Exp 6: Surface Analysis

BY YUEJIAN MO

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Introduction

Here, we will study the surface of Huaman hemoglobin, including hydrophobicity, conservation and electrostatic potential. We will use APBS to generate a electrostatic potential surface.

> APBS, the Adaptive Poisson-Boltzmann Solver, is a freely available macromolecular electrostatics calculation program released under the GPL. It is a cost-effective but uncompromised alternative to GRASP, and it can be used within PyMOL. PyMOL can display the results of the calculations as an electrostatic potential molecular surface.

Methods

1. Load human hemoglobin structure (PDB id: 4HHB) in PyMOL

   ```
   PyMOL> fetch 4HHB
   ```

2. Create new objecs containing beta-subunit.

   ```
   PyMOL> select beta, chain B
   ```

3. Separate human hemoglobin and hemo group into two objects

4. Generate the surfarces to display the two different properties of the protein

   - Hydrophobicity

     ```
     PyMOL> set surface_color, white, beta,

     PyMOL> show surface, beta

     PyMOL> select hydrophobicity, resn Ala+Val+IIe+Leu+Met+Phe+Tyr+Trp in
     beta

     PyMOL> remove backbone in hydrophobicity

     PyMOL> set surface_color, blue, hydrophobicity

     PyMOL> show surface, hydrophobicity

     PyMOL> set transparency, 0.2
     ```

   - Conservation

     I aligned protein 4HHB-B, 4BJA-A,4MPM-A,4MPM-B using structural information by tcoffee. The identical residues were marked as red, and highly similar residues were marked as respberry, and similar residues were marked as warmpick.

5. Generate the electrostatic potential surfaces for the tetrameric hemoglobin

Action $\longrightarrow$ generate $\longrightarrow$ vacuum electrostatics $\longrightarrow$ protein contact potential (local)

6. Use APBS program to calculte the electrostatic potential surface

7. Study the muatation E6V.

   Wizard$\longrightarrow$ Mutagenesis$\longrightarrow$ E6 $\longrightarrow$ V6

Results

1. Hydrophobicity

   Save the result in Hydrophobicity.pse sence 003. The hole which heme insert is hydrophobicity, in oder to keep heme inside hemoglobin.(Figure 1) Many sunkens surface is hydrophobic(Figure 2), which suggestes show the folding occuar to decrease system energy. However, here are raised surface is hydrophobic.(Figure 3) The hydrophobic environment between subunit interacte edge may explain this exception.

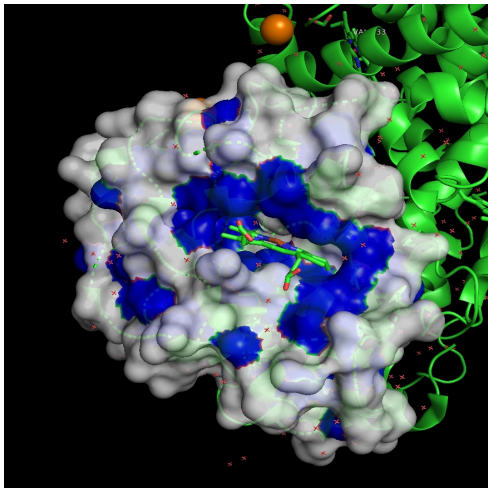

Figure 1. Heme insert to a hydrophobic hole

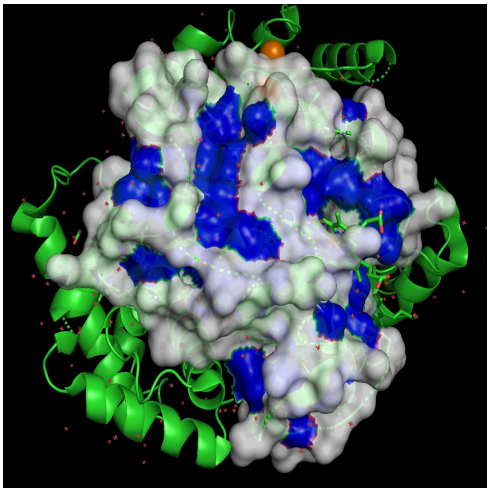

Figure 2. Much sunken surface is hydrophobic

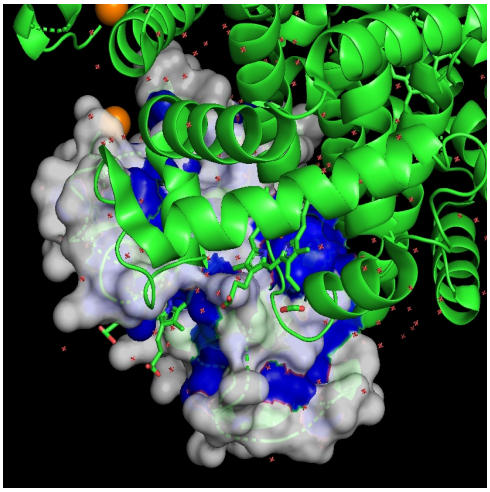

Figure 3. Subunit interacte surface is more hydrophobic

2. Convsersion

I find following :

i. The hemo binding sites are identical convservation.

ii. Most interaction edge is similar residues.

iii. At non subunit interaction surface, most loops and trun are conservation

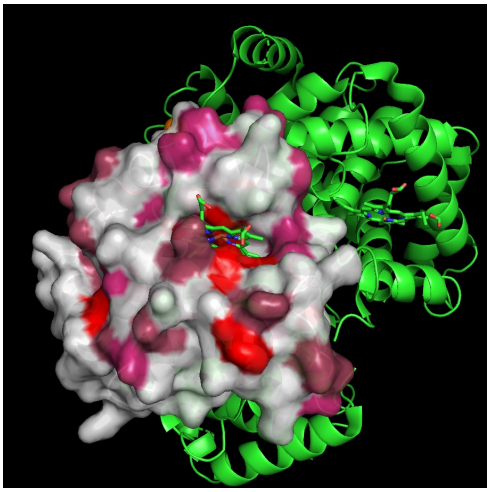

Figure 4. The Hemo binding site is identical convservation

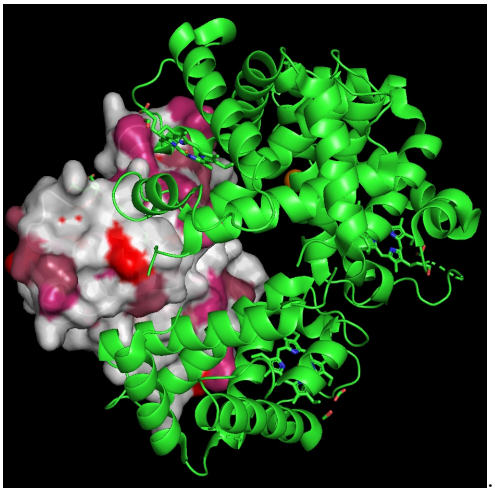

Figure 5. Most interaction edge is similar residues

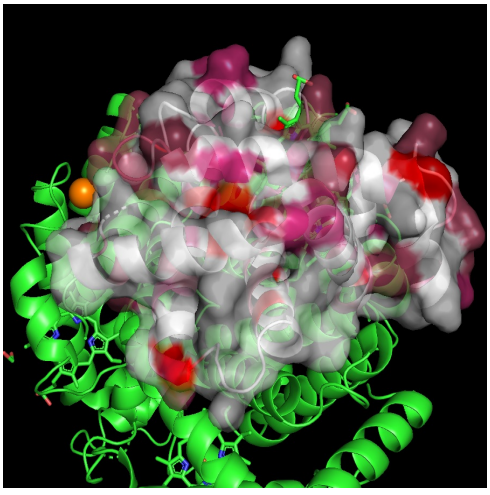

Figure 6. At no interaction surface,most loops and turn are conservation

3. Electrostatic in vacuum

Because 2,3-BPG is negative charge, I find a positive charged surface between two β subunits.(Figure 4)(Reference 4)(electrostatic.pse sence 3). I find that four Heme interactioal surface on hemoglobin are positive charged (electrostatic.pse sence 4). However, Heme doesn't show much negative feactures.

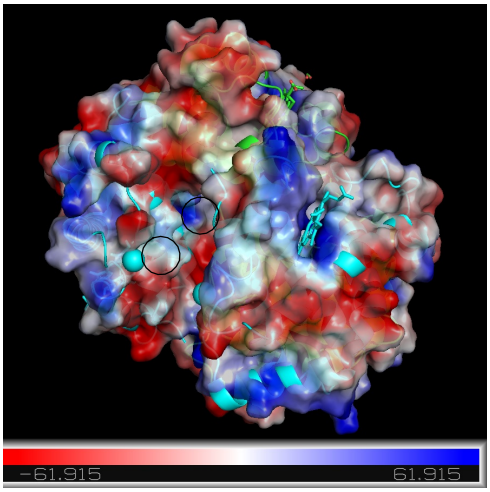

Figure 7. The positive charged pocket for 2,3-BPG binding. Butoom balck circle is a PO^{4-}, and top black circle is responding positive surface to hold PO^{4-}. (Two β subunits are colored by cyans.

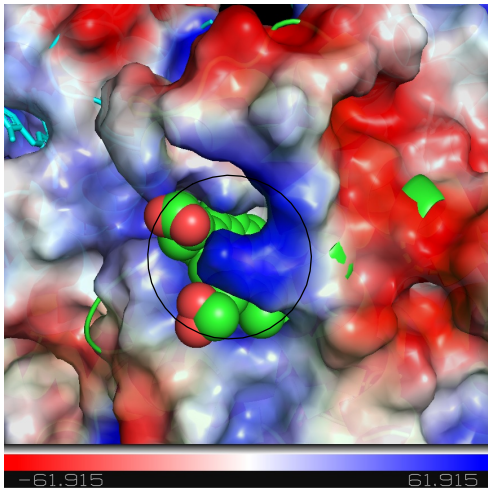

Figure 8. Here are positive charged surface on homoglobin around a Heme (Black circle).

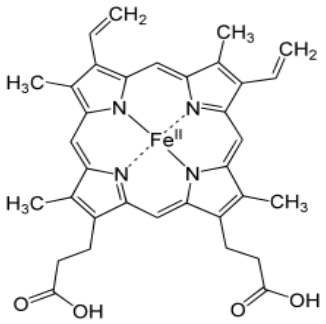

Figure 9. Heme b group (Ref 2)

4. Electrostatic potential surface using APBS program (APBS.pse)

   The 2,3-BPG binding surface show more charged than surrounding, which show high charge constract and more reasonable biogical function than simple calculation. Also, the charge distruction is more smooth and continue than simple calulation.

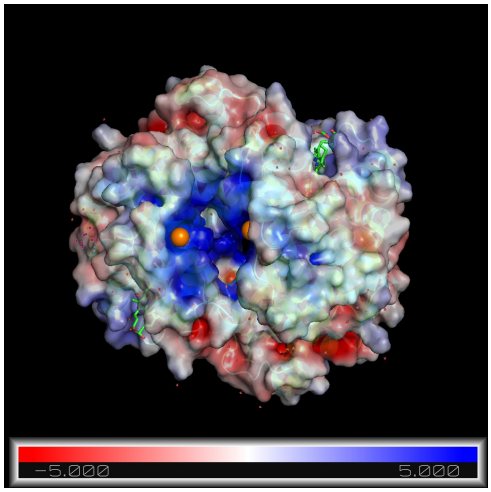

Figure 10. The 2,3-BPG site show smooth positive charged surface

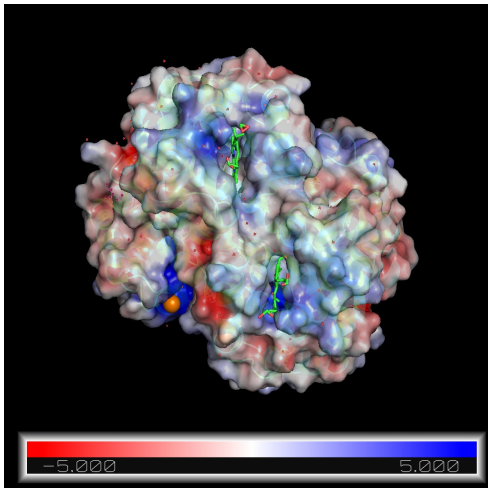

Figure 11. Surface arounds Heme

5.

E6V change this surface from hydroph to hydrophobic. Then E6V beta interacts weakly with the beta globin chain in an adjacent sickle hemoglobin molecule. The complex twisting, 14-strand structure of the bundles produces multiple interactions and cross-interactions between molecules. The mechaism is similar to fibrous proteins.

Conclusions

Powerful.

References

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