Exp 7 Identification of functional sites

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Introduction

POCASA is a program used for prediction of ligand binding sites of protein. CAVER is a software tool for analysis and visualization of tunnels and channels in protein structures.

We will predict pockets and tunnel inside protein using these programs.

Methods

1. Open human hemoglobin structure with 2,3-BPG bound(PDB id: 1B86)
2. Explain following questions based on surface property analysis
   - What is the Heme group binding environment, why?
   - What is the 2,3-BPG molecule binding environment, why?
   - Why does the sickle-cell mutation (E6V) lead to filament assembly?
3. Use POCASA serve to predict pockets in 1B86

   Enter http://altair.sci.hokudai.ac.jp/g6/Research/POCASA_e.html, then using following parameters:

   ```
   Input as file: 1b86_pure_simple.pdb
   Chain ID     : ABCD
   Probe radius : 1
   SPF          : 16
   PDF          : 18
   Top N        : 6
   grid size    : 1 A
   atom type    : protein
   ```

4. Use CAVER to predict tunnel in potassium channel(PDB id: 1BL8).

   Install plugin as https://pymolwiki.org/index.php/Caver3.

   Paremeters:

   ```
   Maximum Java heap size(MB): 6000
   Minimum probe radius      : 0.9
   Shell depth               : 4
   Shell radius              : 3
   Clustering threshold      : 3.5
   Number of approximating balls : 12
   Input atoms               : 20_AA, K
   Starting point            : (69.473, 33.257, 19.894)
   ```

Results

1. Surface property analysis

   Heme are in hydrophobic and little postive charged environment. May Heme's hrdrophobic bandbond and negative charged oxygen result in this.

   2,3-BPG localate in obvious postive charged environment, because 2,3-BPG are negative charged.

   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.(More stimulation are need)

2. POCASA

   The four pockets of HEME are predicted corretly. The the prediction of 2,3-BPG get into trouble. When I set `Top N: 5`, here are 5 pocket are found. Four pocket belong to HEME, and one pocket is in center of hemoglobin. Then I set `Top N: 6`, here are one more predict pocket far away 2,3-BPG. I don't know why.

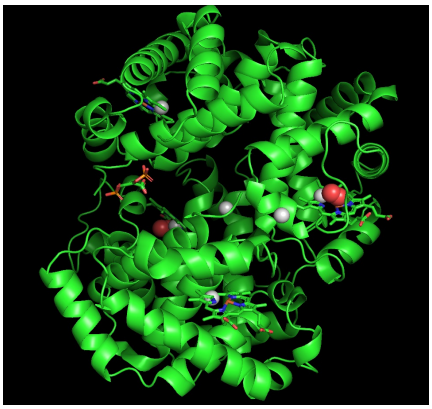

Figure 1. Human hemoglobin(1B86) with predict pockets center(white balls).

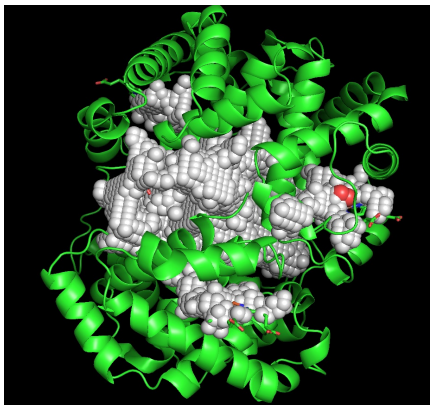

Figure 2. Human hemoglobin with predict pockets

3. CAVER

   CAVER success predict the tunnel in potassium channel. Although here are many predcit tunnels, I choose the most reasonable one.

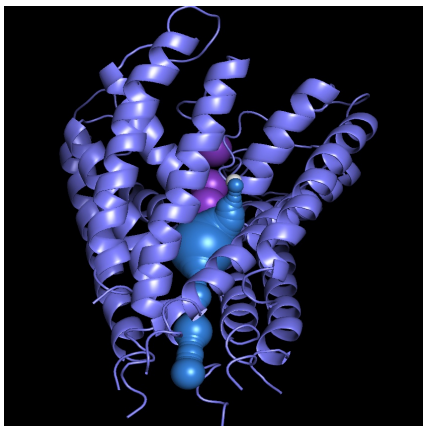

Figure 3. The predict tunnel(blue) in potassium channel(1BL8),White ball is start point

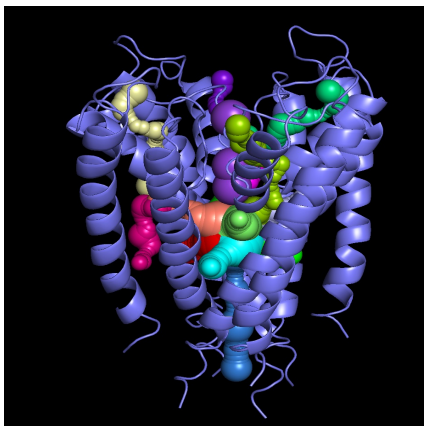

Figure 4. All predict tunnels in potassium channel.

Conclusions

POCASA and CAVER can help us to predict unknown protein's tunnel and pocket.

References

http://altair.sci.hokudai.ac.jp/g6/service/pocasa/manual.html

http://pdb101.rcsb.org/motm/38

Kozlíková, B., Šebestová, E., Šustr, V., Brezovský, J., Strnad, O., Daniel, L., Bednář, D., Pavelka, A., Maňák, M., Bezděka, M., Beneš, P., Kotry, M., Gora, A. W., Damborský, J., Sochor, J.: CAVER Analyst 1.0: Graphic Tool for Interactive Visualization and Analysis of Tunnels and Channels in Protein Structures, Bioinformatics, 30(18), 2014.

http://sickle.bwh.harvard.edu/scd_background.html