Impact of template choice on quality of 5-HT6 receptor homology models

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Introduction

Homology modelling is extremely helpful in determining structures of transmembrane proteins, since solving their three-dimensional structures by means of common physical methods is exceedingly difficult.
G-protein coupled receptors (GPCRs) comprise a vast superfamily of transmembrane receptors. In present study we have focused on serotonin (5-hydroksytryptamine) receptor type 6 (5-HT6R). They are expressed in neural tissue, and their dysfunctions contribute to CNS diseases, such as depression and anxiety.
In the present study a number of 5-HT6R homology models was generated on different GPCR 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), dopamine 3 receptor (PDB ID: 3PBL) and histamine H1 receptor (PDB ID: 3RZE). Next, a set of representative ligands, from chemically diversified clusters, was used for selecting the best models. They were used for docking of a complete set of 5-HT6R ligands (obtained from ChEMBL database) to determine the best models for further research.
The scope of this study is to determine whether there is a correlation between evolutionary distance in modeled protein and template, and the quality of produced models.

Homology modeling

The first step of the study was sequence analysis. To perform homology modeling alignments of query sequence (5-HT6R) and each template had to be done. This was achieved using Discovery Studio software by Accelrys.
Two different methods of helix range assumption: prediction using metaservers and alignment to template's ranges. For each of these methods alignments were created both with loops and without them, which resulted in four different approaches to homology modeling. All alignments were confronted with mutagenesis date to confirm their validity. Since models without helix range prediction and with loops presented the best results, the other are not shown.

Models of 5-HT6R were generated using modeler9v8 software. For each template, 200 models were created, which gave a total of 4800 models. To reduce the enormous amount of obtained structures, a pre-training set of 25 known active 5-HT6R ligands was docked.
Docking poses were analyzed in an automated manner, and receptors accommodating less than 13 ligands were rejected along with ones, whose averaged GlideScore was above -3. About 95% of generated models were discarded after pre-training docking.
Further docking study consisted of set of 258 known 5-HT6R-active ligands. Models whose average GlideScore was above -6 were discarded.
The final step included 1298 decoy ligands, and the models which docked the least of those compounds were selected as the best.

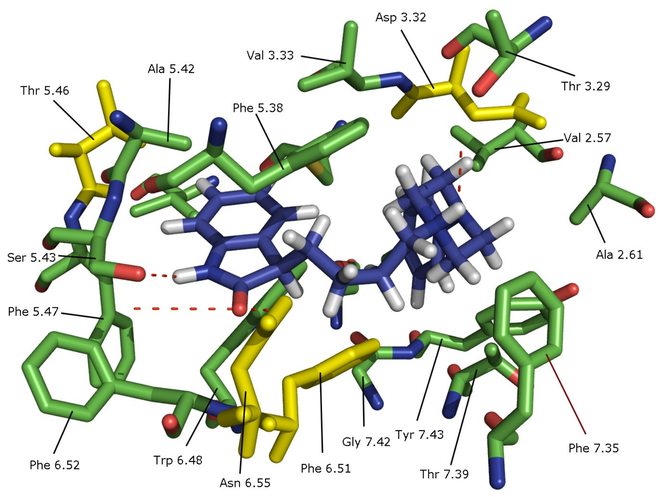

Fig. 1. Ligand binding site. The amino acids from mutation data are shown in yellow.

Results and conclusions

After three steps of model validation through docking studies only a handful of models remained. Comparing their results throughout all the steps allowed to choose the best models acquired in modeling phase.

The results of entire study stand in contrary to common procedures, which assume that the closest relative of modelled protein is the best template. In case of 5-HT6R, after resolving beta2 adrenergic receptor's structure by X-ray diffraction, all other template possibilities were discarded. This study proves that such approach is invalid, since the best models acquired were based on A2A and H1 templates, where both A2A and H1 are more evolutionarily distant to 5-HT6 than favored beta2.
To confirm validity of this research, similar study was performed for 5-HT7R, and the results were analogous to those of 5-HT6R case.

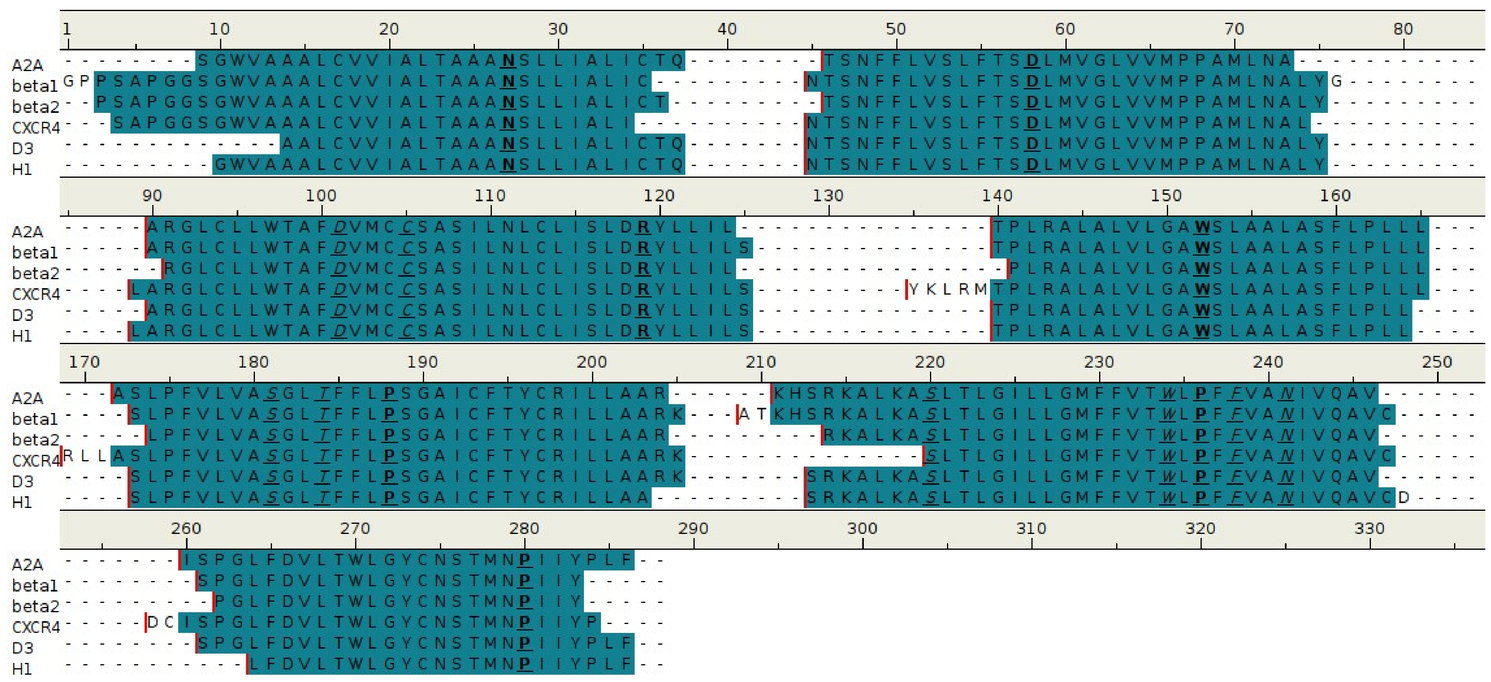

Fig.2. Sequence comparison of 5-HT6R helices based on different templates. Residues with Ballesteros-Weinstein number equal 50, are shown in bold and underlined. Amino acids gathered in mutagenic data are shown in italic and underlined.

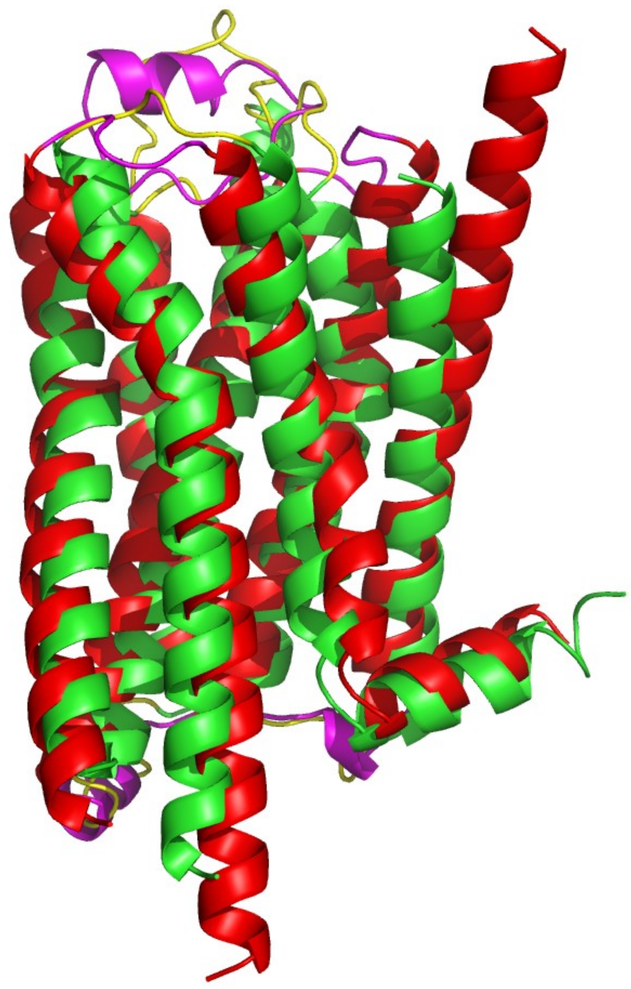

Fig. 3. Comparison of models of 5-HT6R with loops based on different templates. The red one, based on beta1 template, is an invalid model, not meeting screening requirements.. The green one, based on A2A template,. is a valid model, whose screening scores were above average.

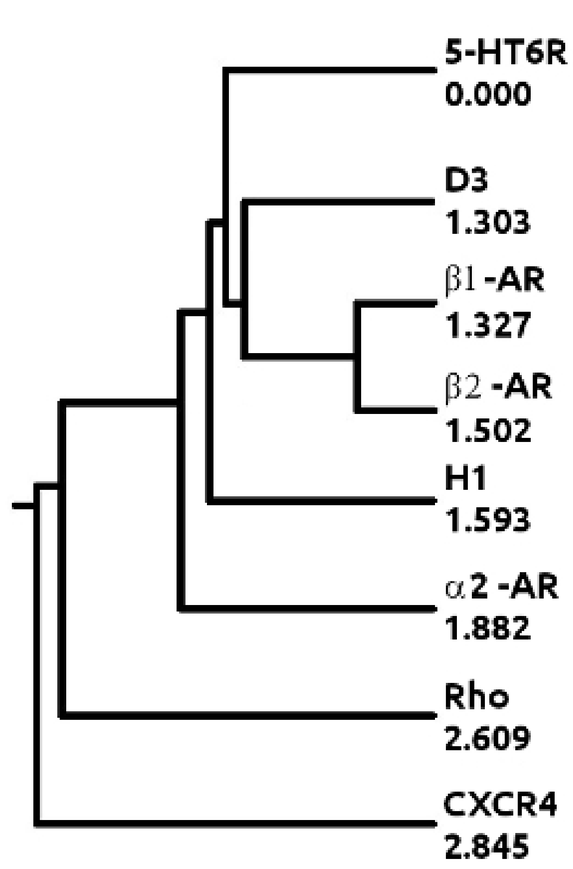

Fig.4. Phylogenic tree of available GPCR templates and 5HT6R with their evolutionary distance to 5HT6. The distance was calculated using protdist application from mobyle@pasteur metaserver. The tree was created using neighbor application. Settings of protdist were set to default, and neighbor's Distance Method was set to UPGMA.

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
H1	2	89	-	-

Literature

- Kevin G. Liu and Albert J., 5-HT6 MEDICINAL CHEMISTRY; Robichaud Lundbeck Research USA, Paramus, NJ 07652, USA
- B.K. Sharmaa*, A quantitative structure-activity relationship study on serotonin 5-HT6 receptor ligands: indolyl and piperidinyl sulphonamides; a Department of Chemistry, S.K. Government College, Sikar-332 001, India; CSIR Lucknow-226 001, India
- Ralph N. Harris III, Highly potent, non-basic 5-HT6 ligands. Site mutagenesis evidence for a second binding mode at 5-HT6 for antagonism; Roche Palo Alto LLC, 3431 Hillview Ave., Palo Alto, CA 94304, United States

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