Section II

The Structure of Macromolecules: *Homology modeling using Swiss-Model- tutorial*

Objective

- To learn how to use Swiss-Model for homology modeling.

Brief info

SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server, or from its user-friendly interface DeepView (Swiss Pdb-Viewer).[1, 2, 3]

To become familiar with basic features and operation modes of these tools, during this tutorial we are going to use a combination of them to generate a three dimensional structure of the human dopamine D2 receptor, a member of G-Protein Coupled Receptors (GPCR).

Before we get started, you have to create your own workspace by accessing SWISS MODEL home page > myWorkspace from Modelling module. Also you have to Download the program DeepView (Swiss Pdb-Viewer) from http://expasy.org/spdbv/; choose "self extracting archive" and follow the directions.

How to

The main steps for building a homology model are template identification, sequence alignment, model building and model evaluation.

1. Template identification

- ✓ Go to SWISS-MODEL home page and launch the *Template Identification* routine from *Tools* module.
- ✓ Paste the amino acid sequence for hD2 receptor (you have saved it during the sequence alignment exercise). Optional, you can give a name to your project or, if you want to receive the results also by email, you may enter a valid e-mail address. Submit your job and wait until it is done.
- ✓ The output of your request is a list of potential templates that can be used to generate a 3D model for human D2 receptor – see Figure 1. Analyzing the results we noticed that the first two crystal structures resulted, 3d4r and 2rh1, belong to the human β2-AR receptor. Scrolling down the page you find out the name of each obtained template structure and its correspondent alignment with hD2 receptor sequence.
- ✓ In general, to select the most suitable template structure for a target sequence you should consider the followed criteria: (i) sequence similarity (ii) the quality of the experimentally determined structure, (iii) the similarity between template's environment and the target's environment. In our particular case, the template structure for hD2 receptor should be one of its two crystal structures 3d4r or 2rh1. Because they are very similar (rmsd 0.53, without T4 lysozome), it is not really important which structure is going be used in the homology modeling process.

Because 2RH1 structure has been elucidated at a lower resolution we will choose this one as template.

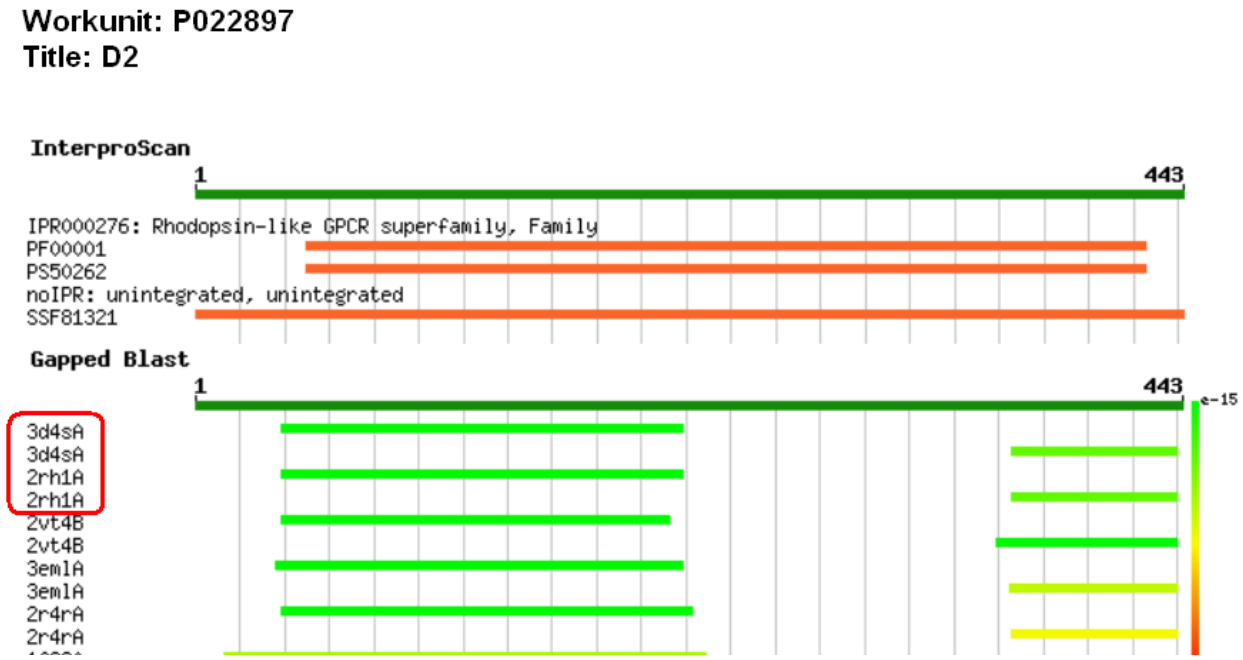

Figure 1

2. Sequence Alignment – Please consult the previous exercise.

3. Model Building

- ✓ Open the Swiss-PdbViewer, go to *SwissModel* > *Load Raw Sequence from Amino Acids* and load the amino acid sequence for hD2 receptor (you have saved it during the sequence alignment tutorial). The protein is built as a long perfect alpha helix, which has nothing to do with its real structure.
- ✓ Go to *Preferences* > *Swiss-Model*. A Swiss-Model Settings window will appear on screen, check if model server and template server fields are correctly completed – see Figure 2; enter your name and e-mail address.
- ✓ Load the 2rh1 pdb file by selecting *Open PDB file* item of the *File* menu.
- ✓ Select the entire sequence: Select > All

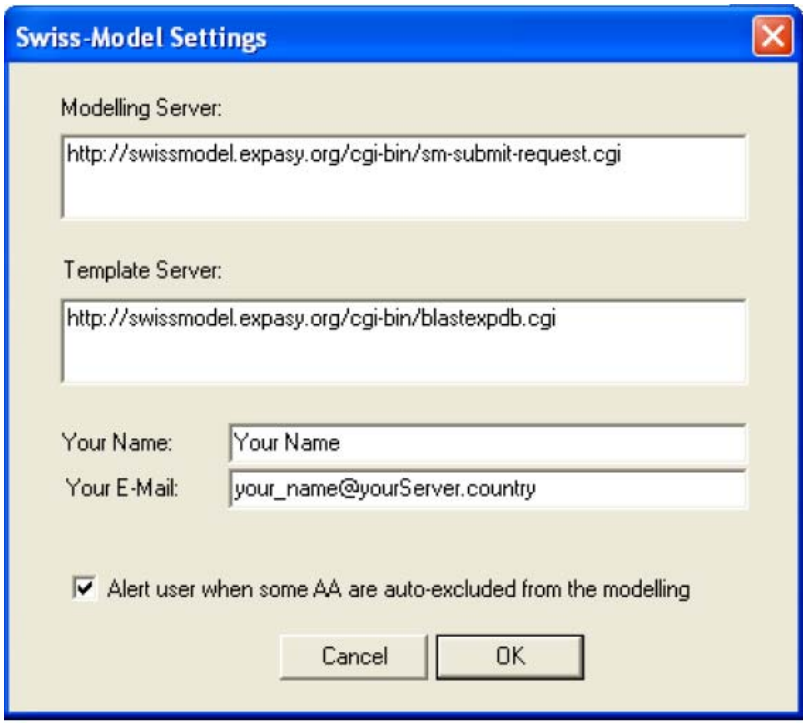

Figure 2

- ✓ Go to *Windows* and activate *Layers Infos* to check if there are two molecules opened. This operation allows choosing which molecule to visualize (vis), to move (mov), etc. The molecule which has the name colored in red is the active one – see Figure 3.

Figure 3

- ✓ Go to *Fit* > *Iterative Magic Fit*. This will perform the sequence alignment. To view the alignment go to *Windows* > *Alignment*.
- ✓ You can proceed further with this automated performed alignment or you can modify it in order to match with our manually refined version that we have already obtained in the previous exercise. The manually refined sequence alignment is highly recommended when best quality models are aimed. To make the alignments' comparison easier you can see a preview of the automated alignment by clicking on the little text icon located on top left corner of the window. To modify the alignment according to the refined one, use the Backspace or Delete keys the mouse or arrow keys. Hold CTRL key when you want to operate just on a single sequence.
- ✓ Submit the modeling request to Swiss-Model server selecting *Swiss-Model* > *Submit modeling request for Raw Sequence*. You will be asked to name the project and then click *OK*. You have now submitted your homology modeling job, and you will receive notification when the results are available. Click *Yes* to replace the coordinates of the associated structure with the model or, you can download the model directly from your workspace. The third intracellular loop between helices 5 and 6 does not represent a

reliable structure because it is was predicted based on T4 lysozome structure, therefore the deletion of this fragment must be performed.

- ✓ In you workspace, together with the modeled structure you will also receive a What Check report which provides the errors or warnings in your structure. In order to obtain a trustful model you have to analyze all of the considerations delivered by What Check.

[1] Schwede T., Kopp J., Guex N., and Peitsch M.C., Nucleic Acids Research 31 (2003) 3381-3385

[2] Vriend G.,J. Mol. Graph. 8(1990)52-56

[3] Hooft R.W.W., Sander C., Vriend G., Proteins, 26 (1996)363-376

[4] Palczewski K., Kumasaka T., Hori T., Behnke C.A., Motoshima H., Fox B.A., Le Trong I., Teller D.C., Okada T., Stenkamp
R.E., Yamamoto M., Miyano M., ***Science,*** 289 (**2000**) 739-745

[5] Teller D.C.,Okada T., Behnke C.A., Palczewski K., Stenkamp, R.E., ***Biochemistry*** 40 (**2001**) 7761– 7772.

[6] Okada T., Fujiyoshi Y., Silow M., Navarro J., Landau E.M., Shichida Y., ***Proc Natl Acad SciUSA*** 99(9) **2002** 5982–5987