AutoDock

AutoDock
Virtual Screening:
Flexible residues

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Introduction

Why flexible residues?

The conformation of a binding site can vary dramatically among structures of the same protein. Very often, the changes are depending on the presence (or not) of a ligand in the crystal structure. The changes induced to the protein structure by ligand binding are often referred to as '**induced fit**'.

When flexible residues are suitable?

Flexible residues can help simulating the induced fit when the changes are mainly occurring in the residue side chains.

When flexible residues are not suitable?

Flexible residues are not able to simulate large protein changes, like the loop conformational changes or protein domain movements.

AutoDock has been successfully applied [1] to simulate the conformational changes of the gate-keeper arginine side chains in HIV protease. The simulation performed was a cross-docking experiment, in which ligands from almost 200 complexes have been extracted and docked against every protein conformation (i.e. every ligand against every protein, 200x200 dockings).

In this tutorial, two ligands (imatinib, B91) will be cross-docked against two protein conformations of Brc-Abl (2x2 dockings), simulating the flexibility of two sidechains.

Bibliography

[1] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. "AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility" Journal of Computational Chemistry, 2009 Dec;30(16):2785-91.

A. Input files preparation   (Raccoon)

1. The input files will be prepared with Raccoon. Open the program and import the two ligands:

> - click on the **[+] Add ligands...** button.

> - browse to the **"input/ligands"** directory

> - import files "**imatinib.pdbqt**" and "**B91.pdbqt**"; it is possible to import multiple files by pressing *Ctrl* while clicking.

2. Import the two conformations of Bcr-ABL:

> - go to the "*Receptor(s)*" tab and select "Multiple targets"

> - click on "**Add a structure...**", browse to the "**input/targets**" directory

> - change the filetype to "PDB" and import files "**1iep_receptor.pdb**" and "**3kfa_ receptor.pdb**"; it is possible to import multiple files by pressing *Ctrl* while clicking.

Water molecules are automatically removed and the PDB files are converted to the PDBQT AutoDock format.

4. Check the "**Flexible residues**" button, select "**From selection**" option:

> - type "PHE359,PHE382" in the entry

> - click on the green "Check" button to check if the residues are correct.

A confirmation message should report that 4 rotatable bonds have been reserved for flexible residues (2 for each Phe side chain).

5. Specify the grid box placement and size:

> -Click on **Load GPF template** the button

> -Browse to the **"input/config"** directory

> -Open the file "**kinase_template.gpf**"

6. Use the AutoDock predefined search parameters:

> -Click on the **"[select docking setup]"** menu and select "From template…" option.

> - generate a default DPF file by clicking on ` default `

7. Revise the virtual screening information to be sure that all required parameters are <u>green</u>, then specify the output directory where the files for the docking will be generated

> -Click on the **"Set directory…"** menu, then browse to the "flexible_residues" directory and specify a new directory called "**abl_crossdocking**"

8. Generate the files by pressing the button "**GENERATE**".

Raccoon will combine input and output files to enumerate all docking jobs between **2** ligands and **2** receptors (with the two flexible Phe sidechains), for a total of **4** docking calculations.

The results of these dockings have already been calculated and are available in the "**results**" directory.

B. Docking results analysis   (Fox)

Fox will be used to analyze the docking results. Each target will be analyzed separately with its own docked ligands.

1IEP crystal structure (Bcr-Abl+imatinib)

9. Select the receptor structure

-Click on　**Choose structure...**　button

-Browse to the **"receptors"** directory

-Open the file "**1iep_receptor_rigid.pdbqt**"

The rigid portion of *1iep* will be loaded in the session and used to calculate interactions. The flexible residues will be loaded as part of the ligand.

10. Load the grid box in the session:

-Click on　**Set grid box...**

-Browse to the **"config"** directory

-Load the file "**kinase_template.gpf**"

11. Import the docking files:

- click on　**Import DLG...**

- click on　**Choose directory**

- select the **"results\1iep_receptor\"** directory

- check "**include subdirectories**" option

- click on the scan icon 🔍

- once the scan is done, press "**Process**"

Results of both imatinib and B91 docked to *1iep* structure will be

imported and clustered.

12. Go to the "Filter & analysis" tab, select the "**Lowest energy**" pose and press the "**Filter**" button.

13. Go to the "Viewer" tab, select imatinib and load the reference poses of the ligand and the flexible residues from the crystal structure:

- Click on the icon in 3D viewer toolbar and select "load",

- Browse to the **"ligands"** directory and load "*imatinib_and_residues_XRAY.pdb*"

- To hide/show the ligand, click on the same icon again and choose hide/show

The ligand is docked correctly and the two Phe side chains don't vary much their position with respect to the experimental pose.

14. Load B91 docking result by clicking on the ligands list then load the reference pose:

- Click on the icon in 3D viewer toolbar and select "load",

- Browse to the **"ligands"** directory and load "*B91_and_residues_XRAY.pdb*"

- To hide/show the ligand, click on the same icon again and choose hide/show

The ligand is docked in a conformation very close to the experimental one, while the two Phe side chains stay in conformations closer to the complex with imatinib (**WHY?**)

15. The imidazole portion of B91 in the lowest energy conformation is distant from the experimental one. Inspect the largest cluster pose:

- Click on the arrows ◀ ▶ near the Score line, or use the keyboard pressing left-right arrow keys

The largest cluster pose is matching the experimental pose with very high accuracy. This is an example in which analyzing more than one

pose is helpful to find the right result.

3KFA crystal structure (Bcr-Abl+B91)

16. Remove the previous docking data. Switch to the Input tab:

-Click on **Remove all** button and press "Yes"

17. Select the receptor structure

-Click on **Choose structure...** button

-Browse to the **"receptors"** directory

-Open the file "**3kfa_receptor_rigid.pdbqt**"

The rigid portion of *3kfa* will be loaded in the session and used to calculate interactions. The flexible residues will be loaded as part of the ligand.

18. Load the grid box in the session:

-Click on **Set grid box...**

-Browse to the **"config"** directory

-Load the file "**kinase_template.gpf**"

19. Import the docking files:

- click on **Import DLG...**

- click on **Choose directory**

- select the **"results\3kfa_receptor\"** directory

- check "**include subdirectories**" option

- click on the scan icon

- once the scan is done, press "**Process**"

Results of both imatinib and B91 docked to *3kfa* structure will be imported and clustered.

20. Go to the "Filter & analysis" tab, select the "**Lowest energy**" pose and press the "**Filter**" button.

21. Load the docking result of B91 by clicking on the list, then the reference pose:

    - Click on the icon in 3D viewer toolbar and select "load",

    - Browse to the **"ligands"** directory and load "*B91_and_residues_XRAY.pdb*"

    - To hide/show the ligand, click on the same icon again and choose hide/show

The B91 is docked correctly in the *3kfa* crystal structure. Compare the the conformations of the flexible side chains with the reference structure.

22. Select the imatinib result from the ligands list. Compare the conformation of the phenilalanine side chains and compare them with the reference structure of the *3kfa* structure. Note the <u>overlap</u> between experimental pose of Phe359 sidechain and the piperazine of the ligand.

23. To compare the predicted conformation of Phe359, load again the reference structure of imatinib in *1iep*.

    - Click on the icon in 3D viewer toolbar and select "load",

    - Browse to the **"ligands"** directory and load "*imatinib_and_residues_XRAY.pdb*"

    - To hide/show the ligand, click on the same icon again and choose hide/show

The ligand is docked in a conformation very close to the experimental one, and AutoDock predicted successfully the movement of Phe359 to allow the binding of imatinib and its position with very high accuracy.