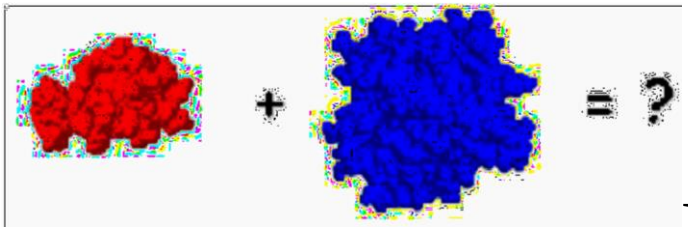


Hands-on Practice

PatchDock

In this exercise we will investigate two protein complexes using PatchDock's rigid docking.

Alpha-Amylase Enzyme Inhibitor Complex[1]

Alpha-Amylase catalyzes the hydrolysis of starch and related compounds. We will investigate the structure of the enzyme in a complex with its inhibitor.

A. Submitting a PatchDock job:

1. Download and unzip the tutorial directory and look at the files in the "Ex1" sub-directory:
   1JAE.pdb – The structure of the alpha-Amylases in PDB format.
   1B1U.pdb – The structure of the inhibitor in PDB format. *1TMQ.pdb* – The structure of the correct native complex.
2. Go to the web server of PatchDock:
   http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html
3. Fill out the following fields in the web-server:

Receptor Molecule:	Upload the *1JAE.pdb* file
Ligand Molecule:	Upload the *1B1U.pdb* file
e-mail address:	Fill out your e-mail, to which the results will be sent.
Clustering RMSD:	4.0
Complex Type:	Choose "Enzyme-Inhibitor"

4. Submit the form.
5. This run will take some time, so meanwhile view the structures using Chimera or Pymol. (For a clear visualization with Pymol press in the "all" line: S (show)→ As →Cartoon).

B. Checking the results:

If PatchDock is still running, use these results:
http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1JAE.pdb_1B1U.pdb_21_45_7_1_11_114/

This example belongs to a special kind of docking category called 'EnzymeInhibitor', where one of the proteins is an enzyme, and the second binds to it and inhibits its activity. Often in complexes that fall into this category the inhibitor (ligand) finds a cavity on the enzyme surface and binds to it. When we ran PatchDock, we entered the category of "Enzyme-Inhibitor". In this category, PatchDock only looks for binding sites that are inside a cavity.

1. In your email, check that you have received a link to the server predictions. Go to this link.
2. Look at the output page and examine the results.
3. Download the top 10 solutions (You can do it easily by downloading a zip file containing the best ten solutions). View them with a molecule visualization program (Pymol/Chimera). Superimpose the solutions to the native structure (See appendix to learn how). 4. Which model is more accurate

References: 1. Strobl S. et al (1998) Structure 6: 911-921

The IkBa/NF-kB transcription factor Complex[2]

NF-κB (p50/p65) is a heterodimer that controls DNA transcription. The IkBa acts as an inhibitor of this transcription factor. The complex of IkBa/NF- κB has been determined (PDB code 1ikn). The complex has three chains; chains A and C are the p65 and p50 subunits of the heterodimer of the NF- κB, and chain D is the inhibitory protein IkBa. The binding of the inhibitory protein blocks the NF-κB DNA-binding cleft, preventing transcription. We will try docking only chains A and C as they are in the IkBa/NF-κB complex.

A. Submitting a PatchDock job:

1. Download and unzip the tutorial directory and look at the files in the "Ex2" sub-directory:
   1iknA.pdb – The structure of the alpha-Amylases in PDB format.
   1iknC.pdb – The structure of the inhibitor in PDB format.
   1iknAC.pdb – The structure of the correct native complex.
2. Go to the webserver of PatchDock:
   http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html
3. Fill out the following fields in the web-server:

Receptor Molecule:	Upload the *1iknA.pdb* file
Ligand Molecule:	Upload the *1iknC.pdb* file
e-mail address:	Fill out your e-mail, to which the results will be sent.
Clustering RMSD:	4.0
Complex Type:	Choose "Default"

4. Submit the form.

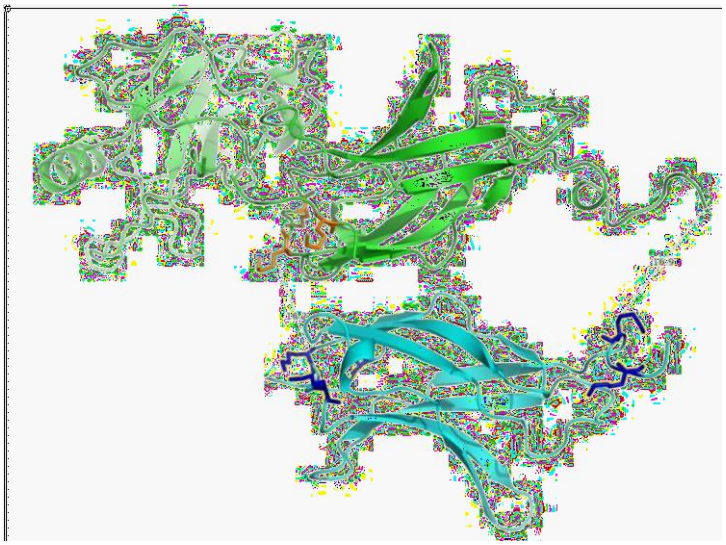
C. Checking the results:

1. In your email, check that you have received a link to the server predictions. Go to this link. If PatchDock is still running, use these results: http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1iknA.pdb_1iknC.pdb_44_45_7_1_11_114/
2. Look at the output page and examine the results.
3. Download the top 10 solutions. View them with a molecule visualization program (Pymol/Chimera). Superimpose the solutions to the native structure (See appendix to learn how).
4. Is there an accurate solution?

References: 2. Huxford et al (1998) Cell(Cambridge,Mass.) 95: 759-770

The IkBa/NF-kB transcription factor Complex using distance constraints

Different experimental data is very helpful in this case. Suppose we have cross linking data. The cross-linker is a molecule with two reactive groups on either end separated by a spacer. These reactive groups usually react with accessible Lysine residues. Determination of the cross-linked residues is done by Mass Spectrometry. Based on the length and flexibility of the cross-linker, one can estimate lower and upper bounds for distances between the cross-linked residues. This way we can have distance constraints of residues in different subunits of the complex. We will use simulated distance bounds.



A. Submitting a PatchDock job:

1. Go to the webserver of PatchDock: http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html
2. Press on "Show" in the Advanced Options.
3. Fill out the following fields in the web-server:

Receptor Molecule:	Upload the *1iknA.pdb* file
Ligand Molecule:	Upload the *1iknC.pdb* file
e-mail address:	Fill out your e-mail, to which the results will be sent.
Clustering RMSD:	4.0
Complex Type:	Choose "Default"
Distance Constraints:	**Upload the CONST file**

4. Submit the form.

B. Checking the results:

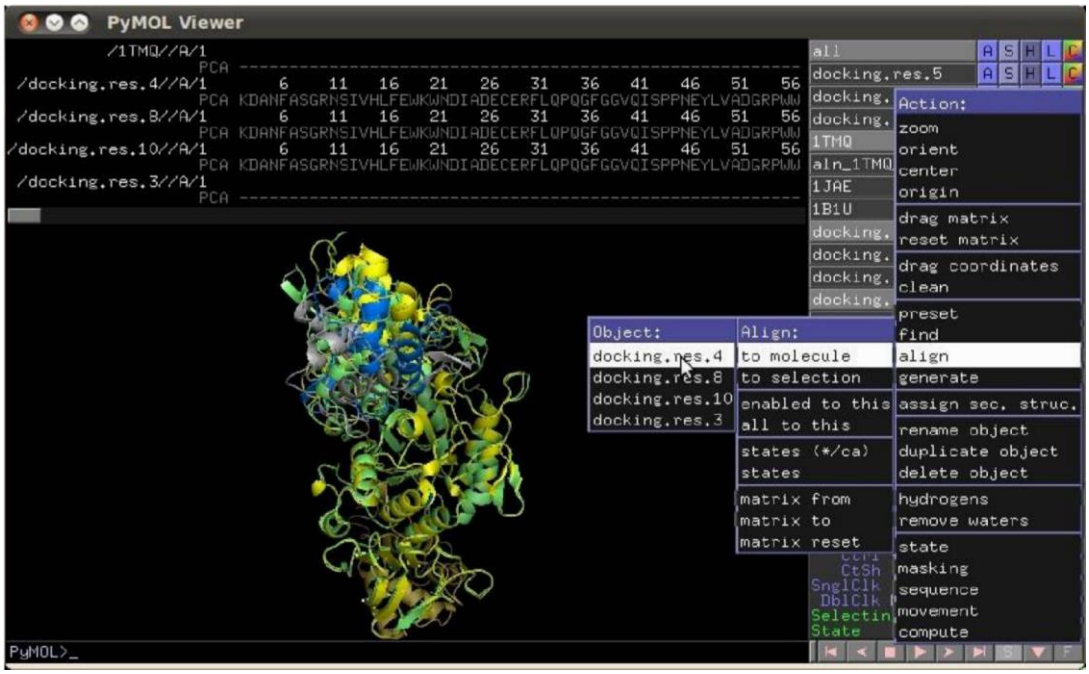
1. In your email, check that you have received a link to the server predictions. Go to this link. If PatchDock is still running, use these results:http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1iknA.pdb_1iknC.pdb_48_46_7_1_11_114/
2. Look at the output page and examine the results.
3. Download the top 10 solutions. View them with a molecule visualization program (Pymol/Chimera). Superimpose the solutions to the native structure (See appendix to learn how).
4. Is there an accurate solution now?

Superimposing structures

As all the solutions are already superimposed to each other, we will superimpose the native structure to one of the solutions.

Superimposing structures with Pymol:

To superimpose model A on model B (in our case: model A is the native structure and model B is one of the solutions):
Press on A (Action) in modelA's line. Select Align → to molecule → modelB.



Superimposing structures with Chimera:

Tools → Structure Comparison → MatchMaker
In the reference structure box select the structure you want to superimpose to. In our case select one of the solutions. In the Structure to match box select the structure to be superimposed. In our case, select the native structure. press OK.