Modeling the Heme-Sensing Region of *Bacillus anthracis* HssS: Docking Studies in Biological Systems

Grace Scofield¹, Michael F. Falcetta¹, Devin L. Stauff², Mark C. Fair³

¹Department of Chemistry, Grove City College, ²Department of Biology, Grove City College, ³Department of Physics and Department of Mechanical Engineering, Grove City College

BACKGROUND

- ❖ *Bacillus anthracis* is the bacterial species responsible for anthrax
- ❖ *B. anthracis* has a two-component signaling system (seen at right) consisting of a heme sensor system (HssS) and a heme response regulator (HssR)
 - ❖ While *B. anthracis* requires high levels of heme to function, too much heme is toxic to the bacteria
 - ❖ Histidine kinase is a transmembrane protein dimer that functions as the HssS in *B. anthracis*
 - ❖ Heme outside of the cell membrane docks to an uncharacterized site in histidine kinase, activating the two-component signaling system
- ❖ The 3D structure of the sensing region and the site where heme binds to histidine kinase have not been experimentally determined
 - ❖ Based on the known amino acid sequences in histidine kinase that are conserved between species, we can predict that heme (shown below right) will likely bind near a particular loop in the protein (shown below left)
 - ❖ Using this prediction, we can use computational modeling to dock heme in a model of the histidine kinase dimer

The two-component signaling system of *B. anthracis*¹

This is the loop of conserved amino acids shown in shaded region that are conserved between species. We predict that heme will bind in this region.

Heme molecule

QUESTIONS

- ❖ Can we create a realistic model of histidine kinase as a transmembrane protein (including the protein dimer, lipid bilayer, and explicit solvent) using Molecular Operating Environment (MOE)² software and NAMD³ molecular dynamics software and use this to predict heme binding sites for the histidine kinase dimer?
- ❖ Can modeling studies suggest experimental strategies for testing the proposed binding site and mechanism?

METHODS

- ❖ Stauff group provided a model of the histidine kinase monomer created with I-TASSER software, based on structural motifs (PDB code: 4WY9)
- ❖ Forcefield: AMBER10⁴
- ❖ We predicted that both hydrophobic alpha helices would point downward into the lipid bilayer membrane
 - ❖ MOE's Loop Modeler was used to perform a conformational search (left: original conformation, right: conformation after search)

Original histidine kinase monomer model

Histidine kinase monomer model with alternative loop conformation

- ❖ Protein-Protein Docking was performed using MOE to create a histidine kinase dimer model
 - ❖ Protonation states were determined and energy minimization was performed on the dimer model with MOE's QuickPrep tool
- ❖ A lipid bilayer membrane with 64 DPPC lipids and 3864 water molecules was downloaded from University of Calgary (<http://people.ucalgary.ca/~tieleman/download.html>)
 - ❖ The membrane was manually expanded in MOE to better enclose the hydrophobic alpha helices of the histidine kinase dimer
 - ❖ The water molecules were deleted from the system
- ❖ MOE's Solvate feature was used to solvate the entire system in a layer of water without salt (margin of 12.0 Angstroms)
- ❖ Site Finder identified potential binding sites on the protein dimer
 - ❖ We tested all identified heme binding sites based on our previous predictions and retained three most promising sites
- ❖ Docking was performed using MOE's Dock feature
 - ❖ All atoms in the system were chosen for the receptor (dimer, membrane, and explicit water molecules)
- ❖ MD simulations using NAMD and the NVT ensemble were performed on the heme-protein-membrane systems based on docking the heme in each of the three retained docking sites
 - ❖ 100 ps, 0 K minimization
 - ❖ 100 ps, 0-300 K heating
 - ❖ 100 ps equilibration, 500 ps production, and 100 ps cooling
- ❖ Final frame from each simulation was used in re-docking calculations
 - ❖ Rigid receptor refinement and GBVI/WSA dG scoring

RESULTS

- ❖ Final geometry from MD run yielding best docking score

- ❖ Re-docking scores from MOE of -16 to -18 kcal/mol indicate strong ligand-protein attraction

- ❖ Ligand interaction diagram for heme re-docked in site shown above

- ❖ Interactions with highly conserved amino acids Arg 151, Arg 93, and the pocket being formed by residues from the highly conserved range (124-129) are consistent with this site being a critical binding site for the protein

FUTURE WORK

- ❖ Use NAMD to calculate absolute free energy of binding of heme to protein system using approach of Chipot and coworkers.⁵

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