Chapter 19

Small-Molecule Library Screening by Docking with PyRx

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Abstract

Virtual molecular screening is used to dock small-molecule libraries to a macromolecule in order to find lead compounds with desired biological function. This in silico method is well known for its application in computer-aided drug design. This chapter describes how to perform small-molecule virtual screening by docking with PyRx, which is open-source software with an intuitive user interface that runs on all major operating systems (Linux, Windows, and Mac OS). Specific steps for using PyRx, as well as considerations for data preparation, docking, and data analysis, are also described.

Key words Virtual molecular screening, Computer-aided drug design, Molecular docking, PubChem, AutoDock, Vina, Open Babel

1 Introduction

Drug discovery is an attractive research area that enables application of cutting-edge biomedical research to improve health of many people [1]. In the past, medicines were derived from natural products, mostly from plant sources. While natural products continue to be used and researched for medicine, it is now possible to synthesize a large number of chemical compounds that are not readily available in nature. The increased number of possible chemical compounds presents both a challenge and opportunity for the pharmaceutical industry. Testing different drug candidates in human clinical trials is a long and expensive process, which is why phenotypic or target-based screening is so important in the earlier stages of drug discovery [2].

In phenotypic screening, different compounds are tested in cells or organisms to see which compound makes intended changes in the phenotype. When molecular causes of the disease are unknown, phenotypic screening is, in many cases, the only available option for finding life-saving drugs. For diseases that are well studied and understood at the molecular level, altering a single macromolecule can lead to a desired outcome. An example of such

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a macromolecular target for the common flu virus is discussed shortly. In target-based screening, compounds are tested with purified macromolecules (usually a protein) to find lead compounds that make intended macromolecular changes. For a lead compound to become a drug, it needs to be able to reach a site of action in the body, bind to its target macromolecule, and elicit the desired biological effect.

Compared to large biological molecule therapeutics, such as insulin or antibodies, which are administered through injection, small molecules can be taken orally and are better at reaching different sites in the body. This is why the majority of approved and experimental drugs are small molecules. Small molecules are also better suited for virtual molecular screening, which is the main subject of this chapter. With virtual screening, different compounds are docked from a small-molecule library to a target macromolecule (usually a protein) to find compounds with the best binding affinity [3]. Note that virtual screening is not limited to drug targets and it can be used to screen against herbicides, pesticides, or any other target of interest [4]. In all cases, finding the right target is very important for virtual screening campaign to succeed. When the three-dimensional (3D) structure of a target is available, through X-ray crystallography, NMR spectroscopy, or any other means, docking algorithms can be applied to search for the best binding mode between target macromolecule and ligand.

In this chapter, methods for performing virtual screening experiments with PyRx open-source software are outlined. The 3D structure of the influenza virus neuraminidase [5] is used as an example to show how to prepare an input file for the target macromolecule. Influenza virus neuraminidase cleaves sialic acid from the infected cell surface to release newly created viruses. Neuraminidase inhibitors bind to neuraminidase and prevent them from binding to sialic acid. This leaves the influenza virus stuck on the surface of infected cells, so that the influenza virus cannot infect nearby healthy cells [6]. Here, steps to prepare input structures for zanamivir (a neuraminidase inhibitor), sialic acid, and sucrose (table sugar) are described. These small molecules are then used to run virtual screening against influenza virus neuraminidase.

2 Materials

PyRx is written in Python programming language and it can run on nearly any modern computer, from PC (personal computer) to supercomputer. Below details are provided of the Windows PC used in Subheading 3, although similar methods also work on Linux and Mac OS as well.

2.1 Hardware and Software

1. Dell Studio 540S with Intel Core 2 Duo CPU at 2.53 GHz, 4 GB memory (RAM), ATI Radeon HD 3400 series graphics card, and 32-bit Windows Vista operating system.
2. Binary distribution of PyRx version 0.8 for Windows available free from http://pyrx.sourceforge.net.

2.2 Input Files

To start with structure-based virtual screening, structures of the target macromolecule and small molecules are needed as input files. There are a number of publicly available websites where users can download these input files. Used in this chapter are DrugBank [7] to get the structure of zanamivir, PubChem [8] for 3D structure of sucrose, and Protein Data Bank [9] to get 3D structures of influenza virus neuraminidase and sialic acid.

1. Open a preferred web browser and visit http://www.drugbank.ca/drugs/DB00558, click on SDF link next to Download, and save that page as DB00558.sdf.
2. Go to http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5988, click SDF icon on top right, and select 3D SDF: Save.
3. Visit http://www.pdb.org/pdb/explore/explore.do?structureId=2BAT, click on Download Files, and select PDB File (Text).

The reason for choosing these particular molecules is that they are familiar to most of the readers and computations can be run relatively quickly on a PC. To apply the protocol described in Subheading 3 to other binding target and ligands, users would need to obtain input files corresponding to their specific binding target and ligands. Selection of the binding target depends on the biological problem of interest, and it is assumed that the 3D structure of the target is available in PDB format through Protein Data Bank (http://pdb.org) or other sources (*see* **Note 1**). Selection of ligands depends on whether virtual screening is used for lead discovery or lead optimization. For lead discovery, it is advised to include as many ligands with diverse shapes, sizes, and composition as possible. Since individual docking computations are independent from each other, users are practically only limited by computational power available at their disposal. For lead optimization, on the other hand, ligands are selected to closely match the lead compound [10]. One of the advantages of virtual screening is that it is not limited to commercially available compounds; a ligand file for a novel compound not found in any of the databases can also be used (*see* **Note 2**).

3 Methods

3.1 Prepare Input Files for Docking

Before input files can be used for virtual screening, they must be converted to the PDBQT file format suitable for docking with AutoDock Vina [11].

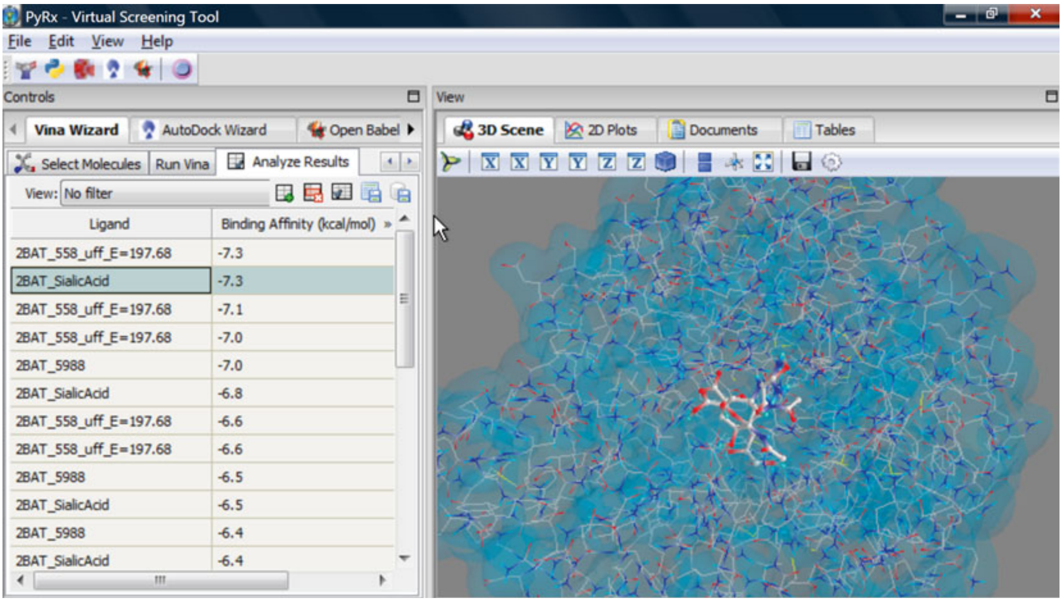
1. Start by double-clicking on PyRx icon on the Desktop.
2. Select Open Babel tab under Controls panel and click on the first icon on its toolbar with plus (+) sign on it. Navigate to the Downloads folder and select CID_5988.sdf (sucrose from PubChem).
3. Click on the first icon on the Open Babel toolbar again, and locate and open DB00558.sdf (zanamivir from DrugBank). 558 is the accession number of zanamivir in DrugBank and it is listed under the Title column in the Open Babel table. If other molecules are to be included in virtual screening, the Open Babel widget can be used to convert them to PDBQT file format (*see* **Note 3**).
4. Select the row corresponding to zanamivir with Title 558, and right-click and use the Minimize Selected option. Click OK and wait for energy minimization to complete. Notice that the title of this molecule has changed to 558_uff_E = 197.68. The _uff part corresponds to the force field used for energy minimization, which, by default, is the Universal Force Field [12] as implemented in Open Babel software package [13]. The _E = 197.68 part corresponds to the energy of the minimized molecule. The precise value for this energy is not important here. However, this notation is helpful to capture changes made to this molecule before conversion to the AutoDock ligand file in the next step.
5. Right-click on any of the rows in Open Babel table and use Convert All to AutoDock Ligand (pdbqt). This will create two pdbqt files corresponding to sucrose and zanamivir molecules under the Ligands folder.
6. Select Documents tab under the View panel, click on the Open icon (second from the left), and open the 2BAT.pdb file. 2BAT is the PDB ID for the structure of the complex between influenza virus neuraminidase and sialic acid [5]. The following steps are specific to this structure. To apply this method to targets which have no ligand attached, please go directly to **step 10** and replace 2BAT with the name of the desired target macromolecule.
7. Next select lines corresponding to sialic acid from 2BAT.pdb. Scroll down, use Ctrl-F or the Find icon on the toolbar to search for SIA residues, and select lines with HETATM 3216–3236. Use Ctrl-C or right-click Copy, click on the New icon, and paste these lines (Ctrl-V or right-click Paste) in a new file. Save this file as SialicAcid.pdb using Save icon (third from the left) on the Documents panel. If working with another target that contains a ligand that is desired for re-docking, the

Documents panel in PyRx can be used or any other text editor (such as Notepad or WordPad) to extract HETATM records corresponding to the ligand of interest. The web page for 2BAT (http://www.rcsb.org/pdb/explore.do?structureId=2BAT) also lists different ligands bound to neuraminidase, including sialic acid, which is listed under Ligand Identifier column as SIA. This web page also offers the possibility to download ligand SDF file for sialic acid.

8. Click on 2BAT.pdb tab under the Documents panel, scroll up, and left-click at the beginning of the line starting with TER 3023. The TER record indicates the end of a list of ATOM records for a chain according to PDB file format specification. In this case, it is desired to keep neuraminidase atom records only and delete all other records that correspond to different ligand and water molecules cocrystallized with this structure of neuraminidase. With the left mouse button pressed, scroll down to the end of the file and click Delete. Save this modified 2BAT.pdb file using the Save icon again.
9. From the menu bar, use File → Load Molecule menu and open SialicAcid.pdb. Right-click on SialicAcid under Molecules panel and select AutoDock → Make Ligand.
10. Use File → Load Molecule menu again and open 2BAT.pdb. Right-click on 2BAT under Molecules panel and select AutoDock → Make Macromolecule.

3.2 Run Virtual Screening Using Vina Wizard

1. Select Vina Wizard tab under the Controls panel and click on the Start button.
2. Select 558_uff_E = 197.68.pdbqt, 5988.pdbqt and SialicAcid.pdbqt under the Ligands folder (use the Shift key for selecting multiple ligands).
3. Select 2BAT under the Macromolecules folder and click on the Forward button on Vina Wizard.
4. Click on the Maximize button under Vina Search Space and then click on the Forward button. This starts AutoDock Vina and docks each ligand, one by one, to neuraminidase (2BAT). It takes less than 20 min to complete this virtual screening on a PC mentioned in Subheading 2.1 (*see* **Note 4**) (Fig. 1).
5. After virtual screening is completed, PyRx automatically advances to Analyze Results page, where results of virtual screening computation can be viewed. AutoDock Vina, by default, outputs the ten best binding modes for each docking run (*see* **Note 5**). Left-click on Binding Affinity (kcal/mol) table header cell under Analyze Results tab to sort this table by predicted binding affinity (*see* **Note 6**).



View: No filter

Ligand	Binding Affinity (kcal/mol)
2BAT_558_uff_E=197.68	-7.3
2BAT_SialicAcid	-7.3
2BAT_558_uff_E=197.68	-7.1
2BAT_558_uff_E=197.68	-7.0
2BAT_5988	-7.0
2BAT_SialicAcid	-6.8
2BAT_558_uff_E=197.68	-6.6
2BAT_558_uff_E=197.68	-6.6
2BAT_5988	-6.5
2BAT_SialicAcid	-6.5
2BAT_5988	-6.4
2BAT_SialicAcid	-6.4

Fig. 1 A screenshot of the PyRx virtual screening tool. The table on the *left* lists predicted binding affinity of zanamivir (2BAT_558_uff_E = 197.68), sialic acid (2BAT_SalicAcid), and sucrose (2BAT_5988) for influenza virus neuraminidase (2BAT). The 3D scene on the *right* shows line drawing and transparent molecular surface of neuraminidase. Ball-and-stick models for zanamivir and sialic acid are also shown on this 3D scene

4 Notes

1. During docking runs, the 3D structure of the target is fixed while the ligand is moved and rotated to find the best binding modes. While it is possible to make some of the side chains flexible during the docking, incorporating full flexibility of the target is still a subject of active research [14].
2. There are a variety of desktop or Web-based molecular editors available that can be used to generate a ligand file for a novel compound not found in any of the databases. The Web-based molecular editors allow users to sketch molecules in 2D, while desktop tools such as Avogadro [15] can draw molecules in 3D.
3. SDF (Structure-Data File) format is commonly used to store multiple structures in a single file. It allows storing arbitrary data together with coordinates and atom types. Oftentimes, small molecules stored in SDF are flat (2D) and energy minimization is performed to get 3D structures with proper bond length between different atoms.
4. The main results from virtual screening runs are the best predicted binding modes and corresponding binding affinity. The negative values for binding affinity (or binding free energy) indicate that the ligand is predicted to bind to a target macromolecule. The more negative the numerical values for the

binding affinity, the better is the predicted binding between a ligand and a macromolecule. In this particular case of screening neuraminidase with zanamivir, sialic acid, and sucrose, Fig. 1 shows that zanamivir (2BAT_558_uff_E = 197.68) and sialic acid (2BAT_SalicAcid) are both predicted to have the best binding affinity of −7.3 kcal/mol, whereas the best binding mode for sucrose (2BAT_5988) is predicted to have binding affinity of −7.0 kcal/mol. In other words, zanamivir and sialic acid are predicted to have better binding affinity to neuraminidase than sucrose. The fact that both zanamivir and sialic acid have the same predicted binding affinity indicates that zanamivir can bind to neuraminidase and inhibit it from binding to sialic acid.

5. PyRx users can also export virtual screening results as CSV (Comma-Separated Values) or SDF files. This is useful for further analysis, filtering, or re-ranking of virtual screening results with third-party packages.
6. There are a number of approximations used to model protein-ligand interactions [16] and there are a number of unknowns when it comes to comparing virtual screening results with experiments [17], not the least of which is that a single protein is being docked with a single ligand. In practice, even with purified samples, it is hard to predict if proteins or small-molecule ligands would aggregate and whether idealistic prediction of binding affinity with single protein-ligand docking applies to diluted samples. Nevertheless, small-molecule virtual screening by docking is a very valuable in silico method that can rank small molecules according to their predicted binding affinity to a target macromolecule. The cost of running virtual screening experiments is minuscule compared to real screening experiments. Virtual screening is also a very good tool for hypothesis generation with which to test modified versions of existing compounds or custom compounds that are not commercially available. With advances in computer software and hardware, and with the increasing number of publicly available bioassay data, virtual screening will continue to remain a vibrant research field.

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