High-Throughput experiments and Rosetta Design

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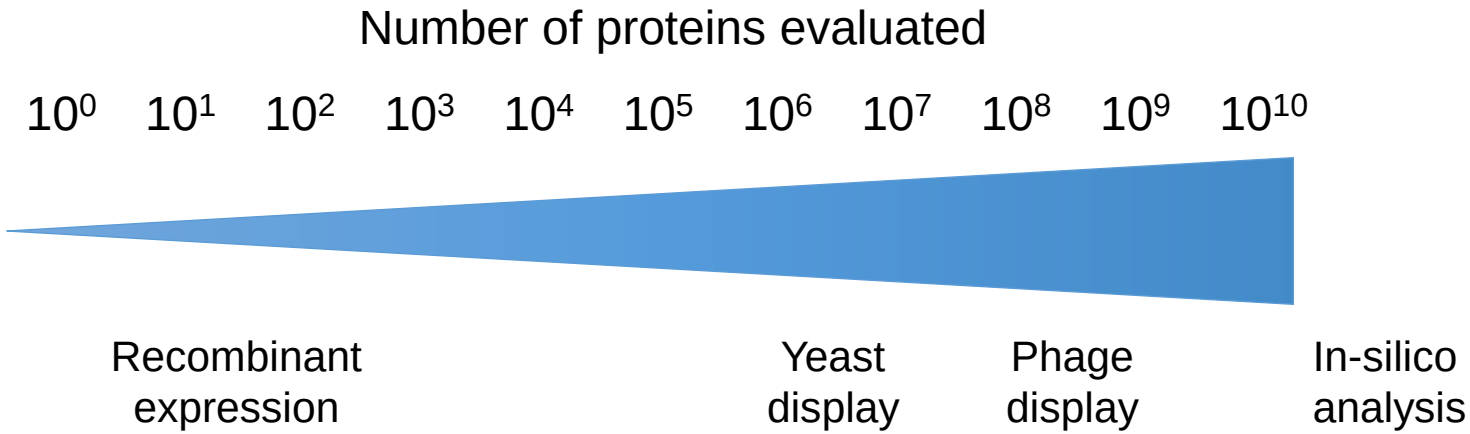
Rosetta Workshop

May 2018

Computation can increase scale of protein engineering



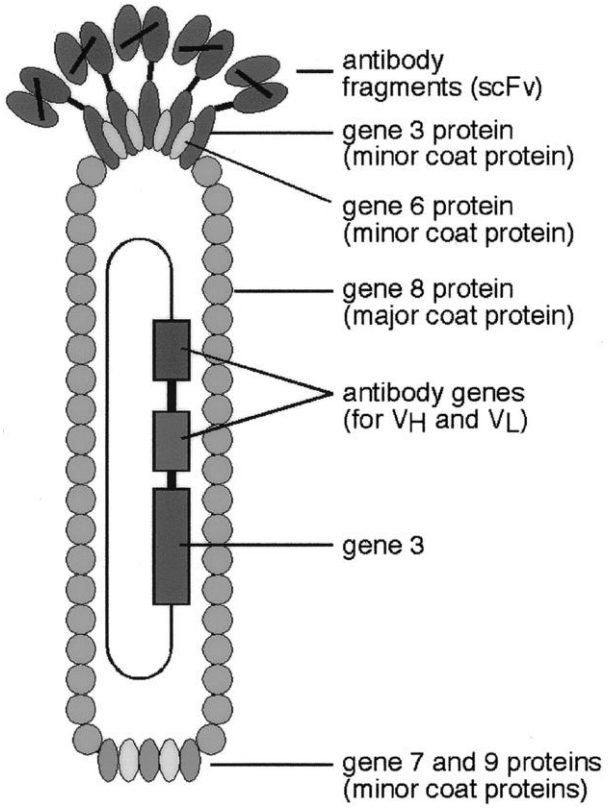
Small protein such as ubiquitin has 76 aa – sequence space 10^{98}
Antibody CDR loop may have 16 amino acids – 20^{16} (~10^{20}) possibilities

How to test a large number of designs?

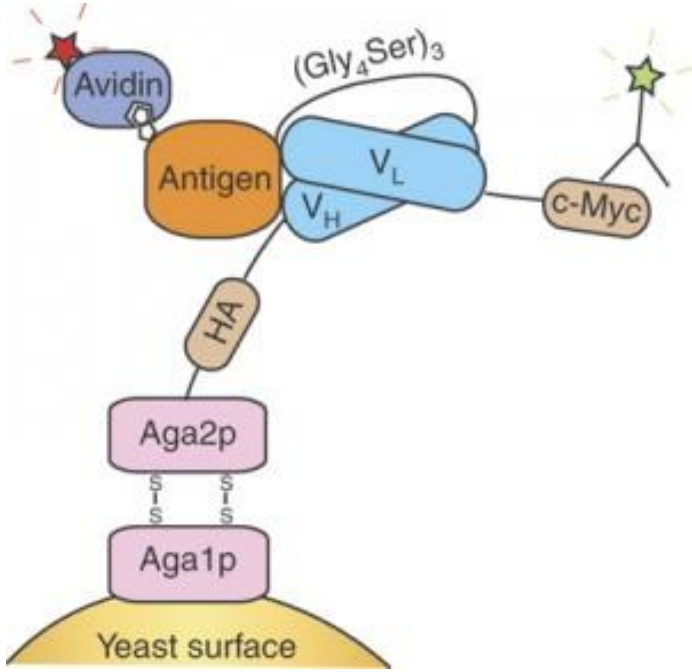
Phage/yeast display

- Protein of interest can be physically linked to the surface of a phage particle or yeast cell which contains its gene
- Functional proteins can then be isolated and sequenced

Phage display



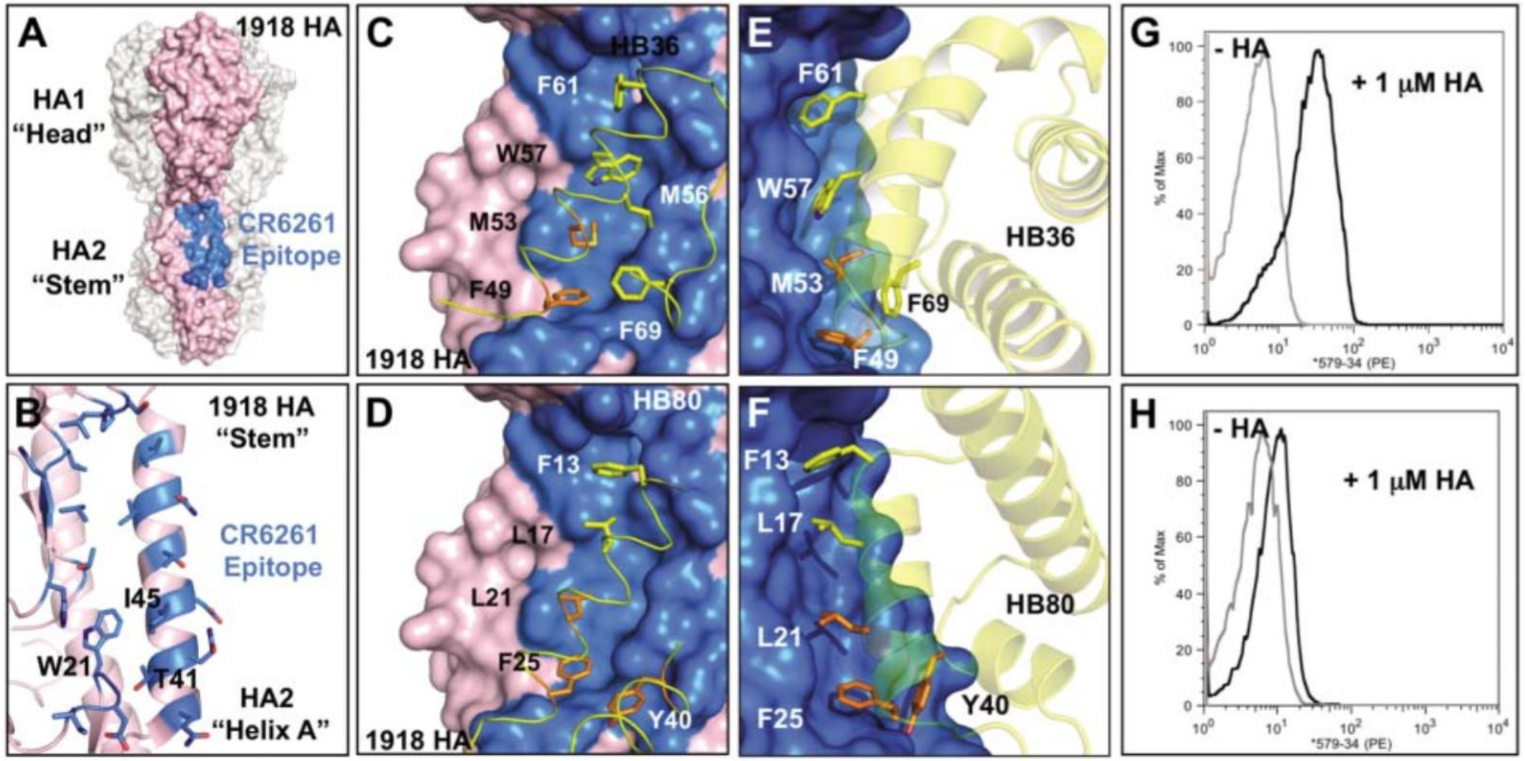
Yeast display



Chao, G. *et al.* Isolating and engineering human antibodies using yeast surface display. *Nat Protoc* **1,** 755–768 (2006).
Smith J, Kontermann RE, Embleton J, Kumar S. Antibody phage display technologies with special reference to angiogenesis. FASEB J. 2005;19(3):331-41.

Affinity maturation of protein binder

- Rosetta-based design of a novel protein targeting the stem region of influenza hemagglutinin
- Initial proteins bound with low affinity – random mutagenesis generated proteins with nanomolar affinity



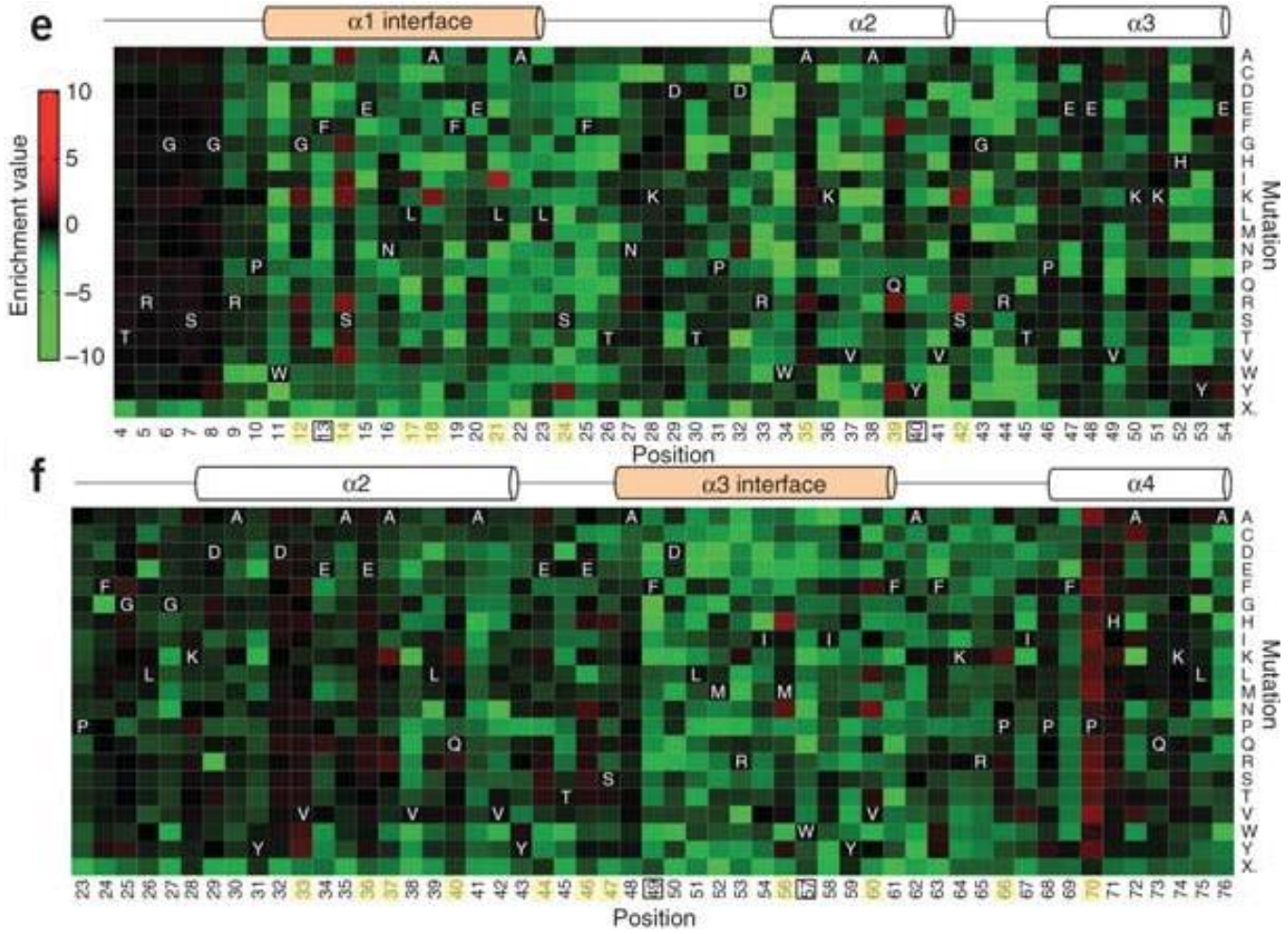
Fleishman, S. J. *et al.* Computational design of proteins targeting the conserved stem region of influenza hemagglutinin. *Science* **332,** 816–821 (2011).

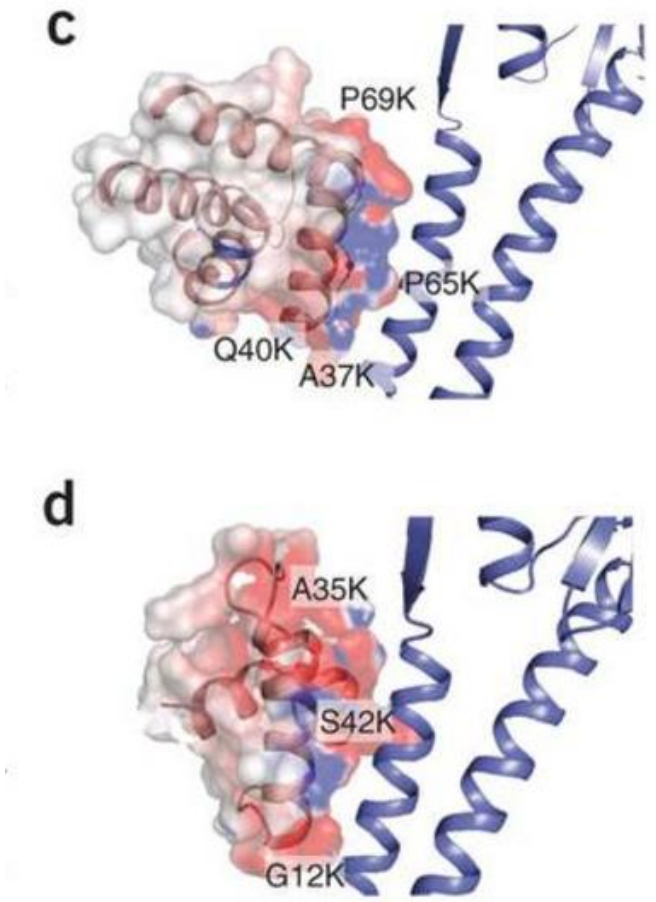
Affinity maturation of protein binder

	Design	K_d (nM)
	1U84 (HB36 scaffold)	NB (NB)
Designed protein	HB36	200 (>2000)
Affinity-matured variants	HB36 D47S	5
	HA36 A60V	8
	HB36.3 (HB36 D47S, A60V)	4 (29)
	HB36.4 (HB36 D47S, A60V, N64K)	4 (22)
	2CJJ (HB80 scaffold)	NB
Designed protein	HB80	>5000
Affinity-matured variants	HB80 M26T	100
	HB80 N36K	300
	HB80 M26T N36K	7.5
	HB80 Δ54-95, M26T, N36K	5
	HB80.3 (HB80 Δ54-95, D12Gly, A24S, M26T, N36K)	3 (38)

Fleishman, S. J. *et al.* Computational design of proteins targeting the conserved stem region of influenza hemagglutinin. *Science* **332,** 816–821 (2011).

Comprehensive mutagenesis of influenza binders



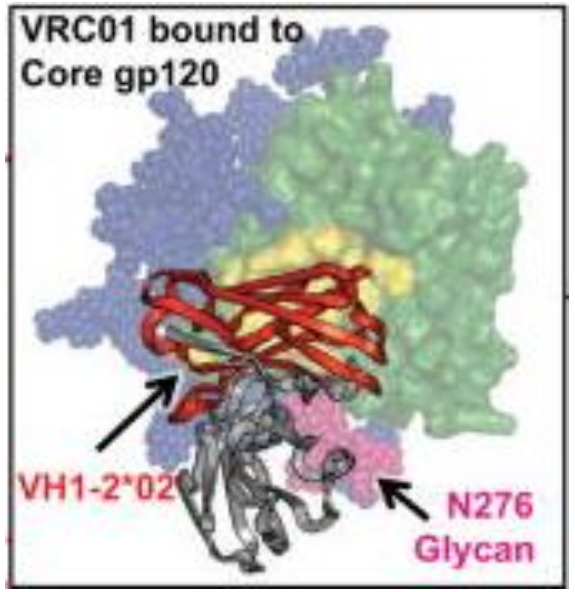


Whitehead TA, Chevalier A, Song Y, et al. Optimization of affinity, specificity and function of designed influenza inhibitors using deep sequencing. Nat Biotechnol. 2012;30(6):543-8.

Experimental mutations improve electrostatics at the interface – Electrostatics were not included in original design calculations

Design of HIV vaccine immunogens

- Used Rosetta to create an immunogen that binds to precursors of known broadly neutralizing antibodies
- Identified computationally designed antigens with μM affinity for target by building targeted libraries
- Further increased affinity by random mutagenesis

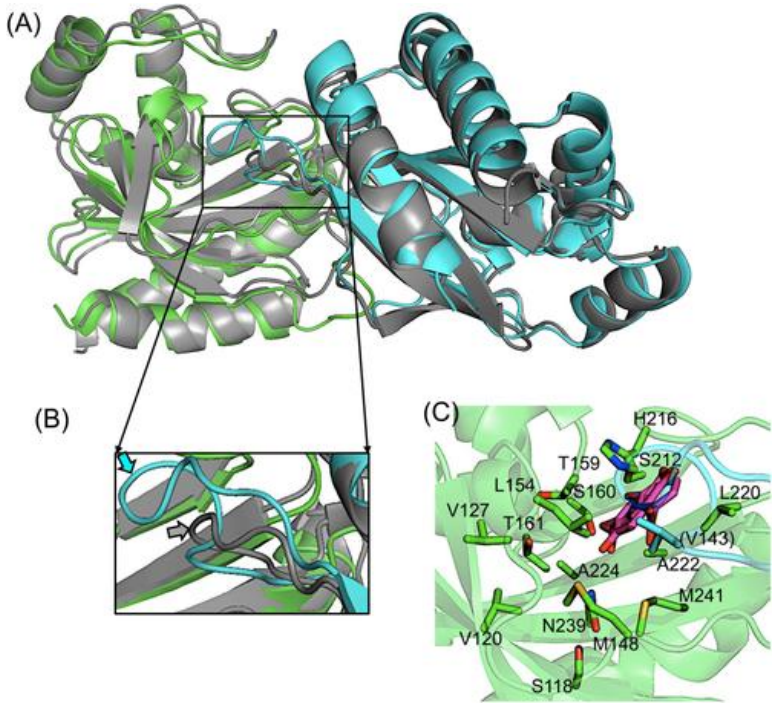


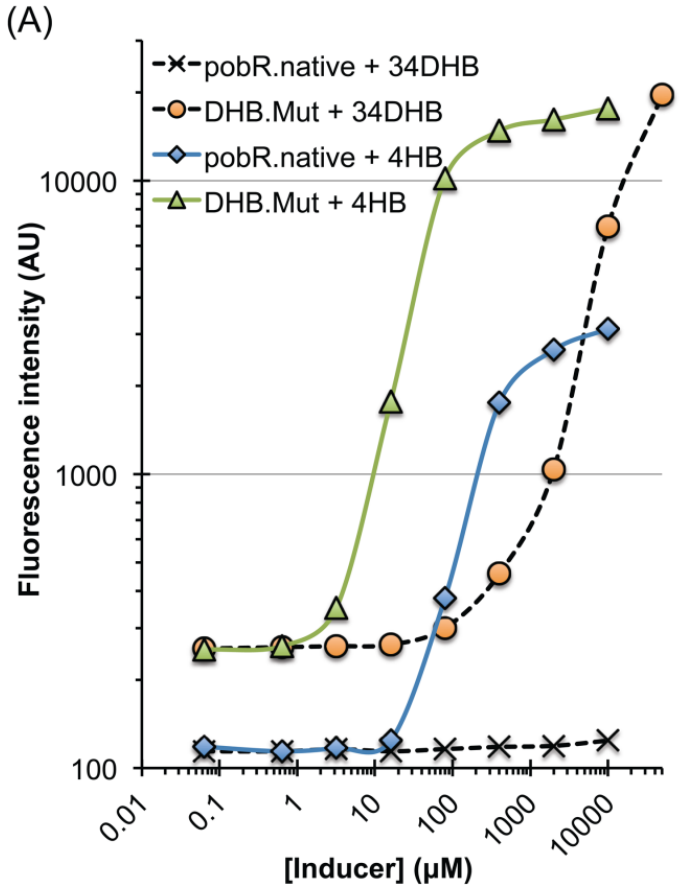
Antigen		Antibody VRC01 GL	Mat
Wild-type	Core.HXB2	$>10^5$	5
Germline-targeted	eOD-Base N276D	$>10^5$	5
	Core.BaL-GT1	1800	0.5
	eOD-GT1	44,000	1
	eOD-GT6	44	2

Jardine, J. *et al.* Rational HIV immunogen design to target specific germline B cell receptors. *Science* **340,** 711–716 (2013).

Rosetta comparative modeling for library design

- Wanted to engineer a transcription factor with no structure for recognition of small molecule
- Created a homology model and docked the ligand
- Made a library mutated at 16 positions predicted to contact ligand



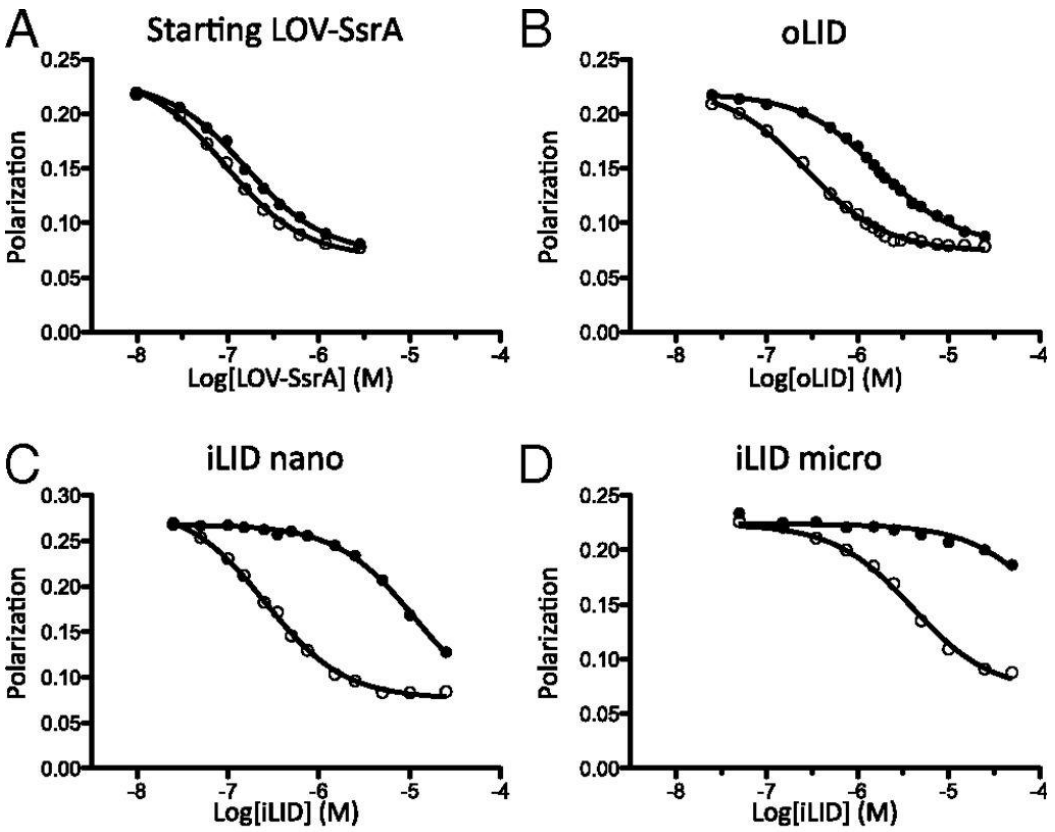


34DHB – ligand target

Jha, R. K., Chakraborti, S., Kern, T. L., Fox, D. T. & Strauss, C. E. M. Rosetta comparative modeling for library design: Engineering alternative inducer specificity in a transcription factor. *Proteins* **83,** 1327–1340 (2015).

Engineering an improved light-induced dimer (iLID)

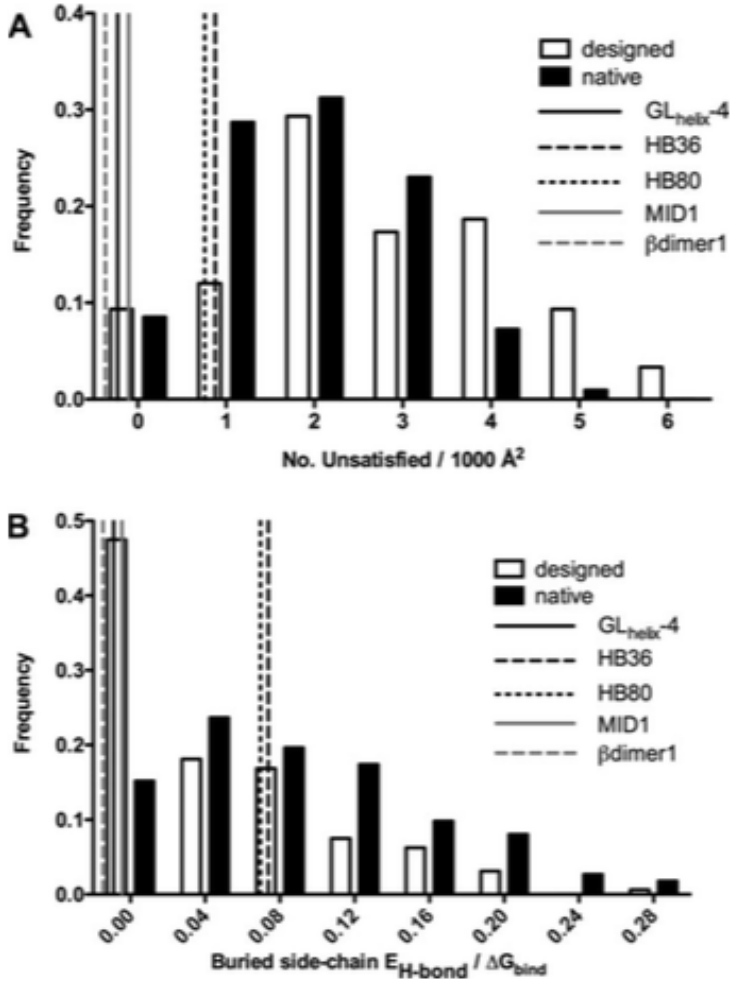
- Design of a protein which heterodimerizes in the presence of blue light
- Initial protein had 2 fold difference between dark and light binding
- Scanned the protein for all point mutants in Rosetta, chose 743 at 49 positions for characterization
- Displayed and screened mutants by phage display



Guntas, G. *et al.* Engineering an improved light-induced dimer (iLID) for controlling the localization and activity of signaling proteins. *Proc. Natl. Acad. Sci. U.S.A.* **112,** 112–117 (2015).

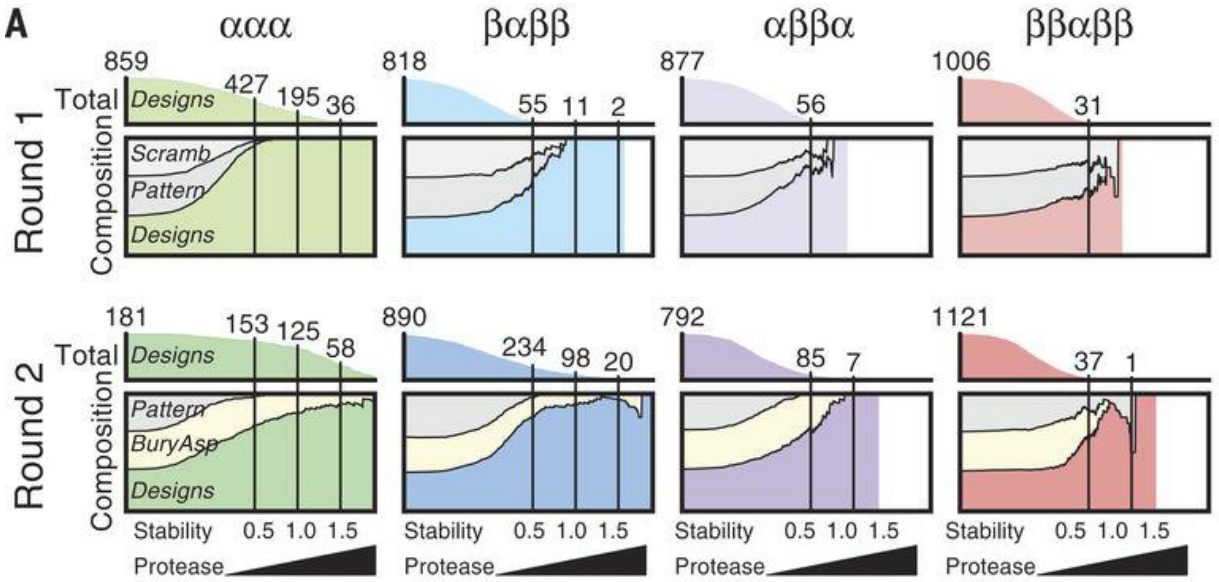
A comparison of successful and failed protein interface designs

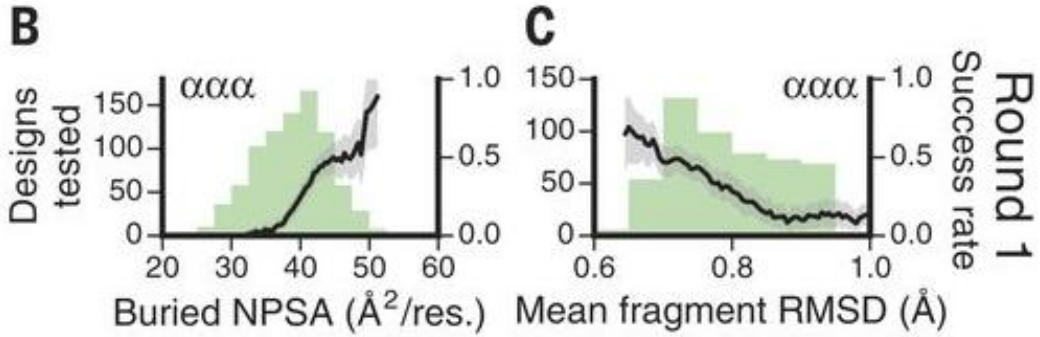
- Comparison of 5 successful de novo interface designs with 158 failures
- Successful designs have fewer polar atoms at the interface
- Predicted hydrogen bond networks at the interface almost never materialize, even though these are common in natural interfaces



Stranges, P. B. & Kuhlman, B. A comparison of successful and failed protein interface designs highlights the challenges of designing buried hydrogen bonds. *Protein Science* **22,** 74–82 (2013).

Global analysis of protein folding using massively parallel design, synthesis, and testing



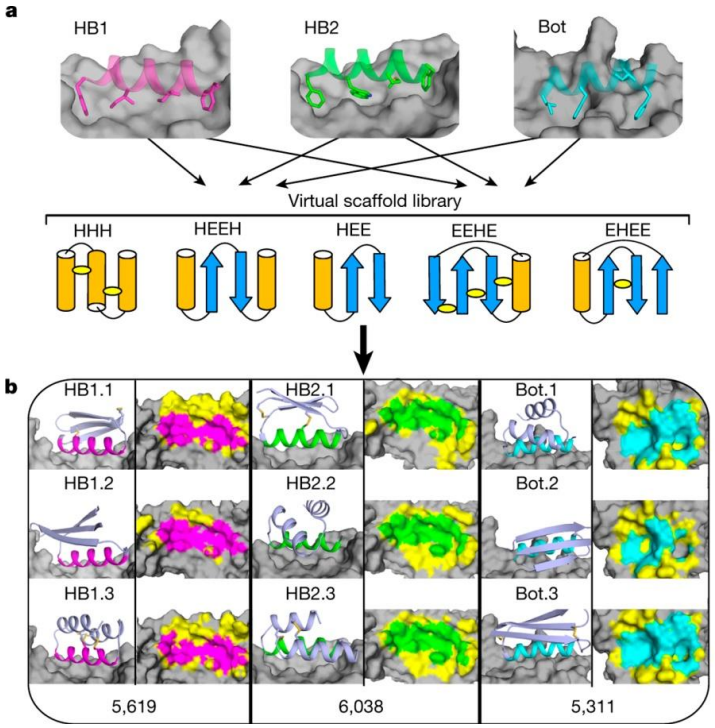


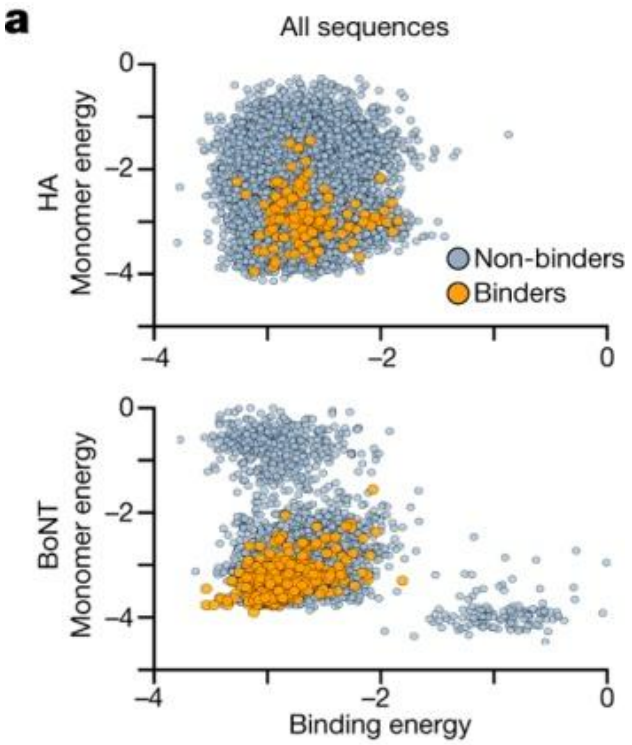
- Expression of massive (10^4) panels of designed small proteins on yeast surface
- Measurement of protease resistant as a surrogate for protein stability
- Identified >2,500 proteins which fold stably

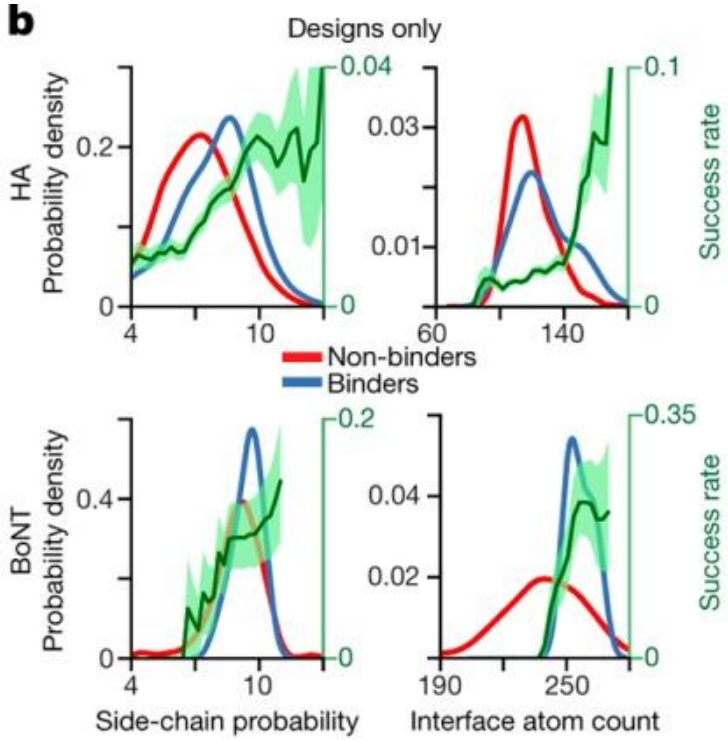
- Buried nonpolar surface area (NPSA) is a major contributor to design success
- Mean fragment RMSD also contributes to successful designs

Rocklin, G. J. *et al.* Global analysis of protein folding using massively parallel design, synthesis, and testing. *Science* **357,** 168–175 (2017).

Massively parallel de novo protein design for targeted therapeutics







- Expression of 22,660 small protein scaffolds designed to bind either influenza HA or botulinum neurotoxin B
- Obtained 115 HA and 2,685 BoNT binders

- Lower monomer energy and binding energy corresponds to experimental success
- Local sequence-structure compatibility and the numbers of contacts across the interface also contributed

Chevalier, A. *et al.* Massively parallel de novo protein design for targeted therapeutics. *Nature* **550,** 74–79 (2017).