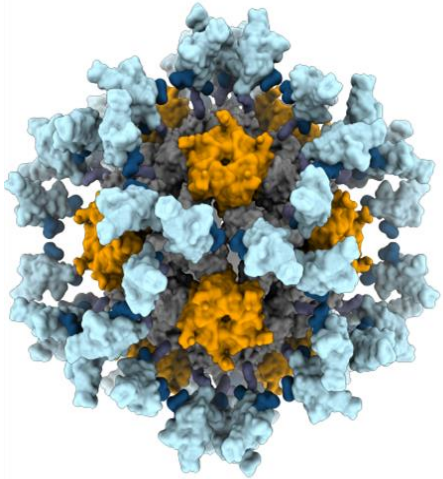


Protein Nanoparticle Vaccines –
A Platform Enabling Rapid Translation from Design to Clinic

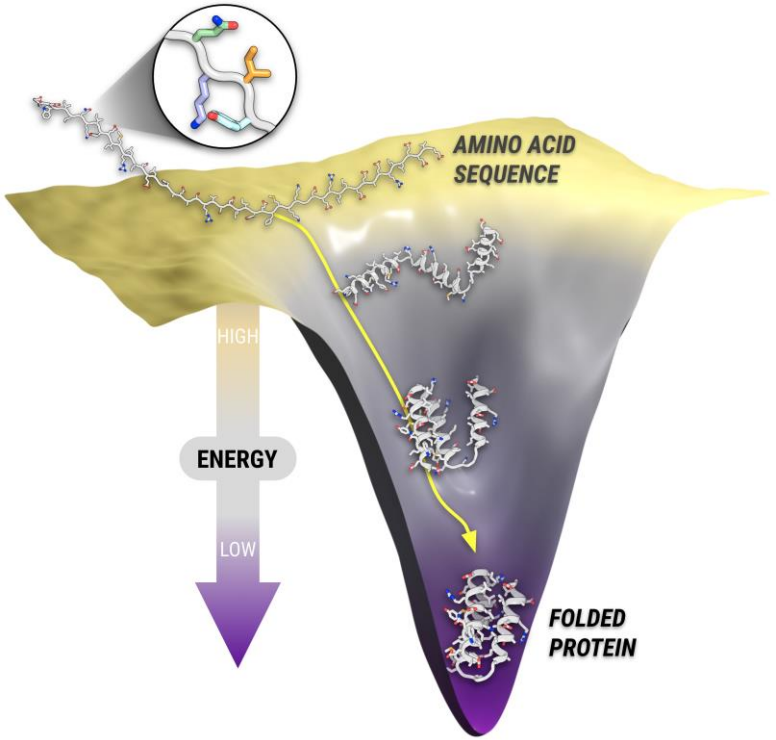
WCPB 2022

Lauren Carter

INSTITUTE FOR Protein Design

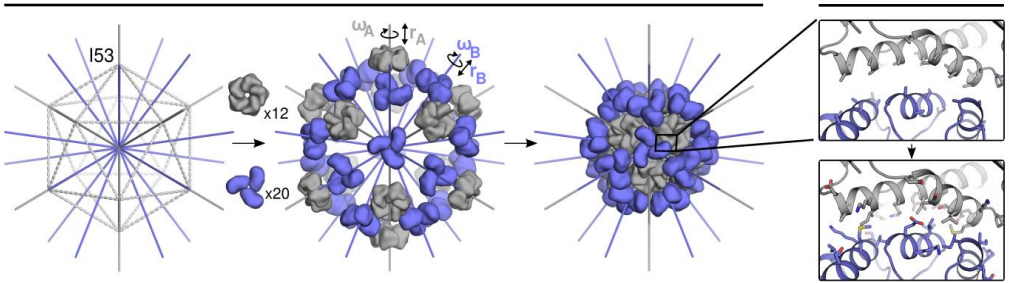
UNIVERSITY of WASHINGTON

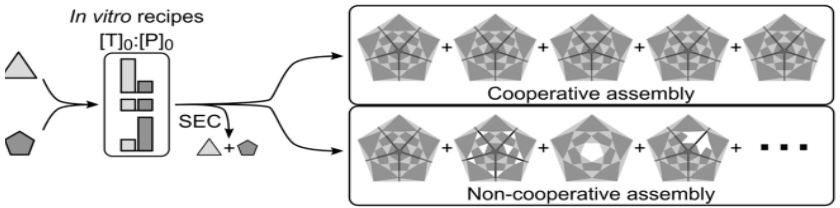
IPD and RoseTTAFold – New Frontiers in Computational Protein Design

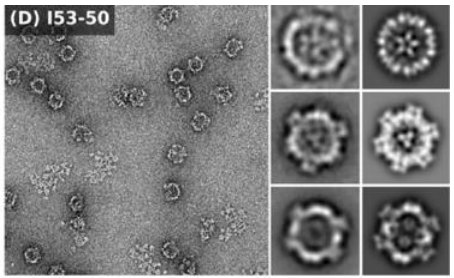


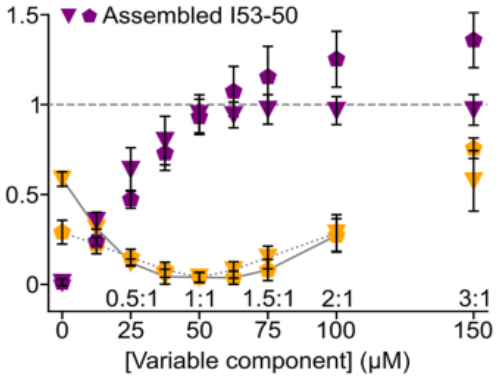
The driving force for protein folding is the burial of hydrophobic residues in the protein's core, secluded from the solvent. Absolute energy minima corresponds to compactly folded structure.

Nanoparticle Scaffolds are Designed to Specifically and Cooperatively Assemble into Well Defined, Highly Stable Particles









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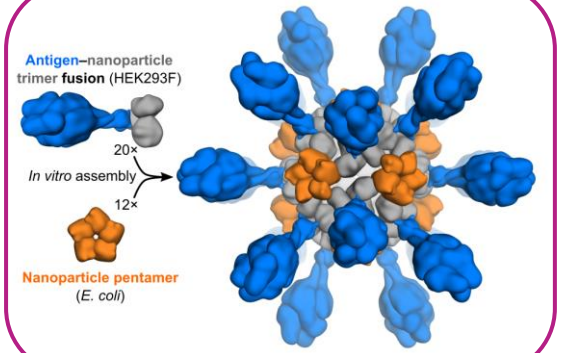
King NP, et al. (2014) *Nature* **510**:103-8.
Hsia Y et al. (2016) *Nature* **535**: 136-9.
Bale J, et al. (2016) Science 353: 389-94.
Ueda G & Antanasijevic A, et al. (2020) eLife 9:e57659.

Complete and cooperative in vitro assembly of computationally designed self-assembling protein nanomaterials. Adam J. Wargacki, Neil P. King . Nature Communications

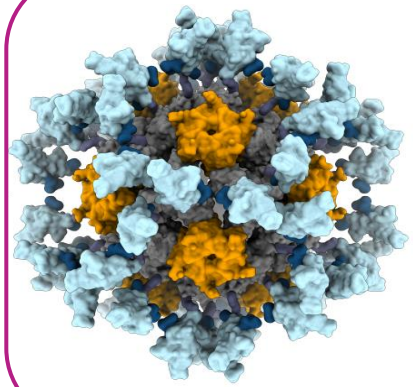
Designed two-component nanoparticles are a robust and versatile platform for multivalent antigen display

RSV



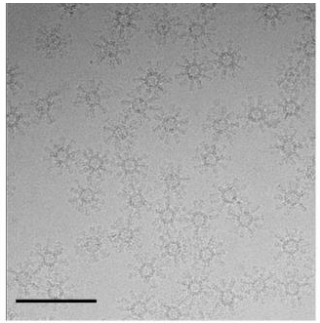
Single Antigen Display
Icosavax

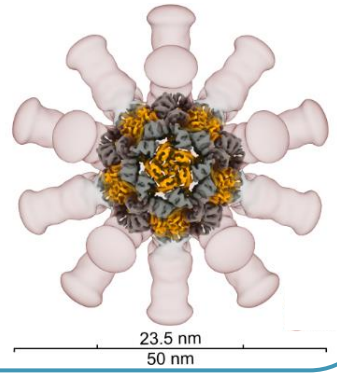
SARS-CoV-2



Mosaic Antigen Display
SK BioSciences
Icosavax

Influenza

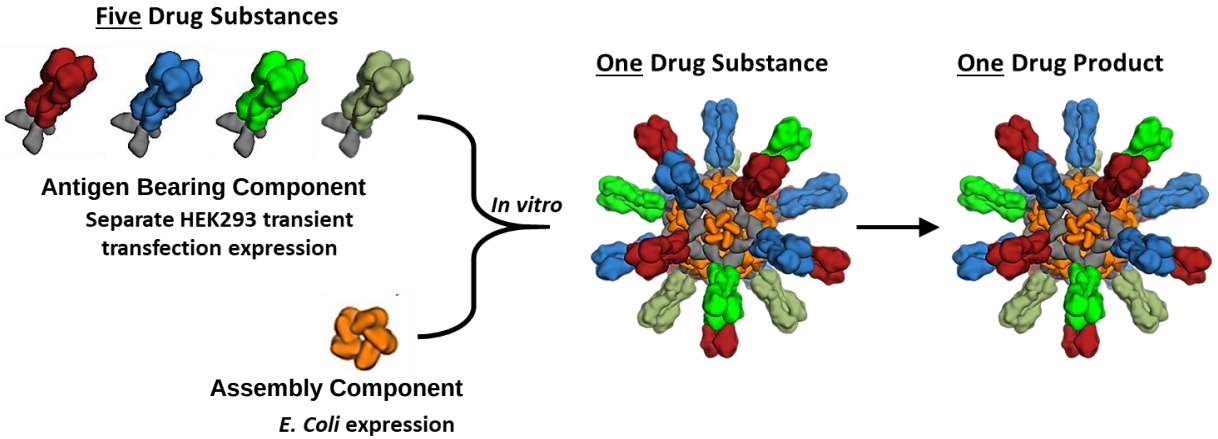




Mosaic Antigen Display
VRC-VPP (NIH)

Marcandalli J, et al. (2019) *Cell* **176**: 1420-31.
Brouwer PJM, et al. (2019) *Nat. Comm.* **10**:4272.
Martin J, et al. (2020) *npj Vaccines* **5**:72.
Antanasijevic A, et al. (2020) *PLOS Path*. 16:e1008665.
Ueda G & Antanasijevic A, et al. (2020) *eLife* **9**:e57659.
Walls AC & Fiala B et al. (2020) *Cell* **183**: 1367-82.
Boyoglu-Barnum S & Ellis D, et al. (2021) *Nature* **592**: 623-628.

cGMP Manufacture of Example Mosaic Nanoparticle Vaccine – Many Drug Substances, One Drug Product



Antigen Bearing Component
- Upstream - Transient or CHO
- Downstream –VI/ Standard Chromatography /VF/UFDF

Assembly Component
- Upstream – E. coli
- Downstream – Standard Chromatography/UFDF

Nanoparticle Assembly
- In Vitro mixture of components at specific molar ratio
- UF/DF

Example of Mammalian-expressed Antigen-Bearing DS Specifications

Release

Attribute	Assay	Target Value
General	Appearance, USP<1>	Clear, colorless, no turbidity
	pH, USP<791>	6.8-7.2
Concentration	A280	10-12
Purity	Gel Electrophoresis (GXII) - Reduced	Report result
Aggregation/Purity	Size Exclusion Chromatography (SEC)	Report result
Identity	ELISA - Ag 1	TBD
	ELISA - Ag 2	TBD
	ELISA - Ag 3	TBD
	ELISA - Ag 3	TBD
Potency	ELISA[2]	TBD
Particle Size	Dynamic Light Scattering	Report result
Safety	Endotoxin, USP<85>	≤ 10 EU/µg
	Bioburden, USP<61>	< 1 CFU/10mL

Characterization

Attribute	Assay	Target Values
Residuals	Host Cell DNA by qPCR (HEK)	Report result
	Host Cell Protein by ELISA (HEK)	Report result
	Valproic Acid	Report result
Molecular Weight	SEC-MALS	Report result
Sequence	Peptide Mapping	Report result

Example of Microbial-expressed Assembly DS Specifications

Release

Attribute	Assay	Target Value
General	Appearance, USP <1>	Clear, colorless, no turbidity
	pH, USP<791>	6.8-7.2
Concentration	A280	10-12
Aggregation/Purity	SEC	Report result
Identity	RPLC	Conforms to Reference
Particle Size	Dynamic Light Scattering	Report result
Safety	Endotoxin, USP<85>	≤ 5.8 EU/µg
	Bioburden, USP<61>	< 1 CFU/10mL

Characterization

Attribute	Assay	Target Values
Residuals	Host Cell DNA by qPCR (E. coli)	Report result
	Host Cell Protein by ELISA (E. coli)	Report result
Molecular Weight	SEC-MALS	Report result
Sequence	Peptide Mapping	Report result

Example of Mosaic Nanoparticle DS Specifications

Attribute	Assay	Target Value
General	Appearance, USP<1>	Clear, colorless, no turbidity
	pH, USP<791>	6.8-7.2
Concentration	A280	2-4
Purity	SEC	>99%
Relative Potency	ELISA (evaluating binding partners)	50-150% of reference
Identity	RPLC (unique chromatography separation with 4 peak profile)	Conforms to Reference
	SEC (size to conform to cage formation)	Conforms to Reference
Particle Size	Dynamic Light Scattering	Conforms to reference
Safety	Endotoxin, USP<85>	<5 EU/ug
	Bioburden, USP<61>	< 1 CFU/10mL

[1]TBD – To be determined

Speed to Patient – Advantages of Protein Nanoparticles

- USP and standard platform analytical methods are applicable
- Upstream and Downstream Processes are exceptionally standard
  - Expression, Viral Inactivation, Chromatography, UF/DF
- Mosaic Nanoparticles tend to be highly potent
  - low dose required → smaller batch size →manufacturing flexibility
- **Well characterized scaffold of Nanoparticle Components allows for rapid iteration of antigens**

Speed to Patient – Rapid Timeline for IPD/SK SARS-CoV2 NP Vaccine

- Feb 2020 – Wuhan1 constructs ordered
- May -Lead Candidate Selection
- June - NHP studies → Dose and Adjuvant
- July - Transfer of WCB to SK
- Aug –Nov - PD
- December 2020 - cGMP manufacture
- January 2021 - Phase 1/2 trials initiated
- June - IND filing (South Korea MFDS)
- July 2021 - Phase 3 enrollment

https://covid19.trackvaccines.org/vaccines/81/

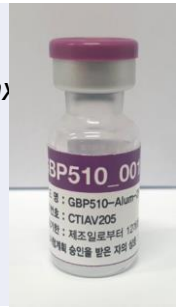
And now for the challenges

- Mosaic Nanoparticle Antigen Content assays are dependent on the antigens
  - Highly similar antigens (SARS-CoV2 VOCs) could be difficult to separate using RP/HIC/IEX methods
- Stability of the Nanoparticle Antigen-Bearing Components, DS, and DP is also dependent on the antigens
- Mosaic Nanoparticles require multiple Drug Substances - increases complexity and risk

Challenges Can Be Overcome, Results Have Potential to Impact Global Health

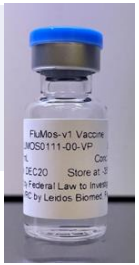
GBP510 **SARS-CoV-2 vaccine**

- *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**

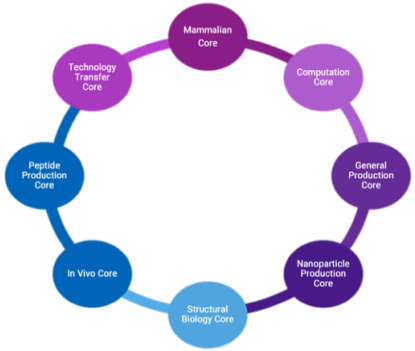
- *Phase 1 (Icosavax)*

Pipeline

Flu – HA mosaic
Pan-coronavirus mosaic
Pan-sarbecovirus mosaic
Malaria – AIV/TBV
ECF
PPRV
RSV
hMPV

Acknowledgements and Thanks

- David Baker
- Lance Stewart
- Neil King
- IPD Cores
- Dan Ellis
- David Veesler



ICOSAVAX

SK bioscience

NIH National Institute of Allergy and Infectious Diseases
Dale and Betty Bumpers Vaccine Research Center

gsk

Seqirus A CSL Company

BILL & MELINDA GATES foundation

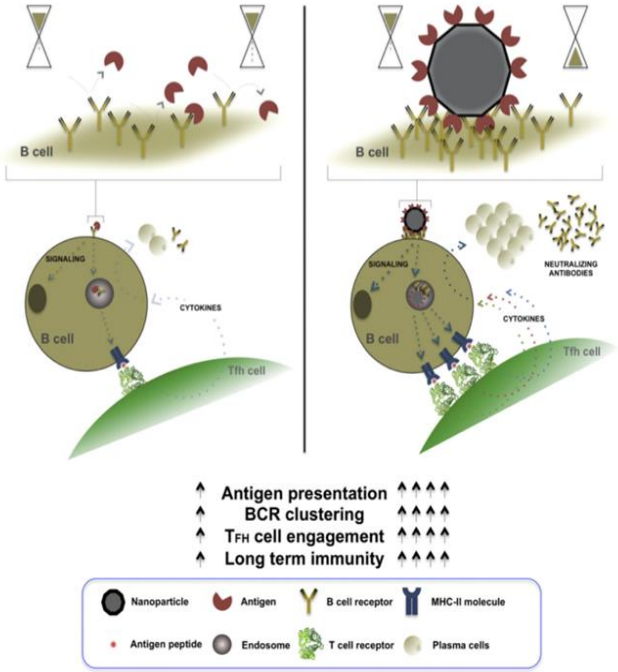
DEFENSE THREAT REDUCTION AGENCY · UNITED STATES OF AMERICA

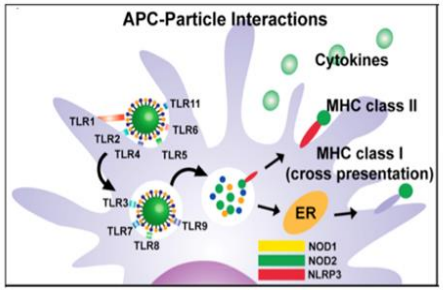
THE AUDACIOUS PROJECT

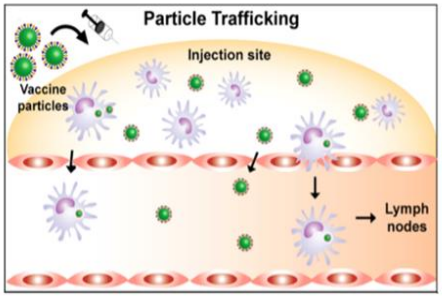
Open Philanthropy Project

Extra slides

Two Display Pathways to Immunity - Mosaic vs

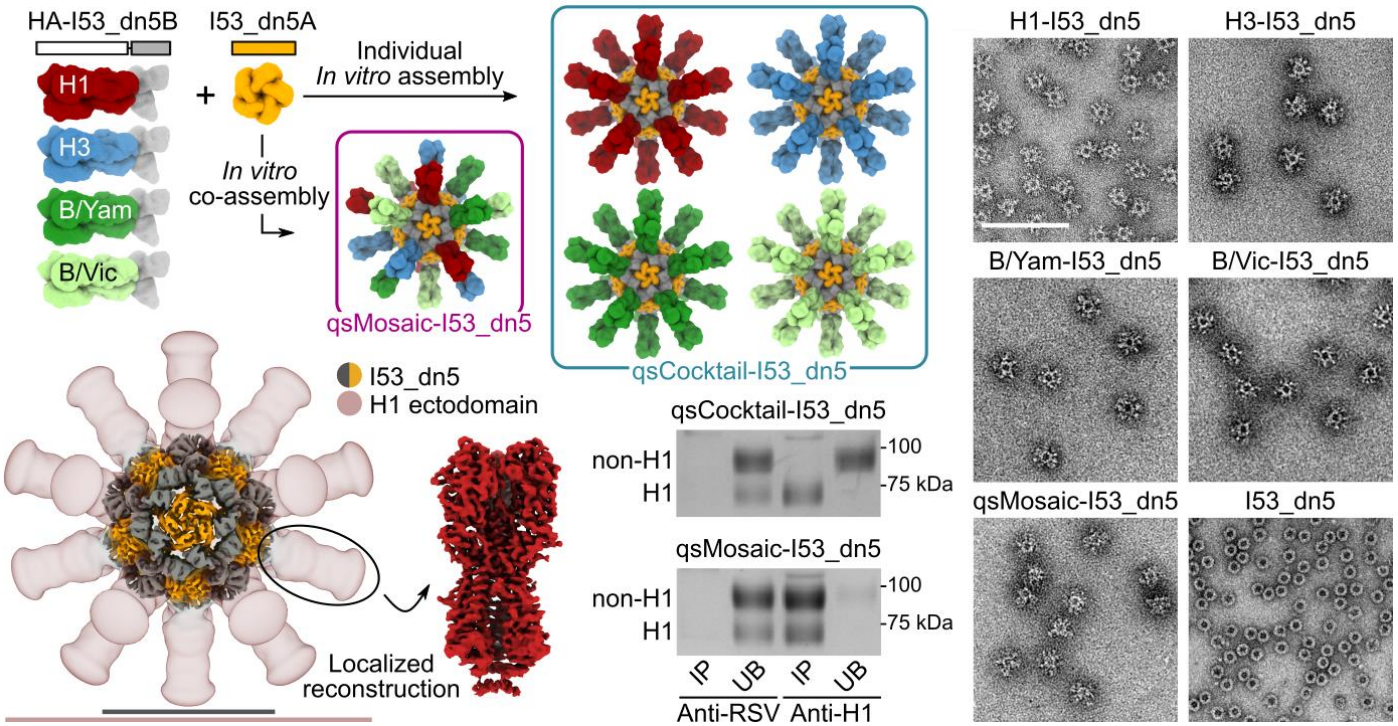




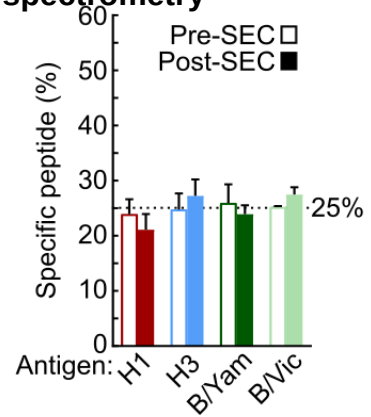


López-Sagaseta J et al. (2016) *Comp. Struct. Biotech. J.* **14**:58-68.
Gause KT et al. (2017) *ACS Nano* **11**:54-68.

Two-component platform allows for modular display of seasonal HA trimers, either as a cocktail of separate particles or as a co-assembled mosaic



Quantification of each HA by peptide mass spectrometry



Boyoglu-Barnum and Ellis et al, Nature (2021)