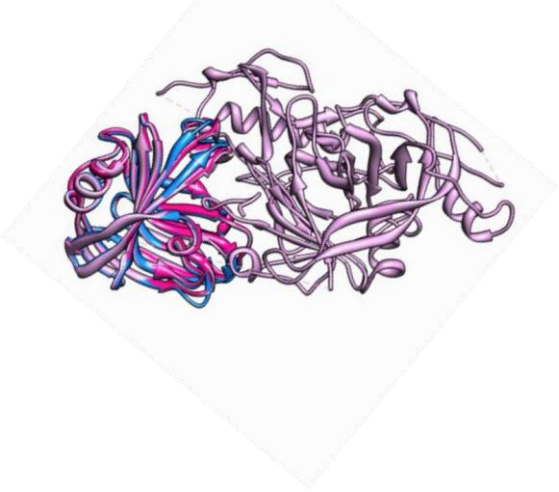


Hands-on Practice

A. *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. We will use a short transformation file that is in the fiberdock directory under the name: ***1t6g_trans.txt***. This file consists of 10 transformations. Open this file and see that you understand the format.
2. Let's run FiberDock.
   Go to: http://bioinfo3d.cs.tau.ac.il/FiberDock/index.html Fill out the following fields in the web-server:

Receptor Molecule:	1ukr:A
Ligand Molecule:	1t6g:B

This specifies to the webserver that we want to dock chain A from pdb 1ukr (this is the unbound enzyme structure) with chain B from pdb 1t6g (this is the bound structure of the inhibitor)

- Upload the transformations file: ***1t6g_trans.txt***
- 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.

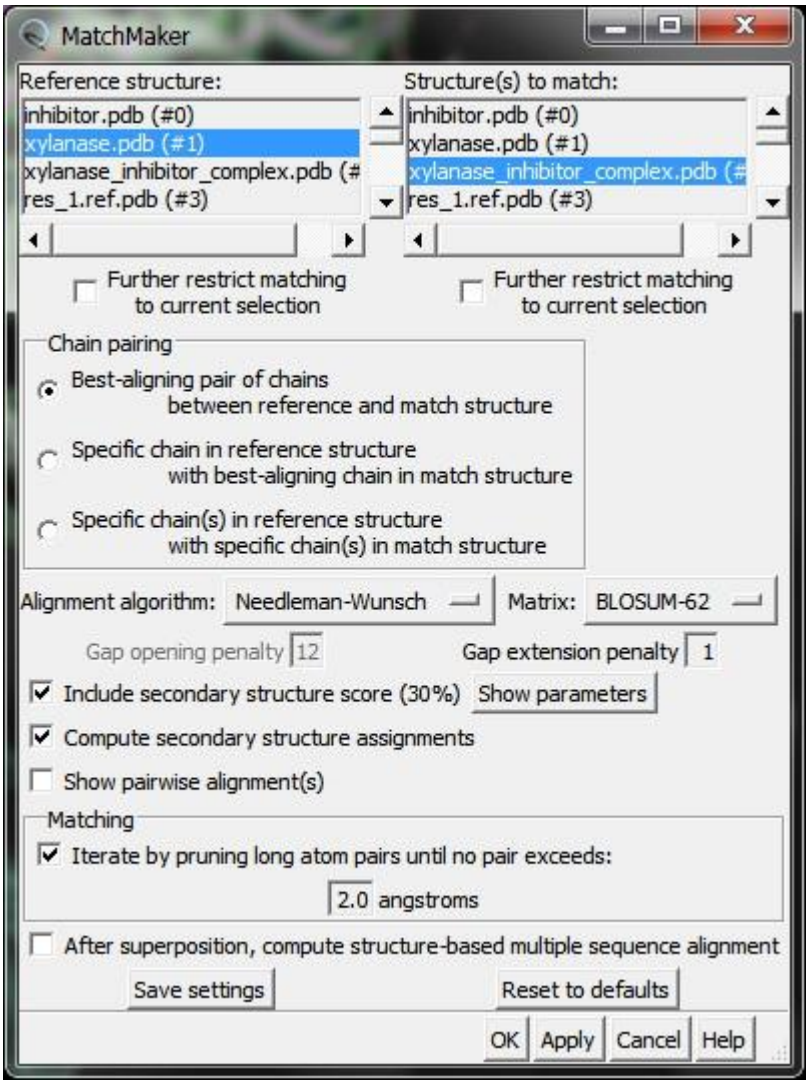
- Press ***Advanced Options – show***
- In ***Side Chain Optimization -> perform SCO for:*** unmark the Ligand
- In ***Scoring -> Complex type:*** choose Enzyme-Inhibitor Press Submit.

3. When the results have arrived, go to the given link or use these results:
   http://bioinfo3d.cs.tau.ac.il/FiberDock/runs/1ukr:a_1t6g:b_37_36_7_1_11_114/
4. Download the structures by pressing on the download best structures link.
5. Open the native complex ***(xylanase_inhibitor_complex.pdb***), the unbound receptor (***xylanase.pdb)*** and FiberDock solutions. Superimpose ***xylanase_inhibitor_complex*** to ***xylanase***. See next page to see how.
6. Can you visualize good solutions? Note that the number of the solution is not the ranking of FiberDock but the index of the transformation in the transformation file you entered. FiberDock's ranking is in the output page. Which complex did FiberDock rank first?
7. Compare the unbound xynalase with the bound one. Can you see conformational change in the backbone? Compare the flexible regions also with the first ranked complex by fiberdock.

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 the xynalase. In the Structure to match box select the structure to be superimposed. In our case, select the ***xylanase_inhibitor_complex***. Press OK.



References:

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