Selected transmembrane receptors – structures, interactions and binding site analysis

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Introduction

G-protein couples receptors (GPCRs), sharing consitution of seven transmembrane helices piercing cell membrane, are largely expressed in mammalian organisms. They are involved in a wide variety of physiological processes, such as autonomic nervous system transmission, immunological response, behaviour and mood regulation.

A number of receptors belonging to GPCR family is present in neural tissue and is considered to be involved in learning and memorizing processes. They can be modulated by exogenous compounds, what makes them a common target in drug research. Acquiring information about protein's structure is a basic step to discover interactions involved in ligand binding, which is crucial in design of potential therapeutic compounds. Since structures of proteins with transmembrane domains cannot be easily determined, it is almost impossible to construct their 3D conformation using physical methods. This is why homology modeling is extremely helpful in determining structures of such proteins. Moreover, application of Structural Interaction Fingerprints (SIFts) and averaged SIFt profiles, enables fast and convenient binding site analysis. Such approach allows to determine residues involved in ligand-protein interaction, reveal its type giving insight into binding site properties.

Receptors

All presented receptors belong to the G–protein coupled receptor class 1. 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptors bind 5–hydroxytryptamine (serotonin), a biogenic hormone that functions as neurotransmitter, a hormone and a mitogen. Those receptors mediate their action by association with G proteins that activate phosphatidylinositol–calcium second messenger system (5-HT_{2A}R) or stimulate adenylate cyclase (5-HT_6R, 5-HT_7R). D2R is a dopamine receptor whose activity is mediated by G proteins, that inhibit adenylyl cyclase. These receptors constitute targets in design of antipsychotic drugs.[3]

Binding site analysis

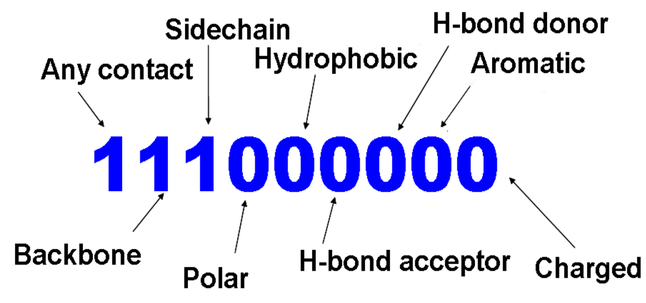
In order to analyze binding site, sets of known ligands active towards each target (retrieved from ChEMBL database), were docked to virtual receptors structures. Afterwards, structural interaction fingerprints (SIFts) were calculated for all ligand-protein poses. SIFts enabled recognition of residues involved in ligand binding, and furthermore, types of interaction. (Figure 1)
Crucial step in interaction analysis was constructing SIFt profiles. They were created by averaging SIFts for each ligand docked to receptor, providing information about the most relevant residues involved in interacion.
Moreover, SiteMap application from Maestro package was used in order to reveal potential ligand binding volumes.

Results and conclusions

Analysis of SiteMap results proved that the largest binding site volumes were present in case of 5-HT_6 and 5-HT_{2A} receptor, and varied from ~300 – 650 cubic Angstroms. 5-HT_7R binding site volume was about 350 cubic Angstroms, while D2 has the smallest binding site – about 240 cubic Angstroms.

SIFt profile analysis proved, that there are always a few residues essential in ligand binding, that all docked ligands interact with.
The most common interactions involved in ligand binding are backbone, sidechain and hydrophobic. In reality many proteins undergo sidechain or backbone movements, or both, upon ligand binding. These changes allow the receptor to alter its binding site so that it more closely conforms to the shape and binding mode of the ligand. Polar and aromatic occur rarely, whilst charged appear only in case of single residues.
Compounds used in case of 5-HT_7R were divided by type into 7 groups[5], each characterized by its own binding mode. (Figure 3)

A few 5-HT_6R residues, revealed as crucial in ligand binding by SIFt profiles (Figure 2), were present in mutagenesis data retrieved from *in vitro* assays, where significant activity decrease triggered by their replacement was reported (Table 1). This proves the fidelity of presented method in prediction of viable binding site.

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...
175     0.6 0 0.6 0 0.6 0 0 0 0
176     0.79 0 0.78 0.78 0 0 0 0 0
177     0 0 0 0 0 0 0 0 0
178     0 0 0 0 0 0 0 0 0
179     1.0 0 1.0 0 1.0 0 0 1.0 0
180     0 0 0 0 0 0 0 0 0
181     0 0 0 0 0 0 0 0 0
182     0.71 0 0.71 0 0.71 0 0 0.71 0
...
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Figure 1. (a) Fragment of SIFt describing bit positions for individual ligand-residue interactions. **(b)** Fragment of SIFt profile.

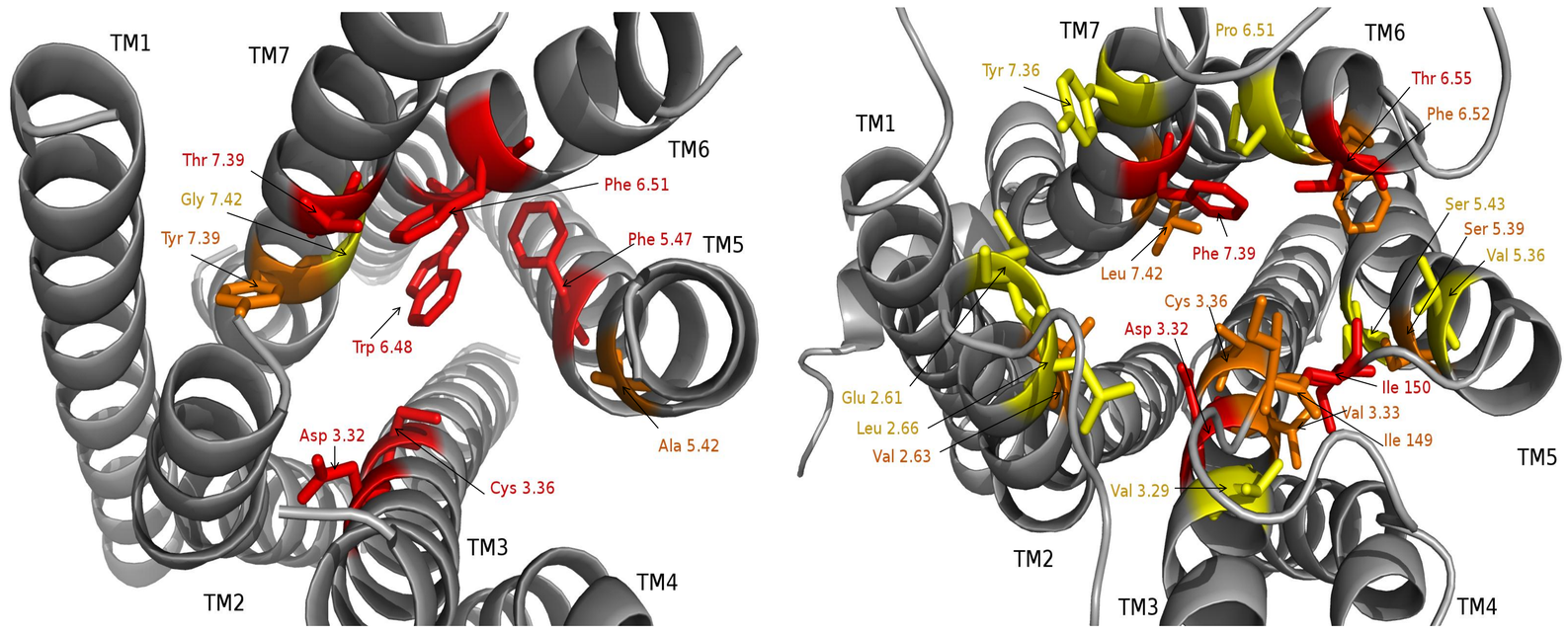
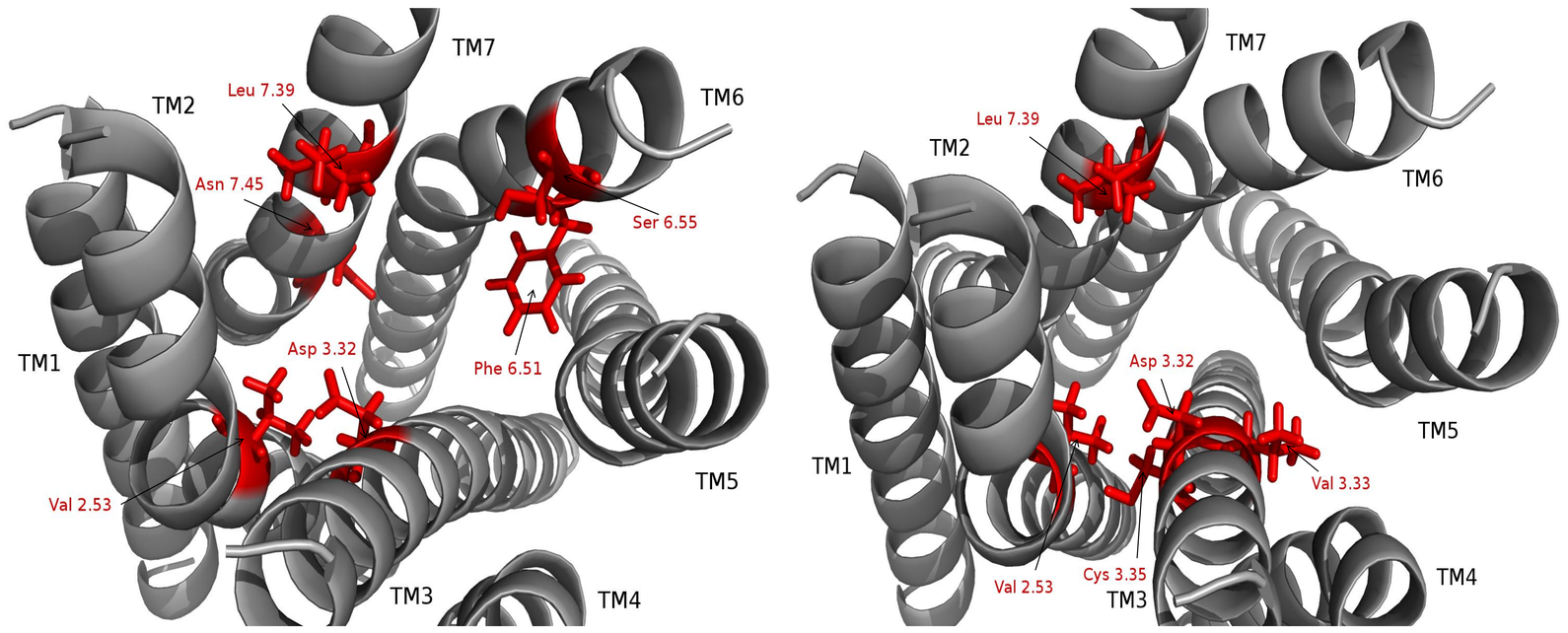


Figure 2. Comparison of 5-HT_6R (left) and D2R (right) binding sites. Residues interacting with 50-70% of ligands are shown in yellow, 71-90% - orange, 91-100% - red.

Table 1. Activity changes in 5-HT_6 receptor triggered by site-directed mutagenesis.

Author, publication year	Mutation	Effect (dKi)
Harris et al., 2010[4]	D32A@TM3	-2,4 × for SB-258585
Harris et al., 2010[4]	C36A@TM3	0,4 × for antagonist -0,5 × for agonist
Harris et al., 2010[4]	W48A@TM6	-2 × for compound 16d, 21d -0,5 × for 5-HT, SB258585, compound 17e



Aporphines	Arylsulfonamidoalkylamines	Ergolines
2.53 3.32 6.52 6.55 7.39	2.53 3.32 6.55 7.39 7.45	3.32 6.52 6.55

LCAP/LCBCs	Tricyclic psychotropes	Diaminopirydines, diaminotriazines and aminotetralines
2.53 3.32	2.53 3.32 3.33 3.35 7.39	2.53 3.32 3.33 3.35 7.39

Figure 3. 5-HT_7R binding site - interaction profiles for distinct groups of chemical compounds (shown as red sticks). Indicated residues interact with 100% of ligands belonging to particular chemical class. Tables underneath figure contain details on the interacting amino acids.

Literature

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Acknowledgments

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