

# Protocol

CityU iGEM 2020

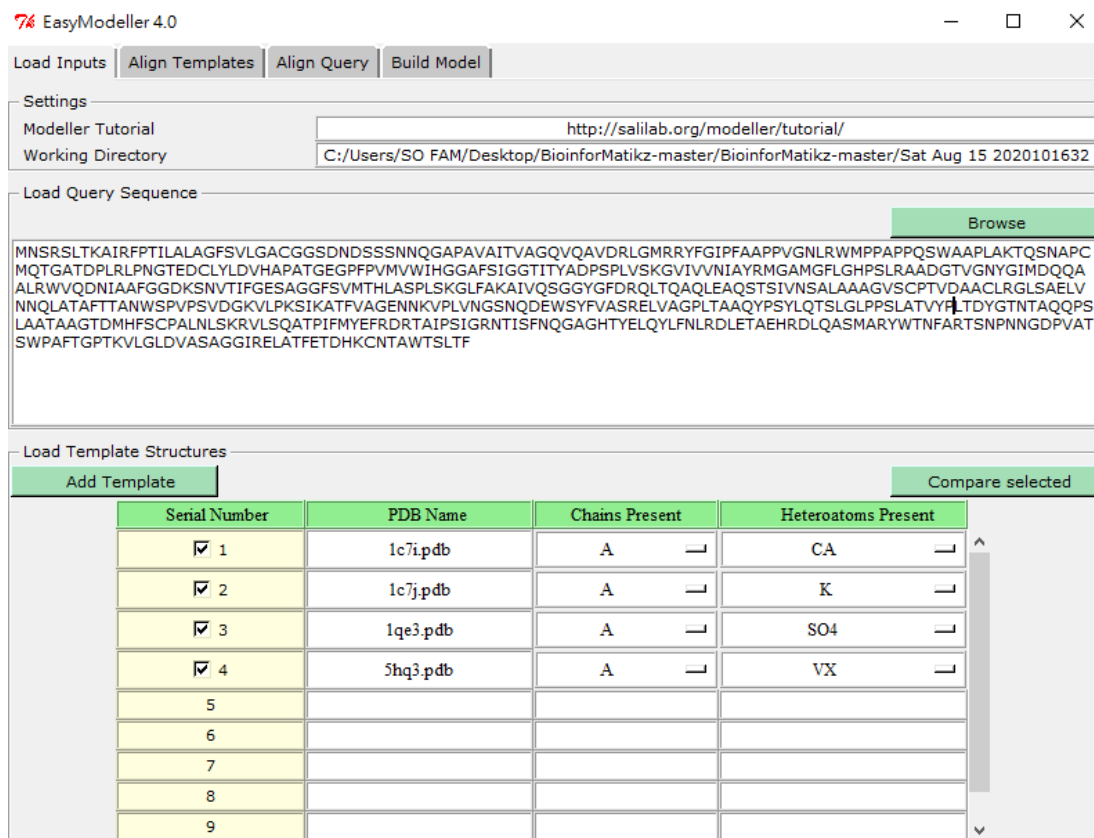
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## Table of Contents

|                                  |    |
|----------------------------------|----|
| Easy Modeller.....               | 2  |
| AutoDock Tools .....             | 4  |
| Swissdock.....                   | 8  |
| PatchDock .....                  | 10 |
| DockThor .....                   | 11 |
| EDock.....                       | 13 |
| LIGPLOT** .....                  | 14 |
| PyMOL Visualization Tool** ..... | 15 |



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



- 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
```

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

# AutoDock Tools

## Purpose

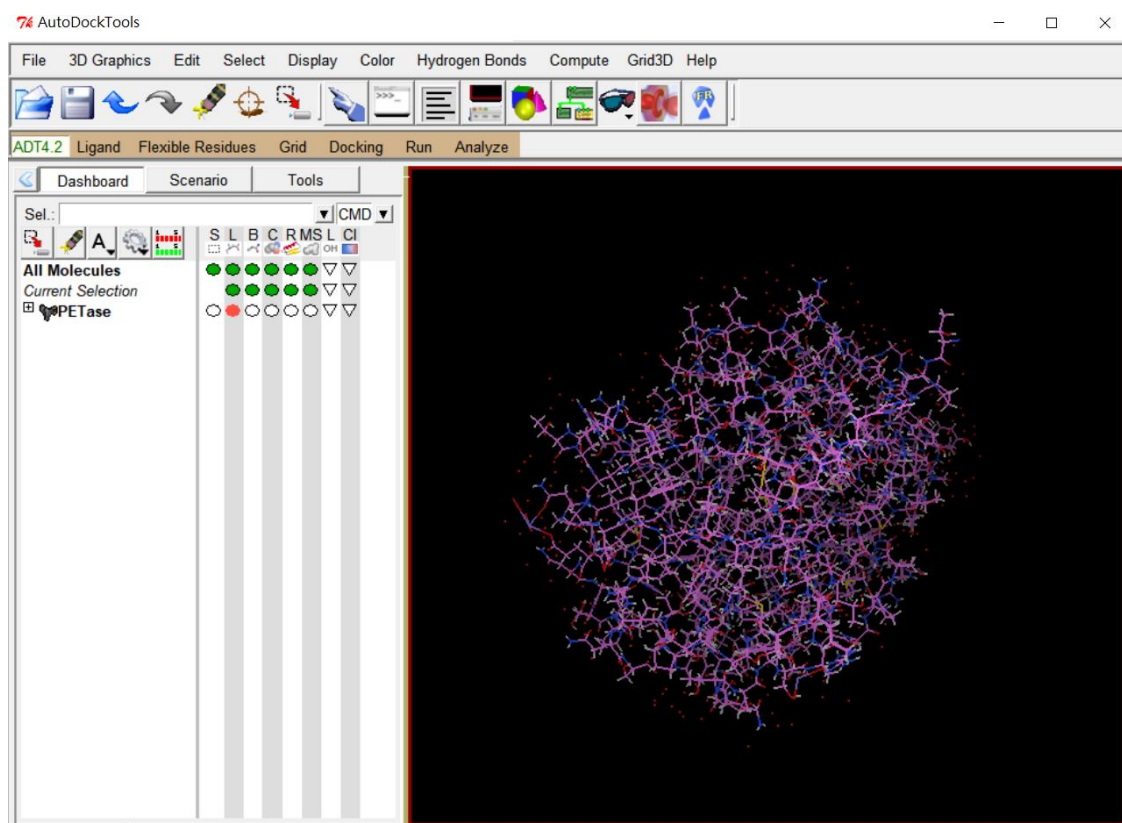
- To perform molecular docking and evaluate the accuracy in terms of actual number binding amino residues

## Computational Configuration Requirement

- Windows 8 / 10

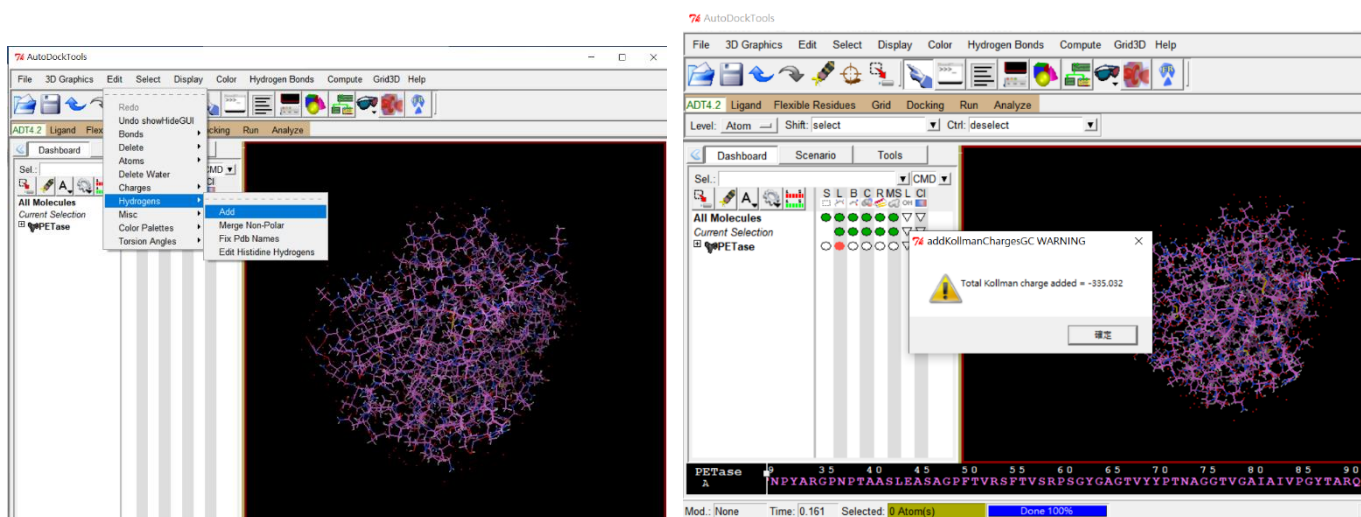
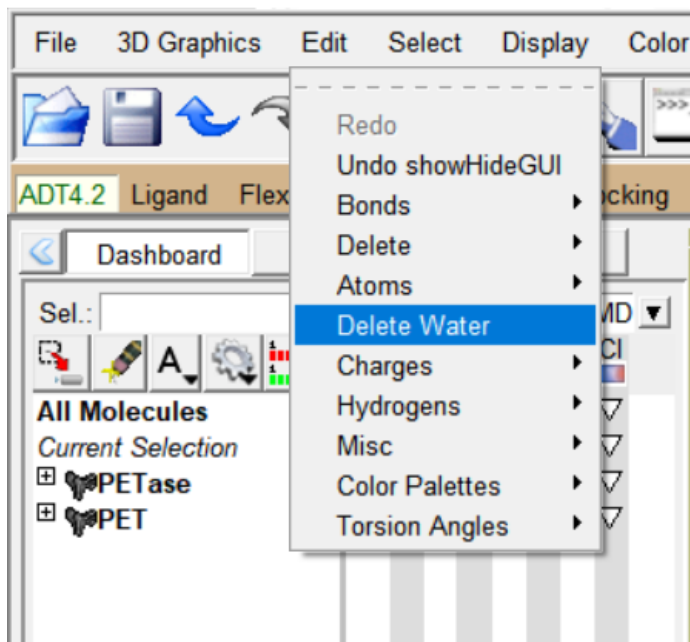
## Protein Preparation

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.

#### 76 Python Molecule Viewer

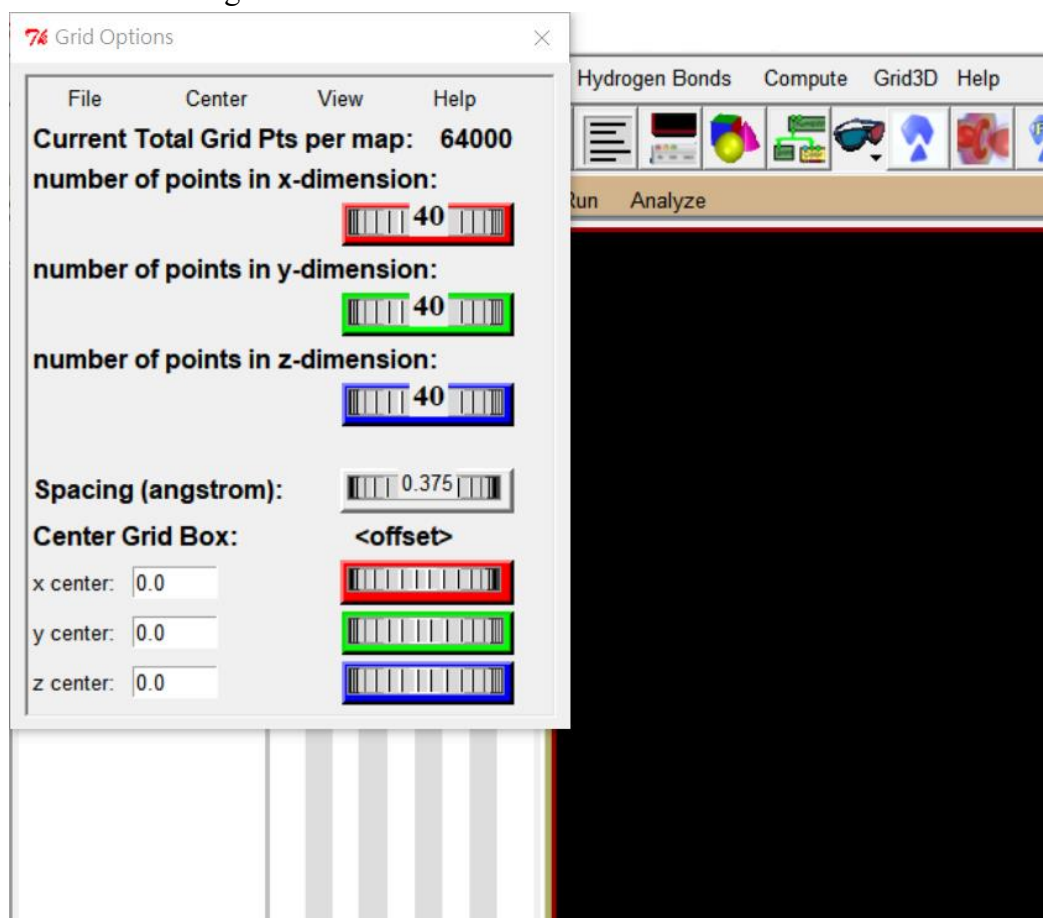


### Ligand Preparation

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

## Grid Setup

- 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

- 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
```

```
C:\Users\SAMSUNG\Desktop\Docking>"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor Pula.pdbqt --ligand pur_tetramer.pdbqt --config config.txt --log log.txt --out output.pdbqt
#####
# If you used AutoDock Vina in your work, please cite:      #
#                                                            #
# 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                                                    #
# DOI 10.1002/jcc.21334                                     #
# Please see http://vina.scripps.edu for more information.   #
#####
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1620709760
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|----|----|----|----|----|----|----|----|----|
*
```

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



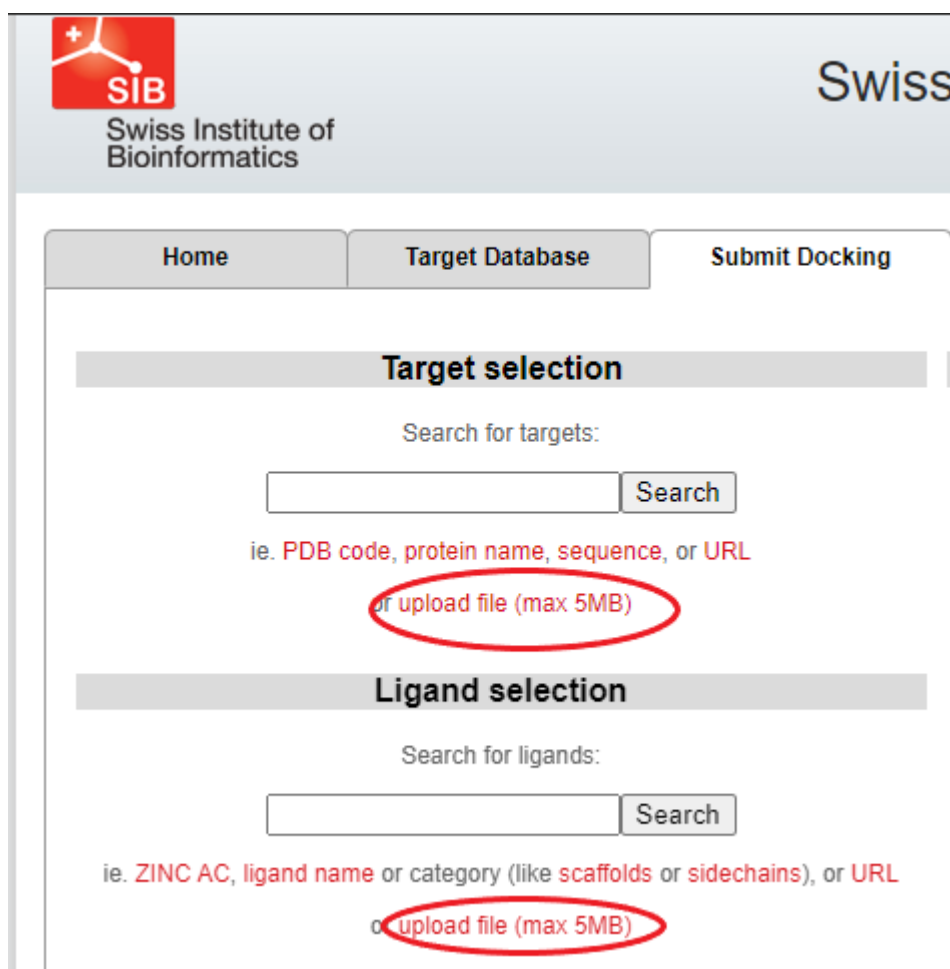
# Swissdock

## 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



The screenshot displays the Swissdock web interface. At the top left is the SIB logo (Swiss Institute of Bioinformatics) and the word "Swiss". Below this is a navigation bar with buttons for "Home", "Target Database", and "Submit Docking". The main content area is divided into two sections: "Target selection" and "Ligand selection".

**Target selection**

Search for targets:

ie. PDB code, protein name, sequence, or URL

upload file (max 5MB)

**Ligand selection**

Search for ligands:

ie. ZINC AC, ligand name or category (like scaffolds or sidechains), or URL

upload file (max 5MB)

In both sections, the "upload file (max 5MB)" option is circled in red.

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

**Target selection**

Search for targets:

ie. PDB code, protein name, sequence, or URL  
or upload file (max 5MB)

**Ligand selection**

Search for ligands:

ie. ZINC AC, ligand name or category (like scaffolds or sidechains), or URL  
or upload file (max 5MB)

**Description**

Job name (required):

E-mail address (optional):

[Show extra parameters](#)

Y  
o  
P  
d  
N  
Y


# 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

**PATCHDOCK** 

Molecular Docking Algorithm Based on Shape Complementarity Principles  
[\[About PatchDock\]](#) [\[Web Server\]](#) [\[Download\]](#) [\[Help\]](#) [\[FAQ\]](#) [\[References\]](#)

---

**Dear users! The server is overloaded, please wait patiently and do NOT submit repeated runs!**  
Type PDB codes of receptor and ligand molecules or upload files in PDB format

**Receptor Molecule:**  (PDB:chainId e.g. 2kai:AB) **or** upload file:  No file chosen

**Ligand Molecule:**  (PDB:chainId e.g. 2kai:I) **or** upload file:  No file chosen

**e-mail address:**  (the results are sent to this address)

**Clustering RMSD:**

**Complex Type:**

Advanced Options:  
[\[Show\]](#)[\[Hide\]](#)

**Be sure to give receptor and ligand in the corresponding order!**

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

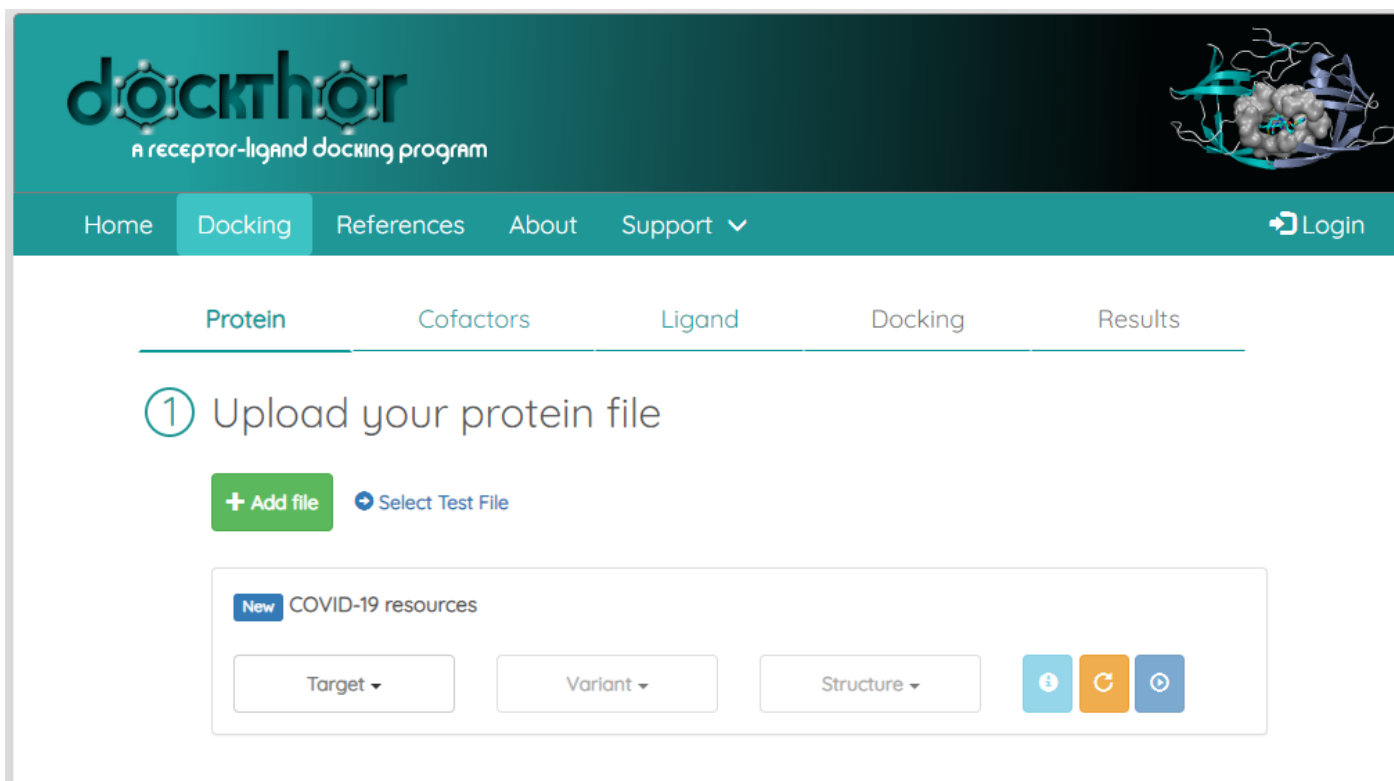
# DockThor

## 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



The screenshot displays the DockThor web interface. At the top, the logo 'dockthor' is shown with the tagline 'A RECEPTOR-LIGAND DOCKING PROGRAM' and a 3D molecular model. The navigation menu includes 'Home', 'Docking', 'References', 'About', 'Support', and 'Login'. Below the menu, the 'Protein' tab is selected, and the main heading reads '1 Upload your protein file'. Two buttons are visible: '+ Add file' and 'Select Test File'. A section titled 'New COVID-19 resources' contains three dropdown menus labeled 'Target', 'Variant', and 'Structure', along with three utility icons (info, refresh, and a circular arrow).

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

The screenshot shows the DockThor web interface. The top navigation bar includes 'Home', 'Docking', 'References', 'About', and 'Support'. The 'Docking' tab is highlighted with a red circle. Below the navigation bar, there are tabs for 'Protein', 'Cofactors', 'Ligand', 'Docking', and 'Results'. The 'Docking' tab is selected. The main content area is divided into two steps: 1. Check your docking input files, and 2. Define the binding site. In step 2, there is a yellow attention box stating: 'Attention: the current version of DockThor (released on April 17, 2020) requires the total size of the grid box instead of the half value on each dimension. For example, now the input grid size for the docking with the test files are X = 20, Y = 20 and Z = 20 instead of 10 Å on each axis.' Below this, there are two tabs: 'User Defined' and 'Blind Docking'. The 'Blind Docking' tab is selected and highlighted with a red circle. The 'Blind Docking' section contains sliders and input fields for 'Grid center' (X, Y, Z) and 'Grid size' (X, Y, Z). The 'Grid center' values are all 0. The 'Grid size' values are all 20. The 'Discretization' is set to 0.25. The 'Total Grid Points' is 531441. A 'Dock' button is visible at the bottom right.

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

The screenshot shows the 'Identify your docking job' step. It features a 'Job name:' label followed by a text input field. Below that is an 'E-mail:' label followed by a text input field with a '+' icon to its right. There are two toggle switches: 'Subscribe DockThor e-Newsletters' and 'Accept terms of use'. At the bottom right, there is a large teal 'Dock!' button with a gear icon.

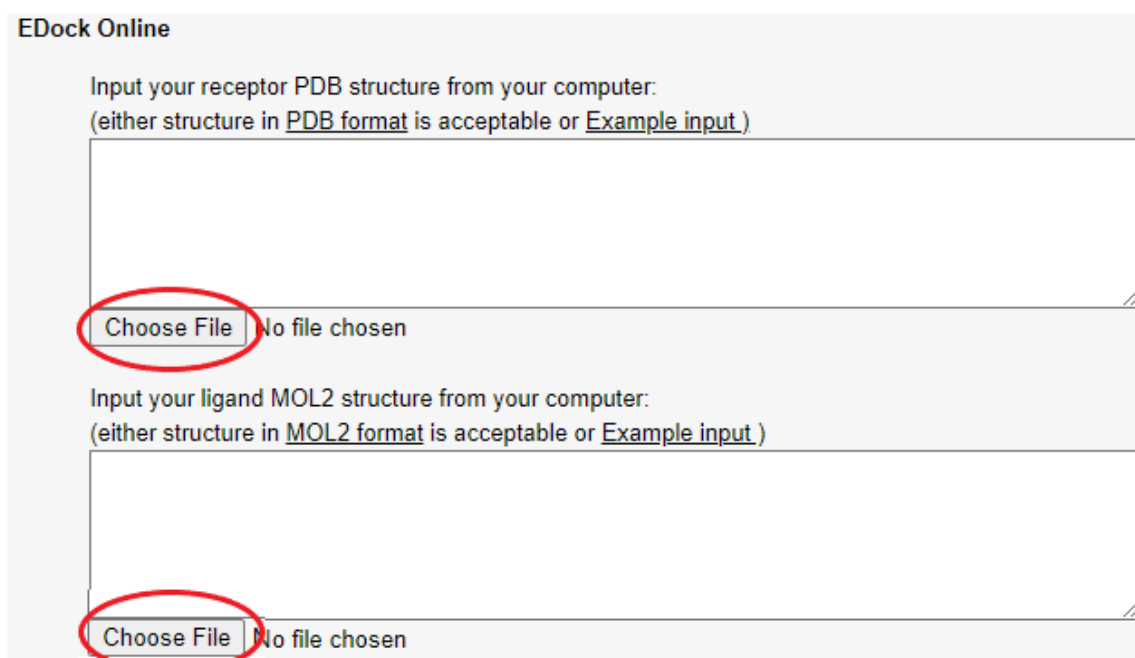
# EDock

## 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



EDock Online

Input your receptor PDB structure from your computer:  
(either structure in PDB format is acceptable or Example input.)

Choose File No file chosen

Input your ligand MOL2 structure from your computer:  
(either structure in MOL2 format is acceptable or Example input.)

Choose File No file chosen

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

Email: (mandatory, where results will be sent to.)

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

► **Advanced options**

- Receptor structure type:
  - The input receptor structure is native 3D structure
  - The input recetor structre is predicted 3D structure

5. Click 'Submit'

Submit Clear Form

# LIGPLOT\*\*

## Purpose

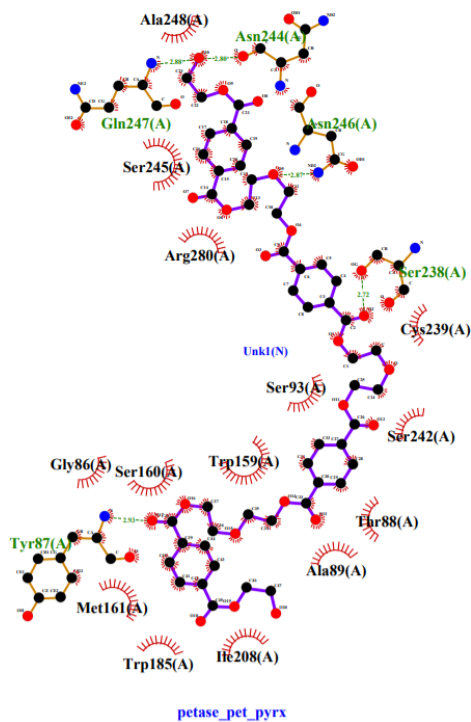
- To generate schematic diagrams of protein-ligand interaction in pdb file format
- The binding site of the enzyme and substrate can then be further determined

## 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

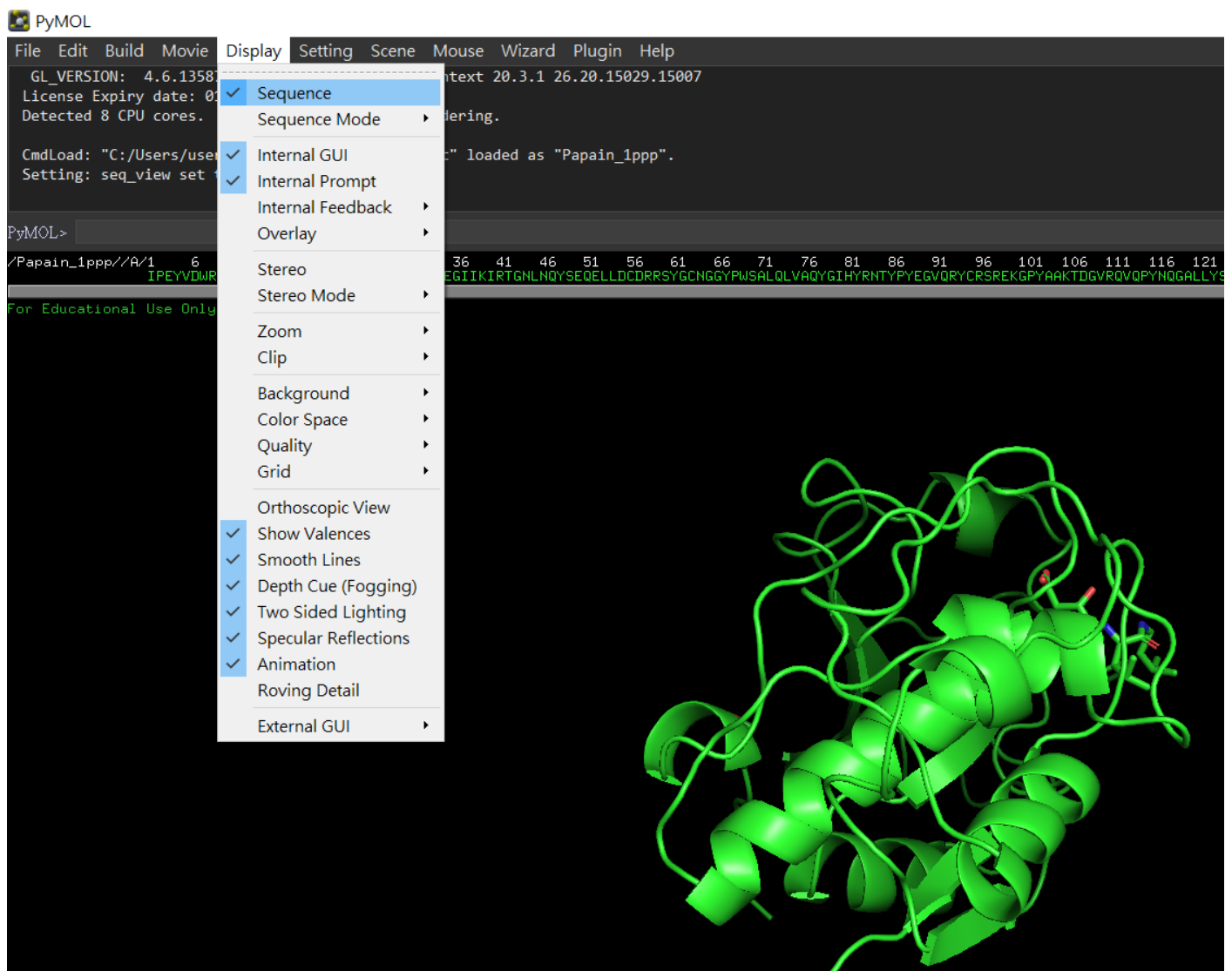
# PyMOL Visualization Tool\*\*

## Purpose

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

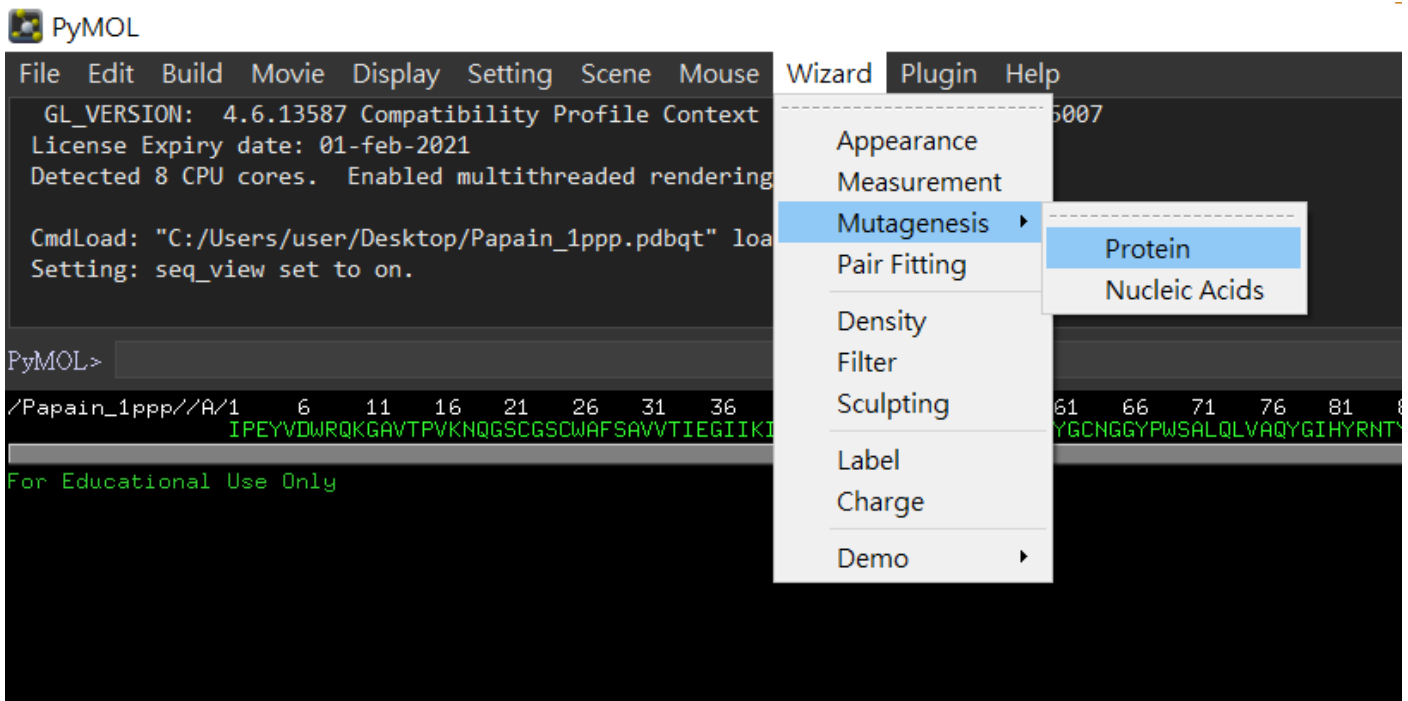
## Procedures

1. Input the protein of interest into PyMOL
2. Use the 'sequence display' feature to view its amino acid sequence

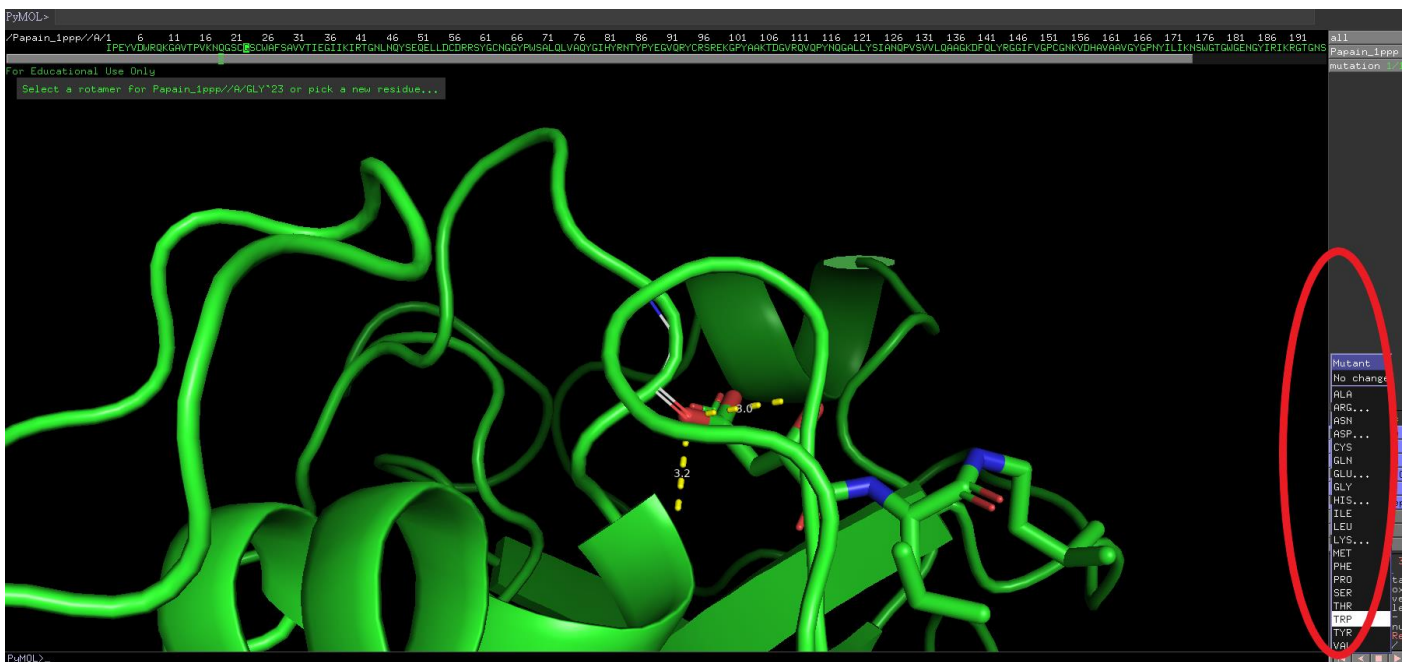




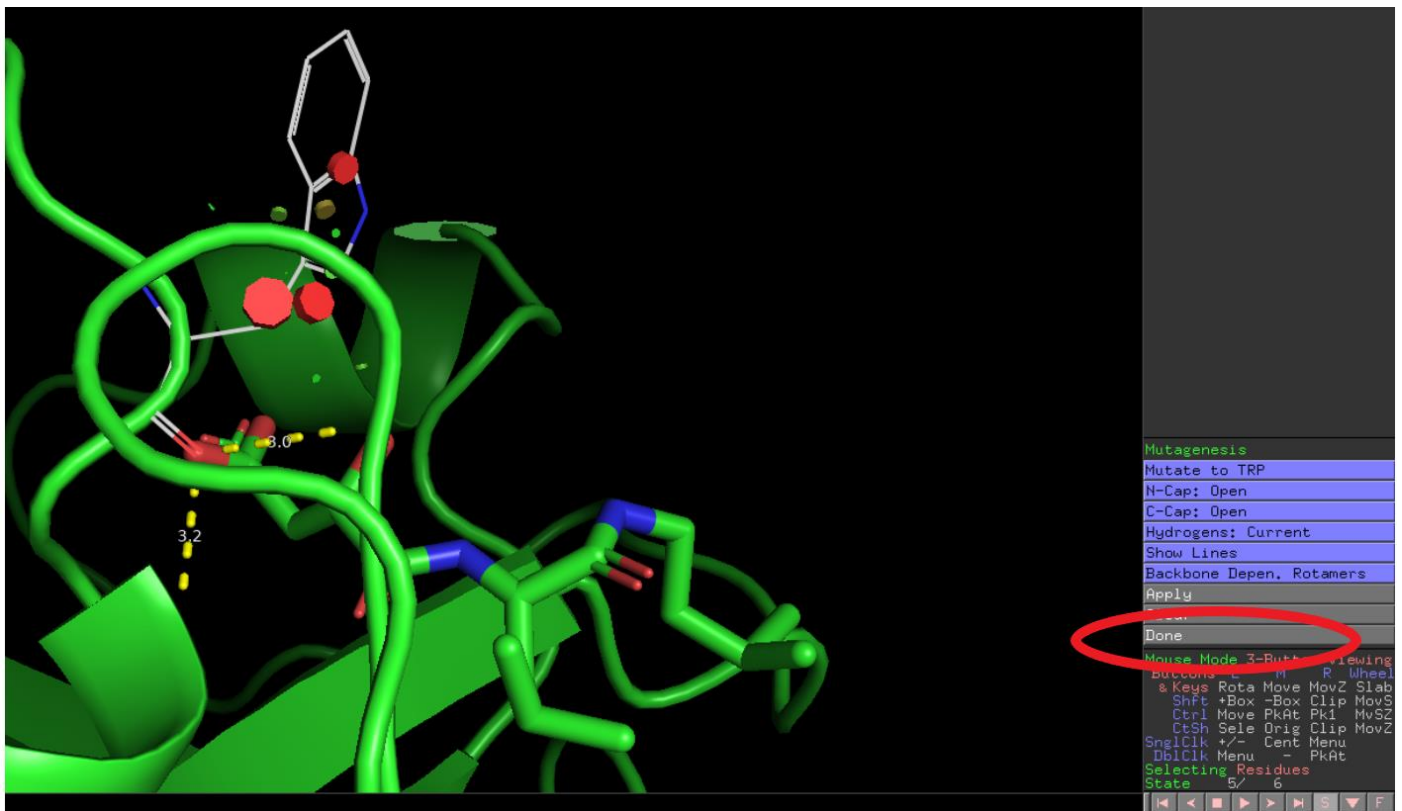
### 3. Locate an amino acid residue on the conserved region



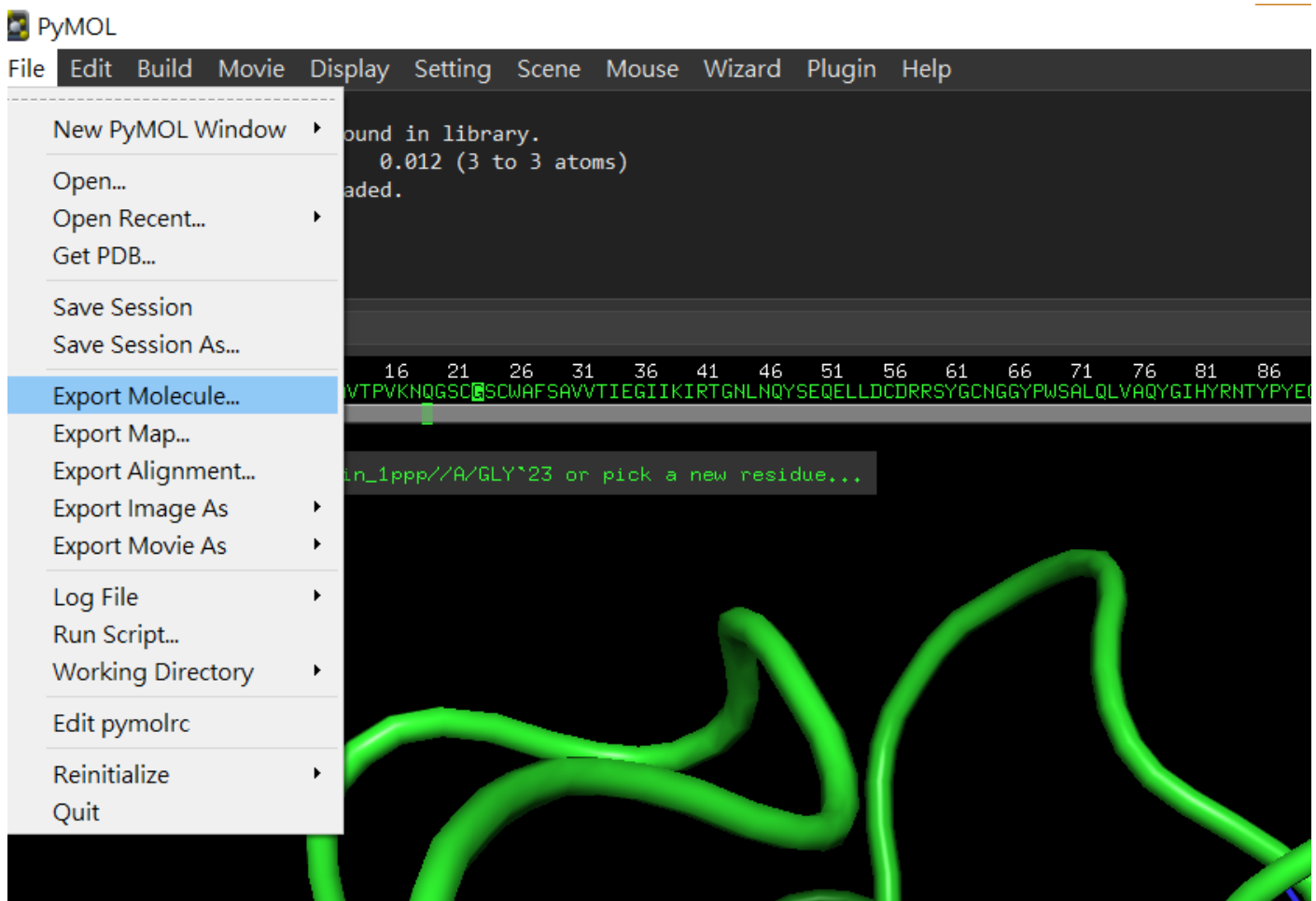
### 4. Alter one amino acid residue to non-polar nature (e.g. Tryptophan) in order to enhance affinity



5. Onclick 'done'



6. Export the molecule



## 7. Save the mutant enzyme in pdb format

PyMOL

The screenshot displays the PyMOL molecular visualization software interface. At the top, a menu bar includes 'File', 'Edit', 'Build', 'Movie', 'Display', 'Setting', 'Scene', 'Mouse', 'Wizard', 'Plugin', and 'Help'. Below the menu, a status bar shows 'Selected!' and several messages: 'Mutagenesis: no rotamers found in library.', 'ExecutiveRMSPairs: RMSD = 0.012 (3 to 3 atoms)', 'Mutagenesis: 6 rotamers loaded.', 'Rotamer 5/6, strain=37.94', and 'Rotamer 5/6, strain=37.94'. The main window shows a protein structure rendered as a green ribbon. A command line at the bottom left contains the path '/Papain\_1ppp//A/1' followed by a sequence of amino acid residues: '6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96 101 106 111 116 121 126 131 136 141'. A specific residue is highlighted with a green box: 'IPEYVDWRQKGAVTVPKNGGSC@SCWAFSAVVTIEGIKIRTGNLNGYSEQELLDCCRYSYGCNGGYPWSALQLVAQYGIHYRNTYPYEGVQRVCRSREKGPYAAKTDGVRQVQPYNQGALLYSIANQPVSVVLQAAGKDFQL'. A dialog box titled 'Save Molecule' is open in the foreground, showing options for 'Selection' (enabled), 'State' (-1 (current)), and checkboxes for 'Original atom order (according to "rank")'. The 'Save...' button is visible at the bottom of the dialog.

\*\*Reference : Bioinformatics Review's Youtube channel