Design of self-assembling protein nanomaterials as next-generation vaccine scaffolds
Protein self-assembly enables specialized functions; our goal is to design new self-assembling molecular machines.
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.
Design allows parts (proteins) to be built for a specific purpose
We have developed a general computational method for designing new self-assembling protein nanomaterials.
The designed interfaces have features resembling natural protein-protein interfaces.
The method enables the design of novel protein nanomaterials with atomic-level accuracy

Backbone RMSD: 1.1 Å

Crystal structure

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

“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.
A wide variety of symmetric architectures can be designed using the approach (e.g., 2D layers)
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.
The 120-subunit, megadalton-scale structures were designed with atomic-level accuracy.
The nanoparticles are highly resistant to thermal stress

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.
Assembly occurs on the timescale of seconds to minutes
Two-component protein nanoparticles: a versatile platform for multivalent display

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

**Expression, purification, and quality control**
- Expression, purification, and quality control can be performed independently on distinct building blocks

**Antigen valency/copy number**
- Antigen valency/copy number can be controlled by including unmodified components during *in vitro* assembly

**Distinct antigens/costimulatory proteins**
- Distinct antigens/costimulatory proteins can be scaffolded in defined ratios

**Complex antigens**
- Complex antigens that fail as genetic fusions to nanoparticle subunits can be labeled with a small adaptor tag

**Adaptor-mediated labeling**
- Post-assembly labeling
- Rapid prototyping of functional domain/nanoparticle combinations

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*In vitro assembly*

1. [Image of nanoparticle assembly]

2. [Image of nanoparticle assembly]

3. [Image of nanoparticle assembly]
An anti-CD20 scFv–I53-50A fusion protein can be produced in good yield.
In vitro assembly allows control over scFv valency

100% scFv-trimer

Nanoparticles
Excess trimer

100% scFv-I53-50A.1
Superose 6

50% scFv-trimer

Nanoparticles
Excess trimer

50% scFv-I53-50A.1
Superose 6

Full-valency nanoparticle

Half-valency nanoparticle

SDS-PAGE

100% scFv-trimer
50% scFv-trimer

scFv-I53-50A.1 (trimer)

I53-50A.1 (trimer)

I53-50B.4PosT1 (pentamer)

J. Burrows
SpyCatcher-SpyTag is a molecular adaptor capable of selective and stable labeling.
Conjugating SpyTag-GFP to purified SpyCatcher-I53-50A enables *in vitro* assembly of nanoparticles with variable GFP valencies.

I53-50A–SpyCatcher genetic fusion + SpyTag-GFP = GFP-labeled trimer

Full valency

I53-50A–SpyCatcher genetic fusion + SpyTag-GFP = Full valency

Partial valency

I53-50A–SpyCatcher genetic fusion + SpyTag-GFP + SpyTag-GFP = Partial valency
Conjugating SpyTag-GFP to purified SpyCatcher-I53-50A enables \textit{in vitro} assembly of nanoparticles with variable GFP valencies.
A pilot immunization study in mice revealed size- and valency-dependent immunogenicity.

Antigen: consensus L2 peptide

HPV 16: GTGGRTGYIPLGTRPPTATDT
HPV 18: GTGGRTGYIPLGGRSNTVVDV
Consensus: GTGGRTGYVPLGTRPPTTVVDV

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
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