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
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.

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: \(20^{100} = 1.3 \times 10^{130}\)

Number of naturally occurring proteins: \(\sim 10^{15}\)

Native sequences

Neanderthal protein design

De novo protein design
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

<table>
<thead>
<tr>
<th>Gene Library Synthesis</th>
<th>Generate Yeast Surface Display Libraries</th>
<th>FACS and Next-Gen DNA Sequencing</th>
<th>Select Individual Designs for Verification</th>
</tr>
</thead>
<tbody>
<tr>
<td>~100,000 genes encoding mini-proteins ~60 aa</td>
<td>Transform yeast with plasmids encoding minibinder design library, and treat with limited protease and / or heat</td>
<td>Identify gene sequences encoding functional designed minibinders</td>
<td>Individual clones expressing designed minibinders are used to verify function</td>
</tr>
</tbody>
</table>

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.
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

Rosetta can Design Peptide Macrocycles with Near Atomic Level Accuracy


L-AA/D-AA computational model / NMR ensemble
Designed proteins show high thermal stability and resistance to chemical denaturation

Immunogenicity ?
Causes of Immune Responses to Proteins

1. Multivalency = Aggregate Large Size Instability
2. MHC-II T-cell Epitopes
3. TCR - MHC-II T-cell Epitopes / Ag Primed B-Cell Synapse

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

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

---

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)

Intravenous

Intranasal

BSA

hIgG

Dosing Protocols

Dosing
3mg/kg i.v.

Blood Draws

Weeks

0

3

6

9

3

1

2

3

1

2

3

Dosing
3mg/kg i.n.

Blood Draws

Weeks

0

2

4

6

2

3

3

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!

Minibinder Dosing
3mg/kg i.v. or i.n

Minibinder
0.3 mg/kg i.n.

Flu virus
2x MLD50 of H1N1 CA09

Weeks
0 3 6 12 13.5 15.5

14 Days Trial

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!

Aaron Chevalier, Daniel Silva, Gabe Rocklin, David Baker et al., Nature 2017
**De Novo Designed Interleukin 2 (IL-2) Mimetics**

- Less toxic than IL-2
- Easier of manufacture
- Heat stable, 90 aa mini-protein
- Wide range of immune-oncology applications
- No immunogenicity observed in mice.

*Mice implanted with CT26 colon cancer cells.*

Daniel Silva, Umut Ulge, Carl Walkey, Alfredo Quijano Rubio, Baker Lab with Michael Dougan and Chris Garcia
De Novo Designed Interleukin 2 (IL-2) Mimetics Have No Anti-Design Antibody Response

C57BL/6 mice were dosed daily with 10 ug for 28 days and then serum collected

- No anti-Neo-2/15 or anti-mIL-2 IgG observed above background / controls
- No anti-KO Neo-2/15 or anti-hIL-2 IgG observed above background / controls

Daniel Silva, Umut Ulge, Carl Walkey, Alfredo Quijano Rubio, Baker Lab with Michael Dougan
De Novo Designed Interleukin 2 (IL-2) Neo-2/15

Mouse polyclonal anti-Neo-2/15 raised with adjuvant do not react with mouse IL-2 or human IL-2

Neo-2/15 still binds hIL-2Rβγ receptor after Incubation @80°C

IgG Responses in ELISA (1:100 serum)

Proteins Plated on ELISA Plates

Daniel Silva, Umut Ulge, Carl Walkey, Alfredo Quijano Rubio, Baker Lab with Michael Dougan
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.

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

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!
Thank You!