Tutorial

Introduction

The Cytochrome P450 superfamily (officially abbreviated as CYP-450) consists of cysteinato-heme enzymes which play a role in oxidative transformation of endogeneous and exogeneous molecules. These cysteinato-heme enzymes consist of the prosthetic group which is constituted of an iron (III) protoporphyrin-IX covalently linked to the protein by the sulfur atom [1].

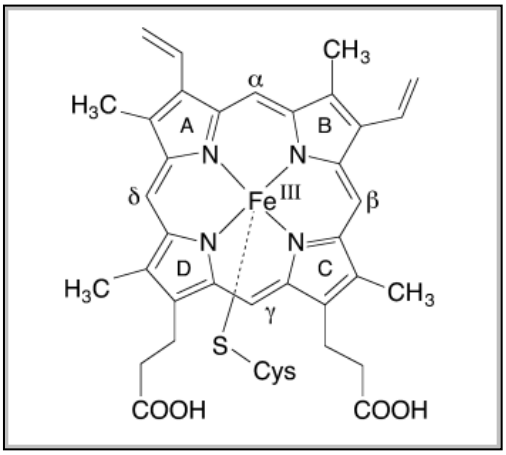


Fig. 1 Prosthetic of cysteinato-heme enzymes: an iron- (III) protoporphyrin-IX linked with a proximal cysteine ligand [1]

These enzymes are potent oxidants and catalyze various reactions such as hydroxylation of saturated carbon-hydrogen bonds, oxidation of heteroatoms, epoxidation of double bonds, oxidation of aromatics, dealkylation reactions etc.

During oxidation, P450 uses molecular oxygen, it inserts one of its oxygen into a substrate (S) and reduces second oxygen to a water molecule; it utilises two electrons that are provided by NAD(P)H via a reductase protein (Eq 1). These P450 are called as monooxygenases as only one of the two oxygen atoms (O_2) remains in the substrate.

$$\text{Substrate-R} + O_2 \xrightarrow[\text{NADPH} \rightarrow \text{NADP}]{\text{CYP450}} \text{Substrate-OR} + H_2O \quad (1)$$

Distance between the iron atom and site of metabolism (SOM) of the substrate is less than 6 Å. This distance is sufficient enough to insert oxygen molecule between substrate SOM and iron atom.

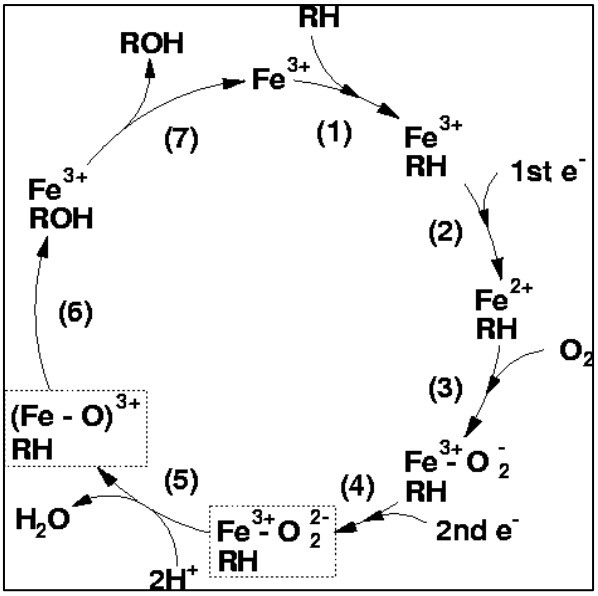


Fig. 2 Electronic mechanism of the oxidation reaction catalyzed by CYP-450 [1]

The electronic mechanism of oxidation reaction which is catalyzed by CYP-450 is shown in Fig. 2.

About DoPoSe-CYP

The expanded form is Docked Pose Selector in CYP-450. It analyzes the results of protein-ligand molecular docking which are carried out in the Glide module of Maestro of Schrödinger [2]. Molecular docking of ligands carried out in active site cavity of CYP-450 generates poses for each ligand. It is tedious to analyze all the poses in order to get the poses of ligand that can be considered as the bioactive conformation of ligand. In case of other receptors, we take care of docking scores and crucial interactions to select poses. The peculiarity of CYP-450 docking is the position of site of Metabolism (SOM) with respect to the catalytic centre i.e. iron is important. The tool DoPoSe-CYP makes this work easy by analyzing all poses of ligand obtained by protein-ligand docking.

For this, three input files are required. First, the protein file (must be in .pdb format), the ligand's poses file (must be in .sdf format) and the site of metabolism (SOM) file (must be in .txt format). It analyzes various poses of ligand based on the criteria that the SOM should be near enough the iron atom of heme moiety (active site of Cyp450 where oxidation occurs) and there should be no obstruction between the iron atom of heme moiety and the SOM of ligand pose. The SOM of all the ligands should be known either through experimental information or can be predicted by various online SOM predictors.

Input files

Protein Input file generation in Glide module of Maestro [2]

The input protein file can be prepared by Protein preparation wizard of Glide which can be exported in .pdb format.

Ligand Input file generation

After molecular docking, results should be sorted out according to title, charge (if ligand can exist as a charged molecule, then sorting should also include charge criteria) and GScore. Then, the ligands should be exported as a .sdf file.

SOM file generation

SOM input file should be generated in .txt format. SOM.txt file should contain two columns, one having the name of the ligand and other carrying the SOM. For preparing SOM.txt input file, SOM information can be taken from experimental information or by using various online SOM predictors.

Note:

The name of SOM input file must be "SOM.txt".

Ligand name written in SOM.txt and ligand .sdf input file (case-sensitive) should be exactly same.

Example of SOM.txt:

Ligand Name	Site of metabolism
Amitriptyline	C5
Celecoxib	C26
Duloxetine	C21
Efavirenz	C18
Fluoxetine	C22
Fluvoxamine	C9

Result Files

The two folders are generated named as “Temporary” and “Final Output”.

The “Final Output” contains two result files:

Satisfactory_poses: This file contain the satisfactory poses (marked by * sign) of ligands.

Good_satisfactory_poses: This file contains the good poses among the various satisfactory poses of ligands.

The “Temporary” folder contains the four output intermediary files which can be seen by the user if he/she wants to know the details:

Distance_between_iron_atom_and_all_atoms_of_ligand: This file contains the information about the iron coordinates and various ligand poses, their atom coordinates, and the distance of each atom of each pose of each ligand with iron.

Distance_between_iron_atom_and_SOM_of_ligand: This file contains the information of ligand’s SOM, iron coordinates, SOM coordinates and the distance of SOM of each ligand’s pose with iron atom.

Atoms_within_6_angstrom_of_iron: This file gives the information of ligand atoms of various poses which are within the six Å of distance from the iron atom.

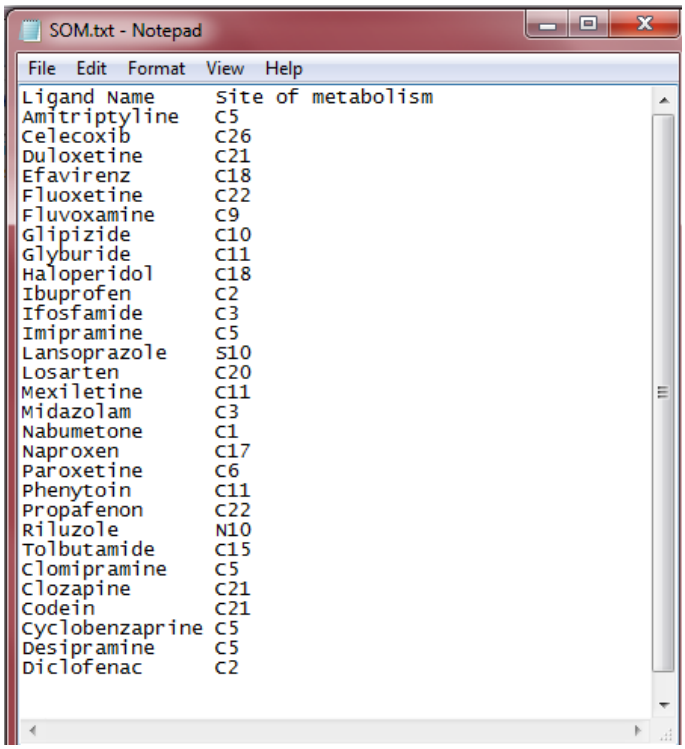
Bad_poses_with_obstruction_between_IRON_and_SOM: This file contains the information about the bad poses among the various satisfactory poses which are provided in “Final Output” folder file named as ‘Satisfactory_poses’.

What a user needs to keep in mind before using this tool

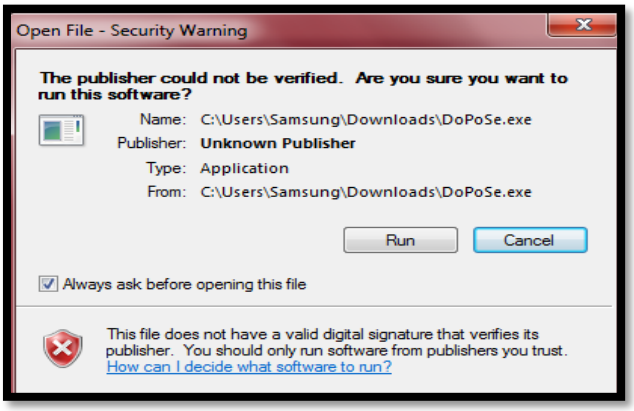
1. The downloaded .exe file and the three input files must be in same folder.
2. This tool has the maximum limits of 500 ligands and 50 poses for each ligand to be incorporated in input files.

Steps after downloading the tool

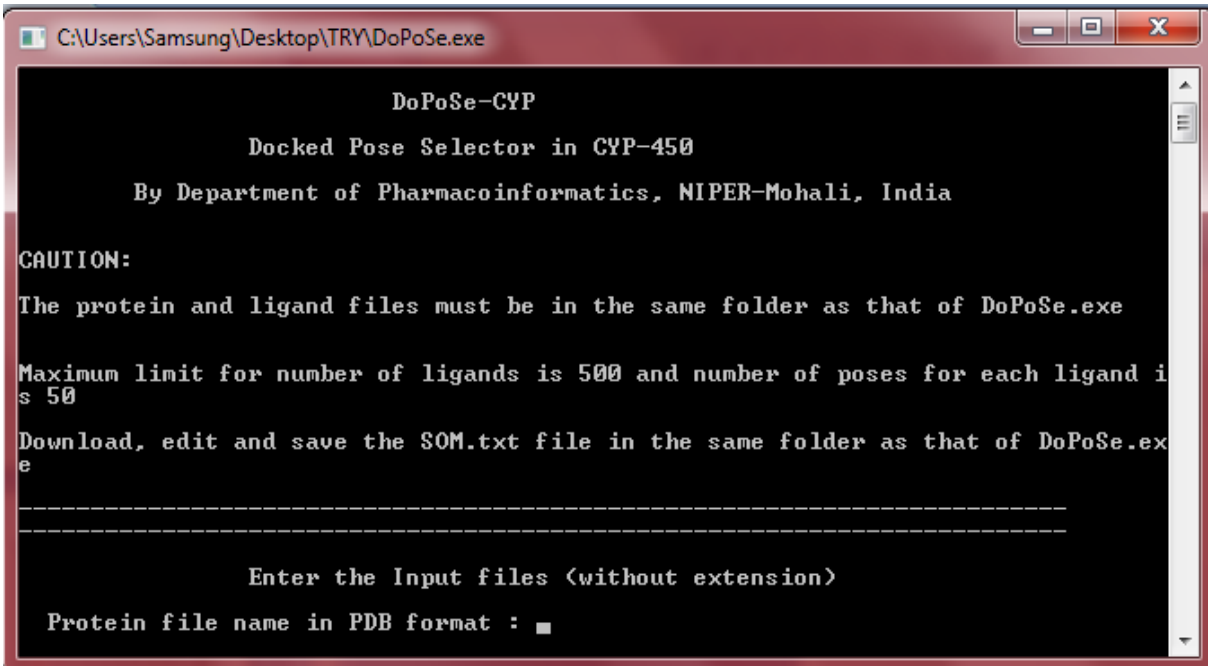
1. Download.
2. Save it in a directory of your choice.
3. In the same directory, keep your protein file (in .pdb format) and ligand file (in .sdf format).
4. Prepare a SOM.txt file in the given format. Remember that the ligand name and Atom symbol are case sensitive and should be same as that of the ligand file. The atom numbering should match with that of ligand file. Therefore a careful examination for the same is needed.



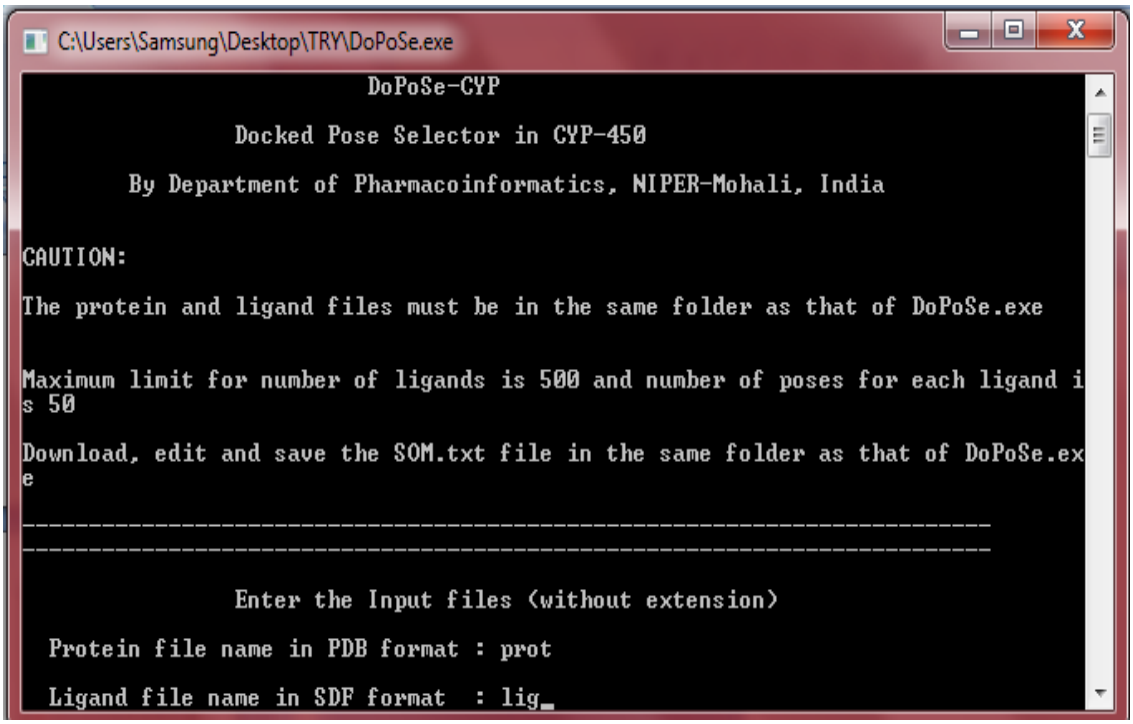
5. Run.



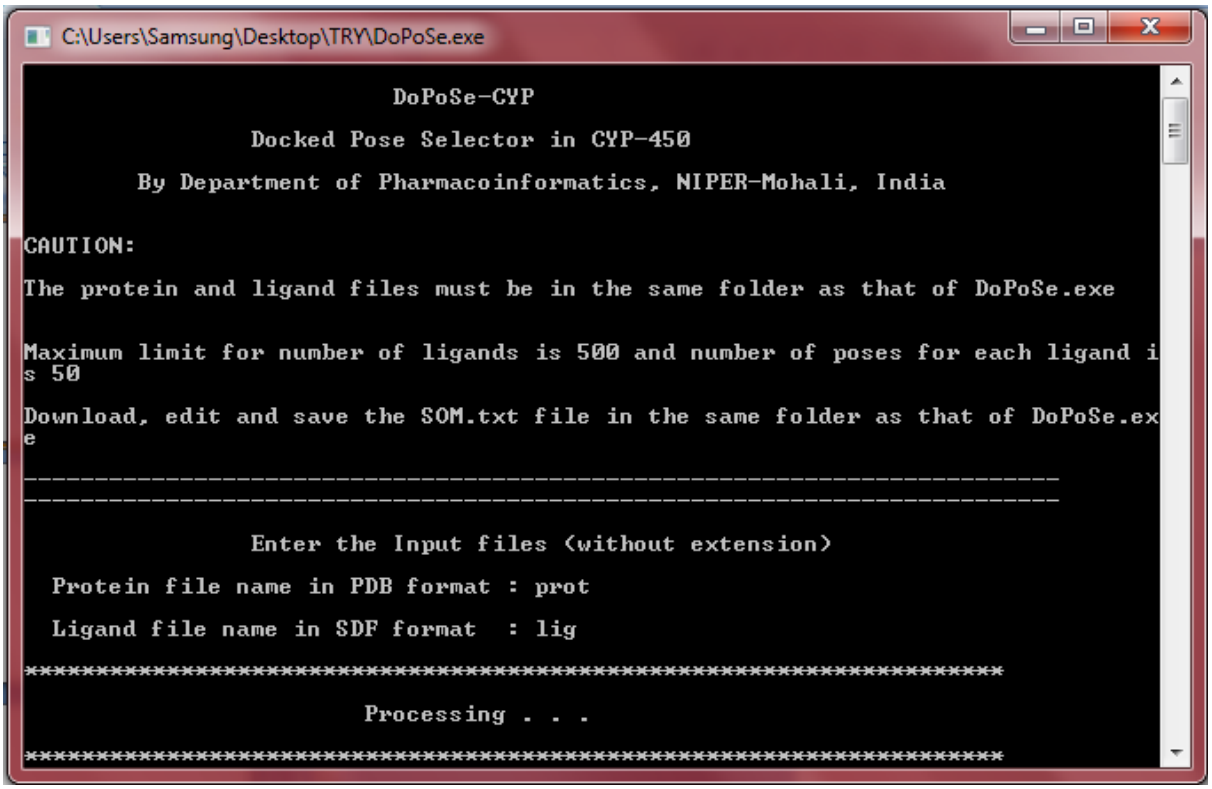
6. Enter the name of protein file without extension (e.g. prot) and ligand file without extension (e.g. lig).



7. Enter the ligand file name.



8. Wait a little for processing and then open the folders for getting the output result files.



References

1. Chem. Rev. 2004, 104, 3947-3980
2. Maestro, version 9.2, Schrödinger, LLC, New York, NY, 2011