Comparison of homology models of 5-HT6R created with different crystal templates

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Introduction
Structures of proteins with transmembrane domains cannot be easily determined, since proper conformation is acquired only in the presence of lipid bilayer, and thus it is almost impossible to construct their 3D structure using common physical methods. This is why homology modeling is extremely helpful in determining structures of such proteins.

5-HT6R (5-hydroxytryptamine receptor 6) is a protein containing 7TM (7 transmembrane helices) domain and is a member of class A GPCR (G-protein coupled receptor) family. It is widely expressed in neural tissue and is considered to be involved in learning and memorizing processes. Blocking the receptor leads to increase in neurotransmission and improves cognition abilities of rodents. The receptor itself is a target for anti-depression drug research.

In the present study a vast number of 5-HT6R homology models was generated on different templates available: adenosine 2 receptor (PDB ID: 3QAK), beta1 (PDB ID: 2Y00) and beta2 (PDB ID: 3P0G) adrenergic receptors, C-X-C chemokine receptor type 4 (PDB ID: 3OE0) and dopamine 3 receptor (PDB ID: 3PBL). Next, a set of representative ligands, from chemically diversified clusters, was used for selecting the best models. They were further used for docking of a complete set of 5-HT6R ligands (over 4000 compounds from ChEMBL database) to determine the best models for further research

Homology modeling
The first step was the analysis of sequences. Knowing the sequences of all templates and the target protein, sequence fitting was performed, using Discovery Studio software. Two different approaches were taken: with and without predicting the ranges of helices. Comparisons of sequences were made both with and without loops. Acquired impositions were confronted with mutagenic data to verify their validity. Since the models without loops and with determined ranges of helices presented the best results, the others are not shown.

Amino acid	B. - W.
Asp106	3.32
Cys110	3.36
Ser193	5.43
Thr196	5.46
Ser267	6.34
Trp281	6.48
Phe284	6.51
Asn288	6.55

Fig. 1. Table containing mutated amino acids with their position (using Ballesteros-Weinstein numeration)

Models of 5-HT6R were generated using modeler9v8 software. For each template, 200 models were generated, which gave a total of 4000 models. To reduce the enormous amount of generated structures, a pre-training set of 30 known active 5-HT6R ligands was docked.
Docking poses were analyzed in the automated manner, and receptors accommodating less than 12 ligands were rejected along with ones, whose averaged GlideScore was above -3. About 130 models were left for further dockings and analysis.

Results and conclusions
After primary screening, about 95% of built proteins were discarded. Remaining models differed largely, in favor of models without loops. This allows to claim, that the best method of homology modelling is determining helix ranges based on templates, and building models without loops.

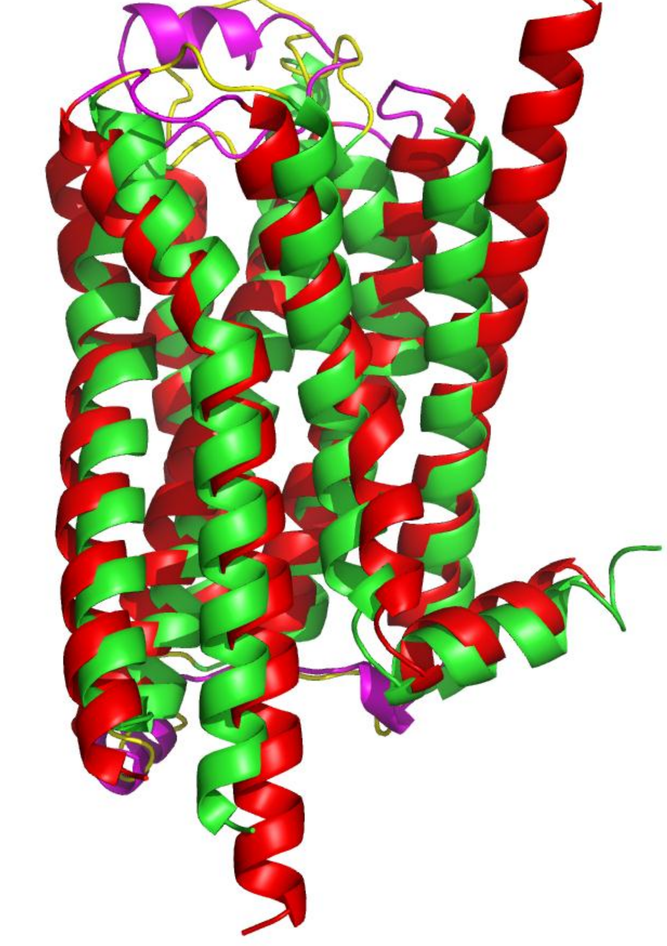

Fig. 4. Comparison of models of 5-HT6R with loops based on different templates. The red one, based on beta1 template, is an invalid model, not meeting screening requirements.. The green one, based on A2A template,. is a valid model, whose screening scores were above average.

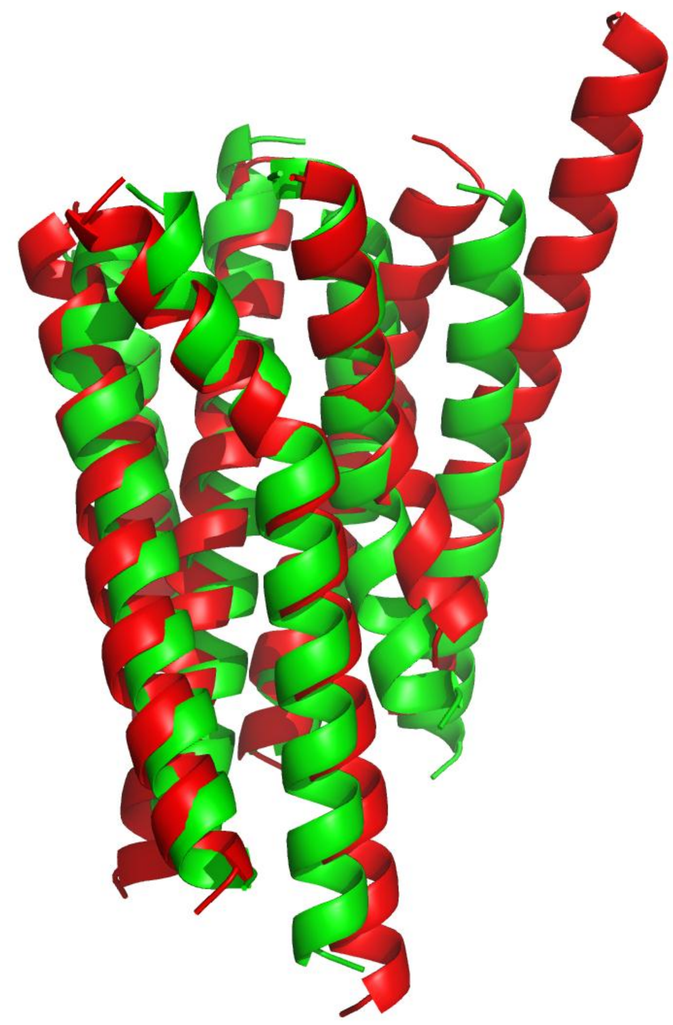

Fig. 5. Comparison of models of 5-HT6R without loops based on different templates. The red one, based on beta1 template, is an invalid model, not meeting screening requirements.. The green one, based on A2A template,. is a valid model, whose screening scores were above average.

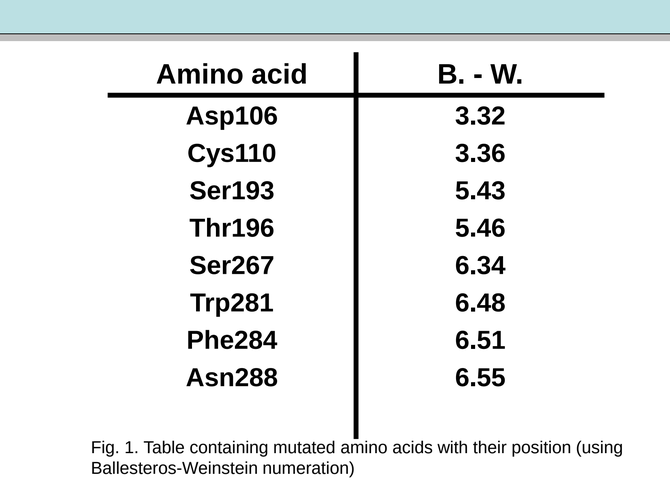

Fig. 2. Ligand binding site. The amino acids from mutation data are shown in yellow.

Table 1. Table containing results of primary screening

Template	Number of receptor models left after primary screening			
	With determined helix ranges		With predicted helix ranges	
	Without loops	With loops	Without loops	With loops
A2A	46	32	16	29
CXCR4	5	21	17	36
D3	4	13	11	9
beta1	0	3	0	2
beta2	2	3	7	5

Literature

- *5-HT6 MEDICINAL CHEMISTRY* Kevin G. Liu and Albert J. Robichaud Lundbeck Research USA, Paramus, NJ 07652, USA
- A quantitative structure-activity relationship study on serotonin 5-HT6 receptor ligands: indolyl and piperidinyl sulphonamides\B.K. Sharmaa*, aDepartment of Chemistry, S.K. Government College, Sikar-332 001, India; CSIR Lucknow-226 001, India
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Acknowledgments

This study is supported by project UDA-POIG.01.03.01-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD); http://www.prokog.pl

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