**PyMOL**

**Aim:**

To visualize the protein and small molecules and give various representation of the small molecules.

**Description:**

Pymol is an open-source tool to visualize the molecules available from web. It runs on windows, linux and MACos. This helps in creating the high-quality images from 3D structures and also helps to visualize the protein, its active site, surface representation of the sites as well as various structural representations of the small molecules. Pymol helps in manipulating structures, mutate the residues and some basic functions are also incorporated to analyze the chemical properties.

**Procedure:**

1. Open PyMoL
2. File Open Select the protein or Small molecule structure
3. Protein is imported in the PyMoL viewer
4. Click S in the viewer panel to change the representation of the protein or small molecules into lines, sticks, ribbon and cartoon.
5. Click A and select the option Preset Ligand Sites Mesh Surface/required style to separate the view of active site.
6. Check Display in the GUI panel and select the Sequence to view the sequence of the protein.
7. Select the residue of the protein in the sequence viewer to label or change the representation of the residue.
8. Check Wizard in the GUI panel and select Mutagenesis. Pick the residue to Mutate. In the Viewer panel Mutagenesis bar will be opened. In the “No mutation” option select the residue to be mutated and apply.
9. Select the Option C in the viewer panel and changes the colour of the selected section.
10. Select option File and save the structure in image or in required format.

**Results:**

**Ligprep**

**Aim:**

To perform minimization of ligand molecules using Ligprep module.

**Description:**

The accurate starting structures are a prerequisite for successful computational drug design. The quality of docking results depends on reasonable structures for both the protein and the ligand. Schrödinger offers a comprehensive ligand preparation facility in LigPrep. The LigPrep process includes addition of missing hydrogen atoms, refinement of bond orders, formal charges, appropriate protonation state and orientation of various functional groups were fixed and eliminated unwanted structures, and finally optimized the given structures. It is strongly recommended that ligand structures should be processed with this facility in order to achieve the best results.

**Procedure:**

1. Open maestro, go to applications (or) task and select LigPrep module.
2. Import set of ligands through Import structures option to the work space or click browse option to find the ligand structures.
3. Set the filtering criteria on the molecular descriptors.
4. Select Generate possible states at target pH 7.2 and use Force field (OPLS-2005).
5. Set proper ionization state and pH and neutralize the ligand by additionally choosing appropriate metal binding states.
6. Use Desalt and Select generate tautomeric states (default option).
7. Choose generate all possible combinations.
8. Click Run and Monitor the Job.

**Results:**

**Protein Preparation Wizard**

**Aim:**

To perform minimization of protein molecules using Protein preparation wizard.

**Description:**

The accurate starting structures are a prerequisite for successful computational drug design. The quality of docking results depends on reasonable structures for both the protein and the ligand. The protein was prepared by a multi-step process through protein preparation wizard module (Schrödinger Suite). The crystallographic water molecules and other chemical components were omitted, right bond orders as well as charges and atom types were assigned and hydrogen atoms were added to the crystal structure during preparation. It is strongly recommended that protein structures should be processed with this facility in order to achieve the best results.

**Procedure:**

1. Open Maestro, go to applications (or) task.
2. Select protein Preparation wizard.
3. In the Import and Process tab, enter the PDB ID of protein into the PDB text box, and click Import.
4. Click Preprocess which will add the hydrogen, remove water beyond 5Ȧ of the heteroatom.

(Choose default options for preprocessing the protein structure).

1. Go to Review and Modify tab and remove unwanted chains, het atoms and water molecules in the crystal structures and also generate the ionization of the inhibitor by generate state at a pH range 6.7 to 7.3.
2. Go to Refine tab and click Use Crystal Symmetry and Optimize the H-bond network in the Refine tab.
3. Click optimization and click Minimize to run a restrained minimization on the structure to a RMSD of 0.3 Å for the final refinement.
4. Finally click Run and Monitor the Job.

**Results:**

**Protein-Ligand Docking**

**Aim:**

To perform molecular docking to predict the interaction energy between molecules using SWISS-DOCK

**Description:**

Docking procedures aim to identify correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. Schrödinger implements docking using Glide (Grid-based Ligand Docking with Energetics). Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Glide is designed to assist you in high-throughput screening of potential ligands based on binding mode and affinity for a given receptor molecule. You can compare ligand scores with those of other test ligands, or compare ligand geometries with those of a reference ligand. Additionally, you can use Glide to generate one or more plausible binding modes for a newly designed ligand. Glide docking will performed in two steps: 1. Receptor grid generation, 2. Ligand docking. The receptor grid can be set up and generated from the Receptor Grid Generation panel. It calculates the electrostatic and Vander Waals potentials of the binding pocket using grid based method. In ligand docking step, it generates the conformations and orientations inside the binding pocket in the presence of Grid potentials.

**Procedure:**

**Receptor Grid Generation**

1. Open Maestro, go to applications (or) task.
2. Select Glide and choose Receptor Grid Generation.
3. Display the prepared receptor in the Workspace.
4. In the panel, select “Molecules” option to pick the co- crystal ligand to define the grid center.
5. Otherwise, select “Entry” option to pick the binding site of the protein generated using site map.
6. We can also generate the grid center by specifying residues.
7. Start the grid generation job with output file name.

**Docking**

1. Open Maestro, go to applications or task.
2. Select Glide and choose Ligand docking.
3. In settings option, specify the generated grid file in the receptor grid option on the top of the panel.
4. Select the docking precision- standard precision mode (SP).
5. Select Ligand sampling as Flexible and Add Epik state penalties to docking score.
6. Go to Ligands tab and specify the prepared ligands through file or import ligand structures in the project table.
7. Select Write per-residue interaction scores for residues within N Å of grid center if you want to examine interactions of ligand poses with the receptor, and set the cut-off distance.
8. Choose write report file in the output tab.
9. Modify the jobname and Click Start to run the job.

**Results:**