Protocol for Docking with AutoDock

iGEM IISc-Bengaluru

1 Materials

1.1 Software:

1. Linux Machine, Macintosh or Windows PC with Internet access
2. Text Editor
3. AutoDock: Download link
4. AutoDock Vina: Download Link
5. AutoDock Tools: Download Link

1.2 Coordinate Files:

1. Receptor Protein file, preferably in PDB format. Database of protein PDB structures is available at: `https://www.rcsb.org/`
2. Ligand Coordinate file in pdb, sdf, mol2 format. Various sources are available for download. For small molecules, sdf files are available from PubChem.
3. Supplementary files are available here [1]

2 Procedure

2.1 Coordinate Preparation with AutoDock Tools

1. Prepare the ligand coordination file with Hydrogens. Open AutoDock Tools and set the current working directory. If the ligand file does not have hydrogens, click 'File -> Read Molecule', select the ligand coordinate file, and open it. Then click 'Edit -> Hydrogens -> Add' to add Hydrogens.
2. Next, click 'Ligand -> Input -> Open' (or 'Ligand -> Input -> Choose' in case Hydrogens were added in the last step), choose the appropriate file type, and select and open the ligand coordinate file. AutoDock Tools will read the coordinate file, add charges if needed, merge non-polar hydrogens

and assign appropriate atom types. Click ok in the pop-up to continue, and the ligand will be displayed.

3. Now, select 'Ligand -> Torsion Tree -> Detect Root'; this defines the centre of the torsion tree. If the torsional degrees of freedom need to be edited, select 'Ligand -> Torsion Tree -> Choose Torsions'. A window will open, which will allow making the changes. Rotatable bonds are displayed in green, rigid bonds in red, and potentially rotatable bonds are set as 'non-rotatable' in magenta. In order to toggle between setting rotational flexibility on and off, click the bonds. When finished, select 'Done'.

4. After completing the above process, export the processed ligand file as a PDBQT file by clicking 'Ligand -> Output -> Save as PDBQT', name the file appropriately and select 'Save'.

5. Generate the Receptor coordinate file. Ensure that the file has hydrogen atoms. Open the molecule in AutoDock Tools by selecting 'File -> Read Molecule', set the 'Files of Type' to 'all files' and open the relevant PDB file.

6. Remove waters by selecting 'Edit -> Delete Waters'. If the file does not have Hydrogen positions, select 'Edit -> Hydrogens -> Add', select 'All Hydrogens', and click 'Ok' (ADT will automatically merge non-polar hydrogens later).

7. Now go to 'Grid -> Macromolecule -> Choose' and select the protein receptor. AutoDock Tools will add charges, merge non-polar hydrogens and assign appropriate atom types. Click the 'Ok' button to accept changes. A pop-up will show to save the changes. Name the file appropriately and save it.

8. If the Receptor has a metal ion in cofactors (such as Haeme), open the PDBQT file in a text editor and manually assign the metal ions the correct charge. The PDBQT file has a format of the following type:

   (a) ATOM atom_serial_num, atom_name, alt_loc, res_name, chain_id, res_num, ins_code, x, y, z, occupancy, temp_factor, footnote, partial_charge, atom_type

   (b) The second last entry being the partial charge is to be edited.

9. Make the change in the partial charge and save the file.

2.2 Docking Experiment with AutoDock Vina

1. The first part involves generating a configuration file to run the Docking. The configuration file specifies the ligand file, receptor file and other

docking parameters. Restart AutoDock Tools and set the current working directory. Click on 'Grid -> Macromolecules -> Open' and select the PDBQT receptor file saved in the last step. Select 'Yes' to preserve previous changes, and then press 'Ok' to accept the changes.

2. We need to define the Grid Box, which defines the search space. Select 'Grid -> Set Map Types -> Open Ligand' and open the saved ligand PDBQT file. Next, go to 'Gird -> Grid Box', which opens a window allowing you to define the box. In case the probable binding site of the ligand is known, use the ligand to centre the grid box by choosing 'Center -> Center on Ligand'. The grid box can similarly be centred around a particular region using rotating knobs. If the Docking is being done blindly (running the Docking all over the enzyme), enclose the enzyme in the grid box.

3. When the above step has been completed, choose 'File -> Close Saving Current' in the window. Next, go to 'Docking -> Output -> Vina Config' and save the configuration file by naming it and clicking 'Save'.

4. Run AutoDock Vina from the command line. Add Vina to the folder containing all the PDBQT and config files. Exhaustiveness needs to be given, which defines the amount of computational effort to find the binding site (essentially, how exhaustive the search is. Higher is better). The default value is 8; however, higher values like 24 and 32 give more reliable results.

5. To run the Docking, use the command
   `./vina --config config.txt --log log.txt ---exhaustiveness=32`

6. The above command will generate an output file with the name ligand_out.pdbqt. In addition, information about the docking poses and the affinities will be displayed on the command line. The same will be saved in a document called log.txt.

7. To visualise the Docking, open the AutoDock Tools and set the current working directory. Select 'Analyse -> Dockings -> Open AutoDock Vina Results' and choose the output file generated. Select the option of 'Single Molecule with Multiple Conformations' in the pop-up that appears and click 'Ok'. This will show a single molecule for which the different conformations can be toggled by using arrow keys. Click 'Analyze -> Macromolecule -> Open' to choose the receptor molecule. If the crystallographic ligand coordinate file exists, it can also be loaded from 'File -> Read Molecule' and the docking poses can be compared to the crystallographic position. To view interactions, 'Analyze -> Dockings -> Show Interactions' can be used.

8. Other than AutoDock Tools, UCSF Chimera and Pymol are other software which are very useful for viewing Docking poses, along with hydrophobic

and electrostatic mapping of the Receptor and ligand. A different software of choice can be used after performing Docking for visualisation.

2.3 Active Site Prediction using AutoLigand

1. AutoLigand is a part of AutoDock tools and is helpful in identifying the optimal regions for ligand binding by analysing AutoGrid interaction energy maps for three atom types (C, OA and HD) and the electrostatics and desolvation maps calculated with 1 Å grid spacing.

2. Start AutoDock Tools, and set the current working directory. In 'Grid -> Macromolecules', select the coordinate file for the Receptor. In 'Grid -> Set Map Types -> Directly', edit the atom list to be 'C HD OA'.

3. In 'Grid -> Grid Box', set the spacing to 1Å and adjust the Grid Box to contain the whole protein. Save the parameters using 'File -> Close Saving Current', and write the grid parameter file with 'Grid -> Output -> Save GPF' with the name of the Receptor. Run AutoGrid on the command line (make sure the AutoGrid is in the directory containing the files)
`./autogrid4 -p [receptor_name].gpf -l [receptor_name].glg`

4. Load AutoLigand on AutoDock tools by going to 'File -> Browse Commands'. Next, in 'Select Package', select 'AutoDock Tools'; in 'Select a Module', select 'AutoLigand Command'; click 'Load Select Module' and then click 'Ok'.

5. v. Select 'File -> Read Molecule' and read the Receptor PDBQT file and then select 'File -> Read Molecule' and read the ligand PDBQT file. Start AutoLigand Computation by choosing 'Compute -> AutoLigand -> Run AutoLigand'. This will open a window to choose the seed point and envelope size. Use the sliders to place the yellow seed point near the active site, or shift-click an atom to place the marker. Use the default value of 100 for Envelope Volume. Start the computation by clicking ok. When Autoigand finishes, a pop-up will open displaying the results, and the envelope will be saved as a PDB file and will be displayed in the viewer.

6. vi. Alternatively, AutoLigand can also be run from the command line (Which does not require seeding the active site) by using the command:
`python [PATH]AutoLigand.py [Receptor] [Fill points]`
and open the resulting files into AutoDock tools to visualise.

2.4 Docking with Explicit Waters

1. This method adds dummy atoms to the ligand that correspond to all possible hydration sites. A modified AutoGrid map is then used during Docking, giving a favourable score when the water is well placed and

omitting it if it overlaps with the Receptor. A final script analyses the docked results, retaining only those waters in appropriate positions.

2. Prepare the Ligand and Receptor files for standard AutoDock Docking. Add all the required supplementary files to the common directory. Run the command `python wet.py -i ligand.pdbqt`
   This adds dummy 'W' atoms to the ligand PDBQT file and saves it to ligand_HYDRO.pdbqt.

3. Calculate the default atomic grid maps. Start ADT and set the default working directory. Select 'Ligand → Input → Open' and choose the ligand PDBQT file and select 'Grid → Macromolecule → Open' and choose the receptor PDBQT file. Set the map types in 'Grid → SetMapTypes → Directly' and ensure that the 'HD' and 'OA' types are in the list. Use 'Grid → GridBox' to specify the centre and size of the search space. Use 'Center → Center on Ligand' in the pop-up window to define a minimal box. When finished, save the parameters using 'File → Close Saving Current'. Write the grid parameter file using 'Output → SaveGPF' with the name 'protein.gpf.' Run AutoGrid with 'Run → RunAutoGrid' or at the command line with
   `./autogrid4 -p protein.gpf -l protein.glg`

4. Generate the 'W' map. If standard filenames are used for the maps, only the receptor name must be specified for the script that generates the map for the water-energy evaluation:
   `python mapwater.py -r protein.pdbqt -s protein.W.map`

5. Create a modified docking parameter file. Select 'Docking → Macromolecule → Set Rigid File name' and choose the receptor PDBQ. Select 'Docking → Ligand → Choose' and choose the ligand PDBQT file (ligand_HYDRO.pdbqt), and accept the default ligand docking parameters, which will randomise the pose of the ligand before Docking. Set the search parameters in 'Docking → SearchParameters → Genetic Algorithm Parameters', changing 'Number of GA Runs' to 50, and use defaults on the remaining parameters. In 'Docking → DockingParameters', use default parameters for most small ligands.

6. Write the docking parameter file using 'Docking → Output → Lamarckian GA', with the name 'ligand_HYDRO_protein.dpf'.

7. The standard docking parameter file must be modified manually in two ways: 'parameter_file AD4_water_forcefield.dat' must be added near the top, and 'protein.W.map' must be added to the list of maps. Both of these changes are highlighted.

8. Run AutoDock in ADT with 'Run → Run AutoDock' or at the command line with
   `./autodock4 -p ligand_HYDRO_protein.dpf -l ligand_HYDRO_protein.dlg`

9. Extract and score the results at the command line with
   `python dry.py -c -r protein.pdbqt -m protein.W.map -i ligand_HYDRO_protein.dlg`

10. This script will filter the docking results using the Receptor to identify displaced water and the W map to rank the conserved ones as strong or weak. This will write a file called ligand_LELC_DRY_SCORED.pdbqt with the calculated energy.

References

[1] Forli, S., Huey, R., Pique, M. et al. Computational protein–ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc 11, 905–919 (2016). https://doi.org/10.1038/nprot.2016.051

[2] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009) Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry 2009, 16: 2785-91.

[3] O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31 (2010) 455-461

[4] The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

[5] UCSF Chimera--a visualization system for exploratory research and analysis. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.