Protocol

CityU iGEM 2020

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Table of Contents

Easy Modeller

Purpose

- Comparative modelling of unknown protein structure

Enzyme	Source of the structure used in our project
Polyurethanase esterase A (PueA)	A homology or comparative modeling of protein program was adopted to generate the 3D structures of proteins by inputting their amino acid sequence, and corrected and optimized by YASARA and PROCHECK

Procedures :

1. Identify homolog that can be used as template(s) for modelling via Protein BLAST based on target-template identity

2. Choose the first four results with the highest similarity index as the template from the protein bank

3. Input the amino acid sequence information for our target as the input parameter

4. Provide template information for the software using ‘Load template’ feature

5. The ‘Perform Alignment’ feature will align the query sequence (target) with the template(s)

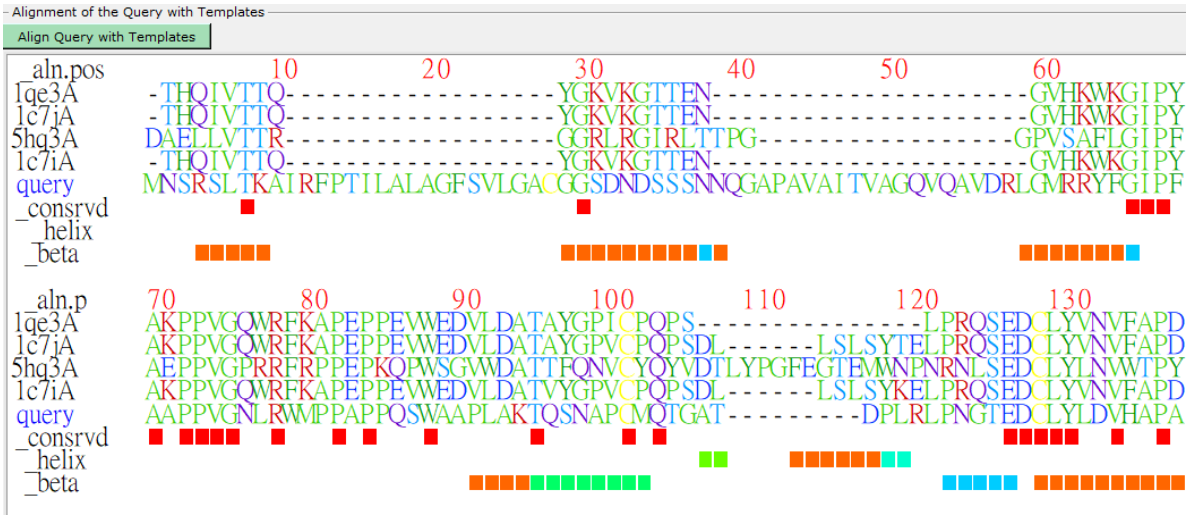
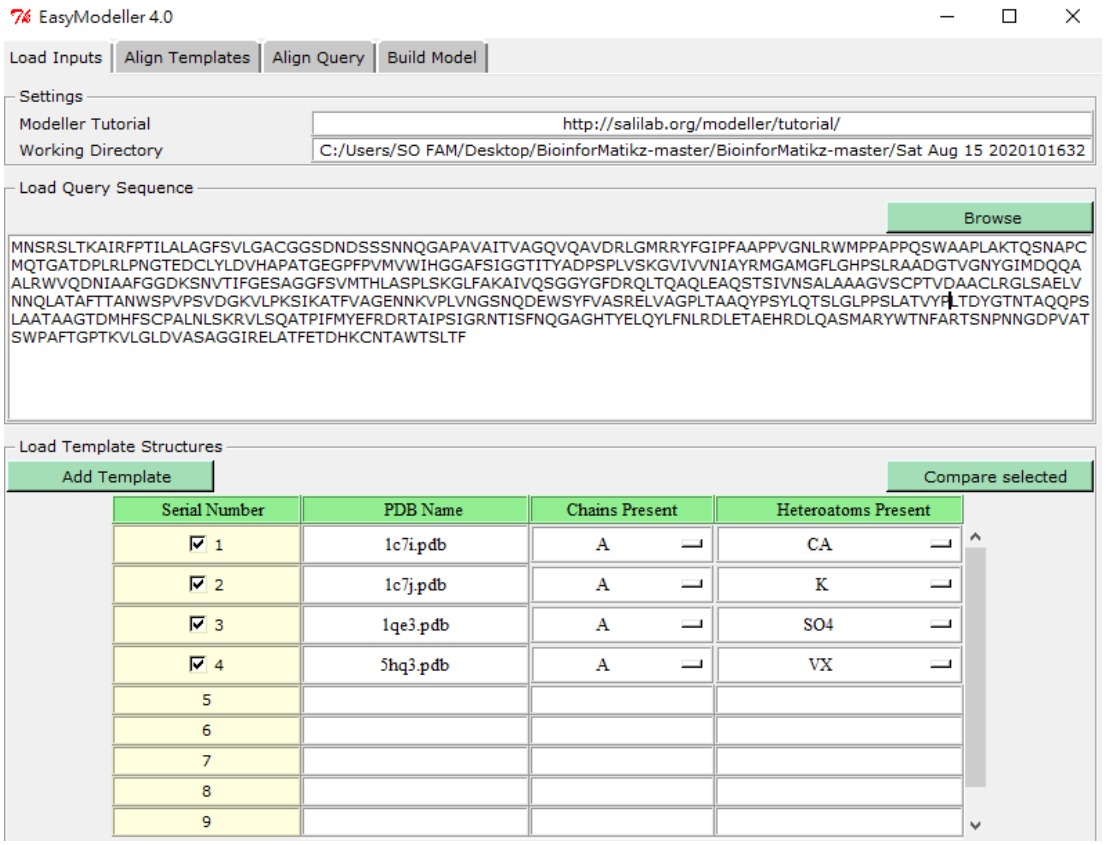


Fig. 1 : Alignment between the sequences of PueA and template

3. Input the templates into EasyModeller Program to generate possible structures of the enzyme molecule.



4. Choose the structure with the lowest discrete optimized protein energy (DOPE) as the best comparative model

```
>> Summary of successfully produced models:
Filename                          molpdf     DOPE score    GA341 score
----------------------------------------------------------------------
query.B99990001.pdb          19438.27539   -54708.65625        1.00000
query.B99990002.pdb          19325.74805   -54823.97266        1.00000
query.B99990003.pdb          19504.75391   -54348.01562        1.00000
query.B99990004.pdb          19814.24805   -54048.32031        1.00000
query.B99990005.pdb          19395.14844   -54562.69141        1.00000
```

5. The pdb files of protein 3D-structures are ready for docking simulations

AutoDock Tools

Purpose

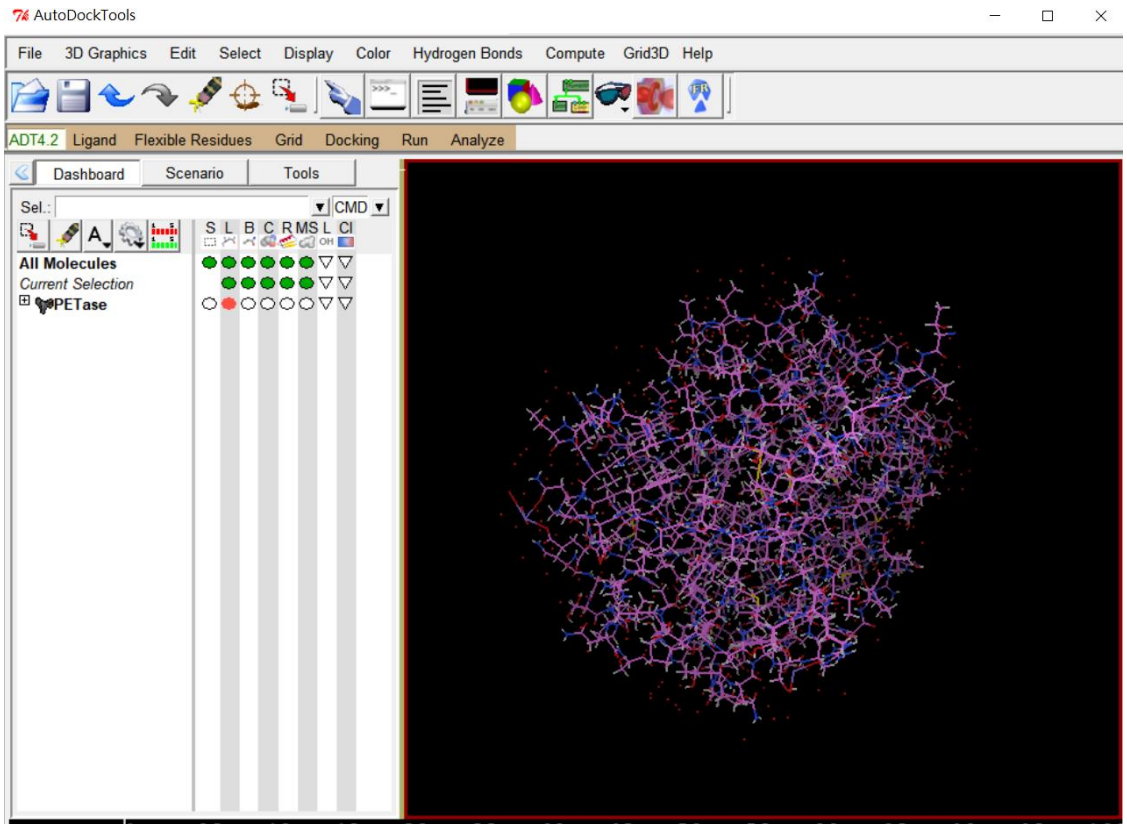
- To perform molecular docking and evaluate the accuracy in terms of actual number binding amino residues

Computational Configuration Requirement

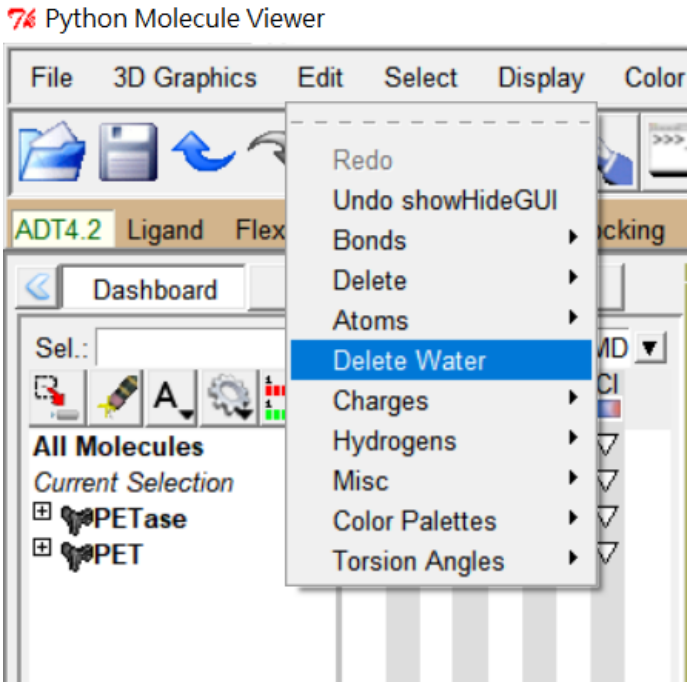
- Windows 8 / 10

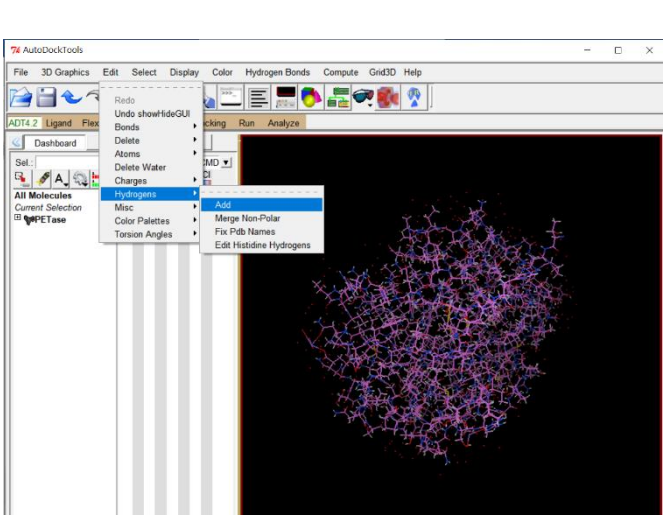
Protein Preparation

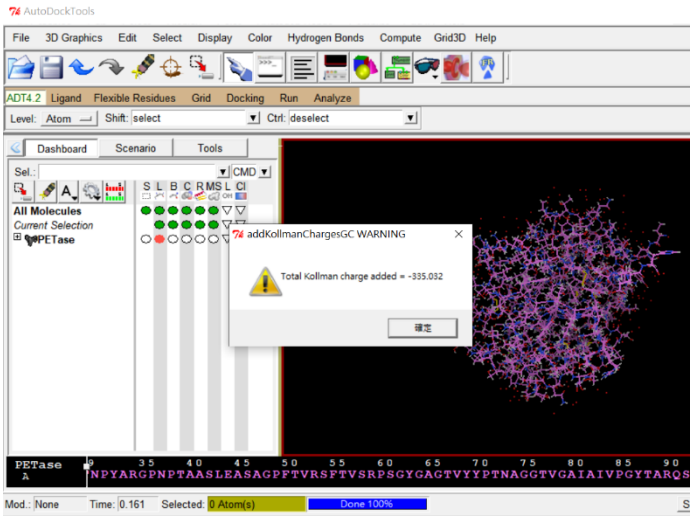
1. Drag the protein molecule and drop it in the tool



2. Delete all the water molecules that may interfere with the docking, and add polar hydrogens and Kollman Charges.



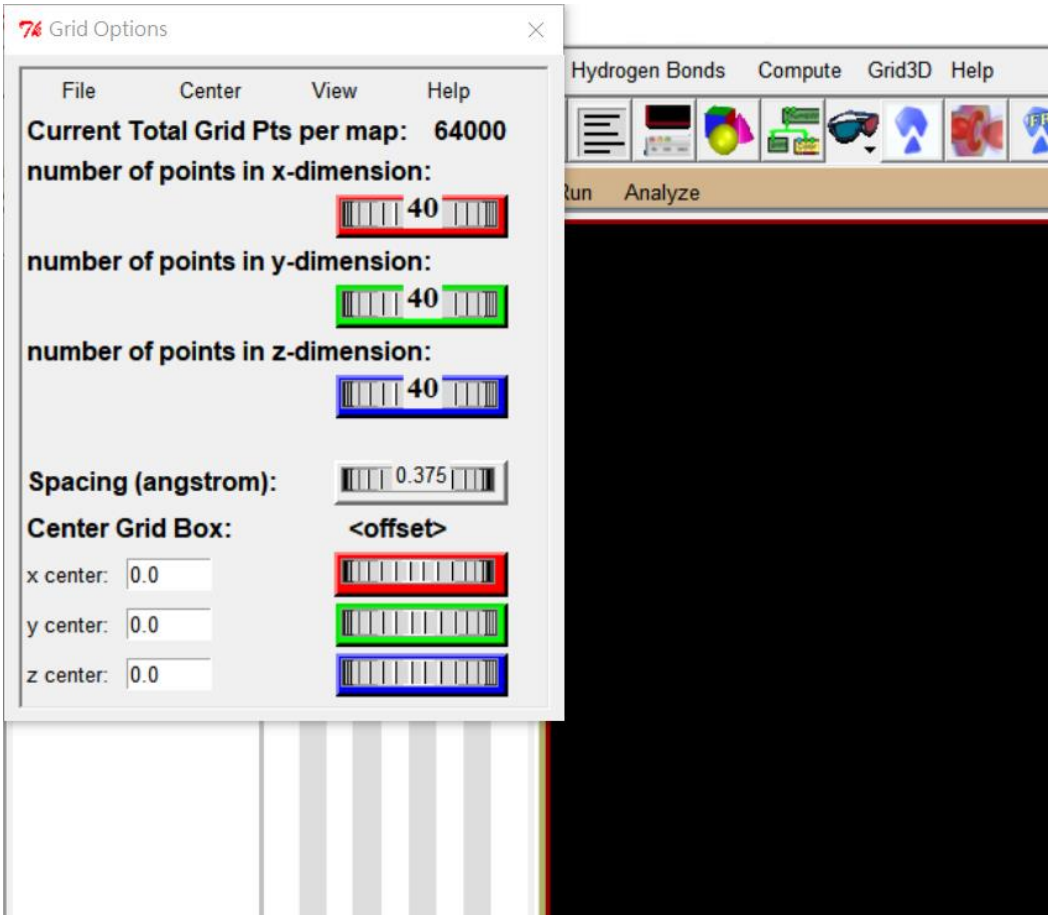




Ligand Preparation

3. Input the ligand primarily obtained from ChemDraw3D into the AutoDock Tools

4. Determine the position where docking happens based on the docking site of the target, and then prepare a grid centered at the binding site



Molecular Docking

5. Prepare a config file with grid parameters

```
receptor = pueA.pdbqt
ligand = pur_tetramer.pdbqt


center_x = 4.953
center_y = 0.175
center_z = 23.559

size_x = 40
size_y = 40
size_z = 40

energy_range = 4
exhaustiveness = 8
```

Fig. 2 : A config file to indicate the position of grid box where docking takes place

6. Input the following command to Autodock Vina to perform molecular simulation.

```
C:\Users\SAMSUNG\Desktop\Docking>"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor PueA.pdbqt --ligand pur_tetramer.pdbqt --config config.txt --log log.txt --out output.pdbqt
```

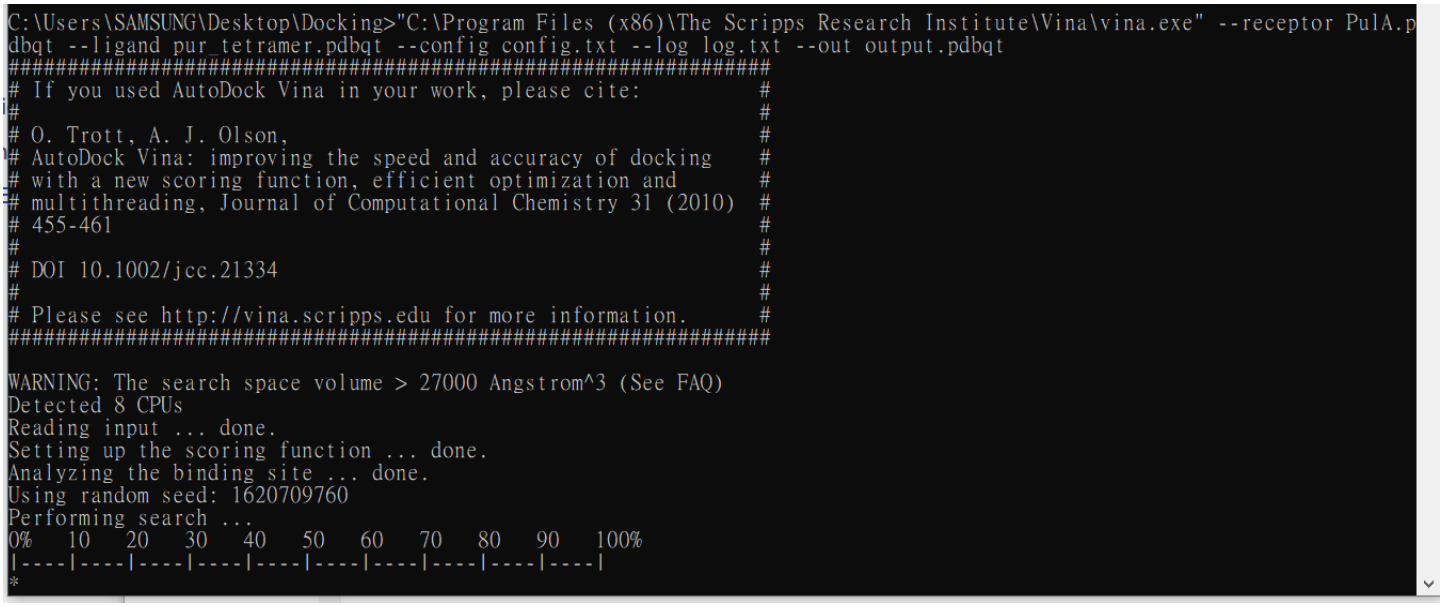


Fig. 3 : The progress bar indicates the program starts docking

Swissdock

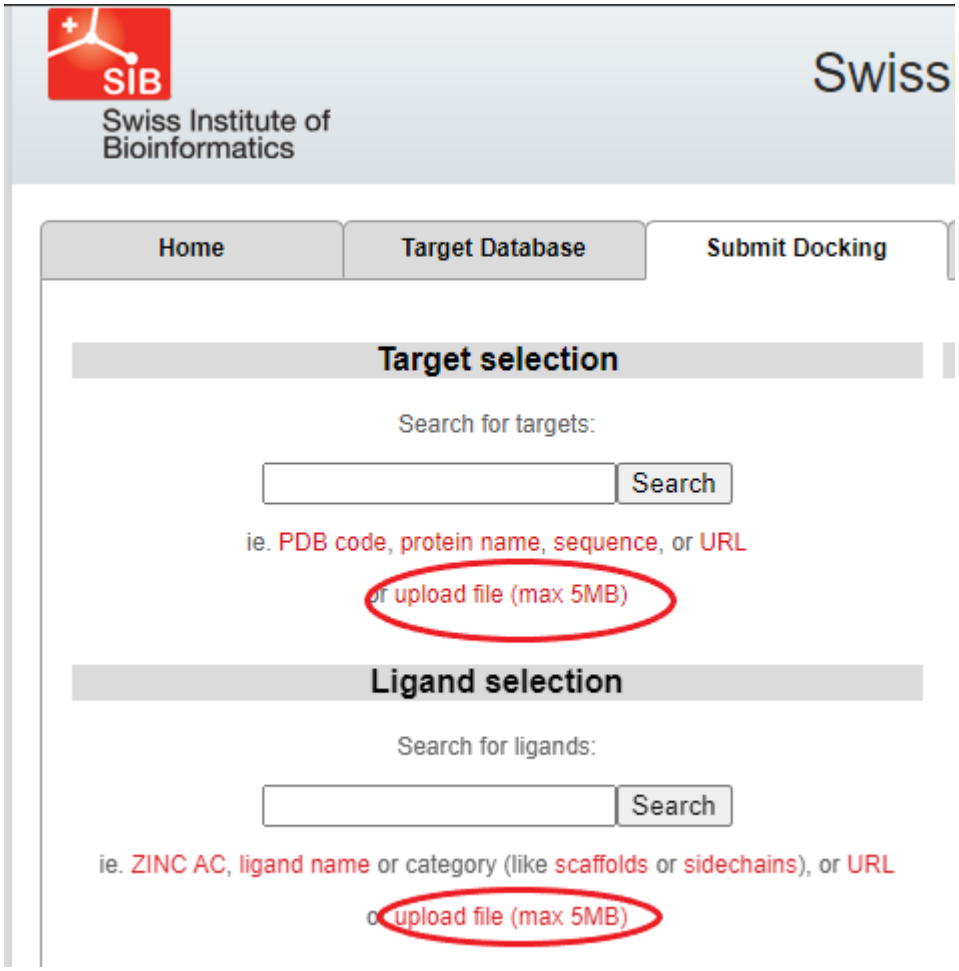
Purpose

- To perform molecular docking and evaluate the accuracy in terms of actual number binding amino residues

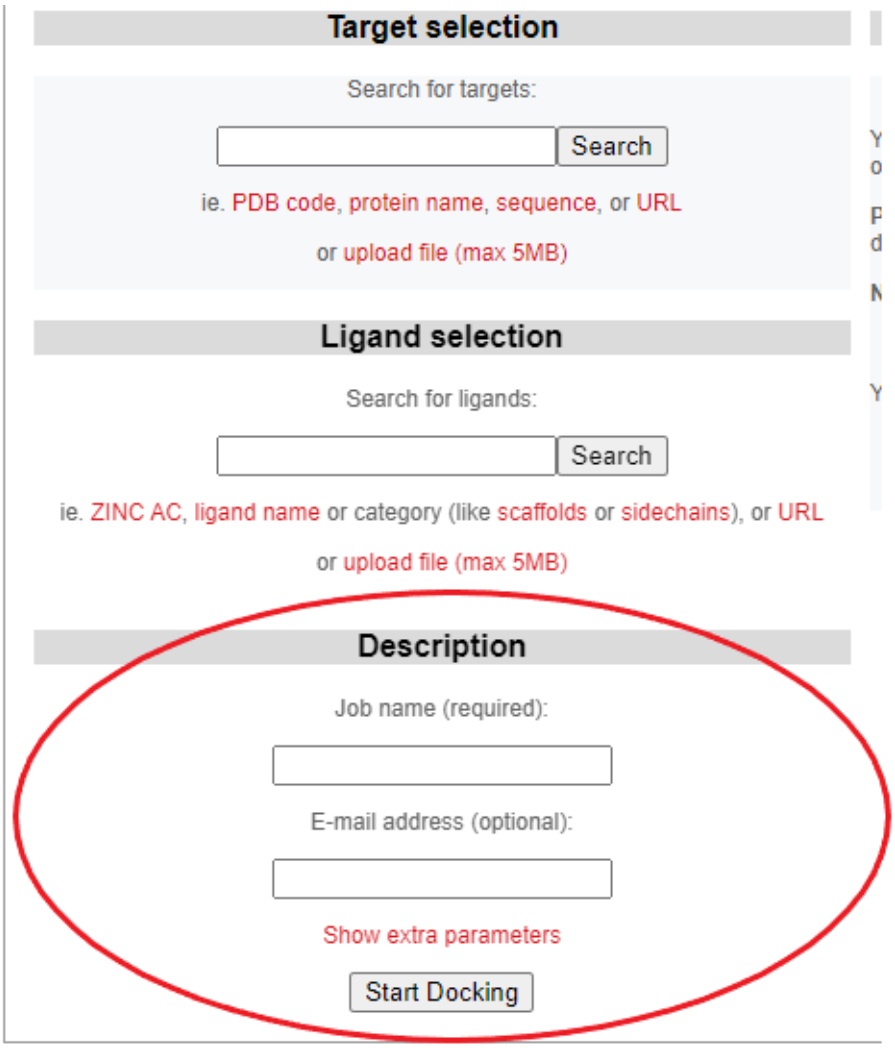
Procedures

1. Access the Swissdock server with the link : http://www.swissdock.ch/docking

2. Upload the files of protein and ligand



3. Input the job name and email address to receive notification upon the docking job is finished



PatchDock

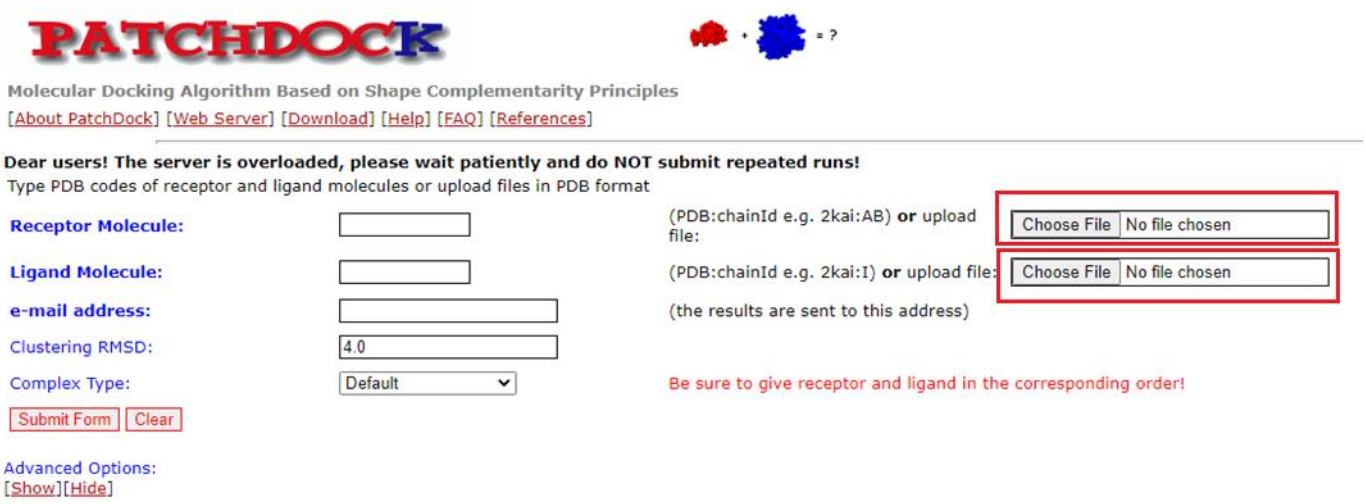
Purpose

- To perform molecular docking and evaluate the accuracy in terms of actual number binding amino residues

Procedures

1. Access the PatchDock server with the link : https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php

2. Upload the files of protein (as receptor molecule) and ligand



3. Input the email address to know the progress of docking simulations

4. Submit the docking action

DockThor

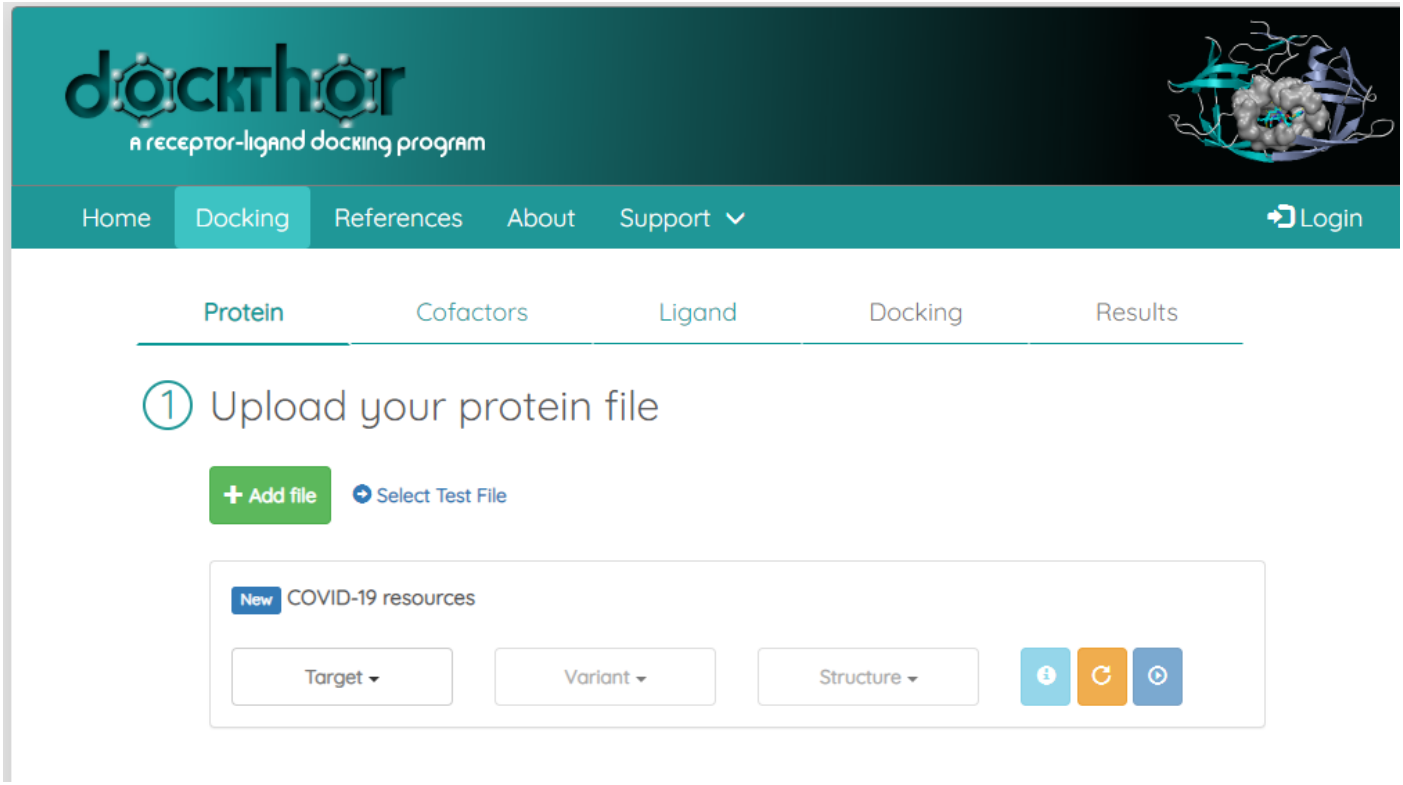
Purpose

- To carry out molecular docking and evaluate the accuracy in terms of actual number binding amino residues

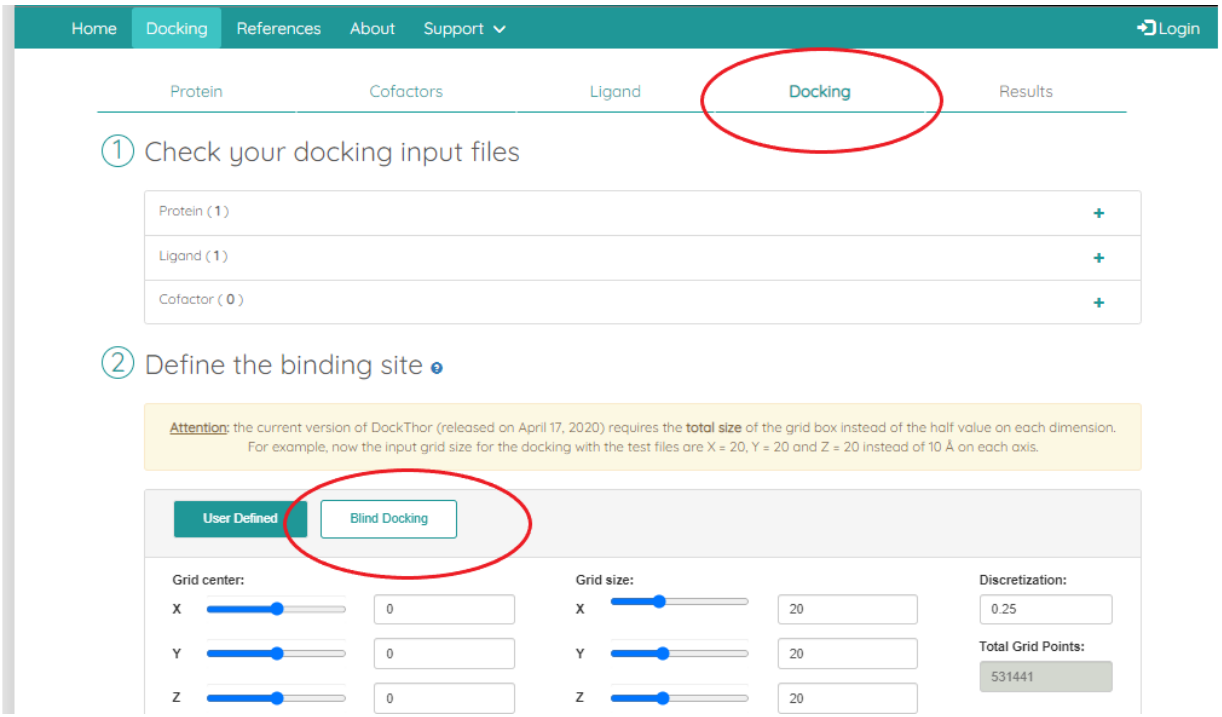
Procedures

1. Access the DockThor server with the link : https://dockthor.lncc.br/v2/

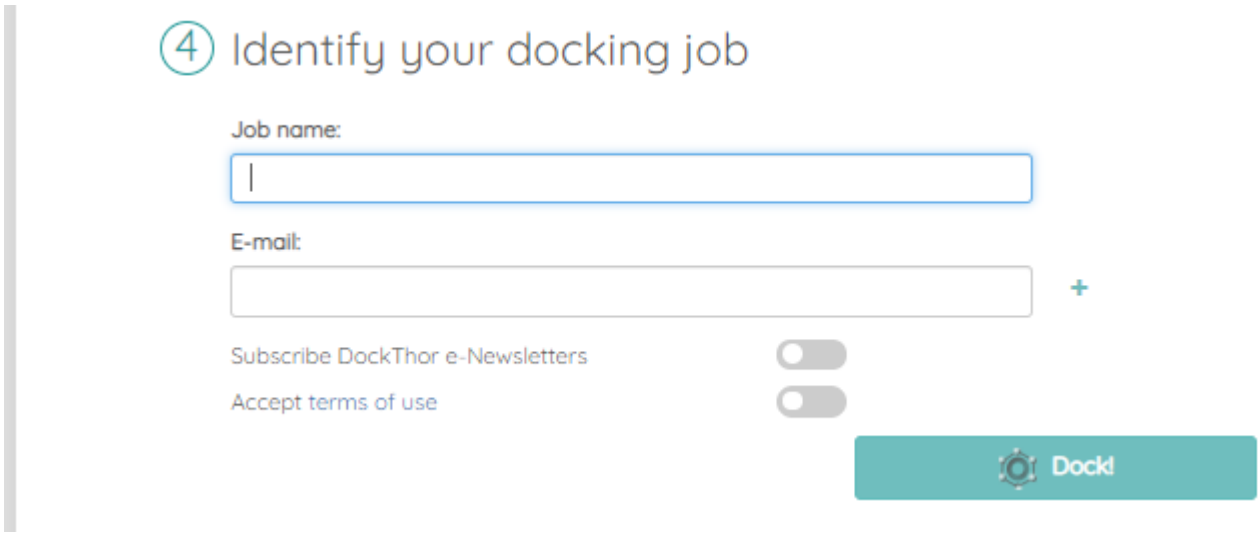
2. Upload the files of protein and ligand but ignore the cofactors



3. Perform blind docking without setting up any defined position of grid box



4. Input the email address to keep an eye on the progress of the job and then onclick ‘Dock’



EDock

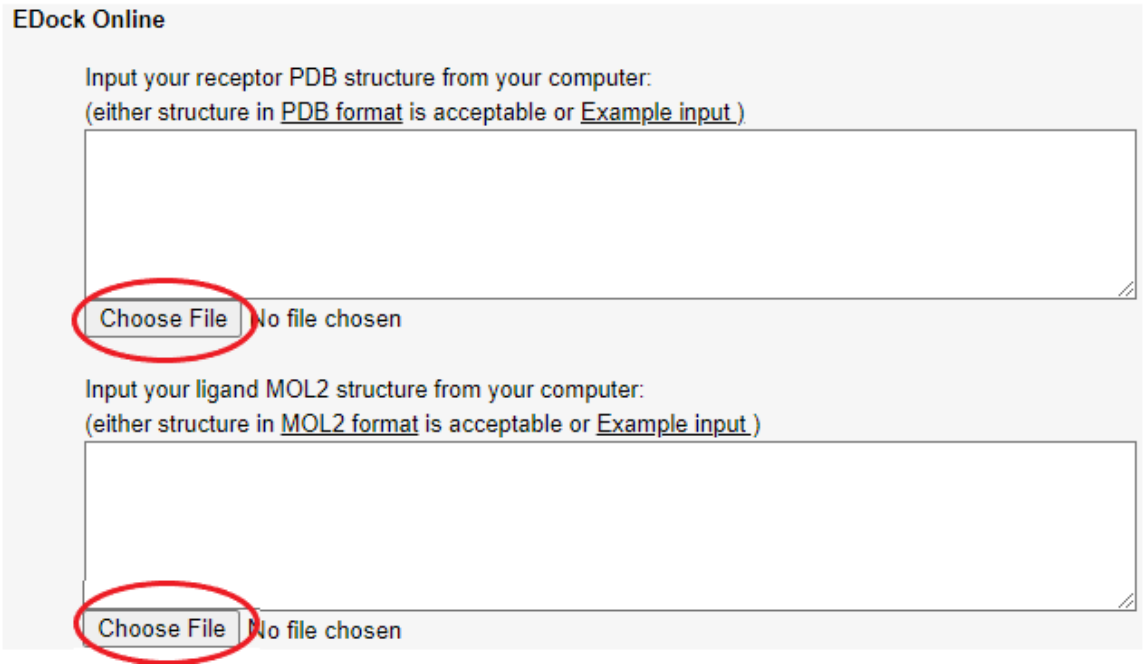
Purpose

- To carry out molecular docking and evaluate the accuracy in terms of actual number binding amino residues

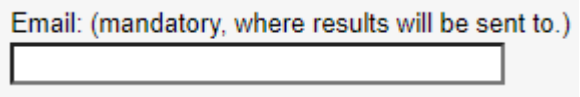
Procedures

1. Access the EDock server with the link: https://zhanglab.ccmb.med.umich.edu/EDock/

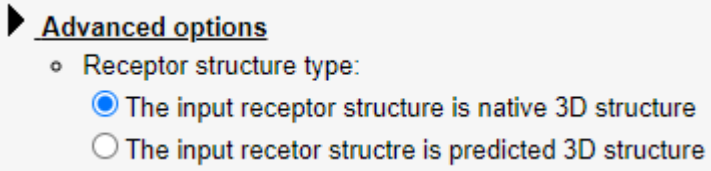
2. Upload the files of protein and ligand



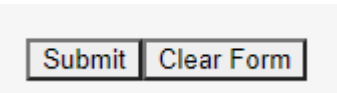
3. Input the email address to know the progress of the job



4. Select 'The input receptor structure is native 3D structure'



5. Click 'Submit'



LIGPLOT**

Purpose

- To generate schematic diagrams of protein-ligand interaction in pdb file format
- The binding site of the enzyme and substrate can then be further determined

Computational Configuration Requirement

- Java
- Windows 8 / 10

Procedures

1. Import the protein-ligand complex in LIGPLOT in pdb format

2. Select 'UNK' mode to run the program

3. Obtain the schematic diagram of the complex showing hydrogen-bonding and hydrophobic interaction between protein molecule and ligand

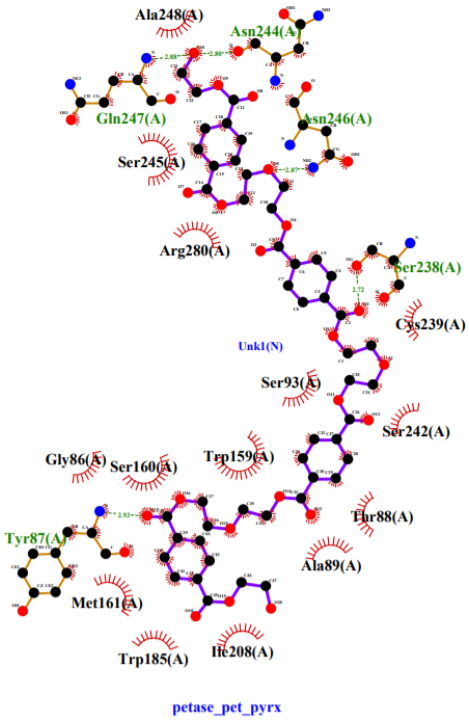


Fig. 4 : Schematic diagram of PETase-PET interaction. It shows hydrophobic interaction (red) and hydrogen bonding formation (green) at specific location

**Reference : Sanket Bapat's Youtube channel

PyMOL Visualization Tool**

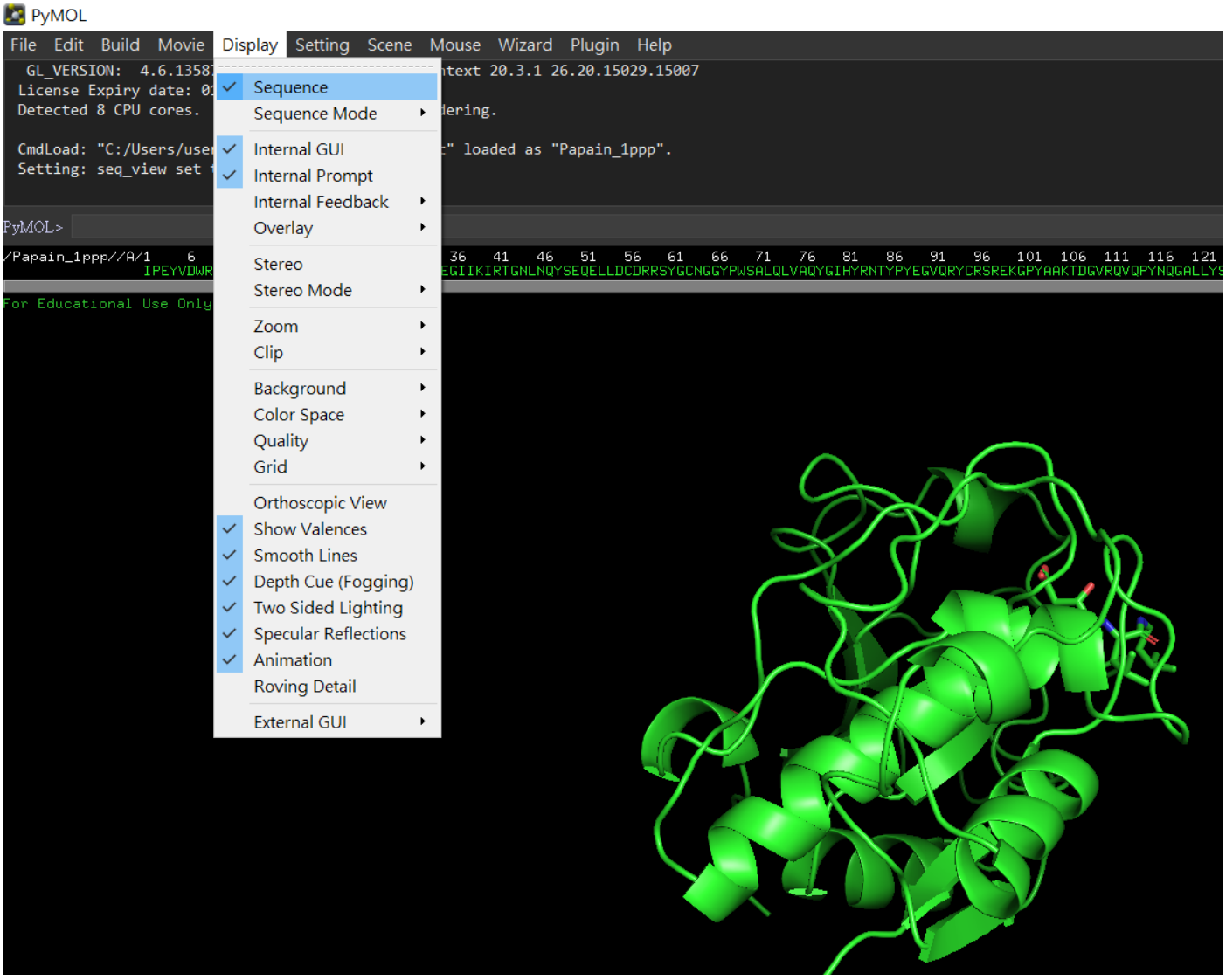
Purpose

- To perform mutagenesis
- To show the crystal structures of enzyme

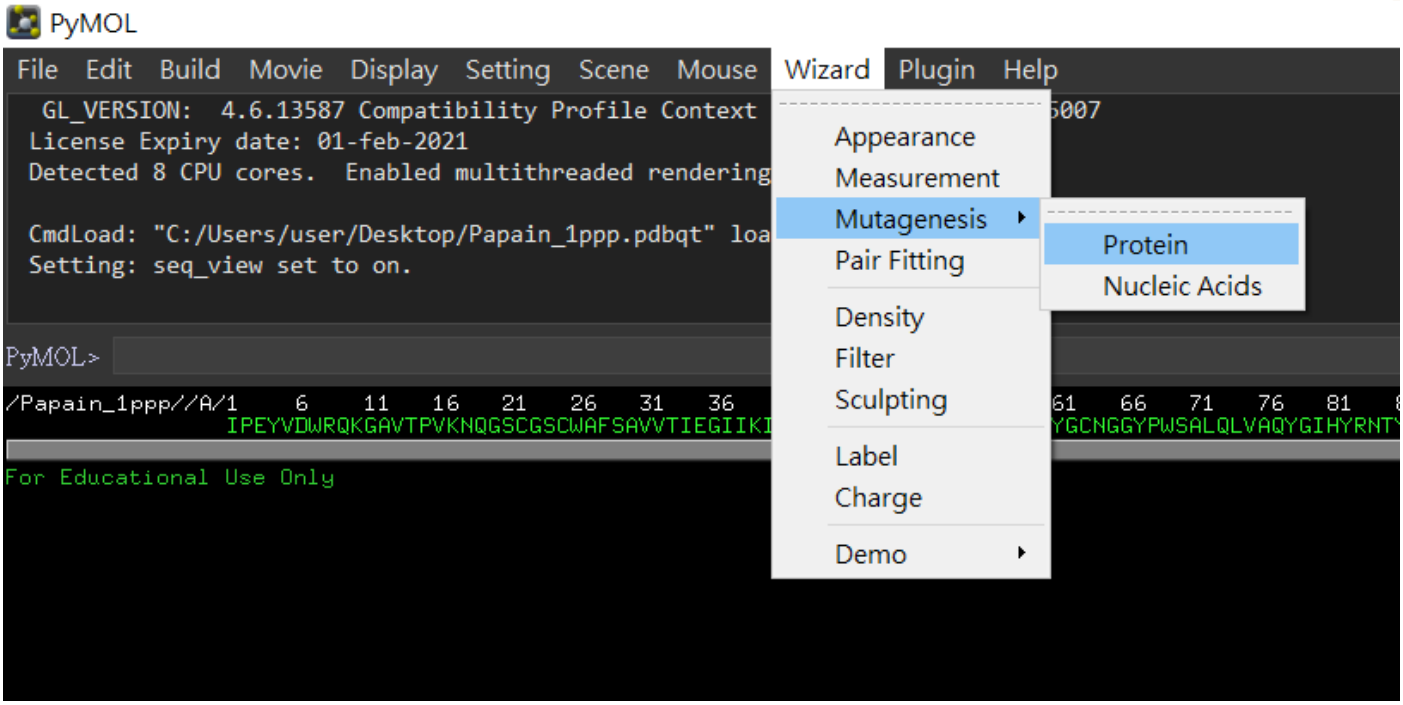
Procedures

1. Input the protein of interest into PyMOL

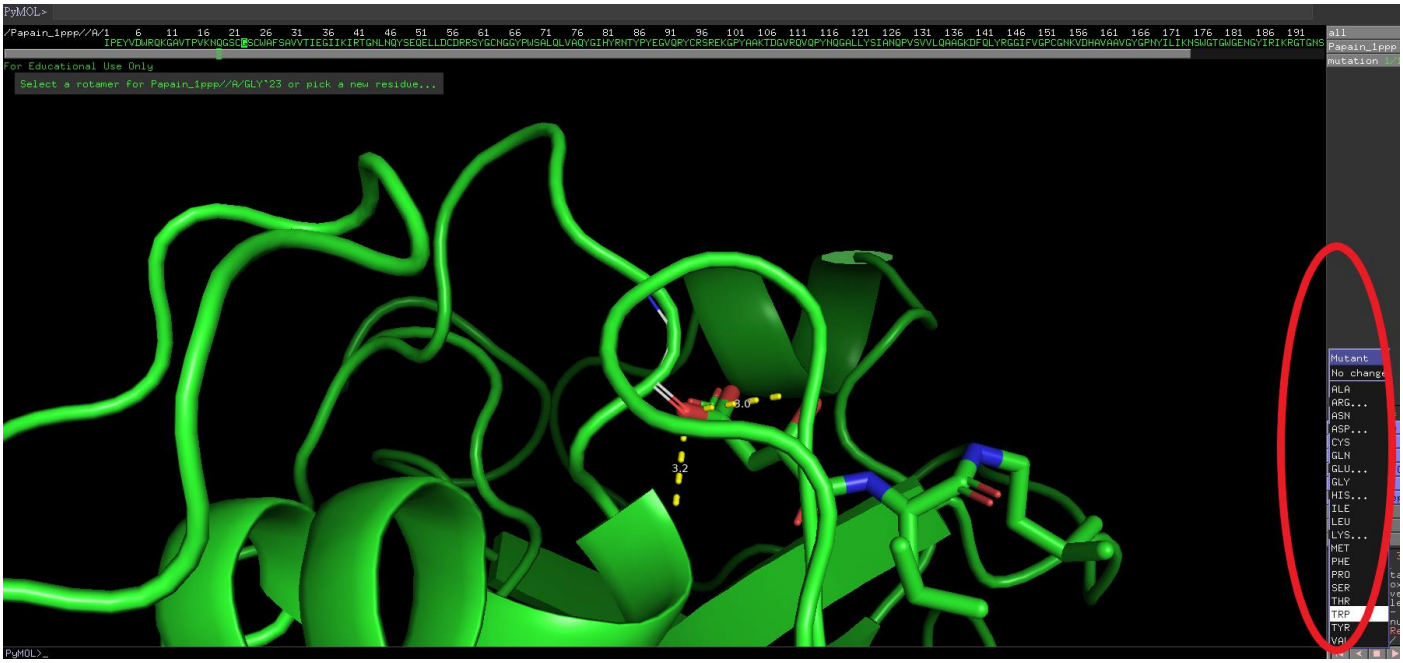
2. Use the ‘sequence display’ feature to view its amino acid sequence



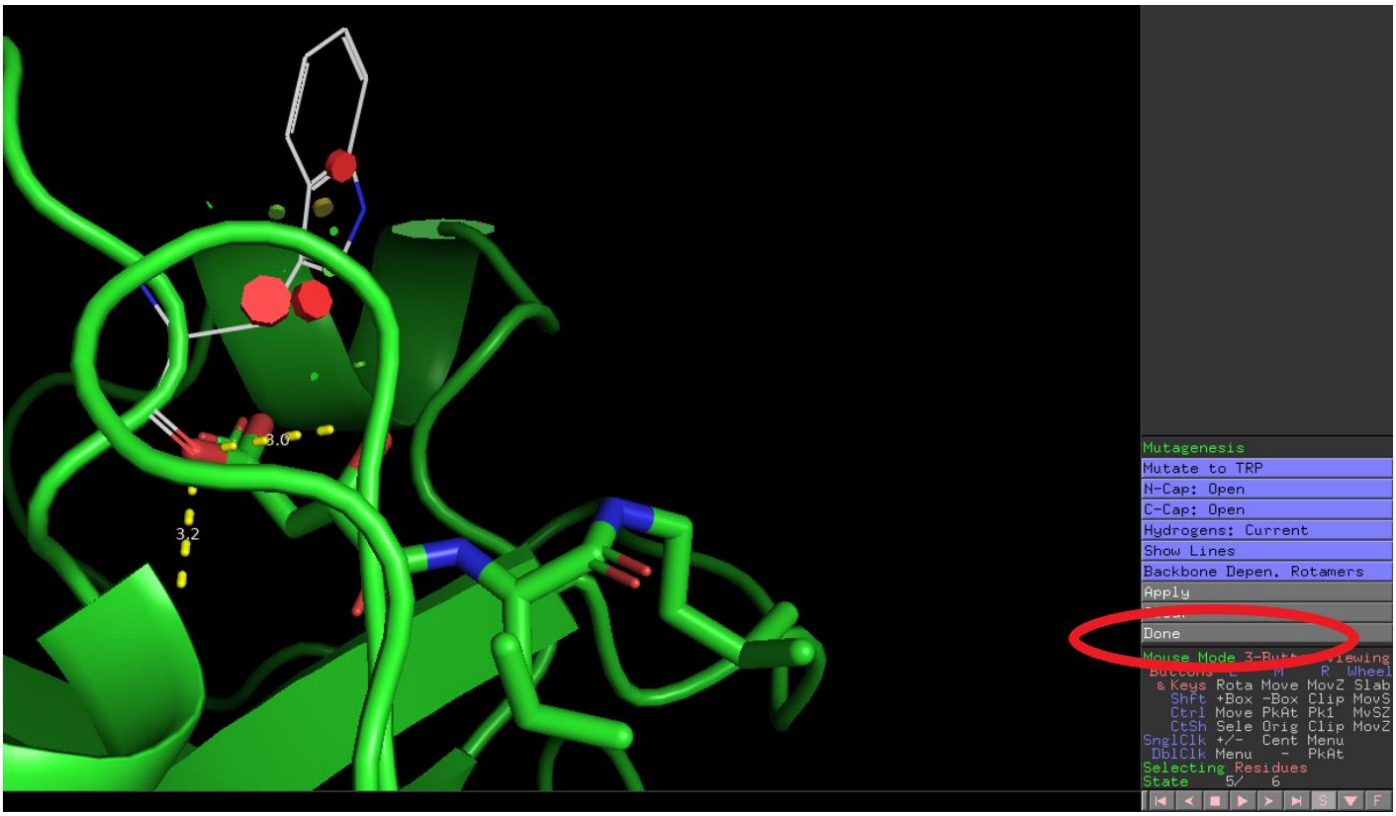
3. Locate an amino acid residue on the conserved region



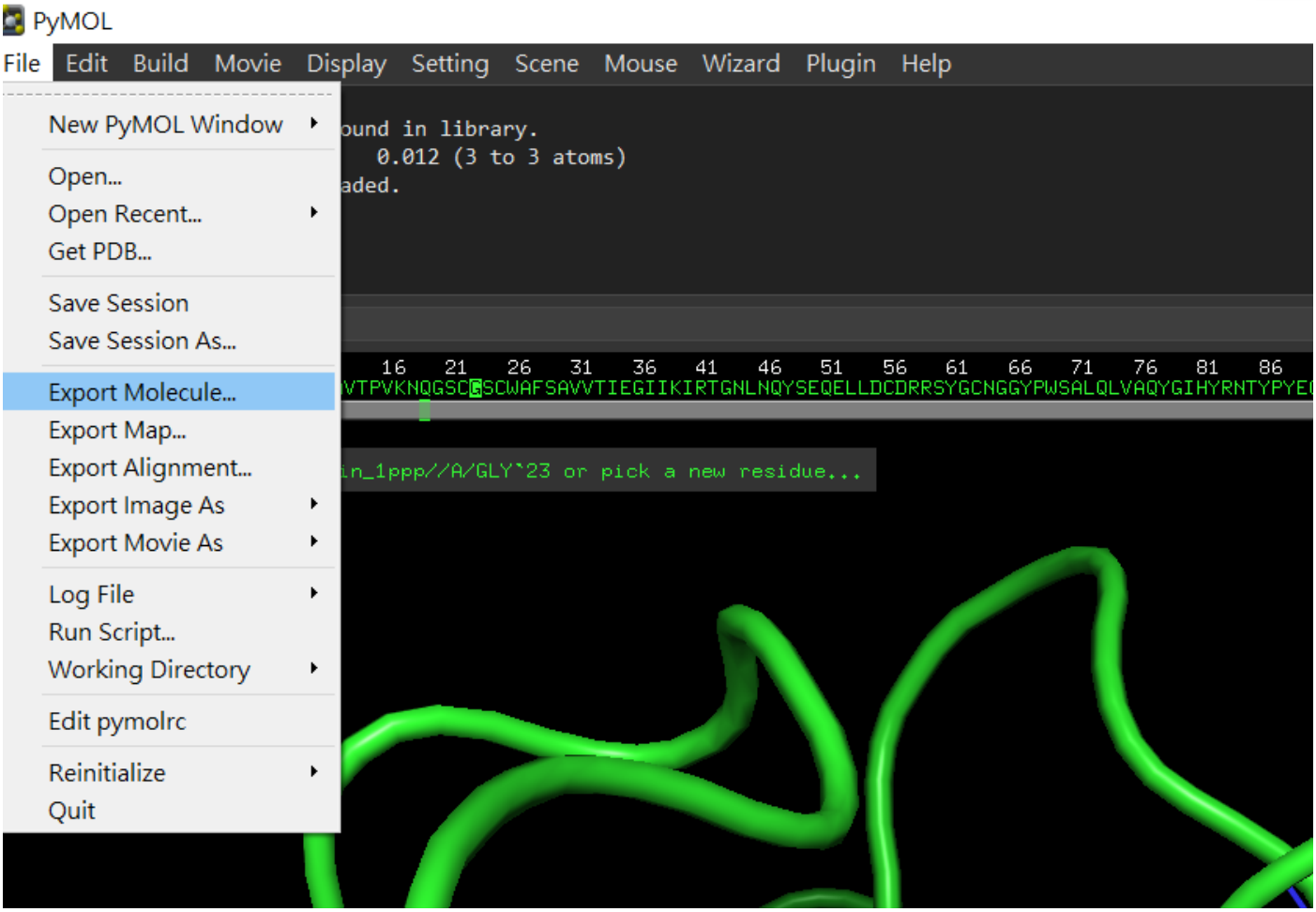
4. Alter one amino acid residue to non-polar nature (e.g. Tryptophan) in order to enhance affinity



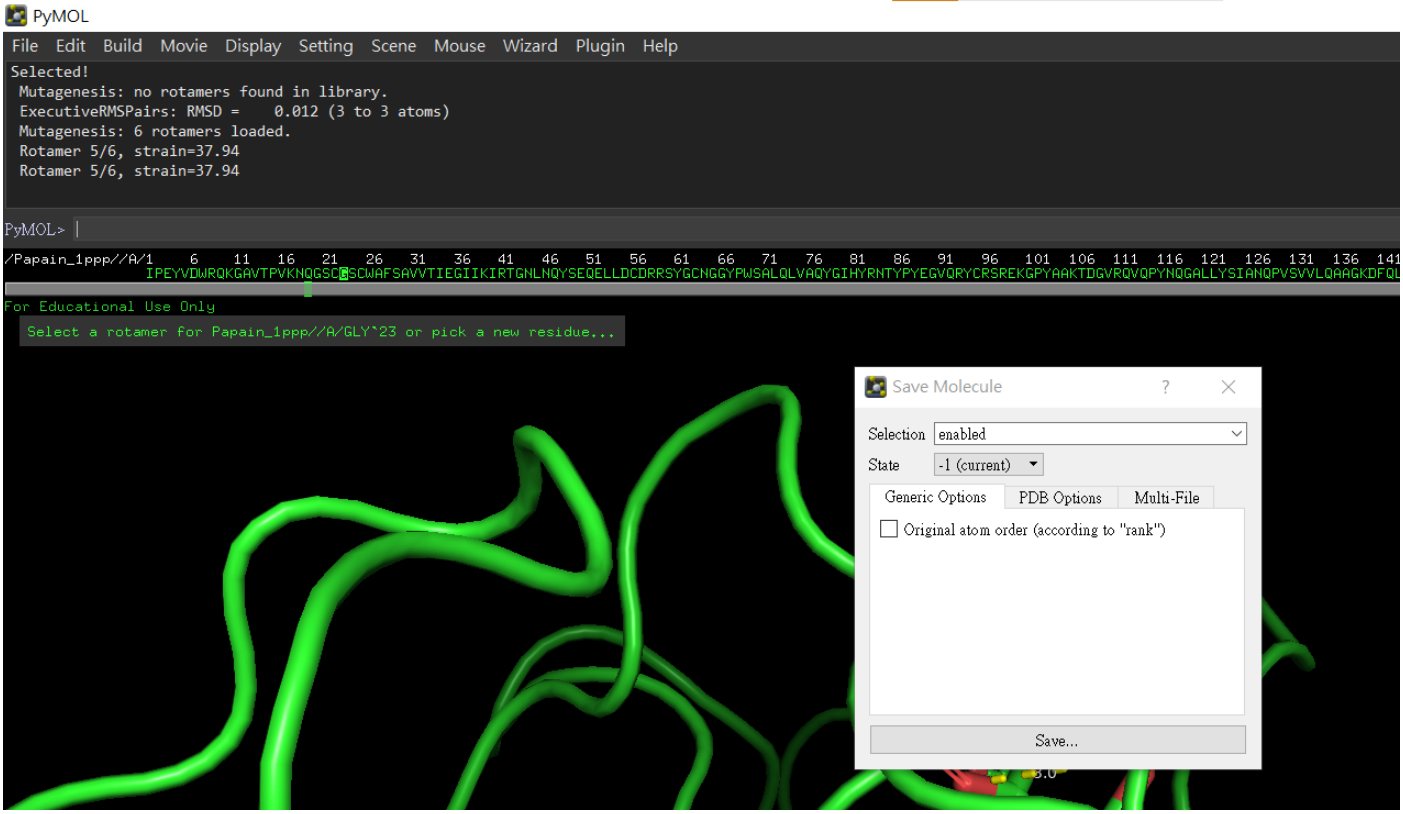
5. Onclick ‘done’



6. Export the molecule



7. Save the mutant enzyme in pdb format



**Reference : Bioinformatics Review's Youtube channel