Prerequisite:

- ➢ Protein-Ligand docking using MOE software.
- ➢ Protein-Protein docking using MOE software.
- ➢ Updated versions of JasMol, JavaScript.

Prerequisite terminologies:

In order to have a thorough understanding of our main topic, you should have the basic concept of the following terminologies:

- ➢ Drug Designing.
- ➢ Ligand.
- ➢ Binding affinities.
- ➢ Biological receptors.

Introduction:

The results we get after docking a receptor molecule against a ligand molecule using any software like MOE are not much reliable to evaluate a particular docking conformation. Also, we get a number of docked complexes from such softwares, so, in order to evaluate and select the best docking complex, we use PDBePISA server. PDBePISA is an interactive tool for the exploration of macromolecular interfaces. Using the PDBePISA,

we can retrieve pre-calculated results for the whole PDB archive and can calculate results interactively for structures uploaded as PDB or mmCIF files.

Steps:

- Click on the link given below to visit the web server page of PDBePISA:
  https://www.ebi.ac.uk/pdbe/pisa/
- Click on the following link to download the PDF copy of the PDBePISA tutorial:
  https://www.ebi.ac.uk/pdbe/docs/Tutorials/workshop_tutorials/PDBepisa.pdf
- From the web server page of PDBePISA, click on the 'Launch PDBePISA' button.
  [It'll open the PISA Query page of PDBePISA, where you have to enter your query to evaluate the docking complex.]
- If you have a PDB ID of your receptor molecule, then enter the PDB ID in the query box and select suitable ligand and then leave the parameters by default and click on the 'Interfaces' button. OR
- If you want to provide the query from your computer, click on 'Coordinate file', then click on the 'Choose file' button and then select the file containing the docking complex on your PC and click on 'Upload'.

Note: Make sure your docking complex file is in .pdf format, otherwise it'll not accept the query file.

➢ **Protein-Protein docking complex evaluation:**

- Before moving on to the next steps, first you need to check the "Analysis" parameter, if it shows a lesser number of amino acid chains then present in your docking complex, you first have to fix that first.
- To do so, open the docking complex file in your Notepad or any other Code Editor.

    - When you open your PDB docking complex file on your Notepad, it'll display the information present in your file in a tabular for.
    - First in the 'Header' section, it'll provide you the information about the 'Helix' and 'Sheets' present in your protein-protein docking complex, which is not of our concern.
    - Then it provides the information about the structure of the complex in the 'Atoms' list, which is our main concern.
    - The first column shows the Atoms list, the second shows the number of atoms, the third shows the symbols of the element (i.e., H, C, N, etc), then in the next column it shows the symbols for the amino acid residues.
        - Then it shows the protein chain (A or B) in the next column, then it shows the residue number in the next column, then in the next three columns it shows the positions of atoms in x, y, and z coordinates respectively.
    - Scroll down the list till the 1st chain terminates, and the 2nd one starts. Since, the first chain(A) represents the ligand molecule, and the second chain (which is also represented as chain A) is the receptor molecule's chain, that is why the PDBePISA is showing the lesser number of chains than present in your complex.
    - So to change the ligand protein chain from A to B, press Ctrl+Alt to select the chain and then after selection of the chain, enter B in upper case, and then save it.

      [In this way, you can manually change the chains present in your docking complex, Chain A as the receptor protein and chain B as the ligand protein.]
- Again click on the 'Choose File' button on the web server page of PDBePISA and select the edited file you've created on the Notepad.
- Then leave other parameters by default and click on 'Interface'.
    - It'll provide you the "PISA Interface list" where it shows the results in tabular form.

- If your complex contains 2 chains, it'll provide you 1 row in the table, and if your complex contains more than 2 chains, the number of rows in the table will be increased accordingly.
- For each of the header (column), click on it (the hyperlinks) to get more details about that particular field:

Column Name	**Description**
NN	➔ No. of rows.
Structure 1	➔ Range. ➔ No.of atoms. ➔ No. of residues. ➔ Surface area in Angstrom units.
x	➔ 'c' in this column represents the covalent bonds. ➔ 'f' in this column represents the ligand that is fixed in the receptor's active site. ➔ Any special symbol in this column represents there are no special properties identified in this complex. ● It also shows the table where it gives the color description of the 3D complex for both the ligand and receptor chains. ● Click on the hyperlink provided in this column to open the 3D structure of the complex. ● You can apply multiple parameters on the structure to analyze it in a better way.
Structure 2	➔ Range. ➔ No.of atoms. ➔ No. of residues. ➔ Surface area in Angstrom units.

- Click on the 'Details' button present below the interface table, to get a detailed description of the 'Interface' parameters in another tabular form.

- On the light hand side of this page, it provides three options to download the files of ‘Structure 1’, ‘Interface’ and ‘Structure 2’. By clicking on any of the respective buttons, it’ll start downloading the respective files on your PC.
- You can also view these files online by clicking on the respective button present against the “View” field.
- Scroll down on this page, it’ll provide you the details about the H-bond and other bonding interactions in the complex.

Note: For an optimal docking complex model, it must contain 5-10 or more than 10 H-bonding interactions in the complex, otherwise it is not acceptable.

- Then below the interacting bonds description, it provides the details about the ‘Interfacing residues’ in the tabular form, which shows the details about the ‘Inaccessible residues’, ‘Solvent-accessible residues’, ‘ residues H-bonding and other bonding interactions’ , ‘Interfacing residues’ and other parameters as well.

★ Upload all of your protein-protein docking complex models one by one and check which one of the models contains a higher number of H-bond interactions.

➢ **Protein-Ligand Docking complex Evaluation:**

- Open the PDBePISA web server and click on ‘Launch PDBePISA’ button to go to the query page of this server.
- Then click on the ‘Choose file’ button and select the file containing the protein-ligand docking complex model in .pdb format and then click on the ‘Upload’ button.
- In the “Analysis” section, it’ll show the number of amino acids chain and ligand molecules present in your query file.
- Then click on the ‘Interface’ button to analyze the details about the interface of the docking complex.
  [It’ll provide the results in the same format as shown in the evaluation of protein-protein docking complex model.]
- Click on the ‘Details’ button present below the interface details table to analyze the results in detail.

[It'll also provide the information in the same format as shown in the evaluation of protein-protein docking complex model, the only difference is that it'll show the 'Structure 1' as receptor protein and 'Structure 2' as ligand molecule.]

Note: For Protein-Ligand interaction, the lesser amount of H-bonds (4-10) are also acceptable, depending upon the size of the ligand molecule.

★ Upload all of your protein-ligand docking complex models one by one and check which one of the models contains a higher number of H-bond interactions.

Summary:

In this video tutorial of PDBePISA web server, we came to know how to upload the query files of both the protein-protein and protein-ligand docking complex models in order to evaluate the models and choose the best among them. We also got to know about different parameters that must be present in an optimal docking complex model.