Exp 6: Surface Analysis

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Introduction

Here, we will study the surface of Huaman hemoglobin, including hydrophobicity, conservation and electrostatic potential.

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.

select hydrophobicity, resn Ala+Val+lle+Leu+Met+PheTyr+Trp

set surface_color, green, phobic

set transparency, 0.2

Results

1.

2.

3.

4. 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.

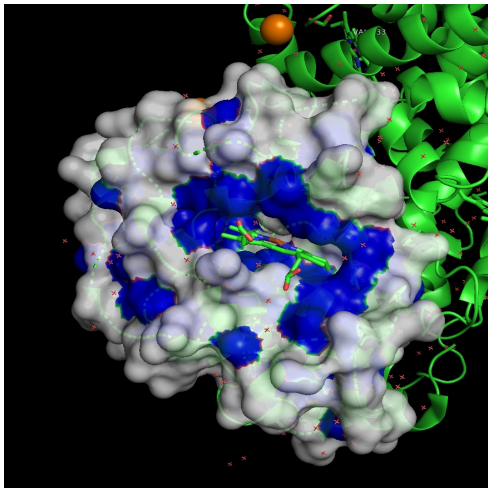

Figure 1. Heme insert to a hydrophobic hole

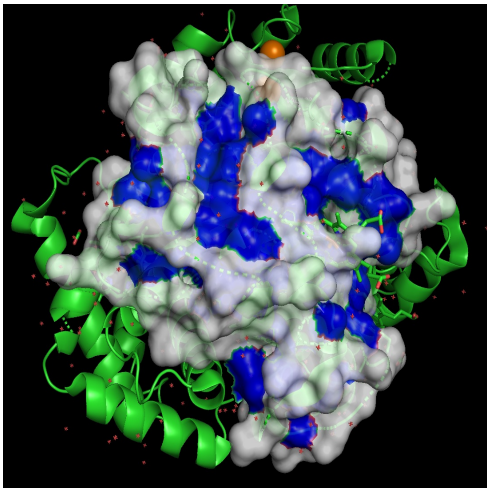

Figure 2. Much sunken surface is hydrophobic

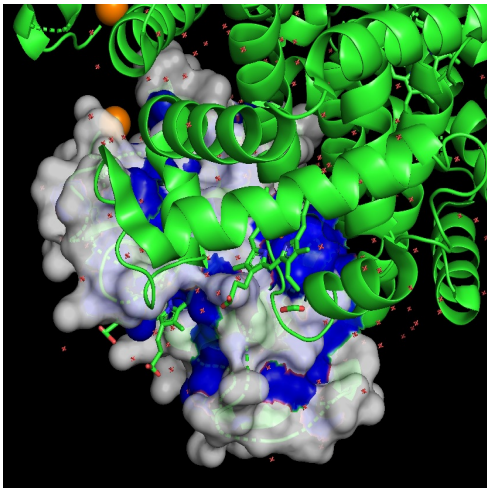

Figure 3. Subunit interacte surface is more hydrophobic

5. 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

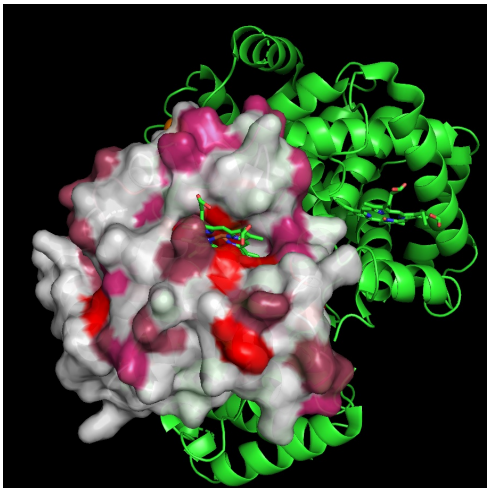

Figure 4. The Hemo binding site is identical convservation

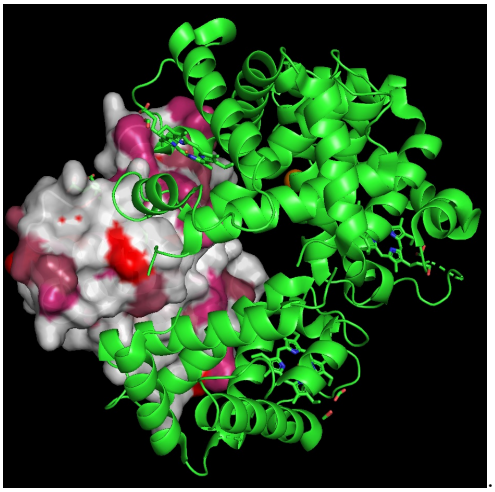

Figure 5. Most interaction edge is similar residues

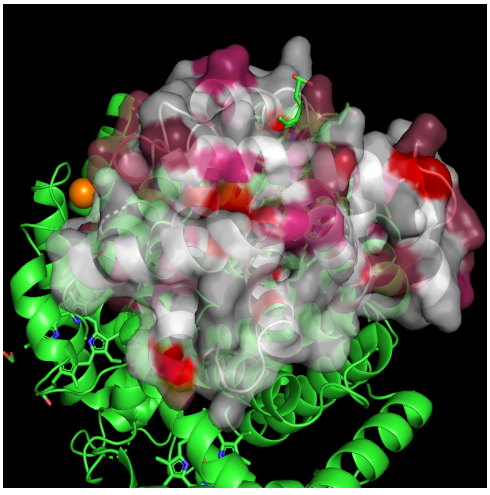

Figure 6. At no interaction surface,most loops and turn are conservation

6. 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.

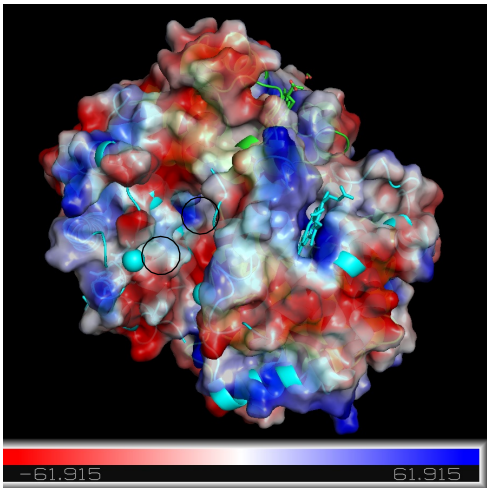

Figure 7. The positive charged pocket for 2,3-BPG binding. Butoom balck circle is a PO^{4-}, and top black circle is responding positive surface to hold PO^{4-}. (Two β subunits are colored by cyans.

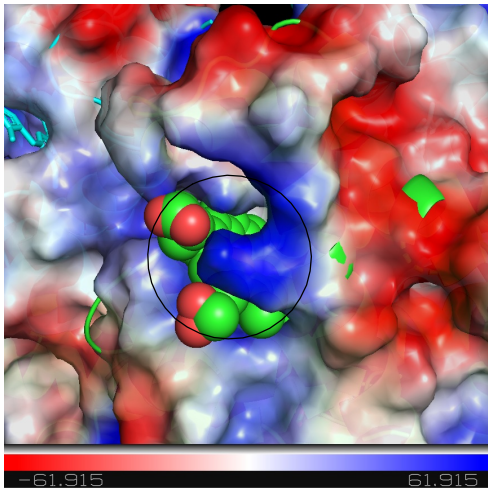

Figure 8. Here are positive charged surface on homoglobin around a Heme (Black circle).

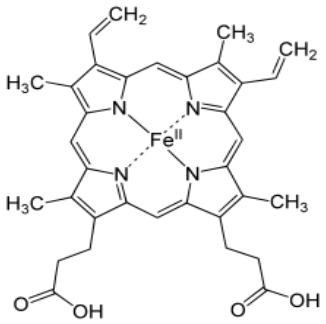

Figure 9. Heme b group (Ref 2)

7. Electrostatic potential surface using APBS program

Conclusions

References

1. https://pymolwiki.org/index.php/Property_Selectors
2. https://en.wikipedia.org/wiki/Heme
3. https://en.wikipedia.org/wiki/2,3-Bisphosphoglyceric_acid
4. http://cbc.chem.arizona.edu/classes/bioc462/462a/NOTES/hemoglobin/hemoglobin_function.htm
5. https://www.rcsb.org/structure/4HHB
6. https://pymolwiki.org/index.php/APBS