Computationally-driven deimmunization of therapeutic proteins

Chris Bailey-Kellogg

Disclosure

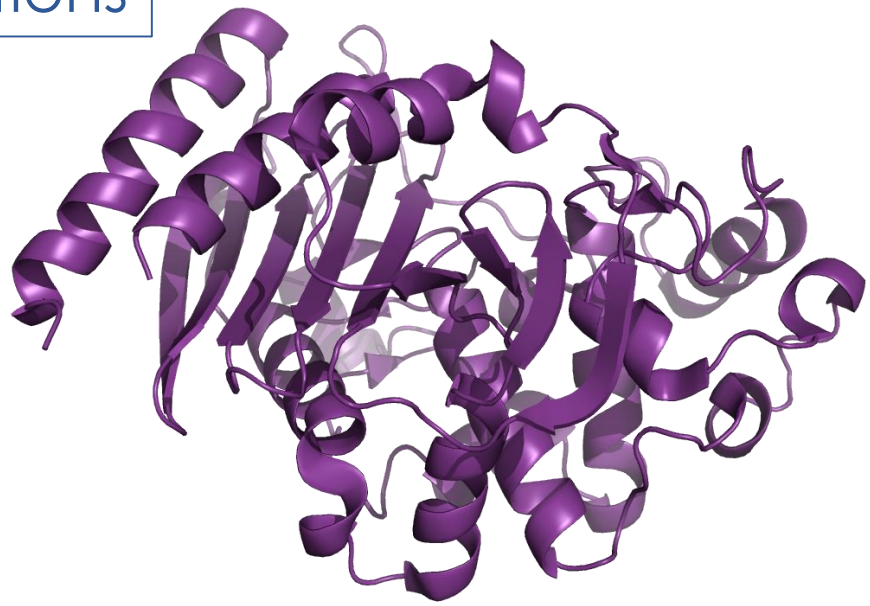
Chris Bailey-Kellogg and Karl E. Griswold are co-founders of Occulo Holdings LLC, Occulo Bio LLC, Stealth Biologics LLC, and Lyticon LLC. These biotechnology companies are pursuing design and development of deimmunized biotherapeutic agents and antibacterial biotherapies. Related research in the Griswold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

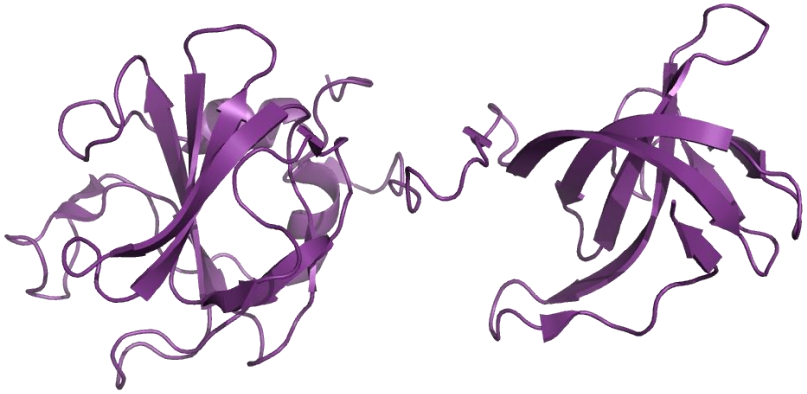
(Non-Ab) protein therapeutics

diverse structures, functions

β-Lactamase

- cleaves lactam ring
- antibody-directed anti-cancer therapies





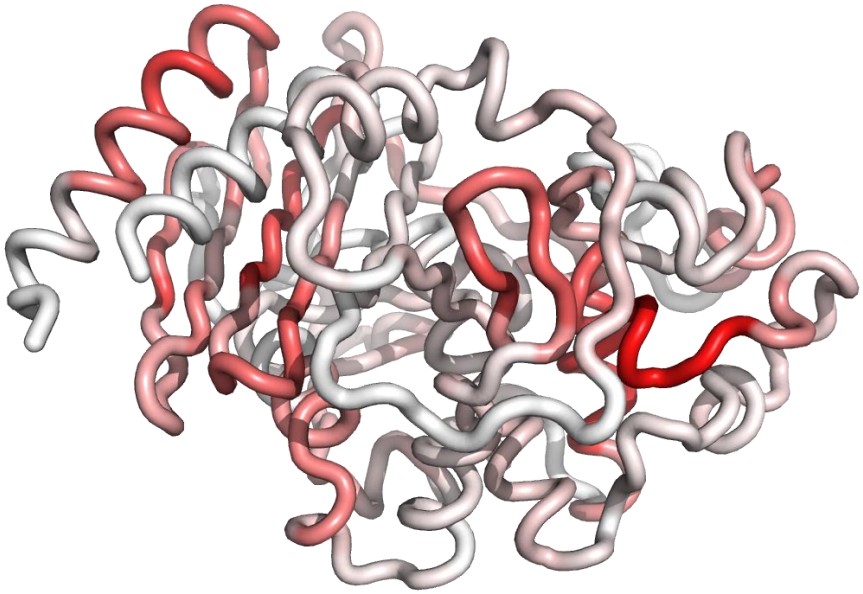
lysostaphin

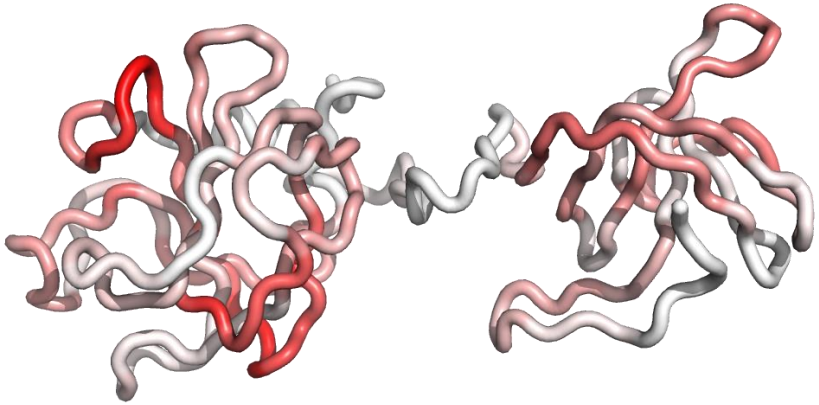
- degrades cell wall
- anti-staph

Protein therapeutics: immunogenicity

red: epitopes

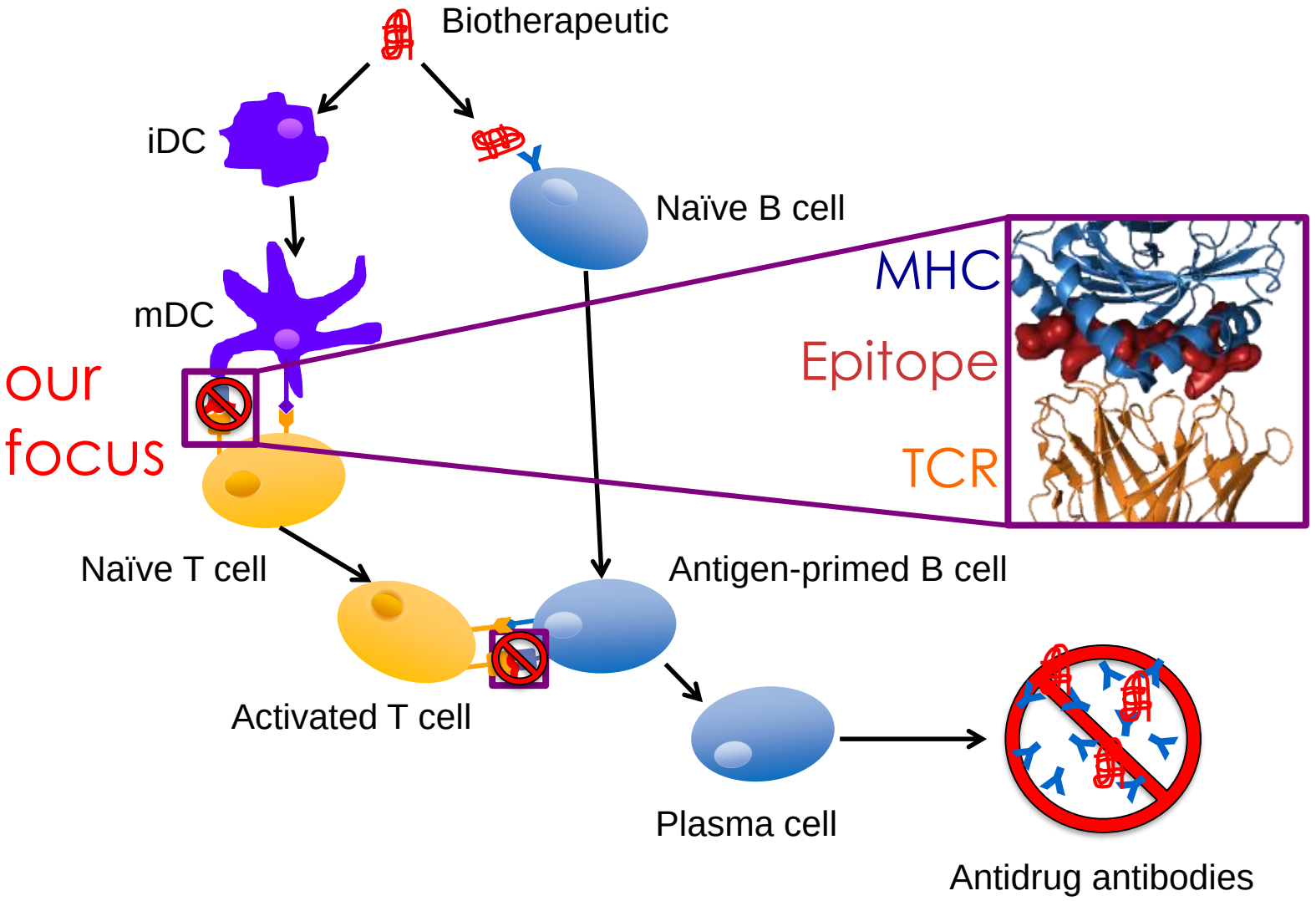
β-Lactamase
- cleaves lactam ring
- antibody-directed anti-cancer therapies





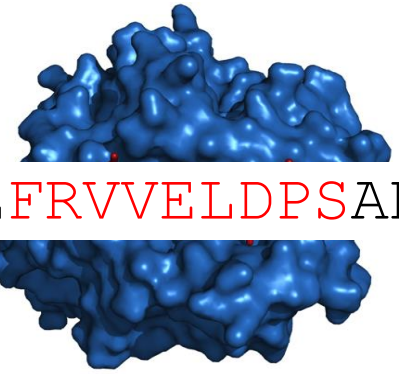
lysostaphin
- degrades cell wall
- anti-staph

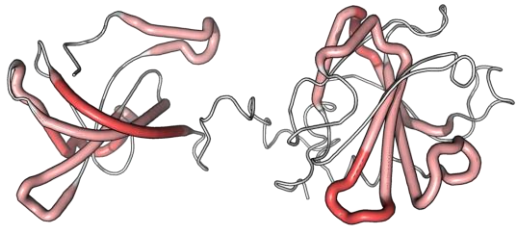
Anti-biotherapeutic immunogenicity



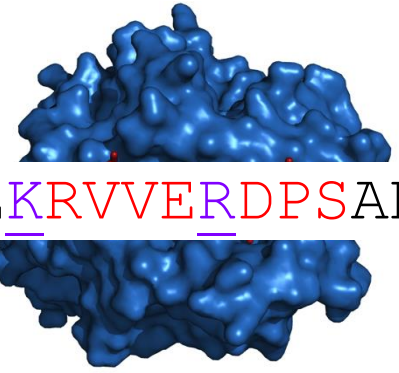
Deimmunization: competing objectives

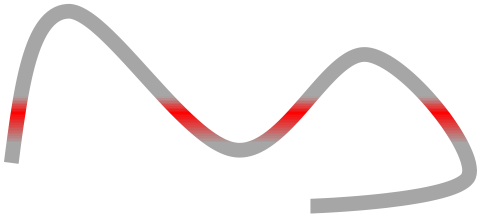
wild-type …TAYKEFRVVELDPSAKI…



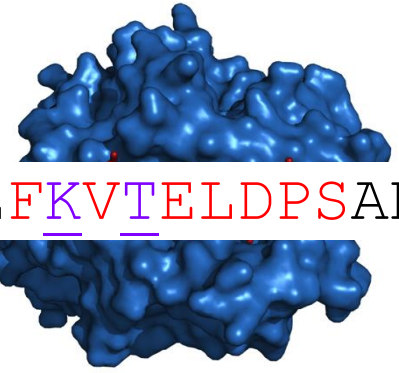


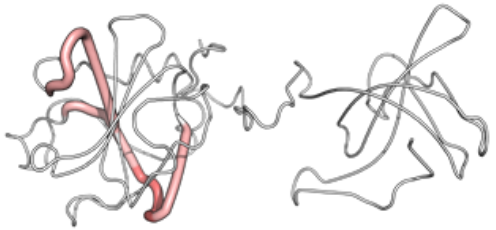
variant 1 …TAYKEKRVVERDPSAKI…





variant 2 …TAYKEFKVTELDPSAKI…

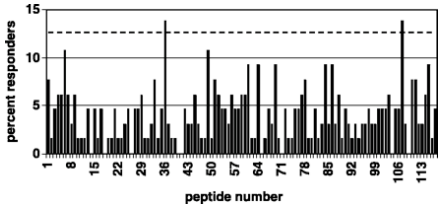




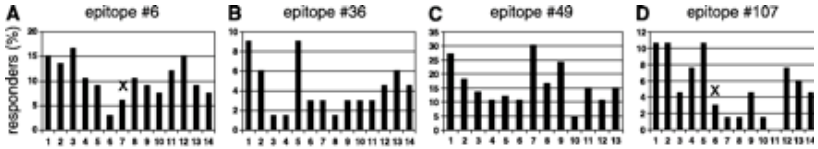
Experimentally-driven approaches

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

immunogenicity screening



alanine scanning



engineer into whole protein

Epitope	Mutation	Expression	Stability
6	M20A	0.05	ND*
6	K21A	1.73	0.53
6	S24A	2.23	1.11
6	V30A	0.12	7.6
36	L107A	0.10	ND*
36	D108A	0.09	ND*
36	T113A	0.21	ND*
36	P118A	0.16	ND*
49	T146A	0.47	0.42
49	T147A	0.43	0.38
49	L149A	0.73	0.2
49	I155A	0.19	ND*
107	S324A	0.80	0.65
107	V326A	0.53	0.50
107	I329A	0.05	ND*
107	P330A	0.06	ND*

possible variants

lots of experimental time and expense

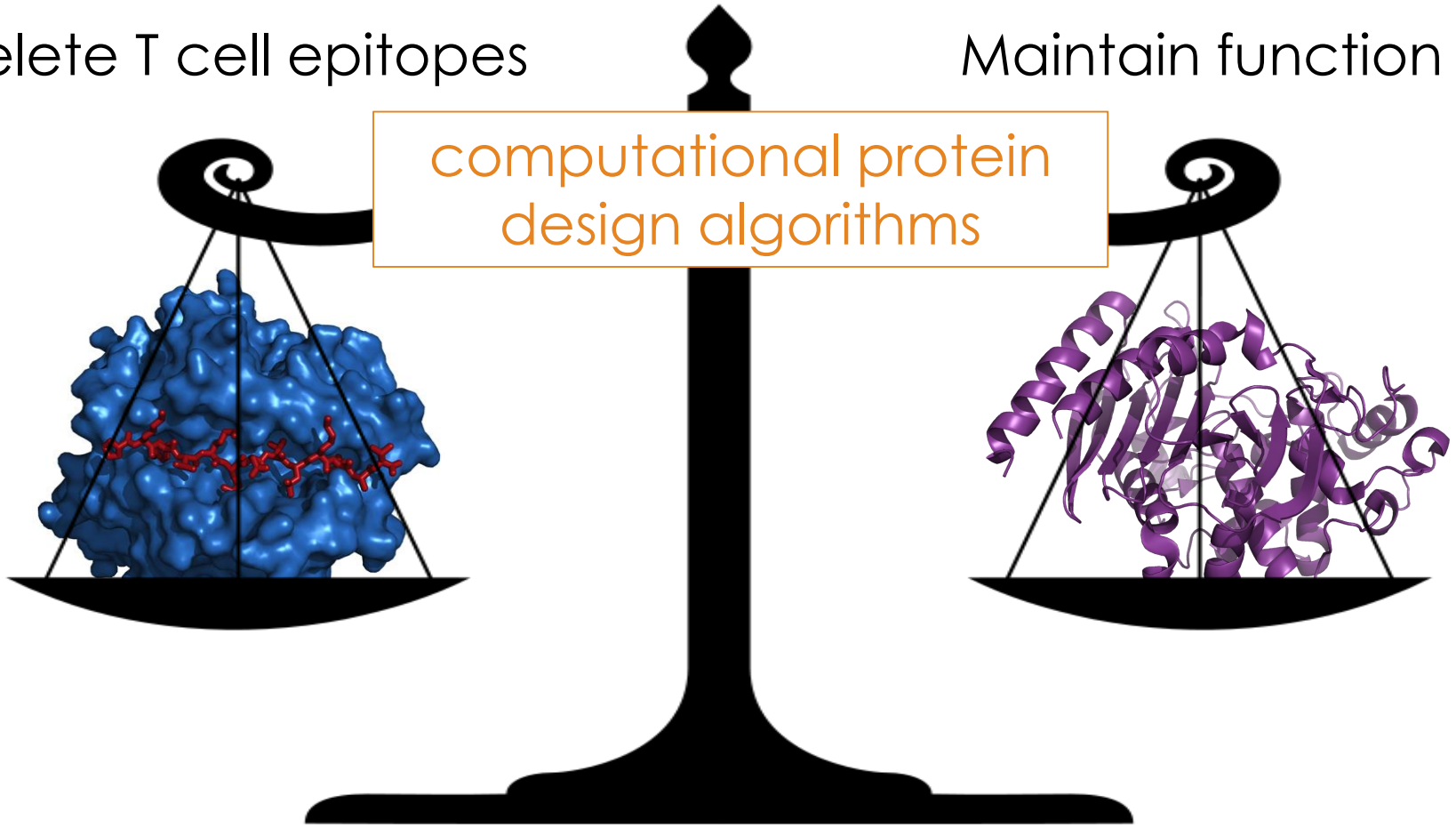
may not consider some good possibilities

K21A, S324A

Our approach:
computationally-driven deimmunization

Delete T cell epitopes

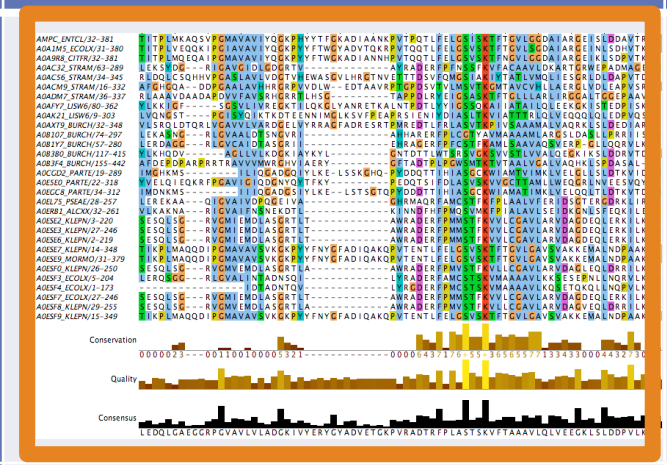
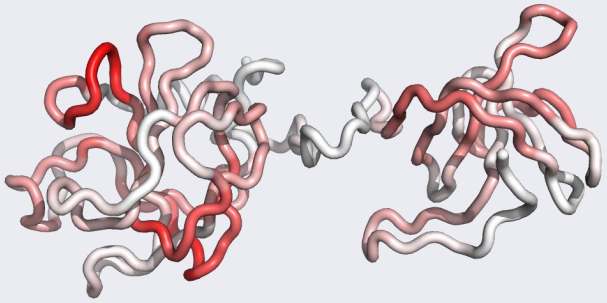
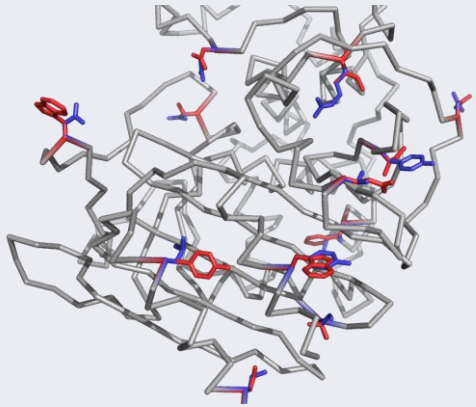
Maintain function



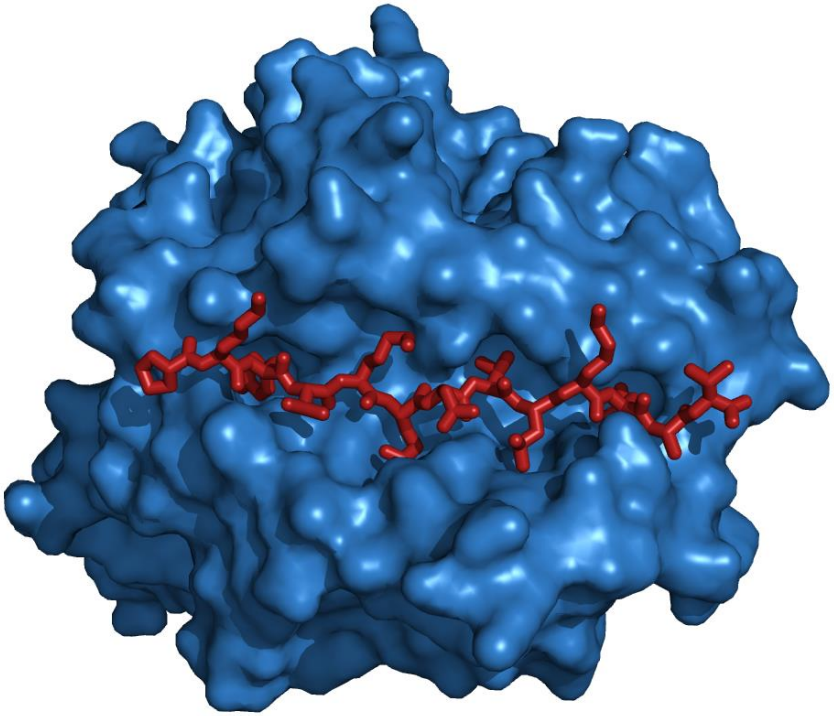
epitope content

stability, activity

Landscape of approaches

	Individual variants	Combinatorial library
Evolutionary		...TAYKEFRVVELDPSAKI... D A E S T ...TAYKDFRVVELDPAAKI... ...TAYKEFRVVELDPSAKI... ...TAYKDFRVVELDPTAKI... ...TAYKEFRVVELDPAAKI... ...TAYKDFRVVELDPSAKI... ...TAYKEFRVVELDPTAKI...
Structural		

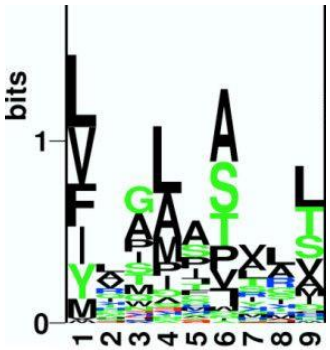
Epitope content



=> different side-chain preferences

Nielsen et al.,
BMC Bioinformatics 2007

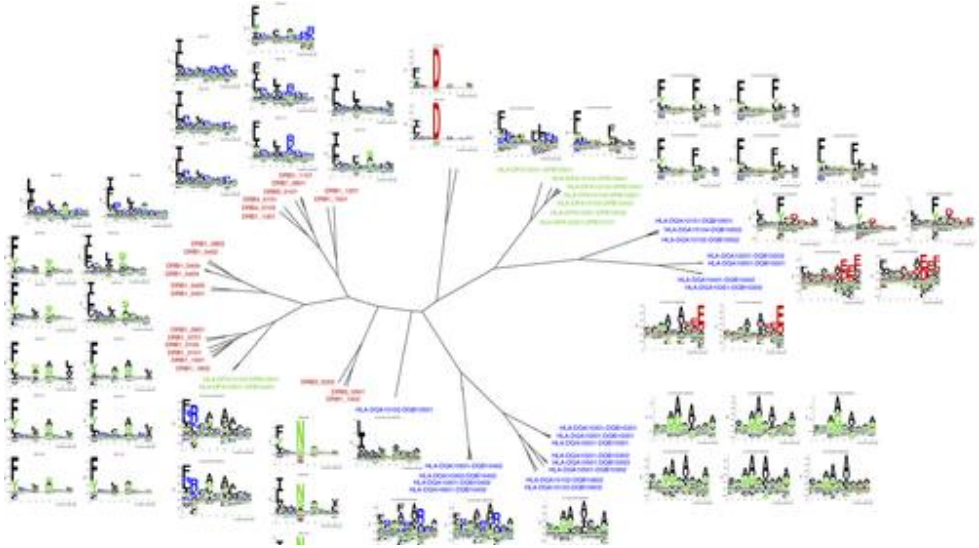
DRB1*01:01



=> predictors for representative alleles

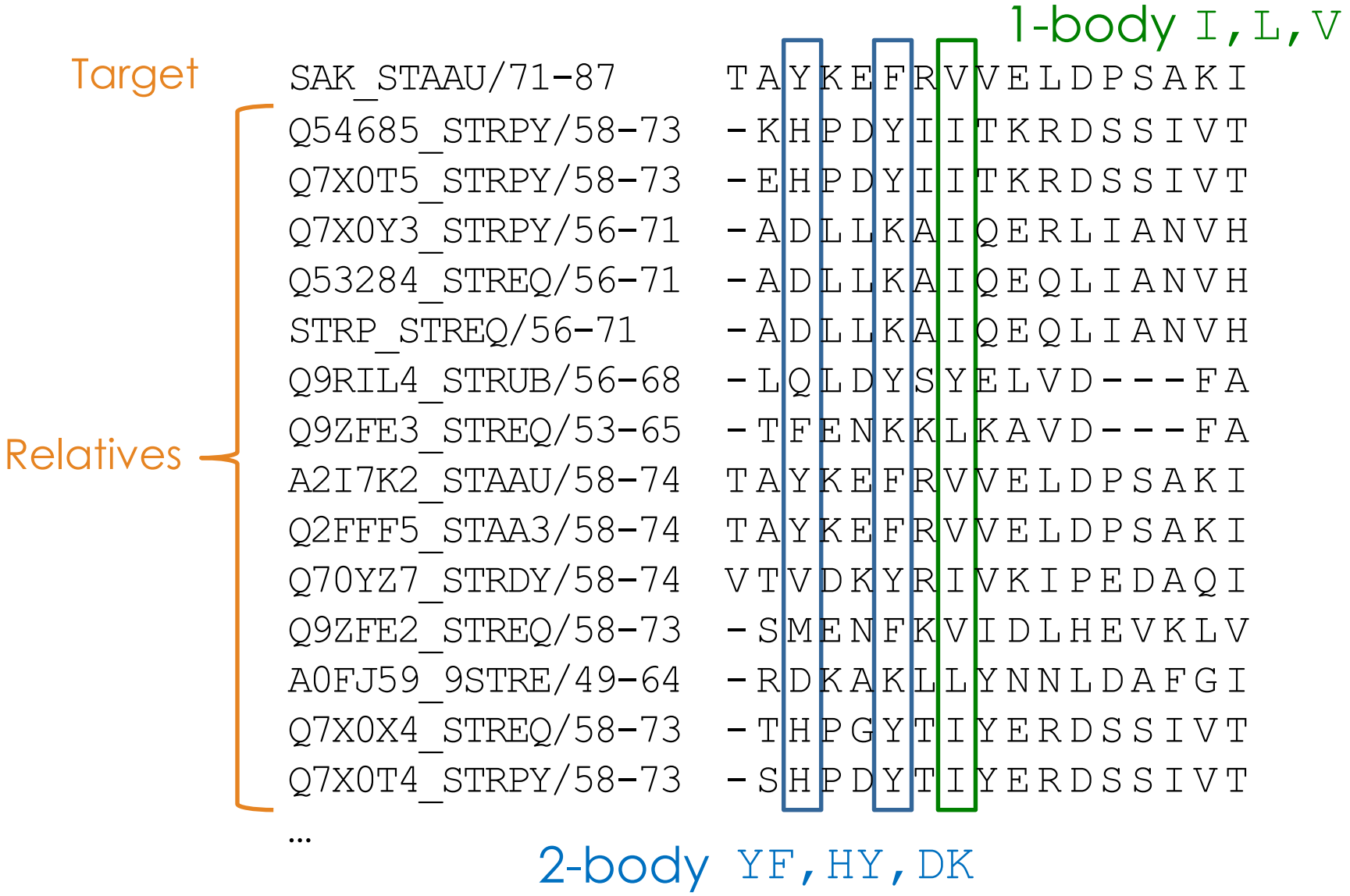
different "pocket" shapes

Sturniolo *et al.*, *Nat. Biotech*. 1999



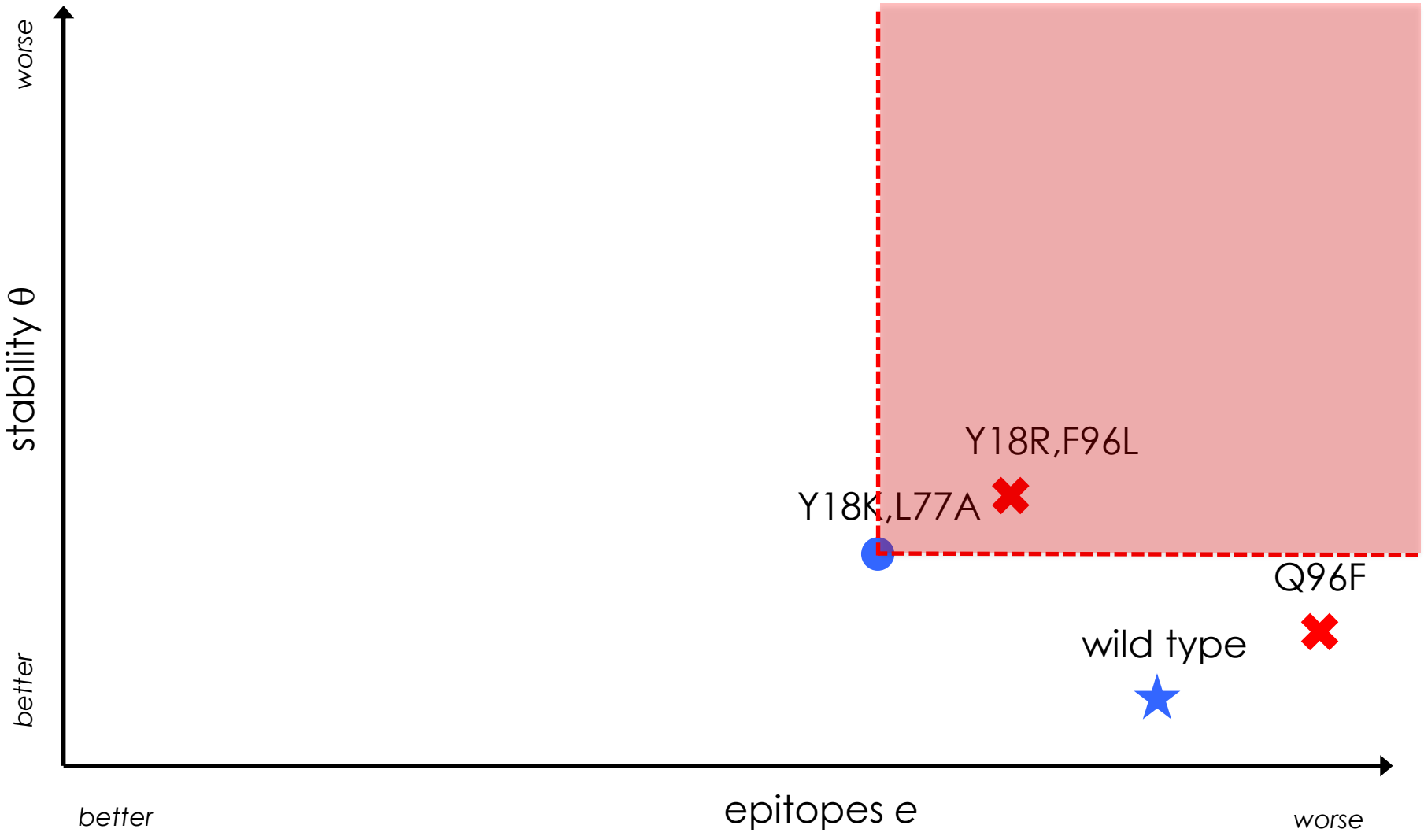
Jensen et al., *J. Immunology* 2018

Function: evolutionary



Trade-offs: EpiSweep

[He et al., *Proteins*, 2011]
[Parker et al., *J Comp Biol*, 2013]



Trade-offs: EpiSweep

[He et al., *Proteins*, 2011]
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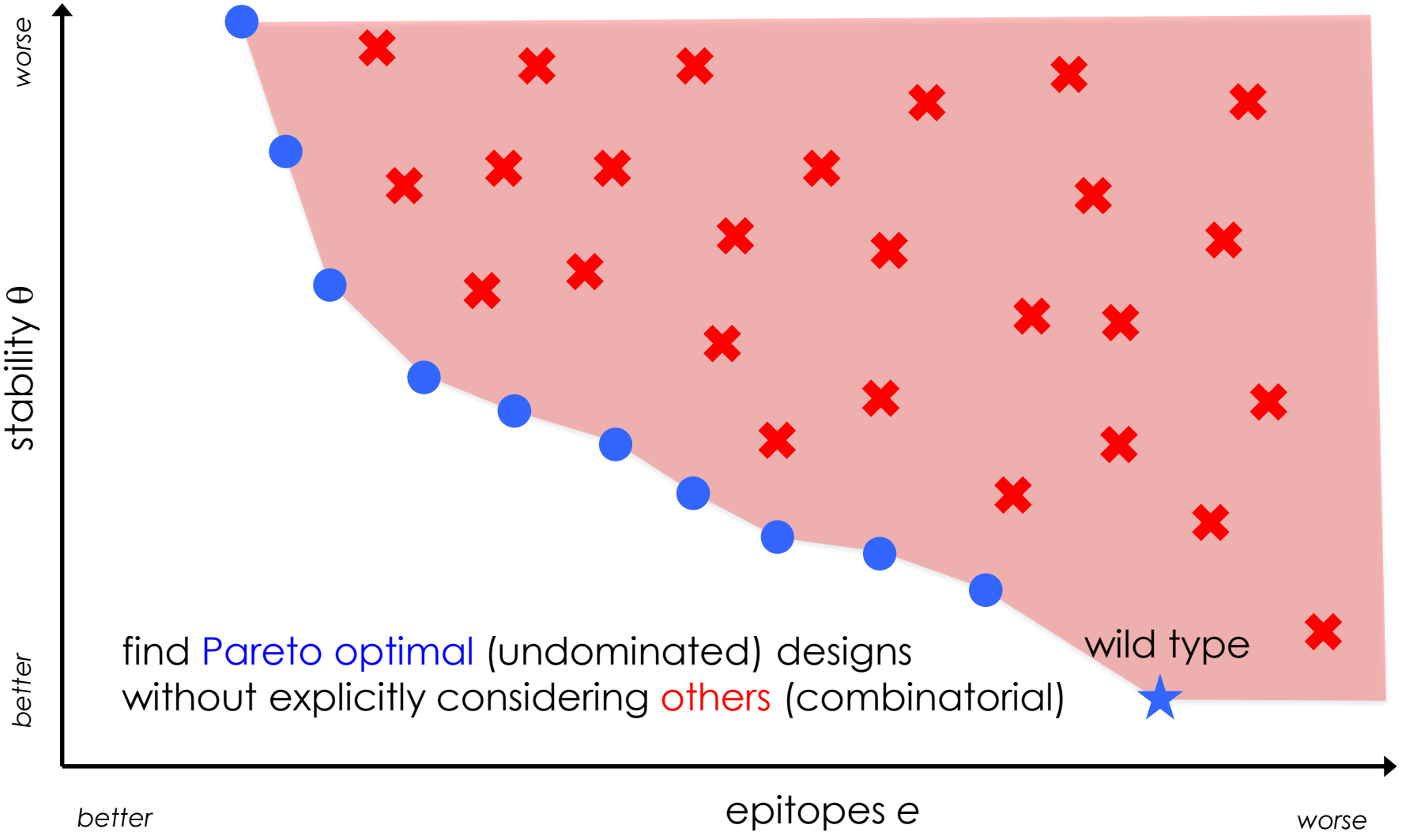
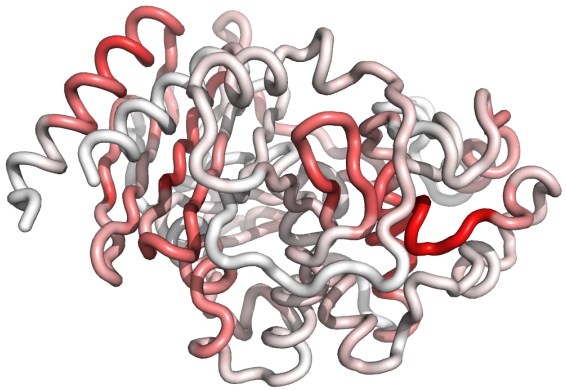
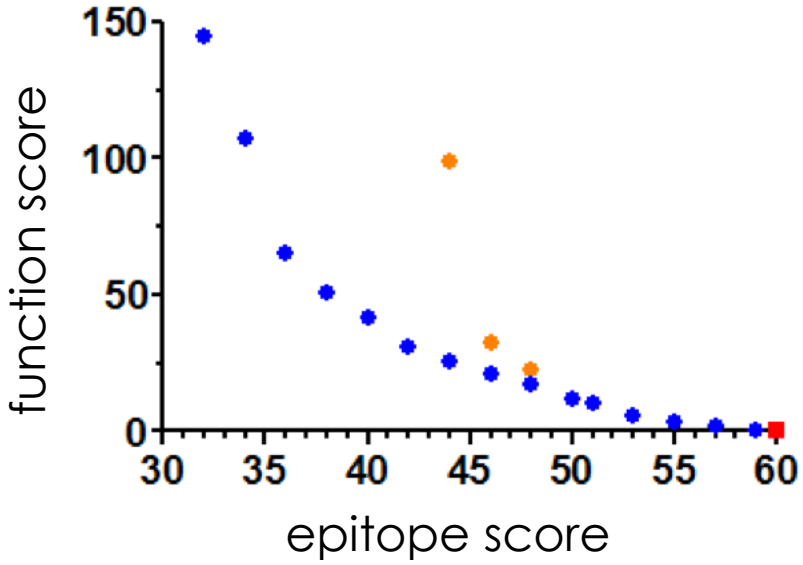


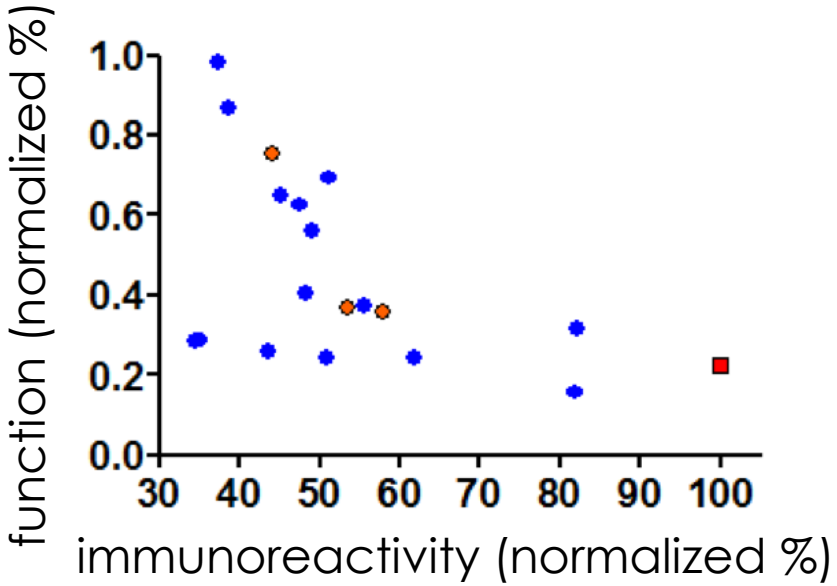
Trade-offs in theory vs. practice

[Salvat et al., *PLOS Comp Biol*, 2015]

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



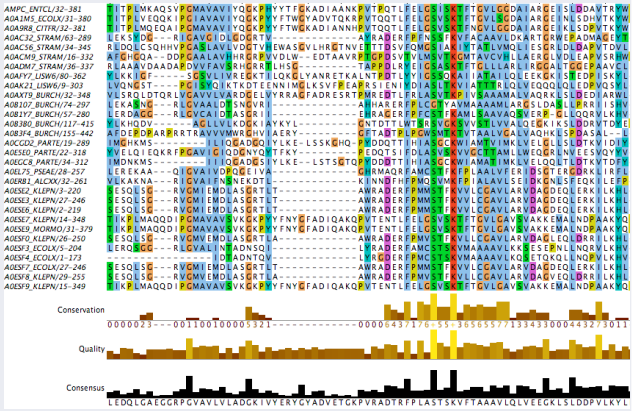
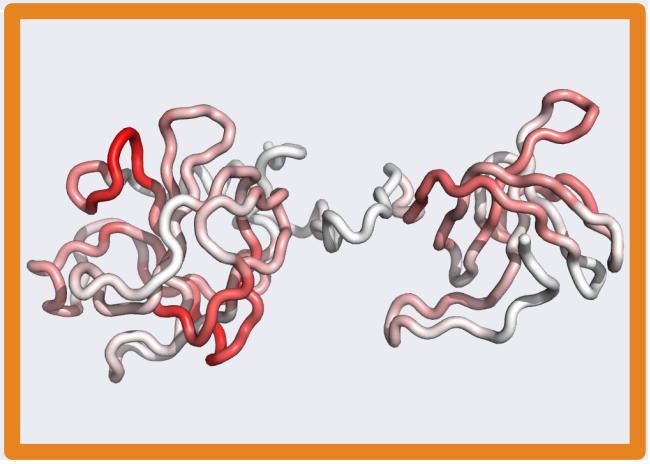
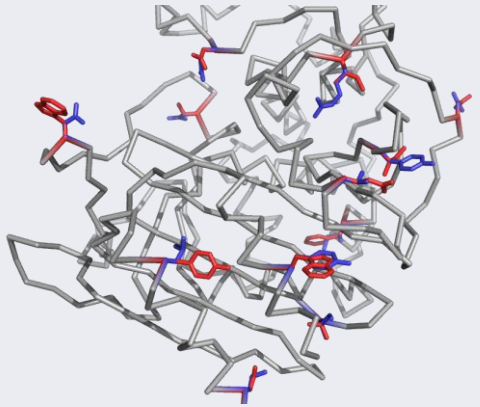




predicted/designed

experimental

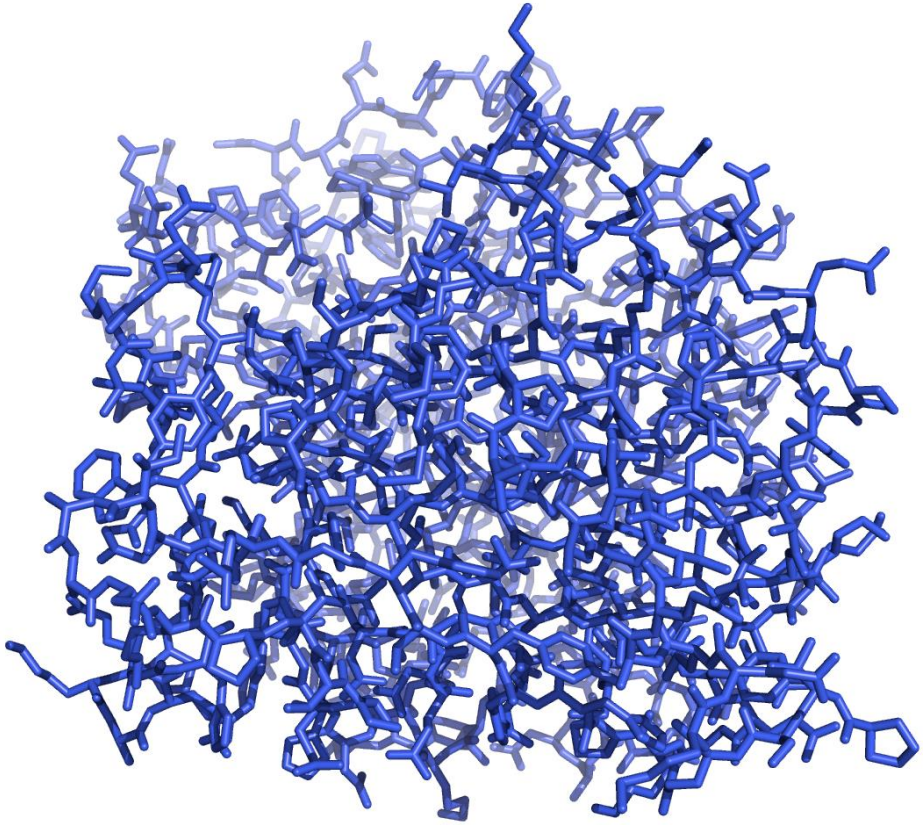
Landscape of approaches

	Individual variants	Combinatorial library
Evolutionary		...TAYKEFRVVELDPSAKI... D A E S T ...TAYKDFRVVELDPAAKI... ...TAYKEFRVVELDPSAKI... ...TAYKDFRVVELDPTAKI... ...TAYKEFRVVELDPAAKI... ...TAYKDFRVVELDPSAKI... ...TAYKEFRVVELDPTAKI...
Structural		

Function: side-chain packing

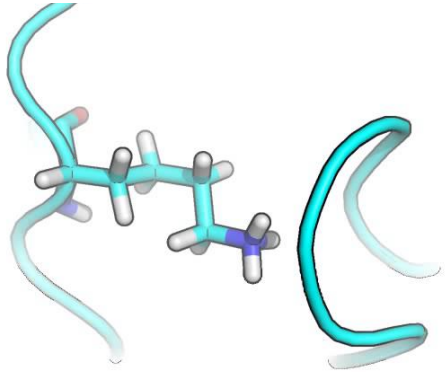
Given primary sequence; take backbone as fixed (target)

Select side-chain conformations to minimize energy



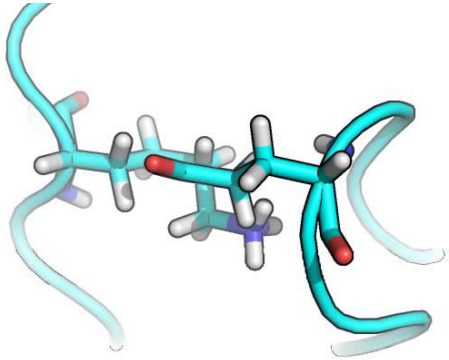
one-body

internal + vs. backbone



two-body

vs. each other



Function: β lactamase

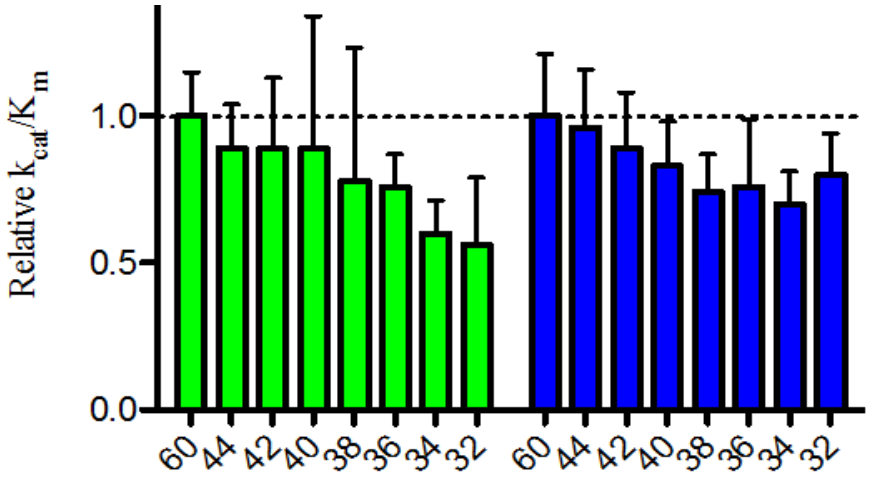
[Salvat et al., *Biotechnol Bioeng* 2015]

8-mutation variants

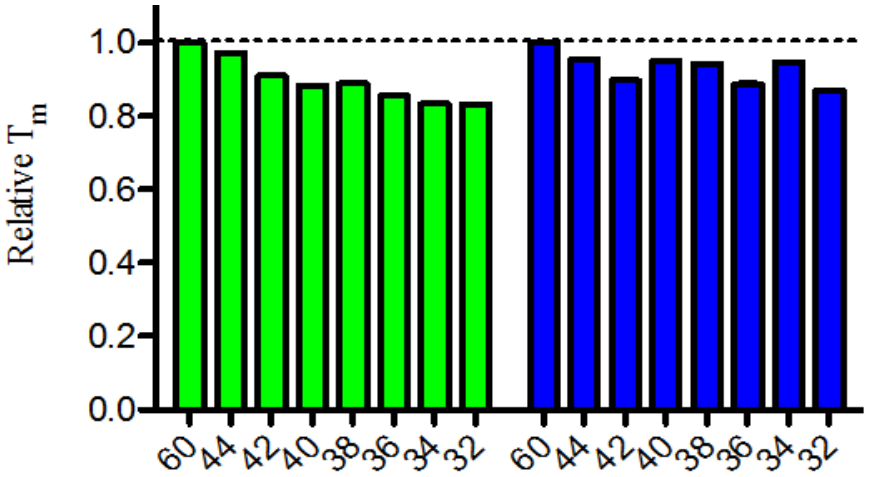
Evolutionary and structural function scores

Matched epitope scores

kcat/Km

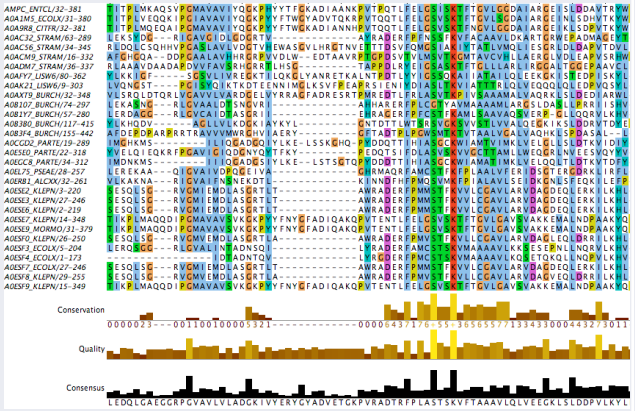
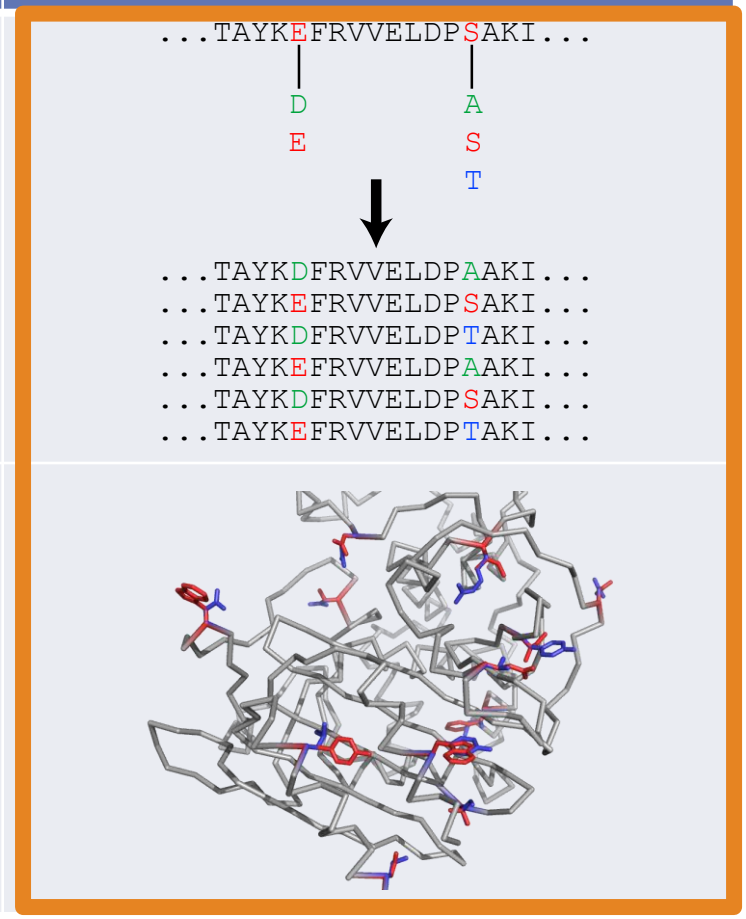
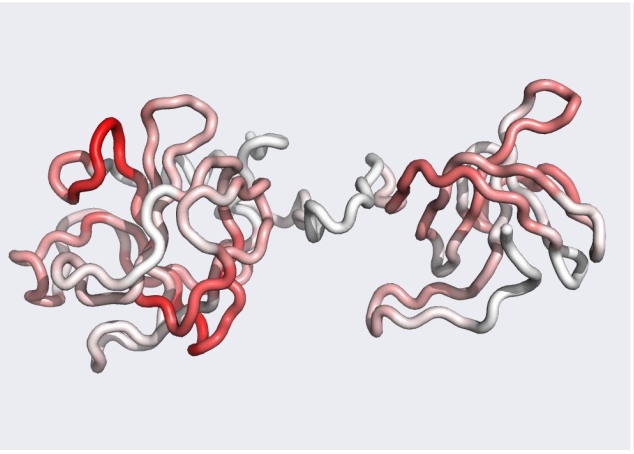


Tm

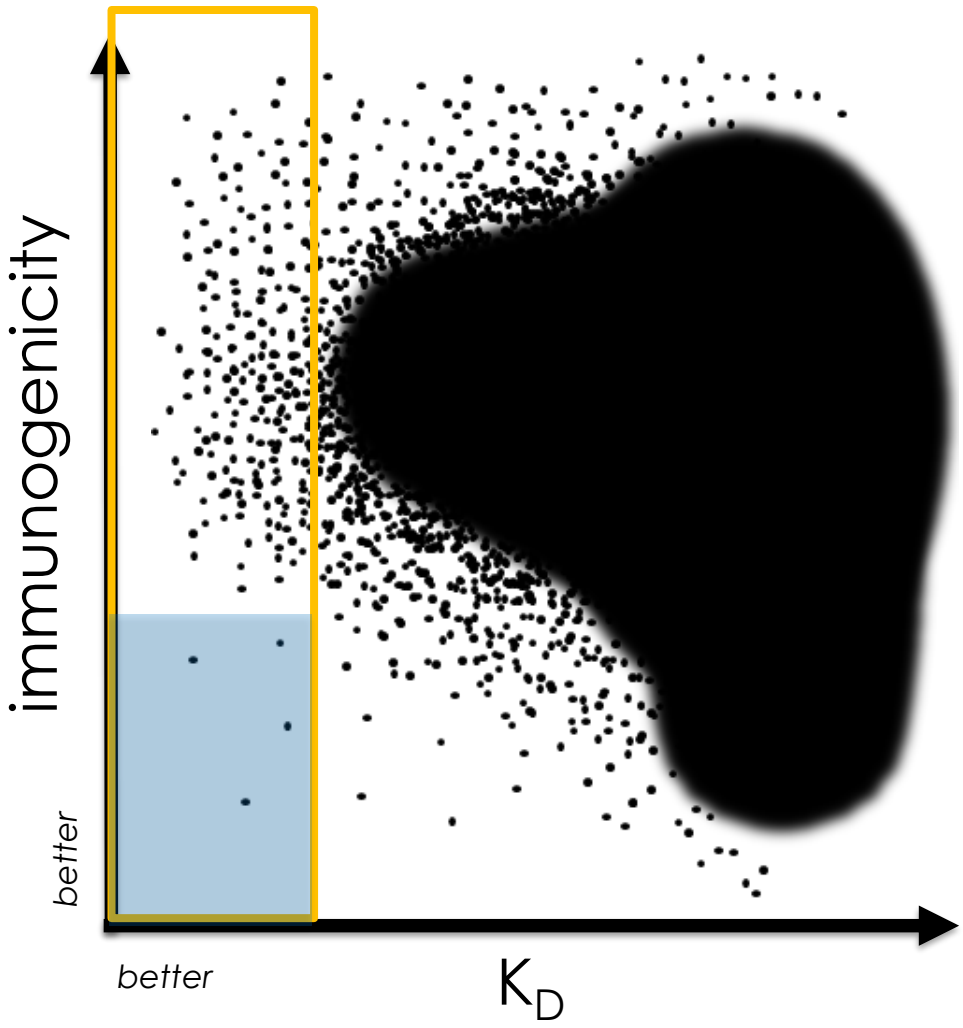


decreasing epitope content

Landscape of approaches

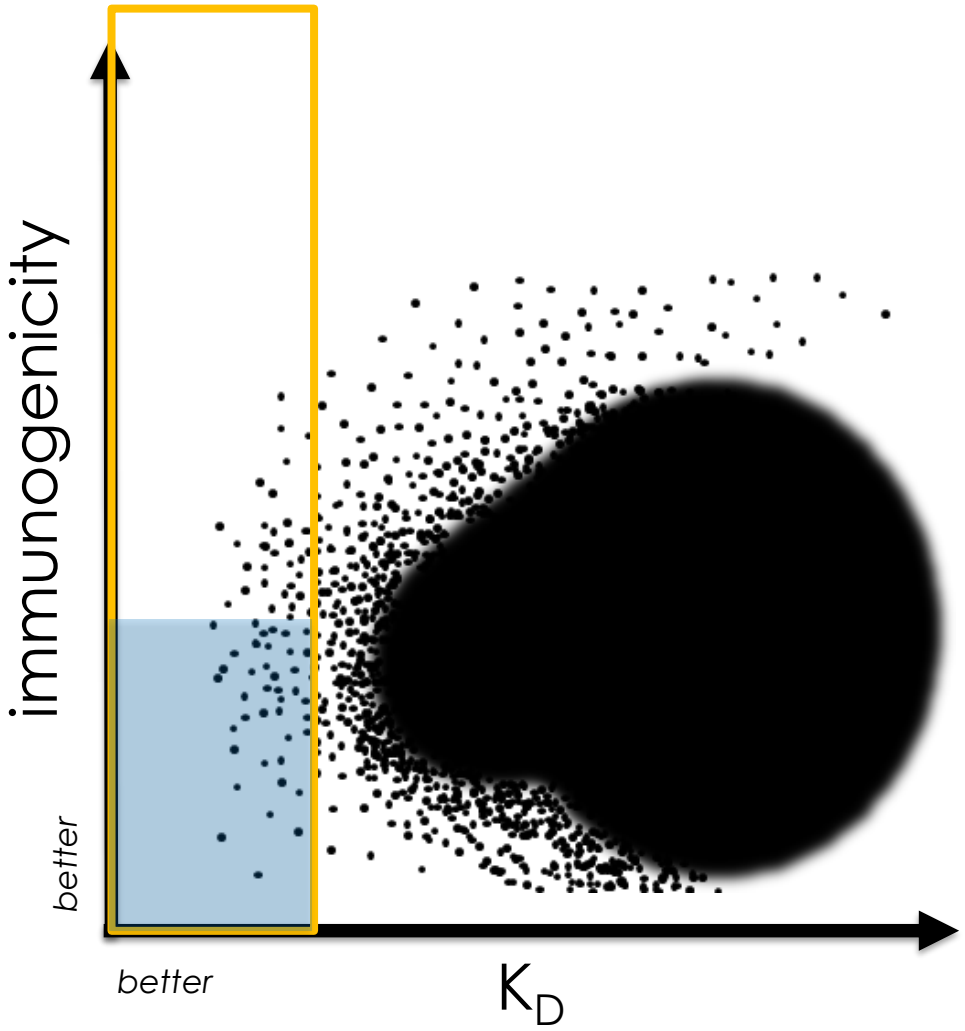
	Individual variants	Combinatorial library
Evolutionary		
Structural		

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



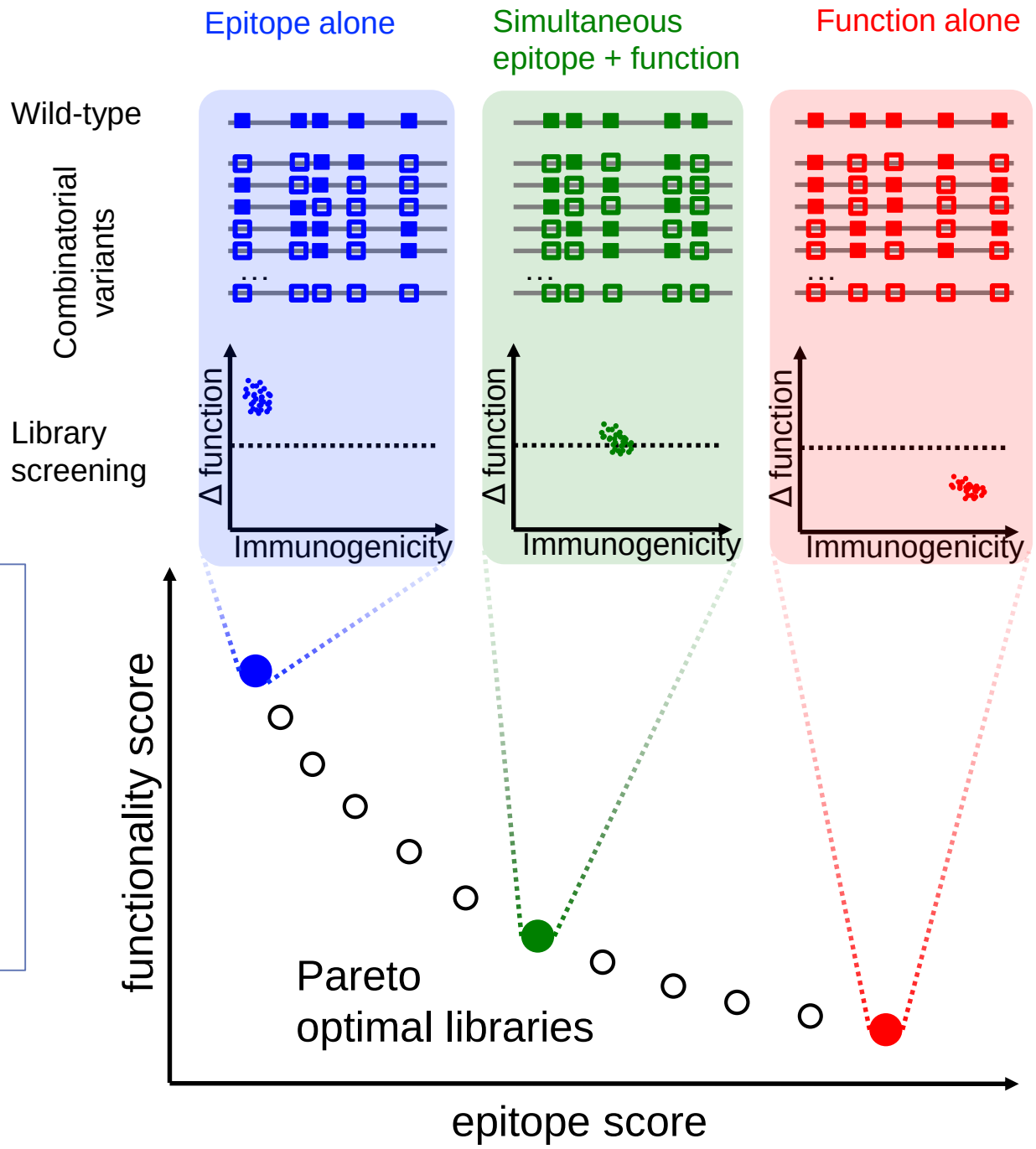
1. Design library to be enriched in low-immunogenicity variants
2. Naïve library
3. High-throughput functional screen

=> many high function clones

=> most also low immunogenicity

Library trade-offs

[Salvat et al., *PNAS*, 2017]

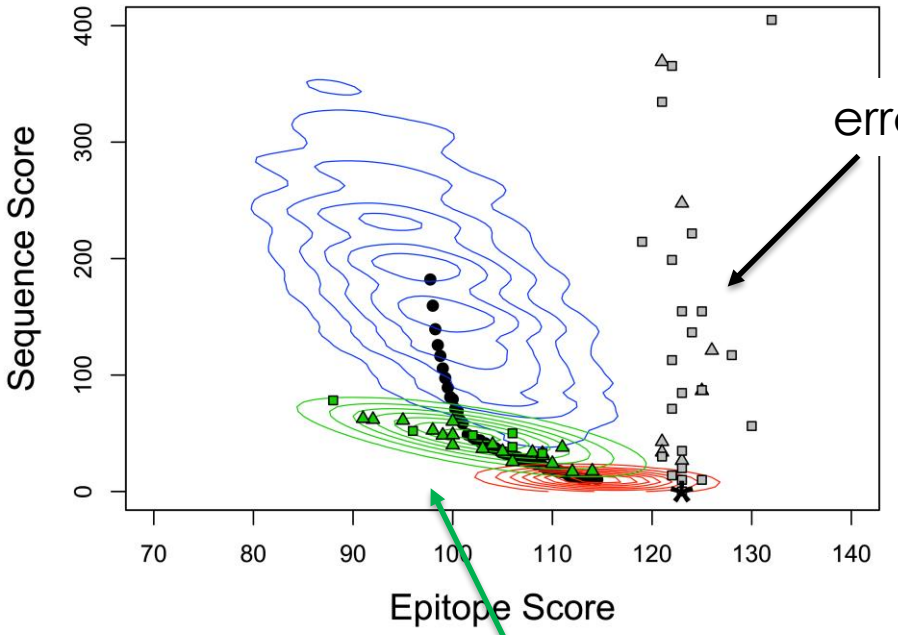


=> design for balance, enrichment of functionally deimmunized clones

Application: β-lactamase

[Salvat et al.. *PNAS*, 2017]

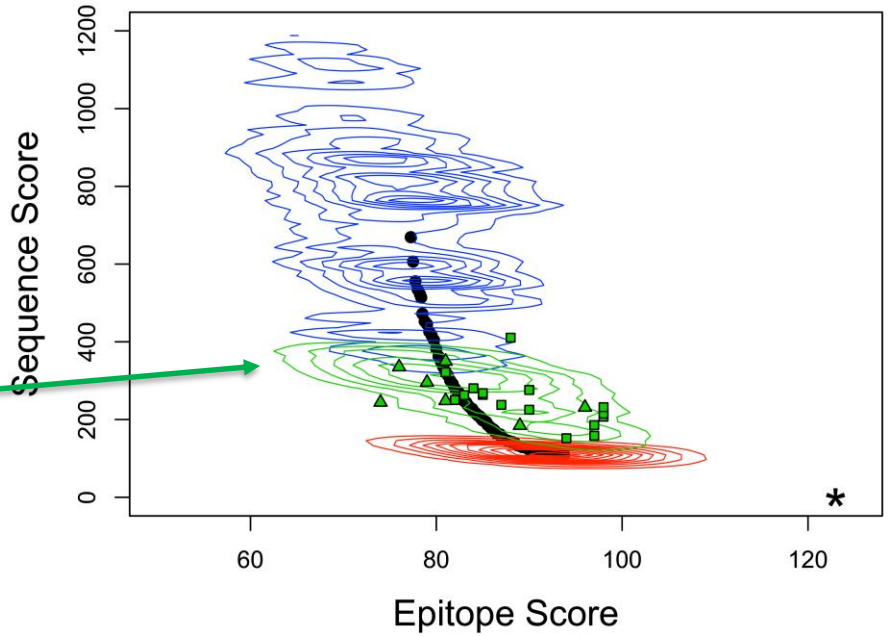
10-site libraries



error-prone

chosen

30-site libraries

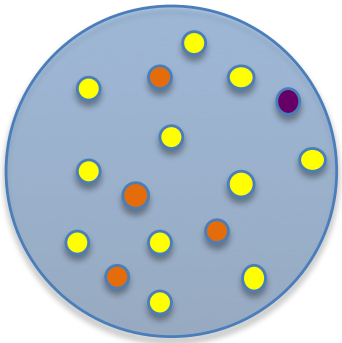


Libraries enriched in high-function variants

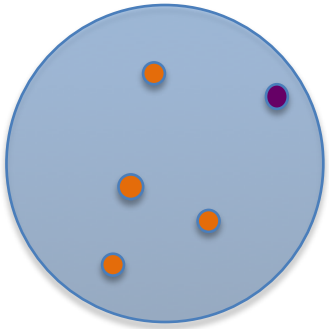
[Salvat et al.. *PNAS*, 2017]

β lactamase => growth selection based on cefazolin

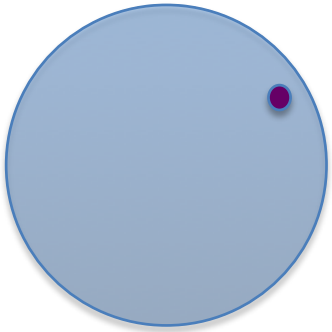
[cefa] = 0 µg/ml

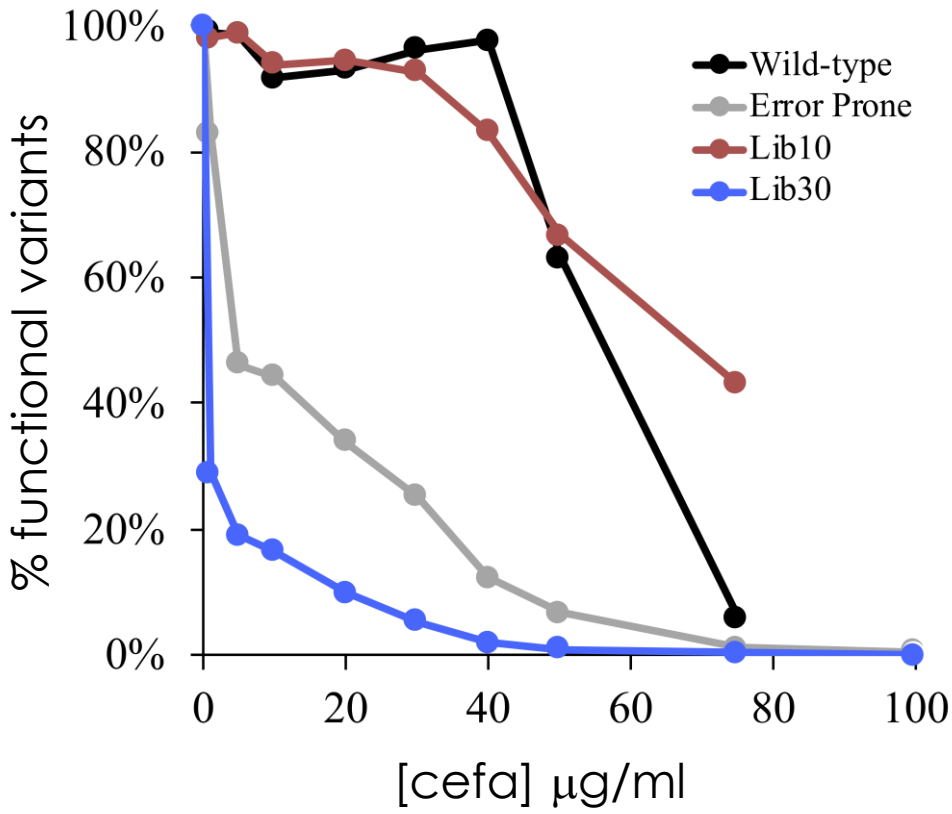


[cefa] = 20 µg/ml



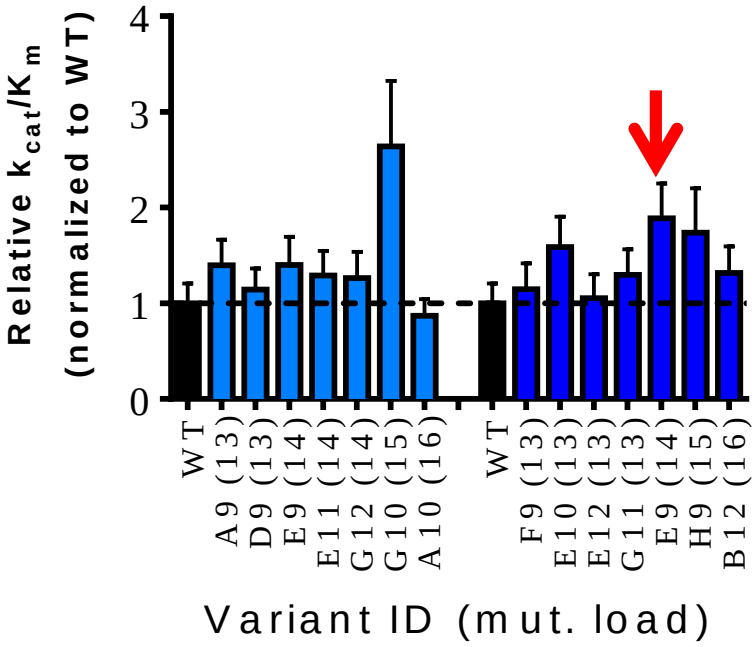
[cefa] = 75 µg/ml

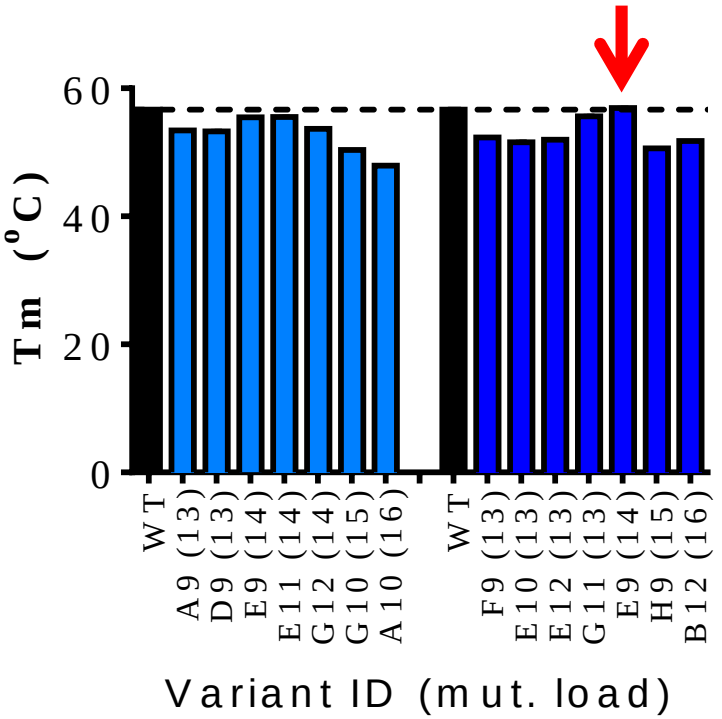




Highly engineered clones -- high activity & stability

[Salvat et al.. *PNAS*, 2017]

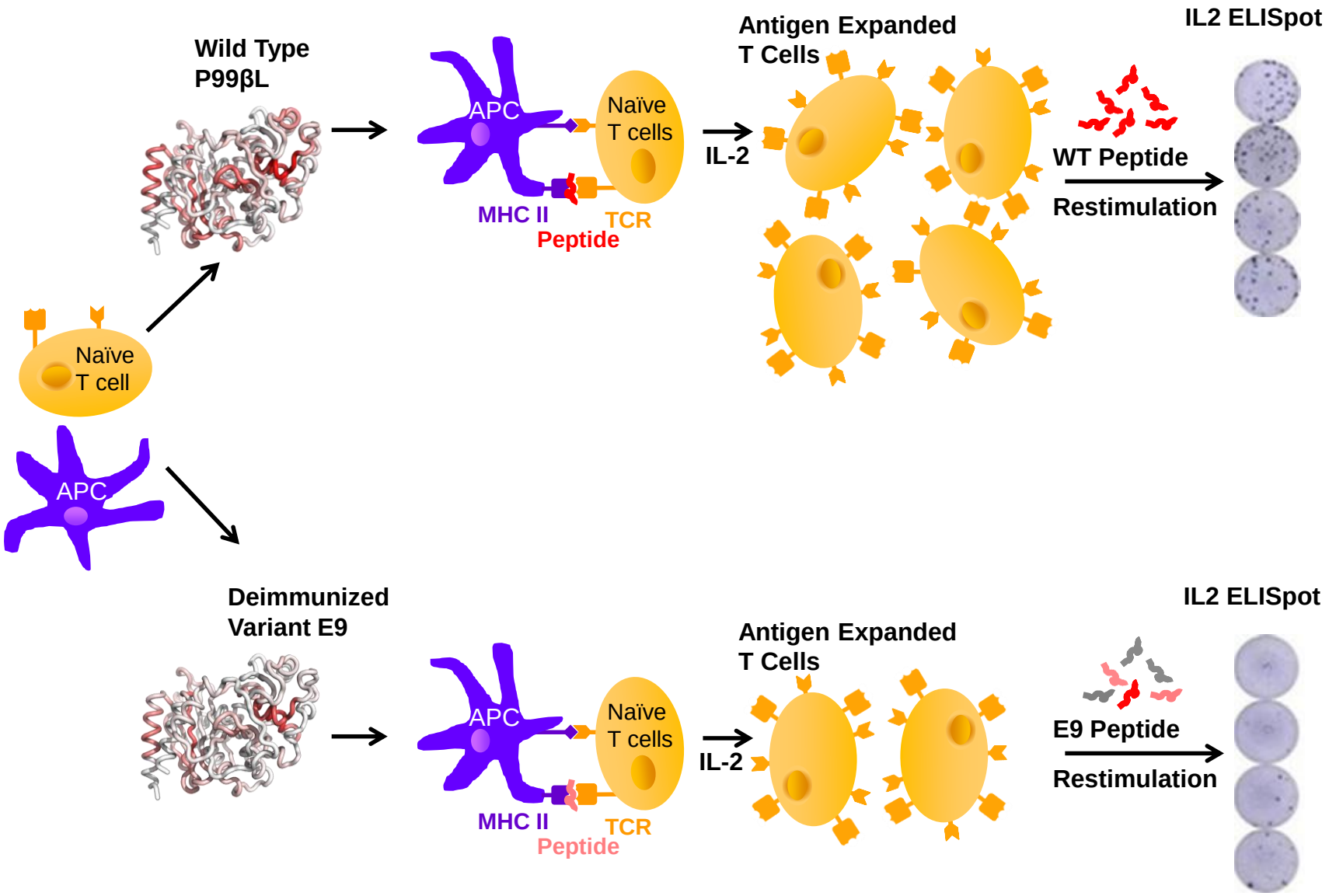




20 µg/ml 50 µg/ml

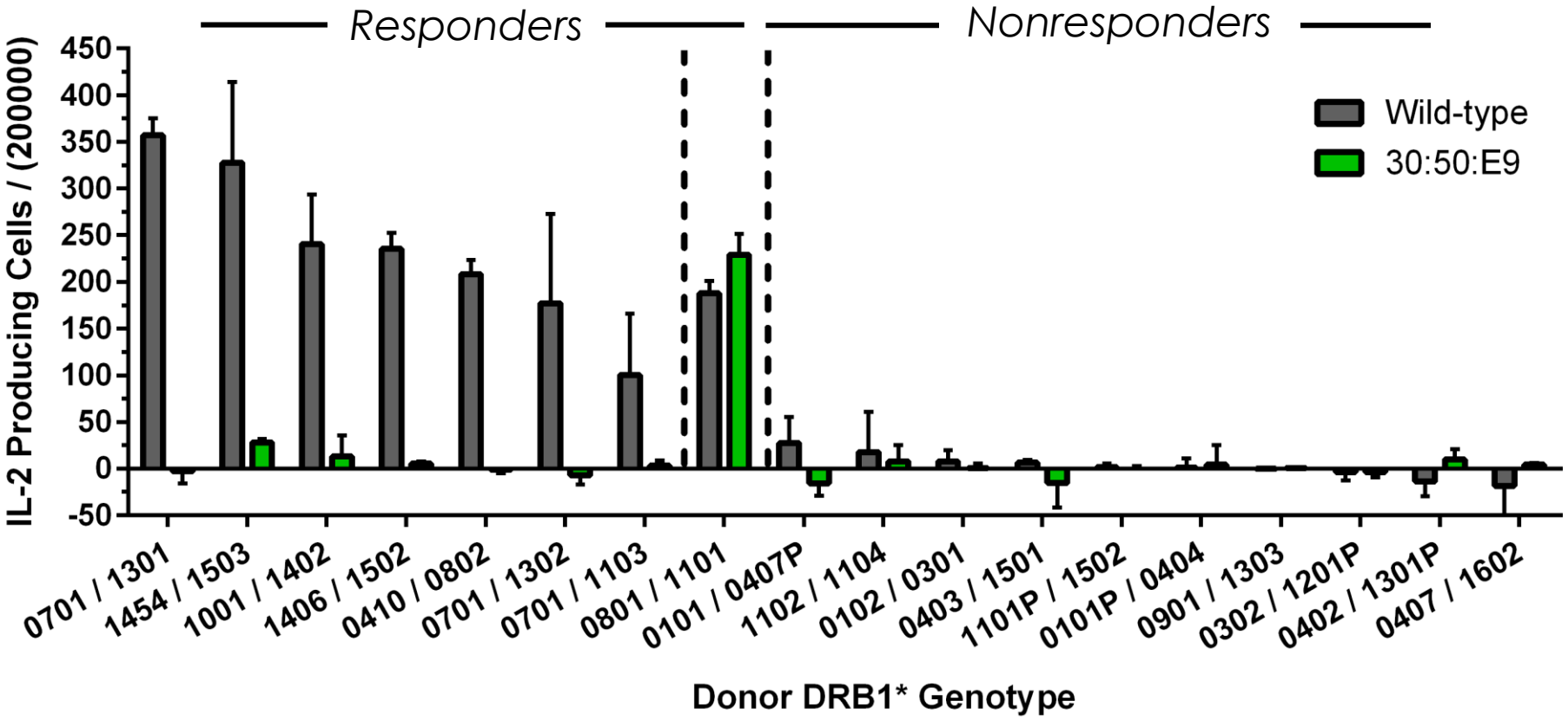
Enzyme	# Mutations	K_m (µM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} μM^{-1})	T_m (°C)
Wild Type	N/A	106 ± 7	200 ± 10	1.9 ± 0.2	56.66 ± 0.03
30:50:E9	**14**	**90 ± 5**	**320 ± 10**	**3.6 ± 0.3**	**56.92 ± 0.06**

PMBC-based immunogenicity



Evades immune recognition

[Salvat et al.. *PNAS*, 2017]

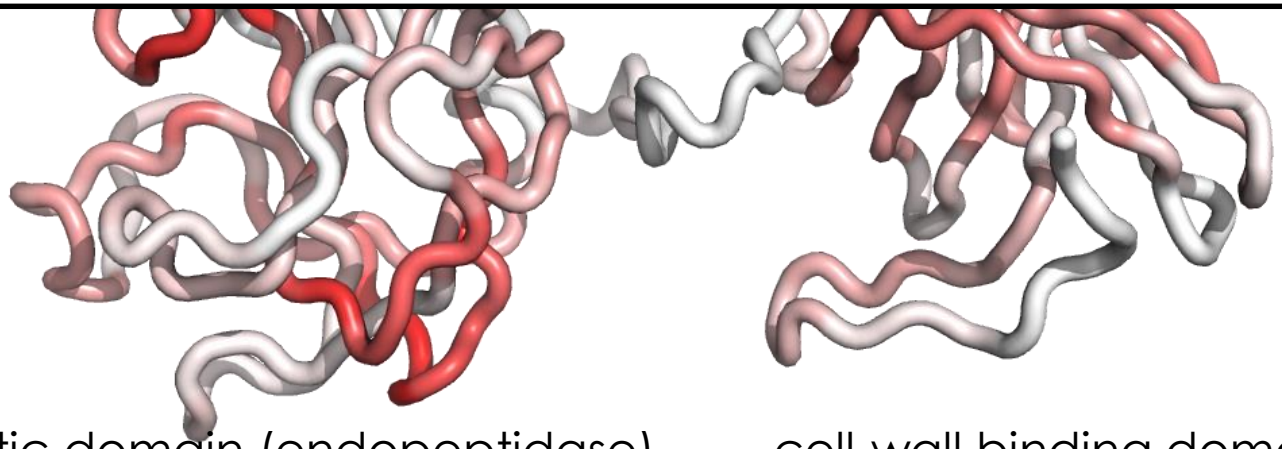


Lysostaphin

anti-staph treatment (incl. MRSA)

+ novel, potent, specific, biodegradable, renewable, less susceptible to resistance, …
- known to be immunogenic

AATHEHSAQWLNNYKKGYGYGPYPLGINGGMHYGVDFFMNIGTPVKAISSGKIVEAGWSNYG
GGNQIGLIENDGVHRQWYMHLSKYNVKVGDYVKAGQIIGWSGSTGYSTAPHLHFQRMVNSFS
NSTAQDPM*PFLKSAGYGKAGG*TVTPTPNTGWKTNKYGTLYKSESASFTPNTDIITRTTGPFR
SMPQSGVLKAGQTIHYDEVMKQDGHVWVGYTGNSGQRIYLPVRTWNKSTNTLGVLWGTIK

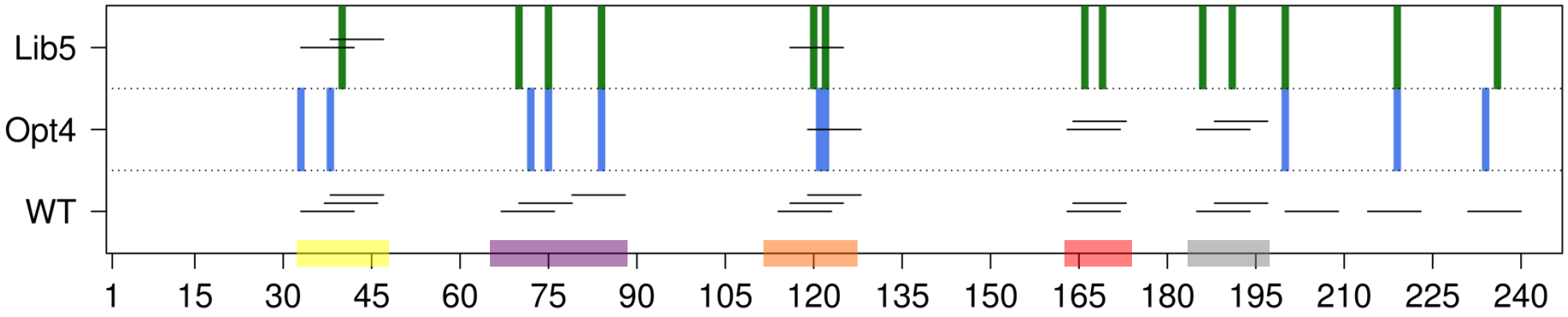


catalytic domain (endopeptidase)

cell-wall binding domain (SH3b)

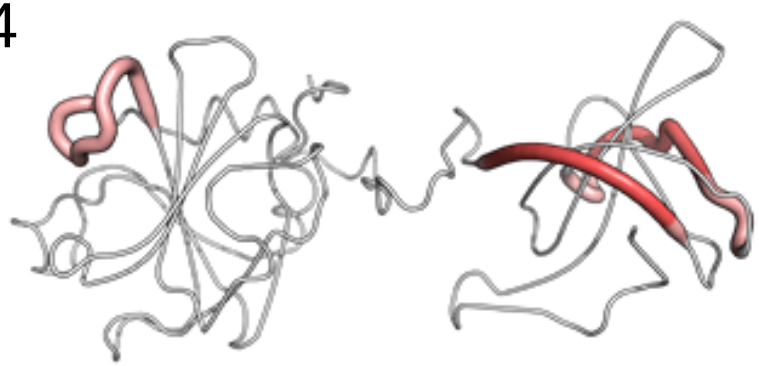
Variant summary

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

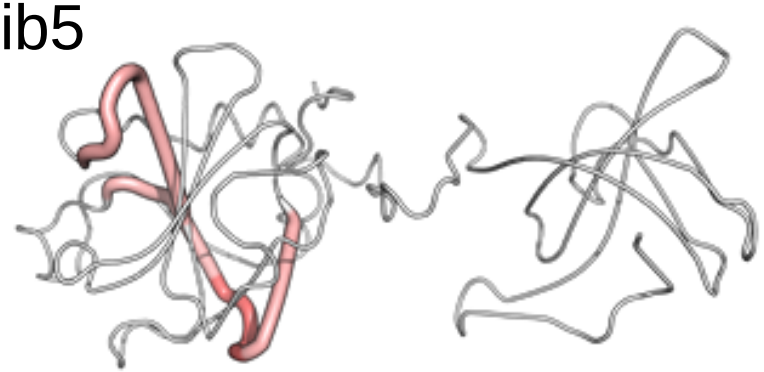


WT

Opt4

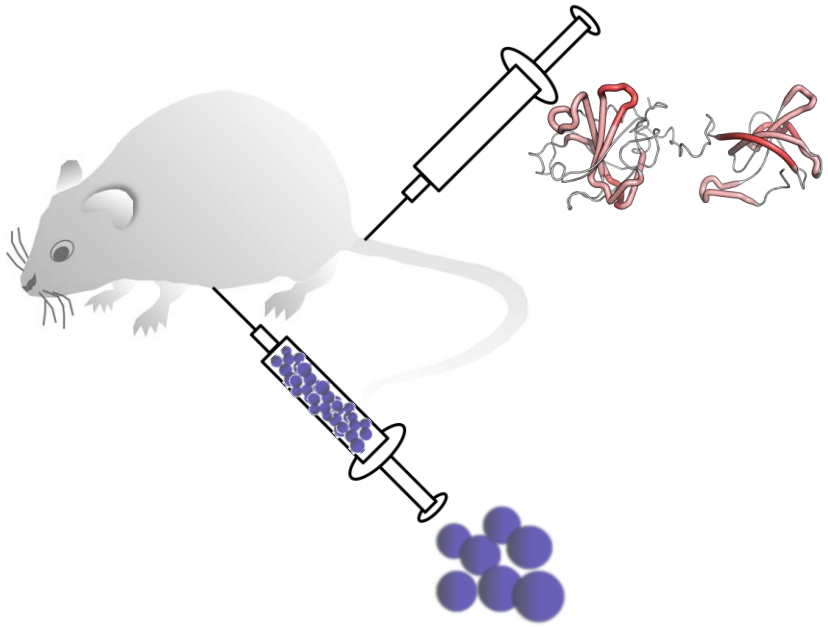


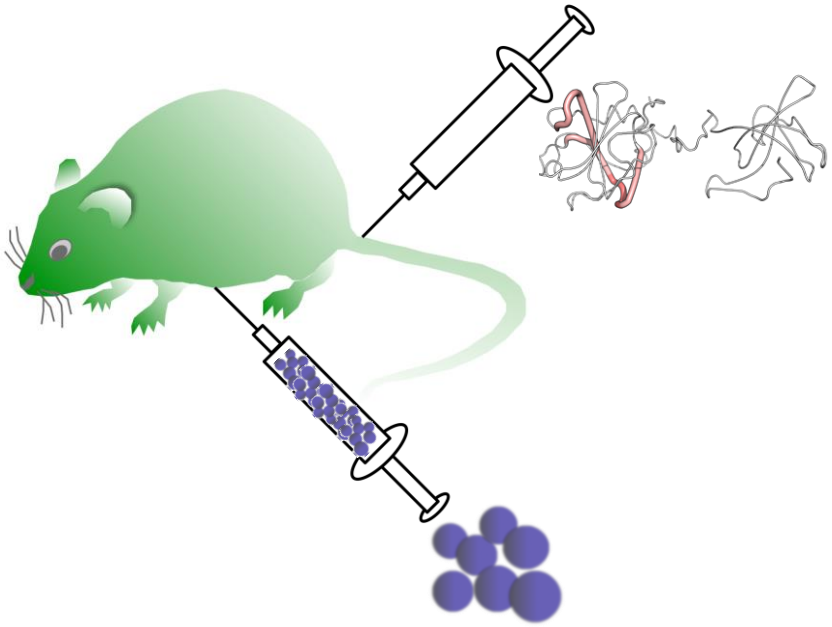
Lib5



In vivo immunogenicity & efficacy

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



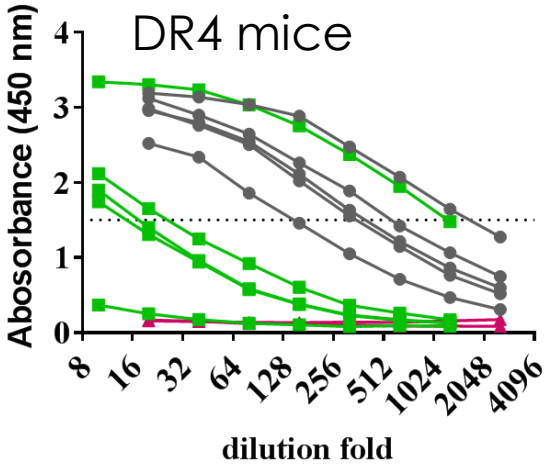


1. Immunized
2. Infected with MRSA
3. Treated according to arm

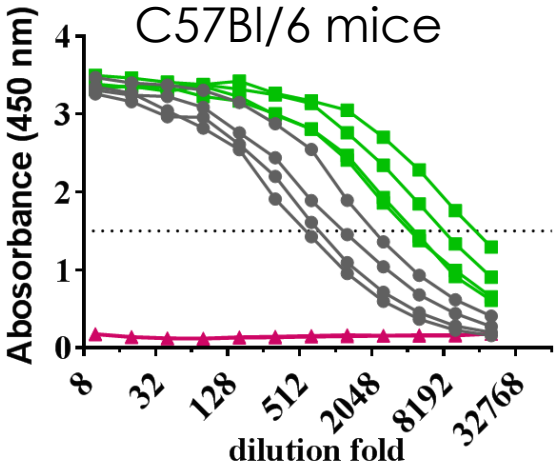
After 3x immunization

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

Higher Ab titers in
wild-type arm vs. variant

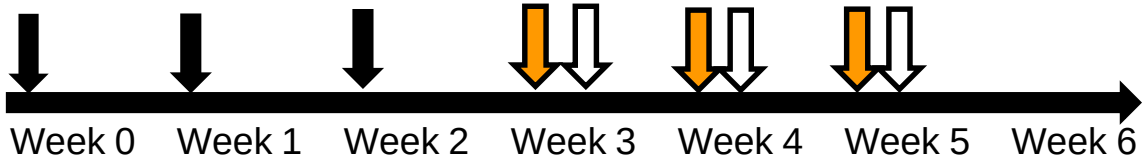


For targeted allele!



Infection & treatment

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



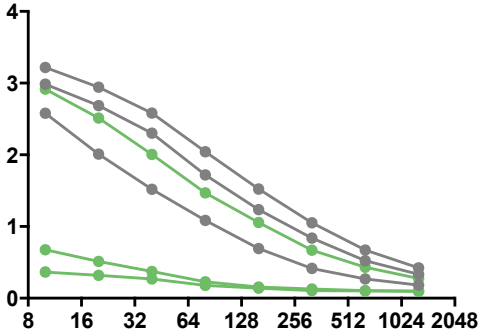
100 μg LST (*s.c.*)

500 μg LST (*i.v.*)

MRSA challenge (*i.p.*)

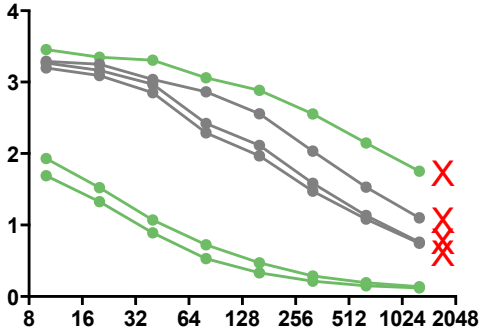
Week 3

All mice recued from initial infection



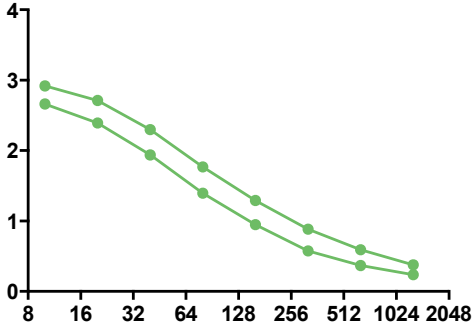
Week 4

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

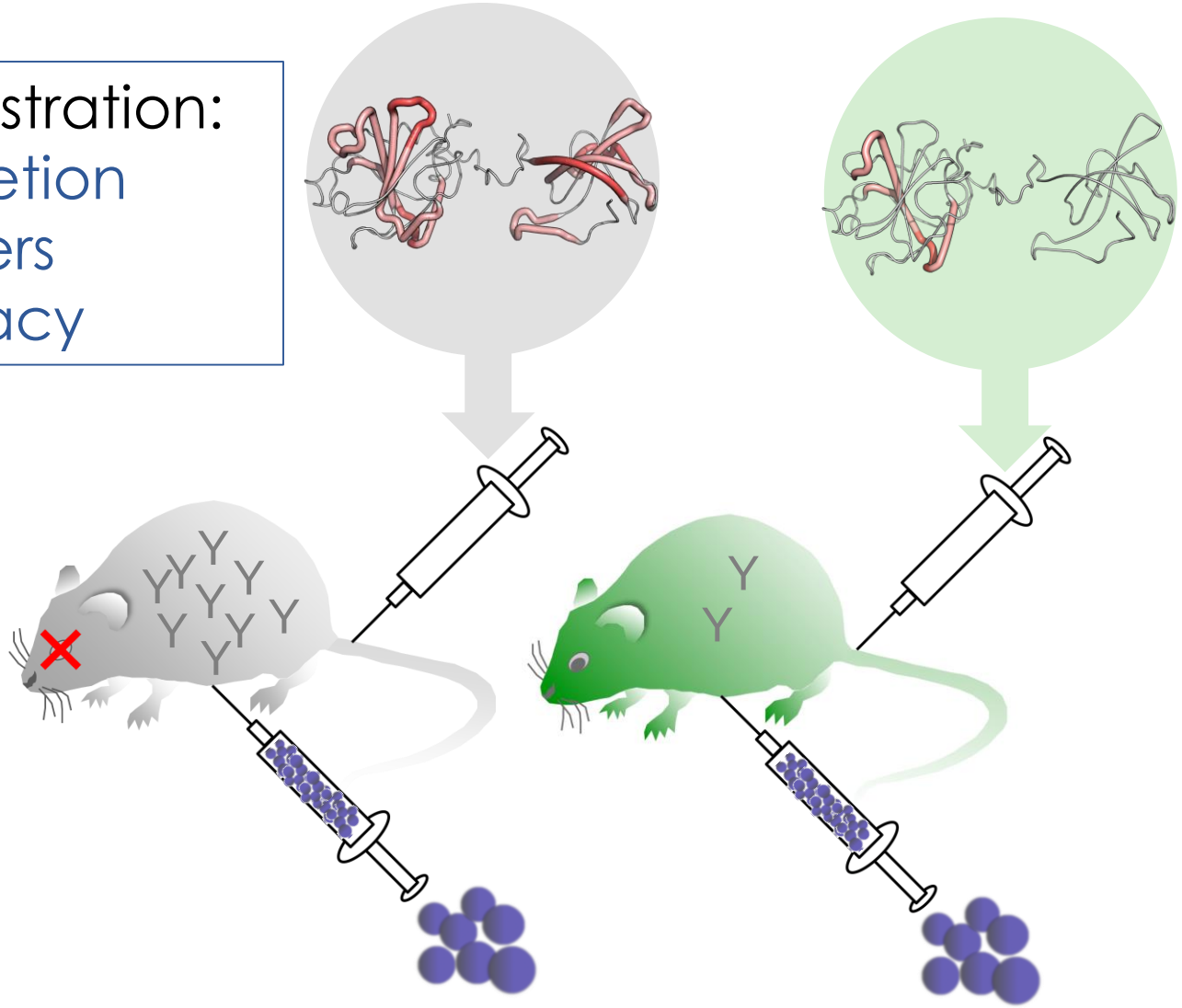


x: serum dilution
y: ELISA absorbance
for wild-type and variant

Epitope depletion summary

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

First direct demonstration:
T cell epitope deletion
⇒ reduced Ab titers
⇒ improved efficacy



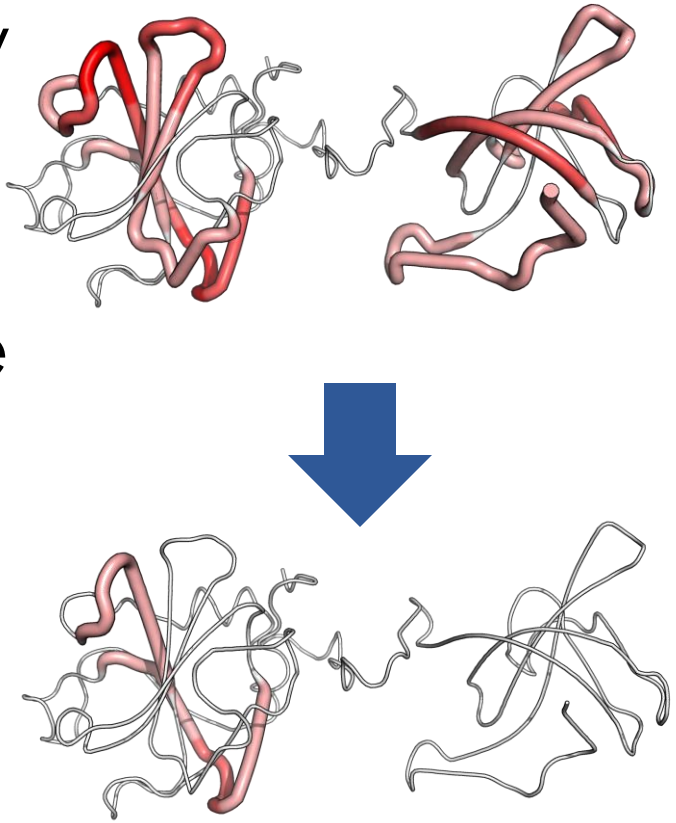
Conclusion

Goal: reduce immunogenicity while maintaining function

Methods: both objectives are predictable and designable

- sequence/structure
- individual/library

Results: reduced immunogenicity & *improved* efficacy



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