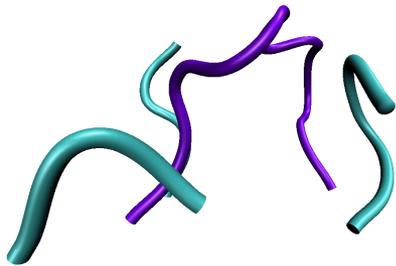


RosettaAntibodyDesign (*RAbD*)



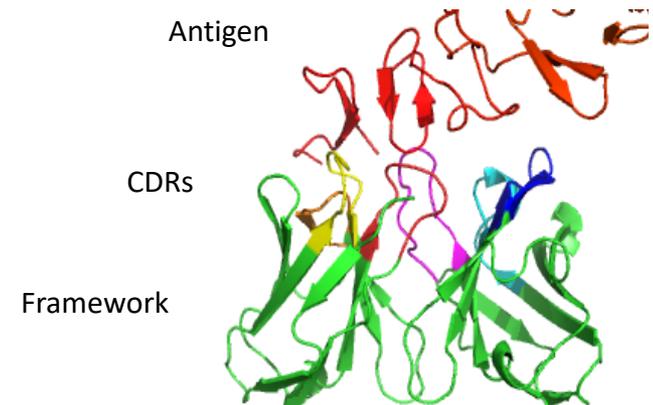
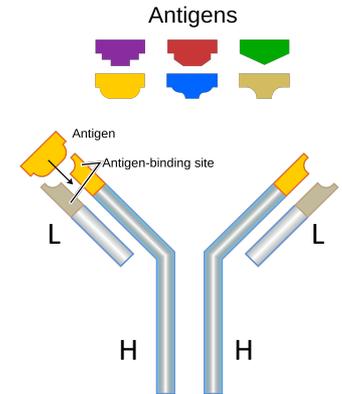
Jared Adolf-Bryfogle, PhD

PhD Advisor: Dr. Roland Dunbrack

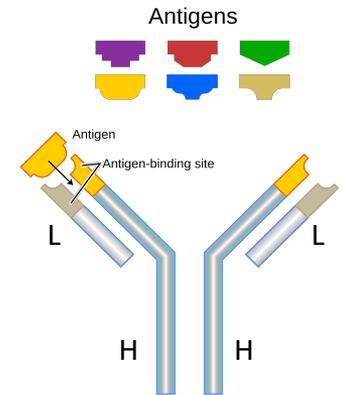
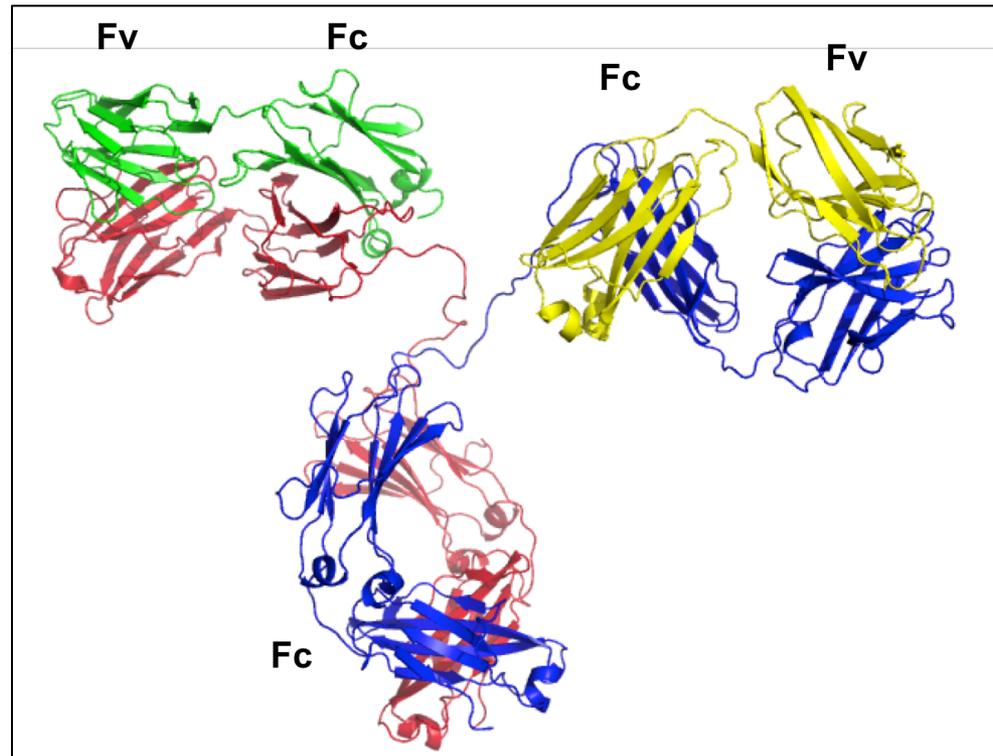
Postdoctoral Advisor: Dr. William Schief

Antibody Structure

- *Two protein chains*
 - **Light (L)** and **Heavy (H)**
 - 1 Gene locus codes for Heavy chain
 - 2 Gene loci code for Light chain (Lambda/Kappa)
- *6 Complementarity Determining Regions (CDRs)*
 - Highly variable in length, structure, and sequence
- *Antibody Framework*
 - Highly conserved for each gene locus



Antibody Structure – Full IgG



Fv - Variable

Fc - Constant

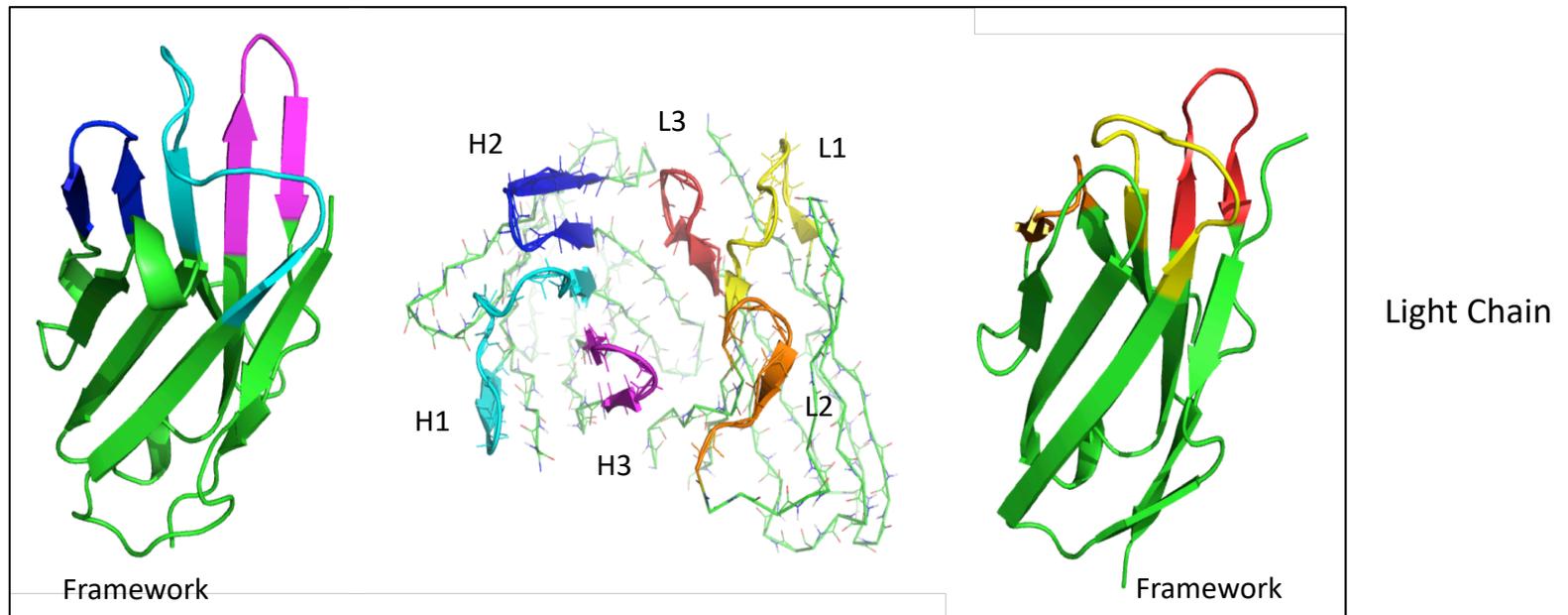
X-ray crystal structure of an antibody, full-length IgG (PDB ID 1IGT).

Light Chain – Green/Yellow

Heavy Chain – Red/Blue

Antibody Structure

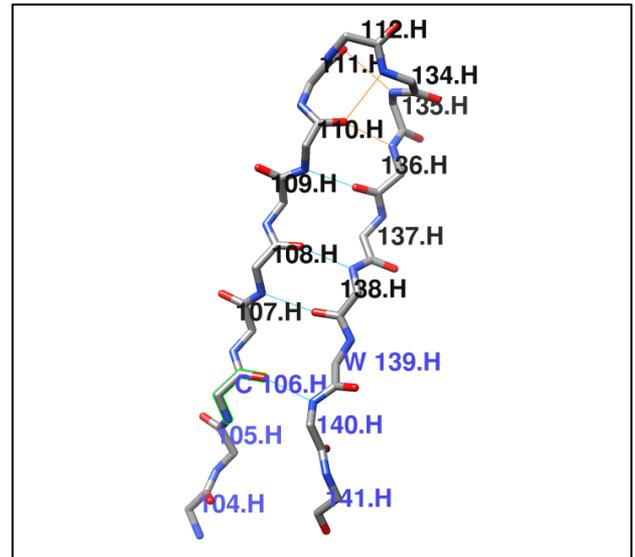
Fv Fragment and CDRs



X-ray crystal structure of an antibody, Fv (PDB ID 2J88).

PylgClassify: Antibody Identification

- Identifies from Sequence:
 - Antibody chains
 - Antibody gene
 - Germline
 - CDR/Framework positions



- Renumbers PDB Structures
 - ***AHo Numbering Scheme***
 - North/Dunbrack CDR Definitions

doi:10.1006/jmbi.2001.4662 available online at <http://www.idealibrary.com> on **IBL**® J. Mol. Biol. (2001) 309, 657–670

JMB



Yet Another Numbering Scheme for Immunoglobulin Variable Domains: An Automatic Modeling and Analysis Tool

Annemarie Honegger* and Andreas Plückthun

PyIgClassify: Server Frontend

PyIgClassify (August 2014) is a weekly-updated web server primarily providing the clusters and associated information of the antibody complementary determining regions (CDRs) in the Protein Data Bank (PDB).

Antibody CDR conformations have been clustered previously by Chothia, Thornton, and others, with the most recent comprehensive clustering appearing in 1997[1][2]. Since then, there has been an explosion of structures in the PDB. Using a dihedral angle metric and an affinity propagation technique, we have clustered the conformations of each CDR and unique loop length of antibody structures in the PDB, at this time, a total of 115 unique clusters [3]. Please browse the **Statistics** page for more information pertaining to each of these clusters. We believe these clusters and their associated structure and sequence data will lead to field-wide improvements in antibody structure prediction and design.

References

1. Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. *J. Mol. Biol.* 1987; 196:901-17.
2. Al-Lazikani B, Lesk AM, Chothia C. Standard conformations for the canonical structures of immunoglobulins. *J. Mol. Biol.* 1997;273:927-48.
3. North, B, Lehmann A, Dunbrack R. A new clustering of antibody CDR loop conformations: *J. Mol. Biol.* (2011), 406(2): 228-256. [pdf](#)

Site developed by the Antibody Team at the lab of Roland Dunbrack. Last updated on August 8, 2014

Contact Us | ProtCID | ProtBuD | Dunbrack Laboratory | Fox Chase Cancer Center

- Qifang Xu
- Updates Bi-Yearly
 - Includes all Antibodies of the PDB
- Submit
- Explore
- Download

CDR Clusters for L1-I1

#Structures = 656; #Chains = 1033; #Clusters = 6

Cluster	Seq	Residues	Gene	CDR Sequence	Frame	Pos (seq)	Pos (auth)	Sequence	Residues	Clusters
11-11-1	IGK1G1	1-2	keappa	Ms_108V1_13*01	Ms_108V1_13*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	7
11-11-1	IGK1G1	1-25	keappa	Ms_108V1_13*01	Ms_108V1_13*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	4
11-11-1	IGK1G1	1-28	keappa	Ms_108V12_14*01	Ms_108V12_14*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	4
11-11-1	IGK1G1	1-3	keappa	Ms_108V1_13*01	Ms_108V1_13*01	26	24	MSAGSITDNDLQ	10AAAGDQDQDFA	4
11-11-1	IGK1G1	1-3	keappa	Ms_108V1_13*01	Ms_108V1_13*01	26	24	MSAGSITDNDLQ	10AAAGDQDQDFA	4
11-11-1	IGK1G1	1-3	keappa	Ms_108V10_16*01	Ms_108V10_16*01	27	24	MSAGSITDNDLQ	10AAAGDQDQDFA	5
11-11-1	IGK1G1	1-4	keappa	Ms_108V10_16*01	Ms_108V10_16*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	5
11-11-1	IGK1G1	1-4	keappa	Ms_108V10_16*01	Ms_108V10_16*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	5
11-11-1	IGK1G1	1-49	keappa	Ms_108V9_12*01	Ms_108V9_12*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	3
11-11-1	IGK1G1	1-49	keappa	Ms_108V12_14*01	Ms_108V12_14*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	3
11-11-1	IGK1G1	1-5	keappa	Ms_108V12_14*01	Ms_108V12_14*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	4
11-11-1	IGK1G1	1-5	Lambda	Ms_108V3_10*01	Ms_108V3_10*01	24	24	MSAGSITDNDLQ	10AAAGDQDQDFA	39
11-11-1	IGK1G1	1-53	keappa	Ms_108V1_13*01	Ms_108V1_13*01	26	24	MSAGSITDNDLQ	10AAAGDQDQDFA	8
11-11-1	IGK1G1	1-53	keappa	Ms_108V1_13*01	Ms_108V1_13*01	26	24	MSAGSITDNDLQ	10AAAGDQDQDFA	8
11-11-1	IGK1G1	1-53	keappa	Ms_108V1_13*01	Ms_108V1_13*01	26	24	MSAGSITDNDLQ	10AAAGDQDQDFA	7

Nucleic Acids Research Advance Access published November 11, 2014

Nucleic Acids Research, 2014, 42, I
doi: 10.1093/nar/gku1106

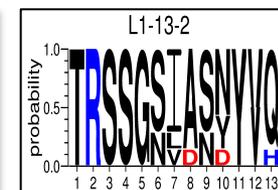
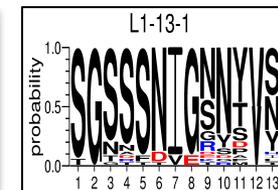
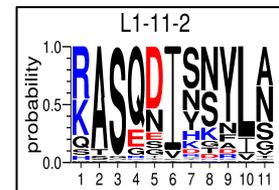
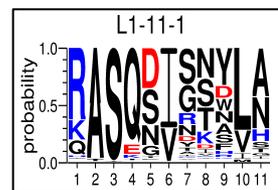
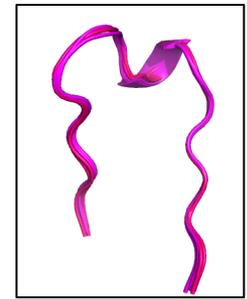
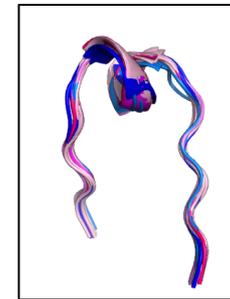
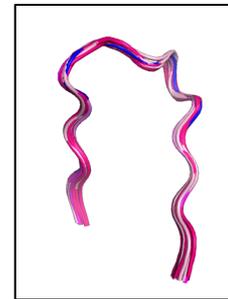
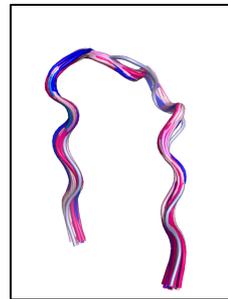
PyIgClassify: a database of antibody CDR structural classifications

Jared Adolf-Bryfogle^{1,2}, Qifang Xu¹, Benjamin North¹, Andreas Lehmann¹ and Roland L. Dunbrack, Jr^{1,*}

<http://dunbrack2.fccc.edu/PyIgClassify/>

CDR Clustering

- **Cluster:** *Group of similar structures*
- Bioinformatic Analysis
 - High quality data set
 - 72 Non-H3 clusters
 - Consensus
 - Sequence
 - Conformation
- Knowledge-Based Antibody Design

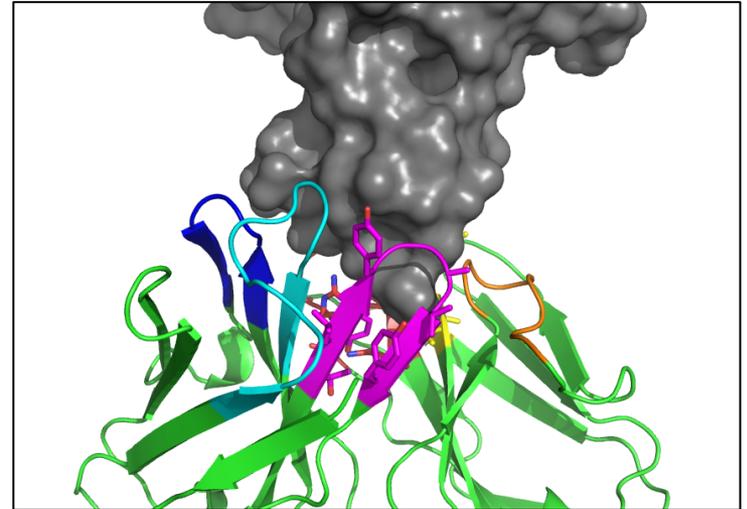


RosettaAntibodyDesign (RAbD)

- Knowledge-Based Antibody Design
- ***Application*** - Application can be used for many antibody design projects
 - ***Affinity Maturation***
 - ***Homologous redesign***
 - ***Stability improvement***
 - ***De Novo design***
- ***Framework*** - set of components we can use in PyRosetta or RosettaScripts

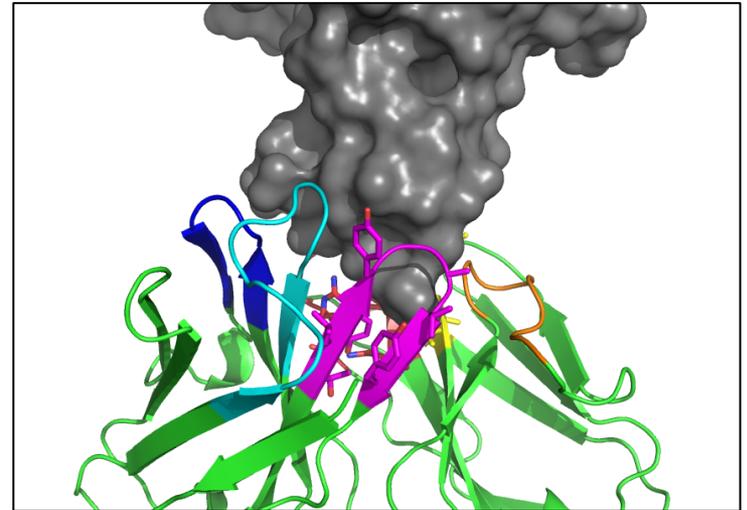
Antibody Designer: Overview

- *Monte Carlo Algorithm.*
- Whole CDR Sampling (GraftDesign)
 - *Sample structures from database*
 - *Insert and optimize peptide bond without CDR disruption*
 - *New Graft Algorithm*
 - *100% Closure*
 - *Roughly mimics Somatic Recombination*
- Sequence Sampling (SeqDesign)
 - *Sample amino acid types*
 - *According to CDR Cluster Profiles*
 - *Roughly mimics Somatic Hypermutation*



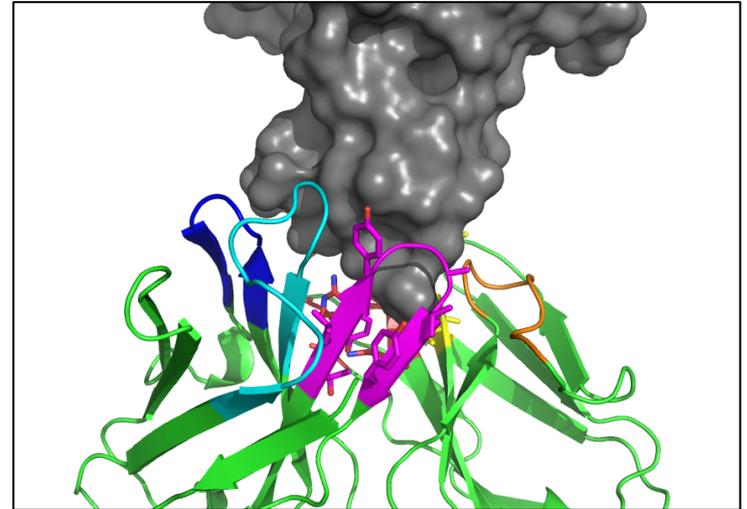
Antibody Designer: Overview

- Structure Optimization
 - Change/Sample DOF to minimize total energy of system
 - Integrated binding energy optimization instead of total energy
- Constraints
 - Dihedral constraints
 - Circular Harmonic
 - CDR Cluster based
 - Epitope/Paratope constraints
 - Site Constraints
- Lambda/Kappa specific design



Antibody Designer: Optional CDR File Overview

- Allows Tailored Design
 - Strategy-based Design
 - Redesigns, *de novo* design, etc.
- Simple Syntax
 - CDR-Level Control



Antibody Designer: CDR Instruction File Example

First Column: *CDR or ALL*

Second Column: *Option Type*

```
ALL GraftDesign ALLOW
```

```
ALL SeqDesign ALLOW
```

```
ALL CDRSet EXCLUDE PDBIDs 1N8Z 3BEI
```

```
L1 CDRSet EXCLUDE Clusters L1-11-1
```

```
L2 CDRSet INCLUDEONLY CLUSTER L2-8-1
```

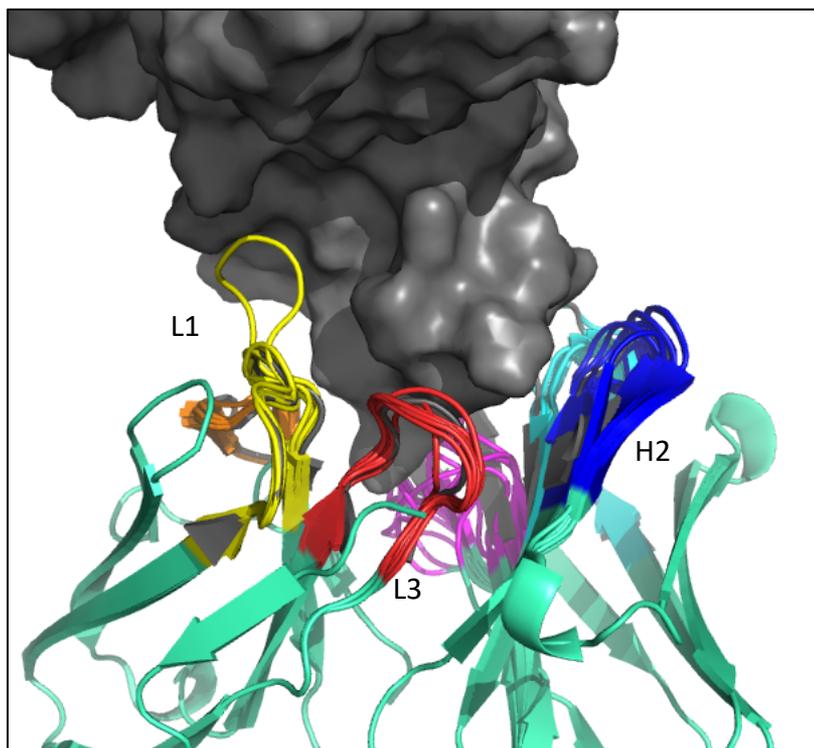
```
L3 CDRSet STAY_NATIVE_CLUSTER
```

Capitalization Ignored

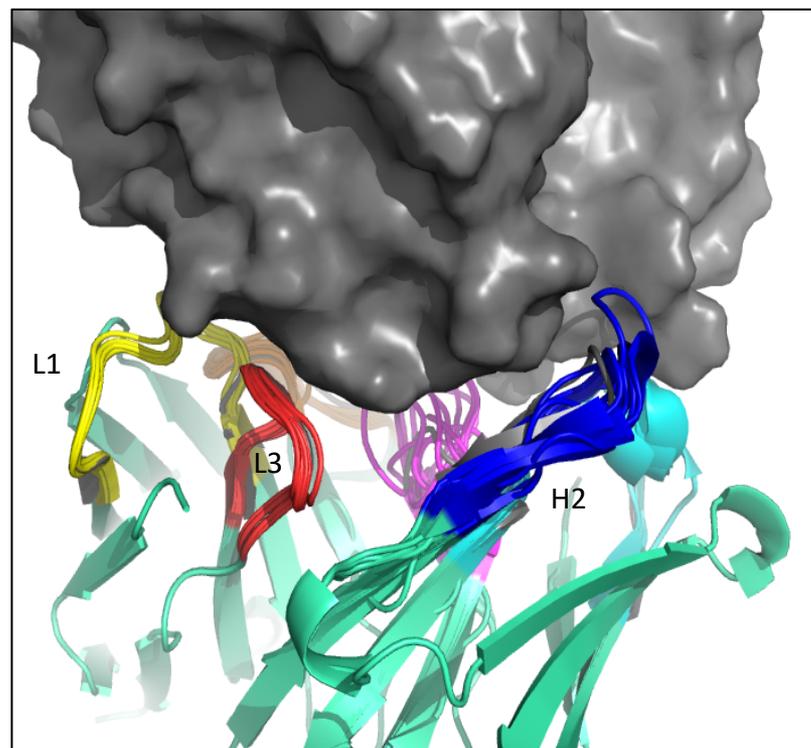
Example Command-Line

```
antibody_designer.default.linuxclangrelease -s  
my_ab.pdb -nstruct 500 -seq_design_cdrs L1 L2  
L3 -light_chain lambda -optimize_dG
```

Top Decoy Examples

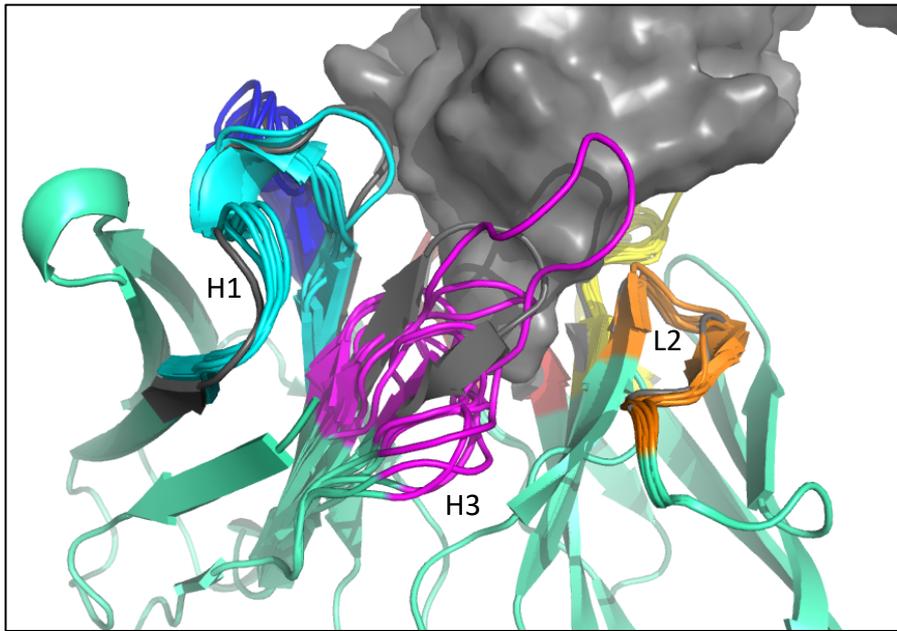


2j88

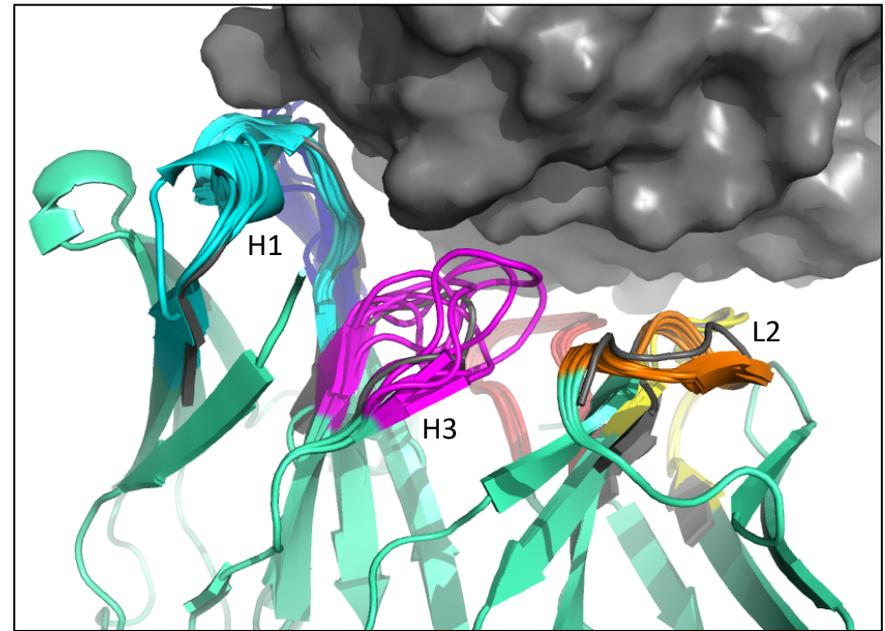


2dqc

Top Decoy H3 Variability

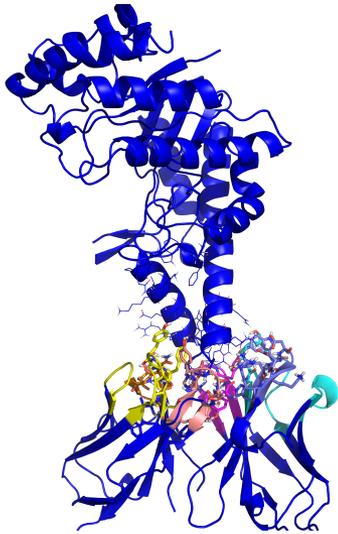


2j88



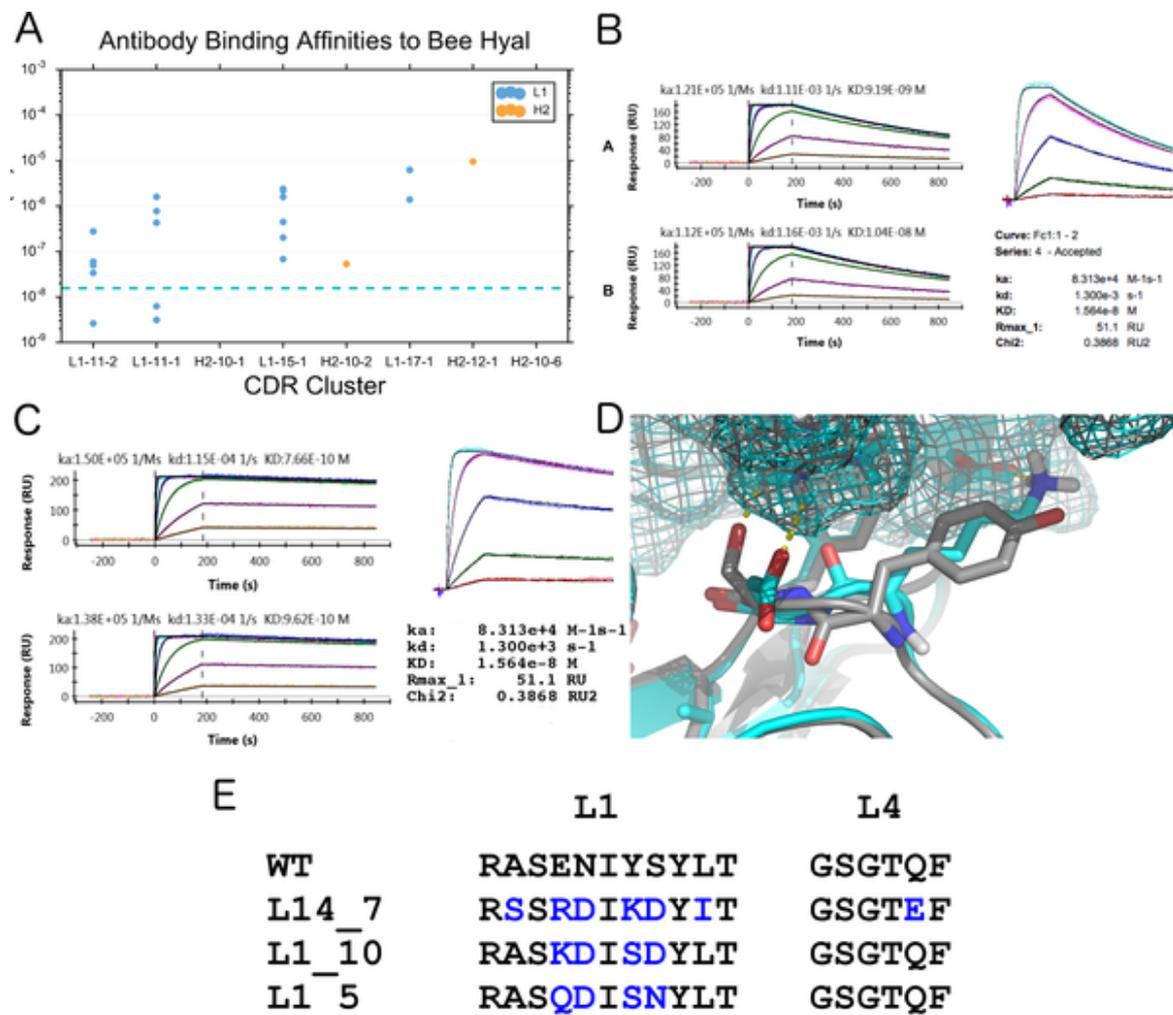
2dqg

2J88 Antibody Improvement



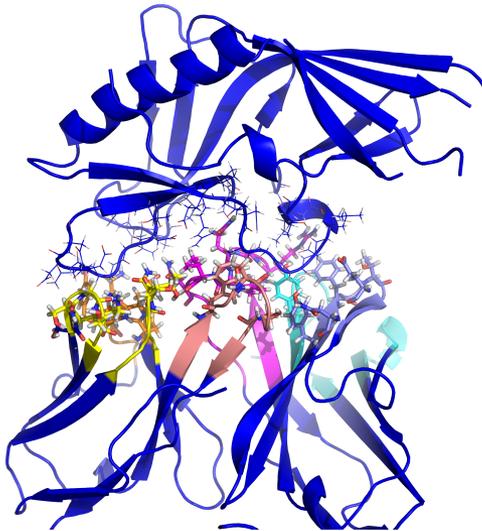
- Antibody binds to Bee Hyaluronidase
 - Major allergen of Bee Venom
- Binds at 15.6 nM
 - Top design binds 2.5 nM
- Designed L1/L4 (DE framework)
- Designed H2
- Dock/No Dock, only CDRs with profiles

Fig 8. Designed antibodies against bee hyaluronidase.



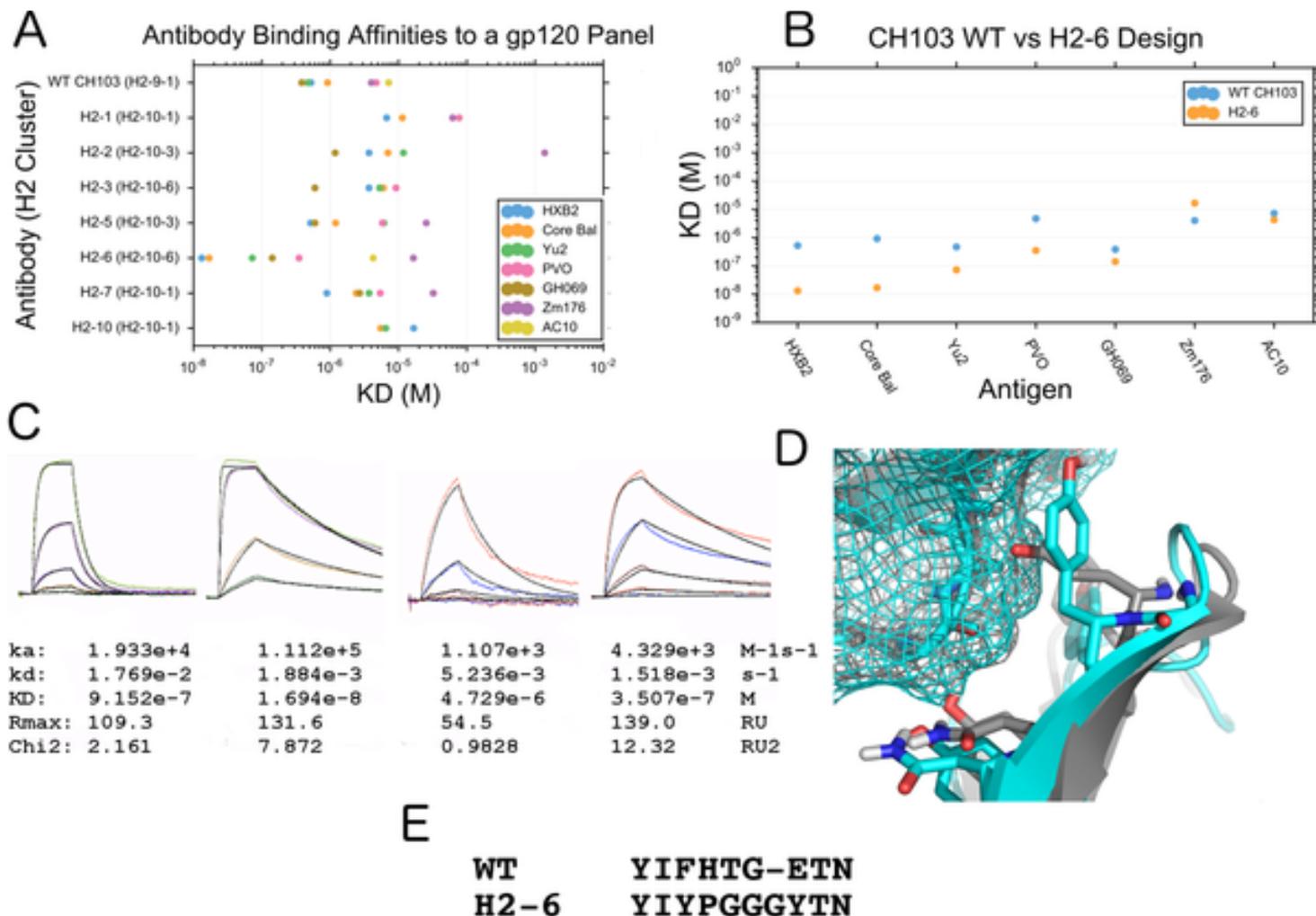
Adolf-Bryfogle J, Kalyuzhnyi O, Kubitz M, Weitzner BD, Hu X, et al. (2018) RosettaAntibodyDesign (RABD): A general framework for computational antibody design. PLOS Computational Biology 14(4): e1006112. <https://doi.org/10.1371/journal.pcbi.1006112>
<https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1006112>

CH103 Antibody Improvement



- Antibody binds to CD4 GP120 binding site
- ZM176 Crystal 4JAN
- CH103 binds 4JAN at **2.30×10^{-6} M**
- Designed L1/L3
- Designed H2

Fig 9. Binding of designed antibodies to HIV gp120.



Adolf-Bryfogle J, Kalyuzhnyi O, Kubitz M, Weitzner BD, Hu X, et al. (2018) RosettaAntibodyDesign (RABD): A general framework for computational antibody design. PLOS Computational Biology 14(4): e1006112. <https://doi.org/10.1371/journal.pcbi.1006112>
<https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1006112>

Tutorial

- 1) ***Affinity improvement/Library Design*** using Sequence Design only
- 2) ***Redesign*** an antibody using **GraftDesign** and **SequenceDesign**
- 3) ***De Novo design*** using All-CDR Sequence Design and GraftDesign of various lengths and clusters
- 4) ***Custom protocol creation using*** RAbD components in RosettaScripts

Thanks!