

Antibody/Antigen Complexes: Prediction of Structure and Design of Function with Rosetta

Jens Meiler

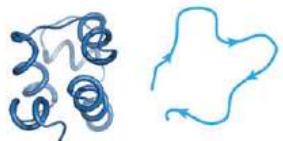


Center for Structural Biology and Institute of Chemical Biology
Departments of Chemistry, Pharmacology, and Biomedical Informatics

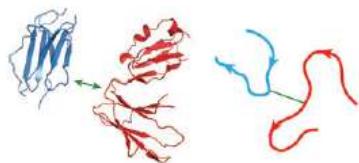
Rosetta: A Unified Framework for Tackling Molecular Modeling



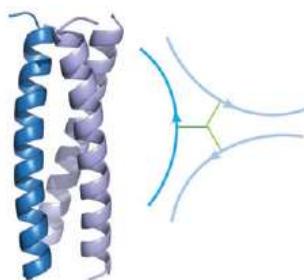
a Protein structure prediction



c Protein docking (fully flexible)



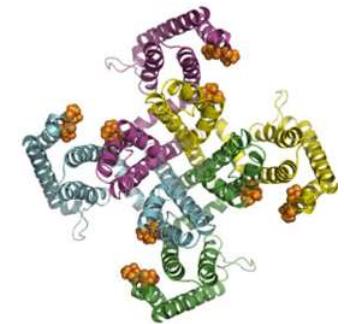
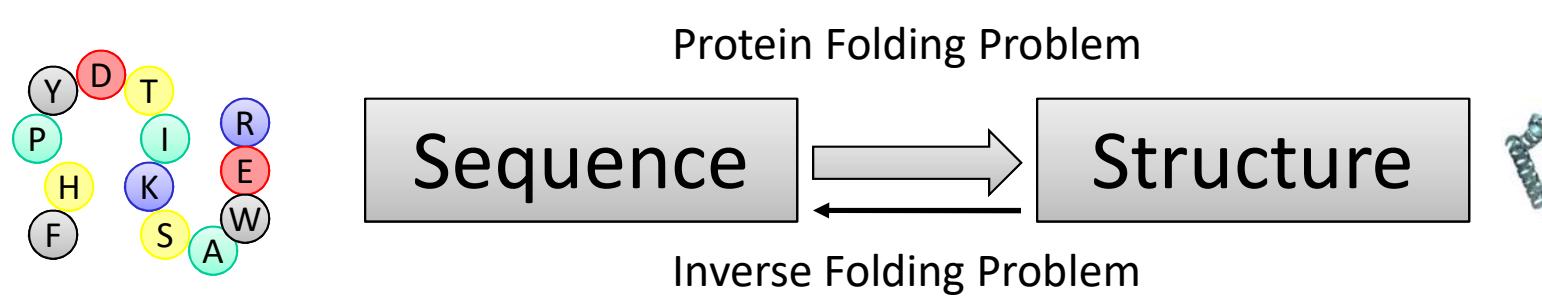
e Symmetric complexes



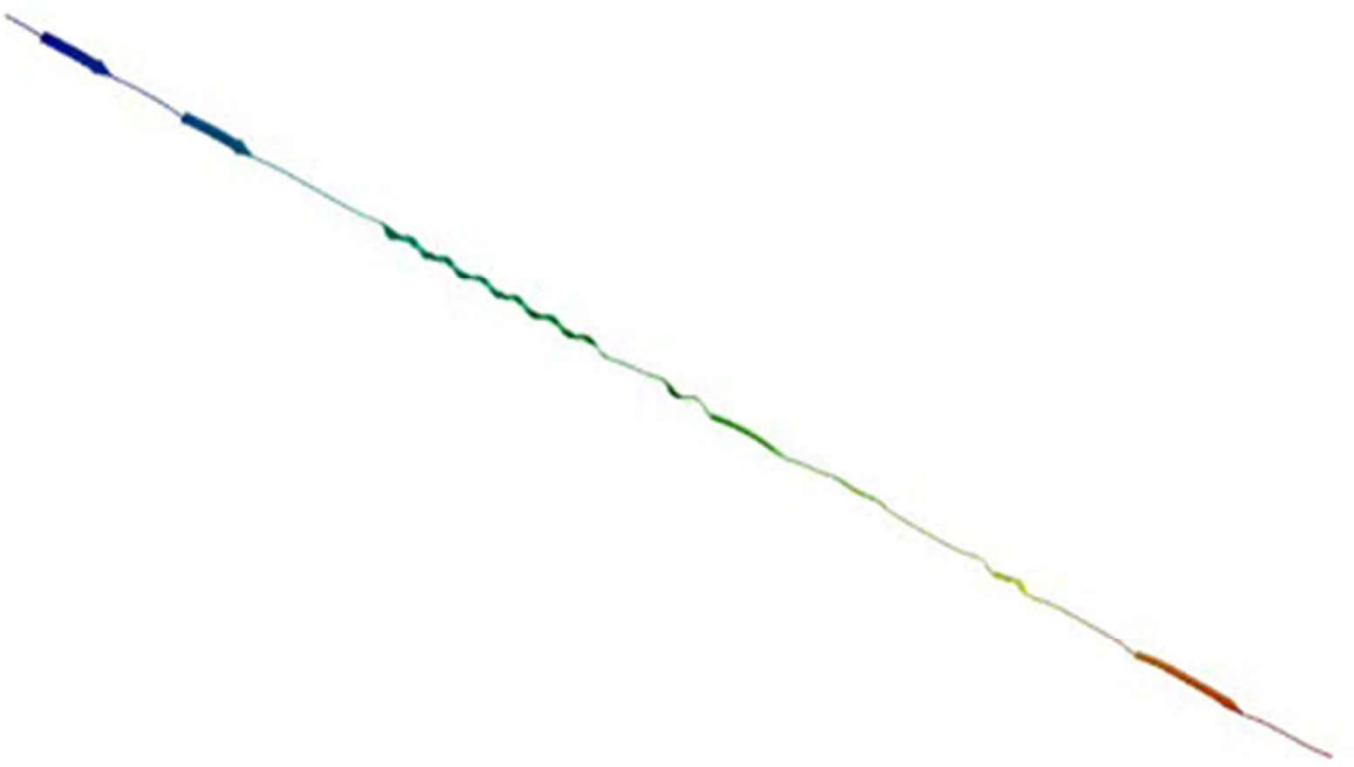
Das, R.; Baker, D. "Macromolecular modeling with rosetta" *Annu Rev Biochem* 2008, 77, 363-82.

(Inverse) Protein Folding Problem

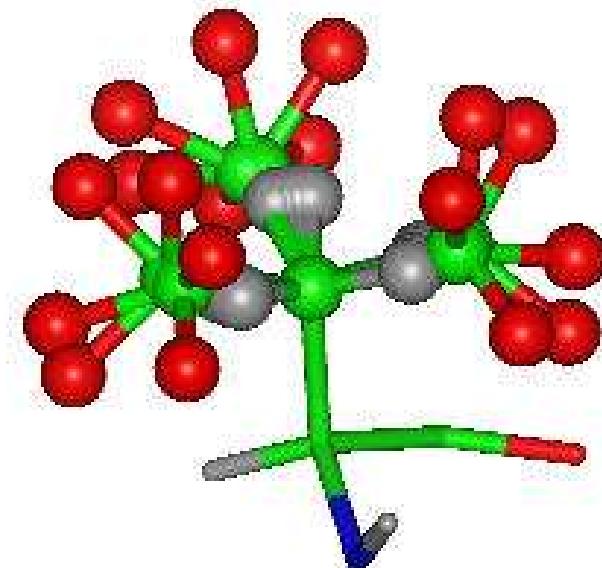
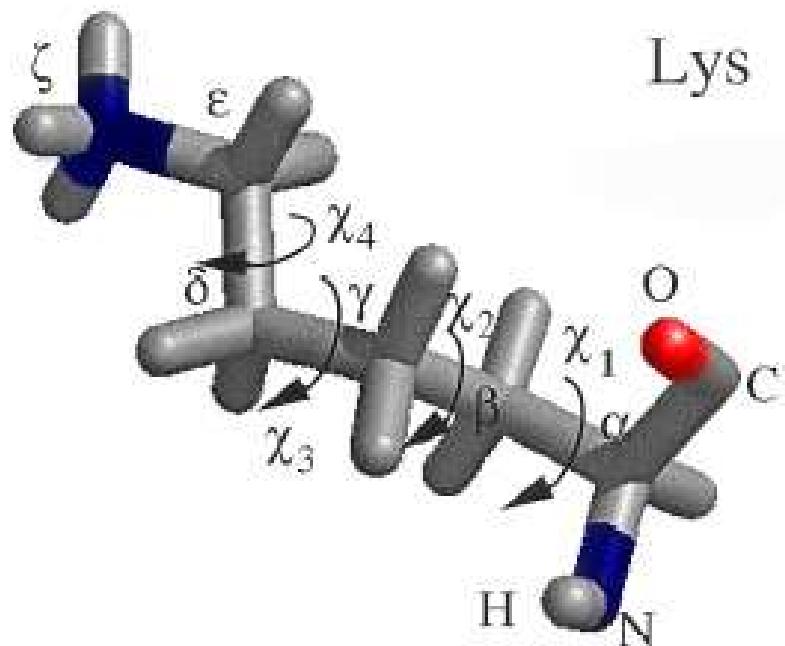
Holy Grail of Comp. Struct. Biology



- Given a protein's AA sequence, what is its 3-dimensional fold , and how does it get there?
- Assume 100 conformations for each amino acid in a 100 amino acid protein $\Rightarrow 10^{200}$ possible conformations!
- Cyrus Levinthal's paradox of protein folding, 1968.



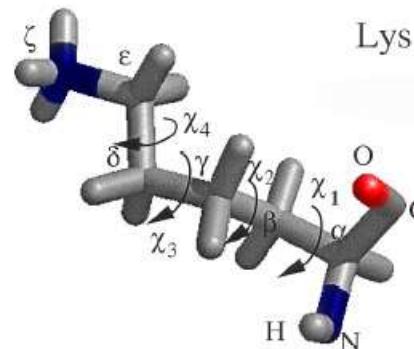
Sidechain Degrees of Freedom



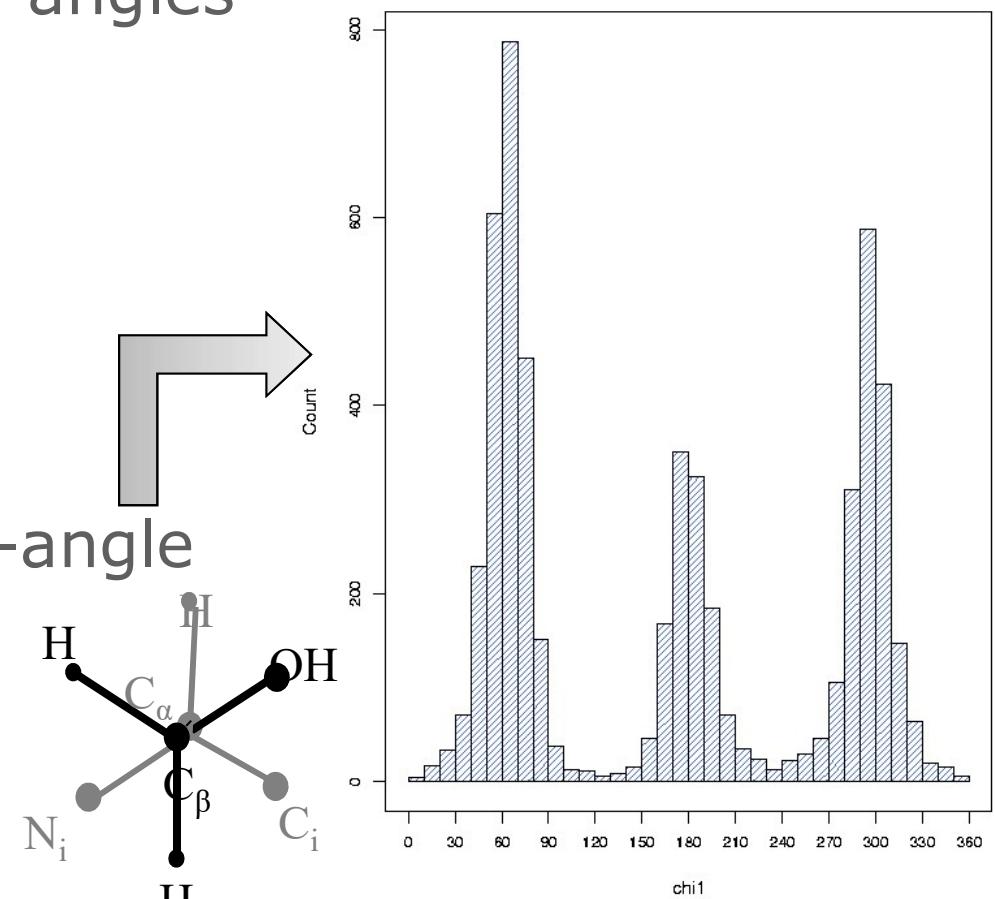
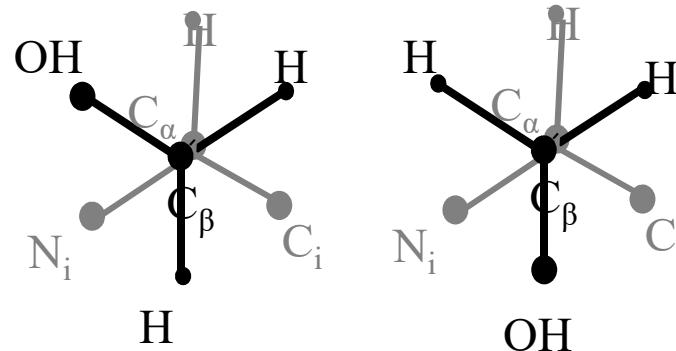
All Likely Side Chain Conformations are Present in the Protein Databank



- Lysine has four side chain χ -angles



- Serine has one side chain χ -angle



“Rotamer” Libraries Encompass all Likely Side Chain Conformations



	No.	χ_1	No.	p	σ	$p \chi_1$	σ	χ_1	σ	χ_2	σ
SER 1 0 0 0	4125	4125		46.61	0.43	100.00	0.00	65.0	10.7		
SER 2 0 0 0	2059	2059		23.27	0.37	100.00	0.00	179.6	11.7		
SER 3 0 0 0	2665	2665		30.12	0.40	100.00	0.00	-64.2	11.0		
THR 1 0 0 0	4165	4165		48.38	0.44	100.00	0.00	61.1	8.8		
THR 2 0 0 0	686	686		7.98	0.24	100.00	0.00	-173.3	12.8		
THR 3 0 0 0	3757	3757		43.64	0.44	100.00	0.00	-60.4	8.2		
TRP 1 1 0 0	337	215		9.56	0.51	63.62	2.13	61.7	9.7	-90.9	9.4
TRP 1 2 0 0	337	16		0.74	0.15	4.92	0.96	65.6	7.5	-16.7	40.9
TRP 1 3 0 0	337	106		4.73	0.36	31.47	2.06	59.4	12.0	88.2	10.1
TRP 2 1 0 0	786	359		15.94	0.63	45.64	1.45	-178.4	12.5	-104.1	15.1
TRP 2 2 0 0	786	139		6.19	0.41	17.72	1.11	-175.5	12.4	18.2	31.0
TRP 2 3 0 0	786	288		12.80	0.57	36.63	1.40	179.8	8.8	84.8	9.7
TRP 3 1 0 0	1127	106		4.73	0.36	9.45	0.71	-70.4	13.2	-91.4	15.4
TRP 3 2 0 0	1127	303		13.46	0.59	26.90	1.08	-68.5	9.9	-2.5	26.8
TRP 3 3 0 0	1127	718		31.86	0.80	63.66	1.17	-67.4	11.3	99.8	16.4

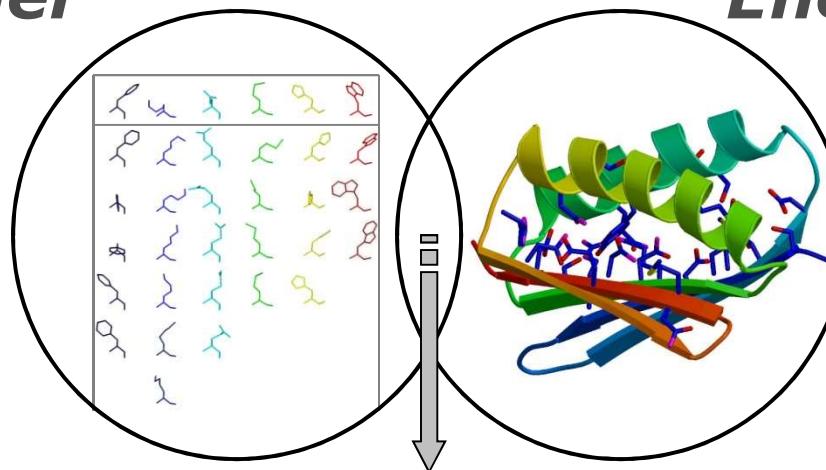
Dunbrack, R. L.; Cohen, F. E. "Bayesian statistical analysis of protein side-chain rotamer preferences." *Protein Sci.* **1997, 6, 1661-1681.**

Sampling and Scoring for Side Chain Repacking and Design



Local Rotamer Bias

Approximate interactions within sidechain using the distribution of sidechain conformations (rotamers) seen in known protein structures

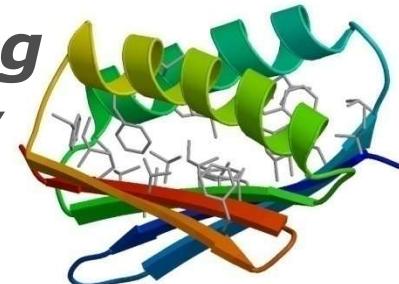


Energy function

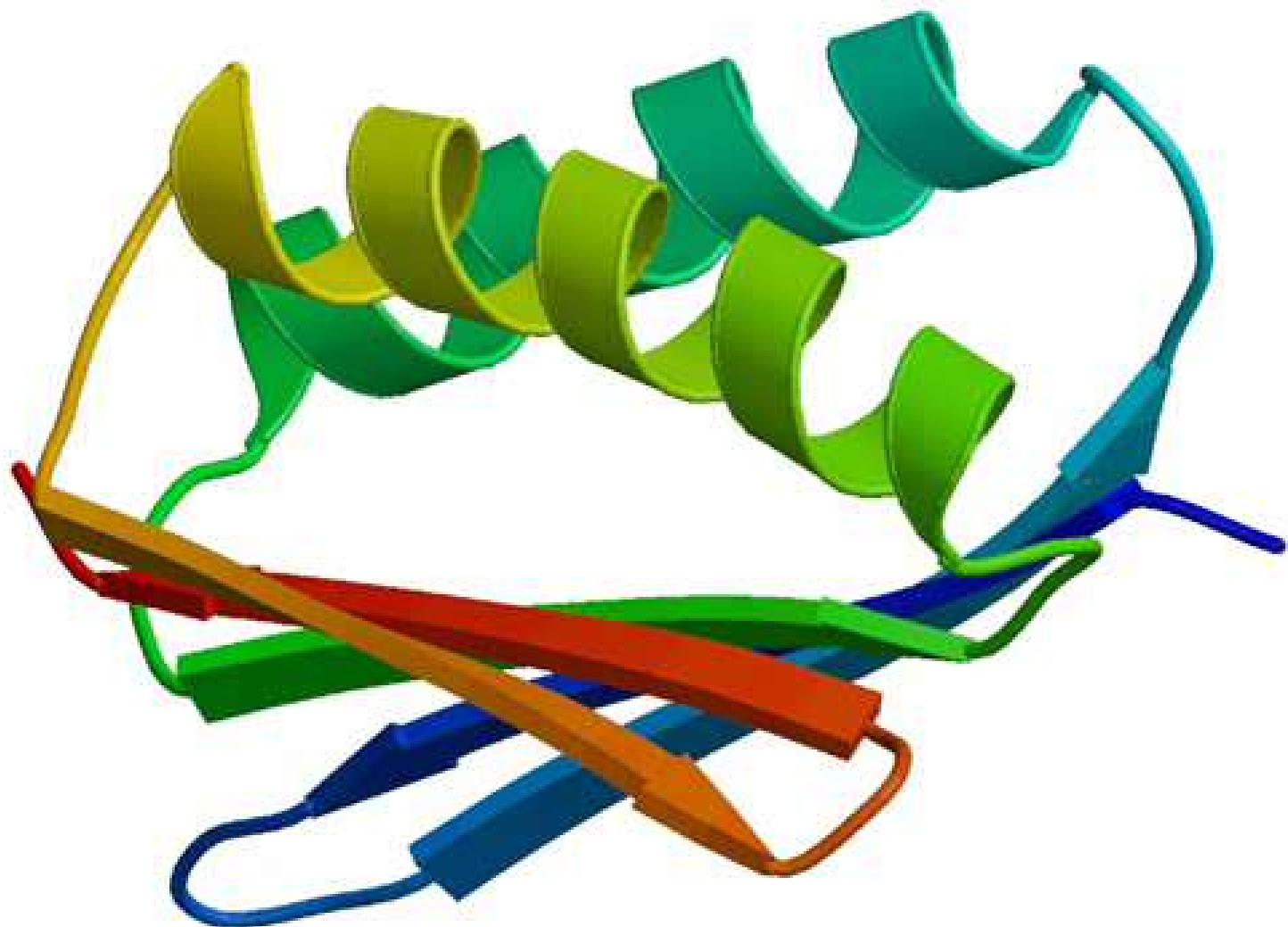
Statistically derived potential function

- VDW interaction
- solvation
- hydrogen bonding potential
- pair wise interactions
- rotamer probability

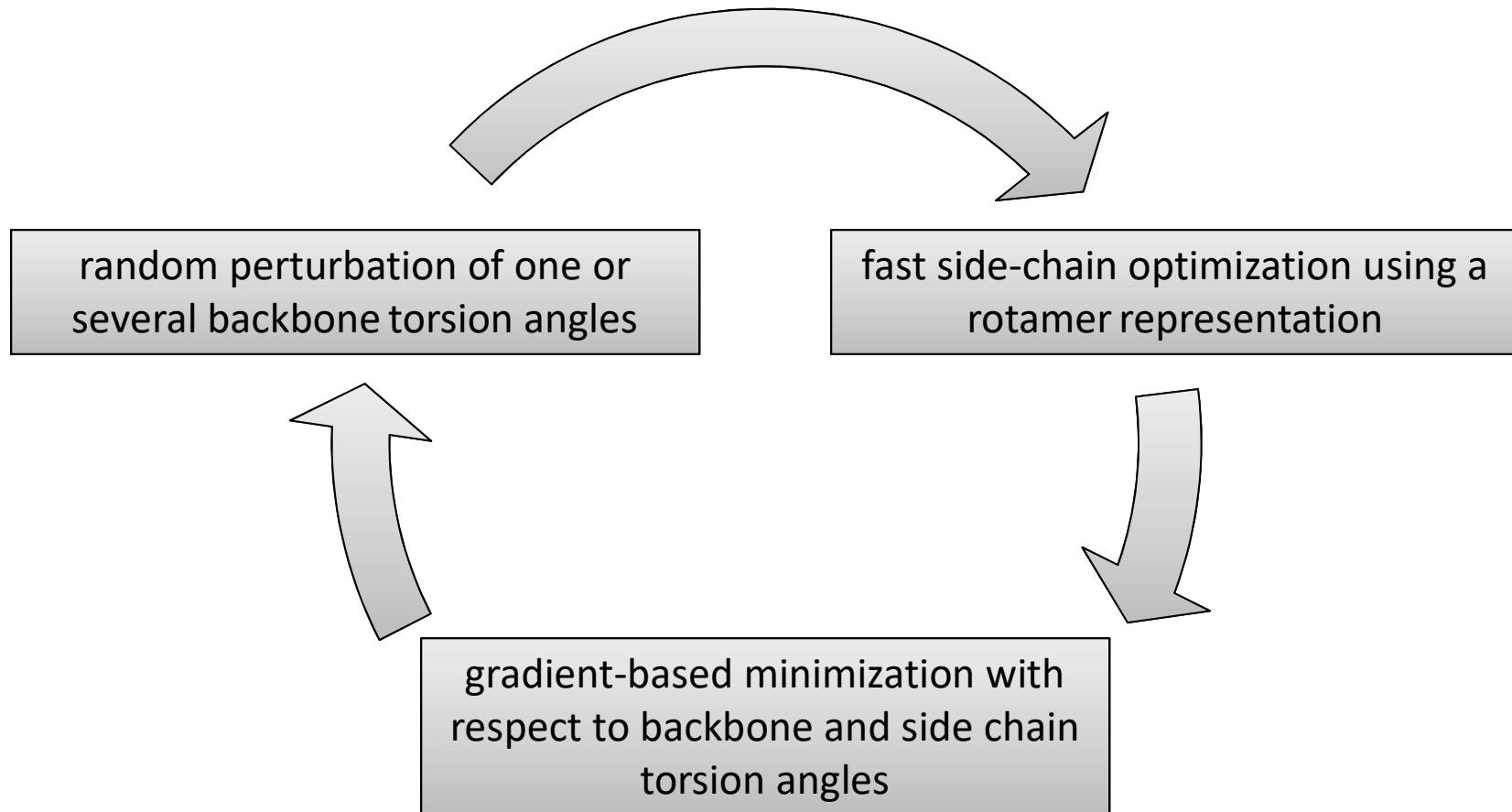
Simulated Annealing Monte Carlo energy minimization



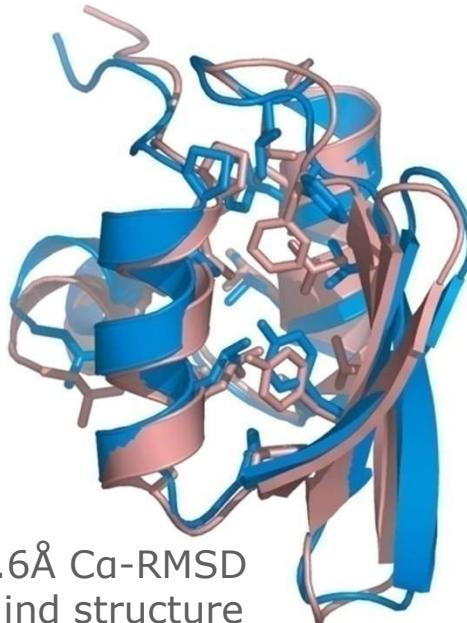
Dahiyat, B. I. and Mayo, S. L. (1997) *Science*, 278, 82-7
Dunbrack, R. L., Jr. and Karplus, M. (1993) *J Mol Biol*, 230, 543-74.
Kuhlman, B., et. al. (2003) *Science*, 302, 1364-1368.



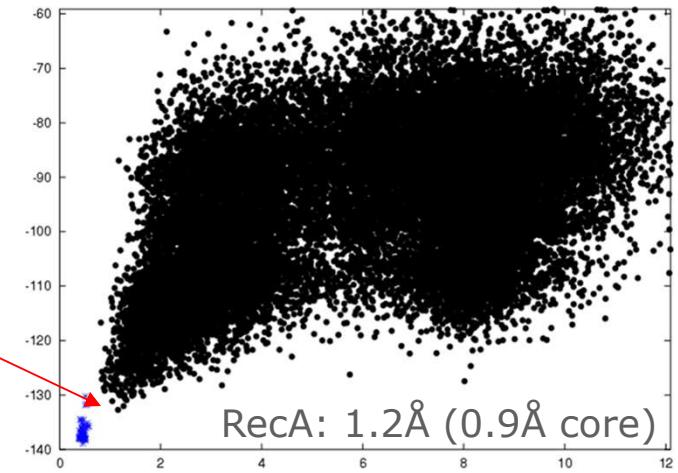
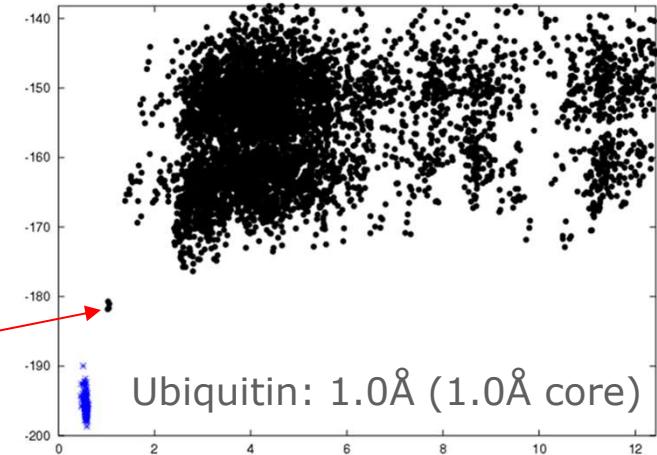
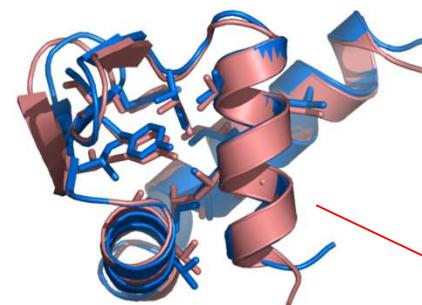
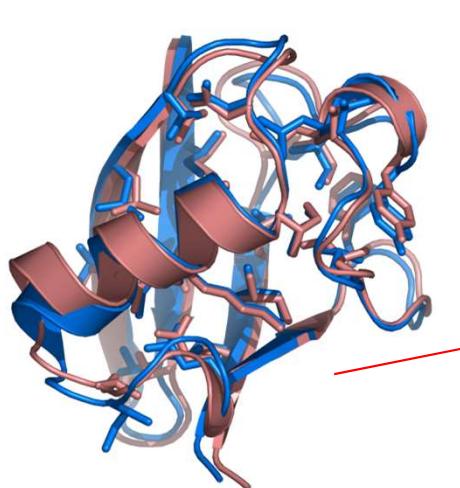
Refinement Cycle with Side Chain Repacking and All Atom Minimization



CASP target T0281 and other Benchmark Examples



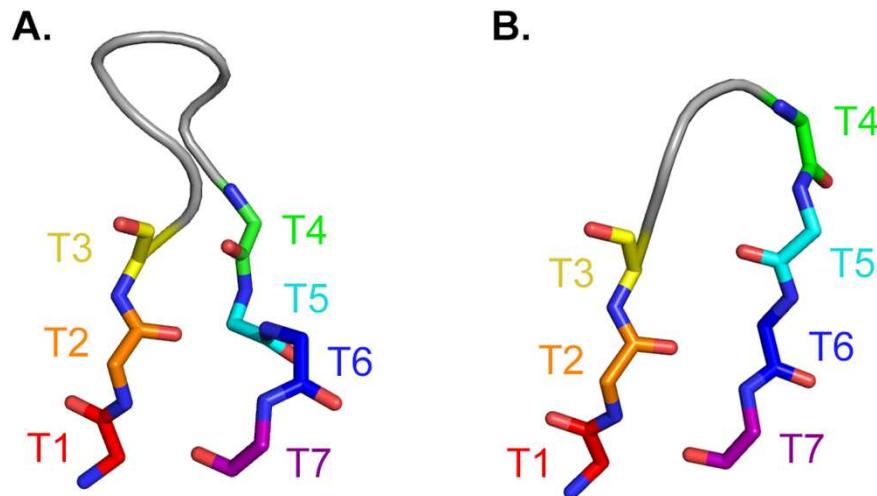
1.6 \AA Ca-RMSD blind structure prediction for CASP6 target T0281, hypothetical protein from *Thermus thermophilus* Hb8. Superposition of our submitted model for this target in CASP6 (blue) with the crystal structure (red; PDB code 1whz)



Structure Prediction of Long HCDR3 Loops in Antibodies



- HCDR3 structures can be grouped into canonical torsos enabling accurate prediction of very long loop conformations



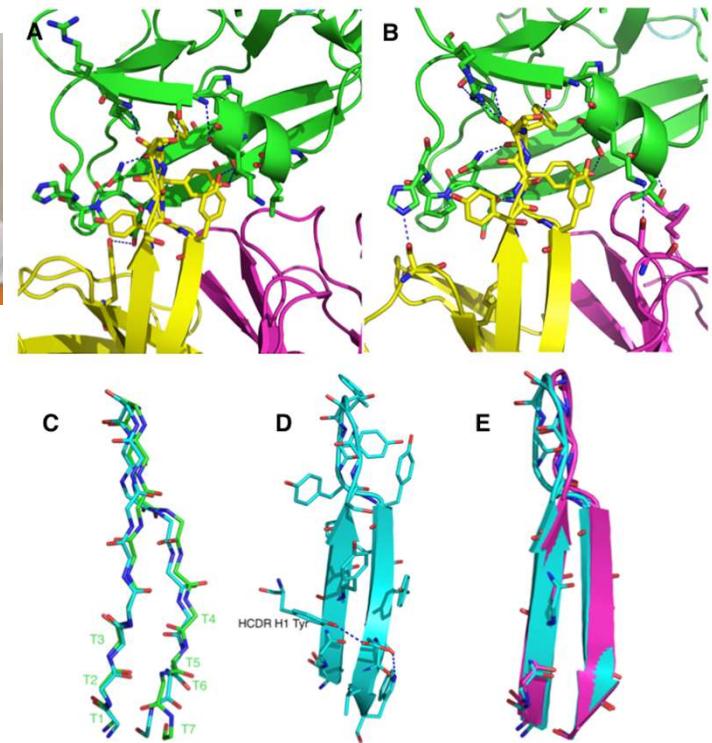
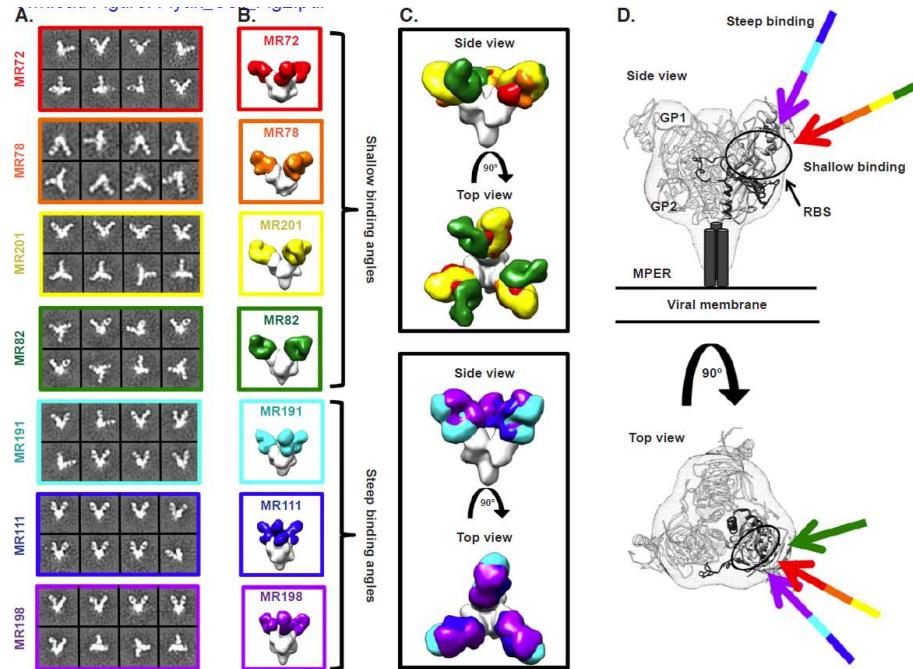
J. A. Finn, J. E. Crowe, J. Meiler; "Improving loop modeling of the antibody complementarity-determining region 3 using knowledge-based restraints"; pending

PDB ID	Length	Without Restraints			With Restraints		
		Best RMSD1 6 sample d	Average RMSD1 6 of top 10 by RMSD1 6	Models below 2Å RMSD1 6	Best RMSD1 6 sample d	Average RMSD1 6 of top 10 by RMSD1 6	Models below 2Å RMSD1 6
1WT5	11	0.72	1.24	31	0.74	0.84	235
2G75	11	0.37	1.11	23	0.48	0.55	449
4G5Z	11	0.61	1.44	13	0.48	0.80	100
3QRG	12	0.72	0.84	26	0.61	0.69	167
4G6K	12	0.93	1.19	50	0.92	1.09	201
4LLU	12	1.16	1.20	44	0.59	0.75	296
1FVC	13	0.81	1.27	37	0.94	1.00	245
3H15	13	1.37	1.58	22	0.95	1.40	90
4HFW	13	2.07	2.24	0	0.64	0.80	99
4FQH	14	0.75	1.98	4	0.46	0.70	25
4NM4	14	0.61	2.03	3	0.64	0.91	38
8FAB	14	1.39	1.52	24	0.95	1.16	146
3G6A	15	1.10	1.77	10	0.77	0.87	99
3TNM	15	0.76	1.80	8	0.76	1.12	30
3W9D	15	1.44	1.92	5	1.33	1.53	34
1AQK	16	1.39	1.78	8	0.96	1.24	65
1DQL	16	1.47	1.91	6	1.44	1.73	22
1OM3	16	1.84	2.14	1	1.10	1.39	31
1U6A	17	2.11	2.40	0	0.91	2.17	1
3AAZ	17	1.40	1.92	5	1.26	1.59	24
4M5Y	17	0.88	1.34	19	0.68	0.94	79
3INU	18	1.73	2.15	2	1.35	1.73	16
3QEHE	18	1.90	2.30	2	1.76	2.05	4
4F58	18	2.15	2.48	0	1.45	2.02	2
1HZH	20	1.71	2.07	4	1.32	1.80	9
4LKC	22	2.04	2.38	0	0.71	1.66	7
1RHH	24	2.62	2.84	0	2.32	2.57	0
4FNL	26	1.75	2.33	2	2.09	2.25	0
Average	16	1.35	1.83	12	1.02	1.33	90

Rapid Screening for Human Anti-bodies neutralizing the Ebola Virus

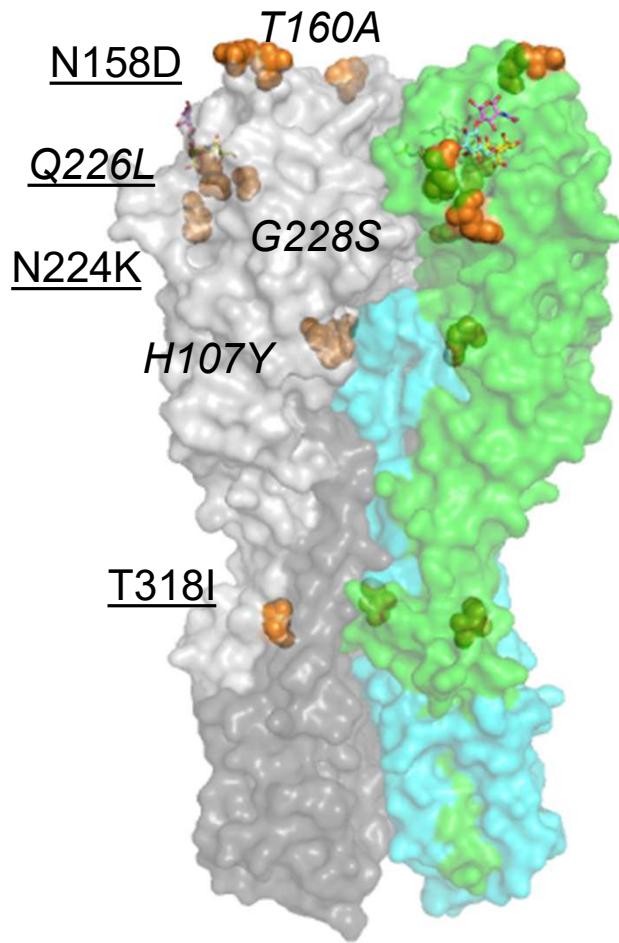


- Towards Therapeutic Antibodies targeting Ebola and Marburg
- Adding Atomic-Detail Accurately to Low-Resolution Structures



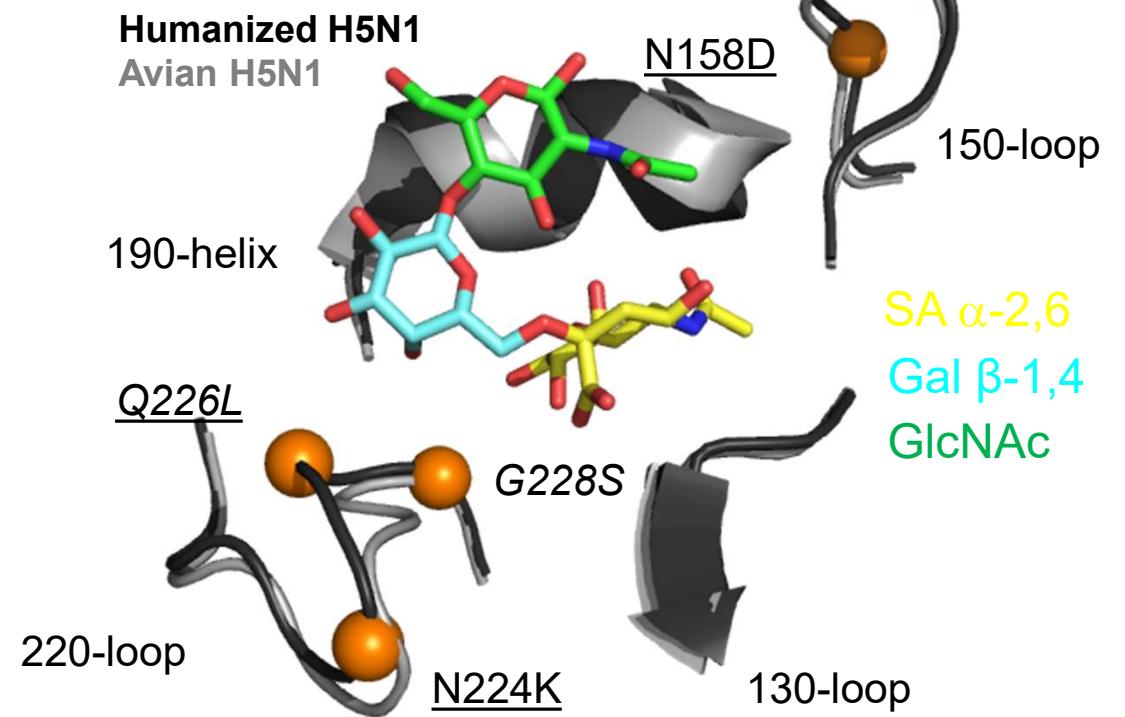
A. Sangha, J. Finn, J. E. Crowe, J. Meiler; "Rapid Structure-Based Screening for Human Antibodies neutralizing the Ebola Virus"; in prep.

Laboratory-generated Mammalian Transmissible H5N1 Avian Flu



- ▶ **Kawaoka Lab**
 - UW-Madison
 - *Nature* 2012, 486 (7403), 420-8
 - A/Vietnam/1203/2004
 - For HA mutations

- ▶ **Fouchier Lab**
 - Netherlands
 - *Science* 2012, 336 (6088), 1534-41
 - A/Indonesia/5/2005
 - Five HA *mutations*



Human Antibodies Isolated from B-cells of Vaccine Participants

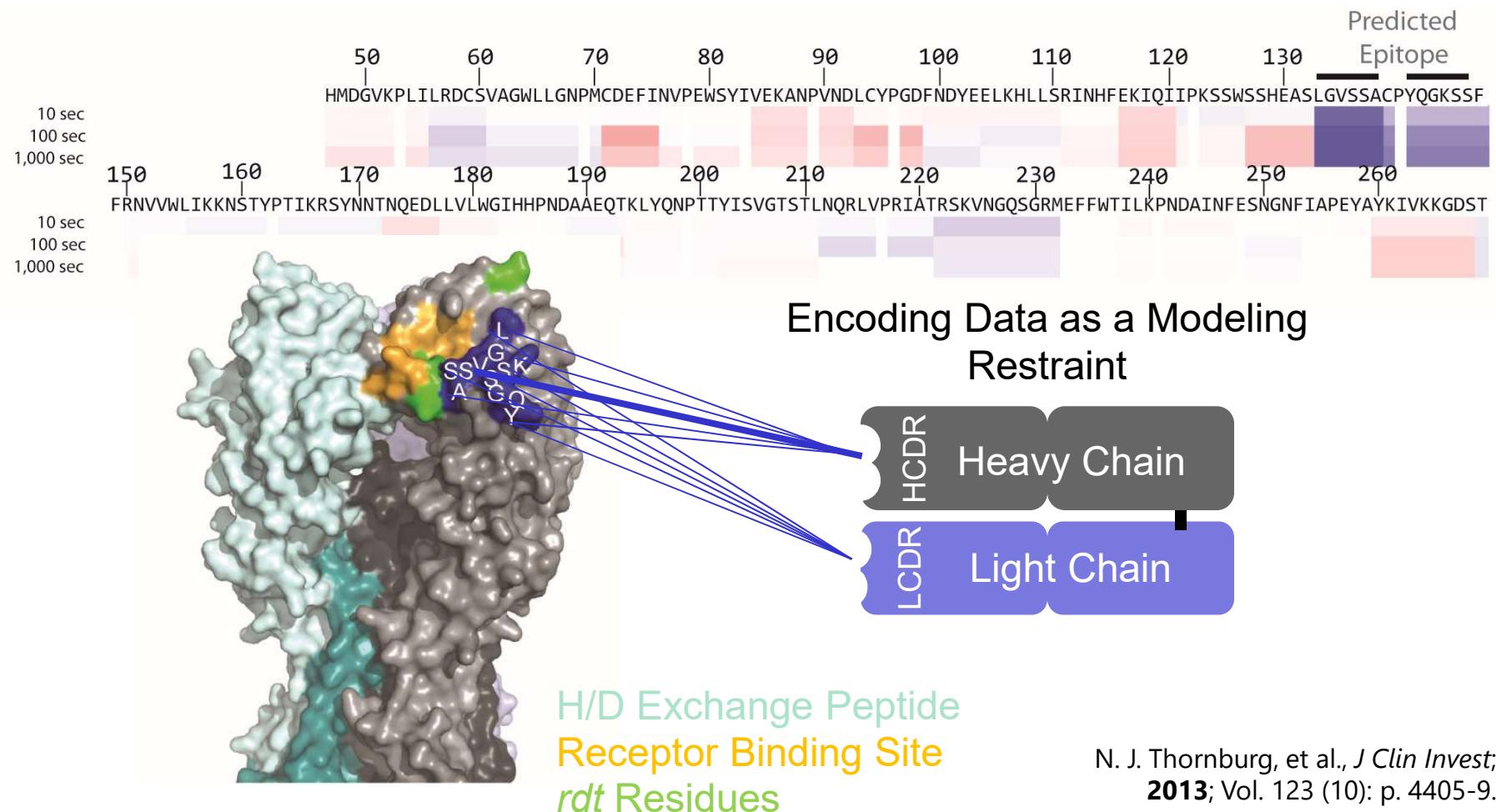


Domain / mAb		EC ₅₀ for binding to HA of indicated H5N1 strain (ng/mL)								
		Wild-type H5N1 field strain							Respiratory droplet transmissible (<i>rdt</i>) variant	
		VN/ 1203	IN	Anhui	Egypt	BHG	HK/ 156	HK/ 213	<i>rdt</i> VN/K	<i>rdt</i> IN/ F
Head	H5.2	106	325	143	22	1,140	14	157	126	410
	H5.3	17	10,000	2,780	> ^a	>	18	>	19	>
	H5.9	200	1,800	376	178	16	22	61	137	>
	H5.13	660	19,900	119	16	>	63	>	363	>
	H5.31	44	>	83	201	>	28	>	4	7
Stem	H5.7	79	156	134	12,800	518	109	275	8	18
	H5.36	31	34	32	36	37	27	53	38	73

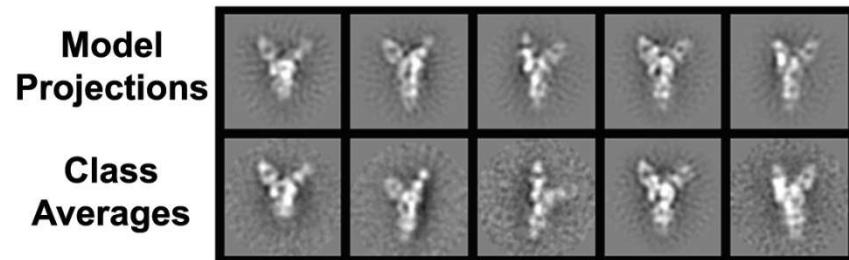
^a. > 50,000

N. J. Thornburg, et al., *J Clin Invest*; 2013; Vol. 123 (10): p. 4405-9.

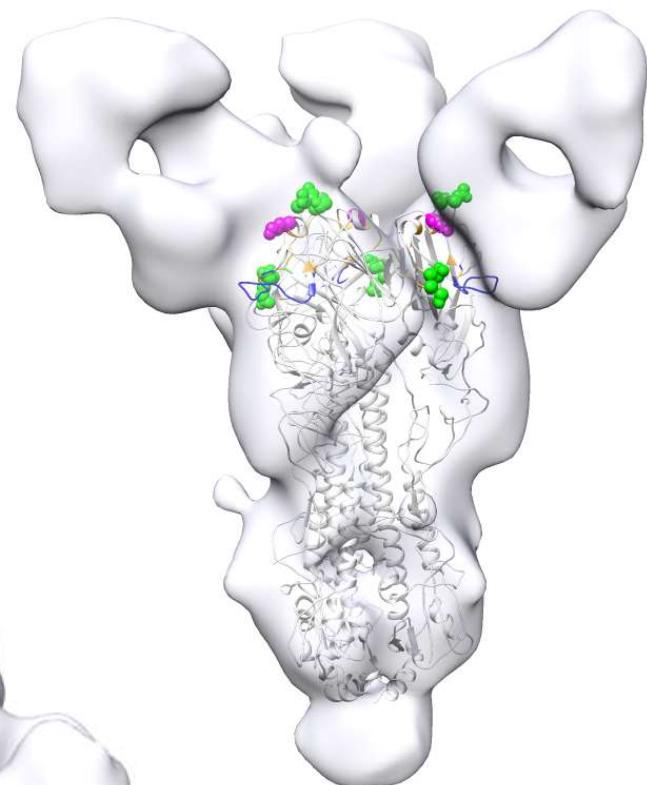
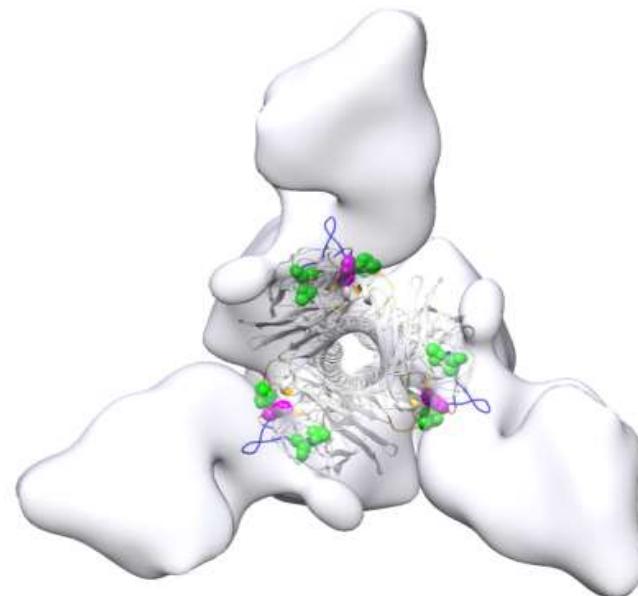
H/D Exchange Implicates the 130-Loop in Binding by mAb H5.3



Single Particle EM Indicative of Binding over Receptor Binding Site



Receptor Binding Site
H/D Exchange Peptide
Lysine-193
rdt Residues

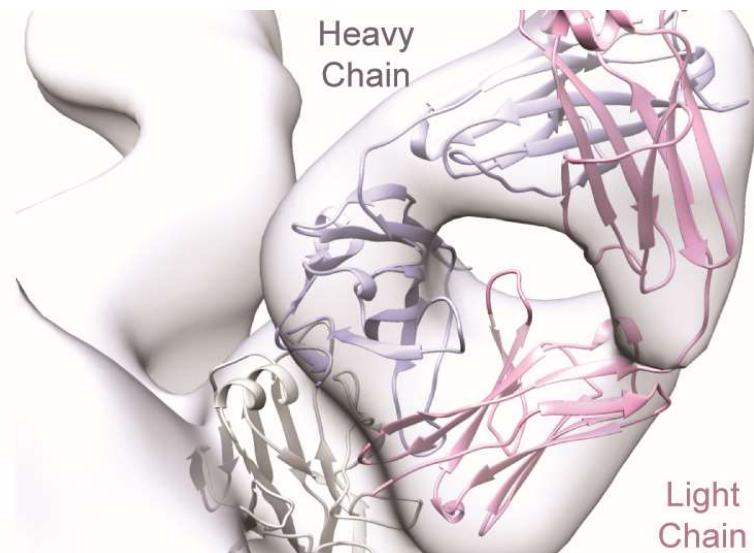


N. J. Thornburg, et al., *J Clin Invest*; 2013; Vol. 123 (10): p. 4405-9.

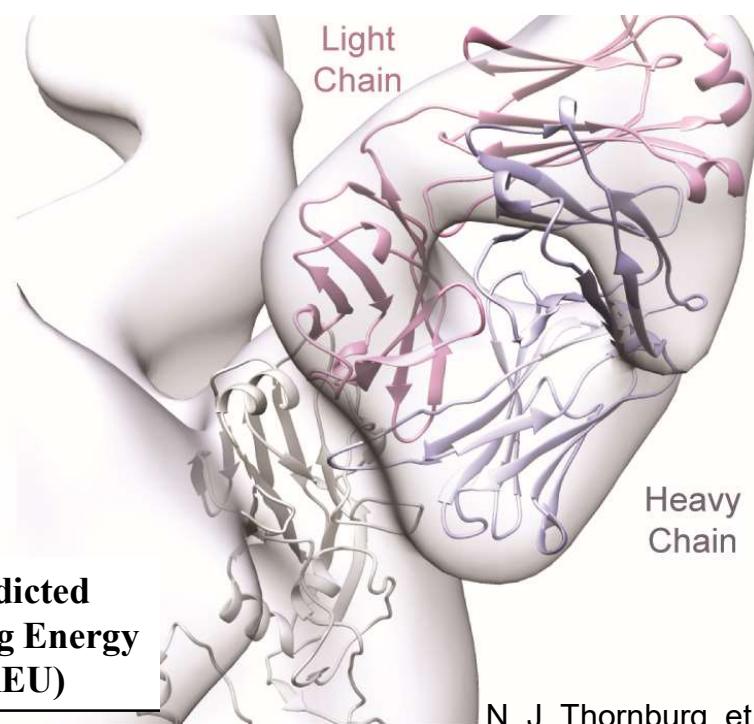
Relative Orientation of H5.3 Fab is Ambiguous by EM Density Fitting



Orientation A
CCC=0.895



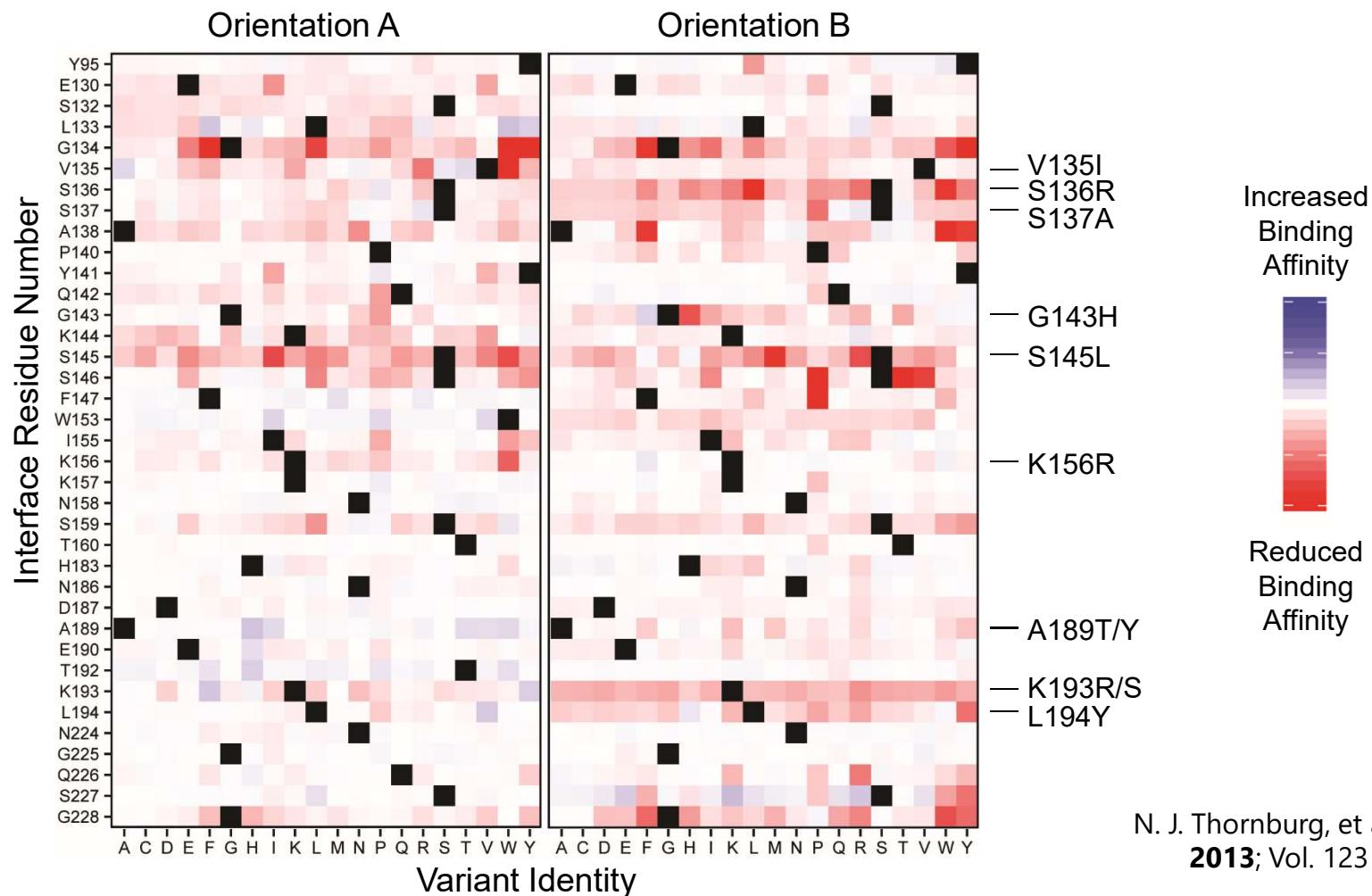
Orientation B
CCC=0.868



Initial Cross Correlation Coefficient	Model Cross Correlation Coefficient	Predicted Binding Energy (REU)
Orientation A	0.895	0.903
Orientation B	0.868	0.911

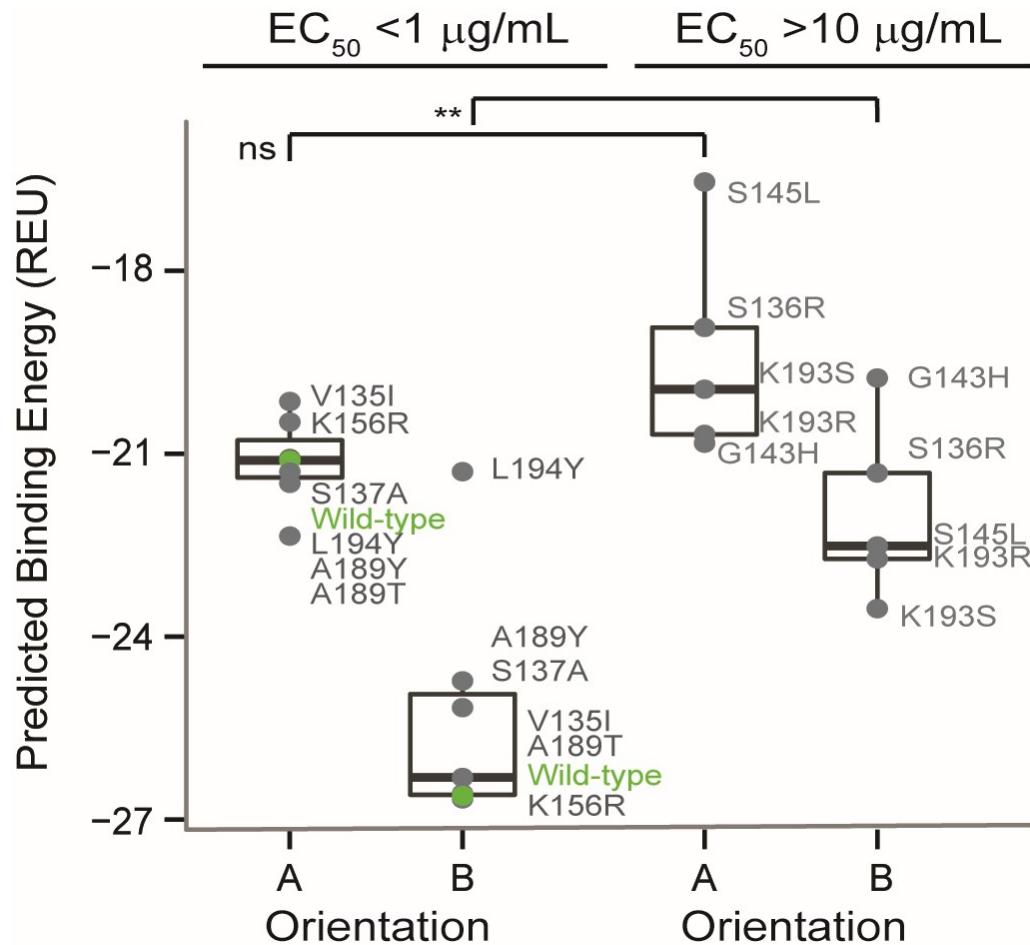
N. J. Thornburg, et al., *J Clin Invest*; 2013; Vol. 123 (10).
A. Sircar and J. J. Gray, *PLoS Comput Biol*; 2010; Vol. 6 (1).

Prediction of Escape Mutants Assesses Model Quality



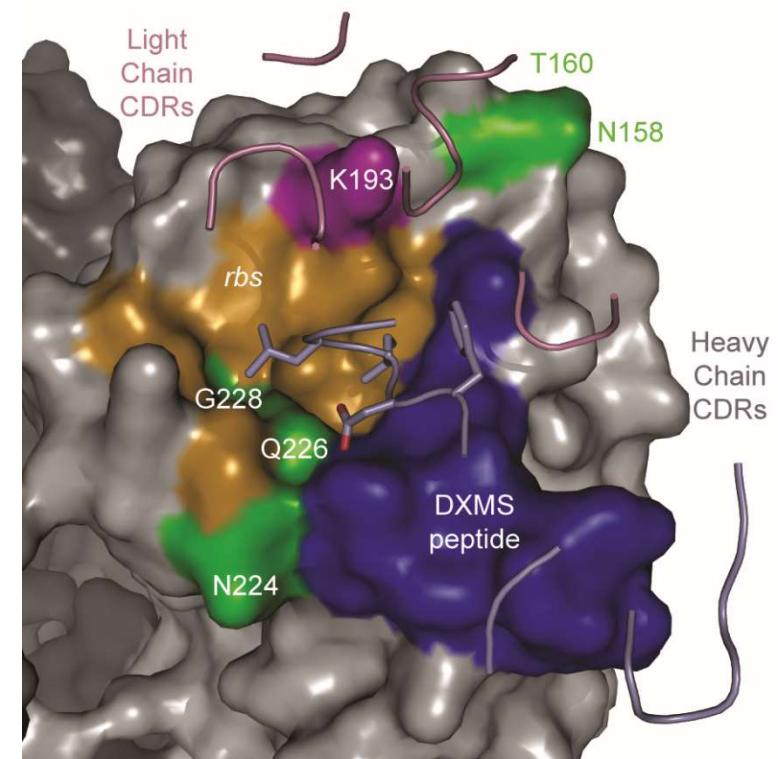
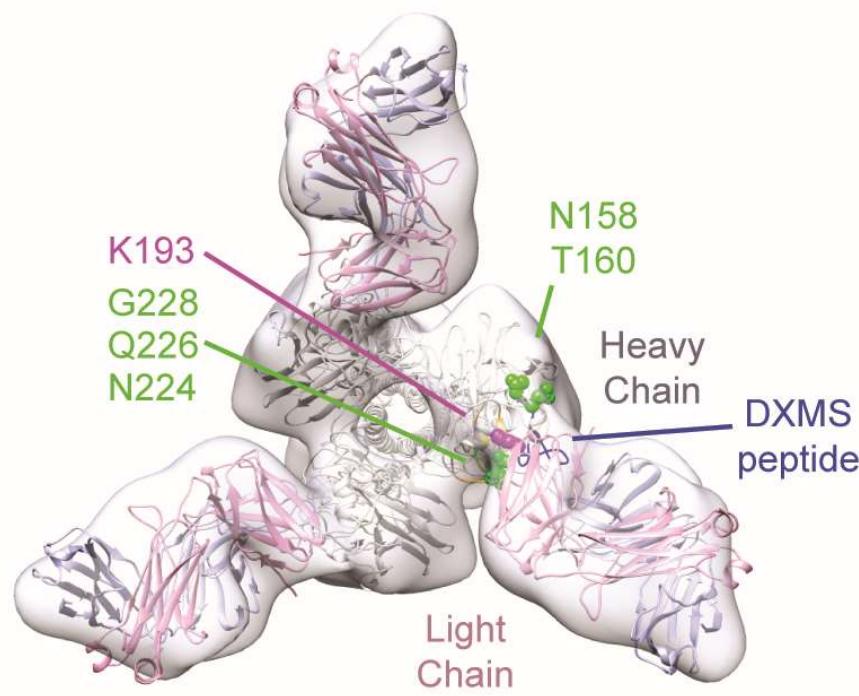
N. J. Thornburg, et al., *J Clin Invest*; 2013; Vol. 123 (10): p. 4405-9.

Mutational Analysis Indicates H5.3 Binding in Orientation B



N. J. Thornburg, et al., *J Clin Invest*; 2013; Vol. 123 (10): p. 4405-9.

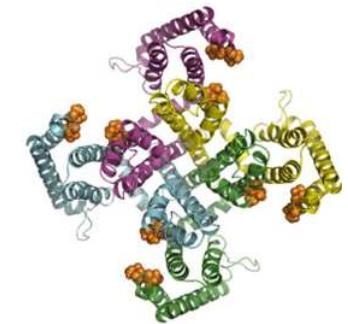
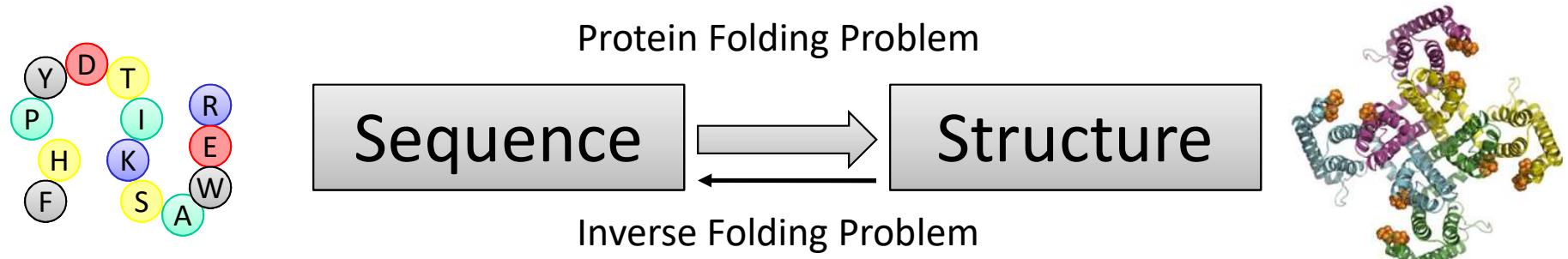
H5.3 Binds Receptor Binding Site and Bisects *rdt* Mutations



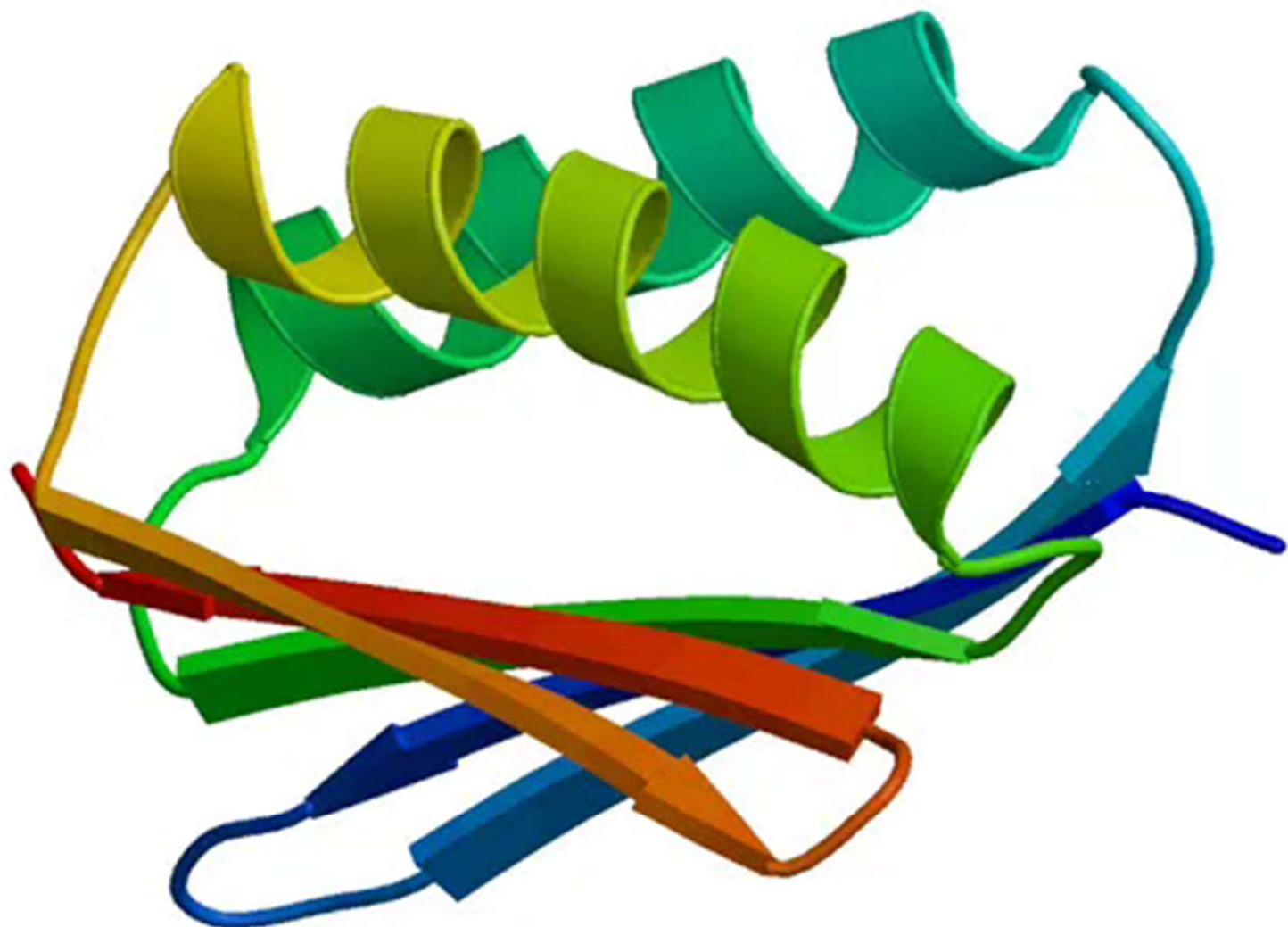
N. J. Thornburg, et al., *J Clin Invest*; 2013; Vol. 123 (10): p. 4405-9.

(Inverse) Protein Folding Problem

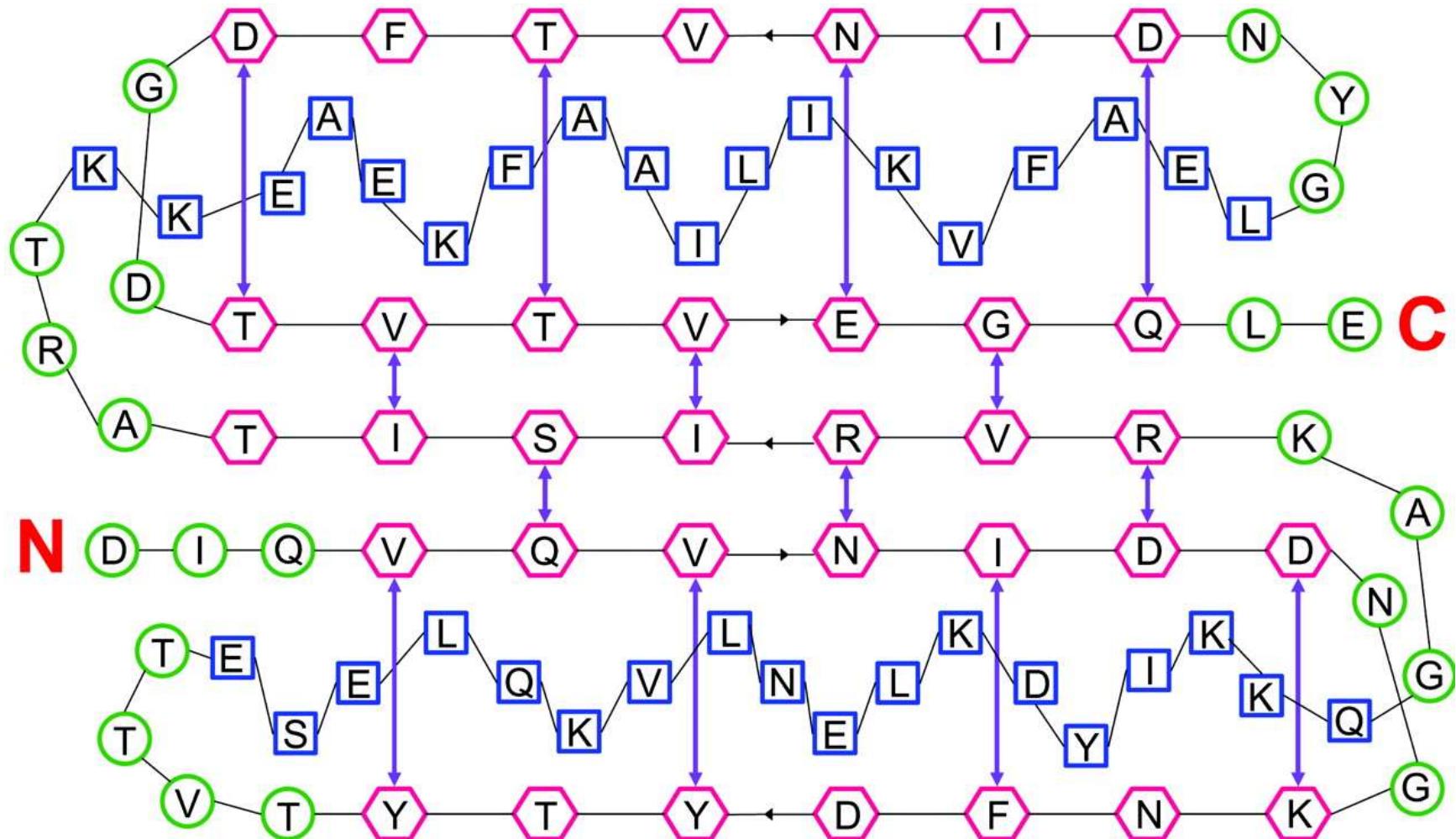
Holy Grail of Comp. Struct. Biology



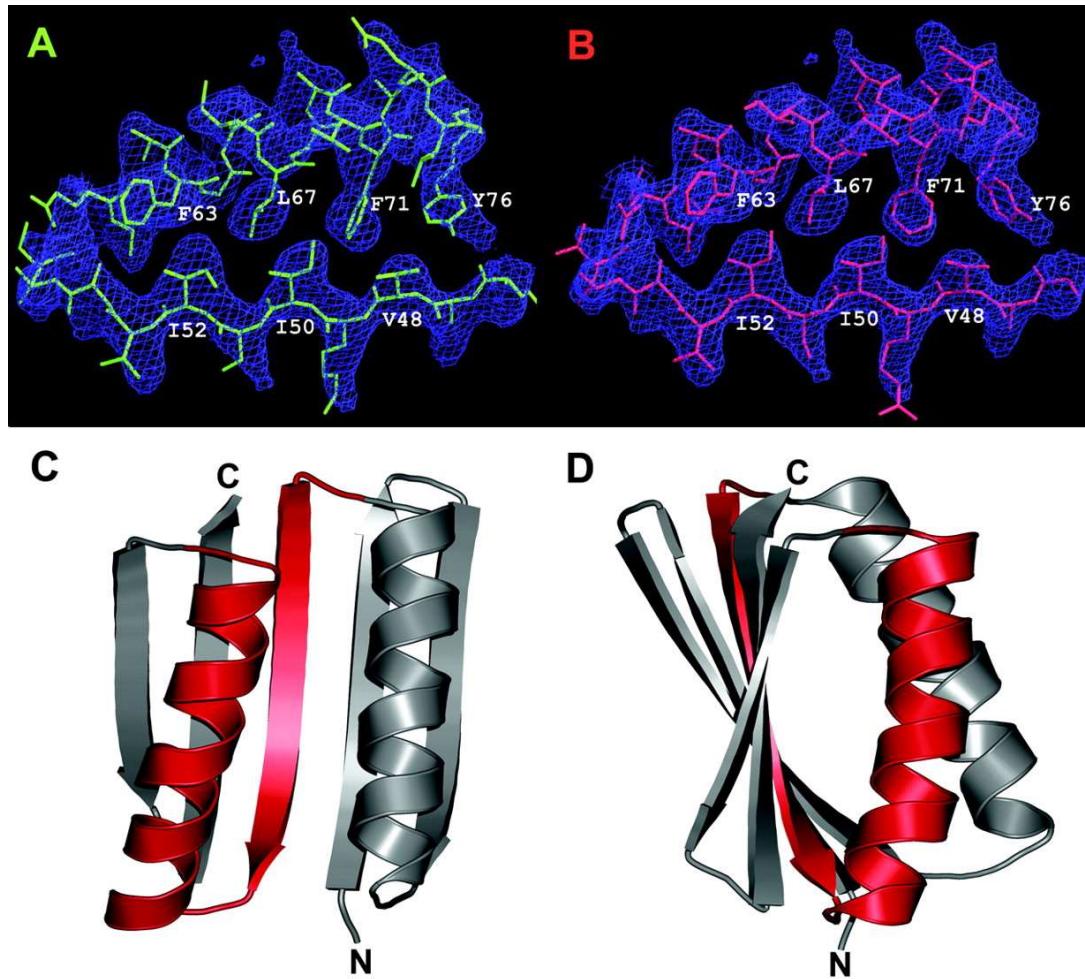
- Given a protein's AA sequence, what is its 3-dimensional fold , and how does it get there?
- Assume 100 conformations for each amino acid in a 100 amino acid protein $\Rightarrow 10^{200}$ possible conformations!
- Cyrus Levinthal's paradox of protein folding, 1968.
- Given a protein fold, which primary sequence(s) fold into it?
- Assume a total of 100 conformations for all 20 natural occurring amino acids side chains in a 100 amino acid protein $\Rightarrow 10^{200}$ possible conformations!
- Earth is less than 10^{10} years old.



A two-dimensional schematic of the target fold

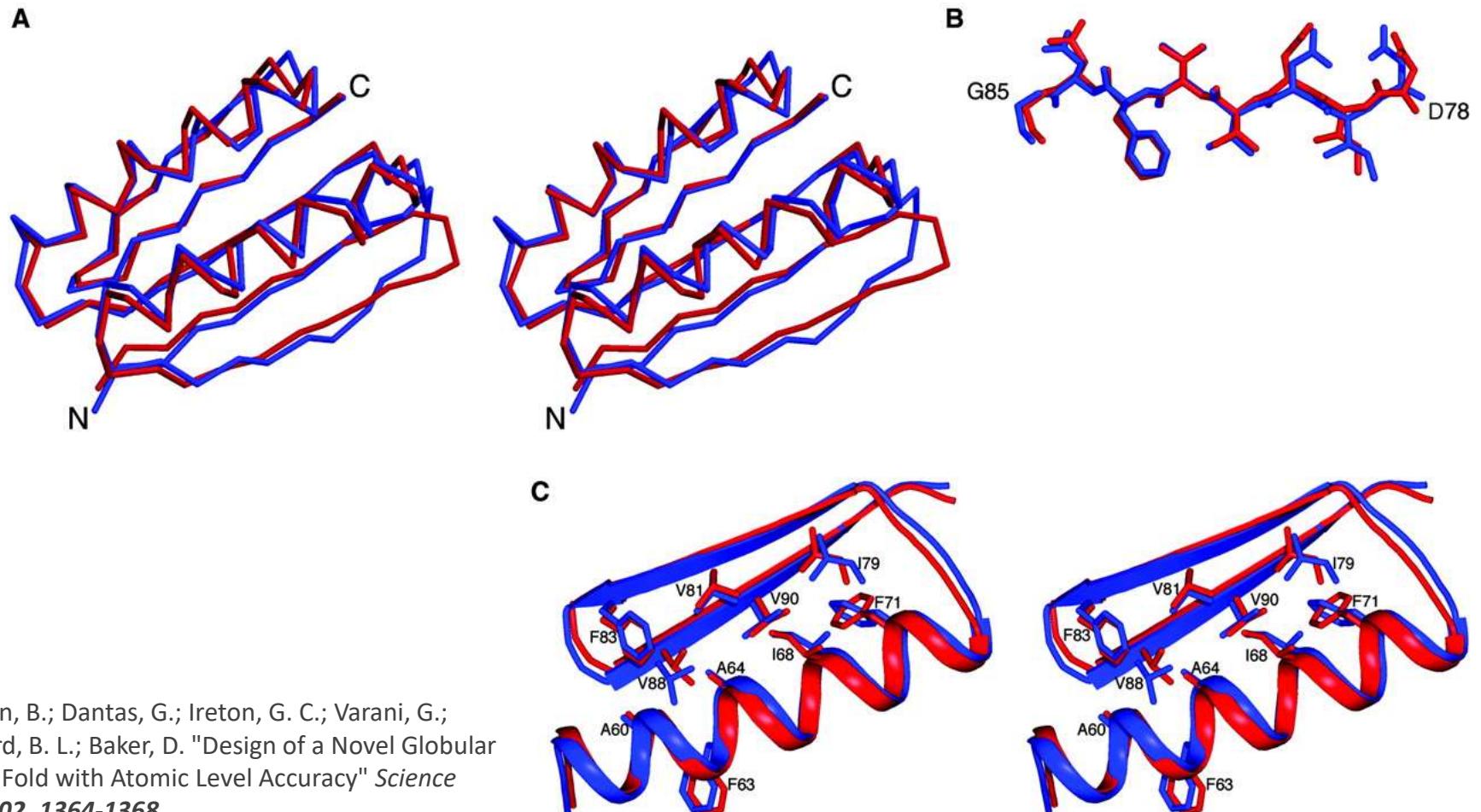


Schematic representation of Top7 residues 46-76



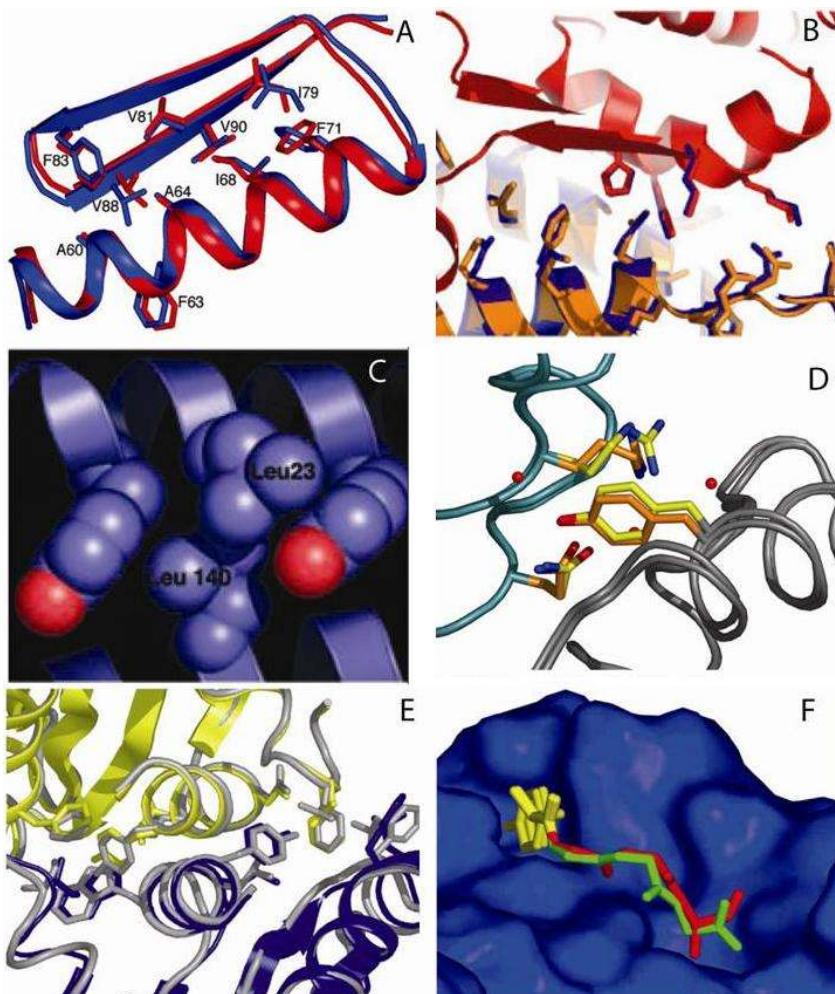
Kuhlman, B.; Dantas, G.; Ireton, G. C.; Varani, G.; Stoddard, B. L.; Baker, D. "Design of a Novel Globular Protein Fold with Atomic Level Accuracy" *Science* 2003, 302, 1364-1368.

Designed Model (blue) and X-ray Structure (red) of Top7



Kuhlman, B.; Dantas, G.; Ireton, G. C.; Varani, G.;
Stoddard, B. L.; Baker, D. "Design of a Novel Globular
Protein Fold with Atomic Level Accuracy" *Science*
2003, 302, 1364-1368.

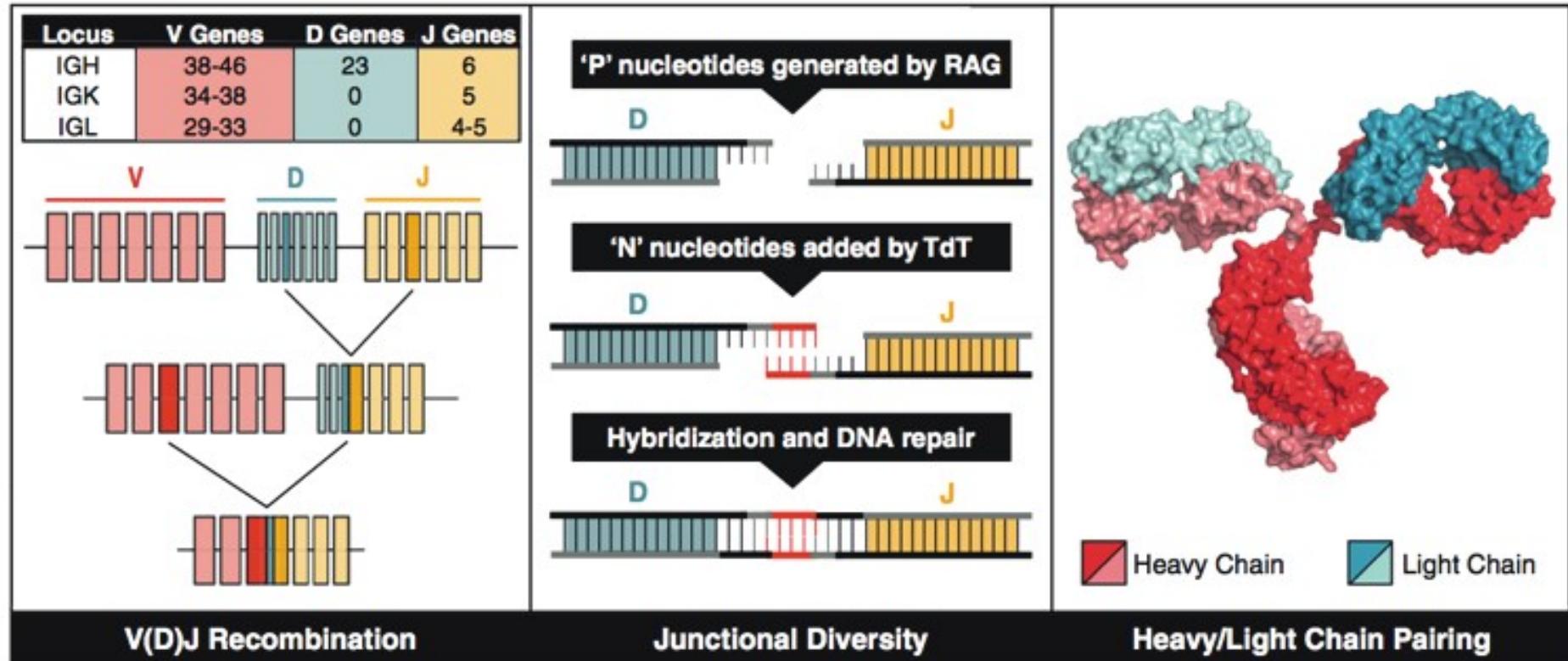
Protein Design With Rosetta



Comparison of predicted ROSETTA models to solved x-ray structures:

- (A) Representation of the effectively superposable side chains in the core of the designed Top7 protein (backbone RMSD 1.17Å) [Kuhlman].
- (B) Detailed view of Colicin D–Immunity protein D. shows overlay of side-chains of the catalytic residues on the solved crystal structure (0.28Å RMSD) [Schueler-Furman].
- (C) High resolution structure of a ROSETTA thermally stabilized hydrolase enzyme [Korkegian].
- (D) Overlay of the E7_C/Im7_C model (orange sidechains) with the experimentally determined structure (yellow sidechains) [Kortemme].
- (E) 2.4Å crystal structure of the redesigned E-DreI homing endonuclease interface (yellow and blue) superimposed with the computational model (grey) [Kortemme].
- (F) Superposition of p56lck bound glycine containing peptide with solved crystal structure [Sood].

Antibody Diversity is Limited to about 10^{11} different unique V-N-D-N-J



Finn et al. *Curr Opin. Immunol* 2013

Simulating Antibody Affinity Maturation in the Computer



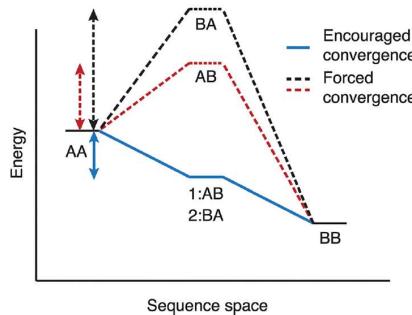
- Rapid Multi-State Design Algorithm for Rosetta

Table 6. Comparison of design-generated sequences to evolutionary sequence profiles of input proteins.

Benchmarkcase	Evolutionary sequence similarity (%) ^a		
	RECON FBB	RECON BBM	MPI_MSD
CheY	56.3	70.5	57.5
Elastase	60.3	70.7	65.9
FYN	87.0	87.0	96.0
PAPD	61.7	65.3	52.4
Ran	76.6	79.3	82.5
V _i 1-69	90.6	91.7	32.0
V _i 3-23	50.7	50.7	36.4
V _i 5-51	69.0	67.0	30.4
Average	69.0	72.8	56.6

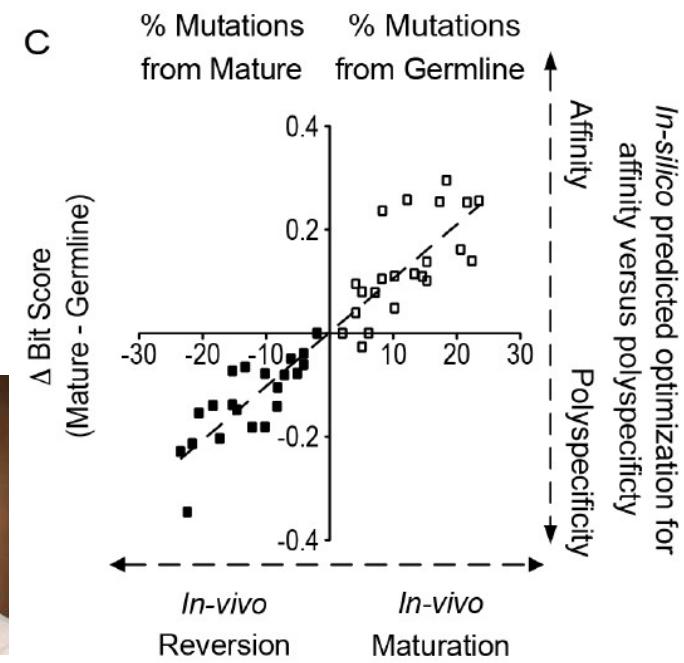
Designs produced by MPI_MSD or fixed backbone (FBB) or backbone minimized (BBM) RECON algorithms were compared to sequence profiles of evolutionarily related proteins at designed positions.

^aSequence similarity is computed as the Sandelin-Wasserman similarity, normalized as a percentage. See [methods](#) for details.



A. M. Sevy, T. M. Jacobs, J. E. Crowe, Jr. and J. Meiler; "Design of Protein Multi-specificity Using an Independent Sequence Search Reduces the Barrier to Low Energy Sequences"; *PLoS Comput Biol*; **2015**; Vol. 11 (7): p. e1004300.

- SSD for Affinity Maturation
- MSD for Broad Neutralization



J. R. Willis, B. S. Briney, S. L. DeLuca, J. E. Crowe, Jr. and J. Meiler; "Human germline antibody gene segments encode polyspecific antibodies"; *PLoS Comput Biol*; **2013**; Vol. 9 (4): p. e1003045.

Sequence of Germline Gene is Altered during Affinity Maturation



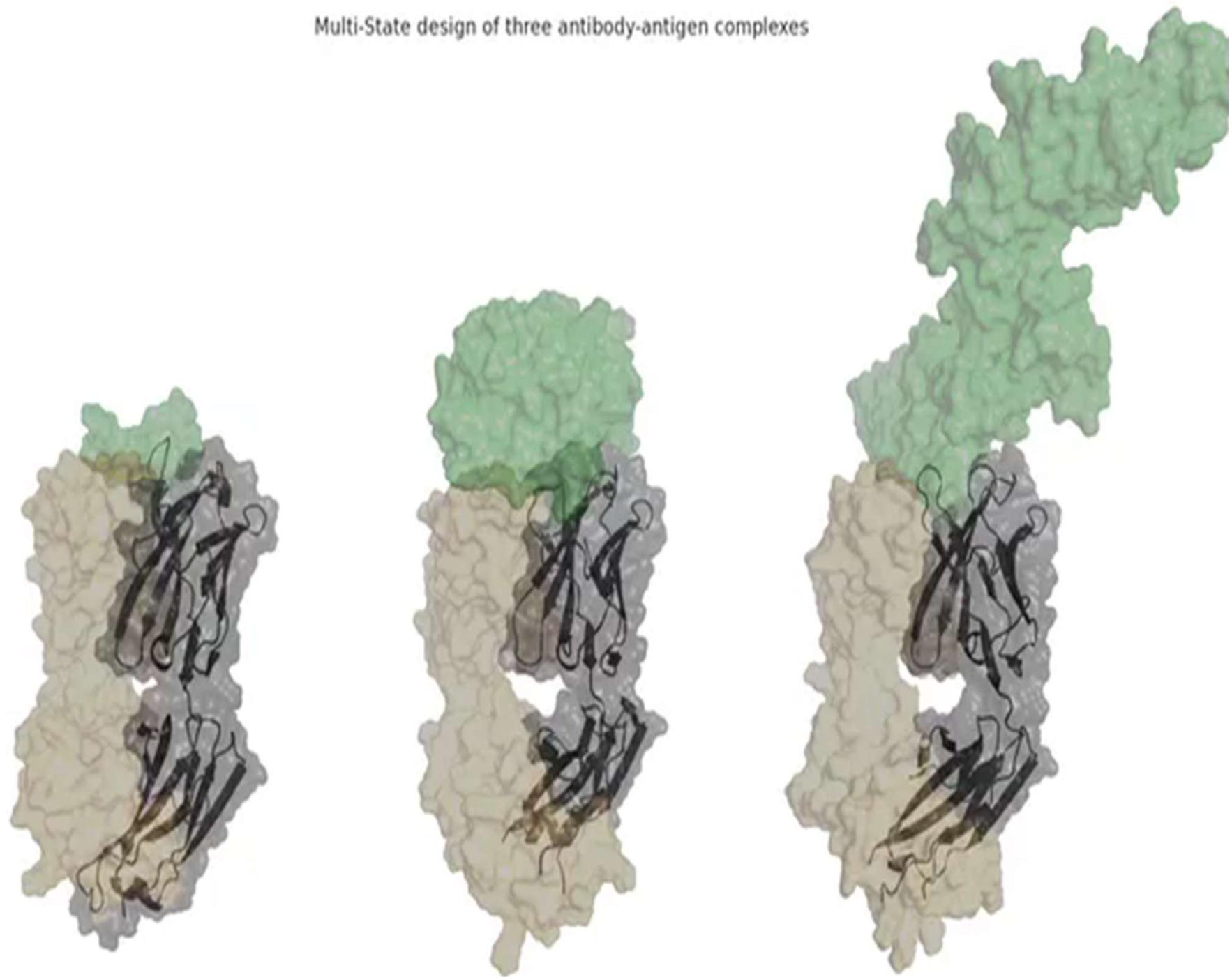
V_H 5-51 Derived Antibodies				
PDB ID	FR 1	CDR 1	FR 2	CDR 2
2XWT	EVQLVQSGAEVKKPQSLKISCKASGYS LTDN WIGWVRQKPGKGLEWMGIIYPGDSDTRY			
3HMX	EVQLVQSGAEVKKPGE SLKISCKGSGYSFTTYWLGWVRQMPGKGLDWIGIMSPVDSDIRY			
2B1A	EIQLEQSGAEVKKSGESLKISCQTSGYSFSDYWIGWVRQMPGKGLEWMGIFYPGDSDSRY			
GERM	EVQLVQSGAEVKKPGE SLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRY *:*** * *****.*:*****: ****: : *:***** *****: *;***: * *** ***	FR 3		
2XWT	SPSF QGQVT I SADKS INT AYL QWSS ILK ASDT A IYY CVG			
3HMX	SPSF QGQVT M SV DKS IT TAYL QWN S I LK ASDT A MYY CAR			
2B1A	SPSF EGQVT M SADR ST NT A H L QWSS ILK P S D T A LY Y CAR			
GERM	SPSF QGQVT I SADKS I ST AYL QWSS ILK ASDT A MYY CAR *****:*****: * . * . * . * . * . * . * . * . * . * . * . *			



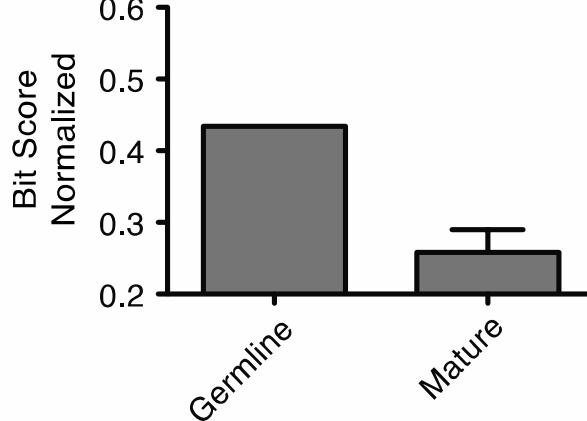
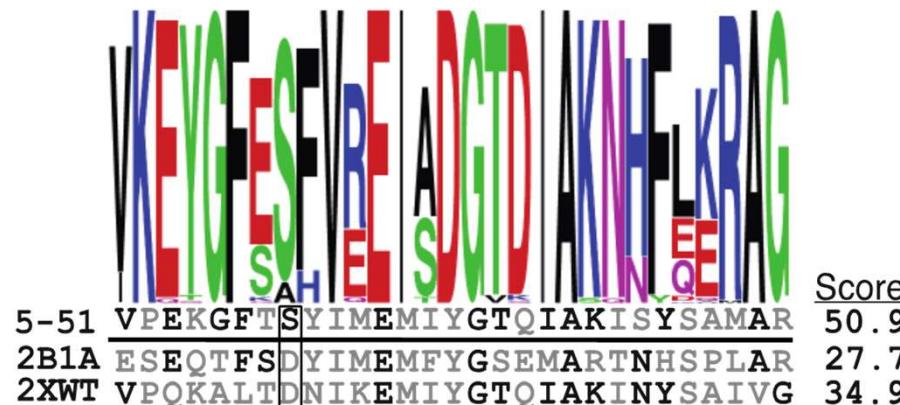
Willis et al. *PLoS Comp* (2013)

M. Babor and T. Kortemme; *Proteins*; 2009; Vol. 75 (4): p. 846-58.

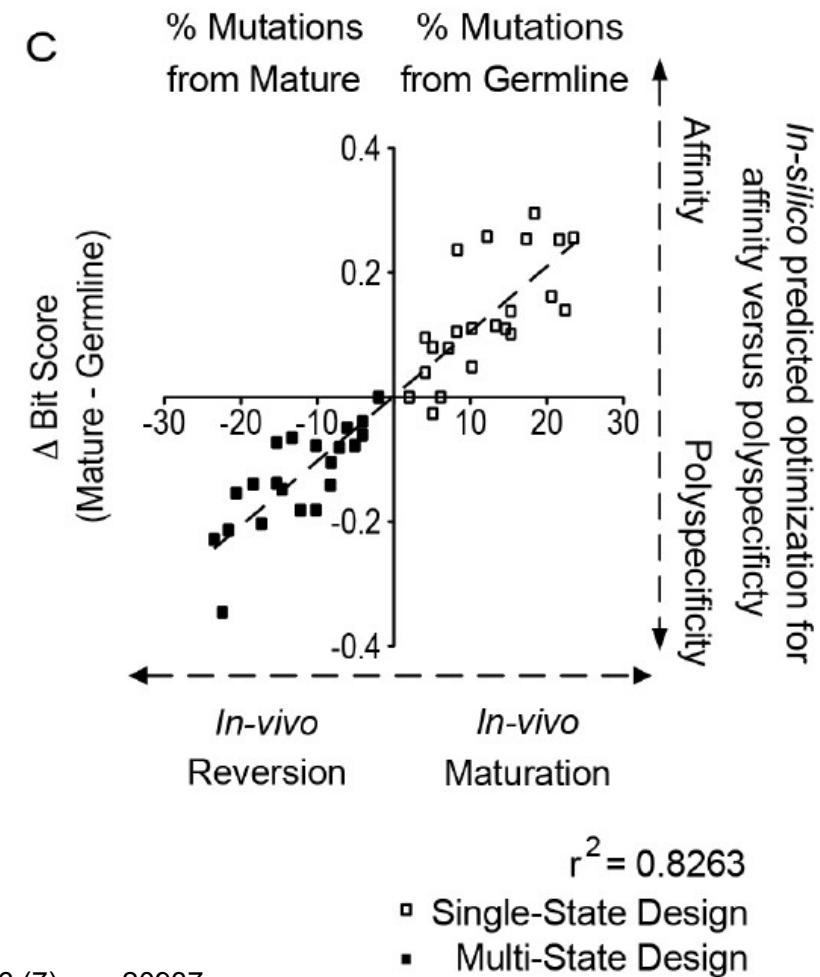
Multi-State design of three antibody-antigen complexes



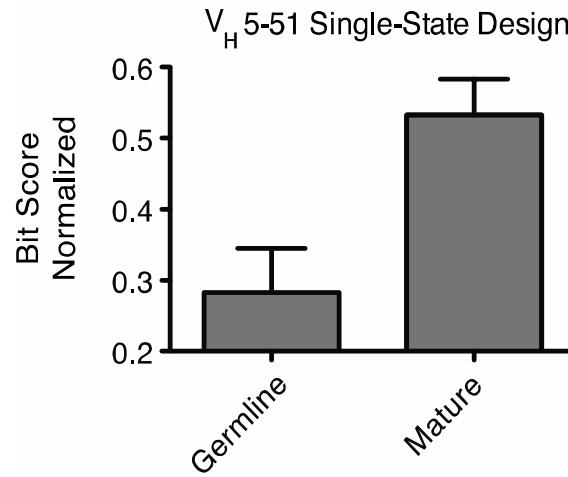
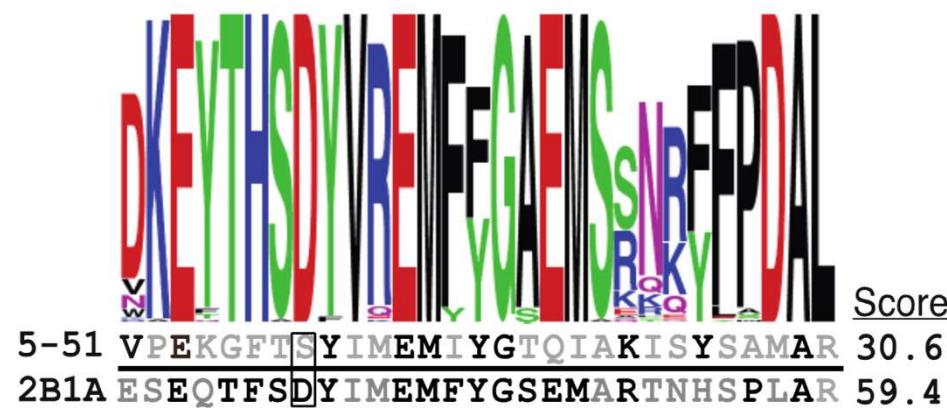
Germline Sequences are Optimal for Polyspecificity



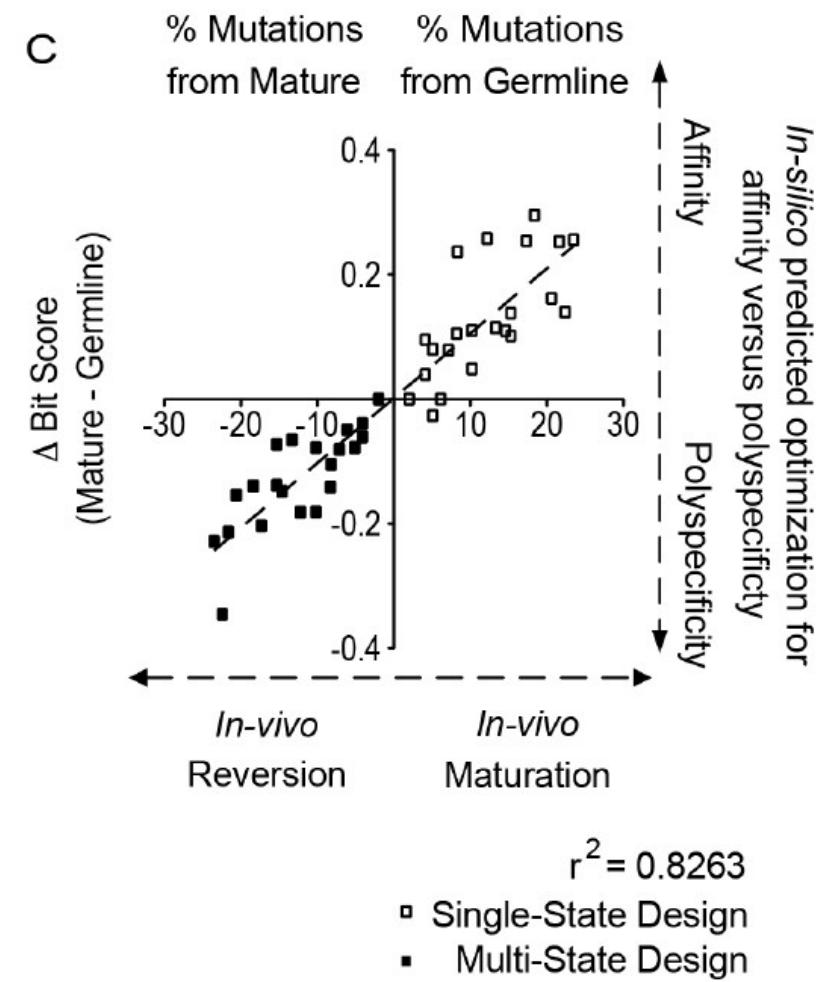
Willis et al. *PLoS Comp* (2013) | A. Leaver-Fay et al., *PLoS One*; 2011; Vol. 6 (7): p. e20937.



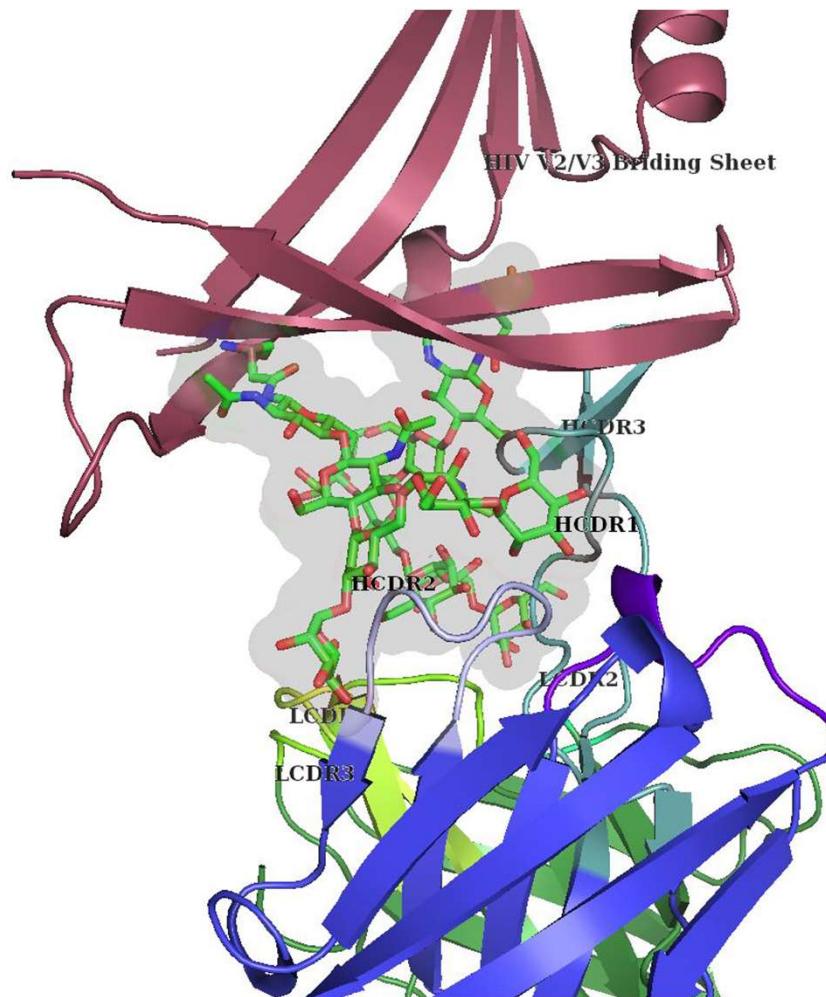
Mature Sequences are Optimal for Affinity



Willis et al. *PLoS Comp* (2013)

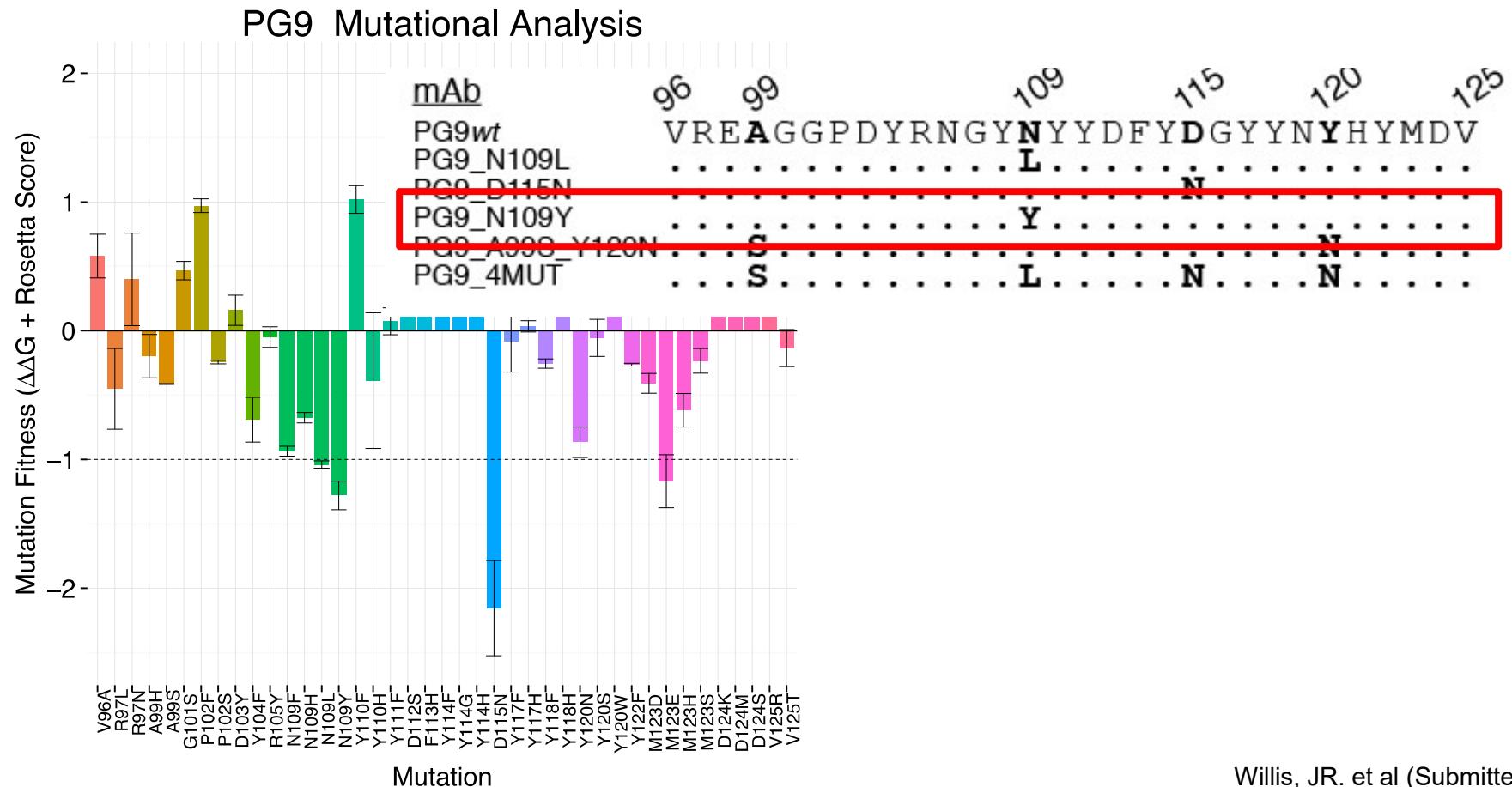


PG9-like is a Broadly Neutralizing HIV Antibody



- 30 residue long HCDR3 loop accounts for neutralization functionality
- Variable chain has few mutations relative to other broadly neutralizing Abs
- Germline reversion of the variable chain retains neutralization

Rosetta-predicted Mutations for Improved of PG9 Antibody



Redesign of PG9 Enhances Binding Potency and Breadth of Neutralization

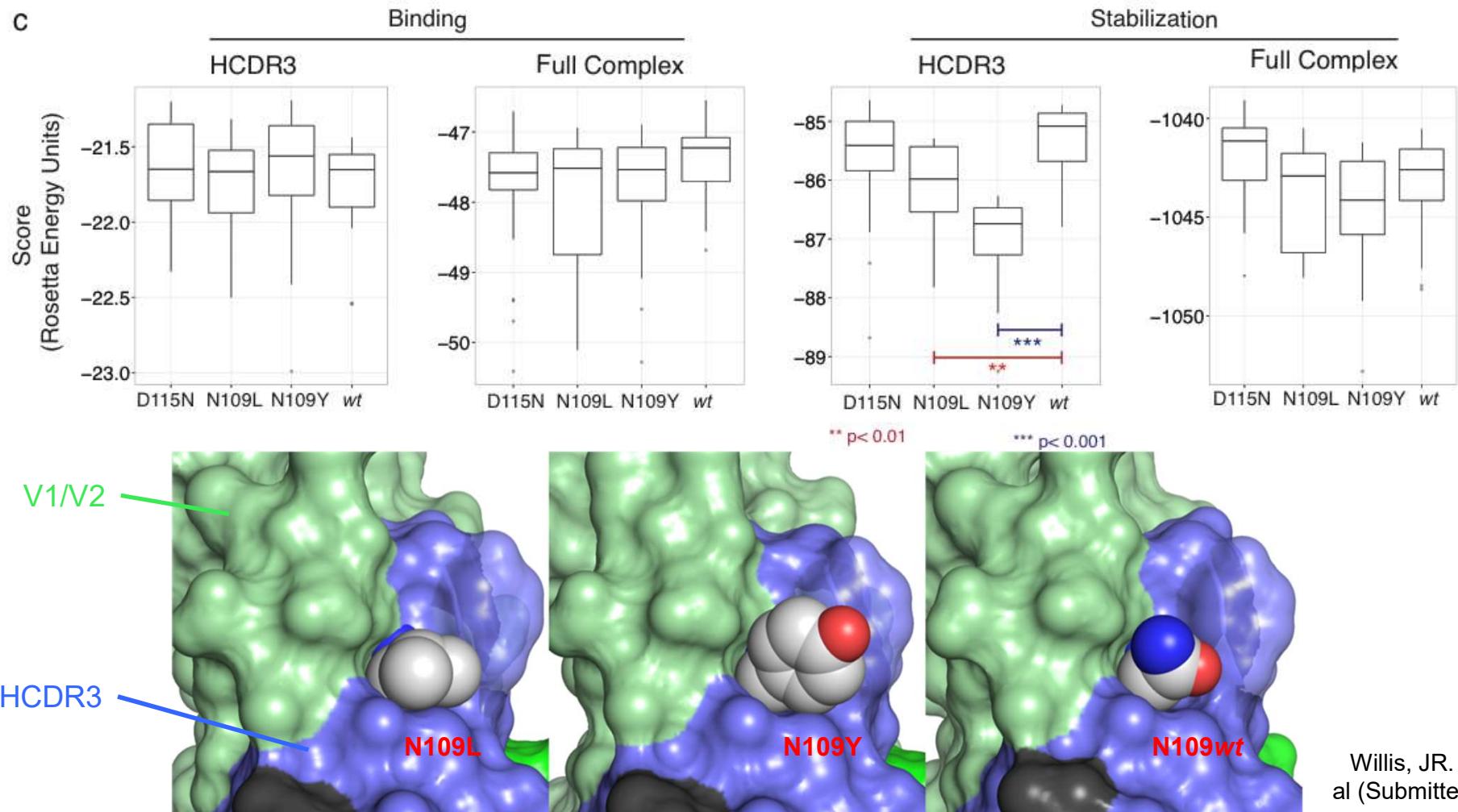


Virus	N160	PG9wt	EC ₅₀ (µg/mL)				IC ₅₀ (µg/mL)
			LEU100F	ASN100L	TYR100F	4MUT	
6535.3	N	0.26	0.10	0.49	0.09	7.74	
7165.18	N	>100	>100	>100	>100	>100	
0260.v5.c36	N	ND	ND	ND	ND	ND	
1054_07_TC4_1499	N	ND	ND	ND	ND	ND	
1056_10_TA11_1826	N	ND	ND	ND	ND	ND	
246F_C1G_S	S	ND	ND	ND	ND	ND	
3016.v5.c45	N	ND	ND	ND	ND	ND	
398_F1_F5_20	N	ND	ND	ND	ND	ND	
7030102001E5(Rev-)	S	ND	ND	ND	ND	ND	
703357.c02	N	ND	ND	ND	ND	ND	
AC10.0.29	N	>100	>100	>100	>100	>100	
BaL.26	N	0.54	0.04	>100	0.05	>100	
BG505.N332	N	1.48	0.42	3.63	0.25	2.86	
BJOX009000.02.4	N	ND	ND	ND	ND	ND	
CAAN5342.A2	N	1.20	0.47	1.06	0.44	2.33	
CAP45.2.00.G3	N	0.03	0.01	0.13	0.01	9.45	
Ce1086_B2	K	ND	ND	ND	ND	ND	
Ce1086_B2.K160N.LucR.T2A.ecto	N	ND	ND	ND	ND	ND	
Ce1086_B2.LucR.T2A.ecto	K	ND	ND	ND	ND	ND	
Ce2010_F5	N	ND	ND	ND	ND	ND	
Ce703010217_B6	N	ND	ND	ND	ND	ND	
CNE55	N	ND	ND	ND	ND	ND	
Du422.1	N	ND	ND	ND	ND	ND	
HIV-16845-2.22	N	ND	ND	ND	ND	ND	
HxBc2P3.2	N	2.41	0.25	0.74	0.49	7.78	
PVO.4	N	>100	>100	>100	>100	>100	
Q461.e2	N	ND	ND	ND	ND	ND	
QH0692.42	S	>100	>100	>100	>100	>100	
R2184.c04	N	ND	ND	ND	ND	ND	
REJO4541.67	N	>100	>100	>100	>100	>100	
RHPA.LucR.T2A.ecto	N	ND	ND	ND	ND	ND	
RHPA/N160.A.5.LucR.T2A.ecto	A	ND	ND	ND	ND	ND	
RHPA4259.7	N	0.66	0.13	1.32	0.16	15.70	
SC22.3C2.LucR.T2A.ecto	N	ND	ND	ND	ND	ND	
SC422661.8	N	2.48	0.25	1.89	0.31	24.87	
TH023.6	N	ND	ND	ND	ND	ND	
TH023.6/N160.A.5	A	ND	ND	ND	ND	ND	
THRO4156.18	N	>100	>100	>100	>100	>100	
TRJ04551.58	N	0.17	0.05	0.39	0.07	>100	
TRO.11	N	>100	>100	>100	>100	>100	
WEAU_d15_410_5017	N	ND	ND	ND	ND	ND	
WITO4160.33	N	ND	ND	ND	ND	ND	
X1632_S2_B10	N	ND	ND	ND	ND	ND	
X2088_c9	N	ND	ND	ND	ND	ND	
X2278_C2_B6	N	ND	ND	ND	ND	ND	
YU2	N	>100	>100	>100	>100	>100	
ZM109.F.B	N	0.02	0.01	0.04	< 0.01	2.27	
ZM214M.PL15	K	ND	ND	ND	ND	ND	

PG9wt	EU100F	ASN100L	TYR100F	4MUT
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
1.69	0.50	2.10	0.30	ND
>33	>33	>33	>33	ND
6.34	1.10	13.60	0.70	ND
>33	>33	>33	2.70	ND
2.46	1.60	9.00	0.20	ND
>33	>33	>33	10.80	>33
>33	>33	>33	12.90	ND
1.16	0.28	19.40	0.23	ND
ND	ND	ND	ND	ND
0.07	0.03	0.52	0.01	>33.3
0.04	0.02	0.11	0.02	0.28
1.73	1.20	>33	1.50	ND
4.60	7.60	>33	1.80	ND
<0.01	<0.01	0.01	<0.01	<0.01
>33	>33	>33	15.40	ND
ND	0.03	0.10	0.04	ND
ND	>33	>33	10.20	ND
>33	>33	>33	15.40	ND
0.02	0.01	0.03	0.01	0.04
1.13	1.80	8.60	0.65	25.20
1.90	0.15	2.80	0.44	5.00
4.40	1.90	17.40	0.80	ND
>3.3	0.59	>33	0.09	>33
ND	ND	ND	ND	ND
1.48	0.80	9.60	0.40	ND
>33	>33	>33	>33	ND
0.28	0.49	17.20	0.11	>33
ND	ND	ND	ND	ND
>10	5.60	>33	2.30	ND
ND	>33	>33	8.40	ND
ND	ND	ND	ND	ND
>33	>33	>33	12.90	>33
1.80	0.30	13.70	0.20	>33
0.11	0.04	1.40	0.05	11.10
>33	>33	>33	3.72	>33
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
4.08	0.06	>33	0.05	ND
0.03	0.01	0.05	0.01	0.07
0.47	0.24	4.20	0.13	19.20
>33	>33	>33	20.70	ND
0.07	0.01	0.35	0.02	2.70
3.09	1.27	3.88	0.66	>33
0.38	0.24	1.57	0.14	>33
>33	>33	>33	13.00	ND

Willis, JR. et al (Submitted)

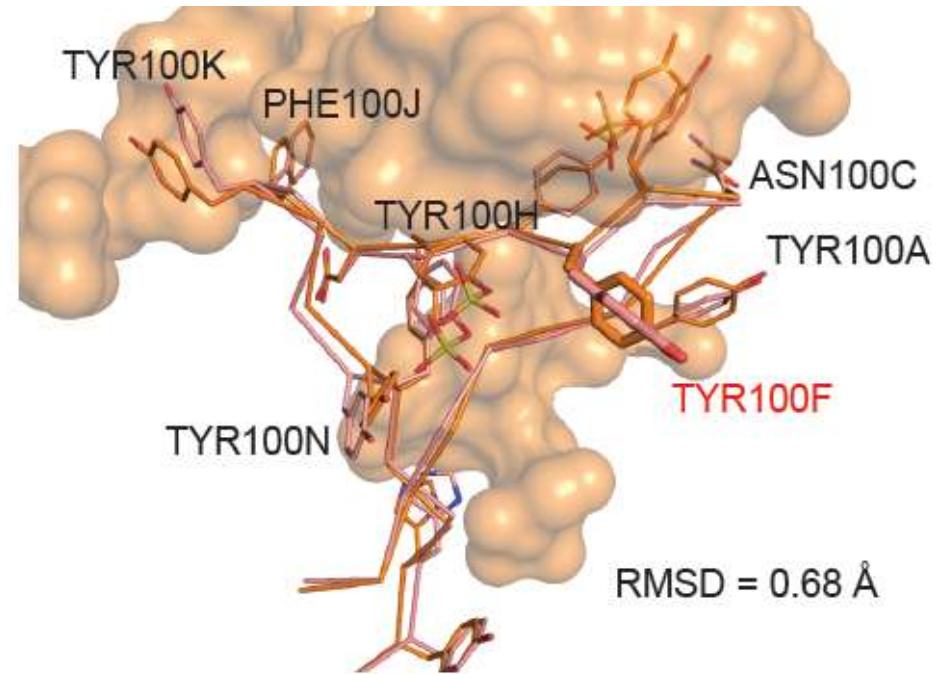
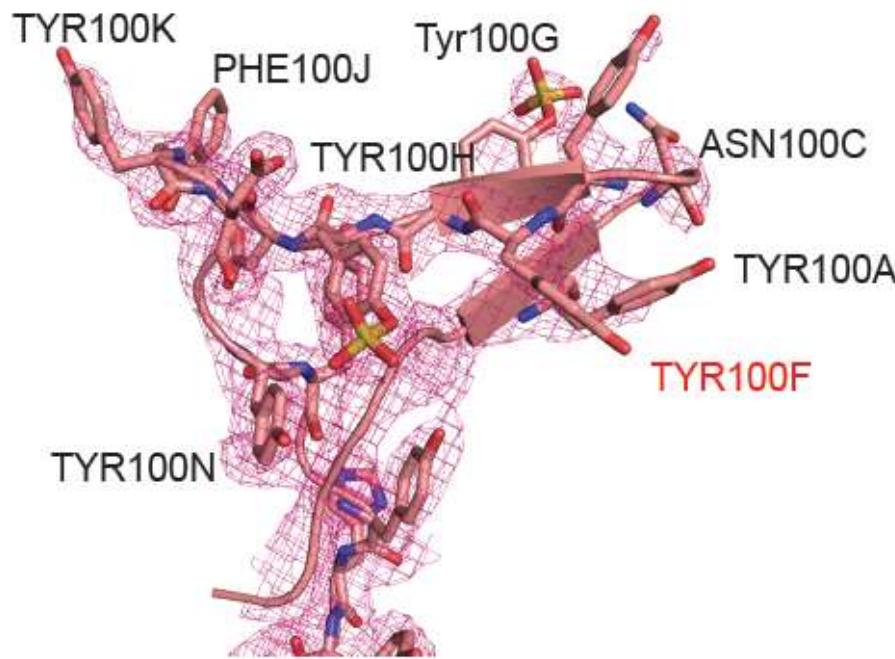
N109Y Mutant is Predicted to Sta-bilize HCDR3 in Active Conformation



Rosetta Design is Confirmed by PG9 N109Y Crystal Structure and DSC

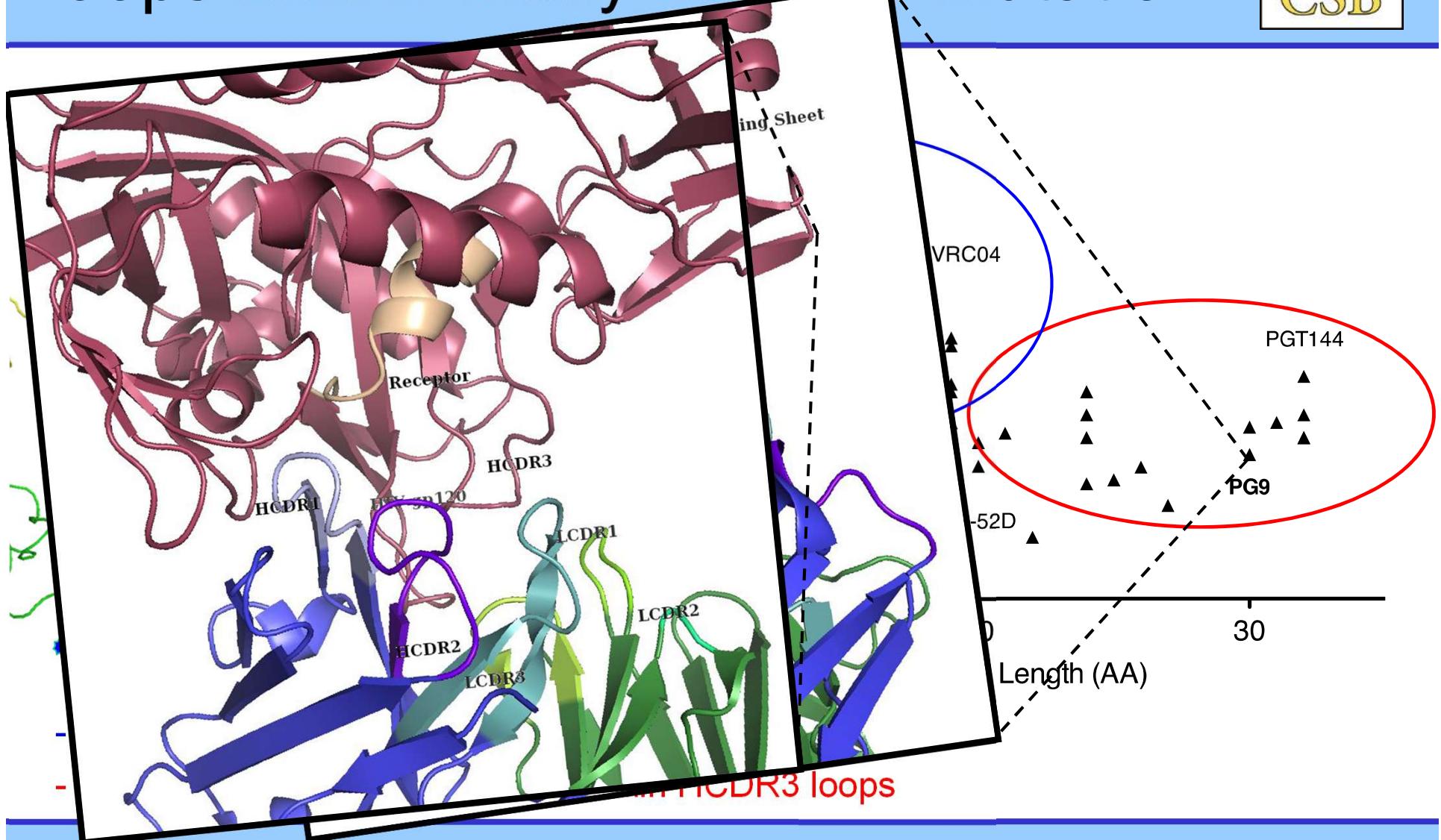


- Crystal Structure of PG9 N109Y apo
- Superimposition with Rosetta Model

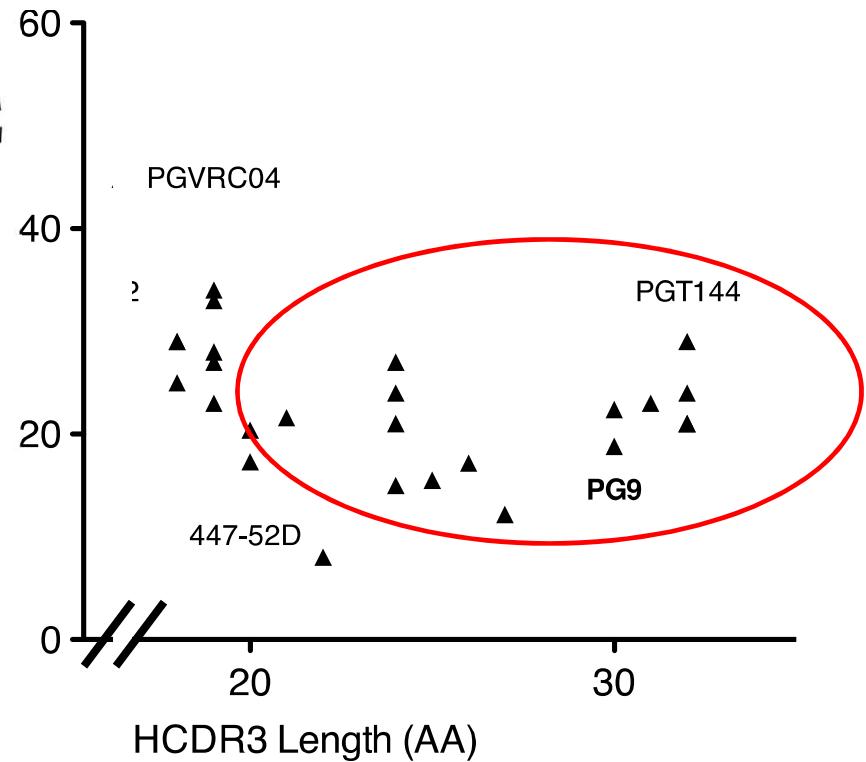
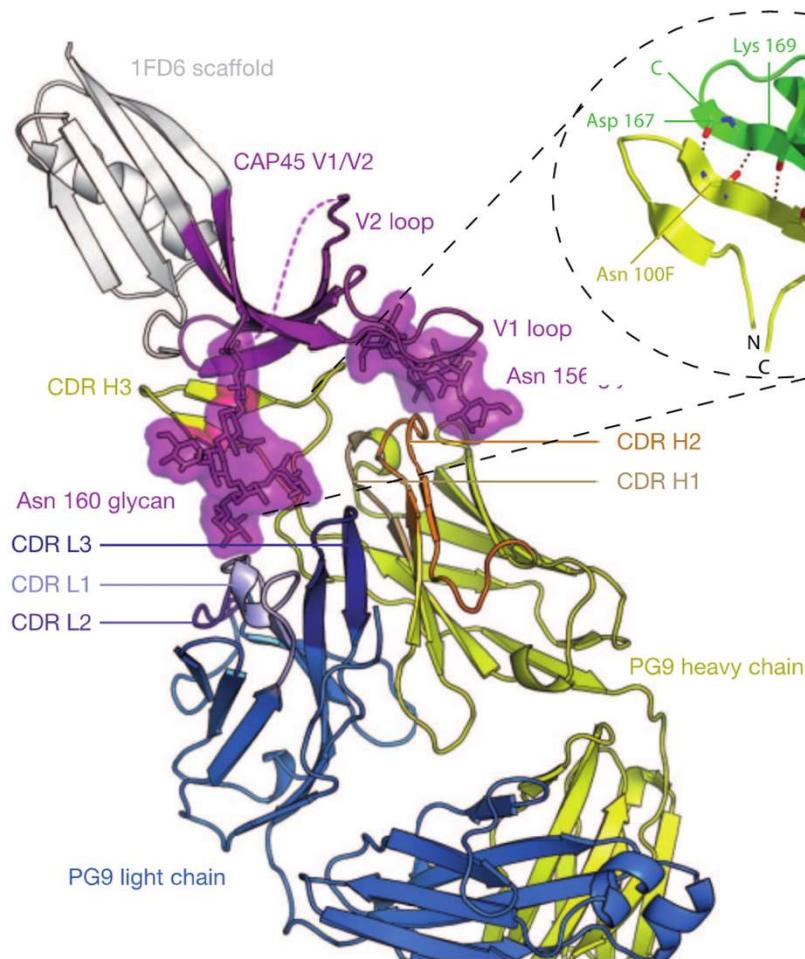


Willis, JR. et al (Submitted)

HIV Antibodies have Long HCDR3 loops and/or many Somatic Mutation



Elicit PG9-like Antibodies through an Epitope-Focused, Designed Vaccine?

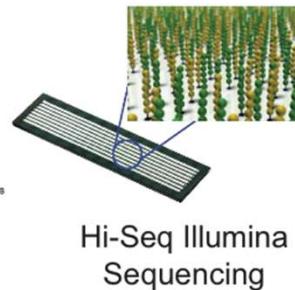
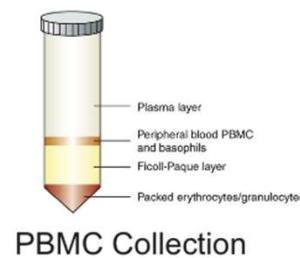


McClellan, Nature 2012

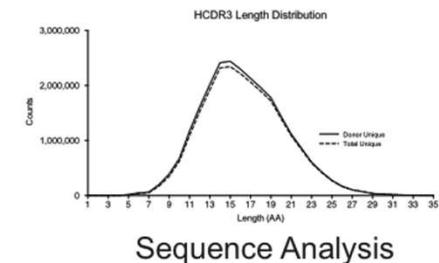
Do HIV-Naïve Humans have PG9-like Antibodies?



A) Sample Collection



B) Bioinformatic Analysis

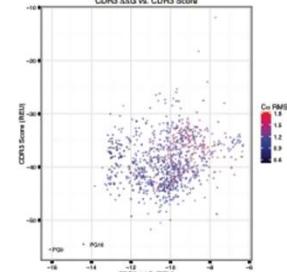
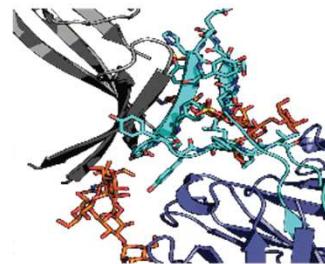
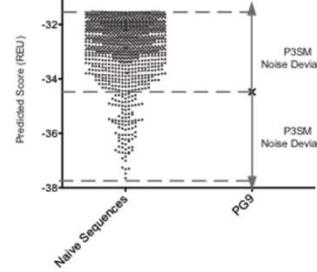


C) Threading and Design

Select random 30-length HCDR3

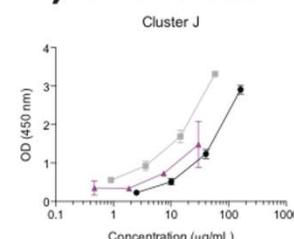
Thread Over PG9 HCDR3

Evaluate Energy of Complex



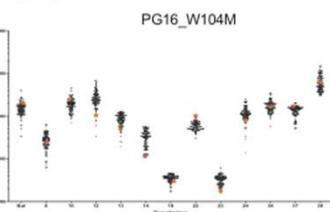
D) Characterization

Cluster J



Biophysical
Characterization

PG16_W104M



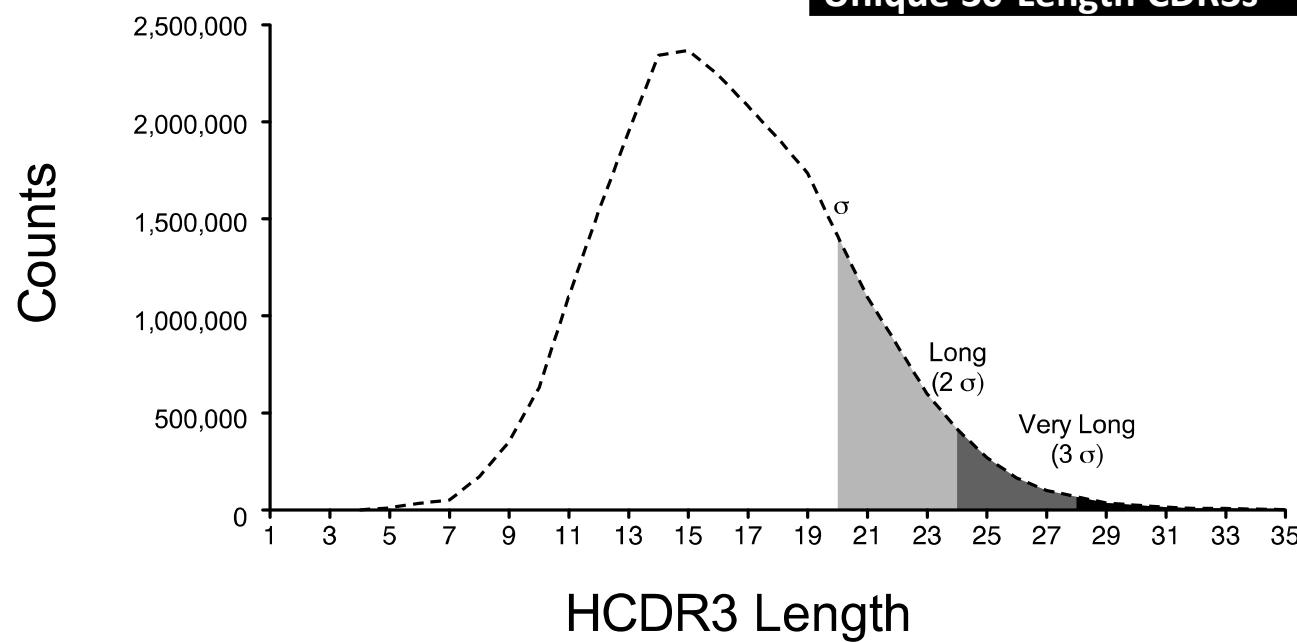
Neutralization
Profile

— Experimental
- - - Computational

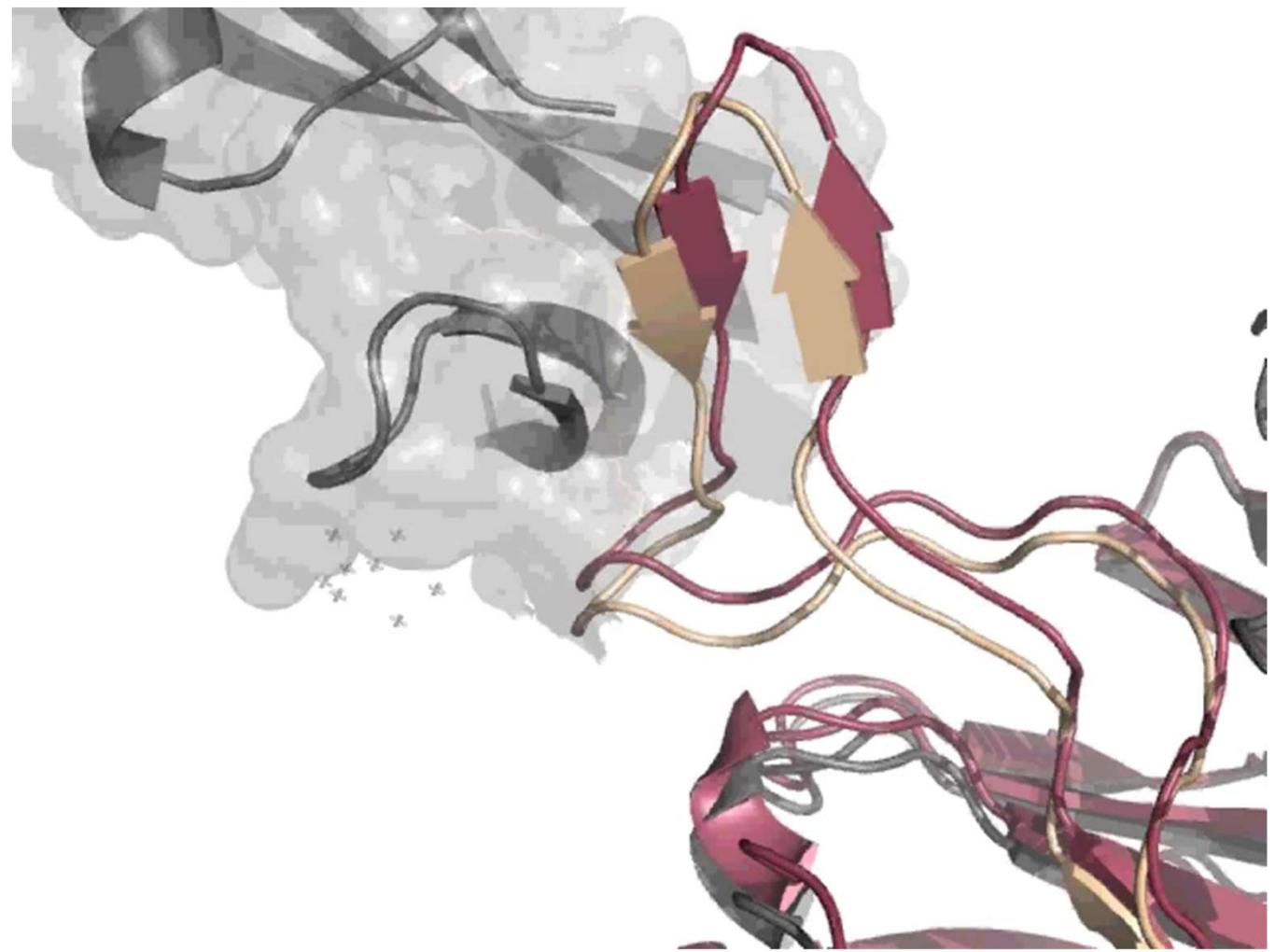
Very Long HCDR3 Sequences are Rare but Present in Naïve Donors



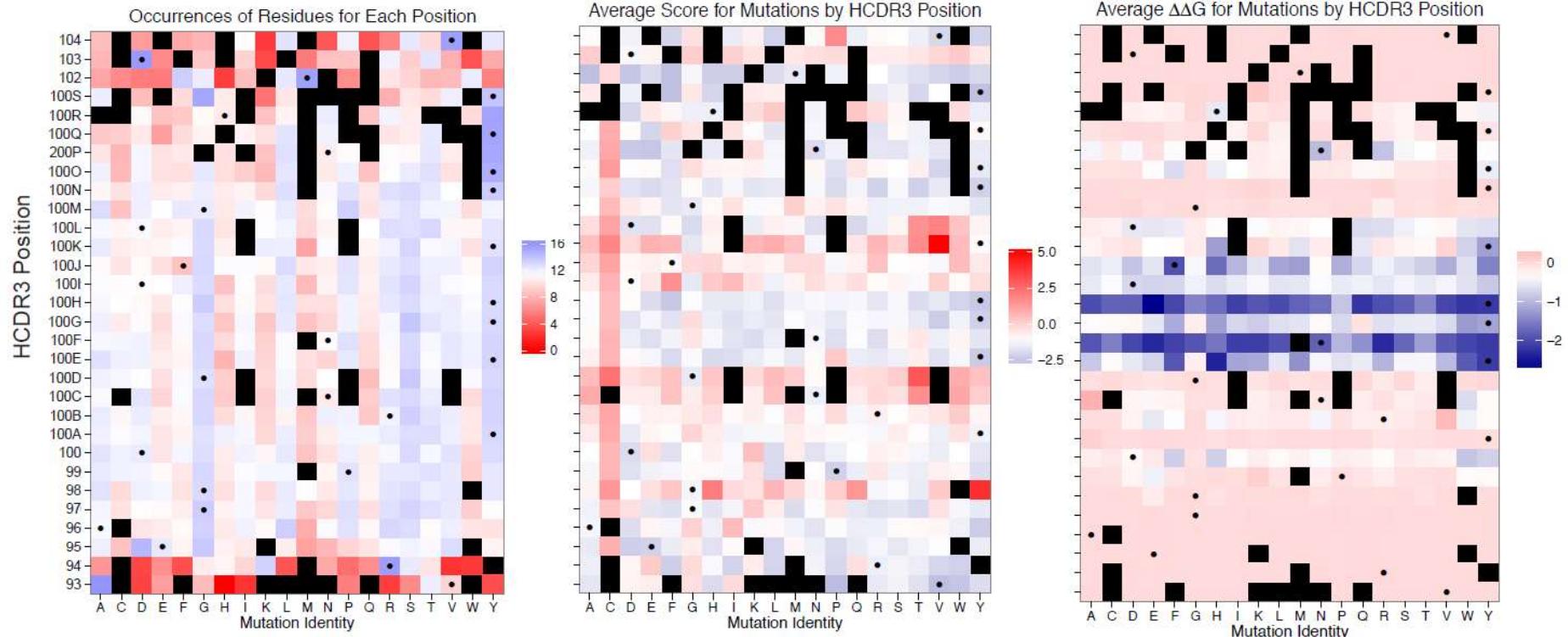
Raw Reads	514,312,664
Unique Reads (Nuc)	167,667,706
Productive CDR3s (no stop)	118,440,255
Unique CDR3s (AA)	23,357,390
30-Length CDR3s	74,457
Unique 30-Length CDR3s	24,917



“Thread” Long HCDR3 Sequences over PG9 co-crystal Structure

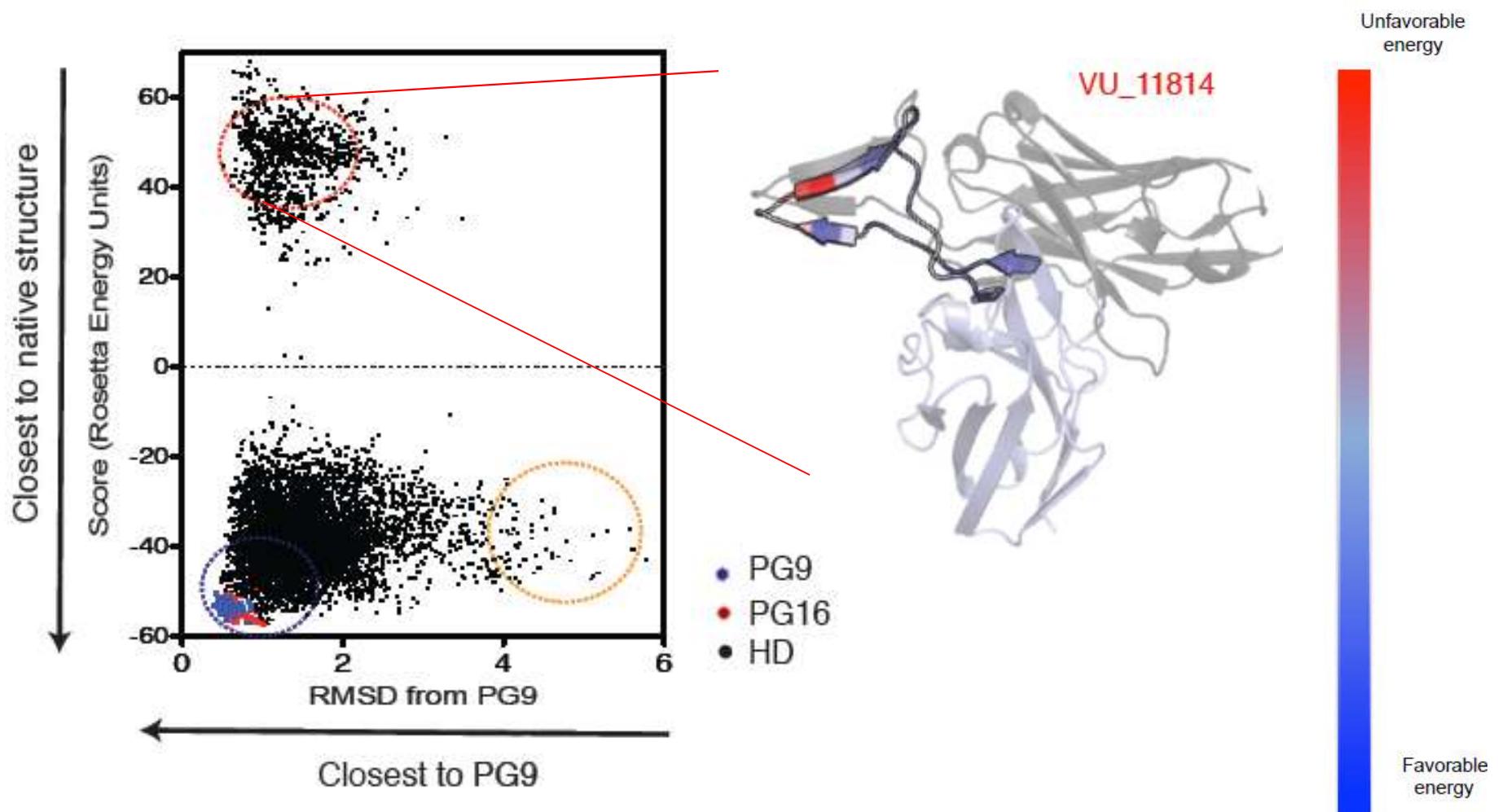


Rapid Screening of 25,000 HCDR3s using Rosetta-Inspired PSSMs

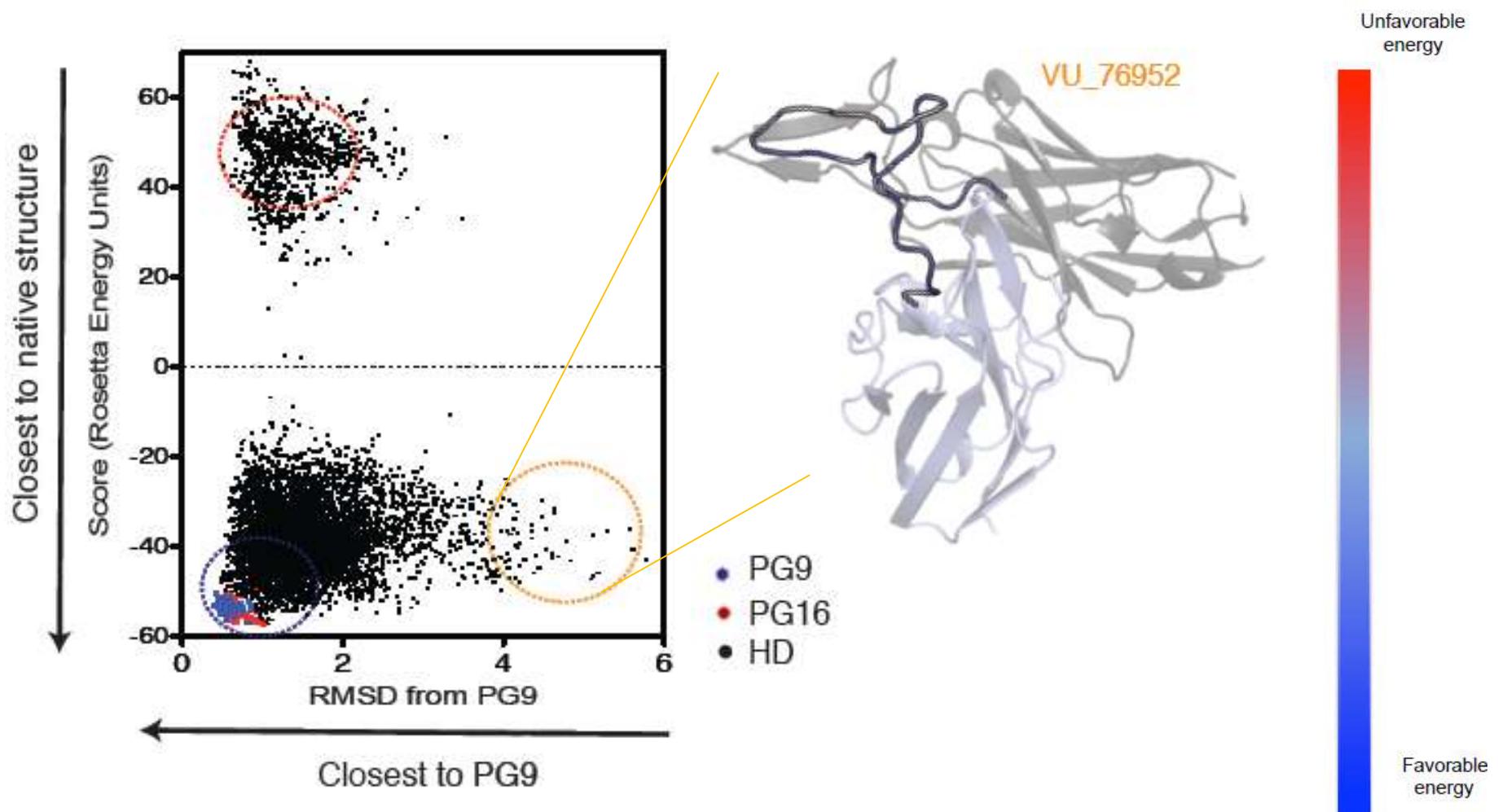


$$\text{Approximated Rosetta Score} = \sum_{i=1}^{30} stability_{i,j} + affinity_{i,j}$$

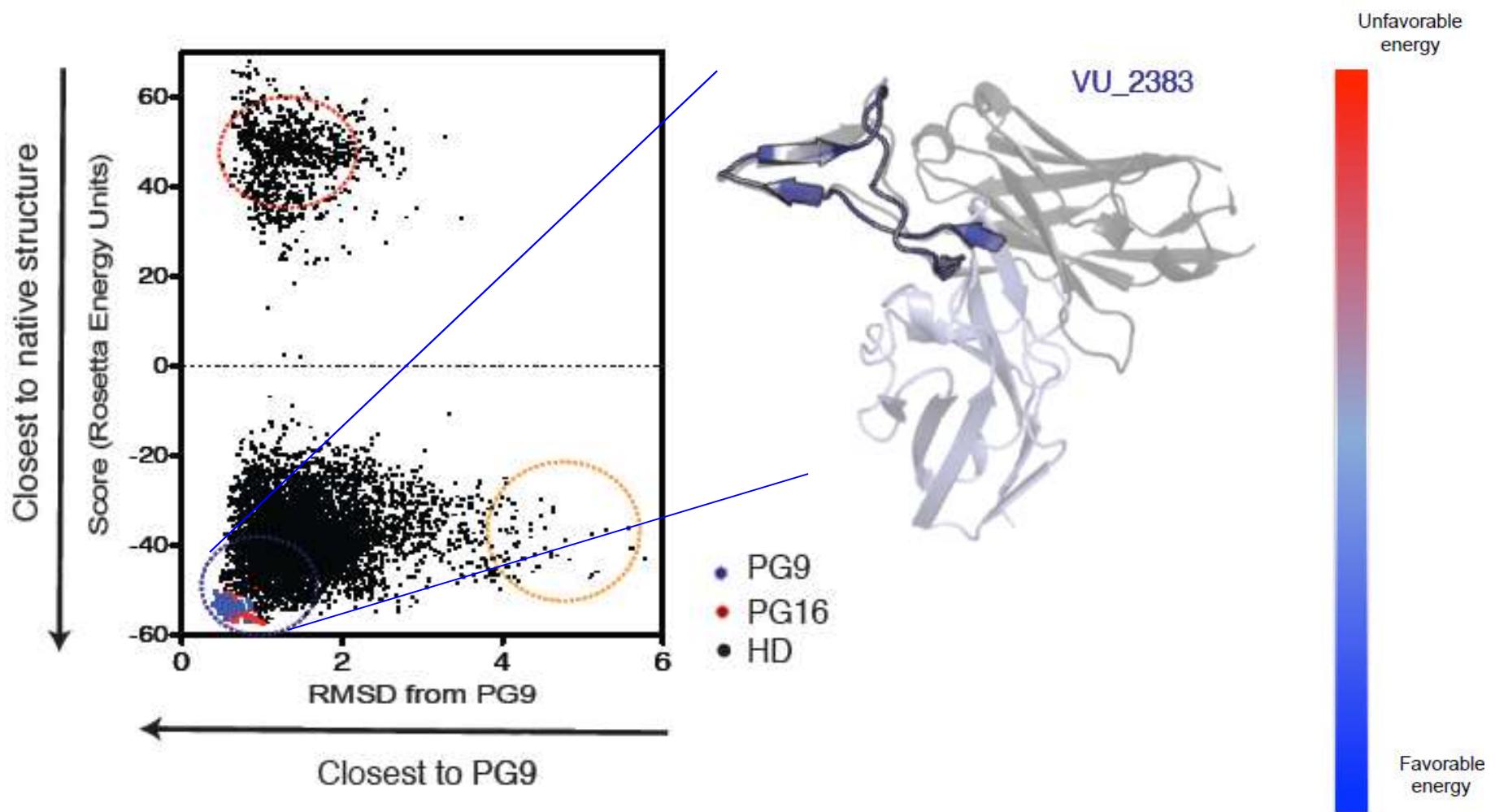
Threading of Top 1000 HCDR3s Discriminates Unlikely Matches



Threading of Top 1000 HCDR3s Discriminates Unlikely Matches



Threading of Top 1000 HCDR3s Discriminates Unlikely Matches



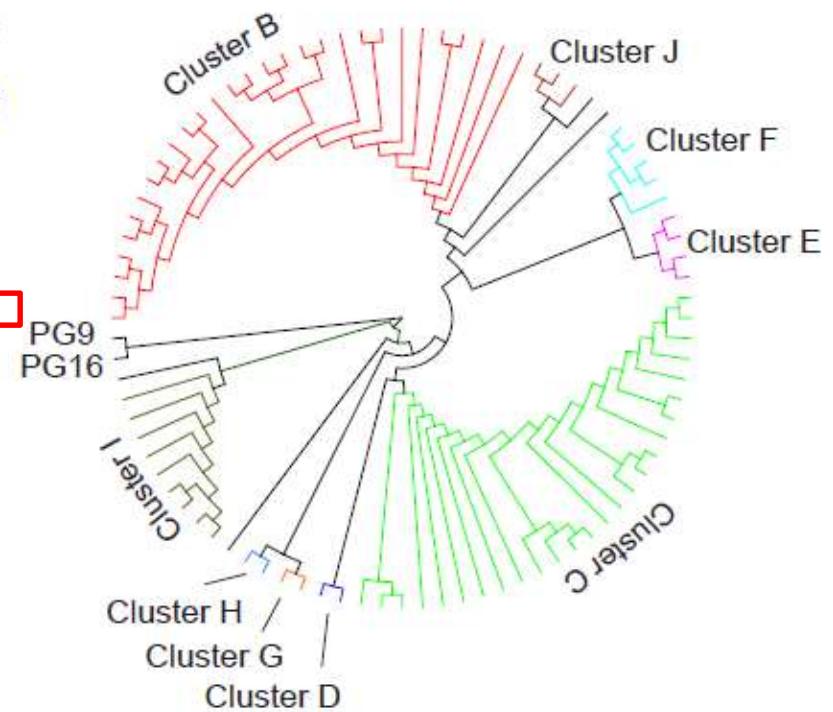
The Top 100 HCDR3 Sequences Cluster into 10 Groups



HCDR3 alignment



Phylogeny of Ab sequences

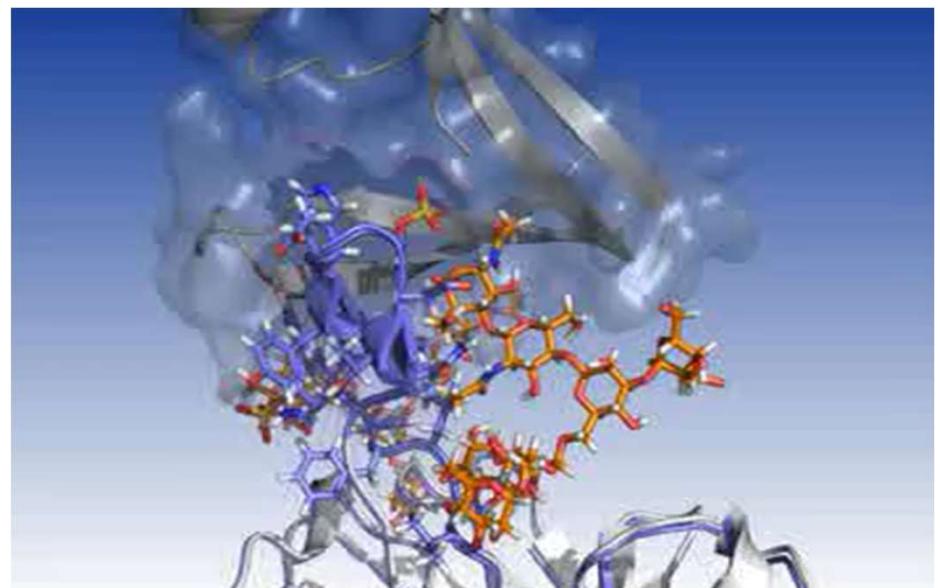
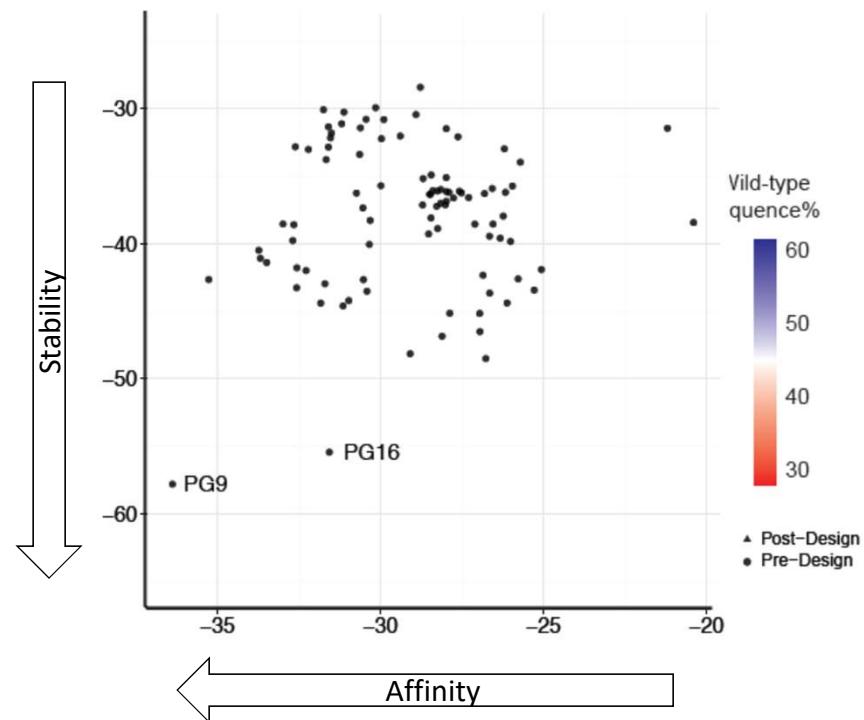


Willis, J. R., et al. 2016 *Proc Natl Acad Sci U S A*, 113(16), 4446-4451.

In silico Affinity Maturation of Candidate Antibody HCDR3s

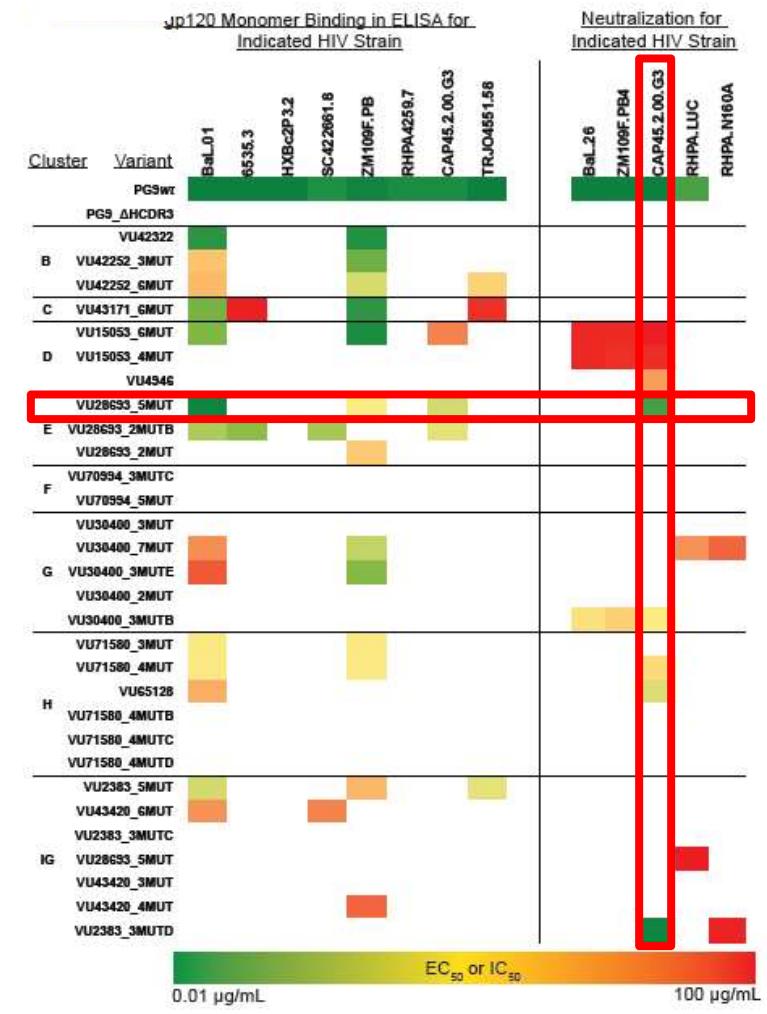
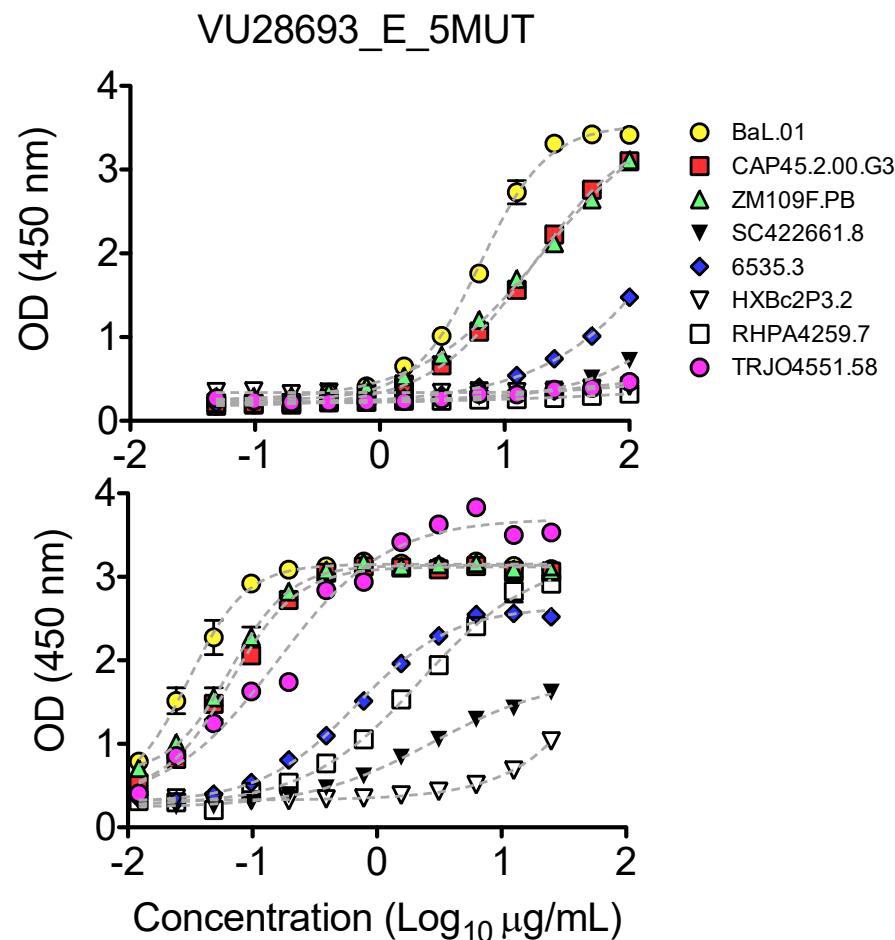


- Rosetta Design



J. R. Willis, J. A. Finn, B. S. J. Meiler, J. E. Crowe; "Long Antibody HCDR3s from HIV-Naïve Donors Presented on a PG9 Neutralizing Antibody Background Mediate HIV Neutralization"; pending

Multiple Sequences Enable Binding and Neutralization to HIV Strains

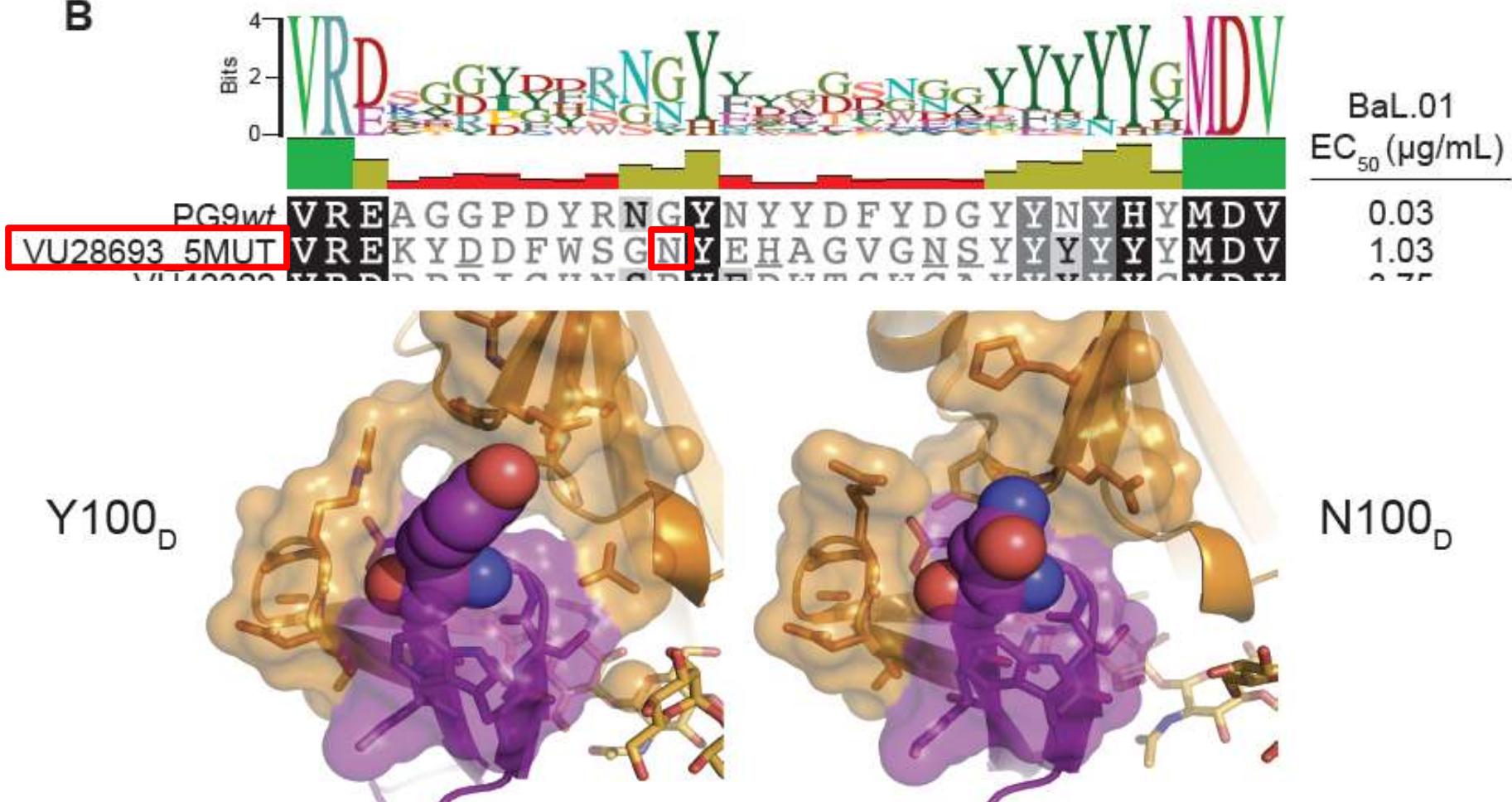


Willis, J. R., et al. 2016 Proc Natl Acad Sci U S A, 113(16), 4446-4451.

Conserved Sequence Positions Correlate with high Binding Affinity



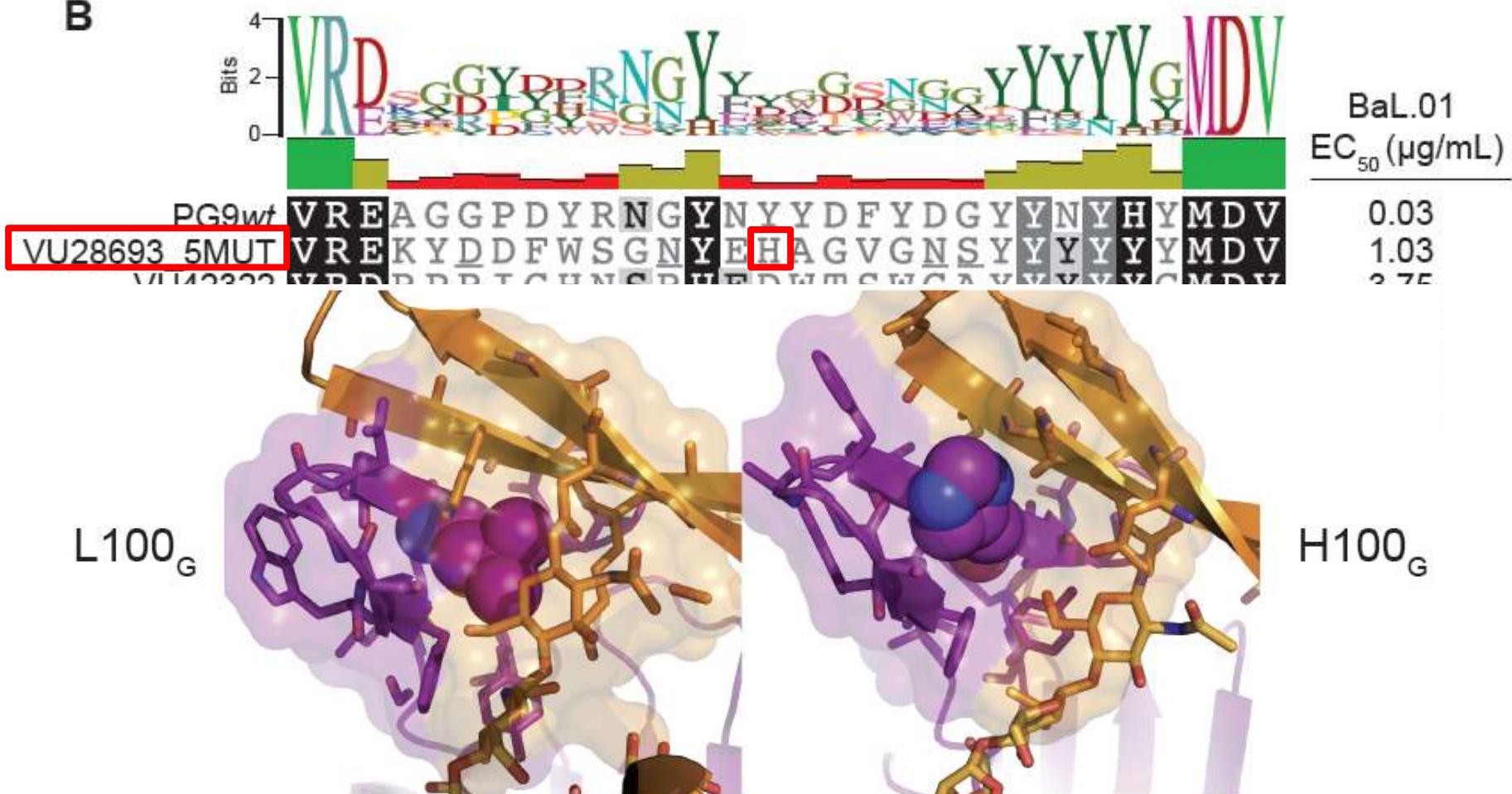
B



Conserved Sequence Positions Correlate with high Binding Affinity



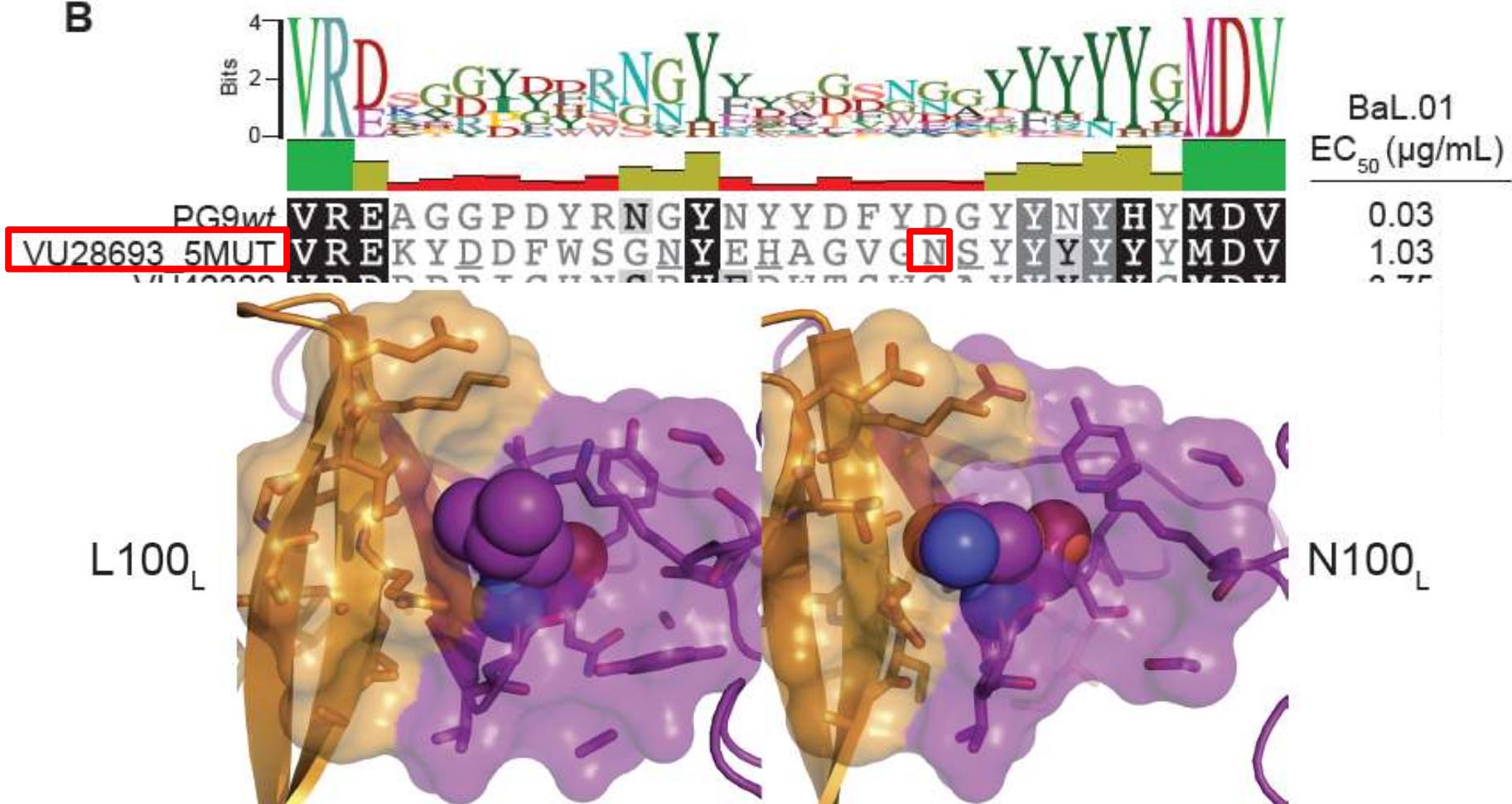
B



Conserved Sequence Positions Correlate with high Binding Affinity



B

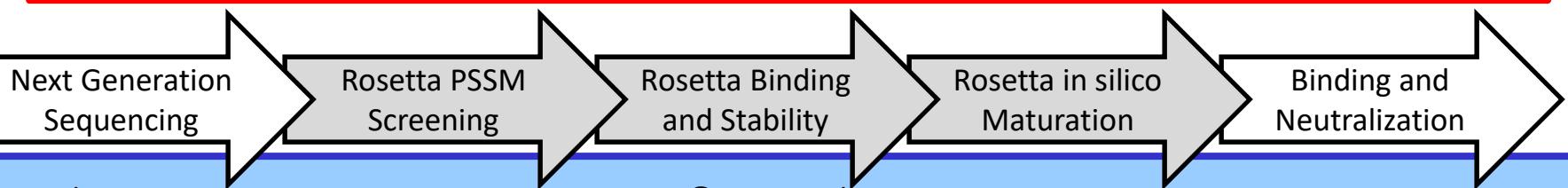


Summary of Neutralization Results after Three (!) Computational Steps



Table x. Neutralizing activity of antibodies with PG9-like HCDR3s derived from healthy HIV-naïve donors

Cluster	Clonal family	Clone	Mutations	Sequence	ID ₅₀ in TZM-bl Cells (μ g/mL) for indicated HIV strain		
					BaL.26 (Tier: 1, Clade: B)	ZM109F.PB4 (Tier: 2, Clade: C)	CAP45.2.00.G3 (Tier: 2, Clade: C)
G	VU29	VU29	3MUT B	VU:30400_E112D_L115E_G118Y	24.1	29.4	21.1
D	VU93	VU93	WT		>91.4	>91.4	46.1
		VU9	4MUT	VU:15053:154715_S106N_E116F_G111S-R115D	89	85.4	86.1
		VU12	6MUT	VU:15053:154715_S106N_E116F_G122H_W105R_G111S-R115D	89.6	87.5	98.3
H	VU60	VU60	WT		>93.2	>93.2	17.7
		VU68	4MUT	VU:71580_Y101G_S105R_S106N_G112D	>91.75	>91.75	26.2
E	V1	V1	WT		ND	ND	ND
		V21	3MUT	VU:2383:160514_S106N_R110I_G111Y	>40	>40	0.7
		V24	5MUT	VU:28693_Y101D_Y107N_L110H_L115N_N116S	>100	>100	5.5

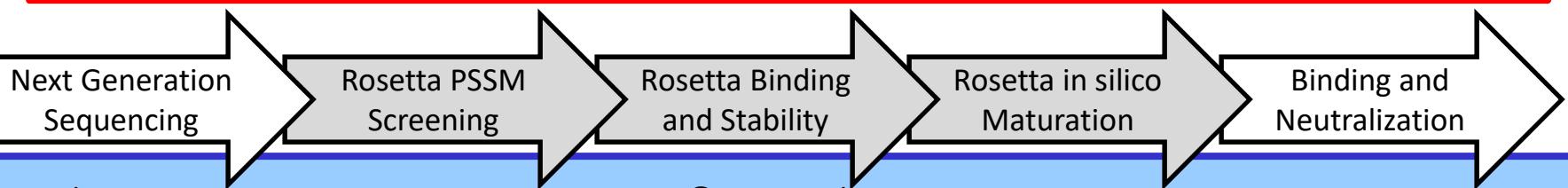


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G	VU29	VU29	3MUT B	VU:30400_E112D_L115E_G118Y	24.1	29.4	21.1
D	VU93	VU93	WT		>91.4	>91.4	46.1
		VU9	4MUT	VU:15053:154715_S106N_E116F_G111S-R115D	89	85.4	86.1
		VU12	6MUT	VU:15053:154715_S106N_E116F_G122H_W105R_G111S-R115D	89.6	87.5	98.3
H	VU60	VU60	WT		>93.2	>93.2	17.7
		VU68	4MUT	VU:71580_Y101G_S105R_S106N_G112D	>91.75	>91.75	26.2
E	V1	V1	WT		ND	ND	ND
		V21	3MUT	VU:2383:160514_S106N_R110I_G111Y	>40	>40	0.7
		V24	5MUT	VU:28693_Y101D_Y107N_L110H_L115N_N116S	>100	>100	5.5



Acknowledgements – Positions and Software @ www.meilerlab.org



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Harikrishna Jayanthan	Yunchao (Lance) Liu
Kaitlyn V. Ledwitch	Naveen Gokanapudi
Nina Bozhanova	Jonathan Sheehan
Rocco Moretti	
Heather Darling	
Jeff Mendenhall	
Benjamin Brown	
Brennica Marlow	
Diego del Alamo	
Elleansar Okwei	
Hope Woods	
Marion Sauer	
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Active: NIH NIGMS R01GM080403, R01GM073151, NIH NIDDK R01DK097376, NIH NIAID U19 AI117905, NIAID HHSN272201400024C, NIH NILBI R01HL122010, NSF CISE 1629811

Completed: NSF MCB0742762 (CAREER), NIH NIGMS R01GM099842, NIH NIMH R21MH082254, R01MH090192, DTRA HDTRA1-10-0067, NSF CHE 1305874

