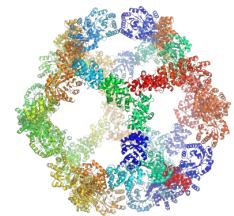
③ Generate predictions for C3 structures
Filters: pLDDT > 85; PAE < 15 →

AF2 Predictions

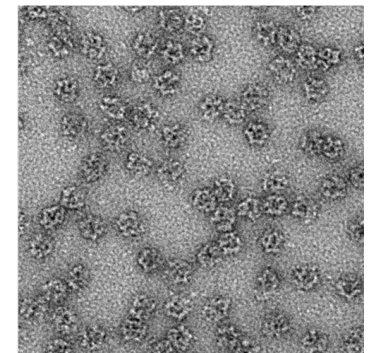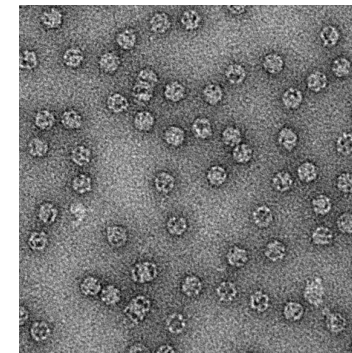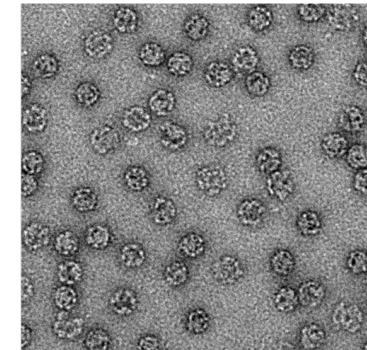④ Use **RPXDock** to dock AF2-predicted oligomers in target geometries. →

⑤ Use **MPNN** to design interfaces that drive nanoparticle assembly.

Larger universe of one-component nanoparticles

N Jasti, C Haas

Three weeks from DNA order to nsEM-confirmed nanoparticles

Order eblocks → Cowboy Biochemistry → SDS-PAGE → HPLC and DLS → nsEM

D12

UV280 Absorbance vs time (min)

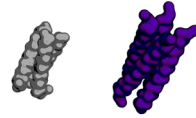*expected; similar to positive control*
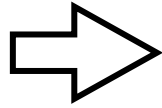
1H7

1D7

1E9
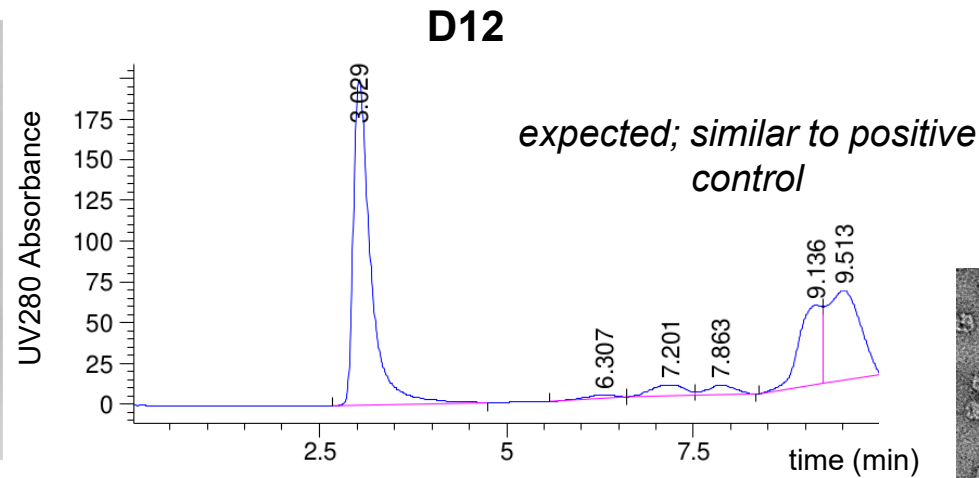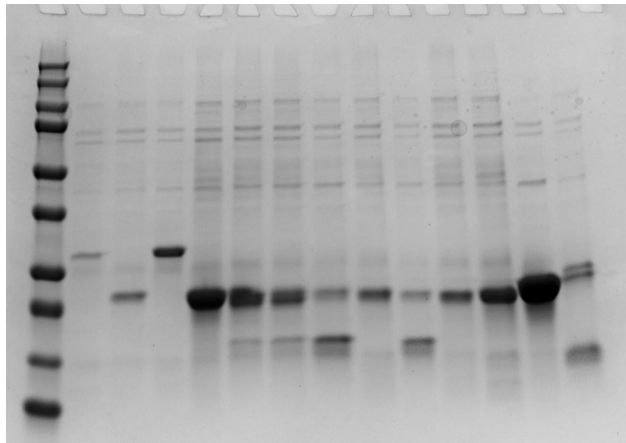
Approach 2: top-down capsid design
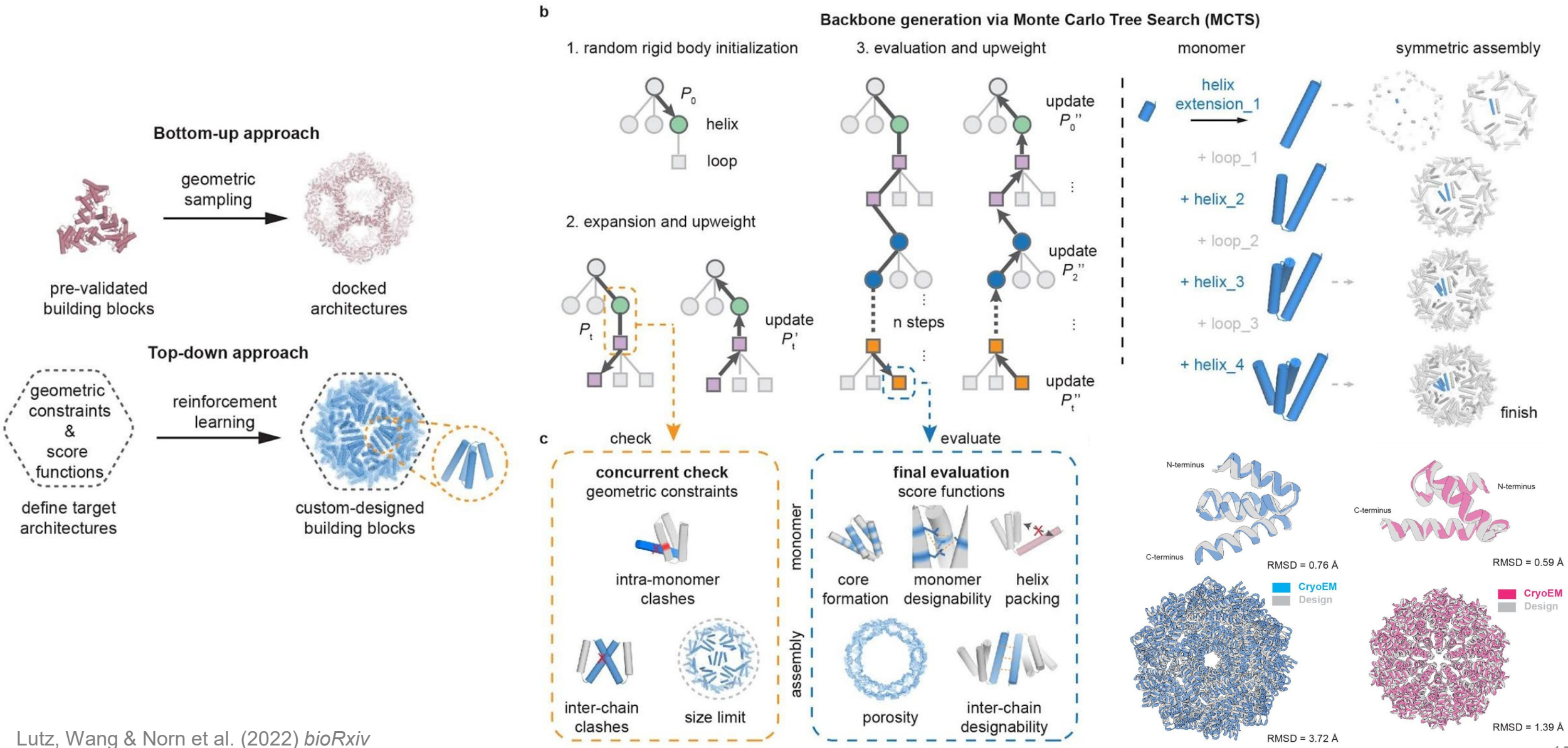
Leveraging symmetry can enable new types of antigen-tailored scaffold design
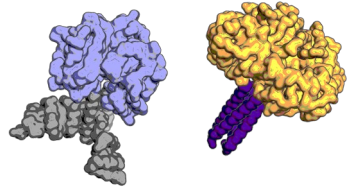
Flu **HA (head)** and **NA** with compatible oligomers

Relevant oligomerization domains
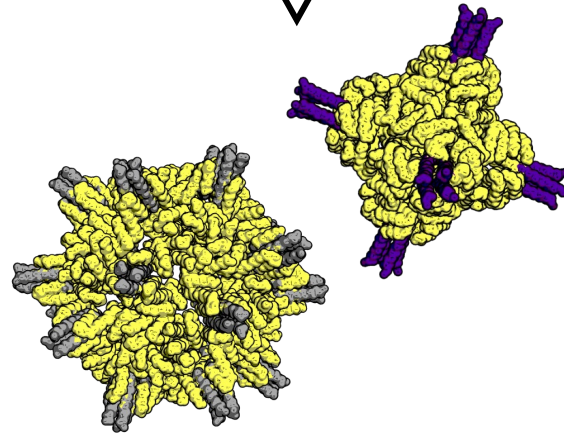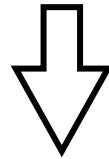
New cages that complete desired geometry
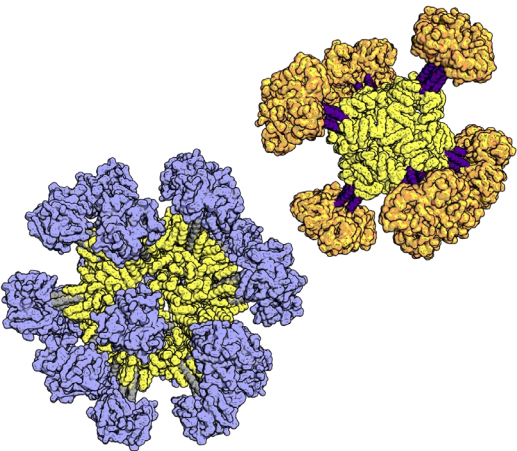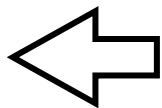
Antigen-tailored mRNA-launched nanoparticle vaccines

- Previously characterized antigen-bearing components serve as starting points

- One-component scaffold design enables straightforward genetic delivery

- Multi-phase characterization (bacterial bare cage, mammalian bare cage, mammalian antigen cage) optimizes throughput

J.Y. Wang, S. Kleinfelter, E. Kinfu

Conclusions and outlook

- Computational protein design allows the generation of novel self-assembling proteins that can be customized at the atomic level

- Computationally designed protein nanoparticles are a clinically validated vaccine platform

- Computational design can be used to generate novel secreted nanoparticle immunogens that elicit potent neutralizing antibody responses

- **We have only scratched the surface**… continued methods development will lead to better and better technology platforms

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Ralph Baric
Tim Sheahan
Alex Schäfer
Victor Tse

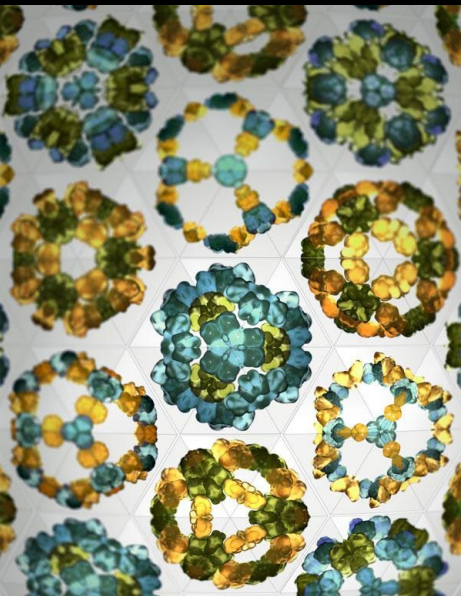**SK bioscience**
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Jin Park

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Ying Tam

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Holger Kanzler
Chris Karp
Harry Kleanthous
Karen Makar
Lynda Stuart

Questions?

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