

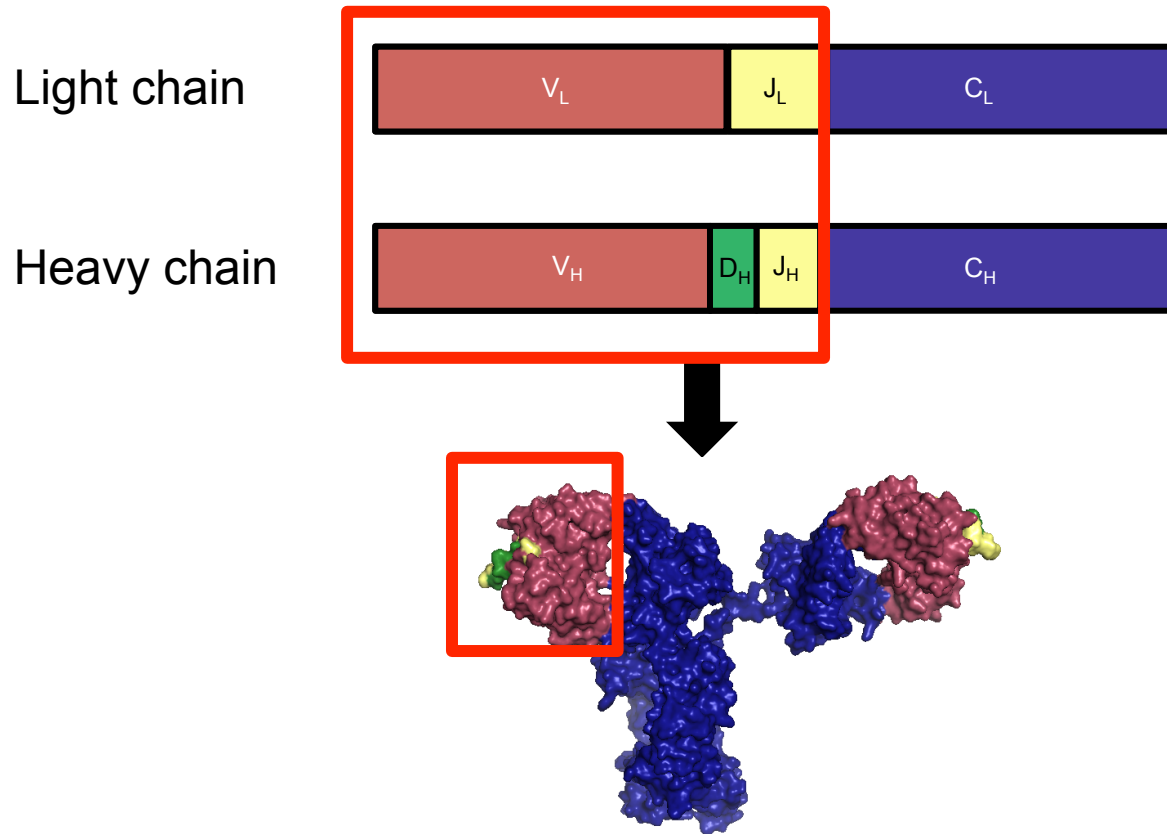
Rosetta Antibody:

Antibody modeling server for predicting F_v
structures

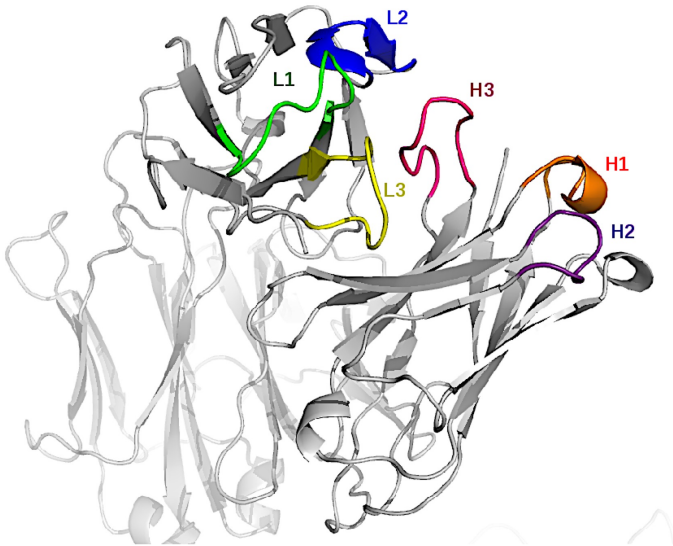
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Predicting F_V structure from sequence



Chothia numbering scheme



CDR	Residues	Hypervariable Region
V _L CDR1	L24 – L34	L31x
V _L CDR2	L50 – L56	L50x
V _L CDR3	L89 – L97	L95x
V _H CDR1	H26 – H32	H31x
V _H CDR2	H52 – H56	H52x
V _H CDR3	H95 – H102	H100x

Defining CDR conformations for grafting

Cluster	Number of structures	Percent loop length	Median PDB entry	Consensus sequence	Number of unique sequences	Percent unique sequences	Species	Gene	Loop conformation	Median angle (°)	Type	Comments
L3-7-1	2	100	1DFBL	qQynSYs	2	100	Hu Mo	κ	BPDADLP	18	III	
L3-8-1	15	68	2G5BG	lQyynlrT	13	68	Hu Mo	κ	BPDABGBB	10	III	
L3-8-2	4	18	1A7OL	qqfwrtpt	4	21	Mo	κ	BBDBGPPB	41		
L3-8-cis6-1	3	14	1E6OL	QqwnyPfT	2	11	Mo	κ	BPABPaLP	4		All of Pro6
L3-9-cis7-1	219	83	1J1PL	qQgss-P1T	182	83	Hu Mo	κ	BBDABppPB	10	II	93% of Pro7
L3-9-1	22	8	1F4XL	alw-snhwv	17	8	Hu Mo	κ,λ	BBPBLBPB	37		88% of non-Pro; All all of λ
L3-9-2	12	5	1KCSL	qQsth-ppT	12	6	Hu Mo	κ	BBDABAPPB	20		
L3-9-cis7-2	8	3	1G7IA	QHfwsTPrT	7	3	Hu Mo	κ	BPDpGBpPB	4		
L3-9-cis7-3	2	1	1L7IL	qQyyiyPyT	2	1	Hu Mo	κ	BBDABPaLP	21		
L3-9-cis6-1	1	<1	2FBJL	QQWTYPLIT	1	<1	Mo	κ	BBBpDBBB	—		Only Pro6
L3-10-1	6	67	3B5GB	qsydss-svv	5	63	Hu	λ	BBBPAALPPB	40	III	All of non-Pro
L3-10-cis8-1	2	22	1I7ZC	lysrefPPwT	2	25	Mo	κ	BBBBApPB	36		All Pro6,7
L3-10-cis7,8-1	1	11	1JGUL	SQSTHVPLT	1	13	Mo	κ	BBDABppPB	—		All Pro6,7
L3-11-1	9	90	1RZFL	aawdssldavv	9	90	Hu	λ	BBPBAADLBPB	12	I	2 with Pro (position 9.10)
L3-11-cis7-1	1	10	2NXYC	QQYNNWPPRYT	1	10	Hu	κ	BPDApPBpPB	—		Pro7.8
L3-12-1	1	100	3C2AL	ATWDSGLSADWV	1	100	Hu	λ	BBBPAPADLPPB	—	III	
L3-13-1	3	100	2OTUG	aawDdsrggpdwV	2	100	Hu Mo	λ	BBBPPAABPBBB	41	III	

Using the Server

1. Enter Job name and FASTA sequences

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 F_y 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!)

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2. Analyze output files

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Antibody Job Rosetta-Workshop-2016_302W_CDR3 [№21247] Detail

Inputs	Status
[Light Chain] ELVMTQTLSLSPVSLGDDQASISCRSSQSLVHNGNTYLEW YLQKPGQSPKFLITKVNRFSGVDRFSGSGSDYFLKI SVVARELQVYFCSSGHFFPFQGGKLEIKRVAAPSV FIFPPSDEQLKSGTASVCLLNNFYPREAKVQMKVDNALQ SGNSQESVTEQDSKDSTYLSSTITLTKADYEKHKVYACE VTHQGLSSPVTKSFRRGEE	Job ID: 21247 Job Name: Rosetta-Workshop-2016_302W_CDR3 Visibility: PUBLIC (you can share this job) Protocol: antibody CPU hours used: 1218.2926408 user: Workshop2016 Status: Finished
[Heavy Chain] QVQLVQSGPELKKPGETVYKISCKASGYMFTNYGMHWKQA PKALKVMGWIHPYTGESTFADDFKGRFAFFLESAFTAY LQIMLNKHEDEATYFCAROTTIVKAFDFWGGQSTVYVSSA STKGPVFPFLAPSSGTAALGCLVKDIFPEPVTYVSNNSGAL TSGVTEFPAVLQSSGLYSLSSVTVPSSSLGQTQYICNVN HKPSTKTKVDKKEF	Description: True Model H3 loop: True Multiple-Template Grafting: 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 F_y Light Chain (V_L) with CDR L1, CDR L2, CDR L3 regions:
ELVMTQTLSLSPVSLGDDQASISCRSSQSLVHNGNTYLEWYLQKPGQSPKFLITKVNRFSGVDRFSGSGSDYFLKISVVARELQVYFCSSGHFFPFQGGKLEIKRVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQMKVDNALQSGNSQESVTEQDSKDSTYLSSTITLTKADYEKHKVYACEVTHQGLSSPVTKSFRRGEE

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

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Analyzing Results

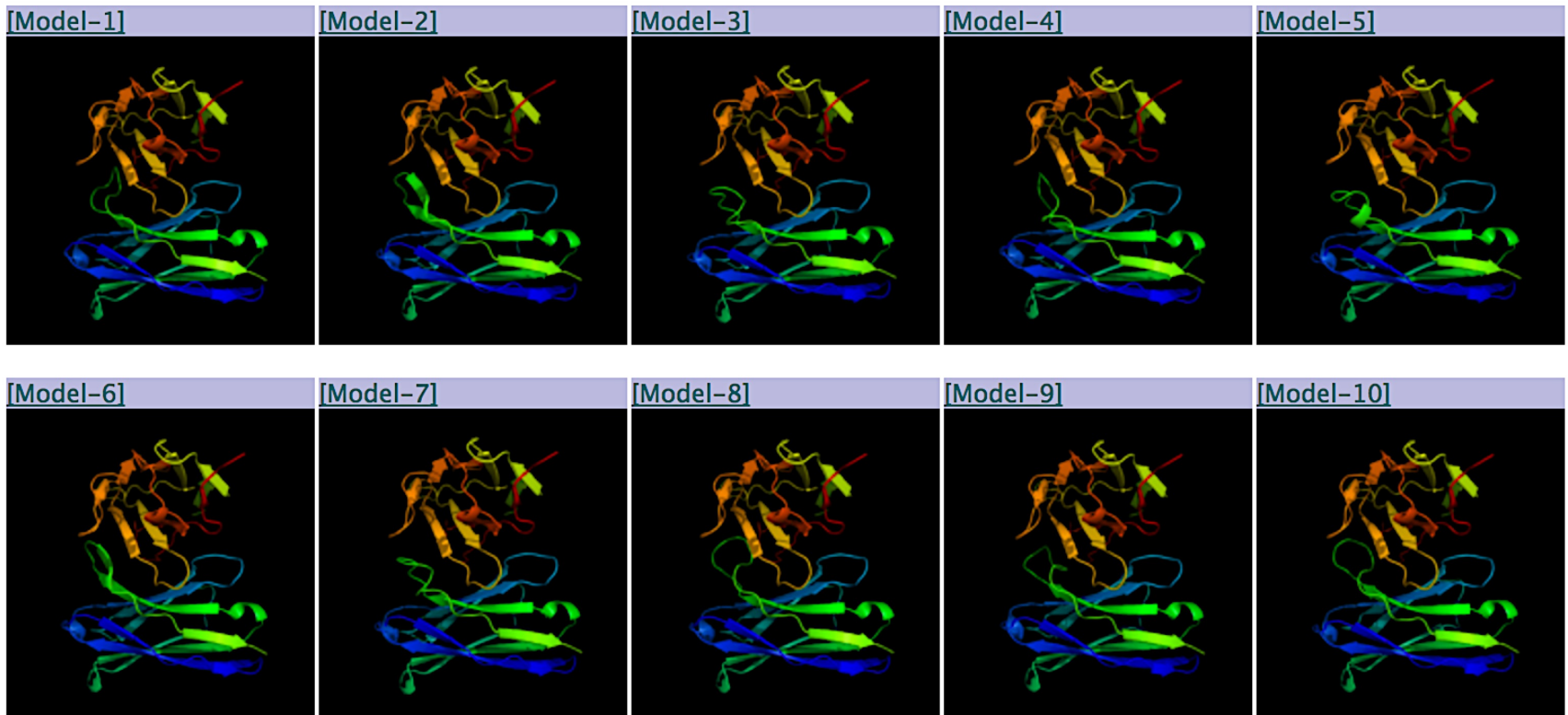
Antibody segment templates

summary	L1	L2	L3	H1	H2	H3	FRL	FRH	light	heavy	light_heavy	
subject-id	resolution	%-identity	alignment-length	mismatches	gap-opens	q.start	q.end	s.start	s.end	evalue	bit-score	
FRL.pdb	1.9	98.28	58	1	0	1	58	1	58	3e-37	115	
FRH.pdb	1.8	88.89	63	7	0	1	63	1	63	7e-38	117	
light.pdb	1.7	94.12	102	6	0	5	106	1	102	3e-68	198	
heavy.pdb	1.8	78.9	109	20	1	5	110	1	109	3e-61	180	
L1.pdb	1.7	87.5	16	2	0	1	16	1	16	4e-07	31.2	
L2.pdb	1.7	100.0	7	0	0	1	7	1	7	0.086	15.9	
L3.pdb	1.9	100.0	9	0	0	1	9	1	9	0.0008	21.8	
H1.pdb	2.0	80.0	10	2	0	1	10	1	10	0.015	19.3	
H2.pdb	1.8	70.59	17	5	0	1	17	1	17	1e-05	29.1	
H3.pdb	2.0	80.0	5	1	0	4	8	4	8	5.9	10.0	
light_heavy.pdb	2.8	79.72	212	42	1	5	216	1	211	5e-123	347	

Loop Modeling of the CDR H3

H3 Loop modeling results:

Ten lowest-energy structures created:



RosettaAntibody Score Table

H3 loop modeling score (with grafted.relaxed.pdb used as a reference structure for RMSD calculations) [Download original score file]										
decoy	total_score †	CDR_SASA	CDR_SASA_HI	CDR_charge	H1_RMS	H2_RMS	H3_RMS	L1_RMS	L2_RMS	L3_RMS
model.4_0034	-233.705	3295.585	1189.063	2	0.198	0.318	1.864	0.392	0.199	0.331
model.4_0032	-232.091	3510.062	1487.697	2	0.293	0.13	1.904	0.266	0.26	0.386
model.4_0134	-232.074	3461.718	1413.34	2	0.268	0.075	7.887	0.296	0.295	0.372
model.4_0111	-231.305	3520.123	1411.613	2	0.209	0.11	6.961	0.254	0.178	0.239
model.4_0186	-231.177	3542.371	1300.824	2	0.469	0.147	8.005	0.255	0.415	0.441
model.4_0057	-230.078	3693.835	1569.021	2	0.29	0.186	2.197	0.288	0.208	0.395

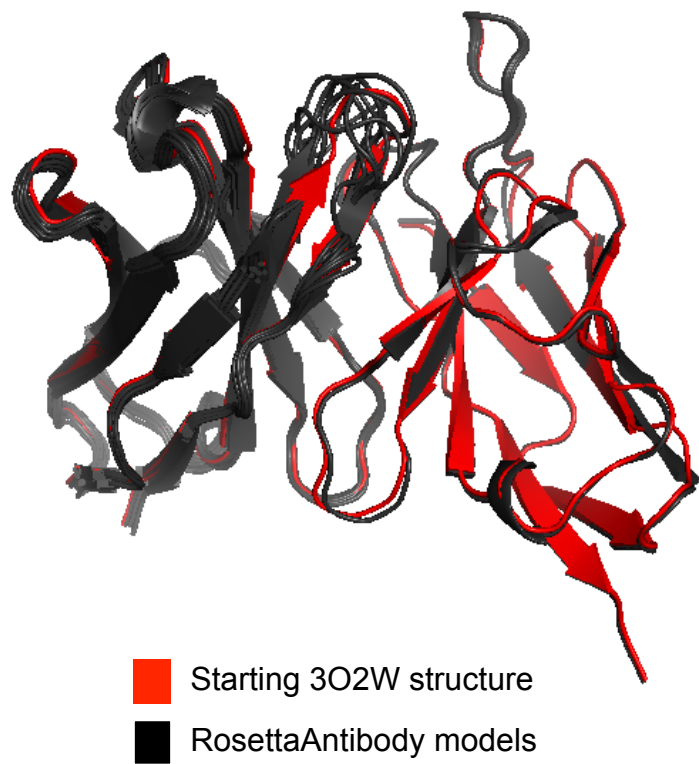
Benefit of modeling H3:

- Minimize steric clashes
- Optimize dihedral bond angles

Drawbacks of modeling H3:

- Increase computation time substantially
- Limited to modeling an H3 loop of no more than ten residues

RosettaAntibody Tutorial



Today's Model: 3O2W

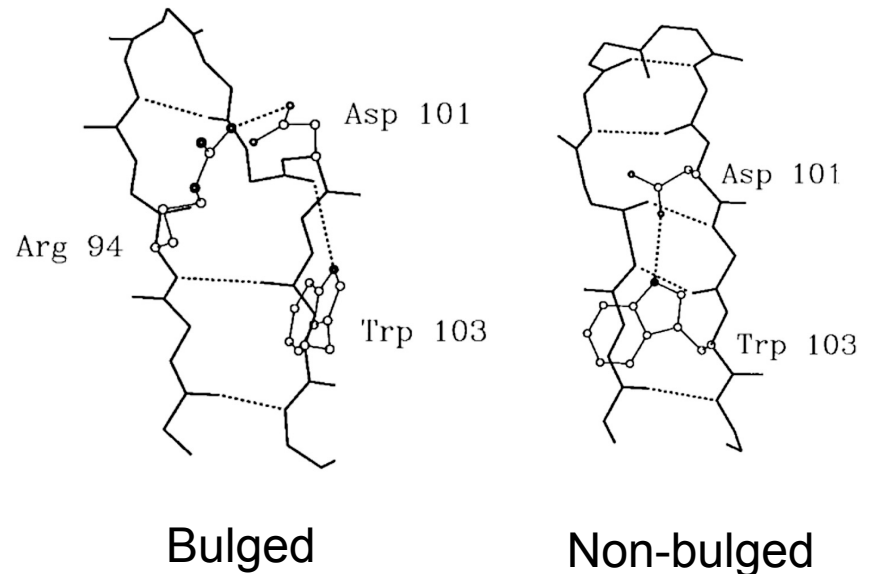
19E F_v mutant in complex with a transition state analogue

First Task: Go through the RosettaAntibody3 downloaded output to assess accuracy of model predictions

Second Task: Go through finished public Rosetta Antibody jobs to understand output

Assessing Model Quality

- RMSD to starting structure
 - Predicted RMSD of $< 1.5 \text{ \AA}$ ideal
- Conserved $V_H - V_L$ interactions
 - L36 interaction with HCDR3
 - L46 or L49 interaction with HCDR3
- Bulged v. non-bulged conformations



Lab 1: RosettaAntibody

Questions?