Computationally-driven deimmunization of therapeutic proteins

Chris Bailey-Kellogg
Chris Bailey-Kellogg and Karl E. Griswold are co-founders of Occulo Holdings LLC, Occulo Bio LLC, Stealth Biologics LLC, and Lyticon LLC. These biotechnology companies are pursuing design and development of deimmunized biotherapeutic agents and antibacterial biotherapies. Related research in the Griswold and Bailey-Kellogg Dartmouth academic labs is subject to a conflict of interest management plan, including public disclosure of financial interests in these companies.
(Non-Ab) protein therapeutics

diverse structures, functions

β-Lactamase
• cleaves lactam ring
• antibody-directed anti-cancer therapies

lysostaphin
• degrades cell wall
• anti-staph
Protein therapeutics: immunogenicity

**red: epitopes**

**β-Lactamase**
- cleaves lactam ring
- antibody-directed anti-cancer therapies

**lysostaphin**
- degrades cell wall
- anti-staph
Anti-biotherapeutic immunogenicity

- Biotherapeutic
- iDC
- mDC
- Naïve T cell
- Naïve B cell
- Antigen-primed B cell
- Activated T cell
- Plasma cell
- Antidrug antibodies

Our focus:
- MHC
- Epitope
- TCR
Deimmunization: competing objectives

wild-type  ...TAYKEFRVVELDPSAKI...

variant 1  ...TAYKEKRVVERDPSAKI...

variant 2  ...TAYKEFKVTELDSLPSAKI...
Experimentally-driven approaches

E.g., Genencor [Harding et al., Mol Cancer Ther 2005]

immunogenicity screening

lots of experimental time and expense may not consider some good possibilities

alanine scanning

engineer into whole protein

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Mutation</th>
<th>Expression</th>
<th>Stability</th>
</tr>
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<tr>
<td>6</td>
<td>M20A</td>
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<td>107</td>
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<td>0.06</td>
<td>ND*</td>
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K21A, S324A
Our approach: computationally-driven deimmunization

- Delete T cell epitopes
- Maintain function

Computational protein design algorithms

Computational models

Epitope content

Stability, activity
Landscape of approaches

<table>
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<tr>
<td><strong>Evolutionary</strong></td>
<td><img src="image1" alt="Evolutionary variants" /></td>
<td><img src="image2" alt="Evolutionary library" /></td>
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<tr>
<td><strong>Structural</strong></td>
<td><img src="image3" alt="Structural variants" /></td>
<td><img src="image4" alt="Structural library" /></td>
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Epitope content

different “pocket” shapes

0401

0405

Sturniolo et al., Nat. Biotech. 1999

=> different side-chain preferences

Nielsen et al., BMC Bioinformatics 2007

=> predictors for representative alleles

Jensen et al., J. Immunology 2018
Function: evolutionary

Target

SAK_STAAU/71-87  TAYKEFRVVELDPSAKI
Q54685_STRPY/58-73  -KHPDYIITKRDSSTIVT
Q7X0T5_STRPY/58-73  -EHPDYIITKRDSSTIVT
Q7X0Y3_STRPY/56-71  -ADLLKAIIQERLIANVH
Q53284_STREQ/56-71  -ADLLKAIQEQLIANVH
STRP_STREQ/56-71  -ADLLKAIQEQLIANVH
Q9RIL4_STRUB/56-68  -LQLDYSYEELVD---FA
Q9ZFE3_STREQ/53-65  -TFENKKLKAVD---FA
A2I7K2_STAAU/58-74  TAYKEFRVVELDPSAKI
Q2FFF5_STAA3/58-74  TAYKEFRVVELDPSAKI
Q70YZ7_STRDY/58-74  VTVDKYRIVKIPEDAQI
Q9ZFE2_STREQ/58-73  -SMENFKVIDLHEVKLV
A0FJ59_9STRE/49-64  -RDKA KL LYNLLDAGFI
Q7X0X4_STREQ/58-73  -THPGYTIYERDSSIVT
Q7X0T4_STRPY/58-73  -SHPDYTIYERDSSIVT

Relatives

1-body I, L, V

2-body YF, HY, DK
Trade-offs: EpiSweep

[He et al., Proteins, 2011]
[Parker et al., J Comp Biol, 2013]
Trade-offs: EpiSweep

- Find Pareto optimal (undominated) designs without explicitly considering others (combinatorial).

[He et al., Proteins, 2011]
[Parker et al., J Comp Biol, 2013]
Trade-offs in theory vs. practice

[Savat et al., PLOS Comp Biol, 2015]

p99 beta lactamase: a component of ADEPT anti-cancer therapy

Predicted/Designed

Experimental
### Landscape of approaches

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Function: side-chain packing

Given primary sequence; take backbone as fixed (target)
Select side-chain conformations to minimize energy

- **one-body**
  - internal + vs. backbone
- **two-body**
  - vs. each other
Function: β lactamase

8-mutation variants

Evolutionary and structural function scores

Matched epitope scores

$k_{cat}/K_m$

$T_m$

decreasing epitope content

[Salvat et al., Biotechnol Bioeng 2015]
# Landscape of approaches

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*1
*2
Library-based deimmunization

1. Naïve library
2. High-throughput functional screen ➞ many high function clones
3. Detailed immunogenicity analysis ➞ few low immunogenicity clones
Libraries optimized for deimmunization

1. Design library to be enriched in low-immunogenicity variants
2. Naïve library
3. High-throughput functional screen

=> many high function clones
=> most also low immunogenicity
Library trade-offs

Wild-type

Combinatorial variants

Library screening

Epitope alone

Simultaneous epitope + function

Function alone

=> design for balance, enrichment of functionally deimmunized clones

[Salvat et al., PNAS, 2017]
Application: β-lactamase

[Salvat et al. PNAS, 2017]

10-site libraries

Error-prone

30-site libraries

Chosen
Libraries enriched in high-function variants

β lactamase => growth selection based on cefazolin

\[ [\text{cefa}] = 0 \, \mu \text{g/ml} \]

\[ [\text{cefa}] = 20 \, \mu \text{g/ml} \]

\[ [\text{cefa}] = 75 \, \mu \text{g/ml} \]

\[ [\text{cefa}] = 0 \, \mu \text{g/ml} \]

\[ [\text{cefa}] = 20 \, \mu \text{g/ml} \]

\[ [\text{cefa}] = 75 \, \mu \text{g/ml} \]

% functional variants vs [cefa] µg/ml

[Salvat et al.. PNAS, 2017]
Highly engineered clones -- high activity & stability

[Salvat et al. PNAS, 2017]

### Enzyme Activity & Stability

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<tr>
<th>Enzyme</th>
<th># Mutations</th>
<th>$K_m$ (μM)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_m$ (s$^{-1}$ μM$^{-1}$)</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild Type</td>
<td>N/A</td>
<td>106 ± 7</td>
<td>200 ± 10</td>
<td>1.9 ± 0.2</td>
<td>56.66 ± 0.03</td>
</tr>
<tr>
<td>30:50:E9</td>
<td>14</td>
<td>90 ± 5</td>
<td>320 ± 10</td>
<td>3.6 ± 0.3</td>
<td>56.92 ± 0.06</td>
</tr>
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20 μg/ml 50 μg/ml
PMBC-based immunogenicity

Wild Type P99βL

Naïve T cell

APC

MHC II Peptide

TCR

Naïve T cells

IL-2

WT Peptide

Restimulation

IL2 ELISpot

Deimmunized Variant E9

APC

MHC II Peptide

TCR

Naïve T cells

IL-2

E9 Peptide

Restimulation

IL2 ELISpot
Evades immune recognition

[Salvat et al. PNAS, 2017]
Lysostaphin

anti-staph treatment (incl. MRSA)
+ novel, potent, specific, biodegradable, renewable, less susceptible to resistance, ...
- known to be immunogenic

AATHEHSAQWLNNYKKGYGYGPYPLGINGGMHYGVDFFMNIGTPVKAISSGKIVEAGWSNYG
GGNQIGLIENDGVHRQWYMHSKYNVKGVGDYVKAGQIIGWGSTGYSTAPHIHQRMVNSFS
NSTAQDPMPLKSAGYGKAGGTVPTPNNTGWKTNKYGTKLYKSESASFPTNDIIITRTTGPF
SMPQSGVLKAGQTIHYDEVMKQDGHVWVGYTGNSGQRRIYLPVRTWNKSTNTLGVLWGTIK

catalytic domain (endopeptidase) cell-wall binding domain (SH3b)
Variant summary

[Zhao et al., Chem. Biol., 2015]
In vivo immunogenicity & efficacy

DR4 transgenic mice (i.e., with human MHC)
Two arms: wild-type, Lib5

1. Immunized
2. Infected with MRSA
3. Treated according to arm
After 3x immunization

Higher Ab titers in wild-type arm vs. variant

For targeted allele!

[Zhao et al., Chem. Biol., 2015]
**Infection & treatment**

[Zhao et al., Chem. Biol., 2015]

**Week 3**
All mice rescued from initial infection

**Week 4**
Avg Ab titers increased rapidly for wild-type, and mice exhibiting high titers succumbed to MRSA challenge

**Week 5**
Variant mice maintained low Ab titers and were rescued from a third MRSA challenge

x: serum dilution
y: ELISA absorbance for wild-type and variant
First direct demonstration:
T cell epitope deletion
⇒ reduced Ab titers
⇒ improved efficacy

[Zhao et al., Chem. Biol., 2015]
Conclusion

**Goal:** reduce immunogenicity while maintaining function

**Methods:** both objectives are predictable and designable
- sequence/structure
- individual/library

**Results:** reduced immunogenicity & *improved* efficacy
Thanks

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