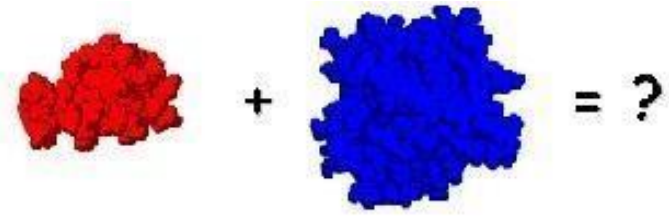


Hands-on Practice

Flexible Docking and Symmetric Docking

Note: We recommend viewing these models via Chimera. Some of the models aren't visualized properly as cartoon in Pymol.

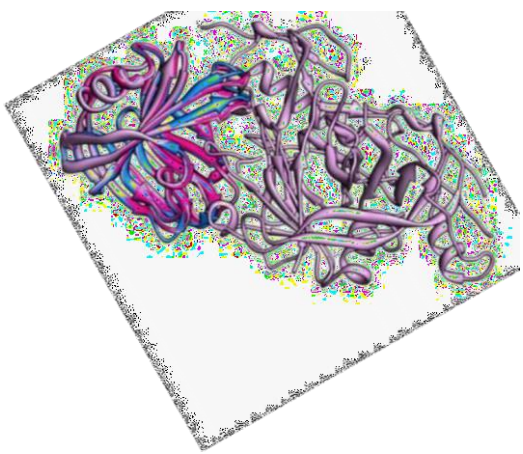
A. Side-Chain Refinement with FireDock

subtilisin BPN' in complex with Streptomyces subtilisin inhibitor[1]

In this exercise, we will examine how side-chain refinement can help in ranking docking results more correctly. For this we will use the subtilisin enzyme inhibitor complex. Subtilisin (serine endopeptidase) is a non-specific protease that attacks the peptide bond through a serine residue at the active site. We will try to model the subtilisin in a complex with a subtilisin inhibitor.

1. In Ex1 file you will find all the pdb files you need for this exercise.
2. Go to the PatchDock server: http://bioinfo3d.cs.tau.ac.il/PatchDock/ 3. Upload molecule ***2SIC_r_u.pdb*** as receptor, and ***2SIC_l_u.pdb*** as ligand.
4. Enter your email address, and change the complex type to Enzyme-Inhibitor, and Submit.
5. When the results arrive to your email, go to the given link or use these results: http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/2SIC_r_u.pdb_2SIC_l_u.pdb_56_50_15_30_10_114/
6. Download the best 10 solutions and check them with Pymol/Chimera.
   The files ***2SIC_r_u.pdb*** and ***2SIC_l_u.pdb*** are in the right transformation.
   Compare PatchDock solutions to this right one.
   Note that PatchDock only moves the ligand as the receptor stays without change.
   As you can see the correct solution isn't among the top ten solutions. While binding, residues from the ligand's and the receptor's interface change their conformation. PatchDock doesn't make these changes and therefore the correct transformation might result in some clashes between the unchanged residues. This might reduce the correct transformation's score so it won't be in the top ten solutions.
7. Try to correct this by running FireDock with the top 1000 best solutions. Go to PatchDock's results page, scroll to the bottom of the page, to "**Refine best solutions with FireDock**" and change **10** to **1000. Press GO.**
8. When the results have arrived, go to the given link or use these results: http://bioinfo3d.cs.tau.ac.il/FireDock/runs/2SIC_r_u.pdb_2SIC_l_u.pdb_7_16_16_30_10_114/

Download the top 10 solutions (link at the bottom of the page) and look at the results. You can see the original solution number by PatchDock ranking, and the new ranking given by FireDock. Solution number 118 of PatchDock should be ranked first by FireDock. This can be due to residues refinement but also due to the fact that we rank according to different measures (energy function and not shape complementarity score)



References:

1. Takeuchi Y. et al (1991) J.Mol.Biol. 221: 309-325

B. *Backbone Refinement with FiberDock*

Complex of ebdo-1,4-beta xylanase I and xylanase inhibitor

The enzyme 1,4-beta xylanase I degrades Polysaccharides. Doing so, it breaks down hemicellulose, one of the major components of plant cell walls. Thus xylanase plays a major role in micro-organisms thriving on plant sources (mammals, conversely, do not produce xylanase). Plants have developed defense mechanisms in response. Their defense arsenal includes inhibitors of cell wall-degrading enzymes, which hinder a possible invasion and colonization by antagonists. [2]

Now, we will see how backbone refinement can help in finding the correct binding site orientation of the xylanase with its inhibitor.

1. First, we need to create the transformation file. Let's do it with PatchDock. Go to: http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html
2. Upload ***xylanase.pdb*** as receptor file, and ***inhibitor.pdb*** as ligand file (all files are in Ex2 directory). Choose the Enzyme Inhibitor complex type.
3. This might take some time. You can use these results: http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/xylanase.pdb_inhibitor.pdb_45_23_16_30_1 0_114/
4. Download the transformations file and the best ten solutions. We will look at them later.
5. FiberDock requires up to 100 transformations, the transformation file might include more than that. Therefor you need to create a file that contains only the top 100 transformations. You can either create this new file or use one that we have already created for you: ***trans_1t6g.txt***.
6. We are ready now to run FiberDock.

   Go to: http://bioinfo3d.cs.tau.ac.il/FiberDock/index.html
   - Upload ***xylanase.pdb*** as the receptor, and ***inhibitor.pdb*** as the ligand**.**
   - Upload the transformations file.
   - Fill in your email address.

   We are now going to tell FiberDock that the ligand is already in its bound conformation, and thus does not need refinement. This will help FiberDock reach the correct solution.
   - In ***Refine ligand's backbone conformation?*** Mark no.
   - Press ***Advanced Options – show***
   - In ***Side Chain Optimization -> perform SCO for:*** unmark the Ligand
   - In ***Scoring -> Complex type:*** choose Enzyme-Inhibitor Press Submit.
7. When the results have arrived, go to the given link or use these results: http://bioinfo3d.cs.tau.ac.il/FiberDock/runs/__9_26_16_30_10_114/
8. Mark the top result in the results page. This result was ranked 4 by PatchDock and reranked as first by FiberDock.
9. Download the structures by pressing on the download best structures link.
10. Open the native complex ***(xylanase_inhibitor_complex.pdb***), the unbound receptor (***xylanase.pdb)*** and ***res_4.ref.pdb.*** The rankings of the files are the original PatchDock ranking. See if you can identify the difference in backbone
11. Open ***docking.res_4.pdb*** from PatchDock's solutions. This solution was modified by FiberDock to ***res_4.ref.pdb***. You can see the changes between the two.

References: 2. Sansen, S. et al (2004) J.Biol.Chem. 279: 36022-36028