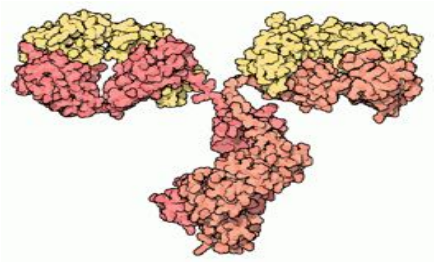
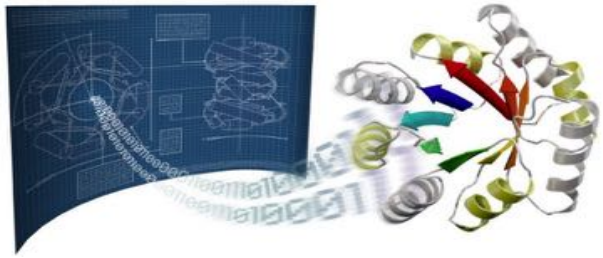




Toward *de novo* design of immune silent protein and peptide therapeutics

Lance Stewart, Ph.D. MBA
Chief Strategy and Operations Officer, UW IPD

FDA – CERSI Collaborative Workshop
"Predictive Immunogenicity for Better Clinical Outcomes"
October 3 and 4, 2018

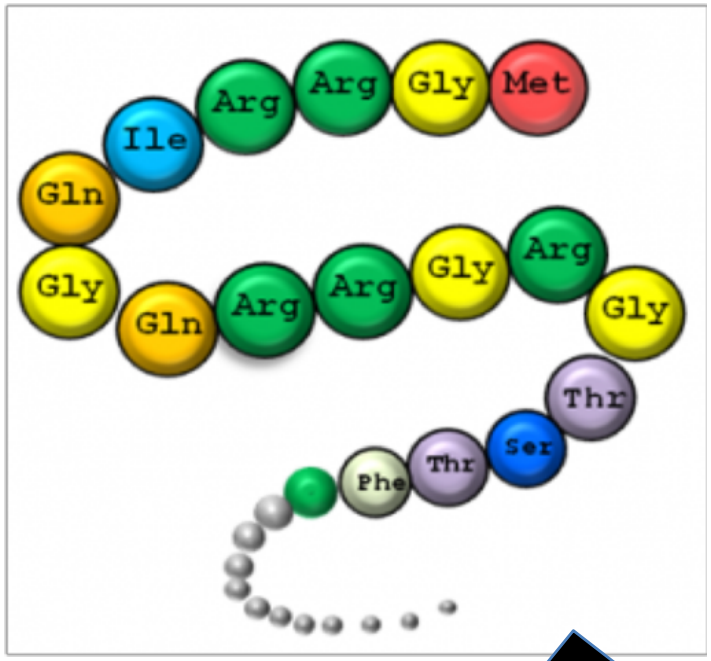
Design a new world of synthetic proteins

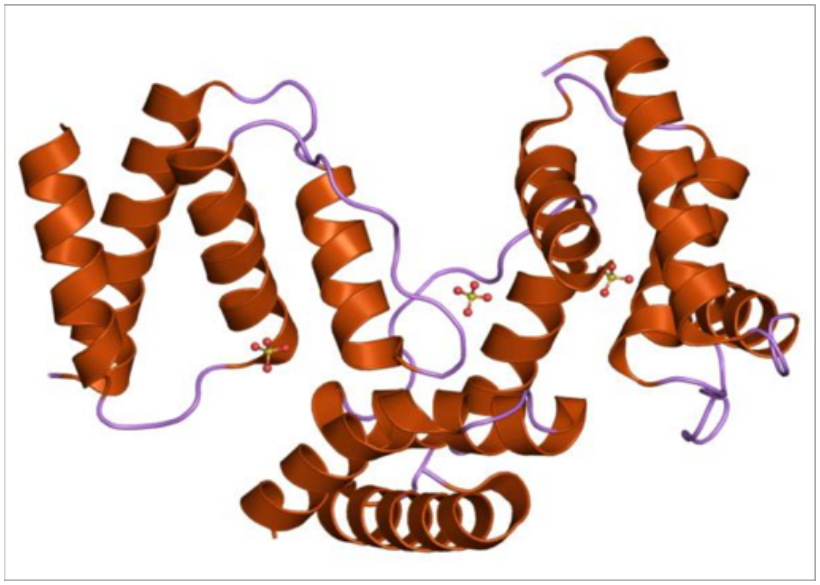
- Founded in 2012 by Dr. David Baker
- Organized within the School of Medicine, Biochemistry
- ~140 Person Umbrella Organization
  - Faculty PIs (Baker, DiMaio, King, Gu, Bradley)
  - WRF Innovation Fellows (21)
  - Translational Investigators (1)
  - Research Staff (17)
  - Postdocs (27)
  - Graduate Students (36)
  - Admin (6)
  - Undergrads (27)
  - High School Student (1)
- Using 10% of all UW Internet Traffic

INSTITUTE FOR Protein Design
UNIVERSITY of WASHINGTON

PROTEIN STRUCTURE PREDICTION

Amino Acid Sequence

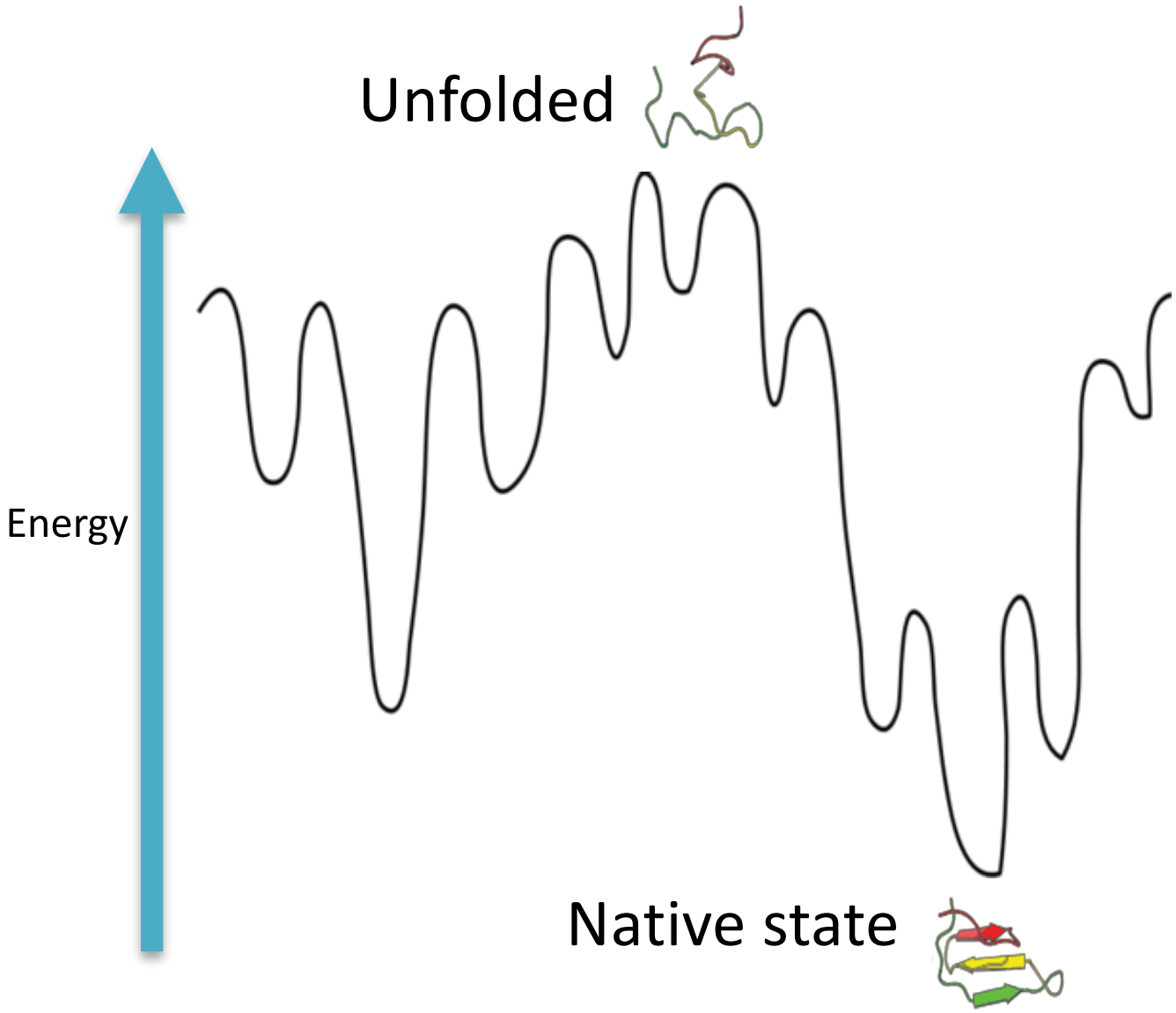




Protein Tertiary Structure

PROTEIN DESIGN

The folded states of proteins are likely global energy minima for their sequences



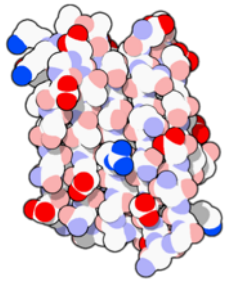
Protein Structure Prediction: find lowest energy structure for fixed sequence

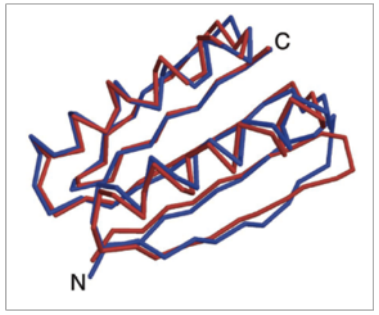
Protein Design: find a sequence for which desired structure has lowest energy

Sample structures and sequences, and evaluate energies using **Rosetta molecular modeling suite**

15 Years Ago (2003): First *De Novo* Design

- First computational de novo design of a novel protein fold (Top 7) with atomic level accuracy.





Model X-ray Structure

RESEARCH ARTICLES

Design of a Novel Globular Protein Fold with Atomic-Level Accuracy

Brian Kuhlman,[1*†] Gautam Dantas,[1*] Gregory C. Ireton,[4] Gabriele Varani,[1,2] Barry L. Stoddard,[4] David Baker[1,3‡]

A major challenge of computational protein design is the creation of novel proteins with arbitrarily chosen three-dimensional structures. Here, we used a general computational strategy that iterates between sequence design and structure prediction to design a 93-residue α/β protein called Top7 with a novel sequence and topology. Top7 was found experimentally to be folded and extremely stable, and the x-ray crystal structure of Top7 is similar (root mean square deviation equals 1.2 angstroms) to the design model. The ability to design a new protein fold makes possible the exploration of the large regions of the protein universe not yet observed in nature.

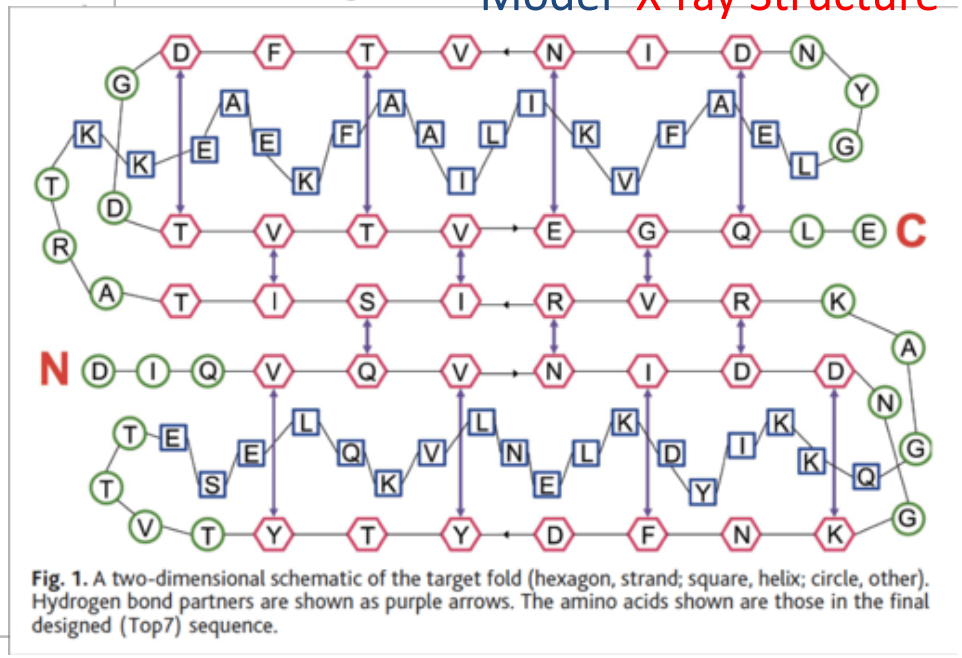
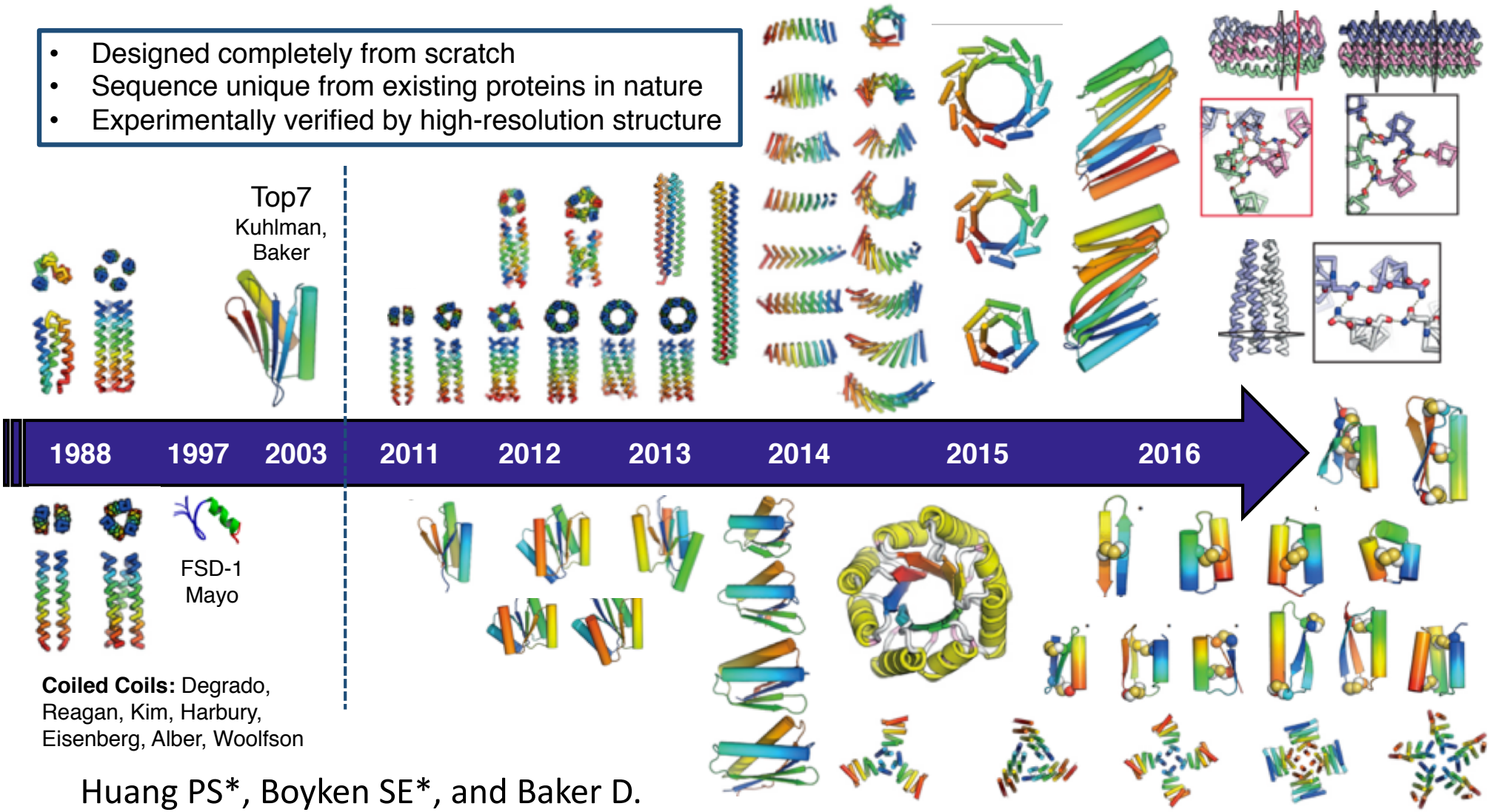


Fig. 1. A two-dimensional schematic of the target fold (hexagon, strand; square, helix; circle, other). Hydrogen bond partners are shown as purple arrows. The amino acids shown are those in the final designed (Top7) sequence.

Kuhlman et al, *Science* 2003

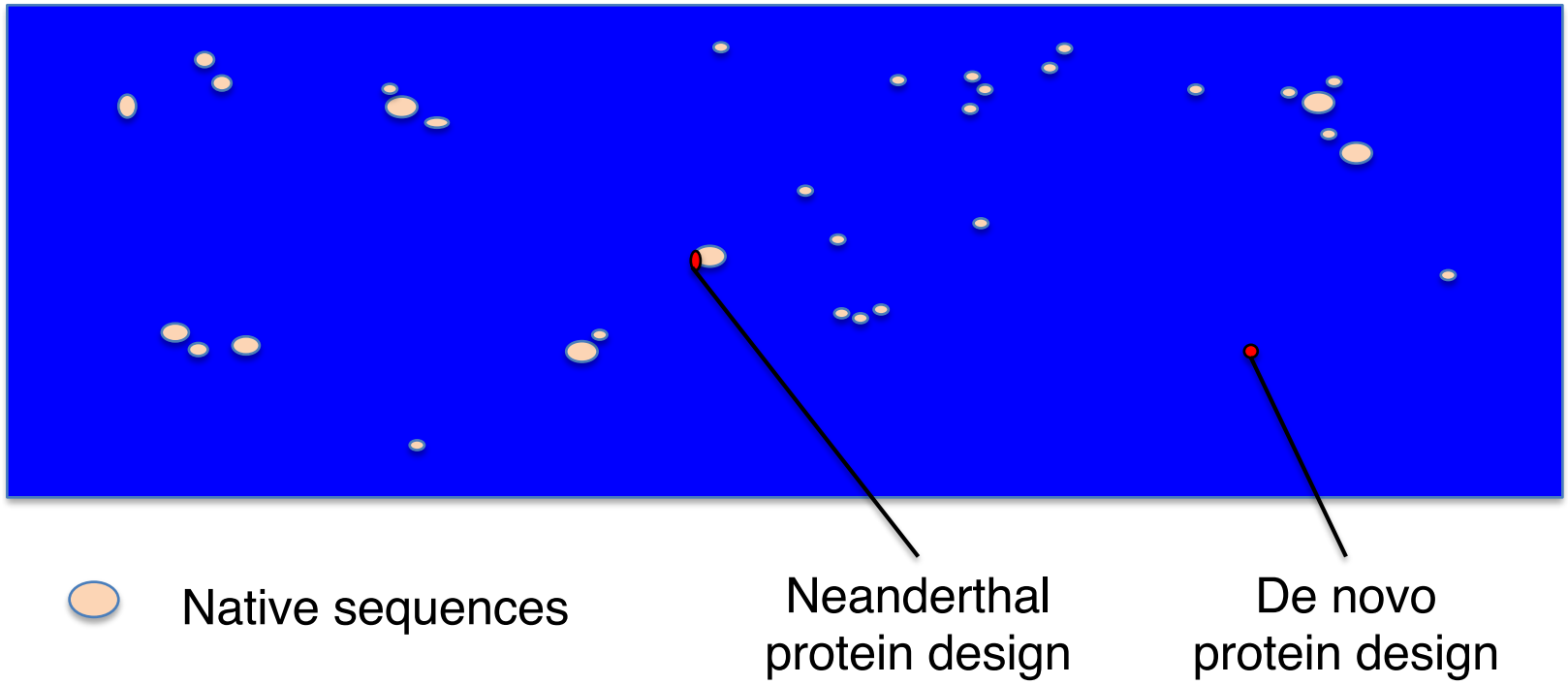
Today: The Coming of Age of *De Novo* Protein Design

- Designed completely from scratch
- Sequence unique from existing proteins in nature
- Experimentally verified by high-resolution structure



Huang PS*, Boyken SE*, and Baker D.
Nature 2016

De novo protein design



Number of 100 residue amino sequences: 20^{100} = **$1.3*10^{130}$**

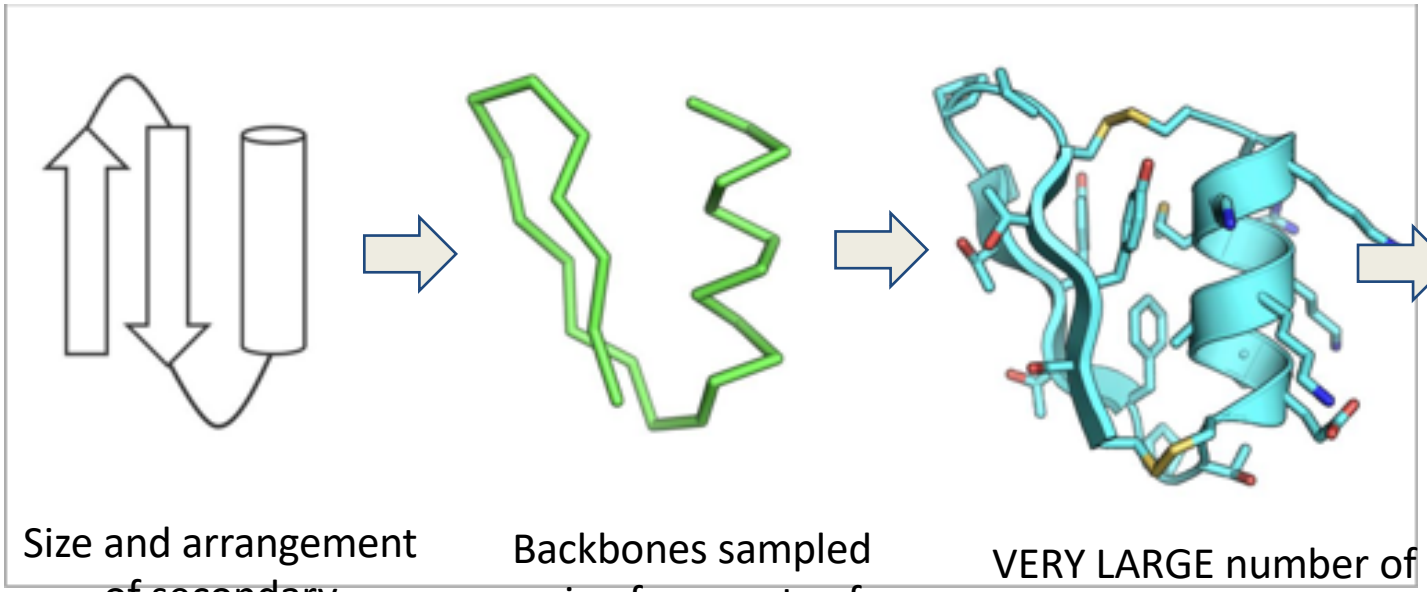
Number of naturally occurring proteins: **~10^{15}**

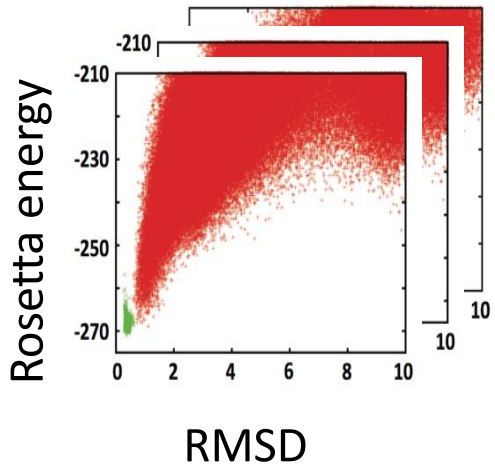
HISTORIC MOMENT IN PROTEIN DESIGN

- We've learned how to design proteins from scratch.
- There is finally enough computing power to do it.
- Genomics enables building and testing designs in the lab.

De novo protein design method

Define Blueprint	Design Strain-Free Backbones	Design a Low Energy Sequences for Backbones	Select Sequences that Fold into Designed Structure





Size and arrangement of secondary structures

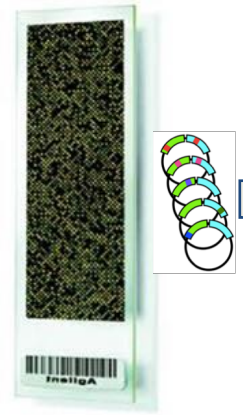
Backbones sampled using fragments of natural proteins

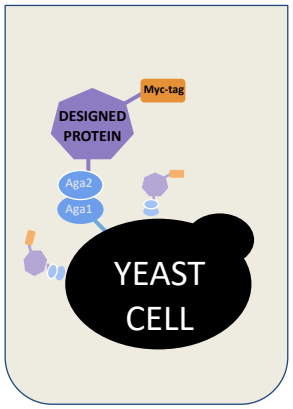
VERY LARGE number of possible amino acid sequences

Selection of designed sequences with lowest energies close to design model

De novo protein design method

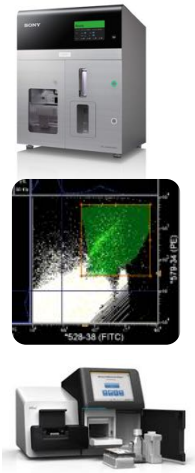
Gene Library Synthesis	Generate Yeast Surface Display Libraries	FACS and Next-Gen DNA Sequencing	Select Individual Designs for Verification

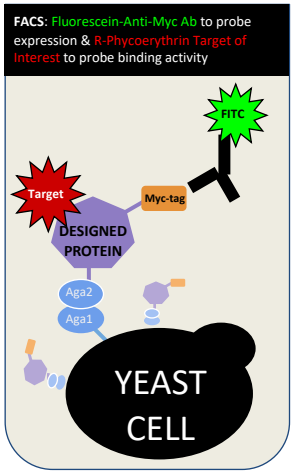


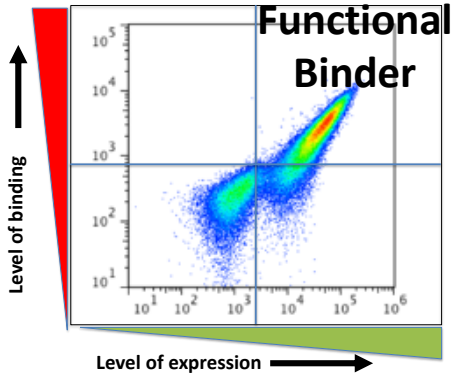


Limited Protease

Limited Heat







~100,000 genes encoding mini-proteins ~60 aa

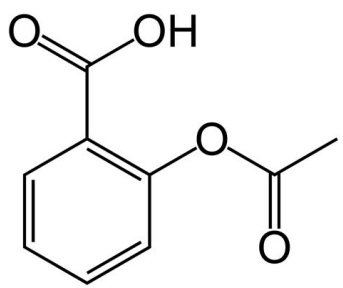
Transform yeast with plasmids encoding minibinder design library, and treat with limited protease and / or heat

Identify gene sequences encoding functional designed minibinders

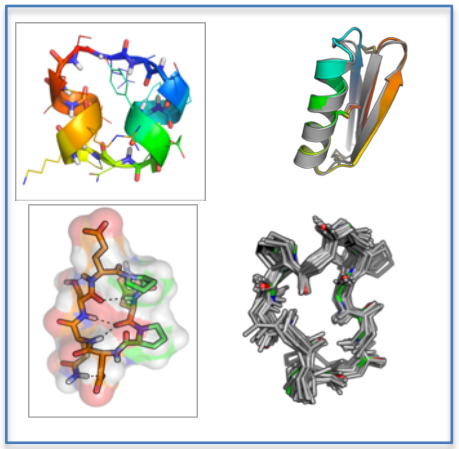
Individual clones expressing designed minibinders are used to verify function

Protein Design Takes Us Beyond Traditional Small Molecules and Antibodies

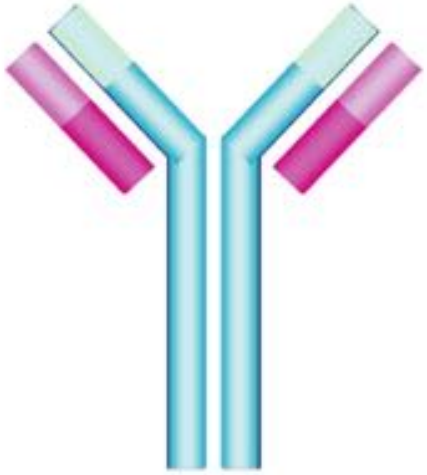
- Computational design enables bottom up creation of totally new functional designer peptides, proteins, and nanomaterials.



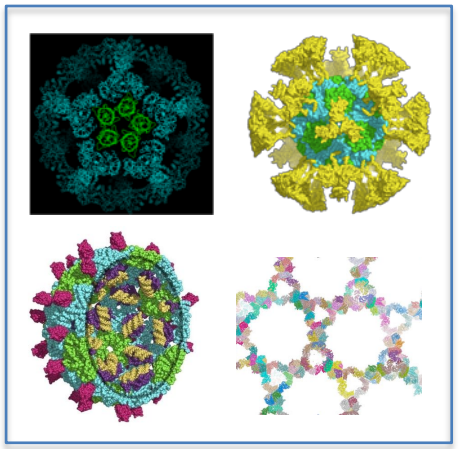
Traditional Small Molecule Drugs <1 KDa.



Computationally Designed Mini-Proteins (<100 aa) Macrocycles (7-16 aa) 1-12 KDa.

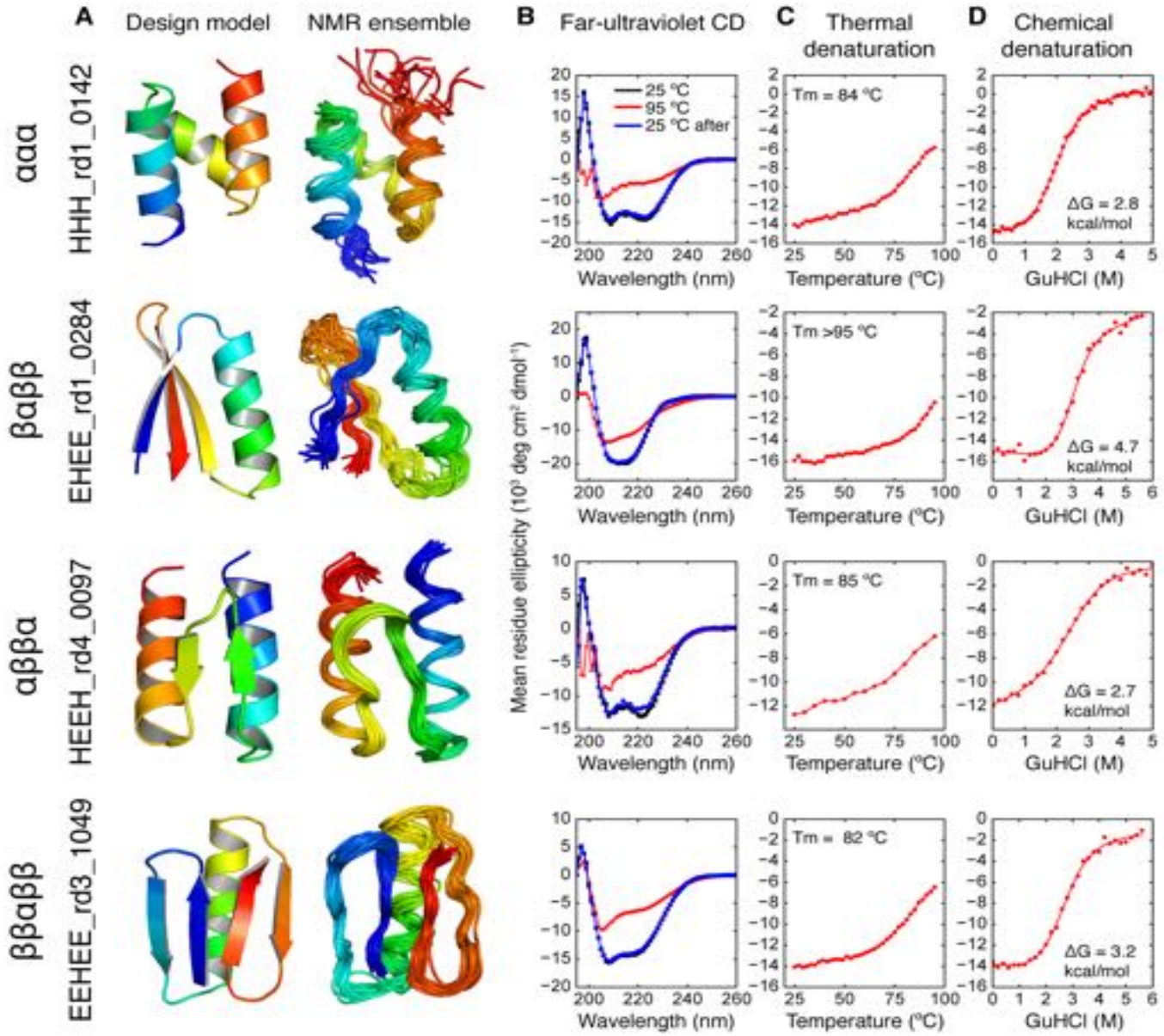


Traditional Antibodies or Smaller Ab Fragments 13-160 KDa.



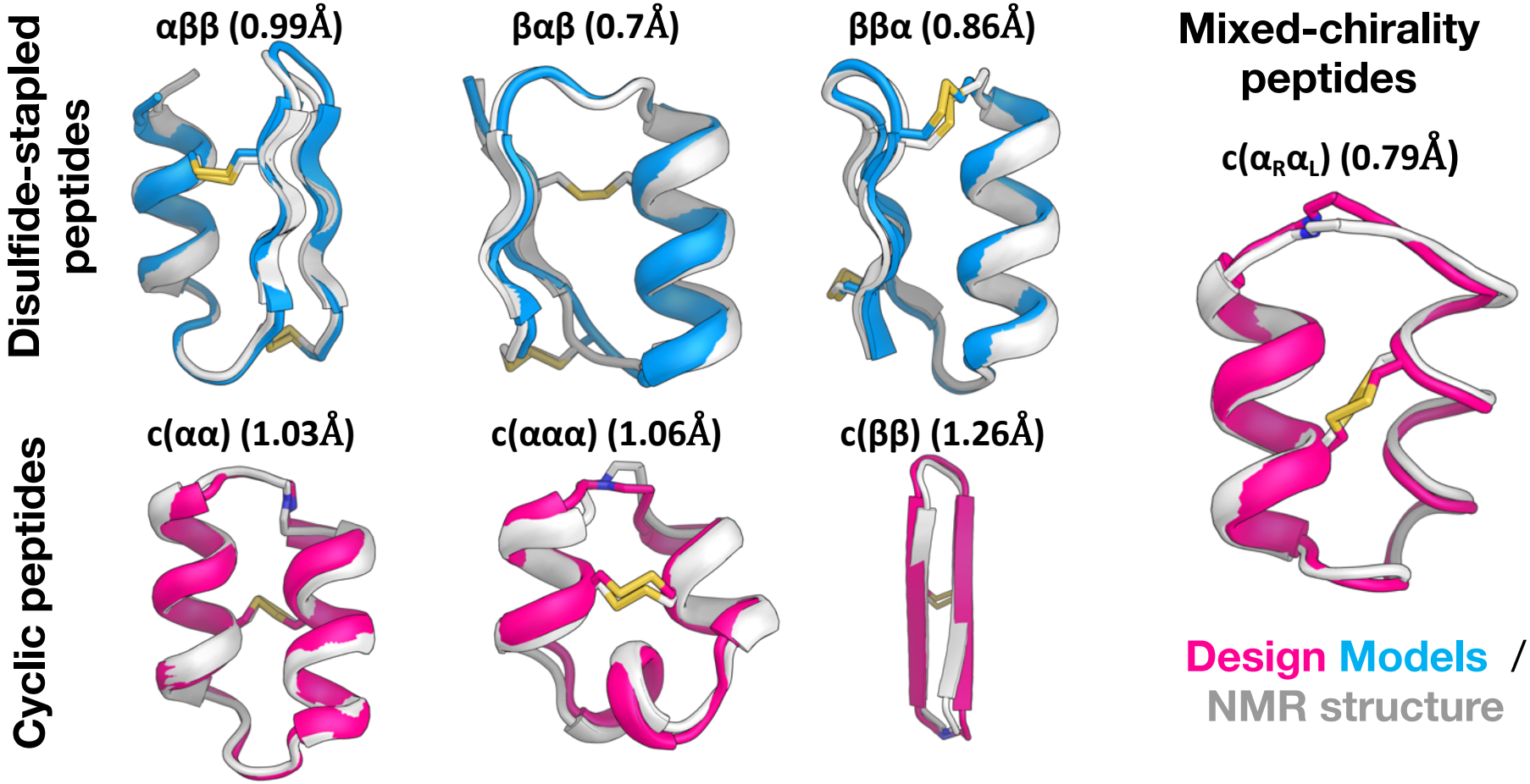
Computationally Designed Smart Nanomaterials 50 KDa. – 3 MDa.

Large Scale Design of Hyperstable Mini-Proteins



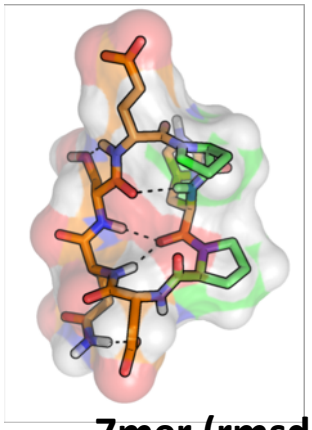
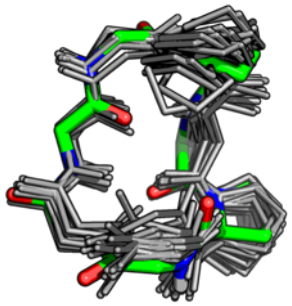
Rocklin et al Science 2017

Design of Disulfide Stapled and Cyclic Mini-Proteins with Precise Control of Shape and Size

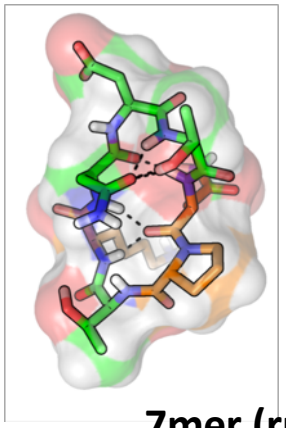
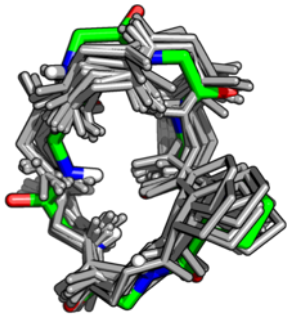


Bhardwaj, G*., Mulligan V.*, Bahl. C* *et al.,* Nature (2016)

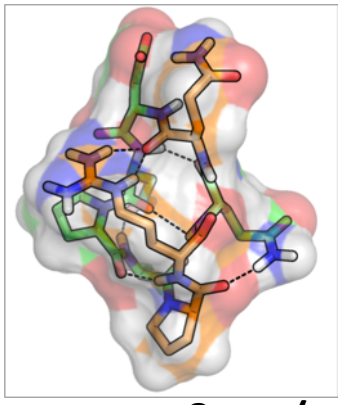
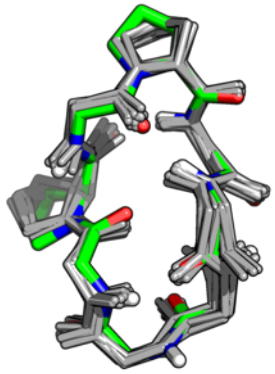
Rosetta can Design Peptide Macrocycles with Near Atomic Level Accuracy

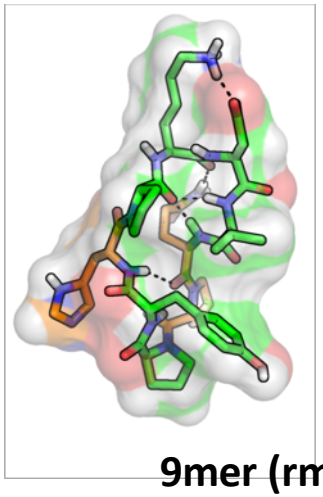
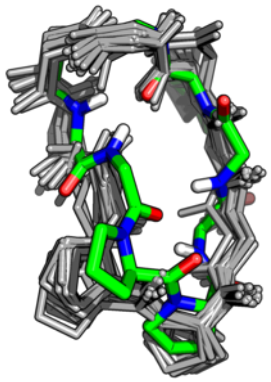
7mer (rmsd: 0.8 Å)

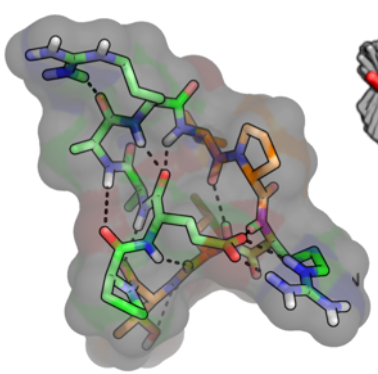
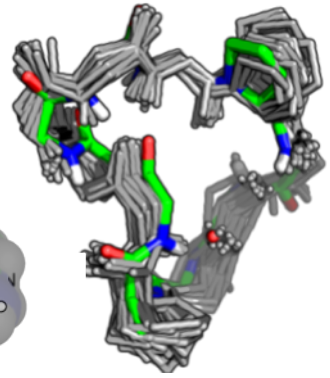
7mer (rmsd: 1.1 Å)

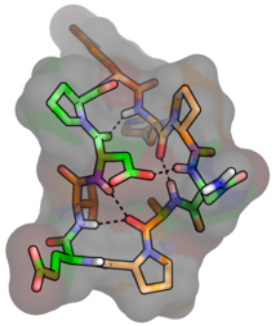
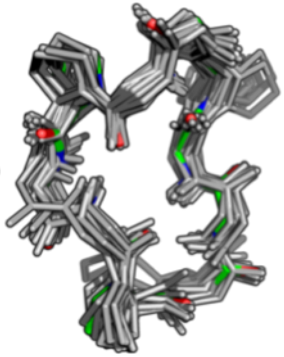
8mer (rmsd: 0.3 Å)

9mer (rmsd: 1.2 Å)

10mer (rmsd: 0.8 Å)

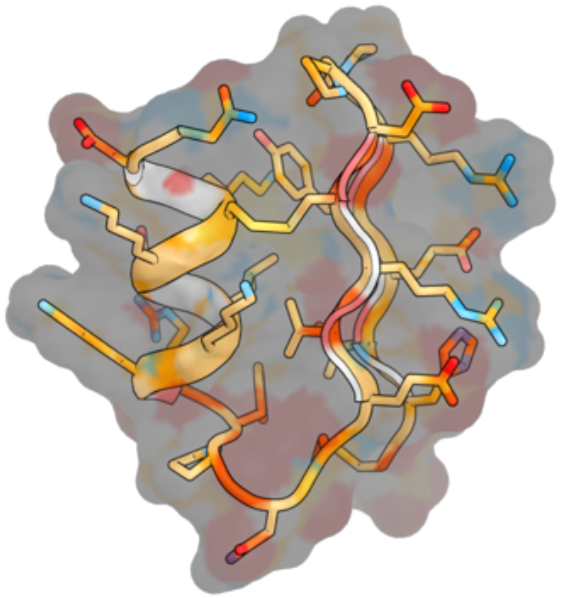



10mer (rmsd: 0.5 Å)

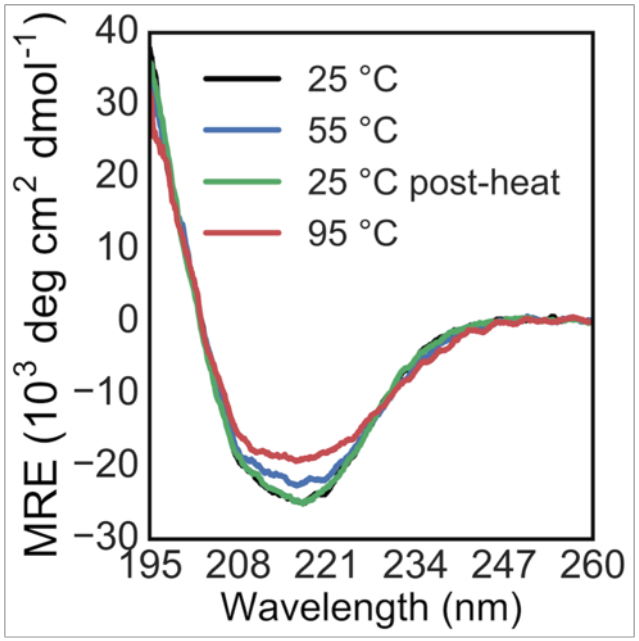
Hosseinzadeh, P. *, Bhardwaj, G*., Mulligan V.* *et al.,* Science *(2017)*

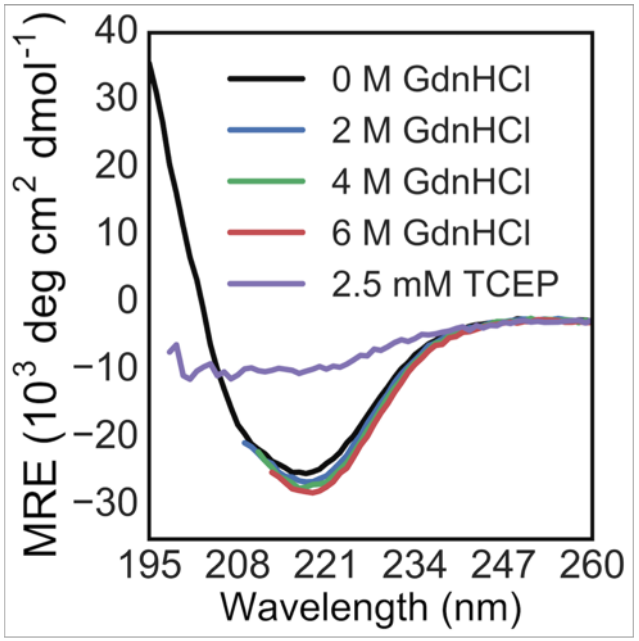
L-AA/D-AA computational model / NMR ensemble

Designed proteins show high thermal stability and resistance to chemical denaturation



NC_HEE_D1

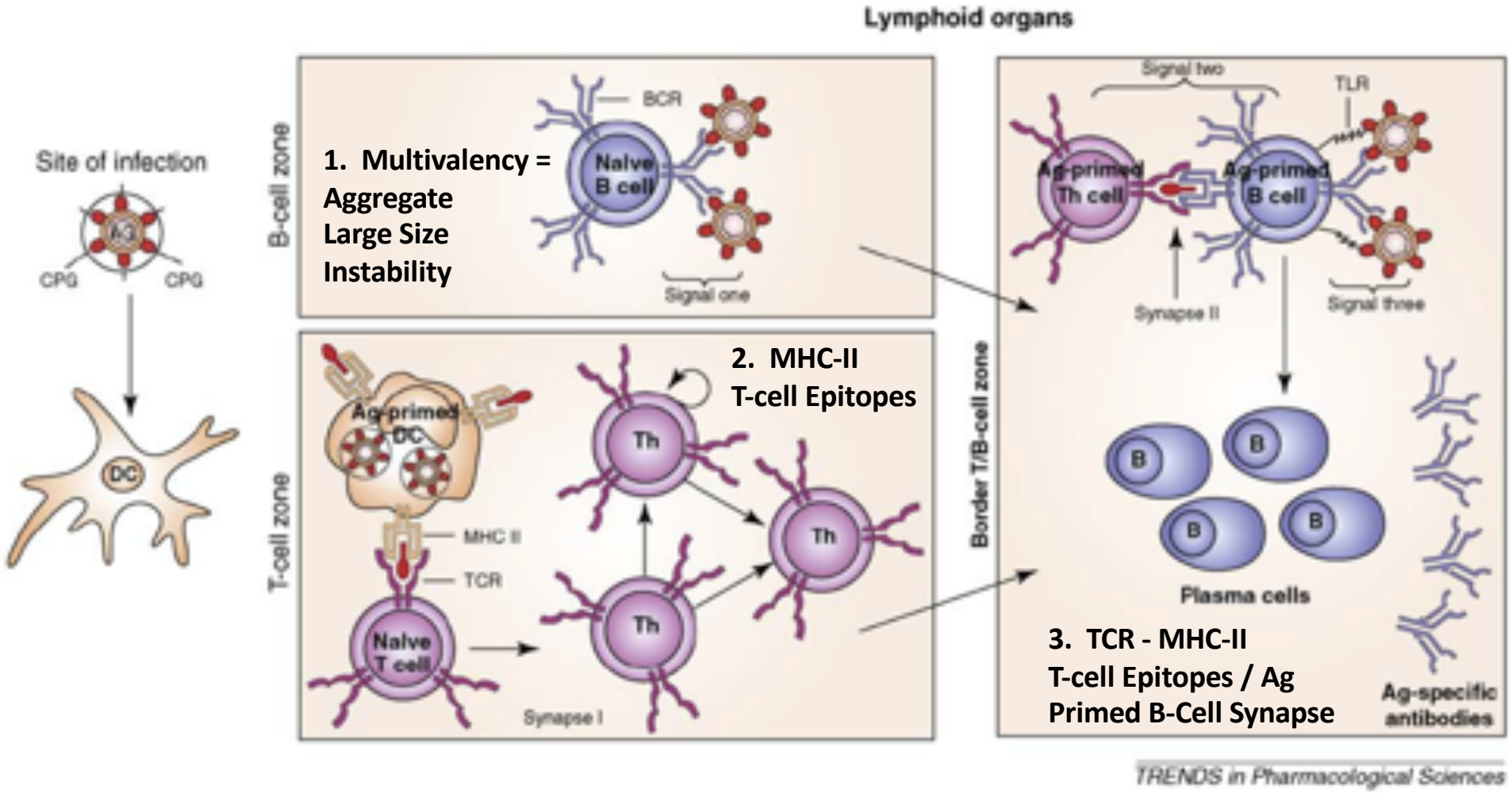




Bhardwaj, G*., Mulligan V.*, Bahl. C* *et al.,* Nature (2016)

Immunogenicity ?

Causes of Immune Responses to Proteins



Sauerborn M, Brinks V, Jiskoot W, Schellekens H. Immunological mechanism underlying the immune response to recombinant human protein therapeutics. Trends Pharmacol Sci. 2010 Feb;31(2):53-9.

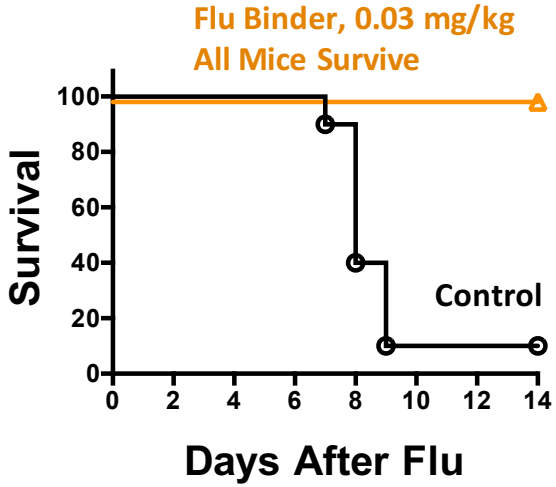
Features of Immunogenic Substances vs. *De Novo* Designed Mini-Binders

Immunogenic Protein	**Designed Mini-Binder / Macrocycle**
Large size (> 10 KDa.)	Small (< 10 KDa.)
Multivalent = B-cell receptor crosslinking	Monomeric
Poor stability = Denaturation = Aggregation = Multivalent	Hyper-Stable (>80 ˚C, Protease resistant)
Not Self	Not Self
T-cell epitopes (MHC-II) require processing and presentation.	Hard to digest or D-handed un-natural amino acids make it hard to process.
Re-design (deimmunize) hard	Re-design (immune silence) easier
Long T1/2 resident time (weeks)	Short T1/2 (minutes to hours)
Delivery is often I.V. or S.C. (systemic)	Delivery options, I.V., S.C., Aerosol (localized)
Excipient formulations	Simple formulations (PBS)

Designed Influenza Therapeutic Mini-Binder

- Low cost potent, inhalable, long-lived, broadly neutralizing anti-viral therapeutic.
- 40 amino acids (synthetic or recombinant)
- 2 disulfide bonds, Tm > 95˚C, Kd > ~5 nM.
- In vitro Neutralization EC50 < 0.003 ug/ml.
- Not immunogenic in mice.

Heat Stable Aerosol



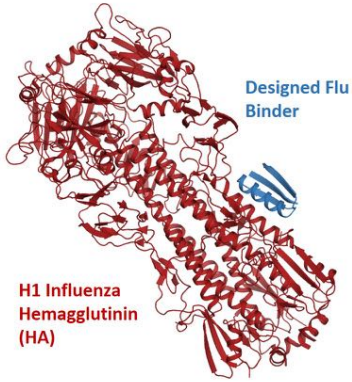
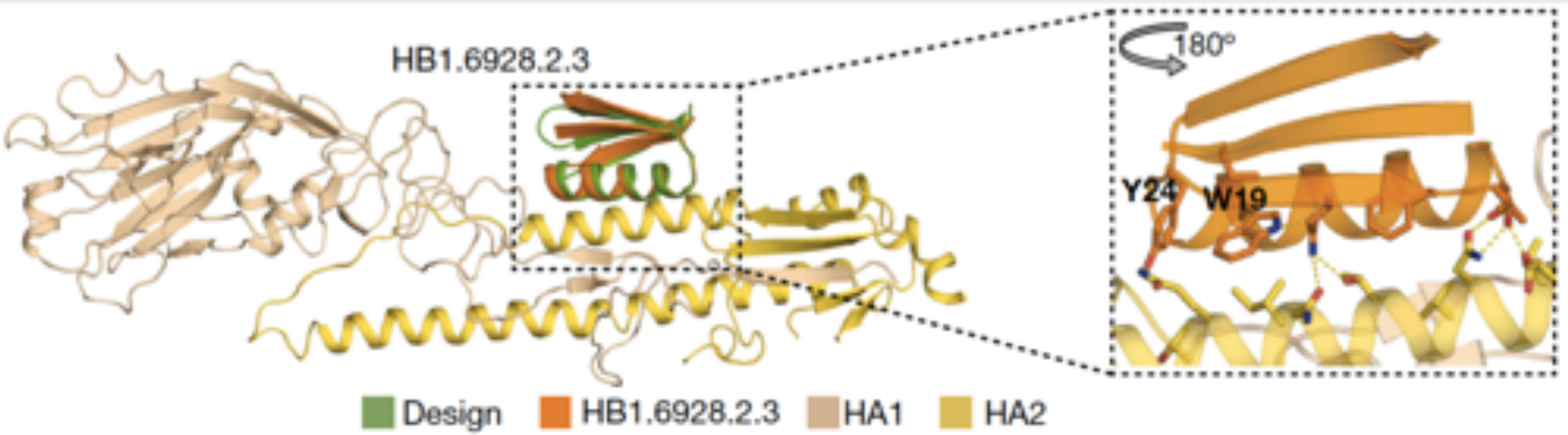


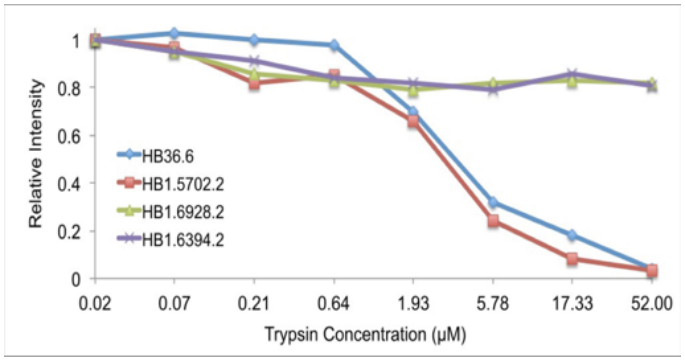
Image by IPD and Cognition Studio

Aaron Chevalier, Daniel Silva, Gabe Rocklin, David Baker et al., *Nature* 2017

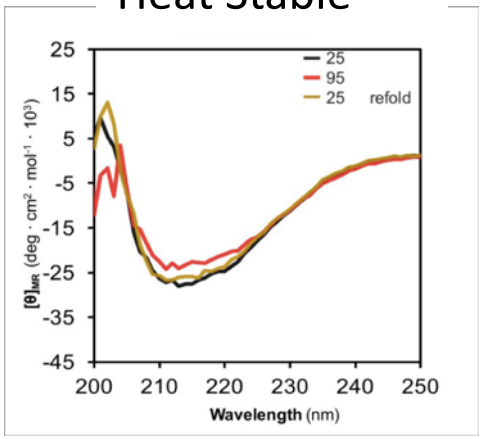
Potent Anti-Flu Mini-binder is Hyperstable



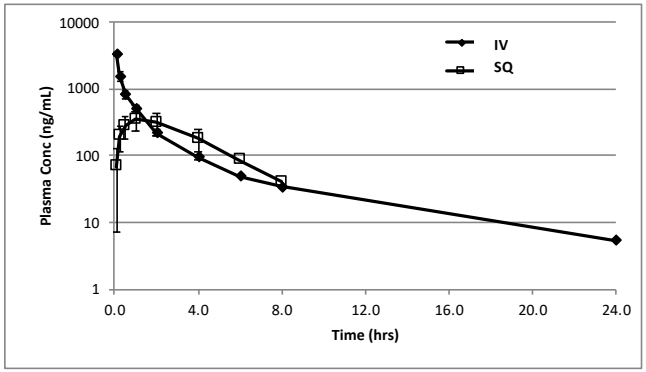
Trypsin Resistant



Heat Stable



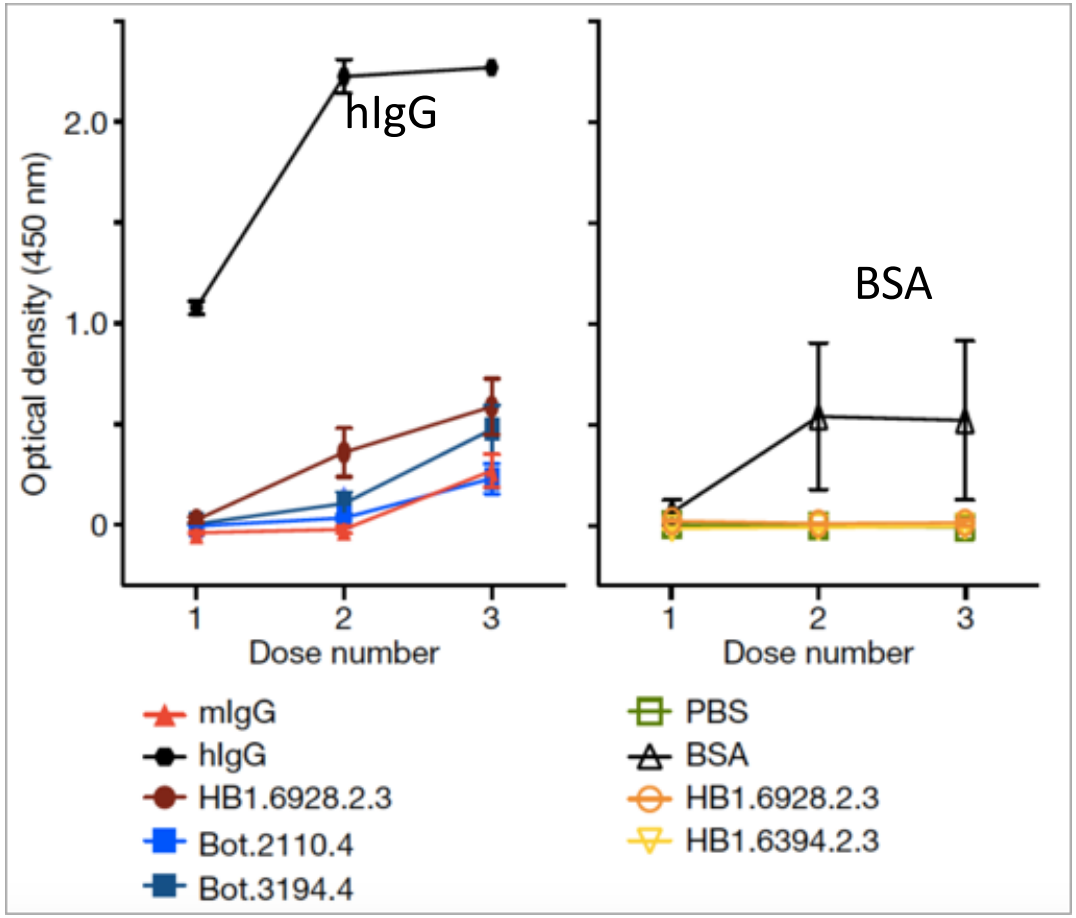
Pharmacokinetics
T1/2 = ~20 min.



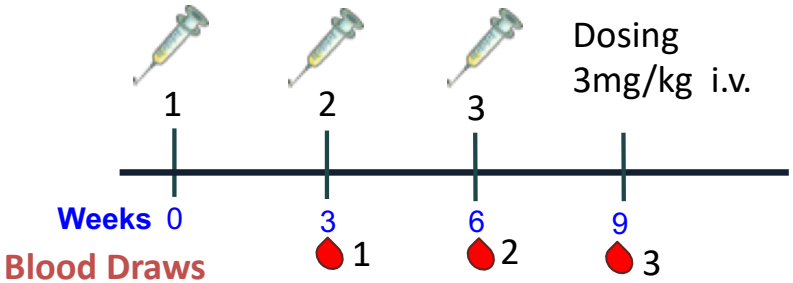
Aaron Chevalier, Daniel Silva, Gabe Rocklin, David Baker et al. , *Nature* 2017

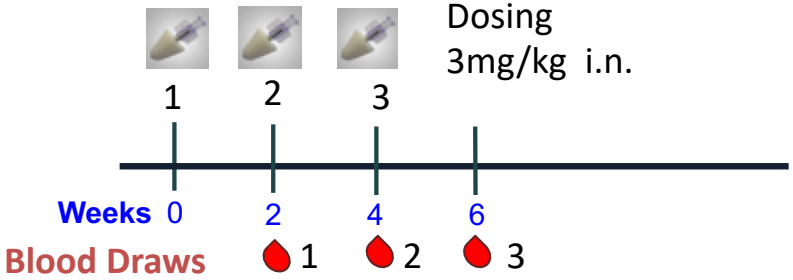
Designed Mini-Binders Elicit Little or No Antibodies in Mice

IgG Responses in ELISA (1:500 serum)



Dosing Protocols

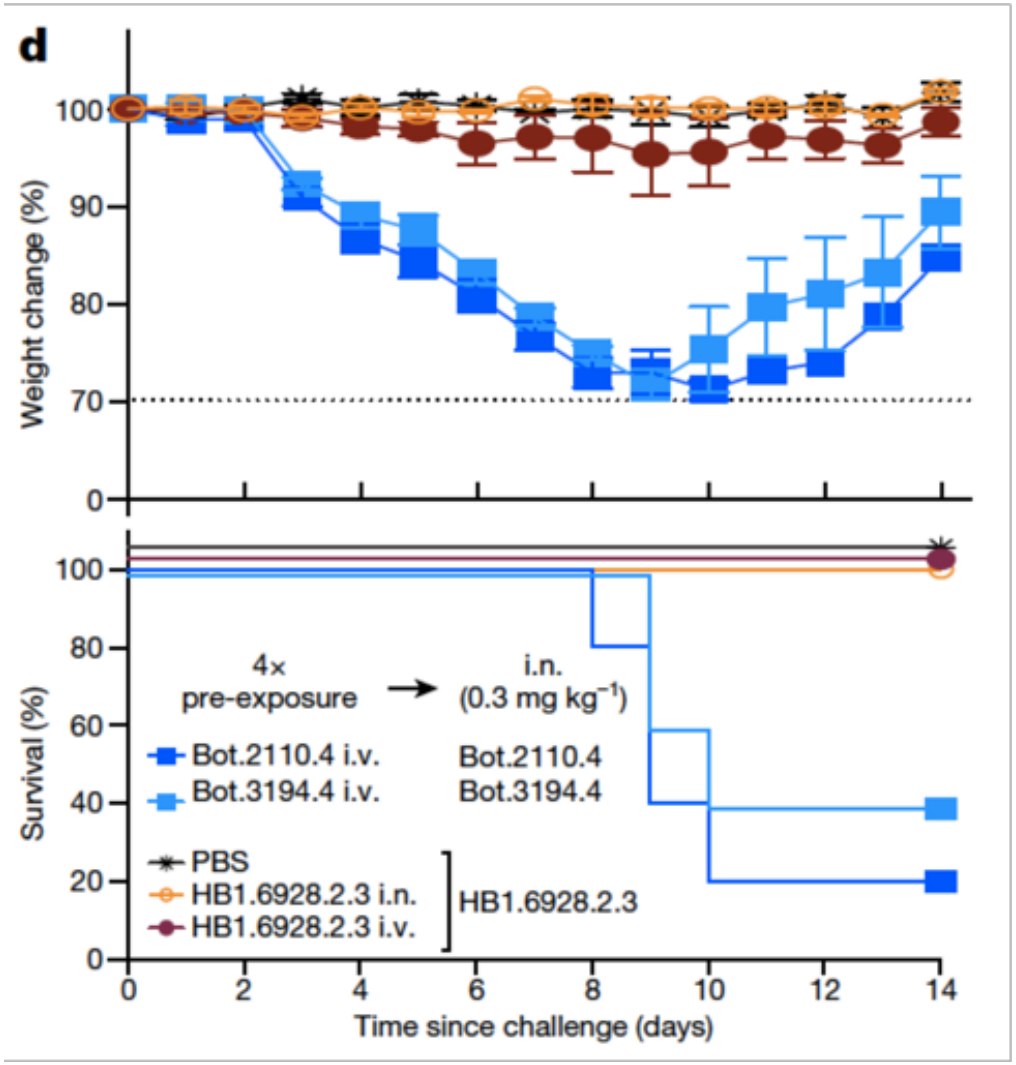


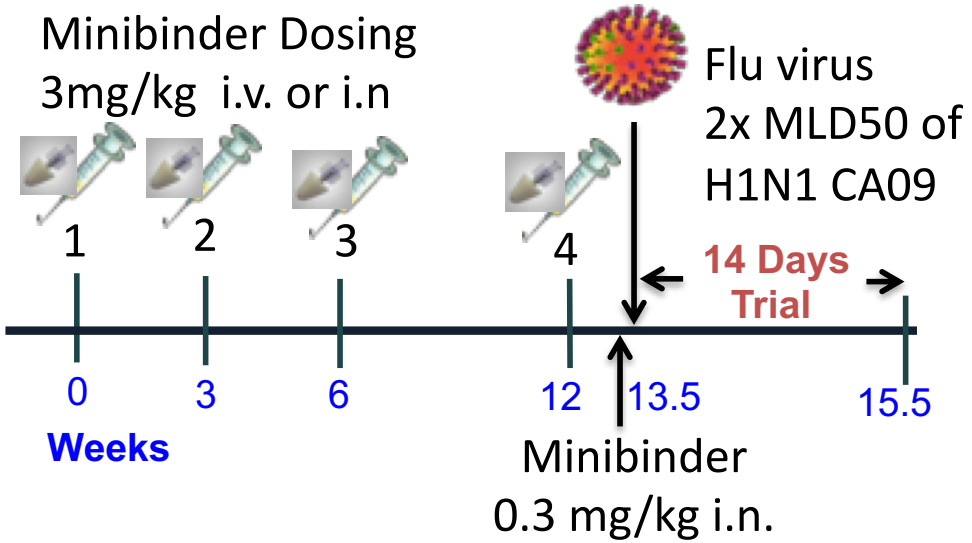


Designs are much less immunogenic than hIgG or BSA in mice !

Aaron Chevalier, Daniel Silva, Gabe Rocklin, David Baker et al., *Nature* 2017

Repeat Dosing of Mini-Binders Does Not Alter Prophylactic Efficacy in Mice

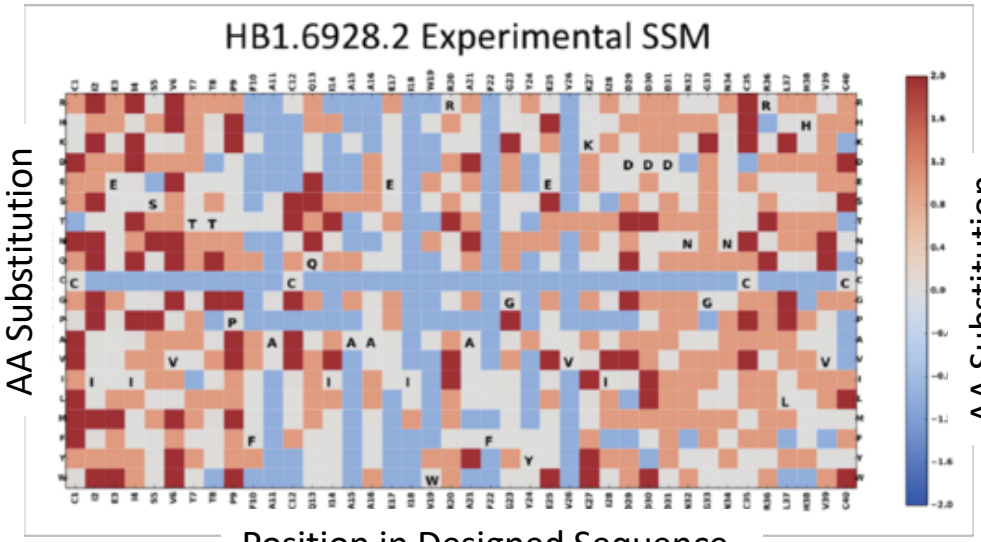


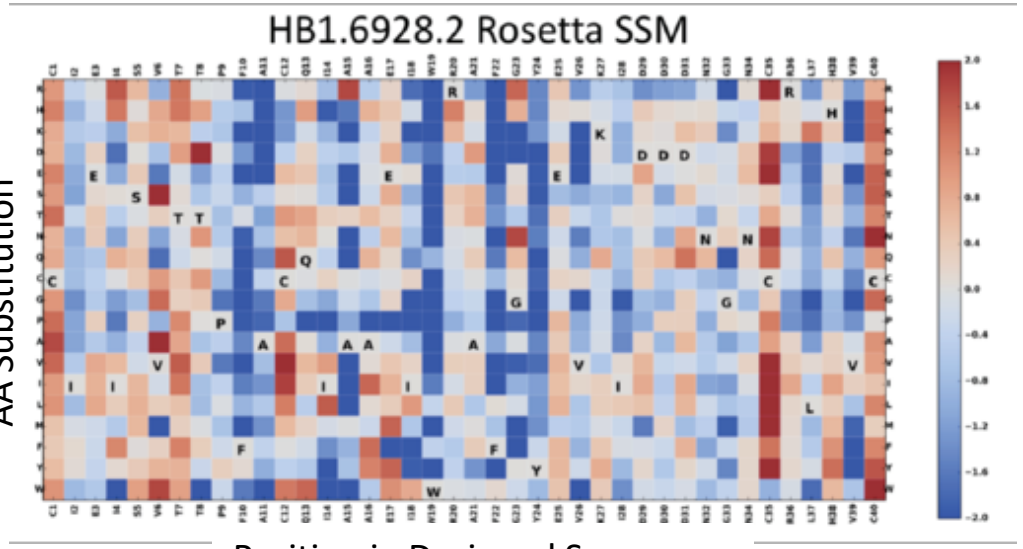


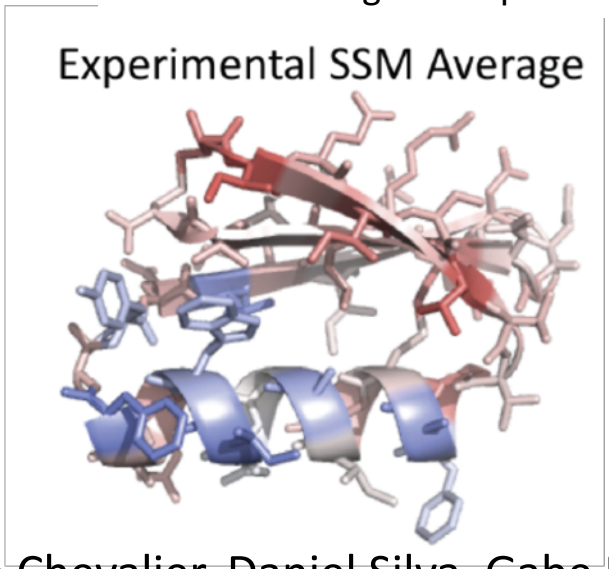
Complete prophylactic protection after 4 repeated doses from 8 weeks to 1 week prior to lethal flu virus challenge !

Aaron Chevalier, Daniel Silva, Gabe Rocklin, David Baker with Deb Fuller's Lab et al. , *Nature* 2017

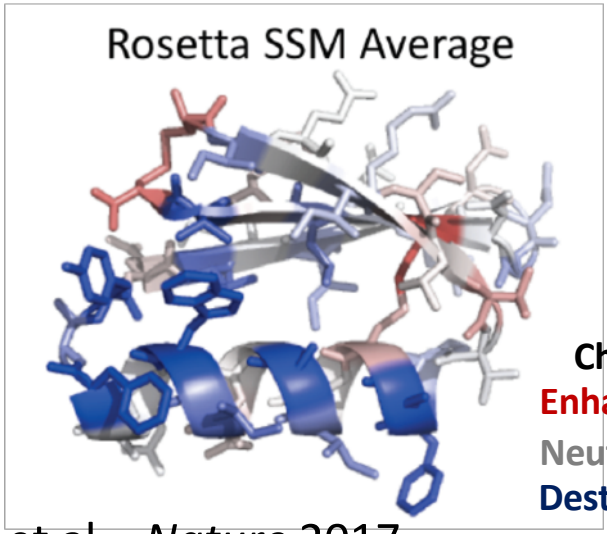
~Congruence Between Computational and Experimental Saturation Site Mutagenesis (SSM)







Can Re-Design Sequence to Reduce T-Cell Epitope Liability if Needed !



Change Effect
Enhance Function
Neutral
Destroy Function

Aaron Chevalier, Daniel Silva, Gabe Rocklin, David Baker et al. , *Nature* 2017

Structure and Function By Design

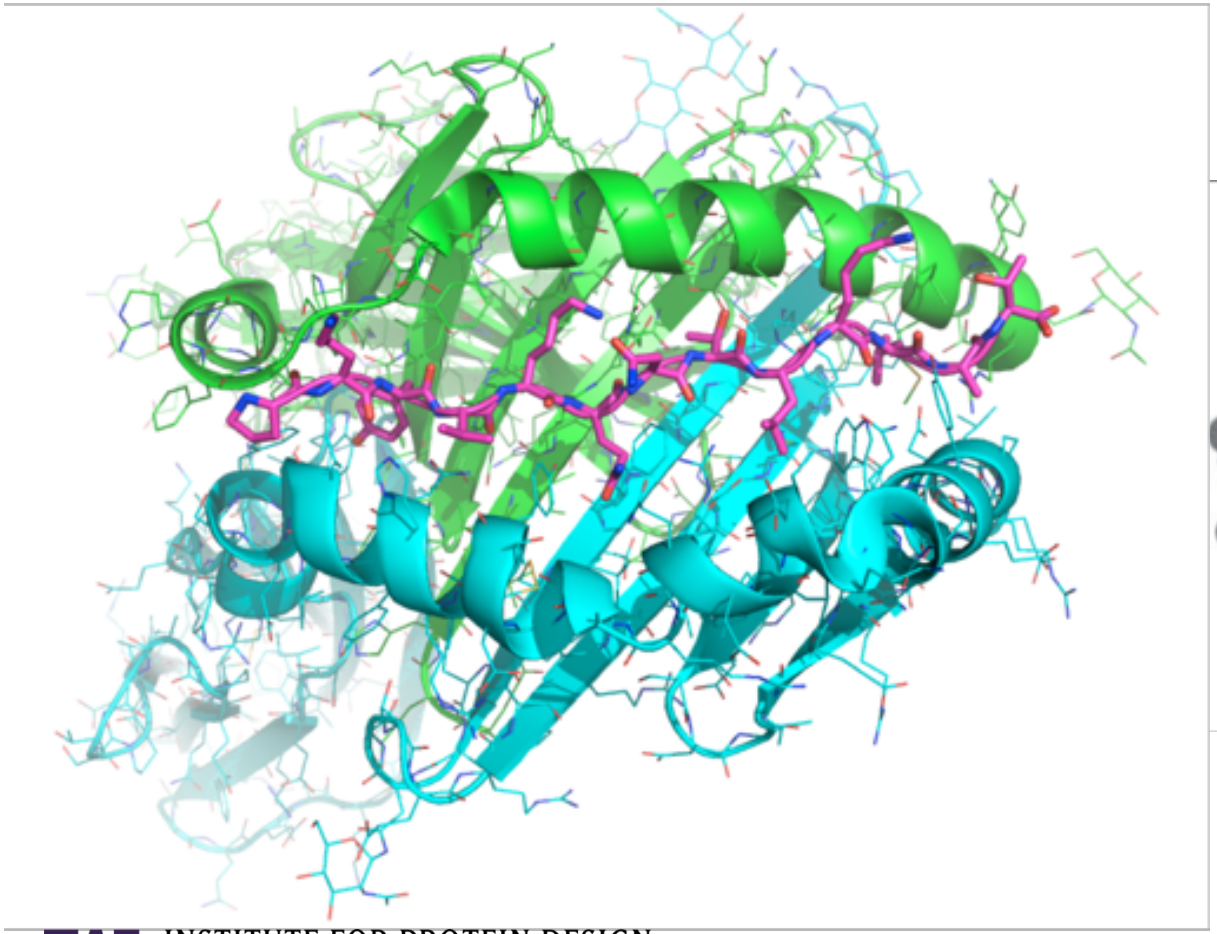
- Through design, we can maintain structural features through design of an astronomical number of different amino acid sequences.

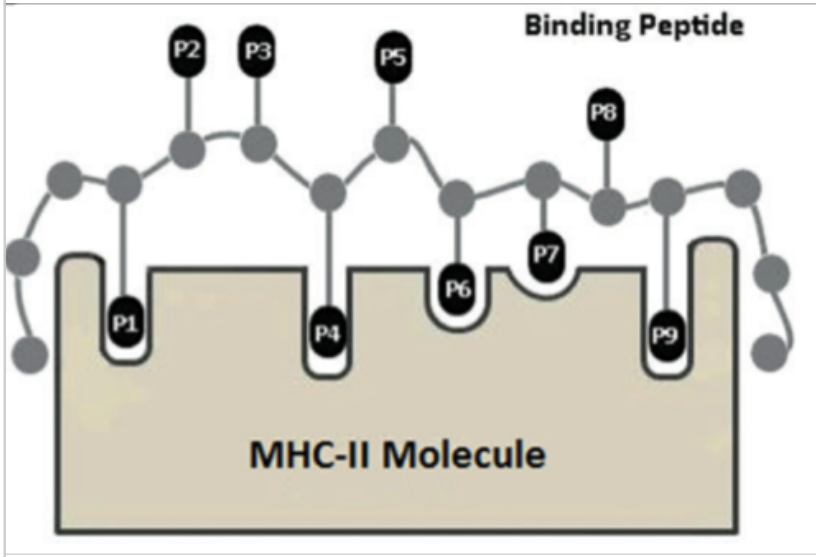
- As such, numerous desired target product profile features are achievable through *de novo* design.
  - Size
  - pI
  - Stability
  - H-bonding networks = hydration sphere
  - Others.

- By definition *de novo* designed protein sequences do not exist in nature and could be recognized as foreign!
- How can we design immune silence ?

MHC-II displays peptides on the surface of cells for T-cell receptors

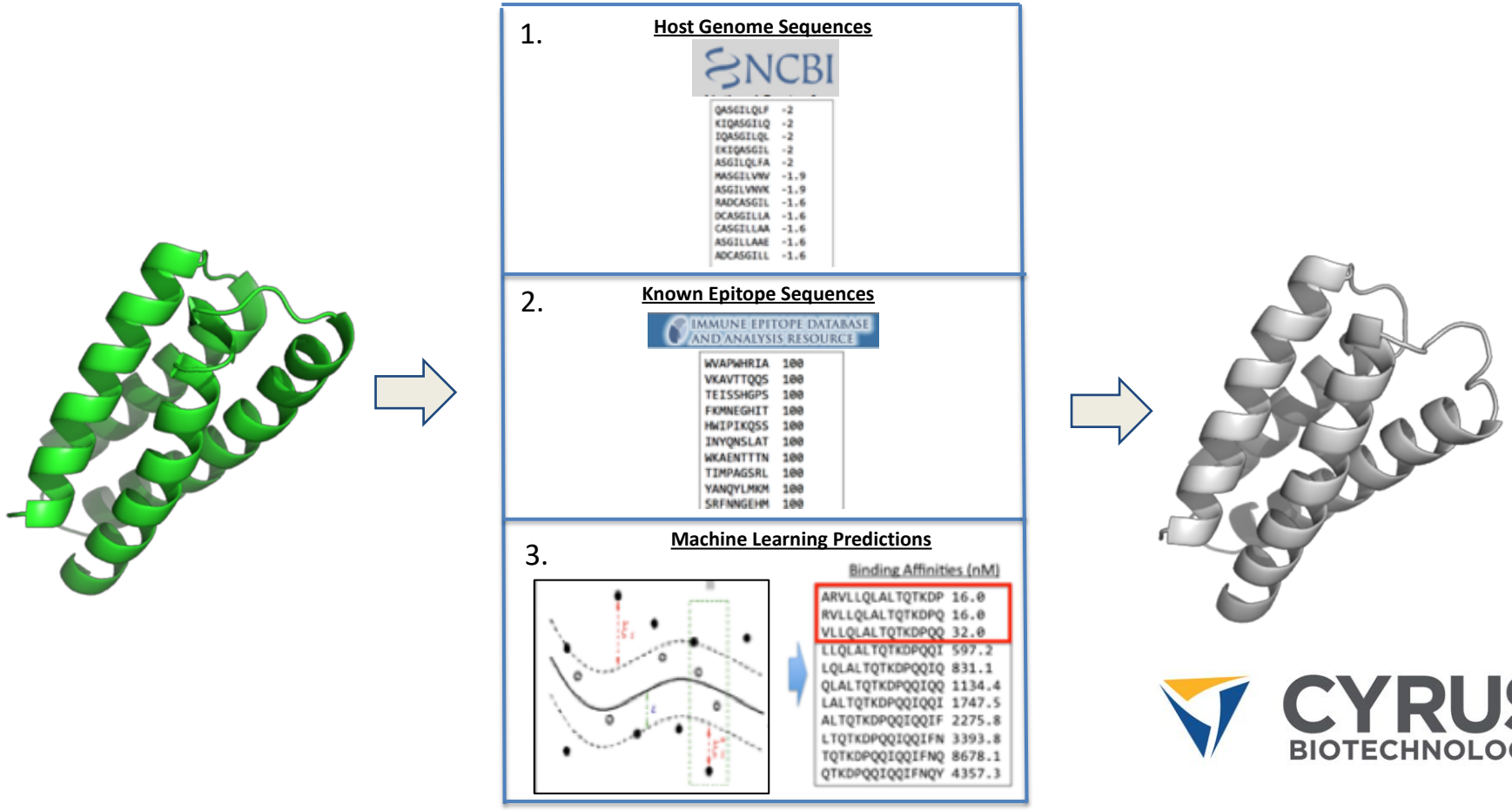
- Peptide binding cleft between 2 domains
- **binds 15-24mers, 9mer core**
- P1,P4,P6,P9 “pocket” positions





Reducing the Liability of T-cell Epitopes by Design

- Through Rosetta design, we can maintain structural features and alter the amino acid sequence to “silence” predicted or known offending T-cell epitopes.



King C, Garza EN, Mazor R, Linehan JL, Pastan I, Pepper M, Baker D. Removing T-cell epitopes with computational protein design. Proc Natl Acad Sci U S A. 2014 Jun 10;111(23):8577-82.

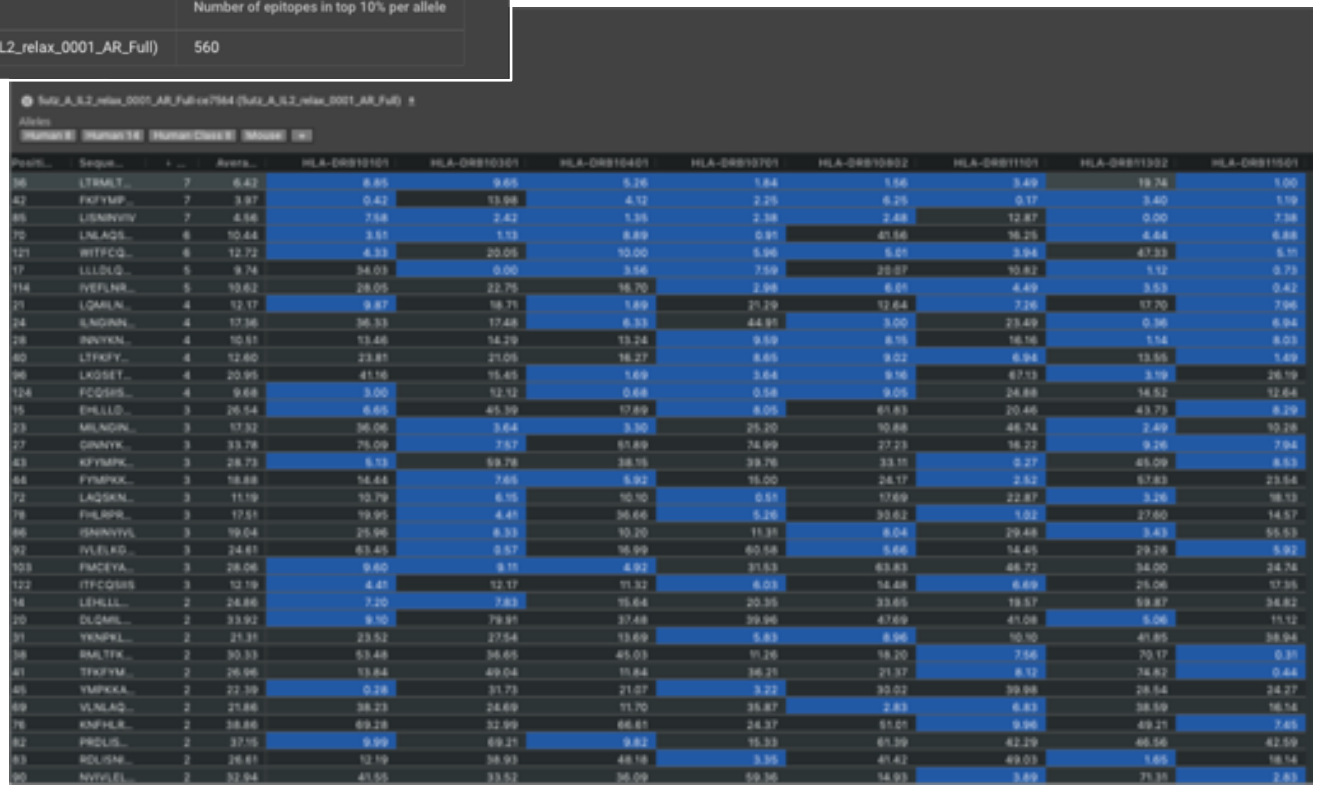
Current Immunogenicity Testing Paradigm for *De Novo* Designed Proteins

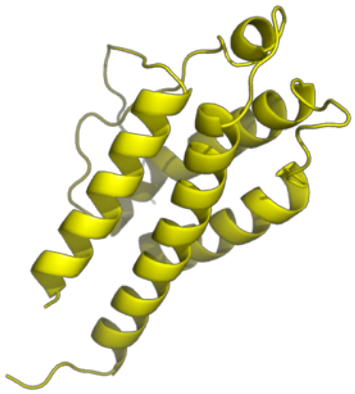
- **Step 1.** Since MHC-II T-cell help is key to IgM to IgG class switching and strong long lived antibody responses, we scan *de novo* designed sequence through T-cell epitope prediction software (~$10 per protein, in compute time).
- **Step 2.** Any T-cell epitopes identified in Step 1 should be synthesized and tested in Naïve Primary T Cell Assay (e.g. Proimmune) which covers 40 human donors looks at CD4+ T-cell responses (~$5K per peptide = expensive).
- **Step 3.** Any T-cell epitopes of Step 2 that are found to activate CD4+ T-cells should be computationally re-designed (Iterative FlexDesign with position specific score matrix and EpitopeScan Indigo King / Cyrus) while preserving structure and function of original design (~$100 per protein, in compute time).
- **Step 4.** Make and test activity of ~10 new "T-cell epitope silenced" *de novo* designed proteins from Step 3 ($5,000 per design). Downselect the best variant(s).
- **Step 5.** Test a limited number of *de novo* designs from Step 4 in pooled donor DC-cell presentation / T-Cell proliferation assay ($30,000 per design, SUPER EXPENSIVE)
- **Step 6.** Repeat Steps 1-5 as needed. But ultimately need to move a candidate into safety / tox and into the clinic.

Caution: Not All T-cell Epitopes Are “Offensive”

- Most Current Computational Methods only “Identify” T-cell Epitopes
- Native human IL-2 has quite a few ”predicted” MHC-II T-cell Epitopes

560 Predicted T-Cell Epitopes in human IL-2





PDBID: 5utz

Presumably central tolerance is dealing with these epitopes

Considering Anti-Drug Antibodies (ADAs) Observed for Approved Biologics vs. *De Novo* Designs

- Most approved / licensed biologics are known to elicit ADAs (Stats)
- Most of these ADA's have no effect on PK / PD of biologic therapy
- Sometimes there is a dangerous auto-immune reaction (e.g. Factor VII) / Rare

- Conclusions:
  - Approved biologics aren't necessarily a good proxy for *de novo* designed proteins
  - Of the approved biologics, it is primarily the foreign (not mAb, not human protein) that are known to elicit ADAs.
  - *De novo* designed mini-proteins tend to be immune silent due to their stability, small size, monomeric nature, short serum half-life.
  - *De novo* designed mini-proteins can be re-designed to reduce the liability of offensive T-cell epitopes.
  - Since *de novo* designed proteins are foreign, but do not have sequence similarity to human proteins it is unlikely an ADA immune response would have an adverse effect (needs to be tested).

Acknowledgements !

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Thank You !