Section II

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

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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 the program DeepView (Swiss Pdb-Viewer). SWISS-MODEL is tightly connected to Swiss-PdbViewer,[1,2,3] an application that provides a user friendly interface allowing to analyze more proteins at the same time. Working with these two programs will reduce the overall work necessary to generate models.

In this tutorial we will learn the basic features and operation modes of this tool by building a 3D structure of the human muscarinic M3 receptor.

How to

- download and install the program DeepView (Swiss Pdb-Viewer) from http://expasy.org/spdbv/ . Just follow the directions and choose "self extracting archive".
- open the Swiss-PdbViewer and go to *SwissModel* > *Load Raw Sequence to Model*
- load the short amino acid sequence for hM3 receptor (you have saved it at the end of T-coffee exercise). 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 1.
- enter your name and e-mail address.

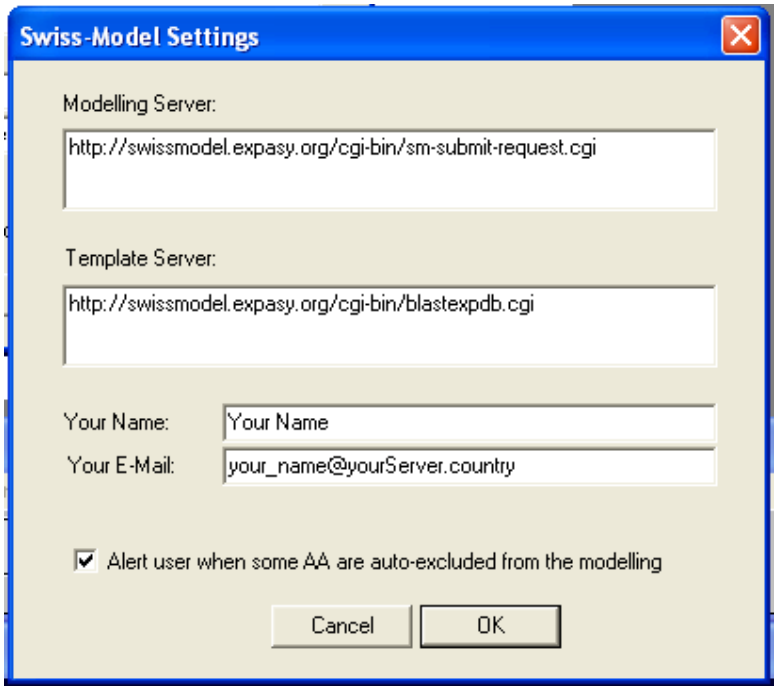


Figure 1

In the next step we have to choose or provide the template structure for hM3 receptor. We know by now that bovine rhodopsin is the only template available for building 3D models for G-protein coupled receptors.[4,5,6] If you already have a PDB file for rhodopsin structure you may load it by selecting *Open PDB file* item of the *File* menu. If you don't have the PDB structure then do the following:

- go to *SwissModel* > *Find Appropriate ExPDB templates*. This will launch automatically a Web browser and fill a form containing the primary sequence of hM3 in Fasta format – see Figure 2.

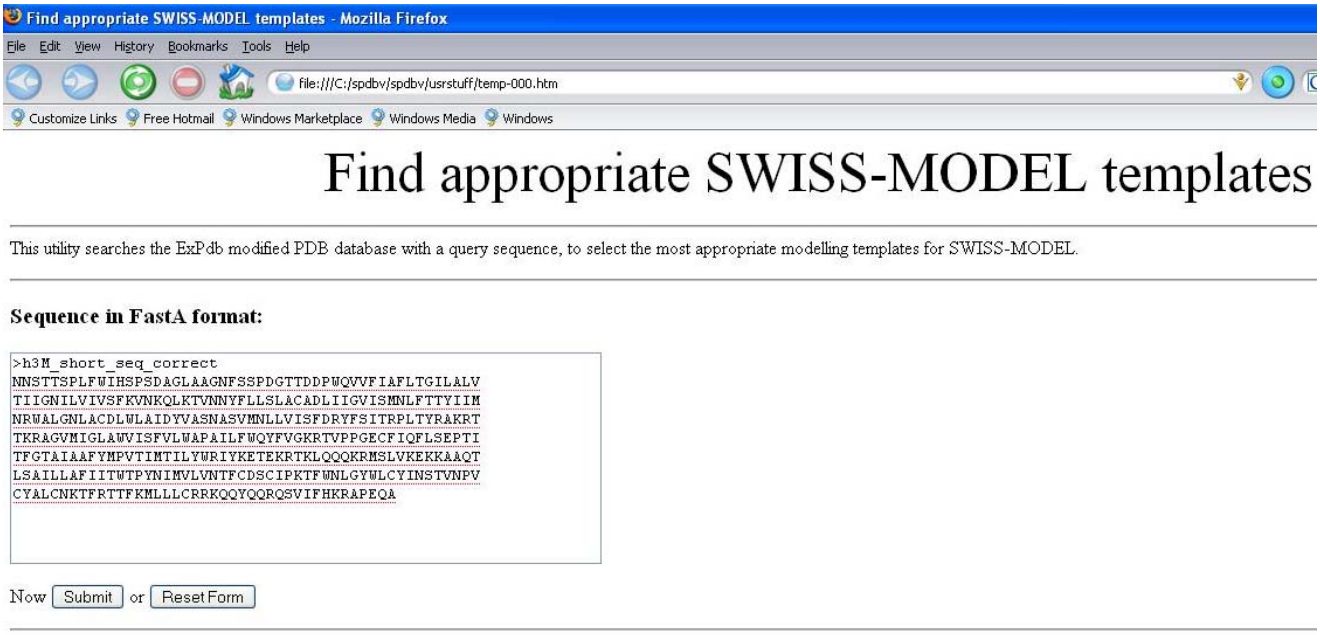
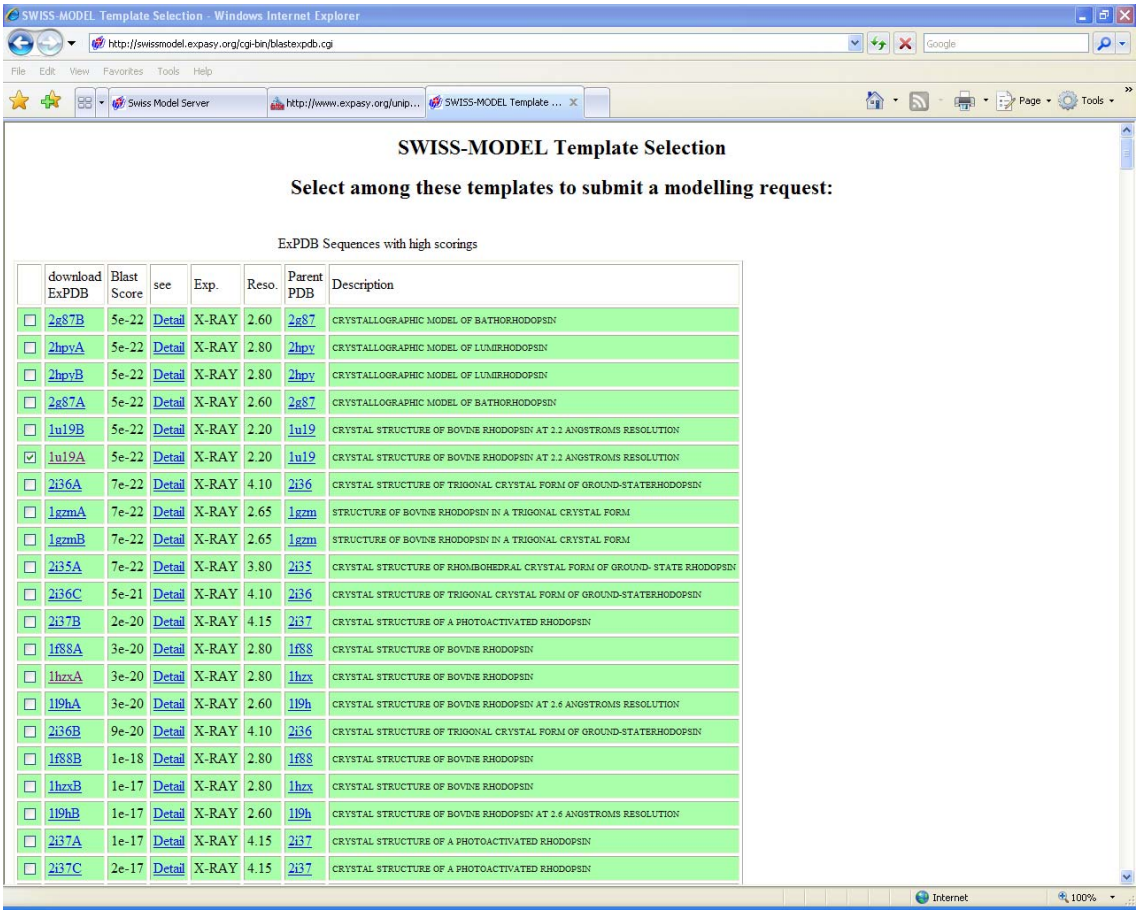


Figure 2

- click the submit button and wait. A page containing the best available templates will appear – see Figure 3.

In the first column are the protein codes from the ExPDB database. The second column contains the Blast score for the alignment between hM3 and each protein listed on the table. The Blast score represents the alignment score and it gives an indication about how good the alignment is. The higher is the alignment score, the better is the alignment. You may see each alignment by clicking on *Details* field in the third column. In the next two columns are given the experimental method used for 3D structure determination and the resolution, respectively. The *Parent PDB* column offers a link to the correspondent PDB structure. In the last column are listed the name of each potential candidate and other related information.

Generally when a list of potential templates is obtained it is necessary to select one or more templates that are appropriate for a particular modeling problem. The template selection rules are as follow: (i) highest sequence similarity, and (ii) highest quality of the experimentally determined structure, but (iii) the similarity between template's environment and the target's environment or, in other words, how they bond similar ligands should also be considered. Because we have obtained a list of bovine rhodopsin structures solved at different resolution, the best one is 1u19A which has the best alignment score and also a lower resolution.



	download ExPDB	Blast Score	see	Exp.	Reso.	Parent PDB	Description
☐	2g87B	5e-22	Detail	X-RAY	2.60	2g87	CRYSTALLOGRAPHIC MODEL OF BATHORHODOPSIN
☐	2hpyA	5e-22	Detail	X-RAY	2.80	2hpy	CRYSTALLOGRAPHIC MODEL OF LUMIRHODOPSIN
☐	2hpyB	5e-22	Detail	X-RAY	2.80	2hpy	CRYSTALLOGRAPHIC MODEL OF LUMIRHODOPSIN
☐	2g87A	5e-22	Detail	X-RAY	2.60	2g87	CRYSTALLOGRAPHIC MODEL OF BATHORHODOPSIN
☐	1u19B	5e-22	Detail	X-RAY	2.20	1u19	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN AT 2.2 ANGSTROMS RESOLUTION
☑	1u19A	5e-22	Detail	X-RAY	2.20	1u19	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN AT 2.2 ANGSTROMS RESOLUTION
☐	2i36A	7e-22	Detail	X-RAY	4.10	2i36	CRYSTAL STRUCTURE OF TRIGONAL CRYSTAL FORM OF GROUND-STATERHODOPSIN
☐	1gzmA	7e-22	Detail	X-RAY	2.65	1gzm	STRUCTURE OF BOVINE RHODOPSIN IN A TRIGONAL CRYSTAL FORM
☐	1gzmB	7e-22	Detail	X-RAY	2.65	1gzm	STRUCTURE OF BOVINE RHODOPSIN IN A TRIGONAL CRYSTAL FORM
☐	2i35A	7e-22	Detail	X-RAY	3.80	2i35	CRYSTAL STRUCTURE OF RHOMBOHEDRAL CRYSTAL FORM OF GROUND- STATE RHODOPSIN
☐	2i36C	5e-21	Detail	X-RAY	4.10	2i36	CRYSTAL STRUCTURE OF TRIGONAL CRYSTAL FORM OF GROUND-STATERHODOPSIN
☐	2i37B	2e-20	Detail	X-RAY	4.15	2i37	CRYSTAL STRUCTURE OF A PHOTOACTIVATED RHODOPSIN
☐	1f88A	3e-20	Detail	X-RAY	2.80	1f88	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN
☐	1hzxA	3e-20	Detail	X-RAY	2.80	1hzx	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN
☐	1l9hA	3e-20	Detail	X-RAY	2.60	1l9h	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN AT 2.6 ANGSTROMS RESOLUTION
☐	2i36B	9e-20	Detail	X-RAY	4.10	2i36	CRYSTAL STRUCTURE OF TRIGONAL CRYSTAL FORM OF GROUND-STATERHODOPSIN
☐	1f88B	1e-18	Detail	X-RAY	2.80	1f88	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN
☐	1hzxB	1e-17	Detail	X-RAY	2.80	1hzx	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN
☐	1l9hB	1e-17	Detail	X-RAY	2.60	1l9h	CRYSTAL STRUCTURE OF BOVINE RHODOPSIN AT 2.6 ANGSTROMS RESOLUTION
☐	2i37A	1e-17	Detail	X-RAY	4.15	2i37	CRYSTAL STRUCTURE OF A PHOTOACTIVATED RHODOPSIN
☐	2i37C	2e-17	Detail	X-RAY	4.15	2i37	CRYSTAL STRUCTURE OF A PHOTOACTIVATED RHODOPSIN

Figure 3

- select 1u19A structure from the list and save it on your computer as PDB file
- open it by going to *File* > *Open PBD file*
- 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 4.

Figure 4

- go to *Fit* > *Iterative Magic Fit*. This will perform the sequence alignment. In order to identify regions that differ between the hM3 and RHO it is possible to color the target and template (go to *Color* > *by layer*).
- to see the initial alignment go to *Windows* > *Alignment*; this is an automatic one so it is better to check if it is identical with the one we have already obtained in the previous exercise. To make the alignments' comparison easier you can see a preview of structural alignment by clicking on the little text icon located on top left corner of the window (highlighted with red in Figure 5). Any modification in the alignment can be easily done with the mouse or arrow keys.

Figure 5

- to submit the modeling request to Swiss-Model server go to *Swiss-Model* > *Submit modeling request*. First you will be asked to name the project and to save it on the hard-disk. A Web browser "*Optimize (project) mode*" will appear – see Figure 6. Follow the directions and then click *Send request*. You have now submitted your homology modeling job, and after a while you will receive an email with the modeled structure for hM3 receptor – see Figure 7.

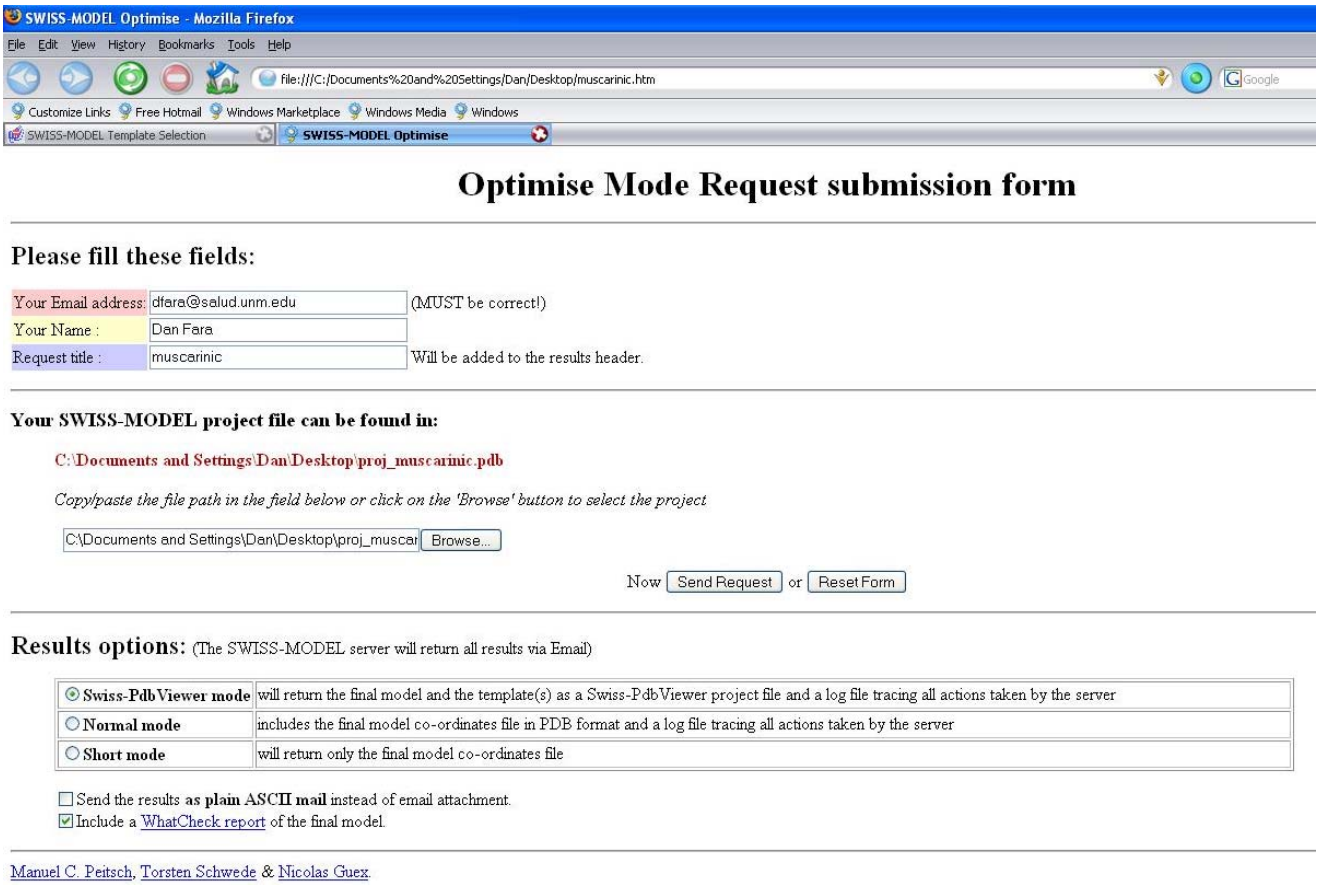


Figure 6

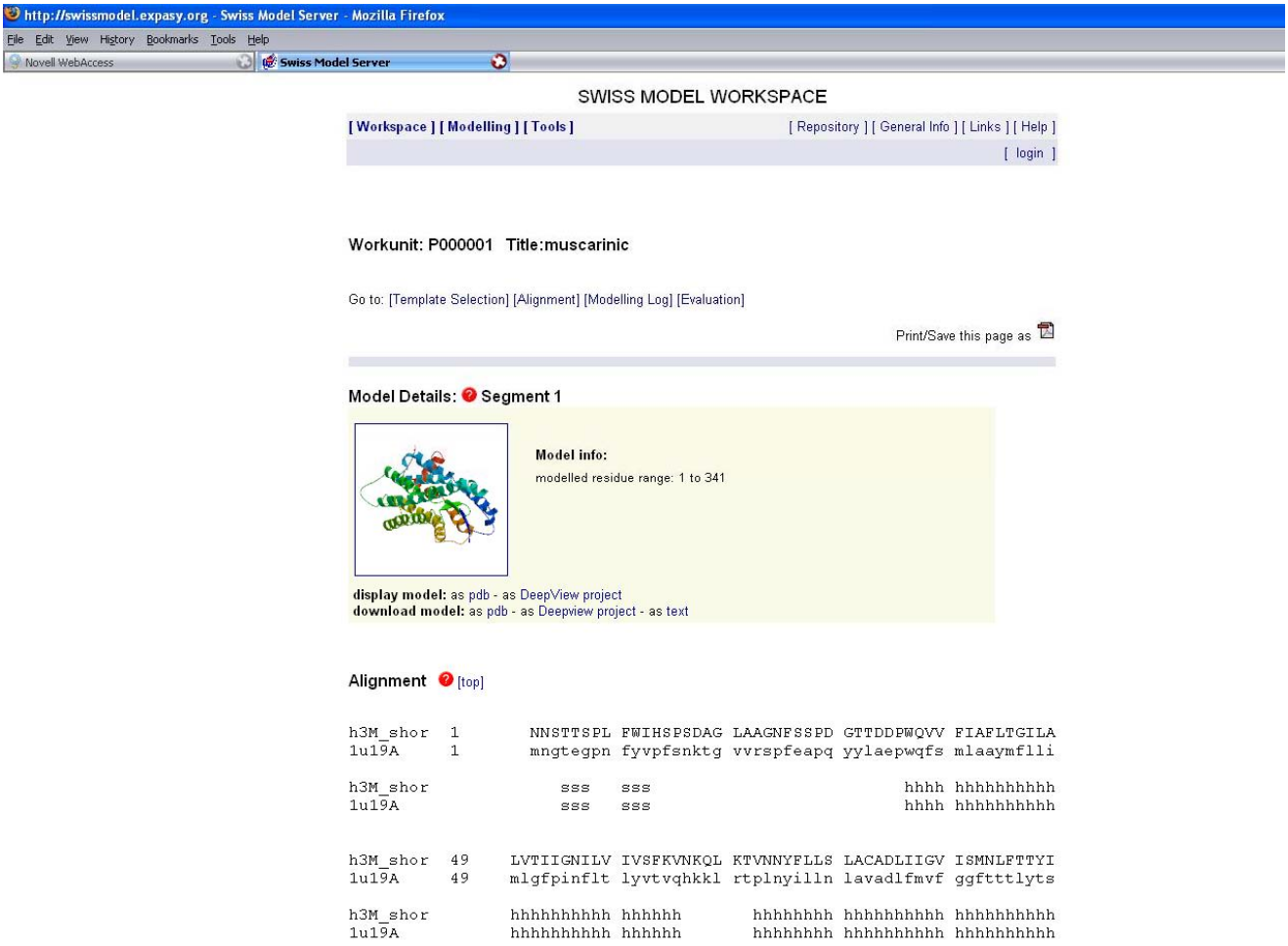


Figure 7

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.

Also you may evaluate your structure by accessing other web pages:

- **ANOLEA:** http://protein.bio.puc.cl/cardex/servers/anolea/
- **BIOTECH:** http://biotech.embl-ebi.ac.uk:8400/
- **PROCHECK:** http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html
- **ProsaII:** www.came.sbg.ac.at
- **VERIFY3D:** www.doe-mbi.ucla.edu/Services/Verify_3D
- **WHATCHECK:** www.cmbi.kun.nl/gv/servers/WIWWWI

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