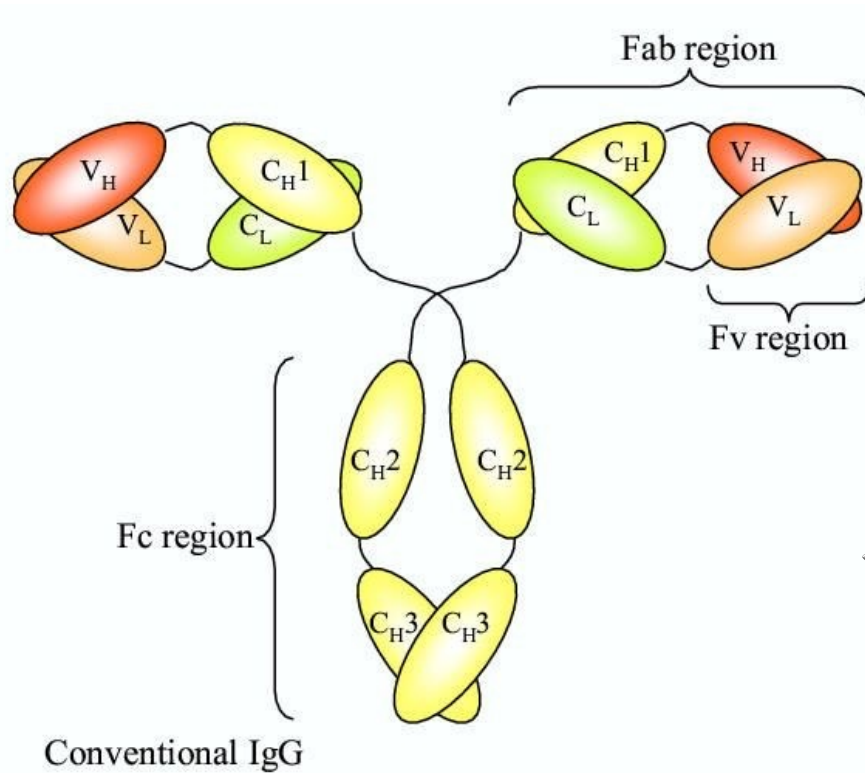


Antibody Modeling with RosettaAntibody

Presented by Taylor Jones
August 2021

Adapted from slides by Nina Bozhanova

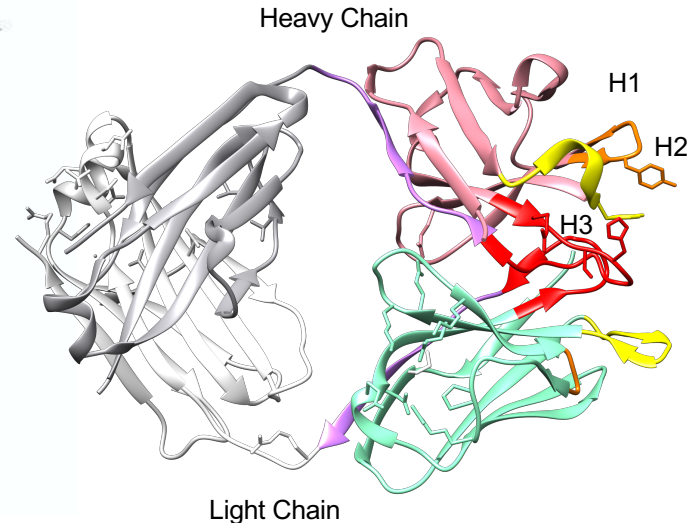
We model the FV region



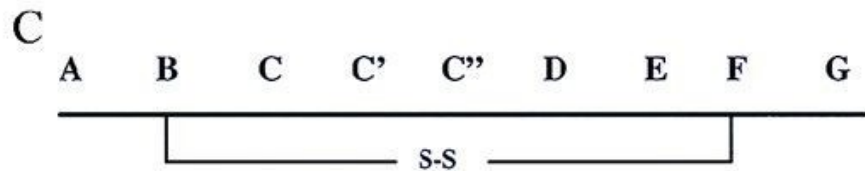
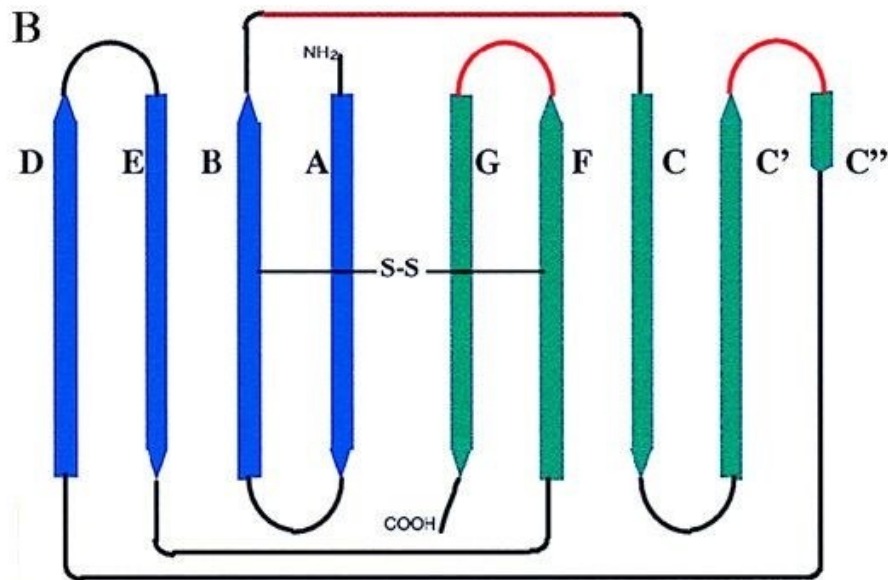
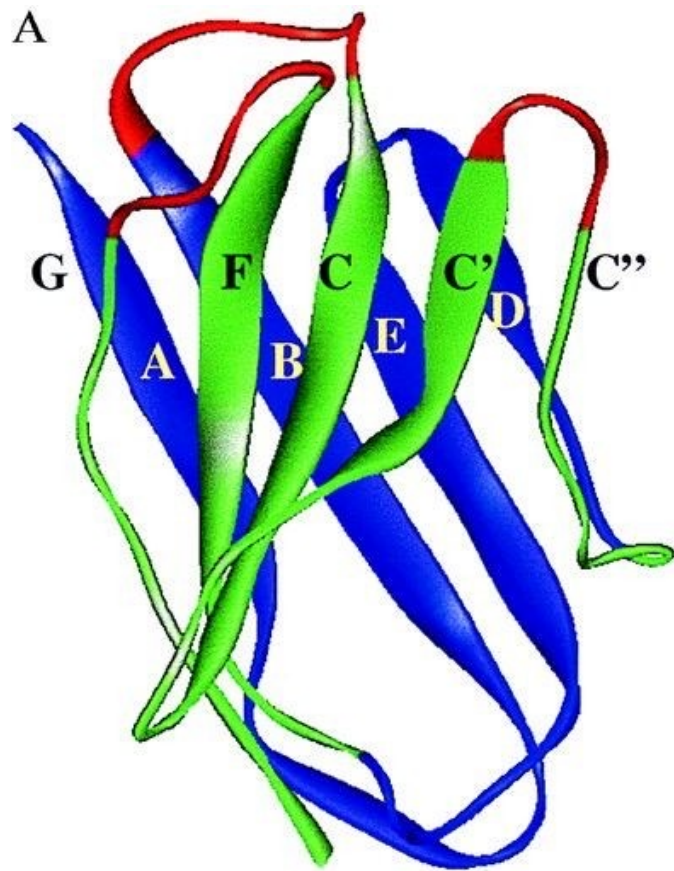
Fc - the crystallizable fragment

Fab - the antigen-binding fragment

Fv - the variable fragment (domain)



Ig fold



Modeling approaches depend upon comparative modeling

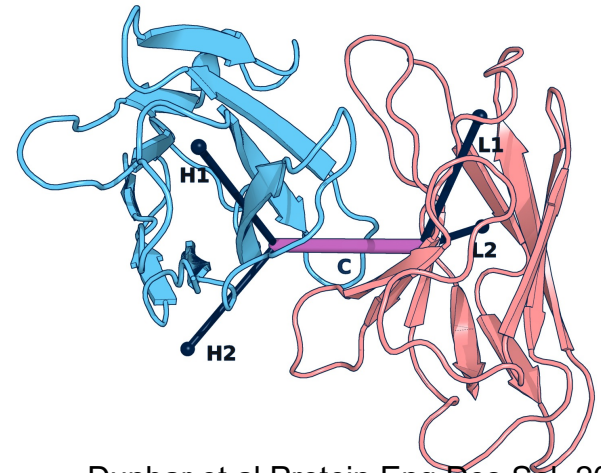
Template search

VH & VL orientation

Canonical loop conformations

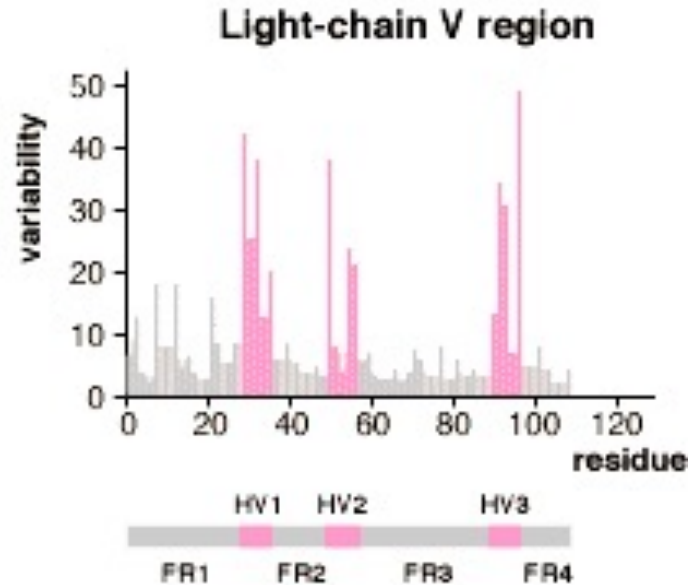
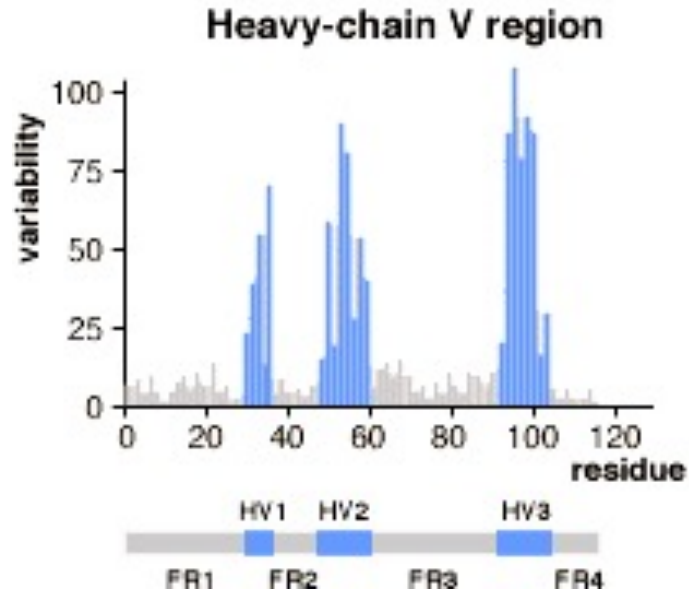
Perturbations & Energy Evaluation

CDRH3 built from scratch due to the high diversity



Diversity is highest in loop regions

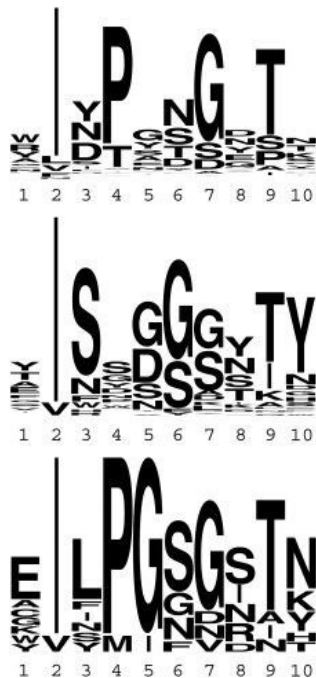
Slide stolen from Rocco Moretti



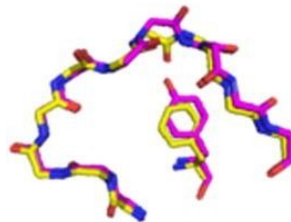
Canonical Loop Conformations

North/Dunbrack Clustering & new clusters

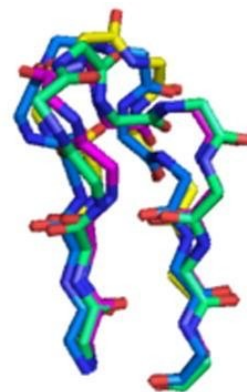
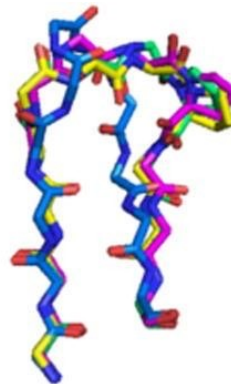
Loop sequence



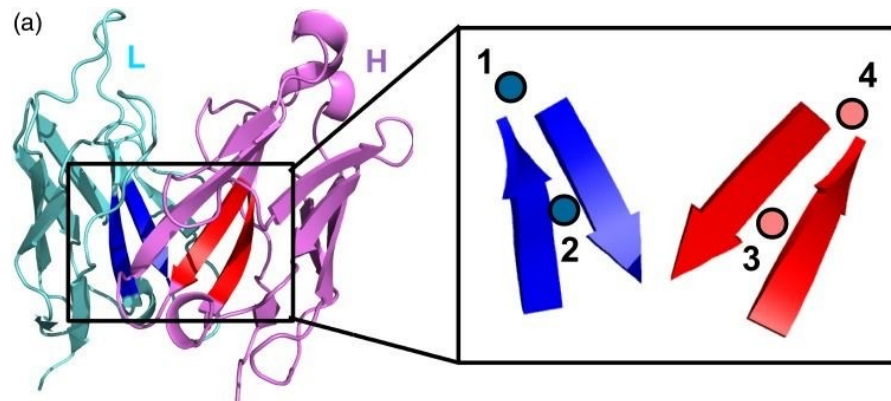
Structure



Left: CDR L1 length 11
Middle: CDR L3 length 9
Right: CDR H2 length 10



VL–VH orientation metrics

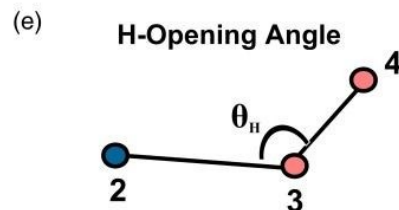
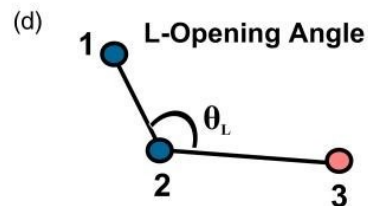
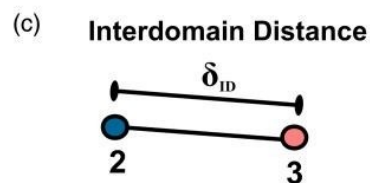
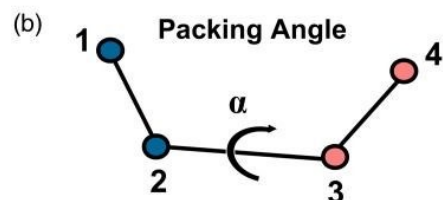


Point 1: located nearer the CDRs than Point 2, along the first principal component line of the coordinate set used to calculate point 2

Point 2: centroid of the α coordinates of residues L35–L38 and L85–L88 (Chothia numbering)

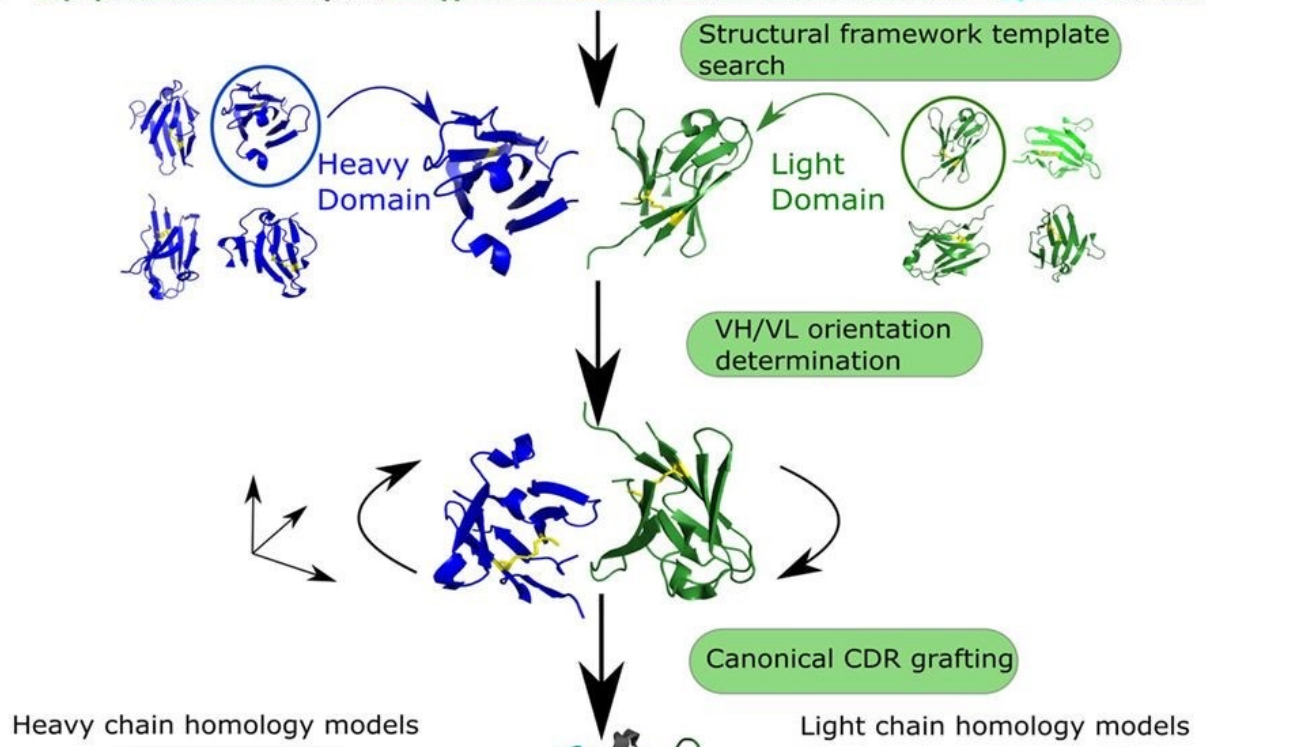
Point 3: the centroid of the α coordinates of residues H36–H39 and H89–H92 (Chothia numbering)

Point 4: located nearer the CDRs than Point 3, along the first principal component line of the coordinate set used to calculate point 3.

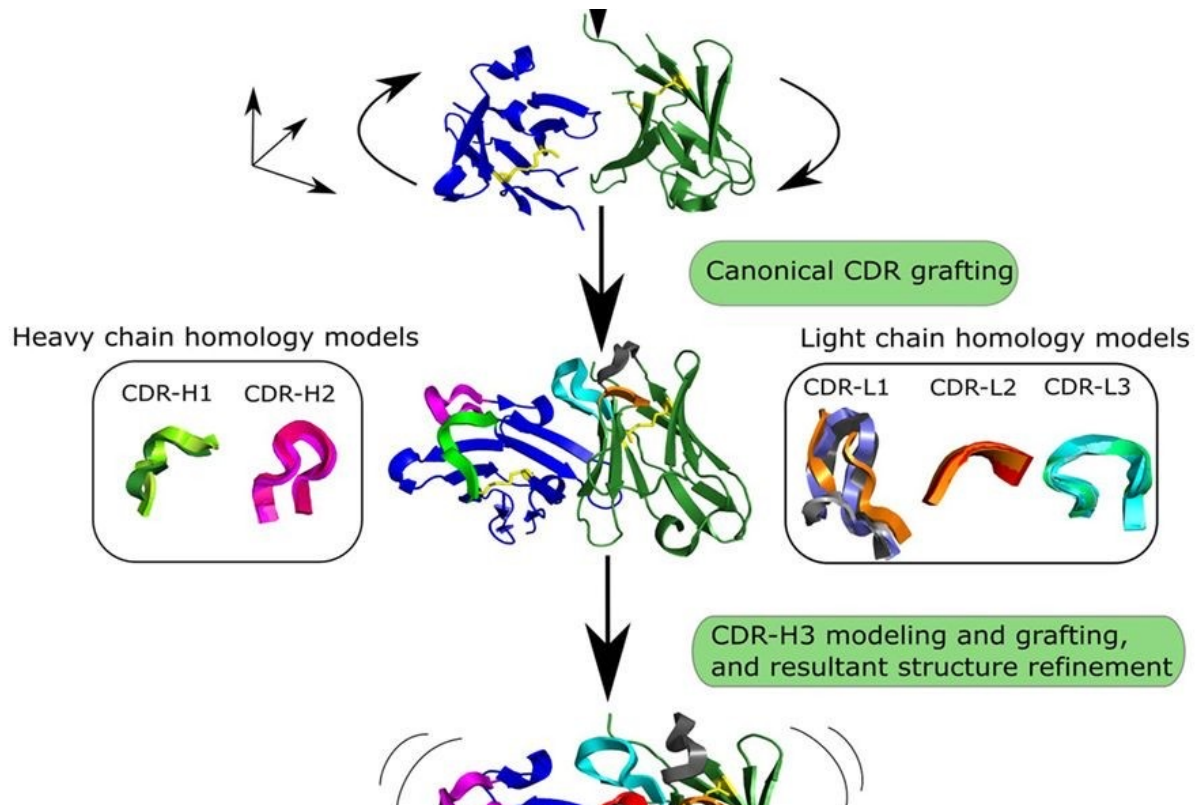


Generalized workflow of antibody modeling

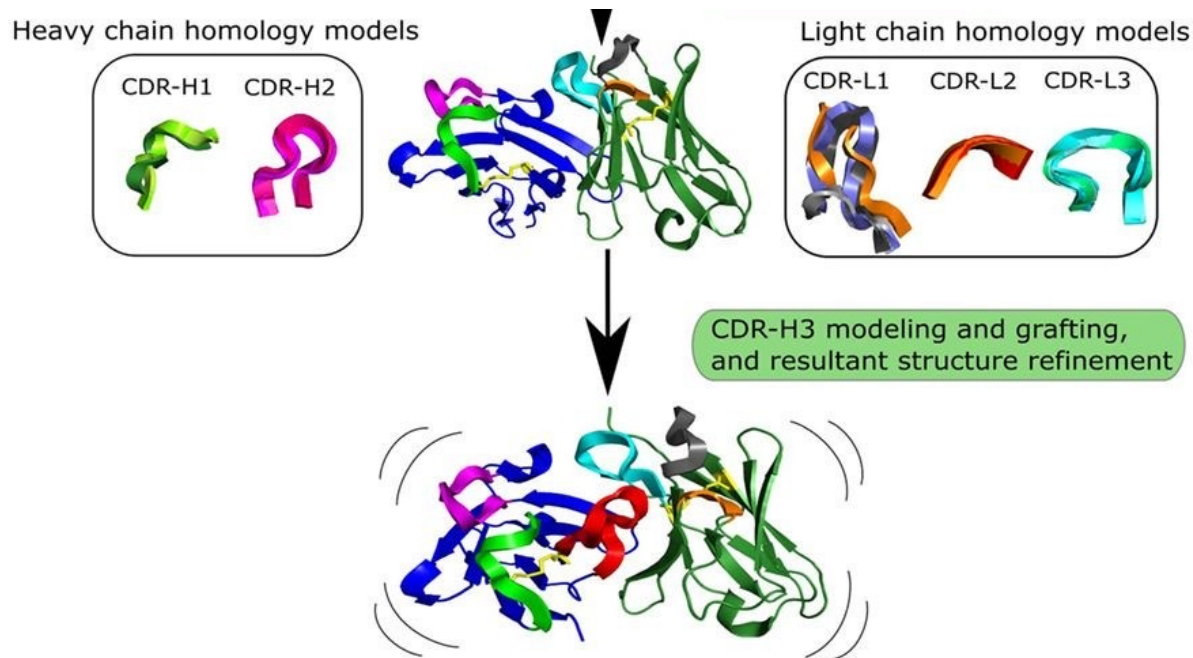
VH QVQLQQSGPELVRPGASVKVSCKASGYAFTTYNIYWLSRGIGRSFRGIGYFDPIYIGGTNTNQNFKD KATLTVDKSSSAAYMHLDSLTSSEDSAVYFCARSGGLADWGQGTLVTVSA
VL DIQLTQSPSSLSASLGERV SITCRASQDIGSNLNLWLQKPKDGTIKRLIYATSSLD SGVPKRFSGSRSGSDYSLTISSLESEDFVDYYCLQYASSPPTFGGGTKLEIK



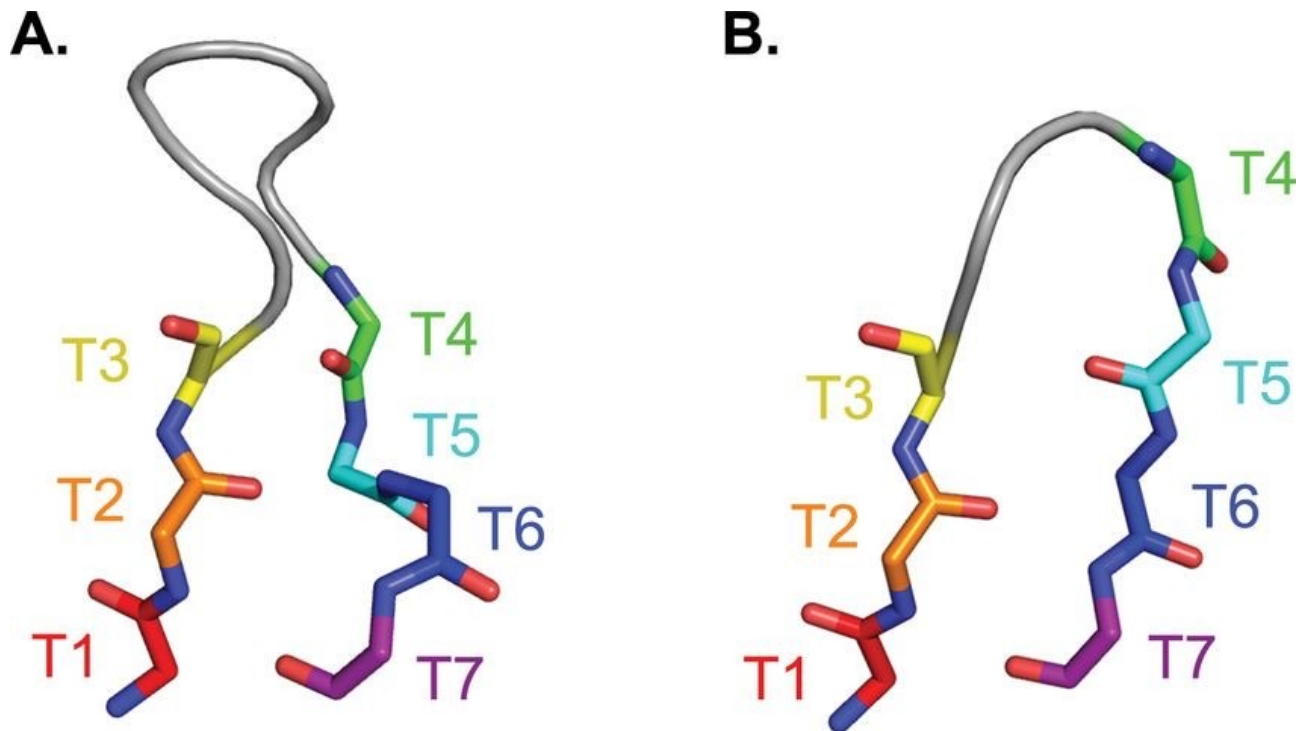
Generalized workflow of antibody modeling



Generalized workflow of antibody modeling



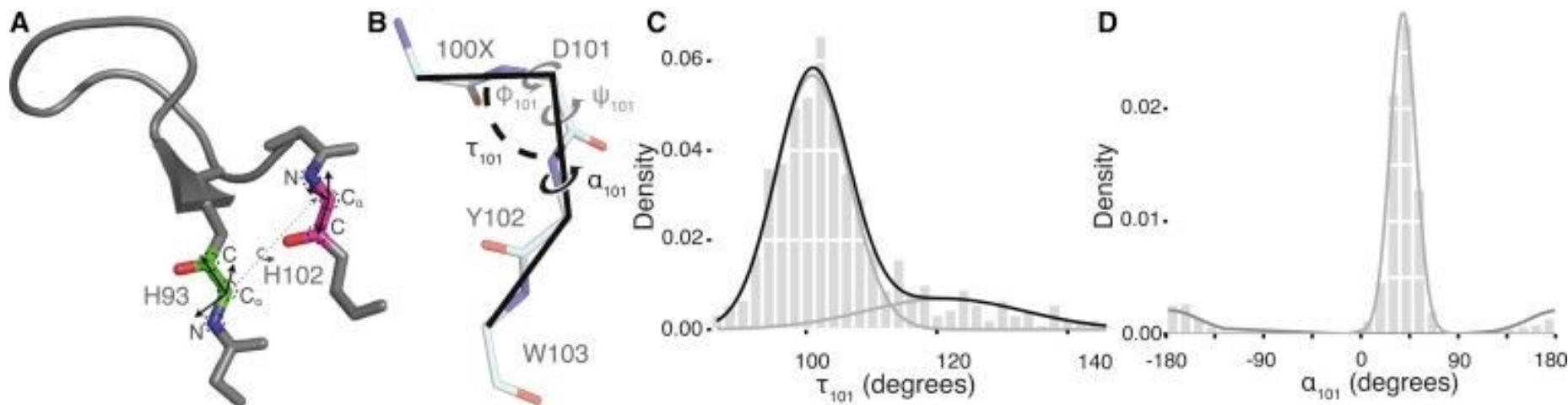
HCDR3 torso



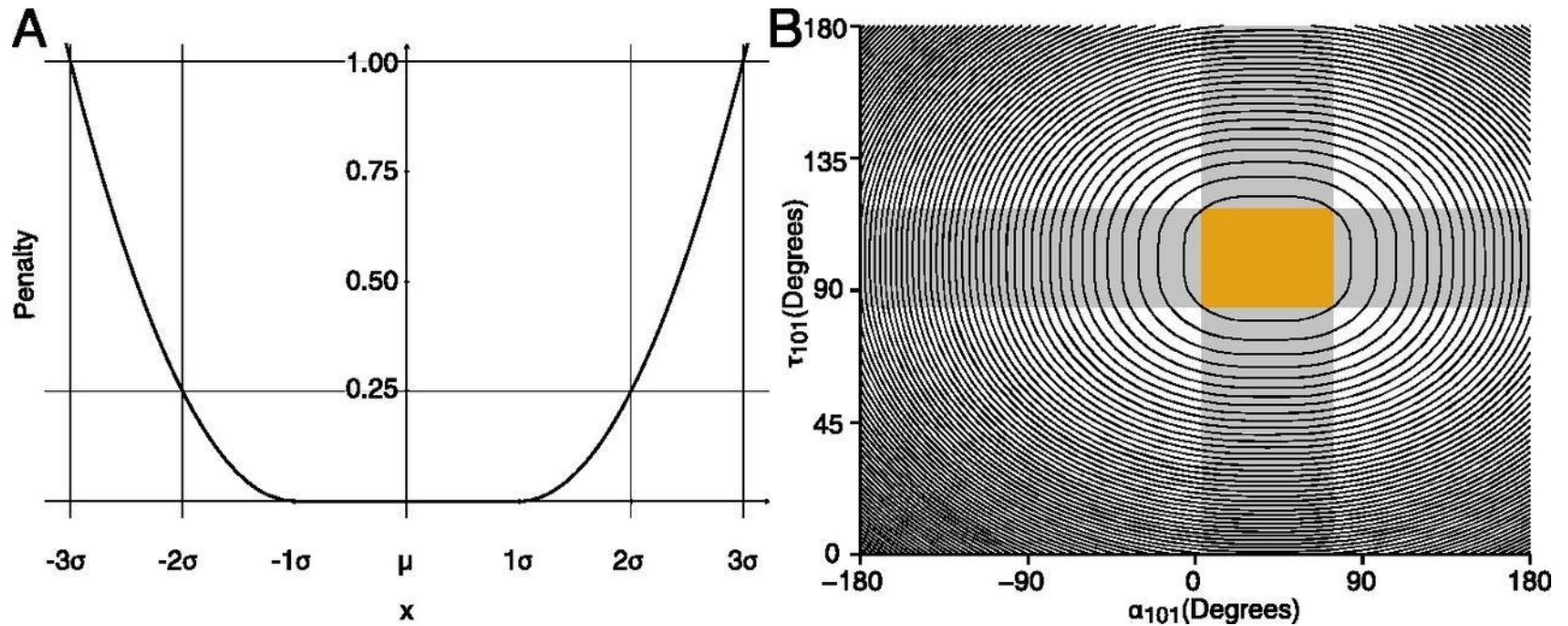
Kink constraints

Traditional Loop prediction algorithms fail to work on CDR3s
Sequence profile alone can't predict kink. North et al 2011

Torso region kink constraints

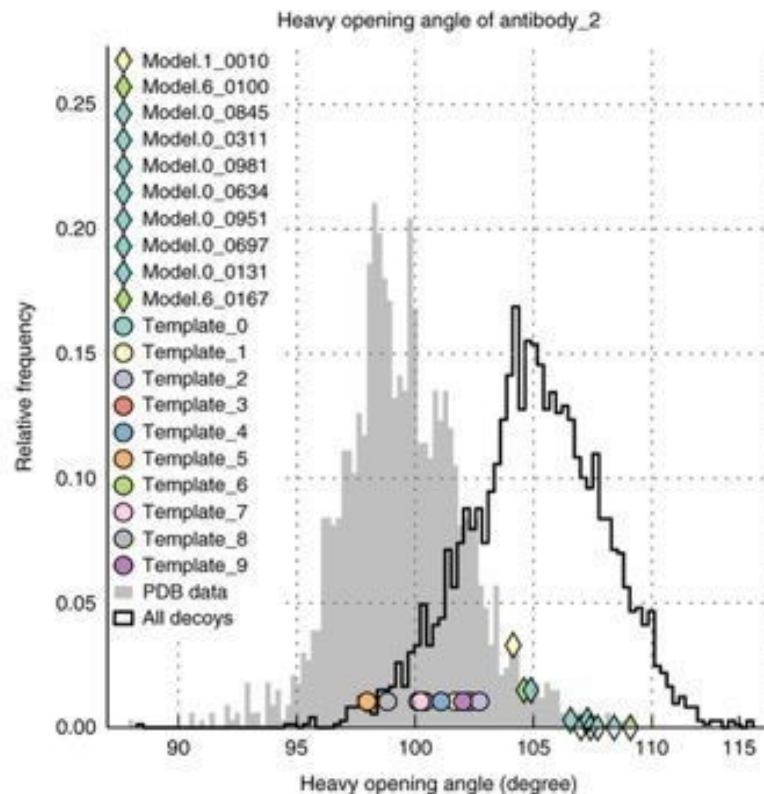
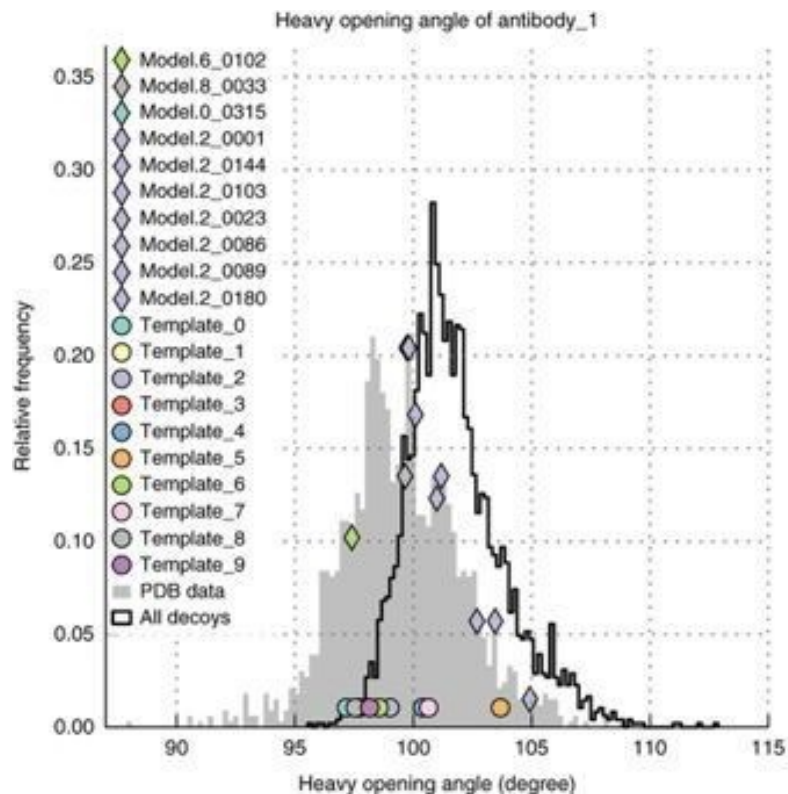


Kink constraints



"We developed new geometric parameters that describe the kink, in particular that the C-terminal loop residues form a pseudo dihedral angle, α_{101} (100X–103 using Chothia numbering), of 39° and a pseudo bond angle, τ_{101} (100X–102 using Chothia numbering), of 101° (2)"

Analysis of Loop design quality

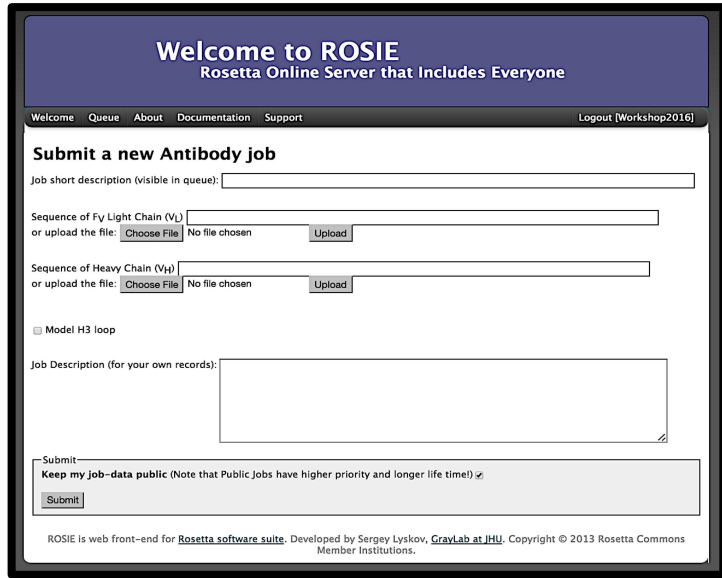


ROSIE web server - entirely automated modeling

1. Enter Job name and Ab sequences

2. Wait

3. Analyze output files



Welcome to ROSIE
Rosetta Online Server that Includes Everyone

Welcome Queue About Documentation Support Logout [Workshop2016]

Submit a new Antibody job

Job short description (visible in queue):

Sequence of Fy Light Chain (V_L)
or upload the file: No file chosen

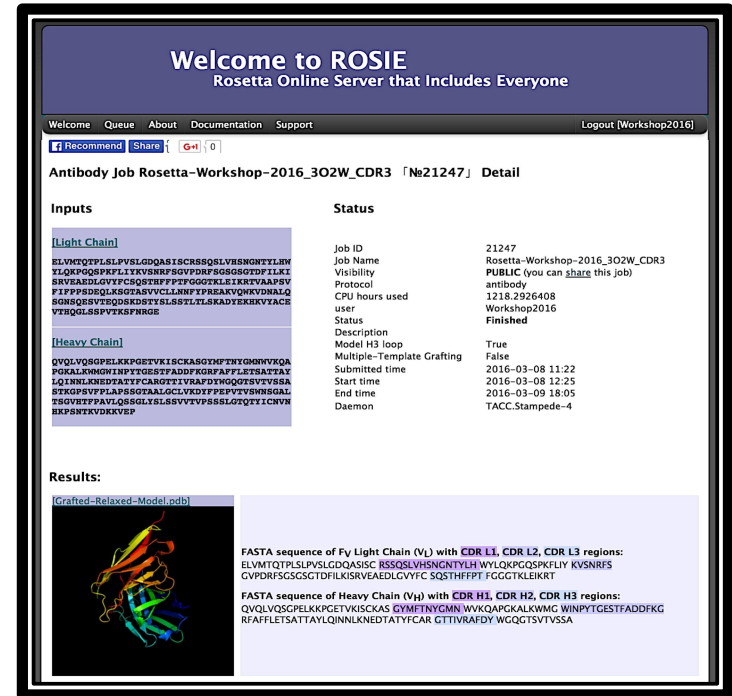
Sequence of Heavy Chain (V_H)
or upload the file: No file chosen

☐ Model H3 loop

Job Description (for your own records):

Submit
Keep my job-data public (Note that Public Jobs have higher priority and longer life time!) ☐

ROSIE is web front-end for [Rosetta software suite](#). Developed by Sergey Lyskov, [GrayLab at JHU](#). Copyright © 2013 Rosetta Commons Member Institutions.



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Welcome Queue About Documentation Support Logout [Workshop2016]

0

Antibody Job Rosetta-Workshop-2016_3O2W_CDR3 「№21247」 Detail

Inputs	Status
[Light Chain] ELVNTQTPLSLSPUSLQDQASISCRSSQELVSNOWTLEW TLQKPGQSPKFLIYKVSRRFSGVDRFSQSGSGTDFILKI SRVAEDLVYFCQSQTFFPTFGGQGLEIKRTVAAPSV FIFPPESQGLSGTASVCLLNNFYPREAKVQGDWNAQ SONSSESVTEQDSKSTYSLSLSTLTKADYKKKVTACE VTBQGLSPVTKSFNRGE	Job ID 21247 Job Name Rosetta-Workshop-2016_3O2W_CDR3 Visibility PUBLIC (you can share this job) Protocol antibody CPU hours used 1218.2926408 user Workshop2016 Status Finished
[Heavy Chain] QVQLVQSGPELKKPGQETVKISCKASGTHFTYGMHWYKA PGALLRNQNIHFTQESTYADQFKGFAPFLKSTATTY LQIHNKNEIDTATFCARQGTIVRAFDTHQGGTSVTYSSA STGPSTVFPFLAPSSGTAALGCLVDTFPEPVTYDHSGL TSQVETFPATLQSGSLSLVSYTVPSSRLQGTICNV RKPENTKVDKVEP	Description Model H3 loop True Multiple-Template Crafting False Submitted time 2016-03-08 11:22 Start time 2016-03-08 12:25 End time 2016-03-09 18:05 Daemon TACC.Stampede-4

Results:

[Grafted-Relaxed-Model.pdb]



FASTA sequence of Fy Light Chain (V_L) with CDR L1, CDR L2, CDR L3 regions:
ELVNTQTPLSLSPUSLQDQASISCRSSQELVSNOWTLEWTLQKPGQSPKFLIYKVSRRFSGVDRFSQSGSGTDFILKISRVAEDLVYFCQSQTFFPTFGGQGLEIKRTVAAPSVFIFPPESQGLSGTASVCLLNNFYPREAKVQGDWNAQSONSSESVTEQDSKSTYSLSLSTLTKADYKKKVTACEVTBQGLSPVTKSFNRGE

FASTA sequence of Heavy Chain (V_H) with CDR H1, CDR H2, CDR H3 regions:
QVQLVQSGPELKKPGQETVKISCKASGTHFTYGMHWYKAPGALLRNQNIHFTQESTYADQFKGFAPFLKSTATTYLQIHNKNEIDTATFCARQGTIVRAFDTHQGGTSVTYSSASTGPSTVFPFLAPSSGTAALGCLVDTFPEPVTYDHSGLTSQVETFPATLQSGSLSLVSYTVPSSRLQGTICNV RKPENTKVDKVEP

<https://rosie.graylab.jhu.edu/antibody>

Literature:

- 1.Weitzner BD, Jeliaskov JR, Lyskov S, Marze N, Kuroda D, Frick R, Adolf-Bryfogle J, Biswas N, Dunbrack RL Jr, Gray JJ. Modeling and docking of antibody structures with Rosetta. **Nat Protoc.** 2017 Feb;12(2):401-416. Doi: 10.1038/nprot.2016.180. Epub 2017 Jan 26. PubMed PMID: 28125104; PubMed Central PMCID: PMC5739521
- 2.Weitzner BD, Gray JJ. Accurate Structure Prediction of CDR H3 Loops Enabled by a Novel Structure-Based C-Terminal Constraint. **J Immunol.** 2017 Jan 1;198(1):505-515. Epub 2016 Nov 21. PubMed PMID: 27872211; PubMed Central PMCID: PMC5173470.
- 3.Finn JA, Koehler Leman J, Willis JR, Cisneros A 3rd, Crowe JE Jr, Meiler J. Improving Loop Modeling of the Antibody Complementarity-Determining Region 3 Using Knowledge-Based Restraints. **PLoS One.** 2016 May 16;11(5):e0154811. Doi: 10.1371/journal.pone.0154811. eCollection 2016. PubMed PMID: 27182833; PubMed Central PMCID: PMC4868311.
- 4.North B, Lehmann A, Dunbrack RL Jr. A new clustering of antibody CDR loop conformations. **J Mol Biol.** 2011 Feb 18;406(2):228-56. Doi: 10.1016/j.jmb.2010.10.030. Epub 2010 Oct 28. PubMed PMID: 21035459; PubMed Central PMCID: PMC3065967.
- 5.Lyskov S, Chou FC, Conchúir SÓ, Der BS, Drew K, Kuroda D, Xu J, Weitzner BD, Renfrew PD, Sripakdeevong P, Borgo B, Havranek JJ, Kuhlman B, Kortemme T, Bonneau R, Gray JJ, Das R. Serverification of molecular modeling applications: the Rosetta Online Server that Includes Everyone (ROSIE). **PLoS One.** 2013 May 22;8(5):e63906. doi: 10.1371/journal.pone.0063906. Print 2013. PubMed PMID: 23717507; PubMed Central PMCID: PMC3661552.

Rosetta Antibody Walkthrough

Resources:

- Antibody applications background:
 - https://new.rosettacommons.org/docs/latest/application_documentation/antibody/antibody-applications
- Antibody Options:
 - <https://new.rosettacommons.org/docs/latest/full-options-list#antibody>

Jumping over to the pdf tutorial

Open the `RosettaAntibody_tutorial.pdf` and walk through the exercise

Arguments in the file

-antibody::snugfit true # Adjust relative orientation of the VL-VH
-antibody::h3_filter false # Filter out things that neither have the kink or extend form
-antibody::h3_filter_tolerance 5 # max number of retries on a filter # not needed if previous value is false
-ex1 - use extra chi1 sub-rotamers for all residues that pass the extrachi_cutoff
-ex2 -use extra chi2 sub-rotamers for all residues that pass the extrachi_cutoff
-antibody:constrain_cter # The option to turn on/off the cterminal constrain penalty in loop scoring function
-antibody::remodel perturb_kic # option removed
-antibody::refine refine_kic # option removed
-antibody::cter_insert false
-antibody::flank_residue_min true
-antibody::bad_nder false
-antibody:constrain_vlvh_qq
-extrachi_cutoff 0 # extra rotamer considerations if x neighbors are met

#these are standard settings for kic/ngk

-loops:legacy_kic false
-loops:kic_min_after_repack true
-loops:kic_omega_sampling
-loops:allow_omega_move true ### remove 'true' and loop::?
-kic_bump_overlap_factor 0.36
-loops:ramp_fa_rep #increase significance of fa repulsive term
-loops:ramp_rama #ramp rama over the outer cycles of refinement
-loops:refine_outer_cycles 5

#These enable the kink constraints. Increase the weight if you want tighter kink constraints.

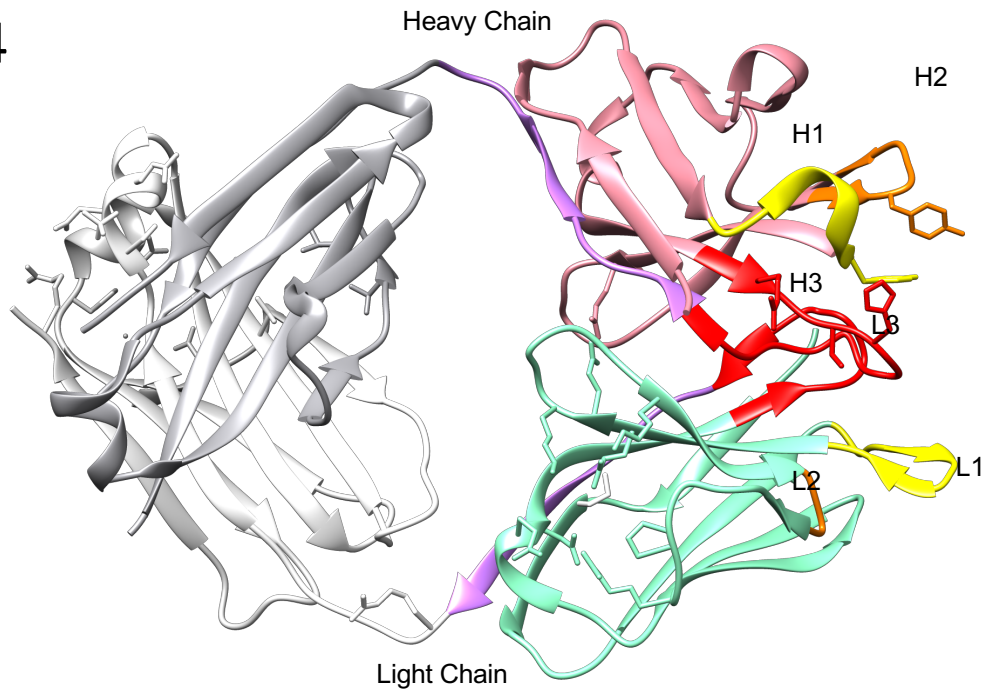
-constraints:cst_weight 1.0 #

Marze, N. A., Lyskov, S., & Gray, J. J. (2016). Improved prediction of antibody VL-VH orientation. *Protein Engineering, Design and Selection*, 29(10), 409–418.
<https://doi.org/10.1093/protein/gzw013>

V(D)J recombination produces diversity

Diversity in humans on the order of 10^{13}

Georgiou et al 2014



EVQLVESGGGLVQPGGSLRLSCAVS **GYSITSGY**SWNWIRQAPGKGLEWVAS **ITYDGGST**NYNPSVKGRITISRDDSKNTFY LQMNSLRAEDTAVYYCAR **GSHYFGHW**FAVWGQGT LVT VSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCHHHHHH