Homology – Modelling– SWISS MODEL

In this exercise we will get to know the basic features and operation modes of the homology model tool SWISS-MODEL.

The SWISS-MODEL is a simple and popular homology-modelling program and one of only few which available on the Internet. It uses the “building by fragments” method to construct the model on the template structures.

The program offers three basic modes

1. Simple first approach mode:

In this mode the user supplies only the primary sequence and the program automatically does all the rest. This mode requires only a web browser.

2. **First approach mode with user-defined templates:**

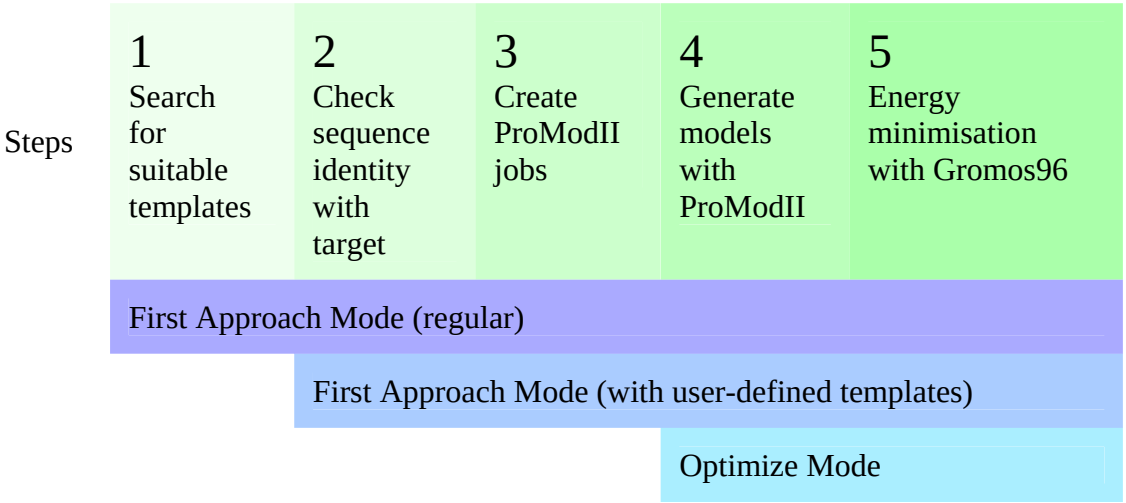
The user supplies the primary sequence and the structures of the template proteins. The template structure might be taken from the public structural database (by specifying a unique ID code) or supplied by the user (uploaded to the SWISS-MODEL server from the user’s computer).

3. **Optimized mode:**

The user can also provide the multiple sequence alignment. The user can either let the program choose the templates or choose them by her-self. All the needed preparations to send a request in this mode are done by the Swiss-PDB-Viewer (Deep Viewer)

program. This is free software available for almost all platforms at: http://www.expasy.org/spdbv/.

SWISS_MODEL homology modeling basic steps:



SWISS_MODEL uses the following tools during the operation:

Step	Program/Method	Database	Action
1	BLASTP2	ExNRL-3D	Will find all similarities of target sequence with sequences of known structure.
2	SIM	-	Will select all templates with sequence identities above 25% and projected model size larger than 20 residues. Furthermore, this step will detect domains which can be modelled based on unrelated templates
3	-	-	Generate ProModII input files
4	ProModII	ExPDB	Generate models
5	Gromos96	-	Energy minimization of models

Output formats

The results of the program are available as a PDB file. This PDB file can be analyzed by any visualization tool (e. g., Rasmol, Swiss-PDB-Viewer, PyMol). Alternatively the results can be returned as Swiss-PDB-Viewer project file, which enables manual corrections for the alignment and quick re-submission by the optimized mode.

Exercise

In this exercise we will be acquainted with the basic operational modes of SWISS-MODEL. We will use the program to build a structure of one of a special protein kinase.

1. Open Swiss-Model at http://swissmodel.expasy.org/.

2. Link to **First Approach mode** (the upper link on the left frame).

3. The first data that we should supply (apart of the personal details) is the primary sequence of the protein we wish to model. In this exercise we will model the structure of a cyclic AMP dependent kinase (PKA). In order to get the primary sequence of this kinase you can enter to the ***Swissprot*** site (http://www.expasy.ch/sprot/), to type the accession number (P05132) and retrieve the entry. Save the sequence into local file in Fasta format. This new file should be later opened as a Fasta format file, meaning that it should begin with description line starting with the character ">". Call this file as **pka.tfa**. Copy and paste this sequence also to the relevant window at the SWISS-MODEL form (without the description line). This is actually all you need to do in order to run SWISS-MODEL in simple first approach mode. However, we **will not run** the program in this mode, instead we will run in **First approach mode with a specific template**. We will supply a specific kinase structures that will serve as templates during the building. These will be the structures of two tyrosine protein kinases (PDB ID 1iep,

chain A and 1k2p, chain A). Under "**Use a specific template**" insert: 1iepA. Send the request.

4. We will now learn how to run the program in optimized mode. **Open Swiss-PDB-viewer.**

5. Choose "**Load Raw Sequence to Model**" item of the "Swiss Model" menu to load the file **pka.tfa** that you previously created.

6. Choose the "Swiss-Model" item of the "Preferences" menu. Enter your name and e-mail address. Make sure that the address of the modeling server is:
http://swissmodel.expasy.org/cgi-bin/sm-submit-request.cgi
and that the address of the template server is:
http://swissmodel.expasy.org/cgi-bin/blastexpdb.cgi

7. **Now we will choose and supply the template structure**. Get the PDB file **1iep** and save it locally on your computer. The program has also an option to choose the template files for you. We will not use this option now. Open the file by Swiss PDB viewer.

8. Choose "**Alignment**" from the "Window" menu.

9. Click on the **pka** name to make this layer active. Choose the "**Magic Fit**" option of the "**Fit**" menu. This will perform the sequence alignment. Choosing "**Improved Fit**" from the same menu will optimize the alignment.

10. Make sure all residues of the 2 proteins are selected. From the "Color" menu choose "color by alignment diversity", so you will be able to identify the conserved regions.

11. Choose the "**Update threading now**" item of the "SwissModel" menu (this item is not accessible if the "Update Threading Display automatically" item is enabled; which is the case by default).

12. After the initial automatic alignment we have now the freedom to change it. This is done with the mouse and the arrow keys. We can also make use of the mean force potential to help threading correctly a protein, although this tool should be used with caution. Make sure the current layer is pka, and click on the little arrow located at the right of the question mark of the Alignment Window. The window expands, and displays a curve depicting how each residue likes it's surrounding. If a residue is "happy", its energy is below zero, whereas unhappy residues will have energy above the zero axis. This is the mean force potential energy. Click on the "smooth" text, and set a smoothing factor of 1. It means that the energy of each residue will be the average of itself plus the energy of 1 flanking residue on each side. You can enable the "Auto Color by Threading Energy" item of the "SwissModel" menu to better see the potential on the structure. Click on the "E= XX" text, this will re-compute the energy for the current layer. **Note:** this tool provides hints and should be used in conjunction with other type of analyses! It works better for displacement of large fragments.

13. You can also evaluate how good your threading is by using the "aa making clashes" items of the "Select" menu. This will allow you to quickly focus on potentially problematic regions. You can then choose the "Fix Selected Side-chains" ("crude") item of the "Tools" menu, which will browse the rotamer library to choose the best rotamer, exactly as during a mutation process. By repeating the "Select aa making clashes" process, you should see that fewer amino-acids are making problems. If not, this is probably a good clue that your threading is incorrect. **Important Note:** Actually fixing the side-chains is just for you, to evaluate prior to submitting the request, it will have absolutely no incidence onto the model building, as side-chains are reconstructed anyway.

14. When everything seems OK, you can submit a modeling request to Swiss-Model simply by choosing the "Submit modeling request" of the "SwissModel" menu. You will be asked to give a project name. By the default, you will get to your email a Swiss-PDB-

Viewer project file, with your model aligned onto the templates you used, and ready for comparison.

15. While the server is working, we will compare the results of the first approach mode with the real structure for that protein. **Open the structural alignment program CE** (combinatorial extension) at the URL: **http://cl.sdsc.edu/ce/ce_align.html**. For the first chain upload the model you obtained from Swiss-Model. Don't forget to mark the "User File" option instead of the "PDB" option. For the second chain, enter 1APM:E which are the PDB code and chain identifier for the real structure exists for PKA. Submit.

16. At the results page, look at the alignment. Notice that this sequence alignment was produced according to the structural information, without considering the sequence. It allows us to judge the structural similarity. Find the regions which were not properly built. What is the overall RMSD of the structural alignment? Is this significant?

17. Save the PDB format of the structural alignment in your computer and open it with **RasMol**. Find the regions not properly aligned by visual inspection and compare to your answer from the previous question.

18. Finally we will take a look at the at the **evaluation** report for this model. Look at the evaluation graphs obtained using Anolea, Gromos and Verify3d**.** Try to find regions which are suspected to be incorrect.

19. To conclude: structural model is basically easy to obtain, but we always be aware of how it was produced, check it with available tools and refine it if necessary.