Protein Docking Study of *Plasmodium falciparum* Plasmepsin I & II to Normal and Sickle Hemoglobin

Sean C Marguet and Victor F Waingeh, Ph.D.
Indiana University Southeast, New Albany, IN 47150

Introduction

Malaria is a major infectious disease affecting millions of people worldwide annually. *Plasmodium falciparum* is the most virulent of the malaria parasites and its resistance to currently available drugs continues to grow, presenting an impediment to attempts to contain the disease.

People with the sickle cell trait have a natural resistance to infection from *P. falciparum*. The sickle cell trait causes the protein sickle hemoglobin to be expressed which has a GLU mutated to a VAL in position six of the beta chain.[1]

Plasmepsin I and II are aspartic proteases found in *P. falciparum* that degrade hemoglobin in the red blood cell as source of nutrients.[2]

Methods

In this study, protein docking was used to identify and explore potential binding modes of *P. falciparum* plasmepsin I & II to normal and sickle hemoglobin. Molecular dynamics (MD) was used to determine the binding energies of the protein-protein complex.

Protein Model:

- The structure of plasmepsin I from *Plasmodium falciparum* was obtained from the Protein Data Bank (PDB ID 3DRV)[3].
- The structure of plasmepsin II from *Plasmodium falciparum* was obtained from the Protein Data Bank (PDB ID 1IF4)[4].
- The structure of deoxygenated normal human hemoglobin was obtained from the Protein Data Bank (PDB ID 2HHB)[5].
- The structure of deoxygenated sickle hemoglobin was obtained from the Protein Data Bank (PDB ID 2HBS)[6].

Docking Studies:

- VMD and Chimera[7,8] were used to prepare the proteins for automated docking. For all the proteins, all hydrogens were added and structures were minimized.
- Plasmepsin I was docked with the dimer of normal and sickle hemoglobin. Plasmepsin II was docked with dimer and tetramer of both normal and sickle hemoglobin.
- Docking was preformed using the ZDOCK Protein-Protein Docking Server[9].
- Top 10 protein-protein complexes were generated and statistically analyzed.
- Namd2[10] was used to minimize the energy and run MD simulations on selected complexes.

Results

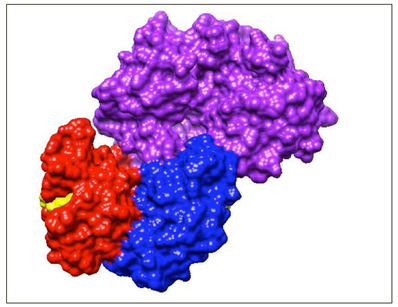

Figure 1: Plasmepsin I with Normal Hemoglobin Dimer Binding Mode with the Beta Chain

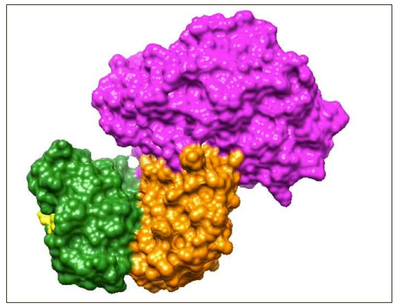

Figure 2: Plasmepsin II with Sickle Hemoglobin Dimer Binding Mode with the Alpha Chain

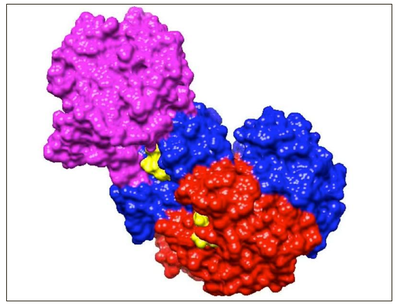

Figure 3: Plasmepsin II with Normal Hemoglobin Tetramer Binding Mode with the Beta Chain

Plasmepsin I	Plasmepsin II
Asn 108	Glu 74
Pro 110	Lys 238
Val 238	Pro 240
Phe 242	Phe 241
Ser 280	Pro 295

Figure 4:Critical Amino Acid Residues in Binding

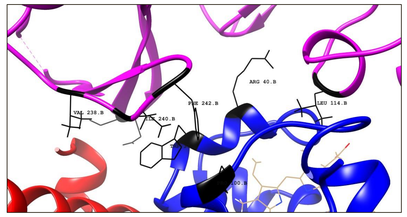

Figure 5: Plasmepsin I with Normal Hemoglobin Dimer Binding Site

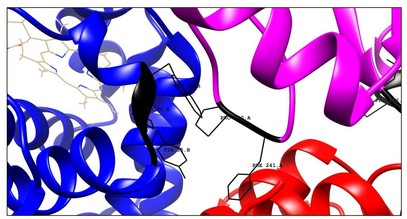

Figure 6: Plasmepsin II with Normal Hemoglobin Dimer Binding Site

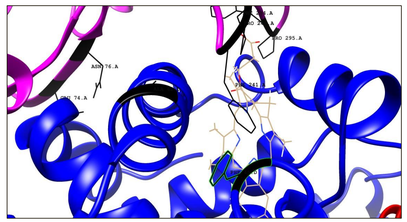

Figure 7: Plasmepsin II with Normal Hemoglobin Tetramer Binding Site

(kcal/mol) x(10^3)	Normal Dimer	Sickle Dimer	Tcal
Plasmepsin I	-0.56	-1.24	7.473
Plasmepsin II	4.64	-2.89	2.585
Tcal	1.785	18.618	-------

Figure 8:Binding Energy in kcal/mol x10^3

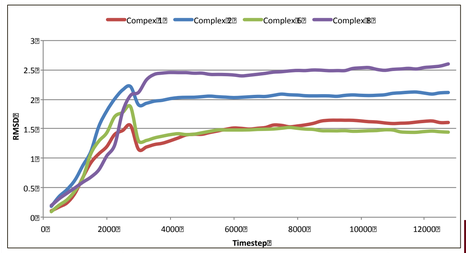

Figure 9:RMSD variation with Time-step Plot for Normal Dimer with Plasmepsin I

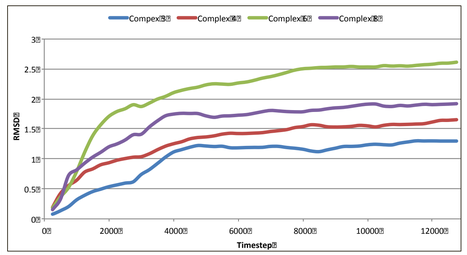

Figure 10:RMSD variation with Time-step Plot for Sickle Dimer with Plasmepsin I

Conclusions

- ✓ Plasmepsin I has potential binding modes with the dimer of normal and sickle hemoglobin which favorer the beta chain of hemoglobin.
- ✓ Plasmepsin II has potential binding modes with the dimer of normal and sickle hemoglobin on the alpha chain of hemoglobin.
- ✓ The binding modes of plasmepsin II to the tetramer of normal and sickle hemoglobin are to both alpha and beta chains.
- ✓ Plasmepsin I shows significantly more favorable binding to sickle hemoglobin dimer.

Future Studies

Docking of plasmepsin I & II to oxygenated normal and sickle hemoglobin.

Mutation study to determine the contribution of the identified critical residues in binding.

Detailed calculations to determine entropy contributions to the binding energy.

References

1. Lauren Gong, Sunil Parikh, Philip J Rosenthal, Bryan Greenhouse. *Malaria Journa*l, **2013**, 12: 317
2. Pandey, Amit; Chaunhan, Virander. *Current Science*, **1998**, 95.9, 911-916
3. Bhaumik, P., Horimoto, Y., Xiao, H., Miura, T., Hidaka, K., Kiso, Y., Wlodawer, A., Yada, R.Y., Gustchina, A. *J.Struct.Biol*, **2011**, 175: 73-84
4. Asojo, O.A., Gulnik, S.V., Afonina, E., Yu, B., Ellman, J.A., Haque, T.S., Silva, A.M. *J.Mol.Biol*, **2003**, 327: 173-181
5. Fermi, G., Perutz, M.F., Shaanan, B., Fourme, R. *J.Mol.Biol*, **1984**, 175: 159-174
6. Harrington, D.J., Adachi, K., Royer Jr., W.E. *J.Mol.Biol*, **1997**, 272: 398-407
7. Humphrey, W., Dalke, A. and Schulten, K., "VMD - Visual Molecular Dynamics", J. Molec. Graphics, 1996, vol. 14, pp. 33-38
8. UCSF Chimera--a visualization system for exploratory research and analysis. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.
9. Pierce BG, Wiehe K, Hwang H, Kim BH, Vreven T, Weng Z. (2014) ZDOCK Server: Interactive Docking Prediction of Protein-Protein Complexes and Symmetric Multimers. Bioinformatics 30(12): 1771-3.
10. James C. Phillips, Rosemary Braun, Wei Wang, James Gumbart, Emad Tajkhorshid, Elizabeth Villa, Christophe Chipot, Robert D. Skeel, Laxmikant Kale, and Klaus Schulten. Scalable molecular dynamics with NAMD. Journal of Computational Chemistry, 26:1781-1802, 2005

Acknowledgments

Support for this work was provided by the IU Southeast Office of Research and Grants.

Sean Marguet was supported by a IU Southeast Student Research Assistant Fellowship.