Homology modelling aspects: template choice. A 5-HT6R-based study.

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Introduction

Over the years, homology modelling has grown into an important part of biochemistry and pharmacology. It allows structure prediction of proteins, which have not yet been resolved with empirical methods. The results, however, are not perfect and the outcome of such research is bound tightly to many factors set during the procedure, the choice of template being a crucial one.

Current paradigm states, that proteins with the smallest evolutionary distance and thus, the highest identity/similarity, to the target, should achieve the highest performance. The goal of this research was to verify the credibility of this paradigm by comparing results of Virtual Ligand Screening using homology models of 5-HT6R based on several templates.
5-HT6R belongs to GPCR-A family, and as a trans-membrane protein, is extremely hard to crystallize or solubilize, maintaining its native form. This makes standard protein structure assessment inexplicably difficult, and, despite major importance in brain functionality and thus specific drug designing, only few members of GPCR-A family had their structure solved. 5-HT6R itself is considered involved in learning, memorizing and overall cognition processes [1], and is a target in anti-depression drug research [2].

This study comprised of homology modelling of 5-HT6R based on seven available GPCR templates (A2AR, beta1-AR, beta2-AR, CXCR4, D3R, H1R, rhodopsin), and further verification of created models by means of ligand docking (Schrodinger Glide).
The quality of generated structures was assessed in three subsequent steps, each consisting of different compounds sets for docking procedure. The final models docked the most of active ligands and the least of decoys. Interestingly, they were based on templates different than the evolutionarily closest ones, therefore putting the existing paradigm into question.

Homology Modelling

To perform homology modelling, alignments between sequences of each of 7 templates and the sequence of 5-HT6R were created. It was done manually, with 4 restrictions:
a) the most conserved amino acids (X.50 position in Ballesteros-Weinstein notation) from both template and the target were aligned for every helix
b) no gaps within helix ranges were allowed
c) the loop regions were aligned with best possible identity/similarity
d) the aminoacids proven to interact with ligands must be within helices [3]
All alignments are gathered and shown on Figure 2. Helix ranges were assessed in 2 different ways: one conserved the helix ranges of the template protein, the second predicted them using metaservers. For each template and for each helix prediction method 200 homology models of 5-HT6R were created, which yielded a total of 6800 models.

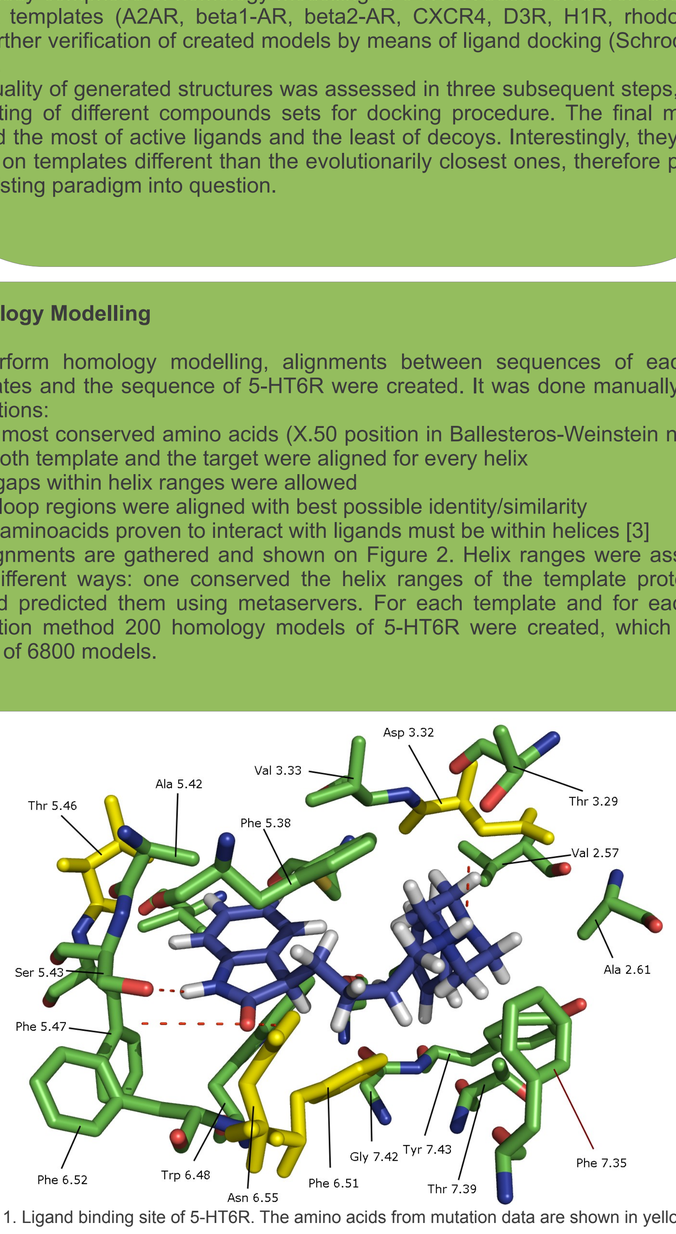


Fig. 1. Ligand binding site of 5-HT6R. The amino acids from mutation data are shown in yellow.

Validation

The quality of models was determined based on 2-step ligand docking procedure.
The first step included docking of 25 structurally diverse highly active 5-HT6R ligands. Models which docked less than 13 models with Glide score lower than -3 were excluded from further verification. The second step comprised of docking 258 active ligands and 1372 decoys in order to calculate Area Under Receiver Operator Characteristic curve (AUROC) for each model.
To ensure the validity of the research, identical procedure was used to create 5-HT7R and 5-HT1A models (see Table 1.).

Results

The results indicate that the best homology models are not necessarily built on evolutionary closest template, and thus the current criteria of template selection should be revised. In fact, this research points out that every modelling study should be performed using multiple templates, in order to guarantee validity of further binding mode studies or virtual screening procedures.

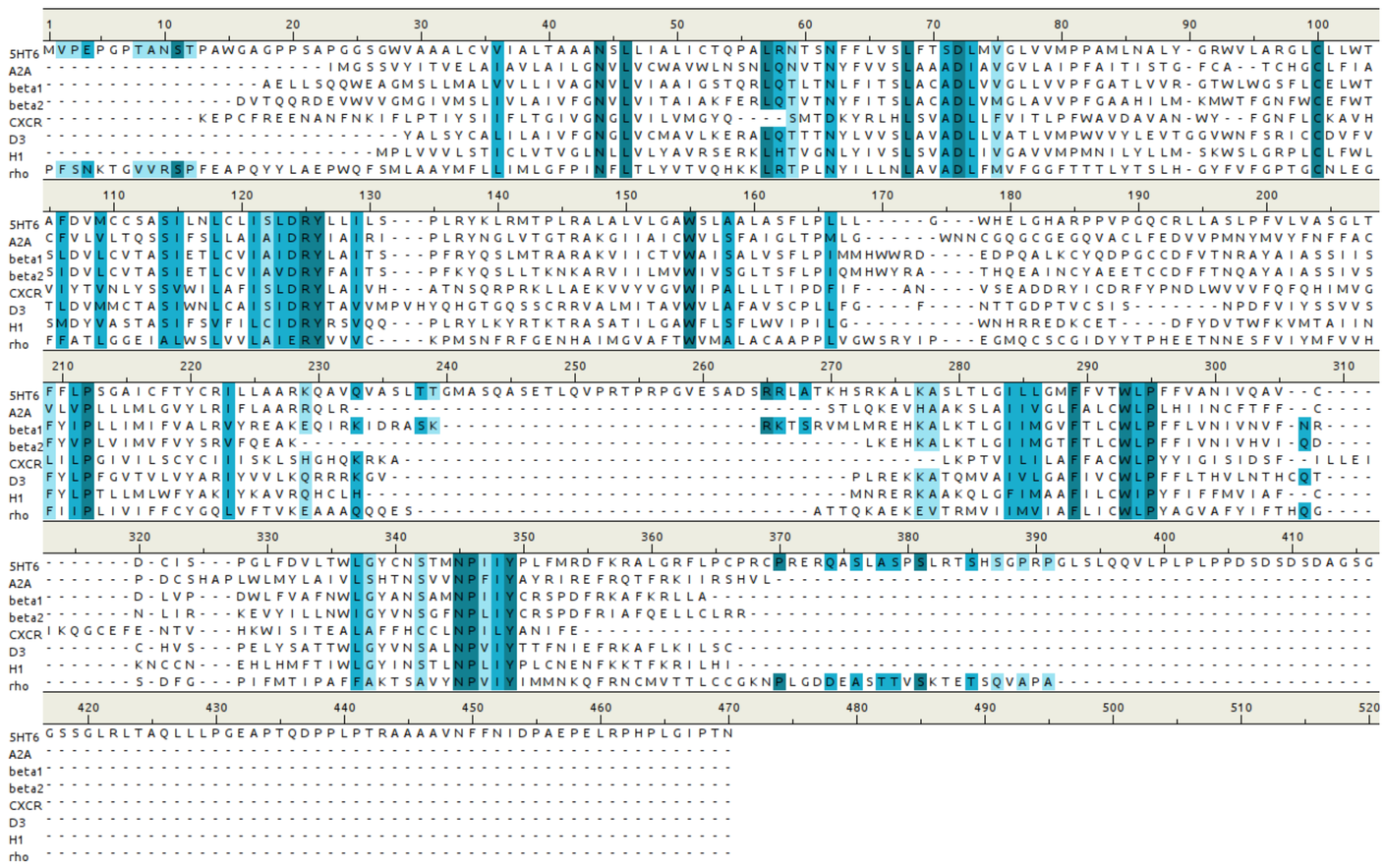


Fig.2. MSA for 5-HT6R. The alignments were hand-made using Discovery Studio software. Residues with Ballesteros-Weinstein number equal 50, are shown in bold.

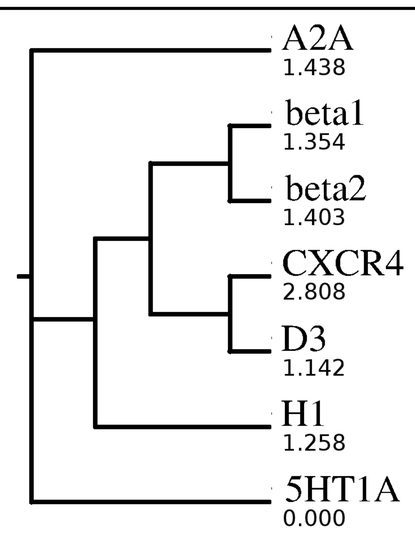
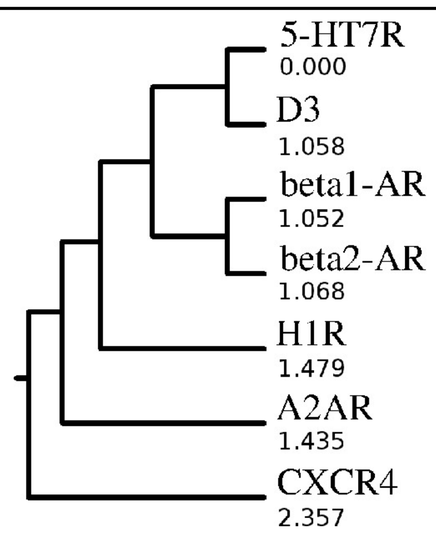
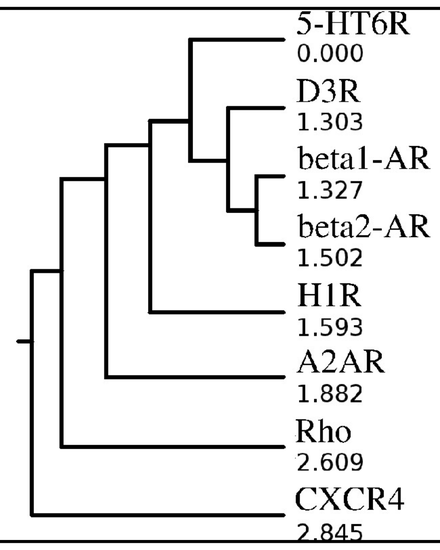
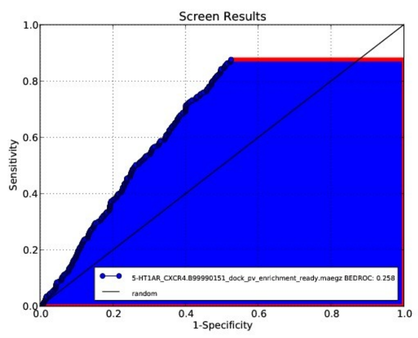
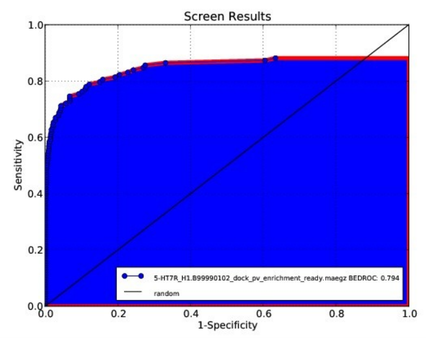
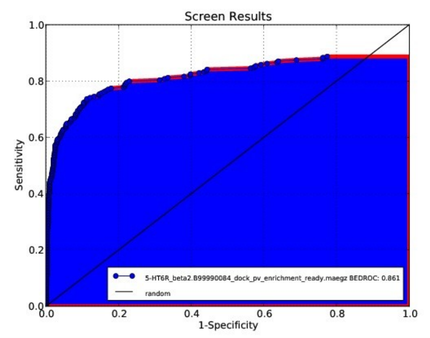
Target protein	5-HT1A	5-HT7R	5-HT6R
Philogenic trees with marked distance			
Best ROC curves			
AUROC values	0.657	0.828	0.772
Template protein	Chemokine receptor 4	Histamine 1 receptor	Beta2 adrenergic receptor

Table 1. Results of docking studies: ROC, AUROC and templates of best models for each protein compared to evolutionary distance between templates and target.

Literature

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2. Hao M., Li Y., Li H., Zhang S. Investigation of the Structure Requirement for 5-HT(6) Binding Affinity of Arylsulfonyl Derivatives: A Computational Study. Int J Mol Sc 2011;12(8):5011-30
3. 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

Acknowledgments

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