Pharmacophore-based Protein Surface Patch Searching and Its Application to *in silico* Antibody Engineering

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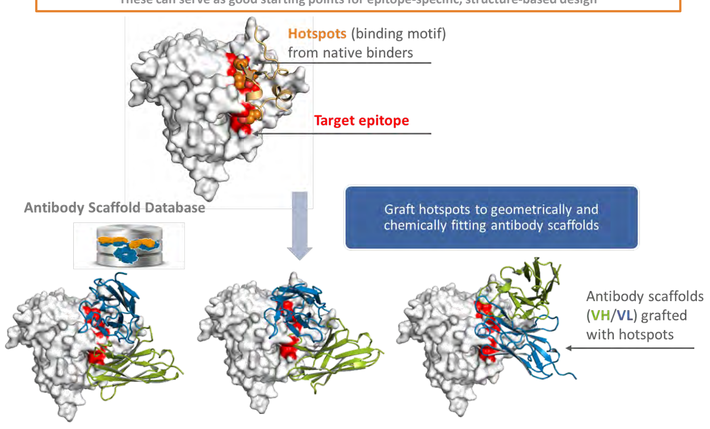
Abstract

A number of successful approaches to the computational design of protein-protein interfaces rely on mimicking native interactions by grafting known hotspots onto alternative scaffolds. Here we present a novel *in silico* approach to the identification of alternative protein scaffolds that can geometrically project multiple hotspots from a known protein-protein interface. As a proof of concept, we demonstrate application of the method to identify antibody scaffolds that have appropriate CDR conformations to support simultaneous grafting of hotspots, which can then serve as good starting points for epitope-specific, structure-based design. In this approach, hotspots are defined as pharmacophore points rather than individual residues, representing different chemical groups such as aromatic, hydrophobic, and charged groups, as well as hydrogen bond donor and/or acceptors. This allows for expansion of the types of amino acids that can be considered hotspots. Pharmacophore matching on proteins is much more computationally demanding than for small molecules given the larger sampling space available to proteins, so here we present a network-based approach which greatly reduces the combinatorial complexity for matching large pharmacophore sets. Finally, we show the success of this approach in identifying antibody scaffolds that share antigen recognizing interfaces with known protein binders.

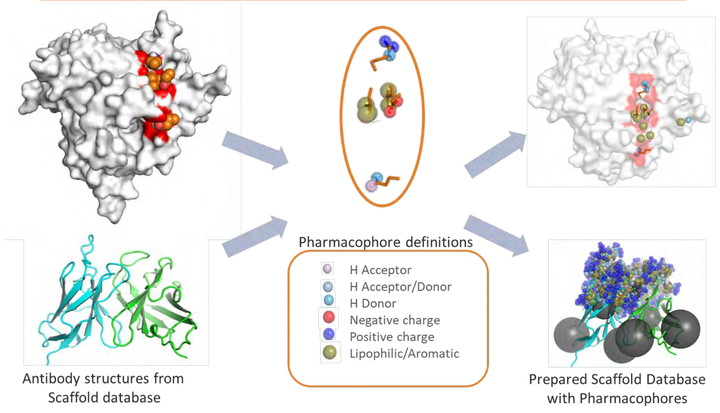
Identify Matching Scaffolds to Enable Hotspot Grafting

Antibody scaffolds are identified that have appropriate CDR conformations to support simultaneous grafting of hotspots.
These can serve as good starting points for epitope-specific, structure-based design



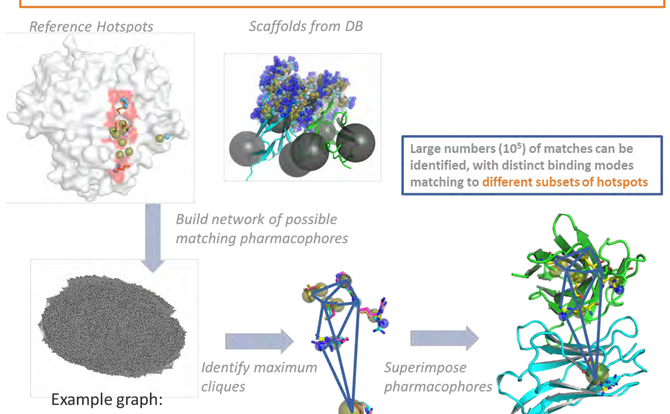
Represent hotspots as pharmacophores

Hotspot residues are represented as pharmacophores describing the chemical groups most responsible for favorable interaction



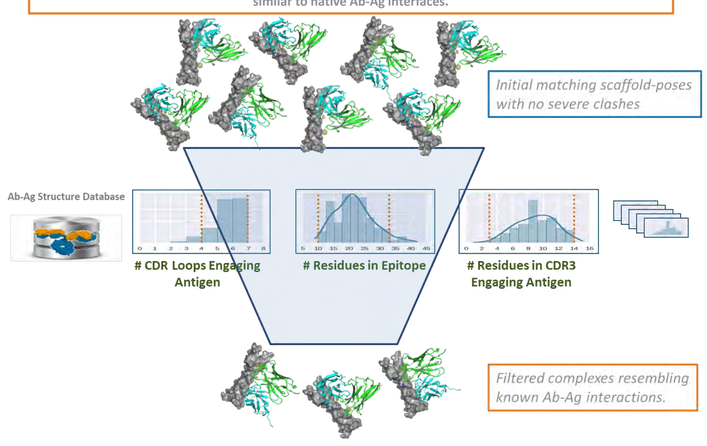
Network-based solution for rapid pharmacophore matching

Matching multiple hotspots can lead to a computationally intractable combinatorial problem. Treating the geometric-matching problem as a graph/network is a much more computationally efficient approach to identification of solutions.



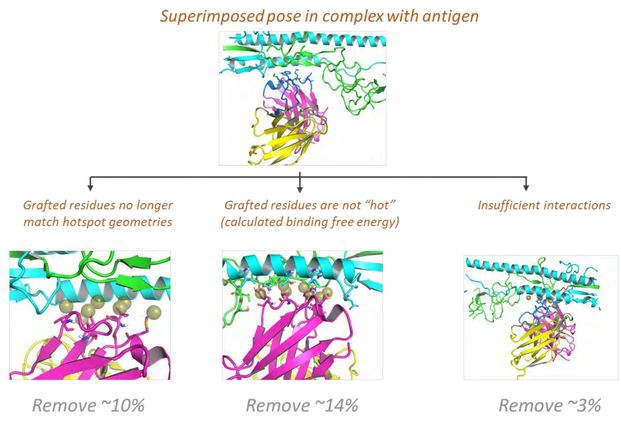
Superposition and initial filtering of matched scaffolds

Multiple structure-based features are determined to identify matching solutions that have binding modes similar to native Ab-Ag interfaces.



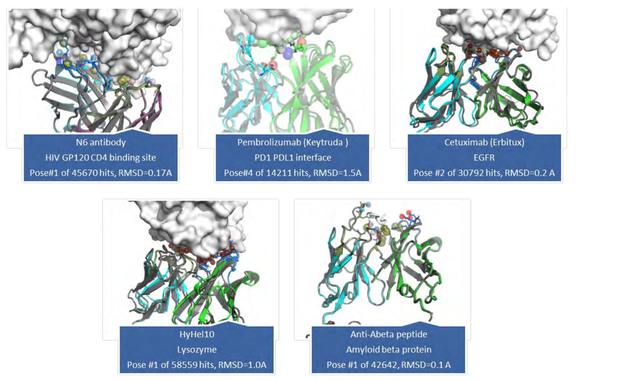
Energetics-based filtering of matches

Resulting poses are energy minimized and further filtered for retention of key binding interactions



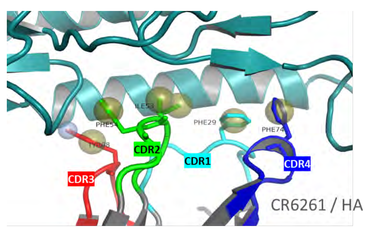
in silico Benchmarking

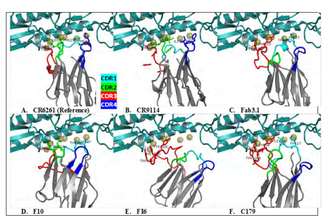
A panel of antibody-antigen complexes with various hotspot compositions were selected as a benchmark set.
Method successfully identifies the native antibody pose with the original or similar hotspot residues grafted.



POC system 1: CR6261's hotspots used to identify known stem-binding HA antibodies

Several stem-binding anti-influenza antibodies are known that share a common epitope region. The matching algorithm successfully identifies these diverse antibodies when matching against a reference stem-binding antibody.



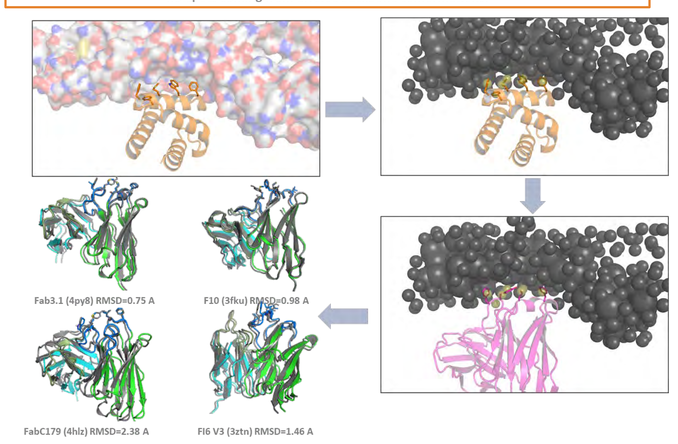


HA stem-binding antibodies use different loops and different AA to target the same epitope

Antibody	Germline	Seq ID to CR6261	HCDR3 Length	Hot spots occupied[1]	CDRs used[2]	Hotspot residues				
CR6261(Ref)	1-69	100%	12	1-5	2,3,4	Y98 (H3)	F54	I53 (H2)	F29(H1)	F74(H4)
CR9114	1-69	73.3%	15	1-5	2,3,4	Y98 (H3)	F54	I53 (H2)	I73	F74(H4)
Fab3.1	3-30	55.7%	13	2,3,4,5	1,3		F104	Y103 (H3)	T28	F27(H1)
F10	1-69	68.7%	15	2,3,4,5	1,3	Y102(H3)	F55	M54 (H2)	T28	V27(H1)
FI6	3-30	48.1%	20	1,2,3	3	W100F L100G	F100D	Y100C(H3)		
C179	Mouse 7-3	45.8%	11	1-5	1,3	Y100A	F99	Y98 (H3)	L29	F27(H1)

POC system 2: HB36 hotspots used to identify known stem-binding HA antibodies

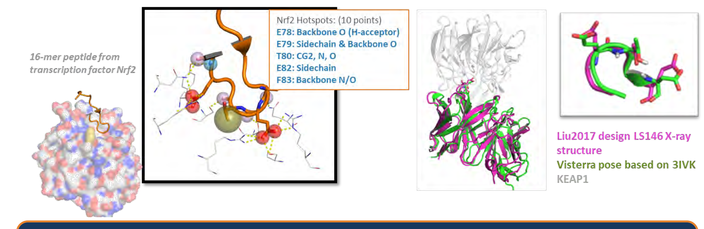
The method identifies stem-binding antibodies Fab3.1, F10, FI6v3, and Fab C179, using hotspots from the *de novo* designed influenza-binding protein, HB36, further demonstrating the robustness of the method to graft hotspots coming from diverse backbone conformations.



POC system 3: Nrf2 hotspots used to find antibody scaffold

Liu, 2017 study used an alternative method to graft 3 hotspot residues of Nrf2 peptide to a list of scaffolds, and demonstrated binding of several of the constructs to KEAP1. Our method was also able to identify these scaffolds as suitable for hotspot grafting.

Design	Scaffold [1]	Hotspots positions	#Mutations from scaffolds (except grafted hotspots)	Fraction of Fab binding sites occupied @500nM Keap1[3]	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_D (nM)	K_D 95% CI[4]
GS3	2YSS[5]	V_H53E, V_L54T, V_L56E	3	0.002	ND[2]	ND	ND	ND
GS3.1			5	0.0009	ND	ND	ND	ND
GS4	3IVK[6]	V_H53E, V_L54T, V_L56E	6	0.01	ND	ND	ND	ND
GS4.1			9	0.468	2.1×10^5	2.6×10^{-2}	126	110-143
GS5	3TCL[1]	V_H102E, V_H102°T, V_H102°E	1	0.015	ND	ND	ND	ND
GS5.1			3	0.016	ND	ND	ND	ND
GS6	3U4B[7]	V_H102E, V_H102°T, V_H102°E	1	0.023	ND	ND	ND	ND
GS6.1			2	0.027	ND	ND	ND	ND
GS8	2JB5[8]	V_H54E, V_L57T, V_L57E	6	0.179	2.3×10^5	4.9×10^{-2}	236	137-405
GS8.1			7	0.171	6.8×10^4	2.3×10^{-2}	341	209-555



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Computational design of an epitope-specific Keap1 binding antibody using hotspot residues grafting and CDR loop swapping

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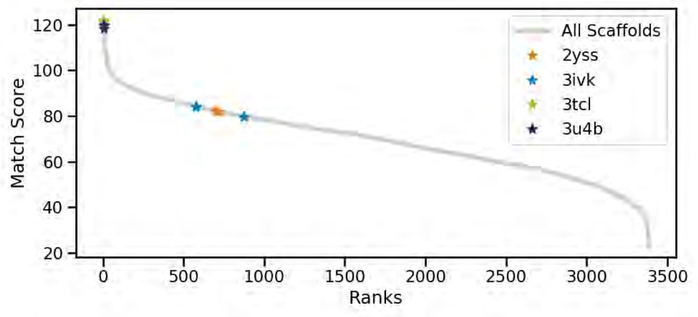
Xiaofeng Liu[1], Richard D. Taylor[1], Laura Griffin[1], Shu-Fen Coker[1], Ralph Adams[1], Tom Cenka[1], Jiye Shi[2], Alastair D. G. Lawson[1] & Terry Baker[1]

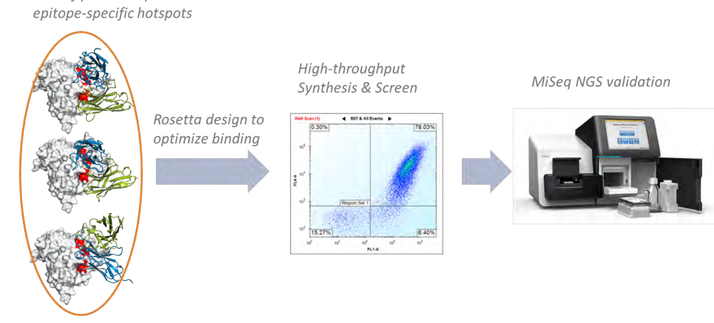
Potential binders are enriched in top-ranked matches

Method identified >3000 matching poses from >500 scaffolds
4 out of 5 binding scaffolds from the Liu 2017 study show up in top 20%
2 out of 5 scaffolds are ranked at the very top by our matching scores
Our superimposed pose based on 3IVK has high structural similarity to the of nanomolar binder LS146, a 3IVK variant
Potential: many more matches could generate binding variants



Prospective: application for epitope-specific antibody design

Antibody interfaces can be redesigned around key grafted hotspots, and converted to libraries enriched in potential binding hotspots at appropriate positions.



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3. Chevalier, A. et al. Massively parallel de novo protein design for targeted therapeutics. Nature (2017). doi:10.1038/nature23912

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