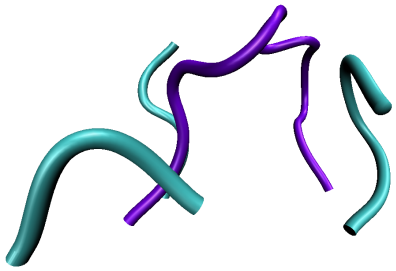
RosettaAntibodyDesign (*RAbD*)

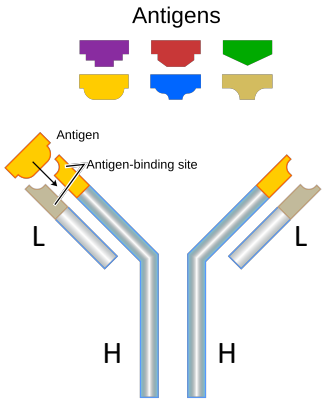


Jared Adolf-Bryfogle, PhD

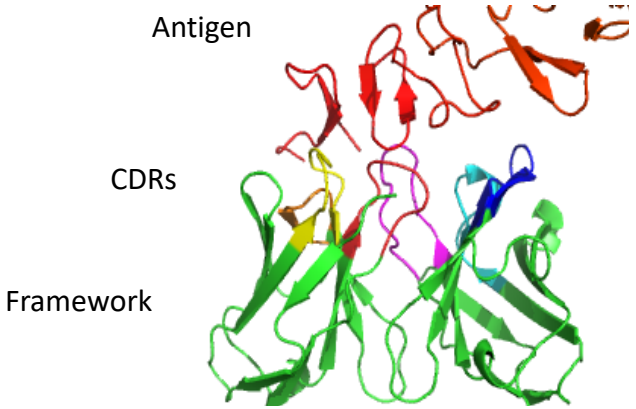
PhD Advisor: **Dr. Roland Dunbrack**
Postdoctoral Advisor: Dr. William Schief

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)

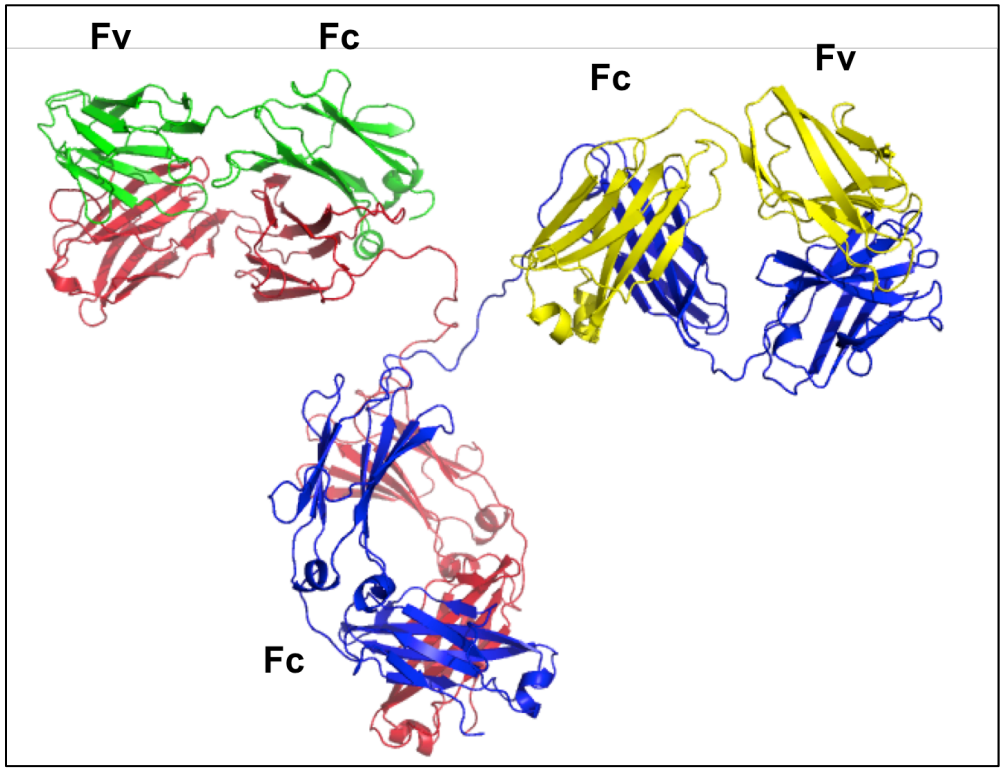


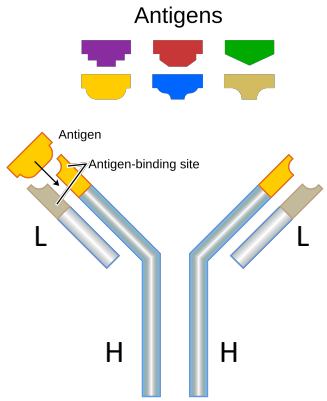
- *6 Complementarity Determining Regions (CDRs)*
  - Highly variable in length, structure, and sequence



- *Antibody Framework*
  - Highly conserved for each gene locus

Antibody Structure – Full IgG





Fv - Variable

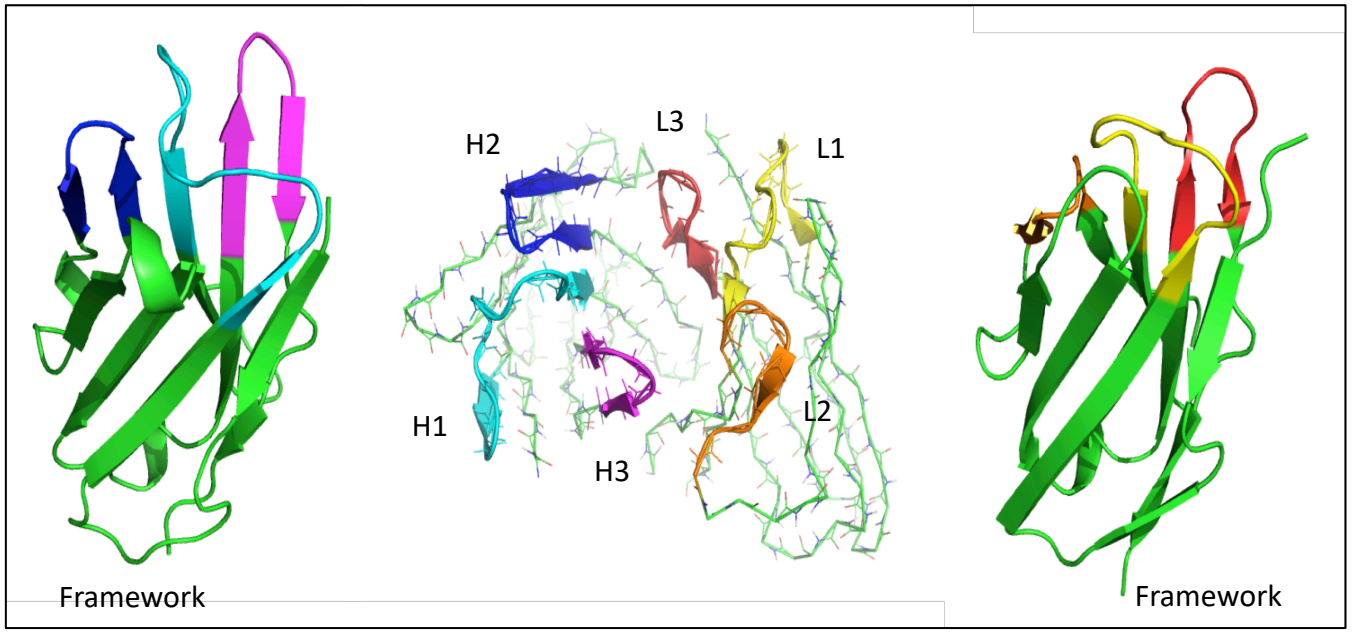
Fc - Constant

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

Heavy Chain

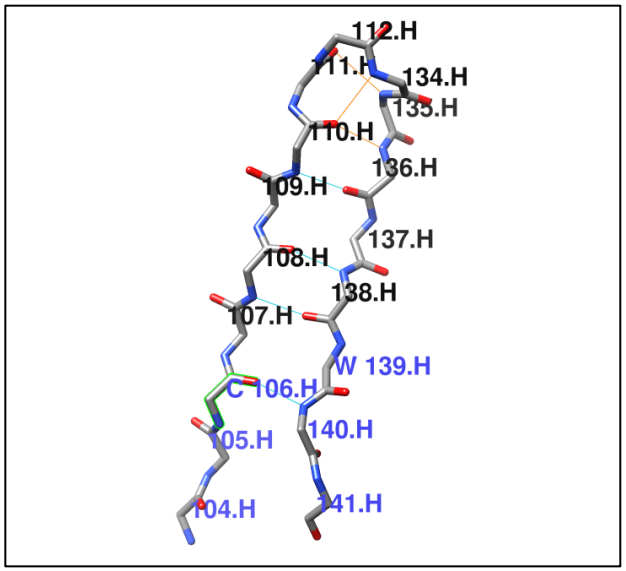


Light Chain

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

PyIgClassify: Antibody Identification

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



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

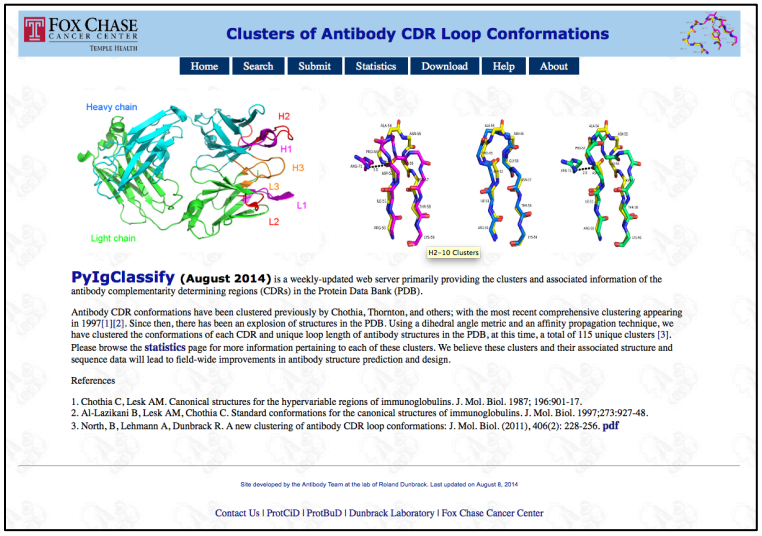
doi:10.1006/jmbi.2001.4662 available online at http://www.idealibrary.com on IDEAL® *J. Mol. Biol.* (2001) **309**, 657–670

JMB

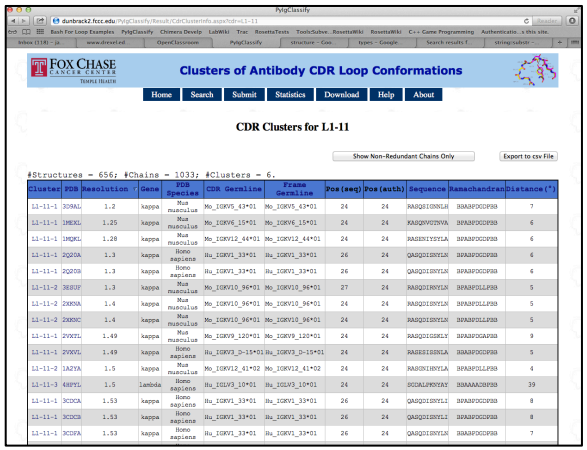
Yet Another Numbering Scheme for Immunoglobulin Variable Domains: An Automatic Modeling and Analysis Tool

Annemarie Honegger* and Andreas Plückthun

PyIgClassify: Server Frontend



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



Nucleic Acids Research Advance Access published November 11, 2014

Nucleic Acids Research, 2014 1
doi: 10.1093/nar/gku1106

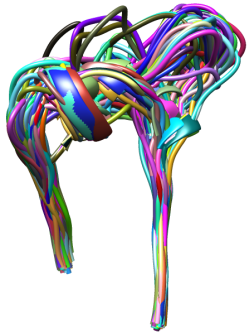
PyIgClassify: a database of antibody CDR structural classifications

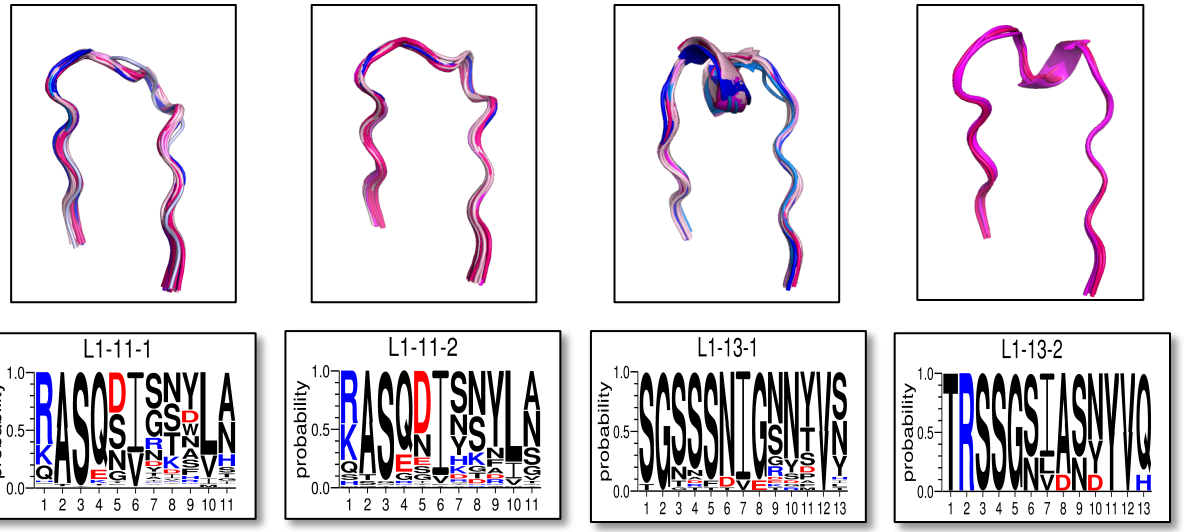
Jared Adolf-Bryfogle[1,2], Qifang Xu[1], Benjamin North[1], Andreas Lehmann[1] and Roland L. Dunbrack, Jr[1,*]

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





A New Clustering of Antibody CDR Loop Conformations

JMB Benjamin North, Andreas Lehmann and Roland L. Dunbrack Jr*

RosettaAntibodyDesign (RAbD)

- Knowledge-Based Antibody Design

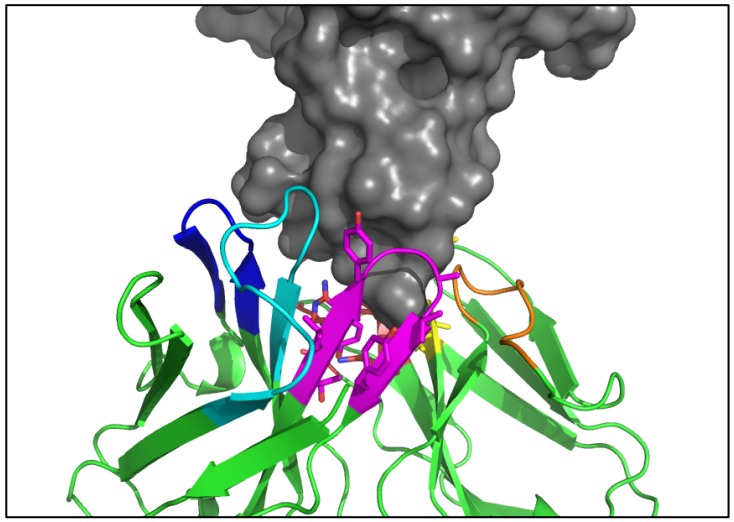
- ***Application -*** Application can be used for many antibody design projects
  - ***Affinity Maturation***
  - ***Homologous redesign***
  - ***Stability improvement***
  - ***De Novo design***

- ***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

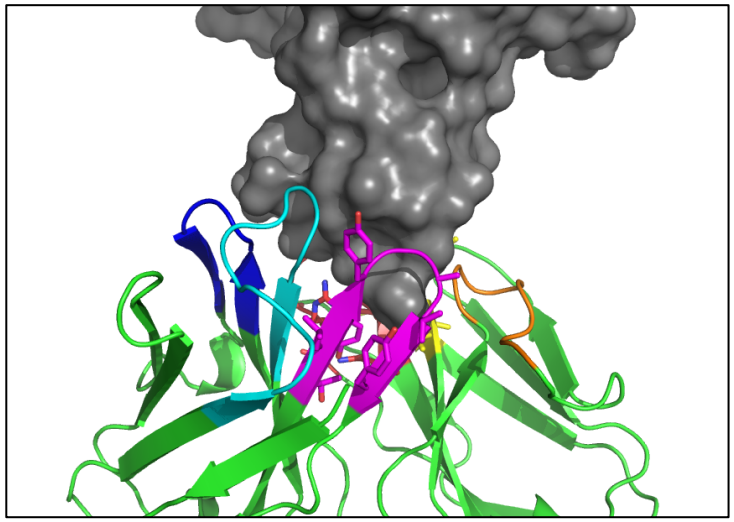


- Sequence Sampling (SeqDesign)
  - Sample amino acid types
    - According to CDR Cluster Profiles
  - *Roughly mimics*

    Somatic Hypermutation

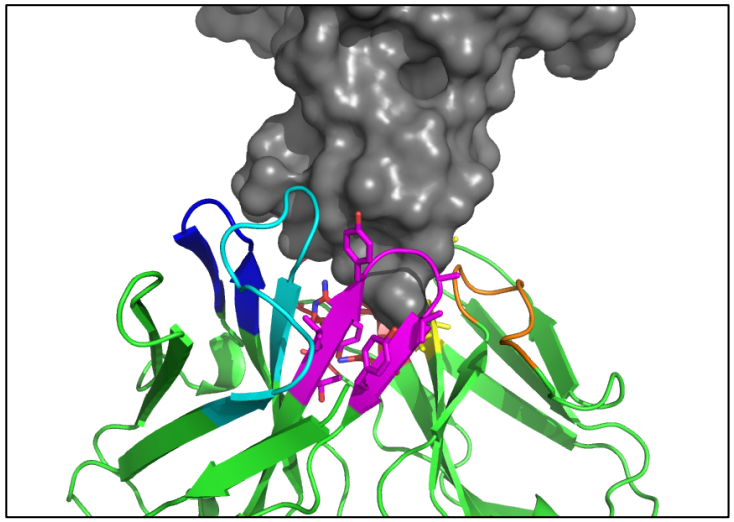
Antibody Designer: Overview

- 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*

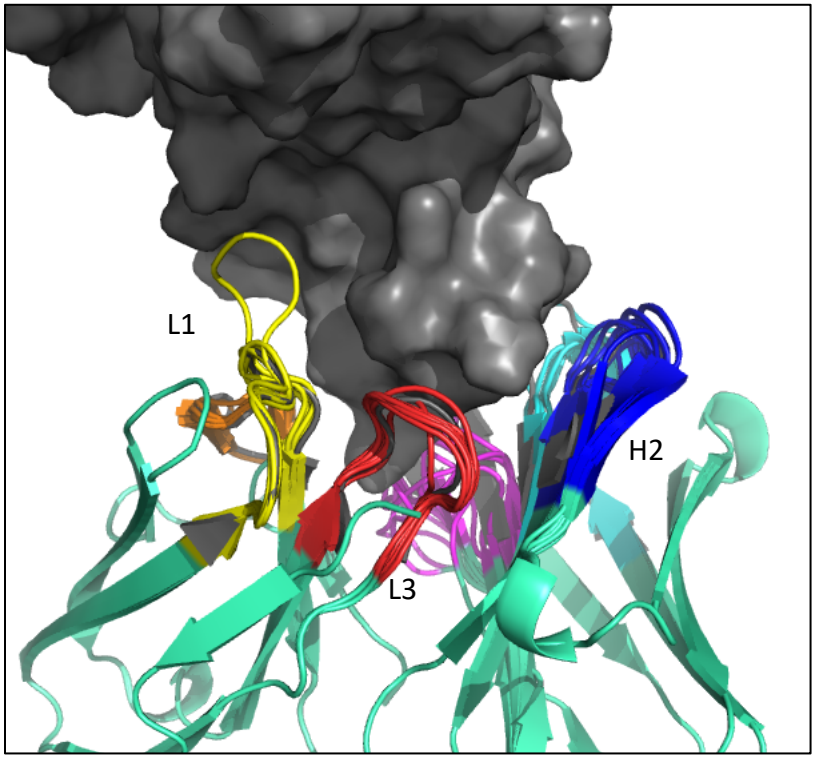
```
ALL GraftDesign ALLOW
ALL SeqDesign ALLOW


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

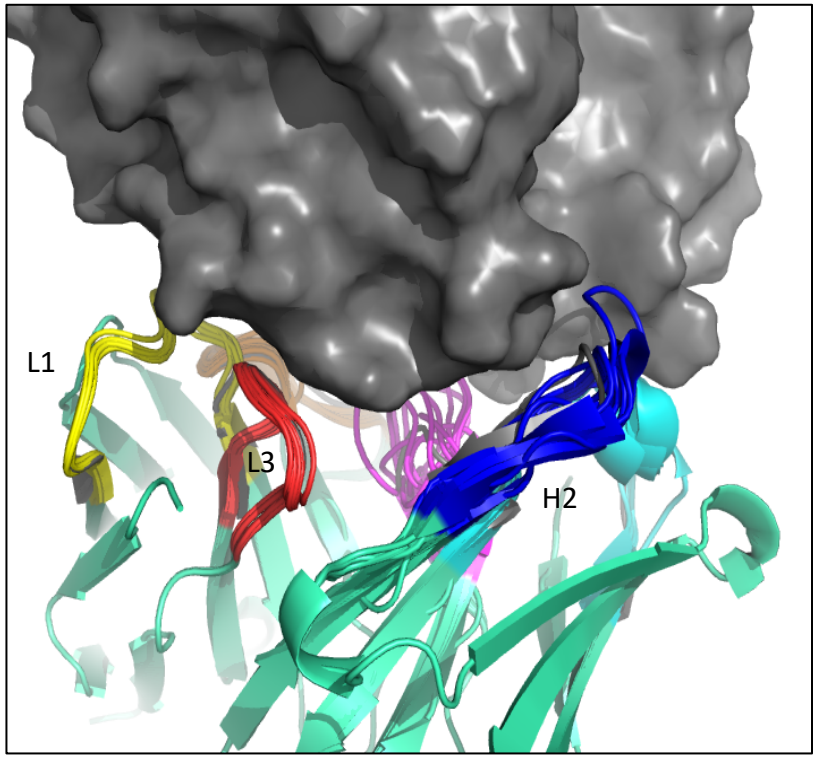
Example Command-Line

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

Top Decoy Examples

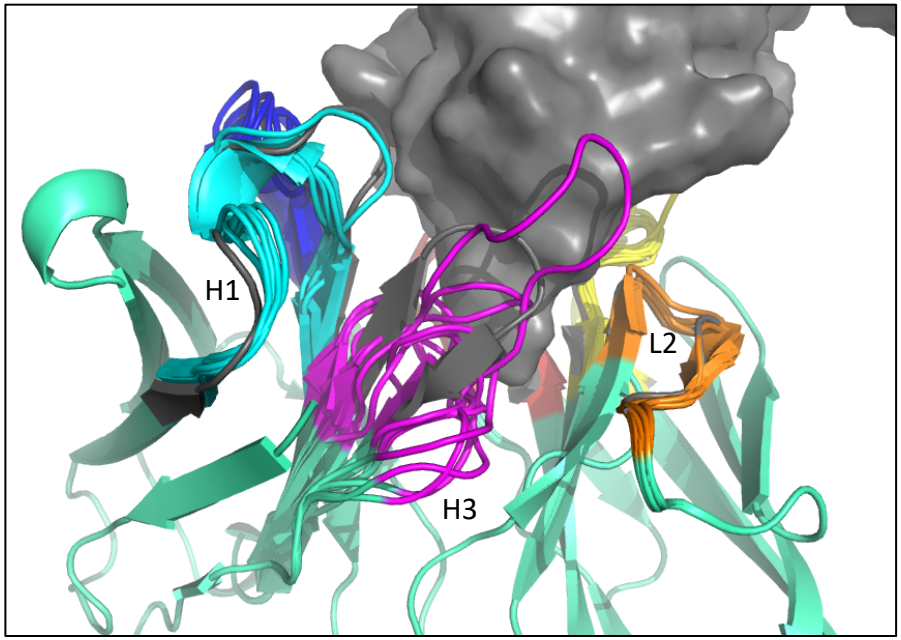


2j88

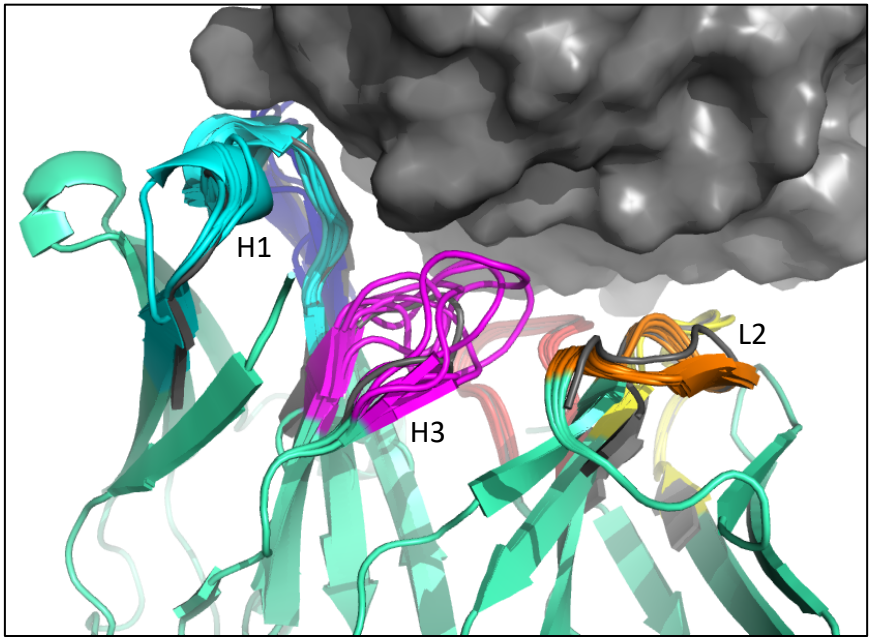


2dqc

Top Decoy H3 Variability

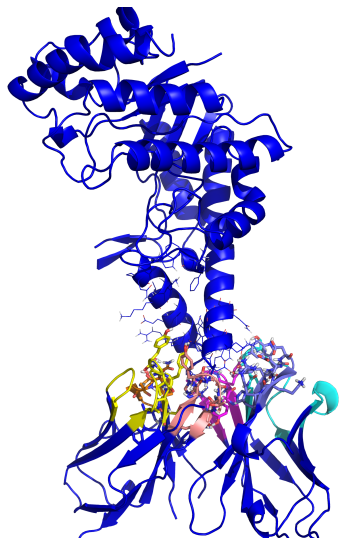


2j88



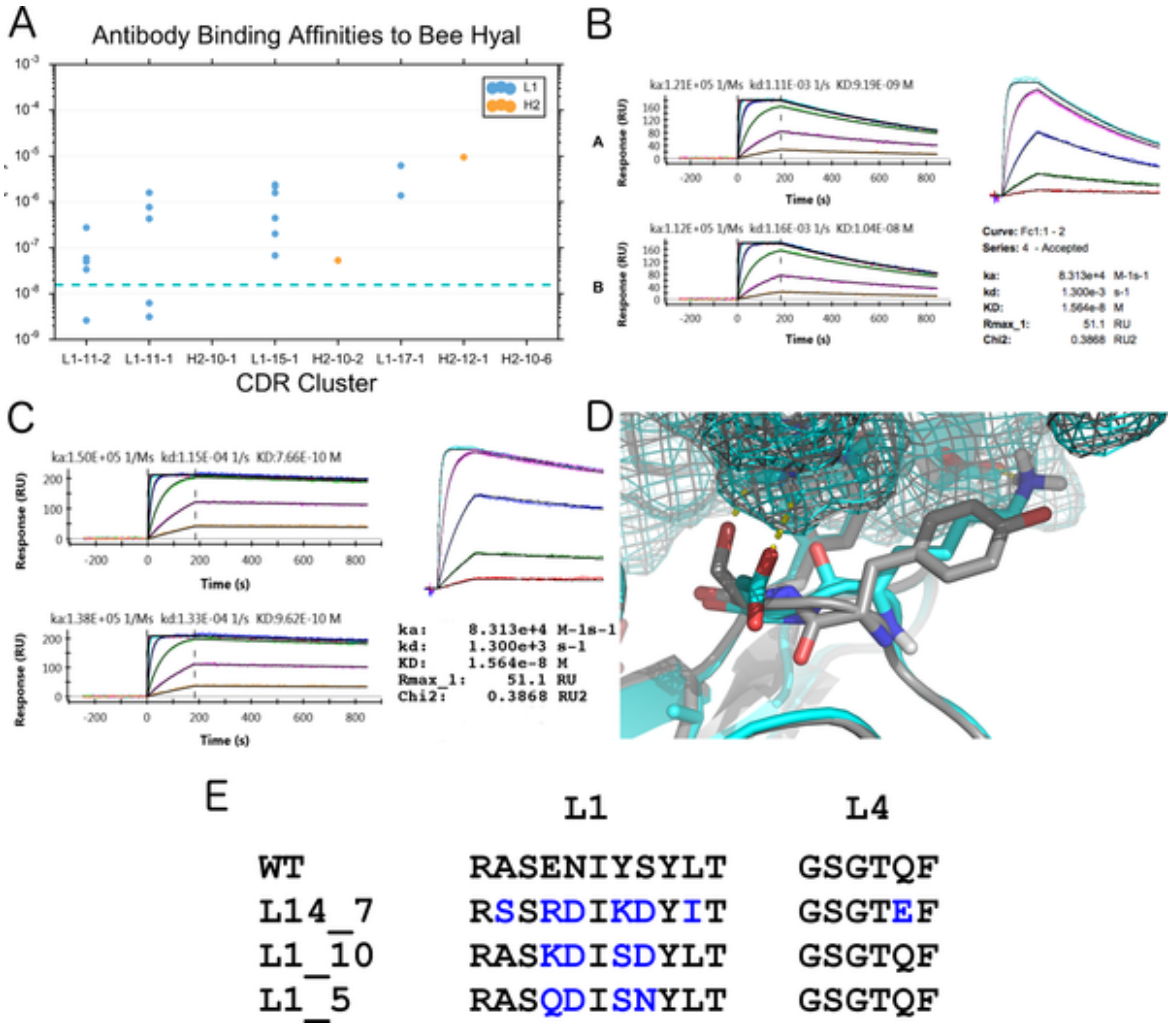
2dqc

2J88 Antibody Improvement



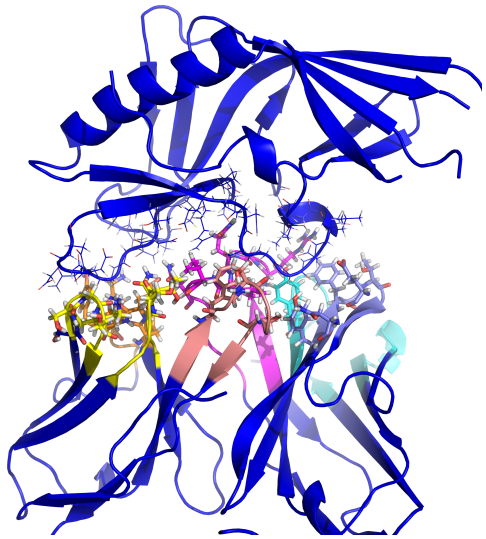
- Antibody binds to Bee Hyaluronidase
  - Major allergen of Bee Venom
- Binds at 15.6 nM
  - Top design binds 2.5 nM
- Designed L1/L4 (DE framework)
- Designed H2
- Dock/No Dock, only CDRs with profiles

Fig 8. Designed antibodies against bee hyaluronidase.



Adolf-Bryfogle J, Kalyuzhniy 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

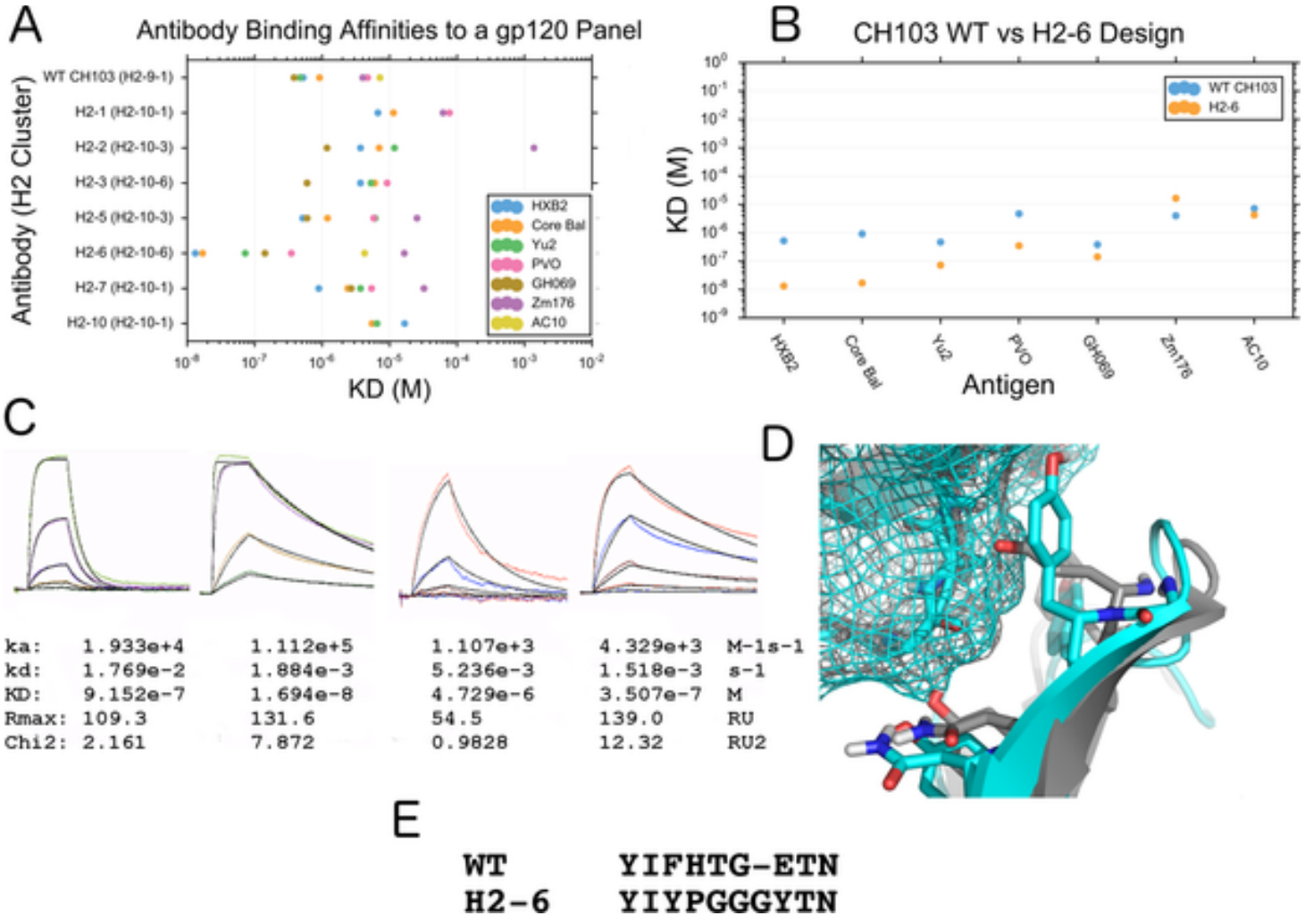
CH103 Antibody Improvement



- Antibody binds to CD4 GP120 binding site
- ZM176 Crystal 4JAN
- CH103 binds 4JAN at **2.30 x 10^{-06}** M

- Designed L1/L3
- Designed H2

Fig 9. Binding of designed antibodies to HIV gp120.



Adolf-Bryfogle J, Kalyuzhniy 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!