Monoclonal Antibodies *in Silico* : Examining the Free Energy of Binding to hemagglutinin

Alexandra DeLaney 2011 PRIME Program

UCSD Adviser — Wilfred Li, Ph.D.
Host Mentor — Habibah Wahab, Ph.D.
Host Site —USM, Penang, Malaysia

Project Outline

Background: Antibodies are produced during infection as a means of targeting foreign bodies. They are an integral component of acquired immunity and have vast utility as biochemical reagents. This project focuses specifically on designing antibodies to increase targeted immunity during influenza infection.

Project: To model a previously identified cross-reactive anti-hemagglutinin monoclonal antibody: mAb S139/1 and to create mutant mAbs that target HA with higher specificity than naturally occurring mAbs.

***In silico* methods:**

- Create high-affinity, cross-reactive mAbs.
  1. Homology modeling (Blast/ClutalW, Rosetta Antibody or WAM web server, MODELLER)
  2. Protein-protein docking system (ZDOCK, ZRANK, RDOCK)
  3. Molecular Dynamics simulation (Amber 11.0)

Background – S139/1

- Cross-reactive monoclonal antibody(mAb) isolated from mice by Yoshida et. al. 2009.
- Conferred protection against challenge with H1, H2, H3 *in vitro* and *in vivo*.
- Key binding site for S139/1: Lys156, Gly158, Ser193.
  - Adjacent to sialic acid binding site on HA
  - Residues may help to recruit antibody to sialic acid binding domain to inhibit endocytotic uptake

Background - Project Aims

1. Model mAb S139/1 *in silico* and determine its binding affinity to H3.

2. Create mutant mAbs with higher binding affinities to specific H1, H2, H3 epitopes.

Background – canonical antibody structure, see Chothia et. al. 1989 for review

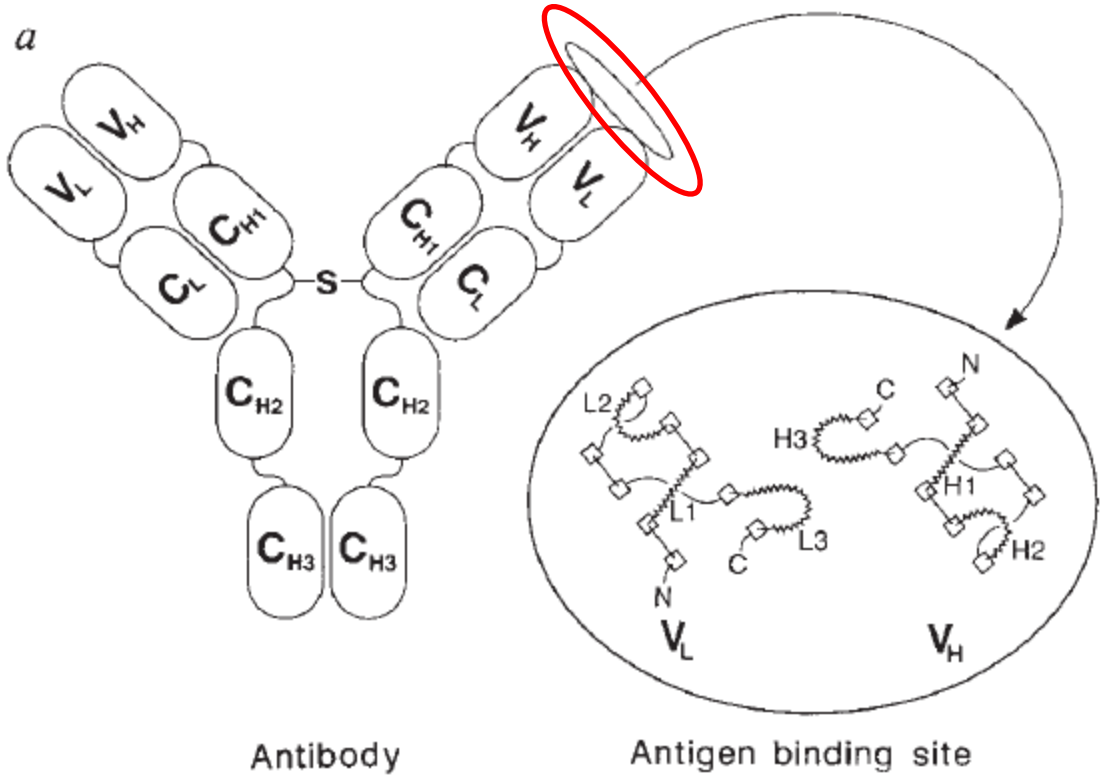


Figure 1: Taken from Chothia et. al. 1989 (*Nature*). A graphical depiction of IgG/IgG2a structural motifs. Complementarity determining regions **(**CDRs) are circled (red).

Obstacles – Constructing the **CDR** of antibody with no crystal structure

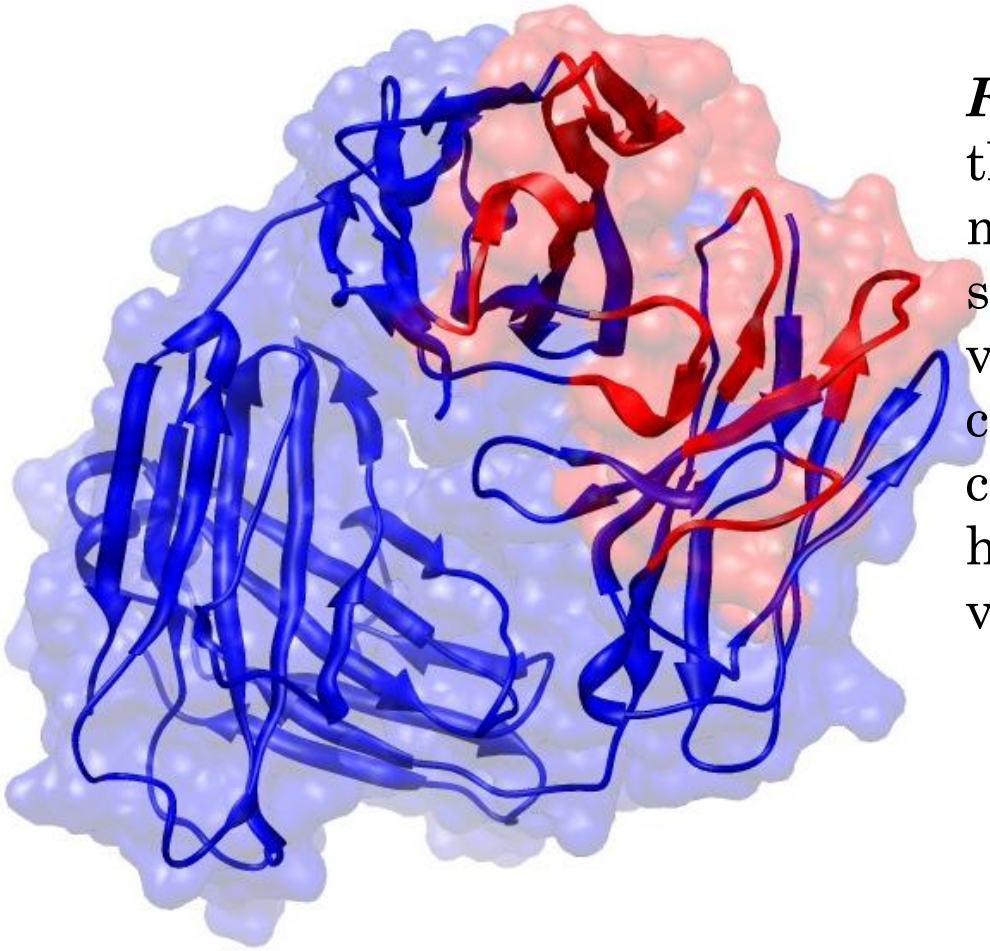
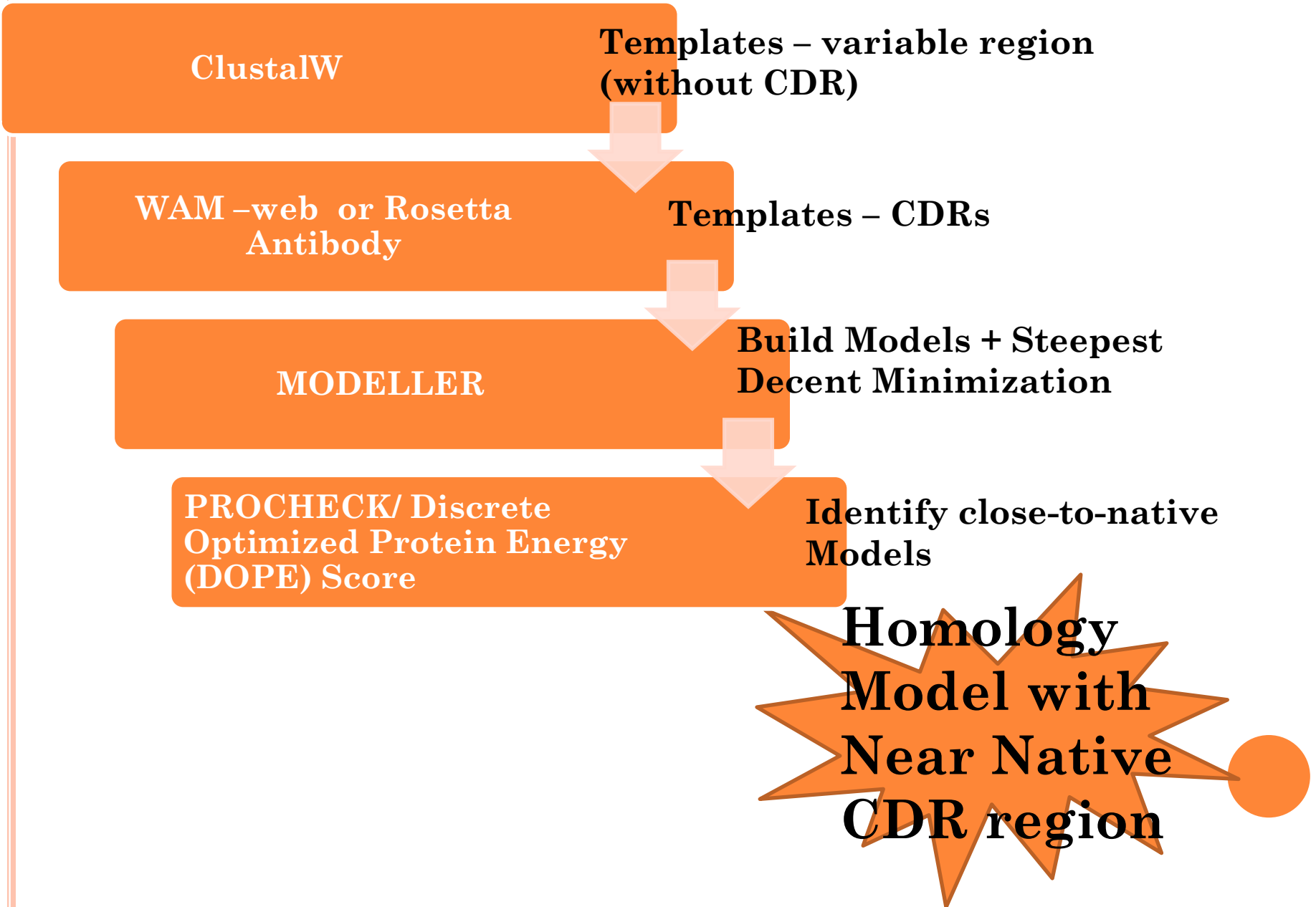


Figure 2: The CDR (red) of the variable antibody binding motif (blue) is subject to structural and sequence variation. The CDR region cannot be accurately constructed using traditional homology modeling due to this variability.

METHODS – HOMOLOGY MODELING



Homology Model

Target (S139) :

Humanized antibody, no secondary structure information

Template Selection:

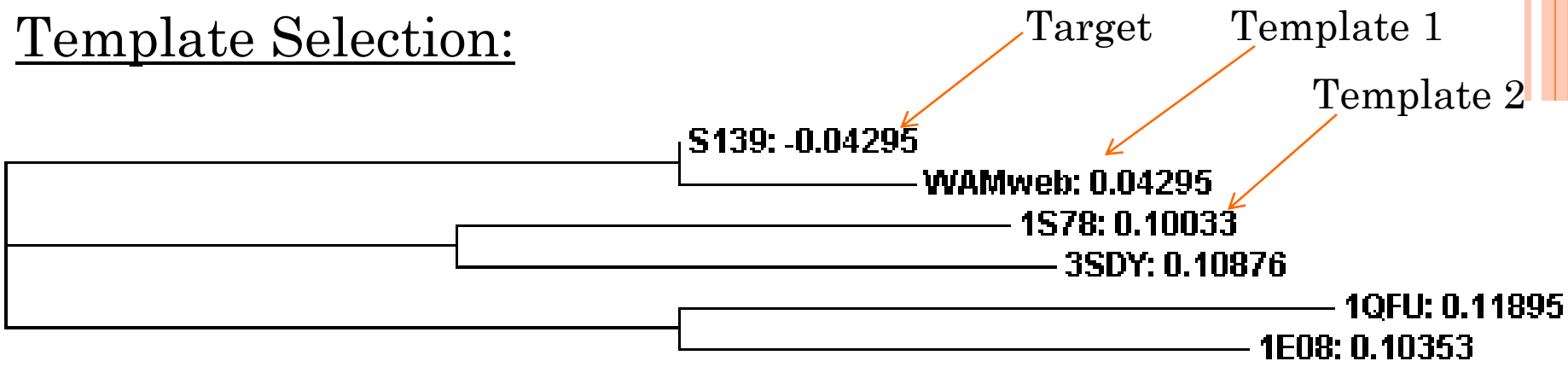


Figure 3. Phylogenetic tree used in template selection. WAM-web model and 1S78, humanized anti-ERb2 antibody, were selected. Other anti- hemagglutinin antibodies, 3SDY, 1QFU, 1E08, did not meet criteria for template selection (sequence identity, clade). Note Rosetta template and WAM template share 100% sequence identity.

Homology Model: S139/1

Web Server	Web address	Numbering	Template selection	L1, L2, L3, H1, H2 templates	H3	RMSD (Å)
Rosetta Antibody Server	http://antibody.graylab.jhu.edu/	Chothia	BLAST (Sequence + Loop length)	Grafted	Ab initio + Monte Carlo	//
WAM web	http://antibody.bath.ac.uk/	Chothia + Extended Version	BLAST (Canonical Class + Sequence Homologs)	Grafted + five rounds of steepest decent	CAMAL [Database search + CONGEN + Final Filter of top 5 structures]	//
						1.137

Figure 4. Table comparison of the servers available for mAb CDR template generation. Both were used to construct the CDRs of S139/1. The RMSD was calculated between the α-carbon atoms of the hypervariable region, and can primarily be attributed to differences in the H3 loop.

Homology Model: S139/1

Web Server	MODELLER	DOPE Score	PROCHECK	RMSD (Å)	RDOCK score (ΔG)	Average RDOCK Score
Rosetta Antibody Server	Aligned to templates + 1 Round of Minimization	-39499.30078	80.10%	//	-15.5572	-5.3868
WAM web	Aligned to templates + 1 Round of Minimization	-41088.45703	86.4.%	//	-15.0288	-8.51736
				19.198		

Figure 5. Table comparison of the mAb homology models produced using WAM web and Rosetta Antibody. DOPE score, PROCHECK evaluation completed with PDBsum, and RMSD of α-carbon atoms from the top scoring models were used to compare the constructs.

Homology Model: S139/1

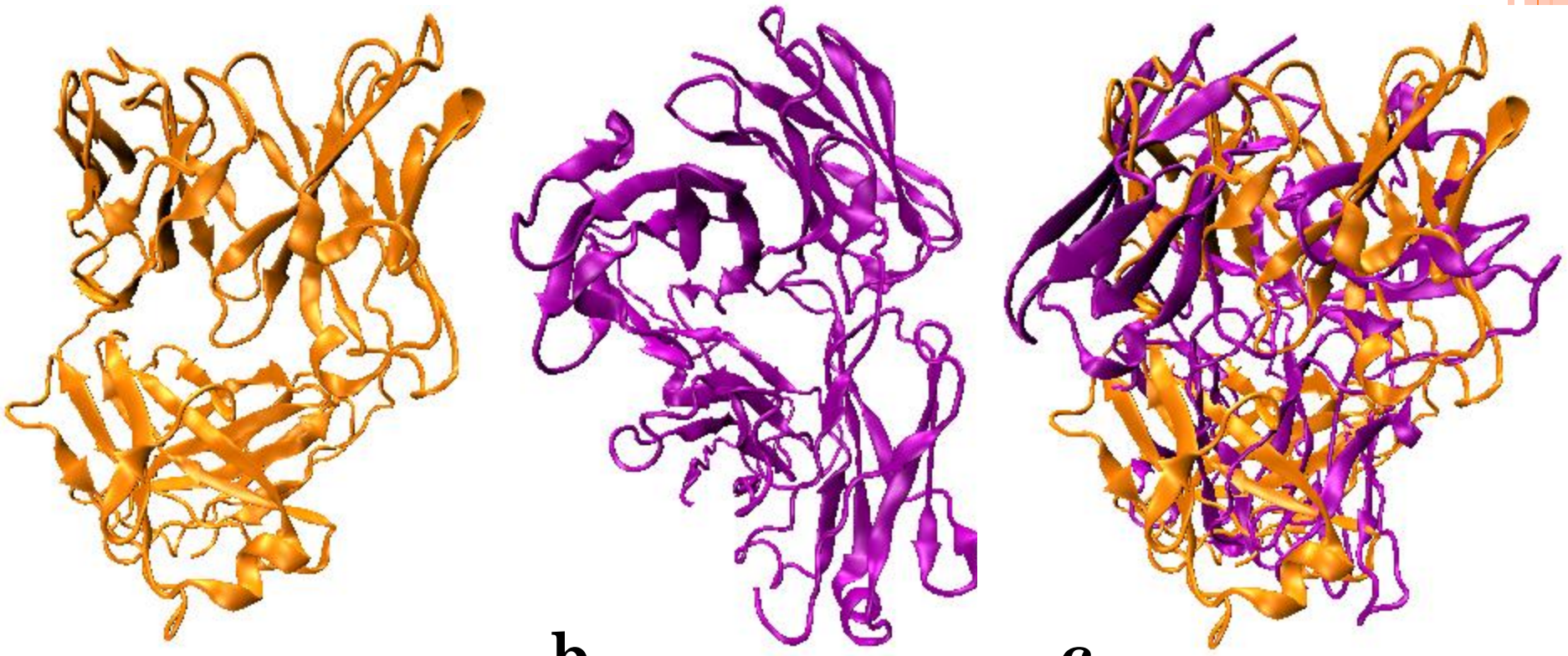


Figure 6. (a) Model produced with WAM template (b) Model produced with Rosetta template (c) Superimposed structures. WAM web model was selected for further docking studies.

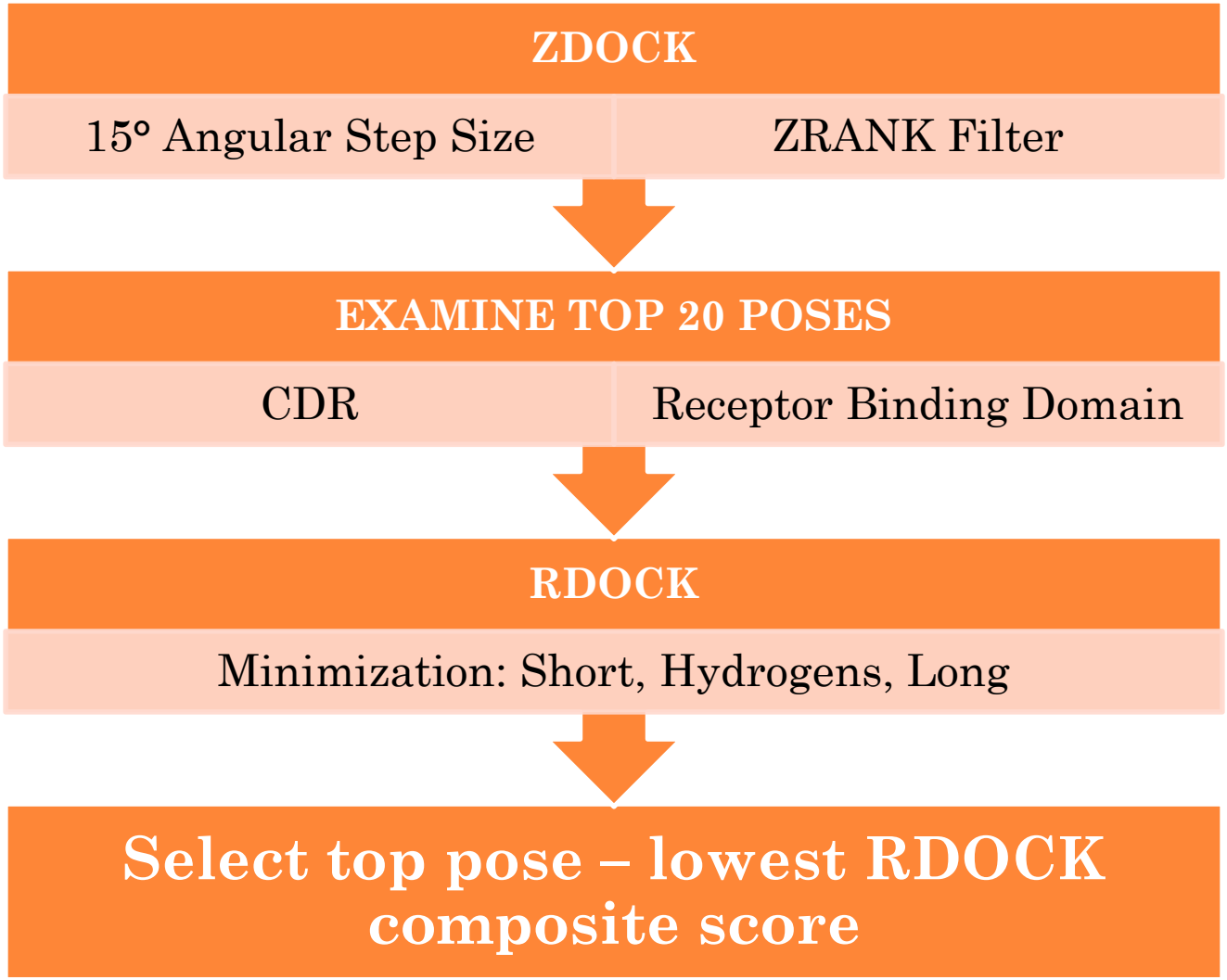
Obstacles – Docking

1. Selecting best rigid docking protocol – antibody-antigen Complexes
2. Minimization of top docked poses

Solution – ZDOCK/RDOCK

1. ZDOCK – developed on antibody-antigen complexes (CAPRI competition)
2. ZDOCK Theory:
   a. Pairwise shape complimentarity
   b. Desolvation
   c. Electrostatics
3. RDOCK – uses CHARMM with polar H for three minimization steps

Methods – docking



Methods –positive and negative control

1EO8 - Positive

- Mouse Fab (1gG2a)
- Known anti-HA antibody
- Co-crystallized structure (HA and Fab of mAb) available

3IET -Negative

- Mouse Fab (IgG2a)
- Known anti-Tn antigen expressed on the surface of tumor cells
- Crystal structure available

Findings - Docking

PDB ID	Pose	ZDOCK Rank	RDOCK Score (ΔG)	Mean
1EO8	Low	4	-22.8113	-18.6771
	High	9	-13.9934	
S139/1	Low	1	-15.0288	-8.51736
	High	11	7.08337	
3EIT	Low	14	1.66651	5.523396
	High	19	9.86859	

Figure 7. RDOCK score of highest and lowest ranked docked poses taken from the ZDOCK results for the positive (1EO8) and negative (3EIT) controls and homology model of S139/1. ZDOCK's minimization/scoring algorithm can semi-quantitatively predict binding affinity for these antibodies.

RDOCK– S139/1

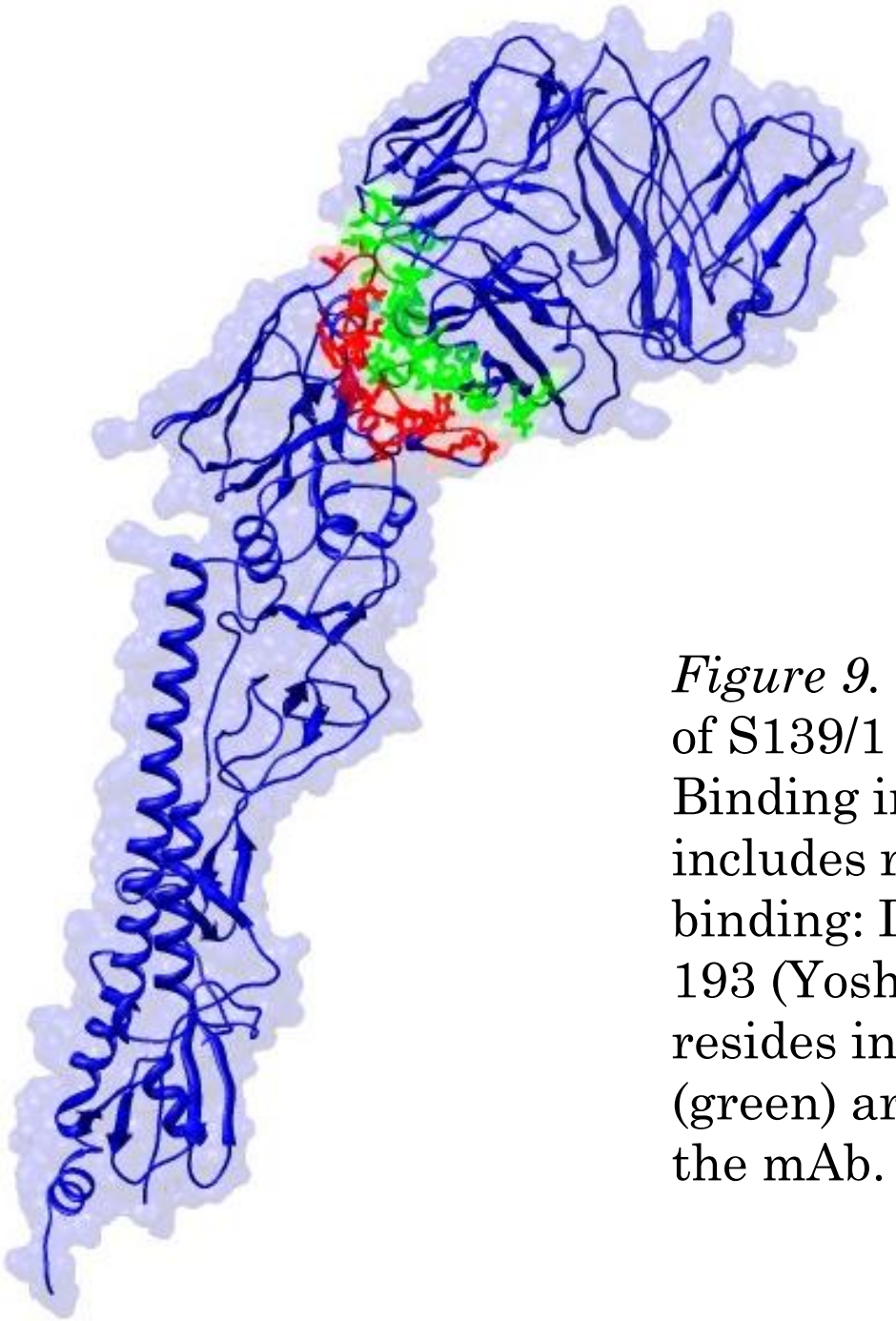


Figure 9. Top ranked docked pose of S139/1 and HA (-15.0288 ΔG). Binding interface of HA (red) includes residues critical for binding: Lys 156, Gly 158, Ser 193 (Yoshida et. al. 2009). 53% of resides in S139/1binding interface (green) are in the CDR region of the mAb.

RDOCK– S139/1

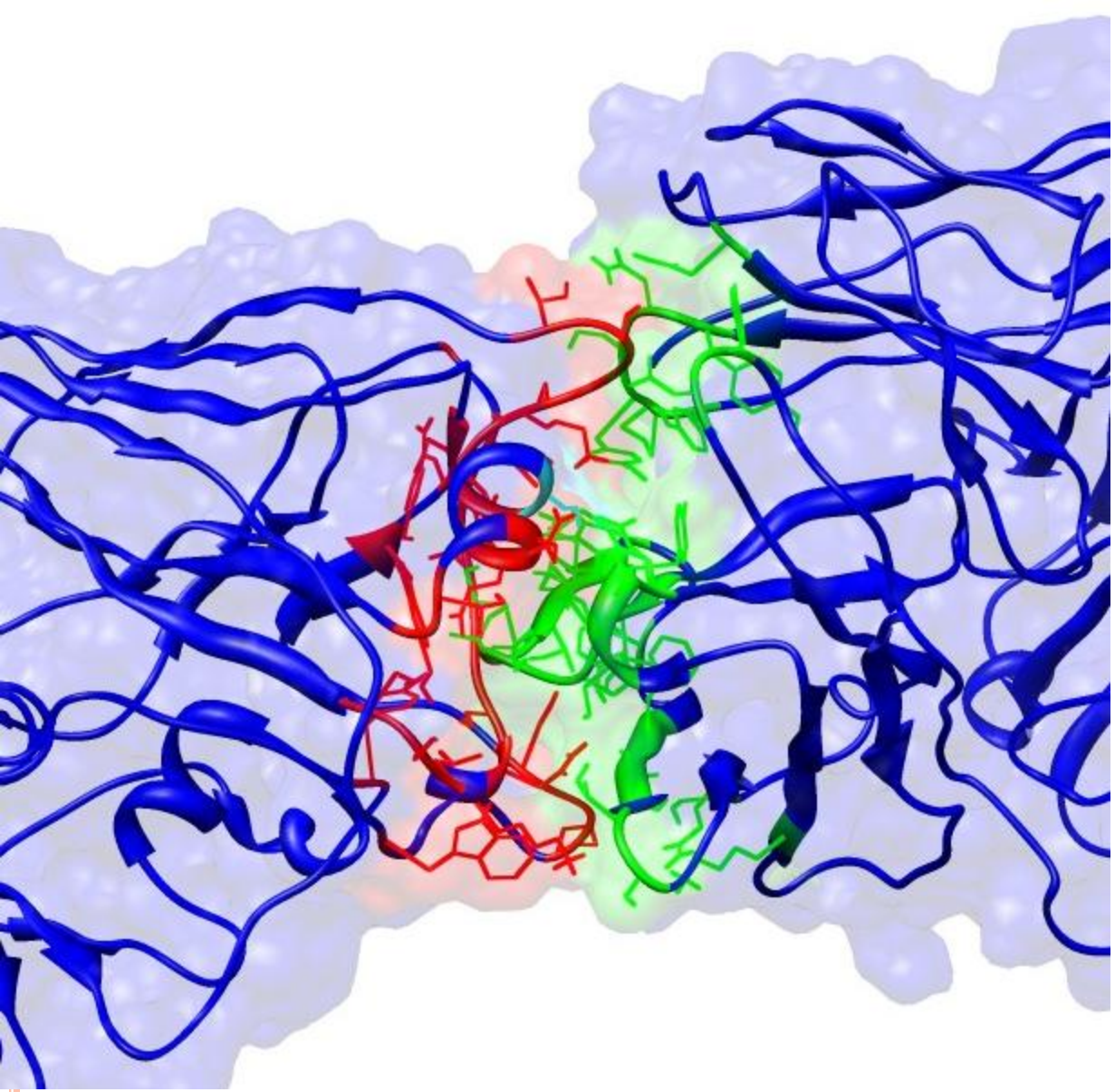
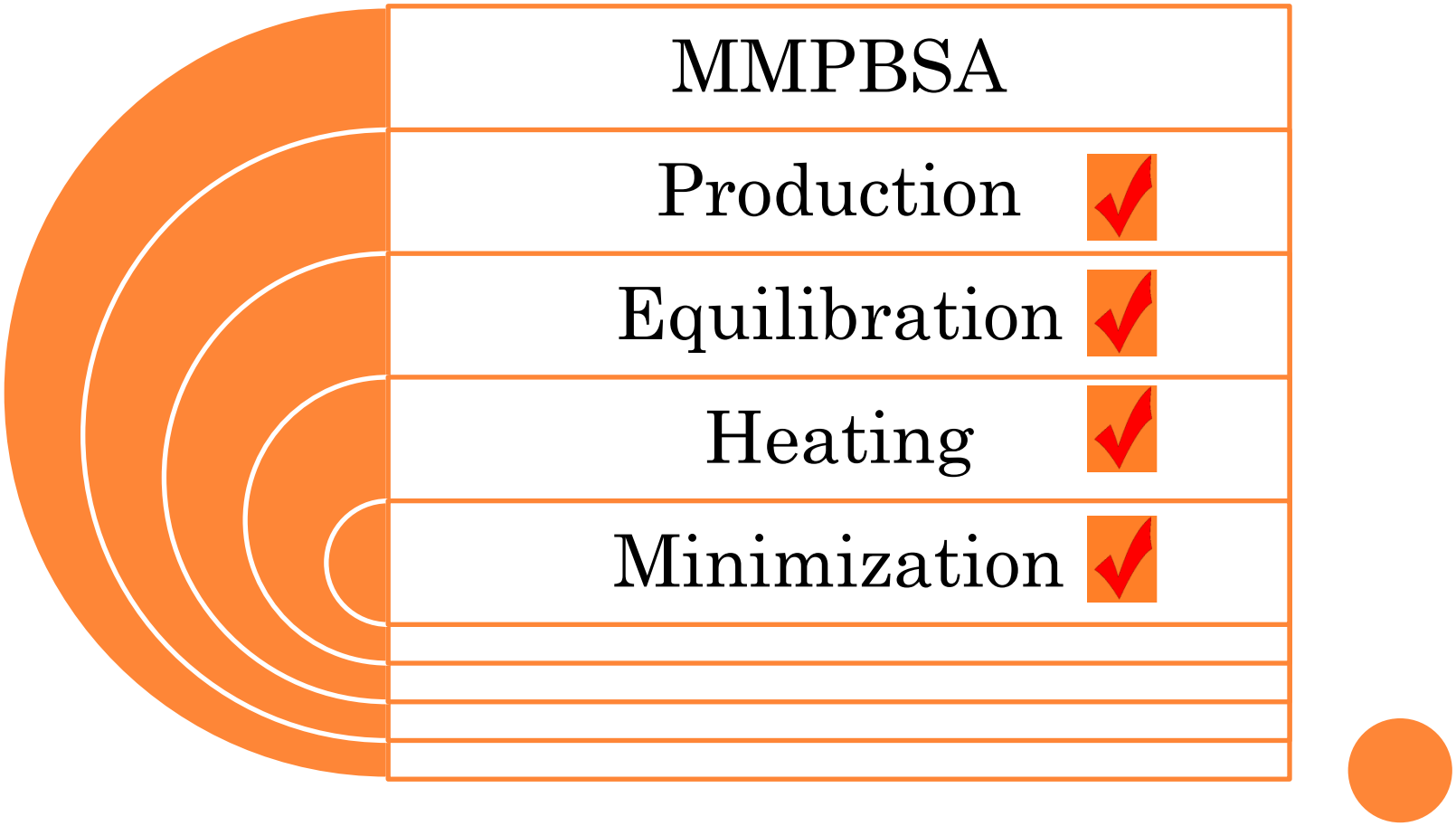


Figure 10. Binding interface of S139/1 that are in the CDR region:

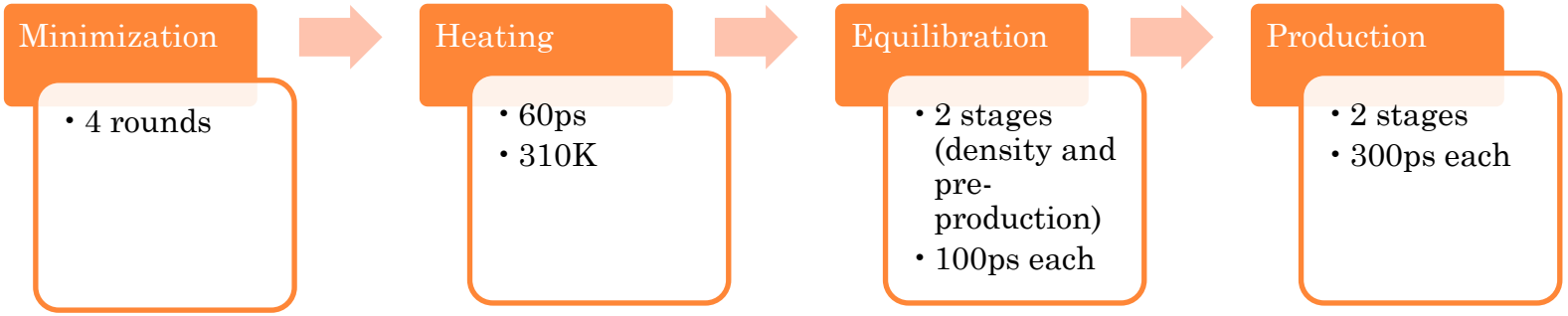
L_1 – Asn32
L_3 – Tyr91, Asn92, Ser93, Tyr94, Pro95, Tyr96,

H_1 – Ser244, Ser245, Trp247
H_2 – Glu268, Ile269, Gly270, Met271, Thr272, Asn273,
H_3 – Asp315, Tyr316

Molecular Dynamics-Current



Methods – MD



Total

- 860ps

Energy – 1E08

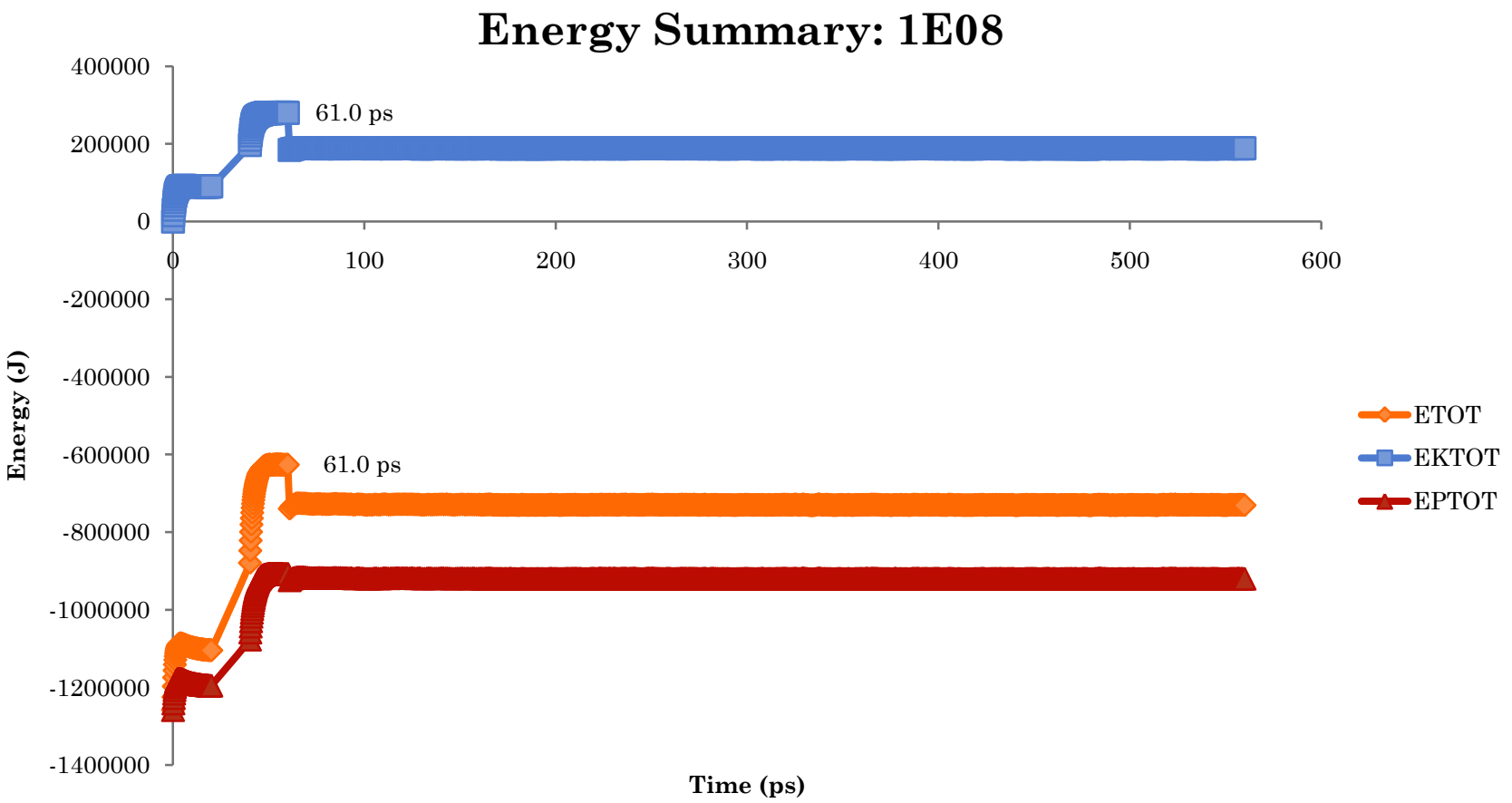


Figure 11. Total (orange), potential (red) and kinetic (blue) energy of the construct during the simulation (560ps). It appears to reach a minima after 61.0ps and remain stable for ~500ps.

RMSD – 1EO8

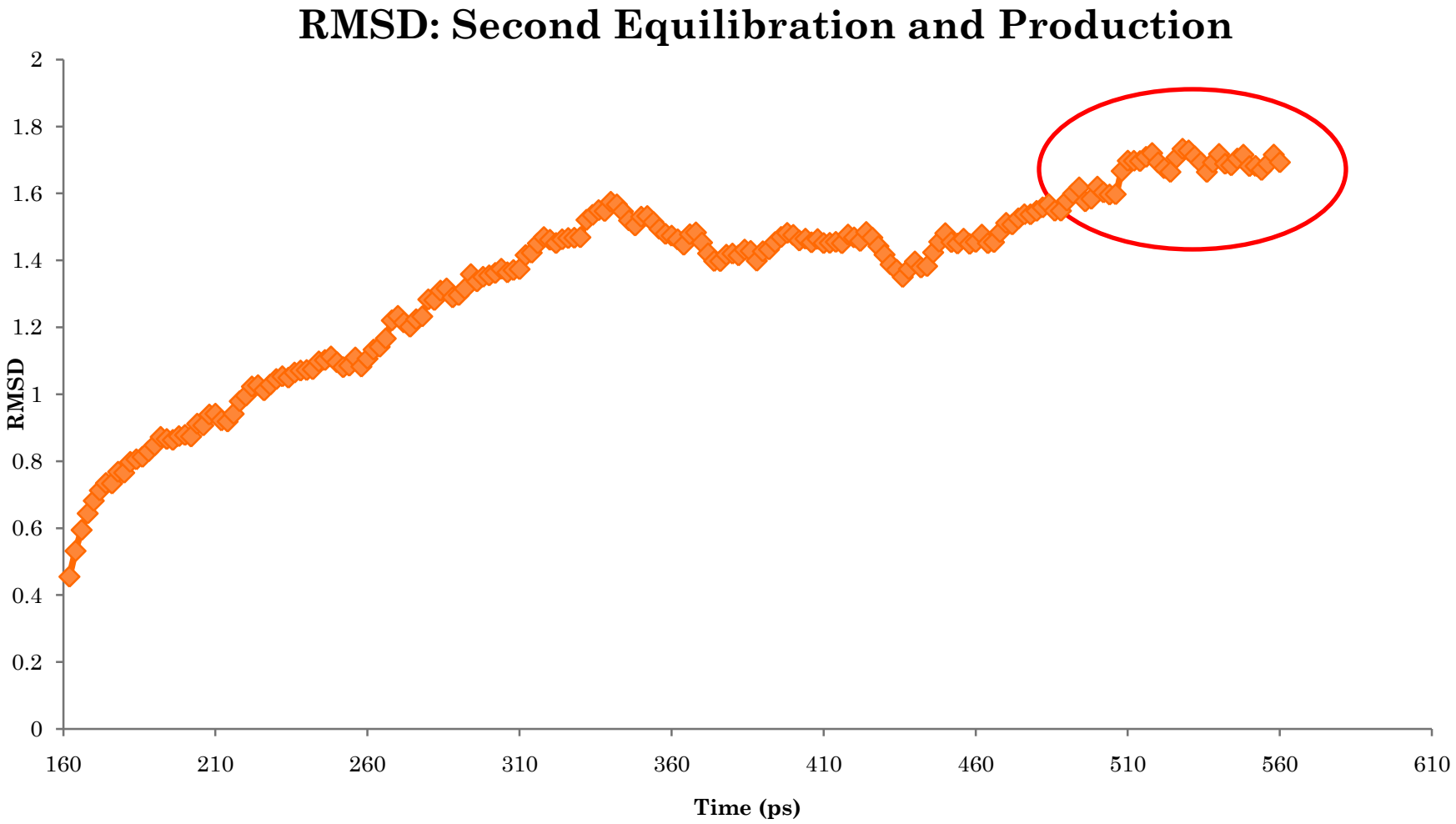


Figure 12. RMSD of main chain atoms during equilibration and production of 1EO8. The trajectory appears to be the most stable after the first 100ps of the first stage of production.

Trajectory – 1E08

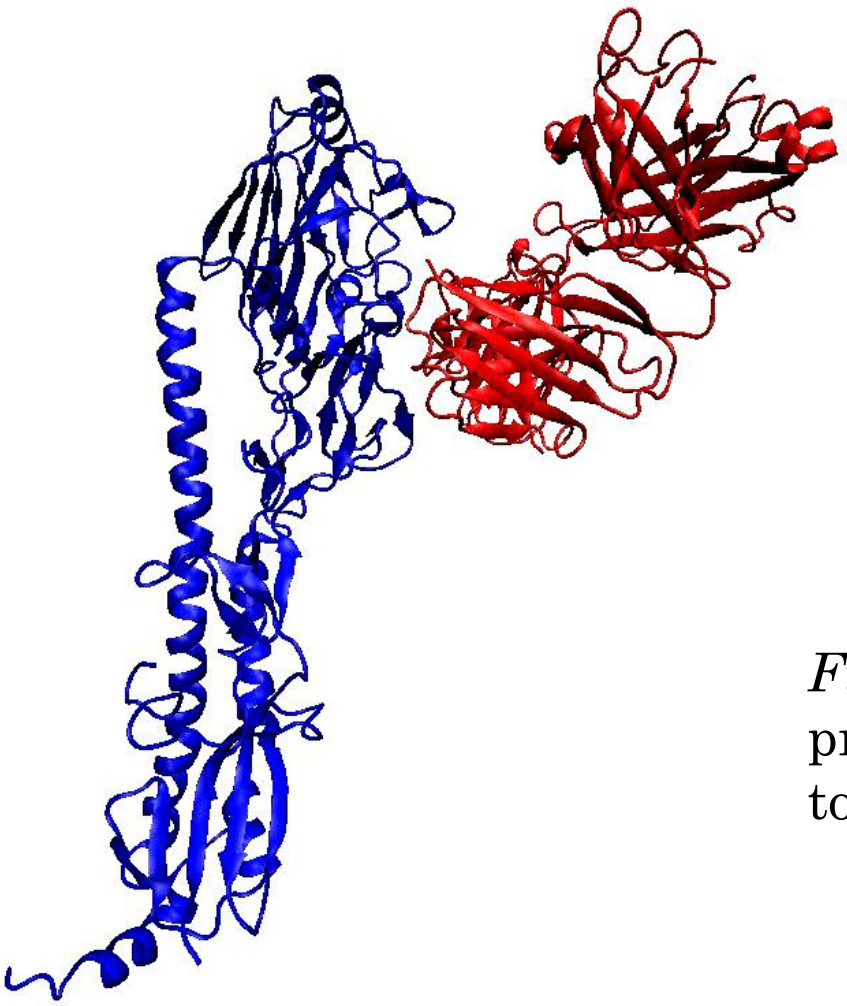


Figure 13. Last 50ps of production 1: 1EO8 (red) docked to H3 (blue).

Energy – 3EIT

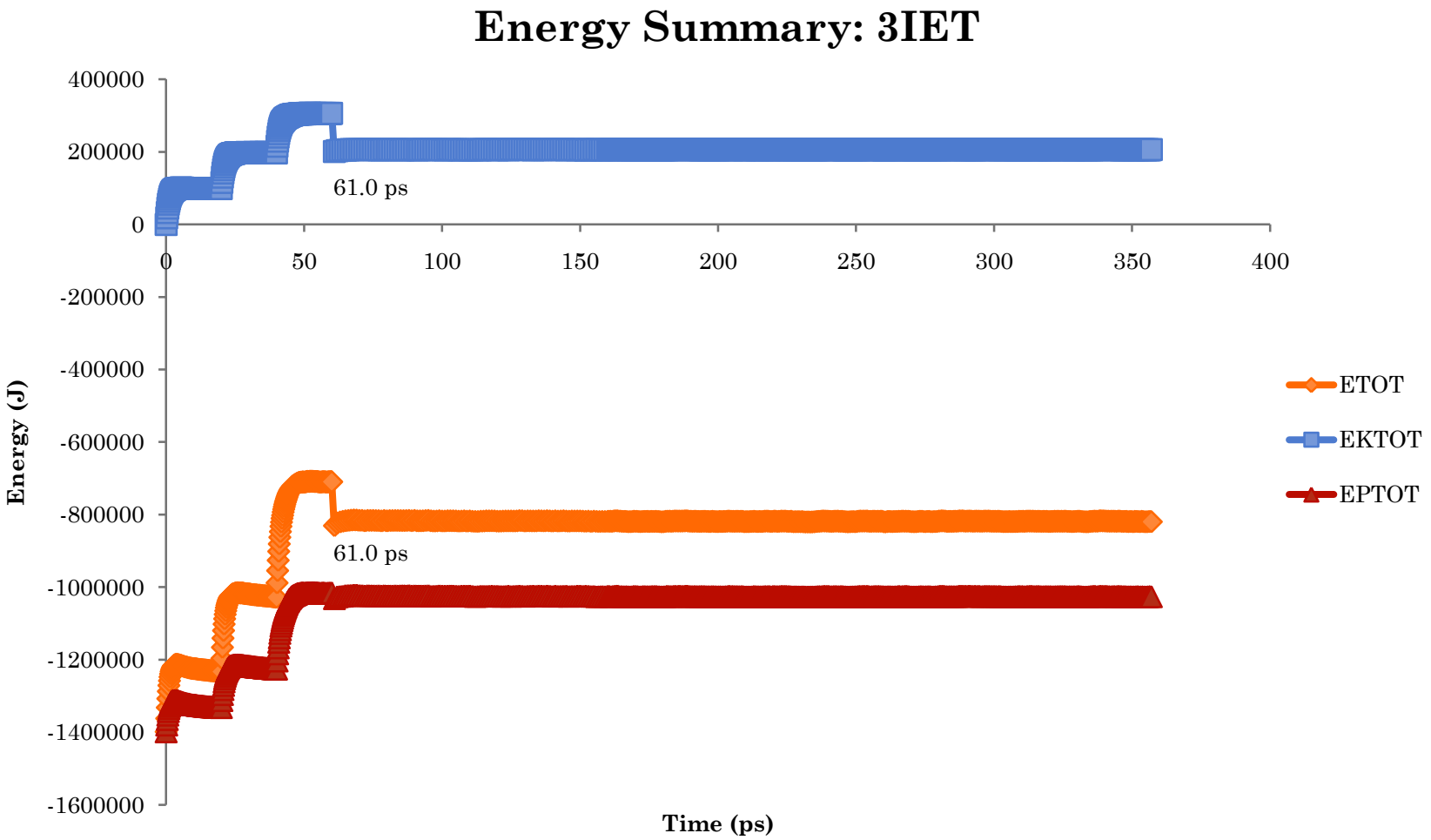


Figure 14. Total (orange), potential (red) and kinetic (blue) energy of the construct during the entire simulation (357ps). It appears to reach a minima after 61.0ps, but production has not been completed.

Trajectory – 3IET

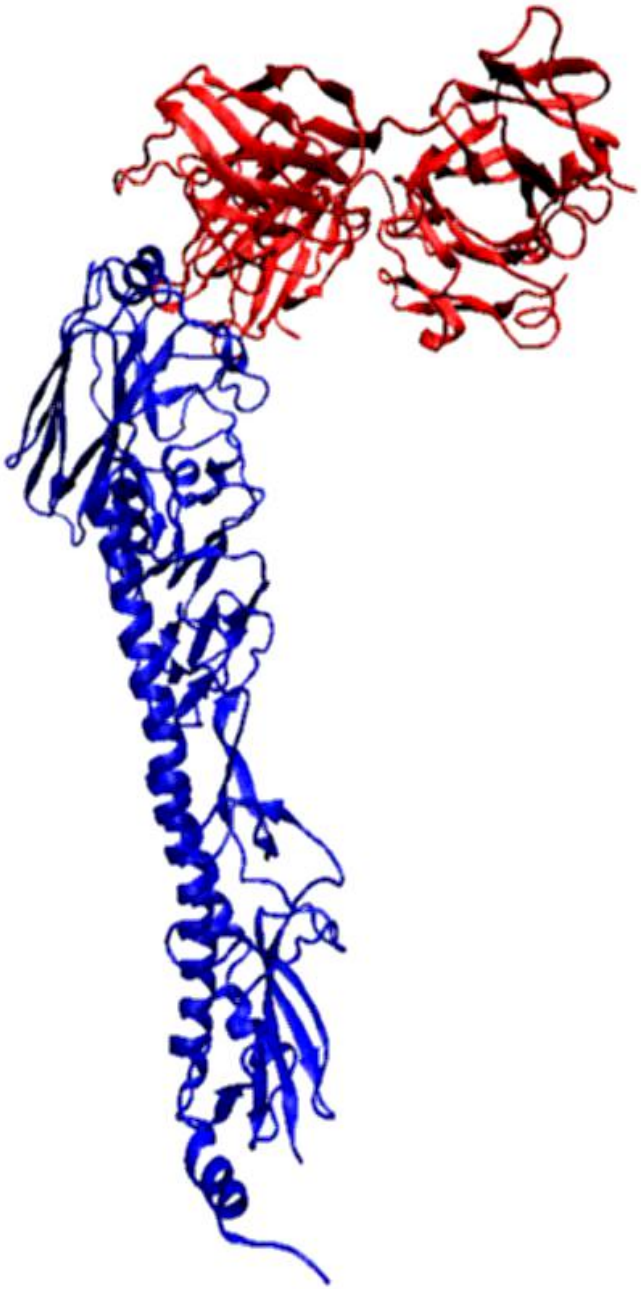


Figure 16. First stage of production: 3EIT (red) docked to H3 (blue).

Further Steps

- Begin MD for S139/1 – This week
- Use trajectory to calculate pair-wise residue interactions (possible with Amber 11.0) – UCSD
- Correlate pair-wise interactions with molecular contacts in RDOCK – UCSD
- Identify key residues for mutagenesis – UCSD

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