

Molecular modeling of the human serotonin_{1A} receptor: role of membrane cholesterol in ligand binding of the receptor†

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Serotonin_{1A} receptors are important neurotransmitter receptors and belong to the superfamily of G-protein coupled receptors (GPCRs). Although it is an important drug target, the crystal structure of the serotonin_{1A} receptor has not been solved yet. Earlier homology models of the serotonin_{1A} receptor were generated using rhodopsin as a template. We have used two recent crystal structures of the human β_2 -adrenergic receptor, one of which shows specific cholesterol binding site(s), as templates to model the human serotonin_{1A} receptor. Since the sequence similarity between the serotonin_{1A} receptor and β_2 -adrenergic receptor is considerably higher than the similarity between the serotonin_{1A} receptor and rhodopsin, our model is more reliable. Based on these templates, we generated models of the serotonin_{1A} receptor in the absence and presence of cholesterol. The receptor model appears more compact in the presence of cholesterol. We validated the stability of ‘compactness’ using coarse-grain MD simulation. Importantly, all ligands exhibit higher binding energies when docked to the receptor in the presence of cholesterol, thereby implying that membrane cholesterol facilitates ligand binding to the serotonin_{1A} receptor. To the best of our knowledge, this is one of the first reports in which lipid-specific receptor conformations have been modeled by homology modeling.

Introduction

The G-protein coupled receptor (GPCR) superfamily is the largest and most diverse protein family in mammals, involved in signal transduction across membranes.^{1–3} Cellular signaling by GPCRs involves their activation by ligands present in the extracellular environment and subsequent transduction of signals to cellular interior through concerted changes in their transmembrane domain structure.⁴ GPCRs are prototypical members of the family of seven transmembrane domain proteins and include > 800 members which together constitute ~5% of the human genome.⁵ They dictate physiological responses to a diverse array of stimuli that include endogenous ligands such as biogenic amines, peptides, glycoproteins, lipids, nucleotides, Ca²⁺ ions and various exogenous ligands for sensory perceptions such as odorants, pheromones, and

even photons. As a consequence, these receptors mediate multiple physiological processes such as neurotransmission, secretion, cellular differentiation, growth, inflammatory and immune responses. It is therefore only natural that GPCRs have emerged as major targets for the development of novel drug candidates in all clinical areas.^{6–10} Interestingly, although GPCRs represent 30–50% of current drug targets, only a small fraction of all GPCRs are presently targeted by drugs.¹¹ This raises the exciting possibility that receptors that are not yet recognized could be potential drug targets for diseases that are difficult to treat by currently available drugs. In spite of an enormous interest in GPCR drug design owing to the pharmacological significance of GPCRs, lack of three-dimensional structural information severely limits GPCR drug design.¹²

Membrane proteins are difficult to crystallize in their native conditions due to the intrinsic dependence on surrounding membrane lipids.¹³ Since a significant portion of any integral membrane protein (such as GPCRs) remains in contact with the membrane lipid environment,¹⁴ membrane lipids are necessary to maintain their structure and function. Purification and crystallization of membrane proteins therefore continue to be challenging in contemporary biology. Crystallization of GPCRs is even more challenging due to the intrinsic structural flexibility (particularly of the loop regions) giving rise to multiple conformational states (conformational heterogeneity).¹⁵ High-resolution structural information of GPCRs is lacking

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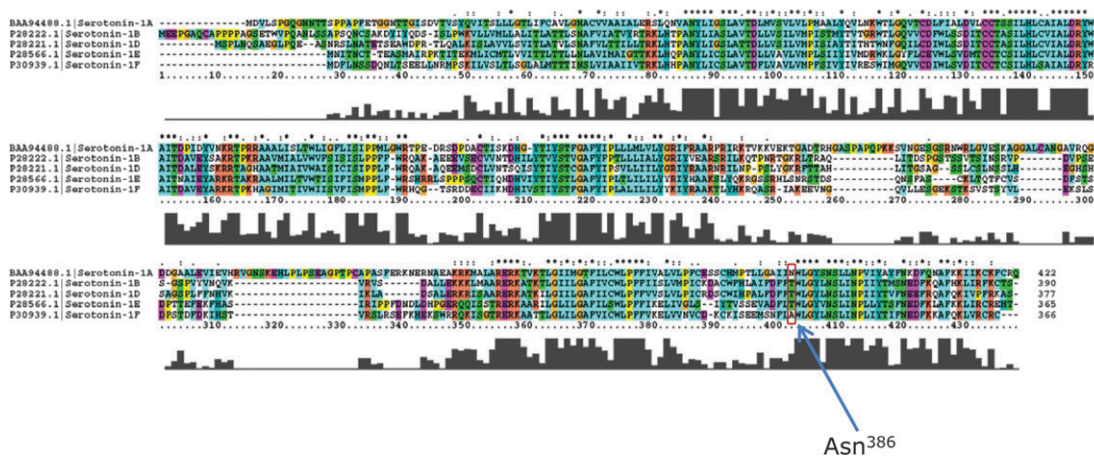


Fig. 1 Sequence alignment of human serotonin-1 receptors. Human serotonin-1 receptor sequences that include receptors of five subclasses (A, B and D–F) have been used for comparison. The amino acid Asn³⁸⁶, crucial for pindolol binding, is highlighted. See text for more details.

for this reason. Crystal structures of only four GPCRs have been solved with atomic resolution to date.^{16–19} Due to improved resolution of modern-day crystallography, some of the solved GPCR crystal structures reveal membrane lipids bound in close proximity to the protein, indicating specific interactions of these proteins with surrounding lipids^{17,20,21} (recently reviewed in ref. 22). Since the crystallization of GPCRs poses considerable challenges, *in silico* approaches for prediction of GPCR structures are being increasingly used for structure-based drug design.^{12,23–25} Homology modeling is a widely used approach in which a known structure of a homologous receptor is used as a template for model building. It is based on the concept that three-dimensional structures of proteins are generally more conserved than their amino acid sequences.²⁶ As a result of this, proteins with homologous sequences are expected to show similar three-dimensional structures.

The serotonin_{1A} (5-HT_{1A}) receptor is an important neurotransmitter receptor and belongs to the superfamily of the seven transmembrane domain GPCRs. It is the most extensively studied of the serotonin receptors for a number of reasons.^{27,28} Serotonin receptors have been classified into at least 14 subtypes on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organization, and second messenger coupling pathways.²⁹ Serotonin_{1A} receptors are known to play a key role in the generation and modulation of various cognitive, developmental and behavioral functions. Agonists and antagonists of the serotonin_{1A} receptor have been shown to possess potential therapeutic effects in anxiety- or stress-related disorders.²⁷ As a result, the serotonin_{1A} receptor serves as an important target in the development of therapeutic agents for neuropsychiatric disorders such as anxiety and depression. Interestingly, mutant (knockout) mice lacking the serotonin_{1A} receptor exhibit enhanced anxiety-related behavior, and represent an important animal model for genetic vulnerability to complex traits such as anxiety disorders and aggression in higher animals.^{30,31} Taken together, these results suggest that the serotonin_{1A} receptor is a central player in a multitude of physiological processes, and represents an important drug target.

In this work, we have used two recent crystal structures of the human β_2 -adrenergic receptor^{17,21} as templates to model

the human serotonin_{1A} receptor. One of these structures shows specific cholesterol binding site(s) in the receptor.²¹ Based on these templates, we generated molecular models of the serotonin_{1A} receptor in the absence and presence of cholesterol. The receptor model appears more compact in presence of cholesterol. We validated the stability of ‘compactness’ using coarse-grain MD simulations. We have further used these models to dock agonists and antagonists to the serotonin_{1A} receptor, in order to identify the role of membrane cholesterol in ligand binding to the receptor. Our results show that the presence of membrane cholesterol facilitates ligand binding to human serotonin_{1A} receptors. To the best of our knowledge, our results represent one of the first reports in which lipid-specific receptor conformations have been modeled by homology modeling.

Results

Sequence alignment and analysis

Human serotonin-1 receptors are divided into five classes which include 1A, 1B, 1D, 1E and 1F subtypes. The serotonin_{1A} receptor is an important member of the serotonin-1 subfamily and is estimated to have differentiated ~650 million years ago from the subfamily during the period when vertebrates diverged from invertebrates.^{32,33} The human gene for the receptor encodes a protein of 422 amino acids. Considering the presence of three consensus sequences for *N*-linked glycosylation in the amino terminus, and the homology of the receptor with β -adrenergic receptor, it is predicted that the receptor is oriented in the plasma membrane with the amino terminus facing the extracellular region and the carboxy terminus facing the intracellular cytoplasmic region.^{27,28,34} The transmembrane domains (I–VII) of the receptor are connected by hydrophilic sequences of three extracellular loops (EC1, EC2, EC3) and three intracellular loops (IC1, IC2, IC3). Such an arrangement is typical for the G-protein coupled receptor superfamily.³⁵

A striking feature of the serotonin_{1A} receptor is the length (~120 residues spanning from 219–343 residues, based on transmembrane helix prediction using TMHMM2²²) of its third intracellular (IC3) loop. The IC3 loop of the serotonin_{1A} receptor is considerably longer than the corresponding loop of

other members of the serotonin-1 receptor subfamily (see Fig. 1) and is important for coupling to G-proteins. Interestingly, it has been previously reported using site-directed mutagenesis that mutations in IC3 loop of the serotonin_{1A} receptor alter G-protein coupling from G_i to G_s in a ligand-dependent manner.³⁶ The serotonin_{1A} receptor was the first of all types of serotonin receptors to be cloned as an intronless genomic clone (G-21) of the human genome, which cross-hybridized with a full length β -adrenergic receptor probe at reduced stringency.^{27,37} Sequence analysis of this genomic clone (later identified as the serotonin_{1A} receptor gene) shows ~48% amino acid similarity with the β ₂-adrenergic receptor in the transmembrane region. A unique pharmacological feature of the serotonin_{1A} receptor is its affinity for the class of drugs called β -blockers.³⁸ Interestingly, sequence alignment of all serotonin-1 receptors shows that Asn³⁸⁶ is present only in the serotonin_{1A} receptor (see Fig. 1), and is also found in β -adrenergic receptors. Importantly, Asn³⁸⁶ is the residue that is involved in the binding of pindolol, a β -blocker (and an antagonist for β -adrenergic receptor).³⁸ The serotonin_{1A} receptor was initially discovered as an ‘orphan’ receptor and was identified (‘deorphanized’) later.³⁹ The sequence alignment of serotonin_{1A} receptors from vertebrates and invertebrates is shown in Fig. S1 and S2†. These figures show that the sequence of serotonin_{1A} receptors is predominantly conserved from fish to humans, while the conservation among sequences of invertebrates is rather poor.

Generation of molecular models of human serotonin_{1A} receptors

As mentioned above, the overall sequence similarity of the human serotonin_{1A} receptor with the human β ₂-adrenergic receptor is ~48% in the transmembrane region. Fig. 2 shows sequences of the template and human serotonin_{1A} receptor to show that the transmembrane regions are predominantly conserved. The homology model generated by using β ₂-adrenergic receptor as template would therefore be more reliable in the transmembrane region. Modeling the transmembrane region assumes significance since the ligand binding pockets of serotonin_{1A} receptors are believed to be present in the

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                TMH1
Serotonin-1A  VSYQVITS-LLGLTILFCAVLGNACVVAAIALERSLQNVANYLIGSLAVT
2RH1         DEVWVVGMIYMSLIVLVAIVFGNVLVITAIKAFERLQTVTNYFITSLACA
                *::: :::: :::: **:: *::: **:: *::: **:: *:::
                TMH2                TMH3
Serotonin-1A  DLMVSVLVLPMAALYQVFNKWTLCQVTCDFIALDVLCCSSILHLCAIA
2RH1         DLVMGLAVVPPGAAILMKMWTFCNFWCFWTSIDVLCVTASIELCVIA
                **::: *::: *::: *::: *::: *::: *::: *::: *::: *:::
                TMH4
Serotonin-1A  LDRYWAITDPIDYVNKRTPRRAAALISLTLIGFLISIPPLMGWRTPEDRS
2RH1         VDRYFAITSPFKYQSLTINKARVILMVVIVSGLTSLPLIQMHWYRATHQ
                :***:***:*. * . * . * . * . * . * . * . * . * . * . * .
                TMH5
Serotonin-1A  DPD-----ACTISKDHGYTIYSTFGAFYIPLLLMLVLYGRIFRAAR---
2RH1         EAINCYAETCCDFEFTNQAYALASSIVSFYVPLVIMVFSRVFQEAQRQL
                :. * . :. * . * . * . * . * . * . * . * . * . * . * .
                TMH6
Serotonin-1A  -----ERKTVKTLGIIMGTFILCWLPFFIVALVLPFCESCHMPFLLGAI
2RH1         KFCLKEHKALKTLGIIMGTFILCWLPFFIVNIHVHVDN--LIRKEVYILL
                * . * . * . * . * . * . * . * . * . * . * . * . * . * .
                TMH7
Serotonin-1A  NWLGYSNLLNPFVIYAYFNKDFQNAFKKIIKC
2RH1         NWIYGVNSGFNPLIYCRS-PDFRIAFQELLCL
                ** . * . * . * . * . * . * . * . * . * . * . * .

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Fig. 2 The final alignment used for modeling the human serotonin_{1A} receptor. Transmembrane helices are highlighted for easy identification. The alignment shows that amino acid residues in transmembrane helices are largely conserved.

transmembrane region.²⁷ We therefore chose to focus mainly on the transmembrane region of the receptor for further analysis. We used two recently published structures of the human β ₂-adrenergic receptor as templates. One of the structures (PDB 2RH1)¹⁷ was used to generate the model in the absence of cholesterol. The other structure (PDB 3D4S)²¹ exhibits bound cholesterol molecules at the Consensus Cholesterol Binding Motif (CCM) in between the helices of a monomer. We used the latter structure to generate a model of the serotonin_{1A} receptor in the presence of cholesterol. The 3D4S structure shows a cholesterol binding site between transmembrane helices I–IV with two cholesterol molecules bound per receptor monomer. The cholesterol binding site appears to be characterized by the presence of a cleft located at the membrane interfacial region.²¹ Both cholesterol molecules bind in a shallow surface groove formed by segments of the above-mentioned helices (I–IV), thereby providing an increase in the intramolecular occluded surface area, a parameter often correlated to the enhanced thermal stability of proteins.⁴⁰ Importantly, we have earlier reported that cholesterol binding sites are inherent characteristic features of serotonin_{1A} receptors and are conserved over natural evolution.²²

The energy-minimized homology models of the human serotonin_{1A} receptor in the absence and presence of cholesterol are shown in Fig. 3. These models were validated using PROCHECK and WHAT IF servers. The corresponding Ramachandran plots for receptor models in the absence and presence of cholesterol are shown in Fig. 4. Analysis using PROCHECK reveals that >98% of the residues are in overall allowed regions (~91% in most favored regions and 8% in additional allowed regions) in case of the model generated without cholesterol (see Fig. 4a). Fig. 4b shows that in case of the model in presence of cholesterol, >98% of the residues are in overall allowed regions (~89% in most favored regions and 9% in additional allowed regions). In addition, models of the serotonin_{1A} receptor in the absence and presence of cholesterol were validated using the ProSA program (Fig. S3†).

Importantly, Fig. 3 shows that the model of the serotonin_{1A} receptor in presence of cholesterol is more compact compared to the model generated in absence of cholesterol. The presence of cholesterol appears to increase the packing of the transmembrane helices. In this context, it is worth mentioning here that calculation of packing values of various helices in the β ₂-adrenergic receptor that are involved in the cholesterol interaction site show that the packing of transmembrane helices II and IV increases upon cholesterol binding. The increased packing would restrict their mobility rendering greater thermal stability to the protein.²¹ Our results from homology modeling of the serotonin_{1A} receptor in the presence of cholesterol is in general agreement with these results with the β ₂-adrenergic receptor. Interestingly, we have recently observed that the thermal stability of the serotonin_{1A} receptor is enhanced in the presence of cholesterol.⁴¹

Validation of the compactness of the receptor model in the presence of cholesterol by MD simulation

Models of the serotonin_{1A} receptor in the absence and presence of cholesterol, mapped to their coarse-grain representations,

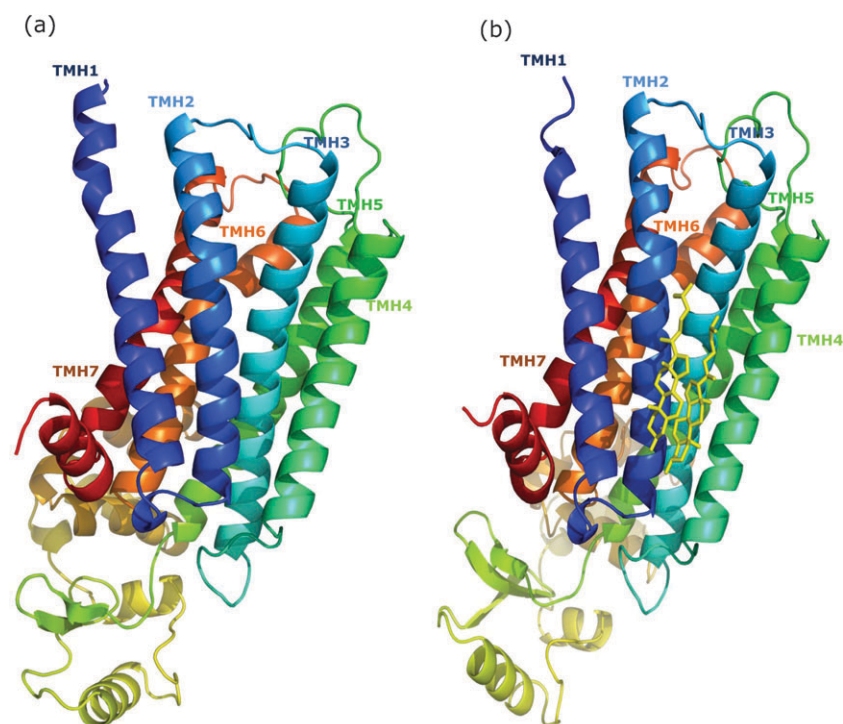


Fig. 3 Energy-minimized final models of the human serotonin_{1A} receptor in the absence (a) and presence (b) of cholesterol. Transmembrane helices I–VII are shown in different colors. Panel (b) shows two cholesterol (yellow) molecules bound to the receptor. The receptor model in presence of cholesterol appears more compact (see text).

were simulated in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and POPC-cholesterol bilayers, respectively. Simulations of 8 μ s were performed for both models. To test the integrity of the models, they were compared to the starting structures. The RMSD of the backbone beads of the transmembrane helices of the receptor in the absence and presence of cholesterol is 0.3 nm and 0.5 nm, respectively. Importantly, the volume of transmembrane domains (considering only the backbone) of receptor models in the absence ($11.6 \pm 0.005 \text{ nm}^3$) and presence ($11.7 \pm 0.01 \text{ nm}^3$) of cholesterol is comparable and remains constant during the simulation (see Fig. 5). In addition, the volume of transmembrane domains of the receptor models in the absence ($24.3 \pm 0.001 \text{ nm}^3$) and presence ($24.7 \pm 0.04 \text{ nm}^3$) of cholesterol is similar, even if side chains are included (Fig. 5).

The deviation of the model of the serotonin_{1A} receptor in the absence of cholesterol from its starting conformation is quite low (indicated by RMSD). The deviation of the model of the serotonin_{1A} receptor in the presence of cholesterol is relatively high but within the average deviation, for coarse-grain protein structures.⁴² During the simulation, a small repositioning of helix II was observed when the receptor model was simulated in the presence of cholesterol. We speculate that this deviation is due to the absence of optimal packing of cholesterol molecules in the starting structure since they were placed randomly in the POPC bilayer. Nonetheless, the volume of the receptor models remains constant, thereby ruling out any substantial deviation of the transmembrane helices from their modeled positions. In addition, the volume of both receptor models is comparable, indicating that the ‘compactness’ of the receptor model in the presence of cholesterol is not due to an

overall decrease in volume, but rather due to better packing of the side chains resulting in a more compact lumen.

Analysis of molecular docking

Our understanding of the structure of the ligand binding site of GPCRs at the atomic level is still emerging.⁴³ Although the crystal structure of the serotonin_{1A} receptor has not been worked out yet, mutagenesis studies have provided useful insight in identifying amino acid residues important for ligand binding and G-protein coupling of the receptor (reviewed in ref. 27). In order to identify residues potentially involved in ligand binding, seven ligands (three agonists and four antagonists) were docked to the receptor in the absence and presence of cholesterol. The agonists used were serotonin, 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) and 5-carboxamidotryptamine (5-CT) and their chemical structures are shown in Fig. 6. The antagonists used were 4-(2'-methoxy)-phenyl-1-[2'-(*N*-2''-pyridinyl)-*p*-fluorobenzamido]-ethyl-piperazine (*p*-MPPF), *N*-(2-(1-(4-(2-methoxyphenyl)-piperazinyl)-*N*-(2-pyridinyl)ethyl)cyclohexanecarboxamide (WAY-100635), *O*-desmethyl derivative of WAY (DWAY) and pyridinyl *N*-oxide derivative of WAY (NOWAY) and their chemical structures are shown in Fig. 7. DWAY and NOWAY have been shown to exhibit high affinity toward the serotonin_{1A} receptor.⁴⁴ The selective agonist 8-OH-DPAT was the first compound that did not contain an indole nucleus as part of its structure.⁴⁴ The availability of 8-OH-DPAT helped in the characterization of the serotonin_{1A} receptor, thereby making this receptor the most studied amongst serotonin receptors.²⁷ Serotonin is the natural ligand of the receptor.

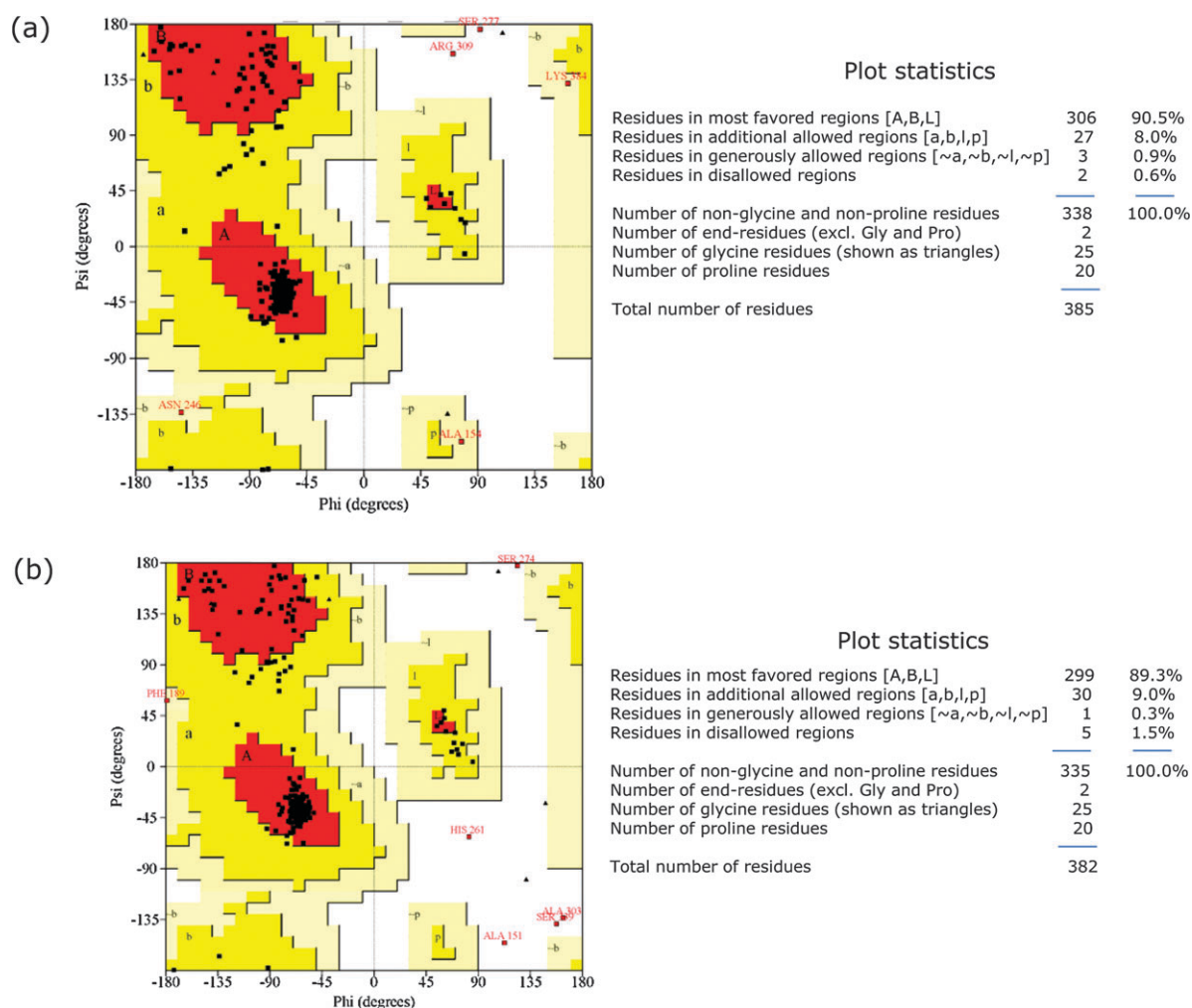


Fig. 4 Ramachandran plots calculated for the human serotonin_{1A} receptor models generated using PROCHECK server. Panels (a) and (b) show Ramachandran plots for models generated in the absence and presence of cholesterol. Fully allowed and partially allowed regions are shown in red and deep yellow, respectively. The analyzed results are shown along with the plots. A vast majority of amino acid residues (> 98%) are within most favored and partially allowed regions in both cases. See the Experimental section for other details.

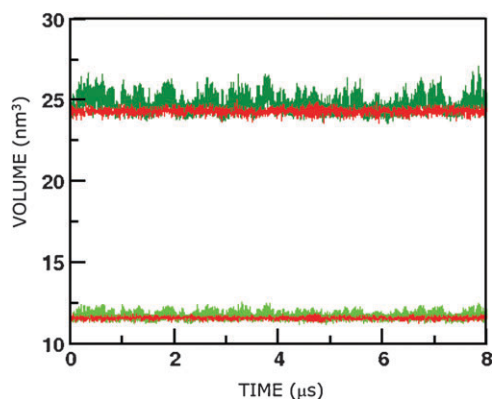


Fig. 5 Plot of volume of transmembrane domains of serotonin_{1A} receptor models in the absence (red) and presence (green) of cholesterol with simulation time. Lower two data sets correspond to the volume of only the backbone, while the upper two traces correspond to volume including side chains.

5-CT has been reported to display affinity similar to that of serotonin.⁴⁴

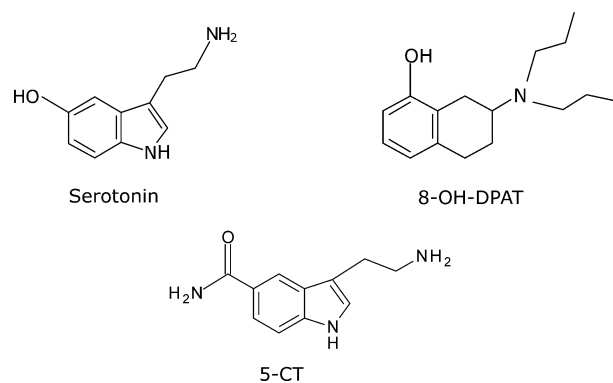


Fig. 6 Chemical structures of agonists for the serotonin_{1A} receptor. Agonists such as serotonin, 8-OH-DPAT and 5-CT are used to dock to the receptor.

Fig. 8 shows the representative docked poses of these ligands to the receptor. The docked poses of ligands to the receptor are the best-energy solutions in the most populated cluster. Clustering analysis with RMSD is shown in Fig. S4†.

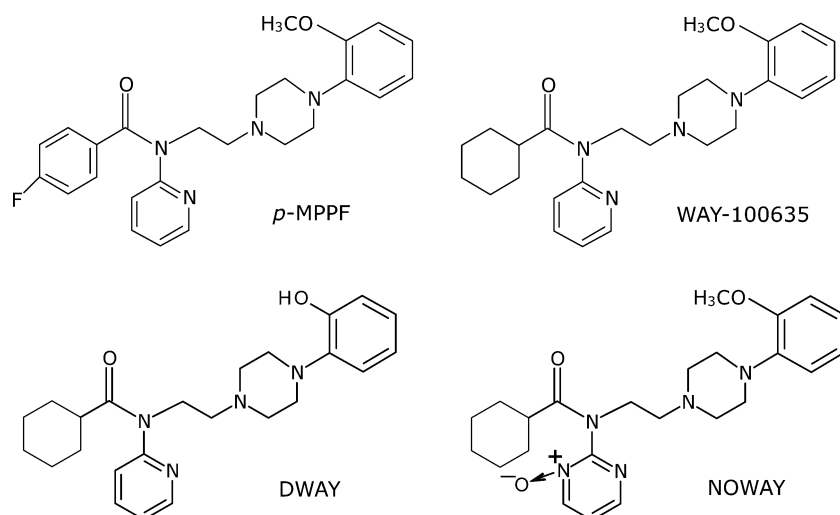


Fig. 7 Chemical structures of antagonists for the serotonin_{1A} receptor. Antagonists such as *p*-MPPF, WAY-100635, DWAY and NOWAY are used to dock to the receptor.

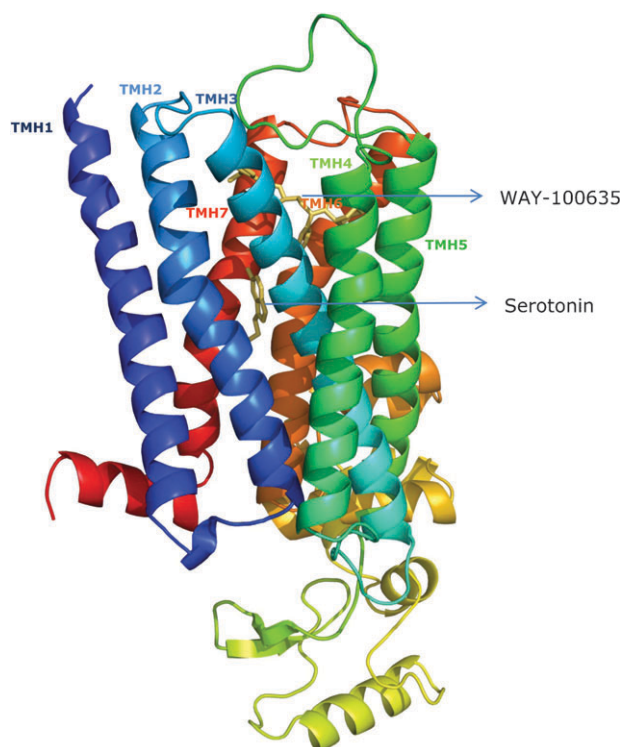


Fig. 8 Model of the human serotonin_{1A} receptor showing docked sites and poses of serotonin (agonist) and WAY-100635 (antagonist). Interestingly, the antagonist binding site is found to be relatively shallow compared to the agonist binding site. Transmembrane helices I–VII are shown in different colors. See the Experimental section for other details.

The docked poses are depicted with the receptor in the absence of cholesterol. Although the docked site remains largely invariant in the presence of cholesterol, the predicted binding energies differ (see later). Docked poses of serotonin and WAY-100635 clearly show that the antagonist binding pocket is relatively shallow compared to the agonist binding pocket.

Interestingly, it was previously suggested by us that the antagonist binding site in the receptor could be at a relatively shallow location compared to the agonist binding site.⁴⁵ This prediction was based on the effect of ethanol on the extent of ligand binding of the receptor.

Although there is no structural data for the serotonin_{1A} receptor, there is considerable experimental data suggesting possible ligand binding sites in the receptor. Site-directed mutagenesis provides a powerful approach to identify the location of the binding pocket in the receptor. Results obtained from point mutations suggest that Asp⁸² and Asp¹¹⁶ in transmembrane helices II and III are crucial for binding of serotonin to the receptor.⁴⁶ It has been suggested that the carboxylate group in Asp⁸² and/or Asp¹¹⁶ probably acts as a counter-ion to the amine group of serotonin, thereby stabilizing its interaction.²⁷ Interestingly, while Asp⁸² is conserved in all cloned GPCRs, Asp¹¹⁶ is conserved only in GPCRs that bind biogenic amines. Another possibility for agonist binding is an interaction between a hydrogen bonding group (*e.g.*, hydroxyl group) of serotonin and the hydroxyl group of serine or threonine residues. In addition, agonist binding could be stabilized by aromatic π - π interactions with the surrounding residues.⁴⁴ Fig. 9 and 10 illustrate agonist and antagonist docking poses on the serotonin_{1A} receptor, and Tables 1 and 2 show amino acid residues of the receptor involved in interactions with ligands. The predicted binding mode of serotonin shows hydrogen bonding with Asp¹¹⁶. Fig. 11 shows the respective predicted binding energy of ligands to the serotonin_{1A} receptor in the absence and presence of cholesterol. Higher binding energy implies better docking of the ligand to the receptor. The predicted binding energy for docked serotonin is found to be ~ 7.25 kcal/mol. Docking of the synthetic specific agonist, 8-OH-DPAT, with the receptor resulted in slightly higher binding energy (~ 7.5 kcal/mol). Synthetic ligands appear to bind to the receptor with higher binding energy. In general, it appears that docked antagonists exhibit higher binding energies compared to that of agonists. *Importantly, both agonists and antagonist show higher binding*

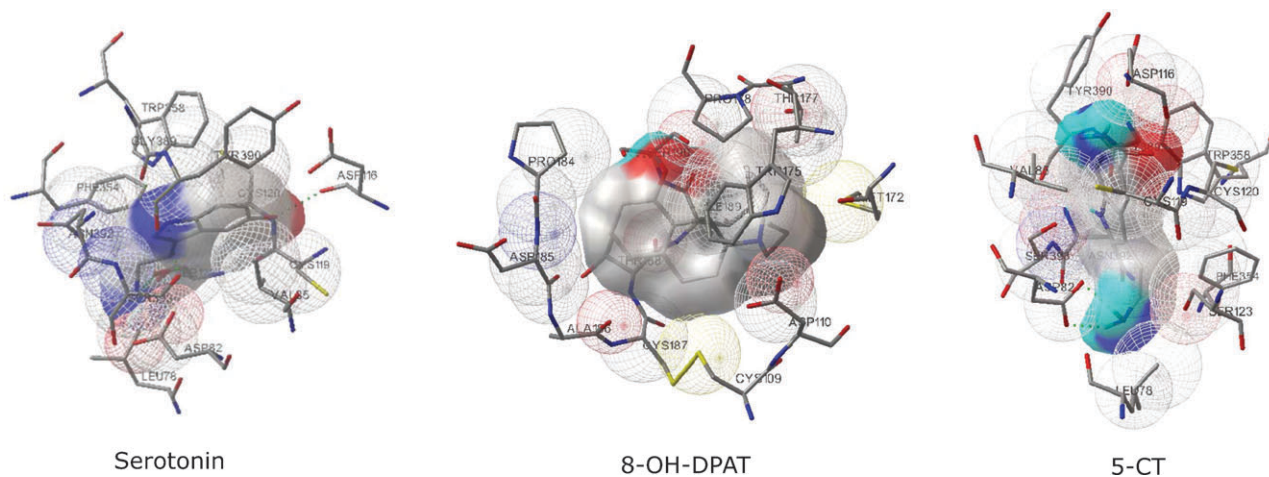


Fig. 9 Representation of docked poses of agonists to the human serotonin_{1A} receptor. Snapshots of interactions of the serotonin_{1A} receptor with various agonists from AutoDock are shown. Agonists include serotonin, 8-OH-DPAT and 5-CT.

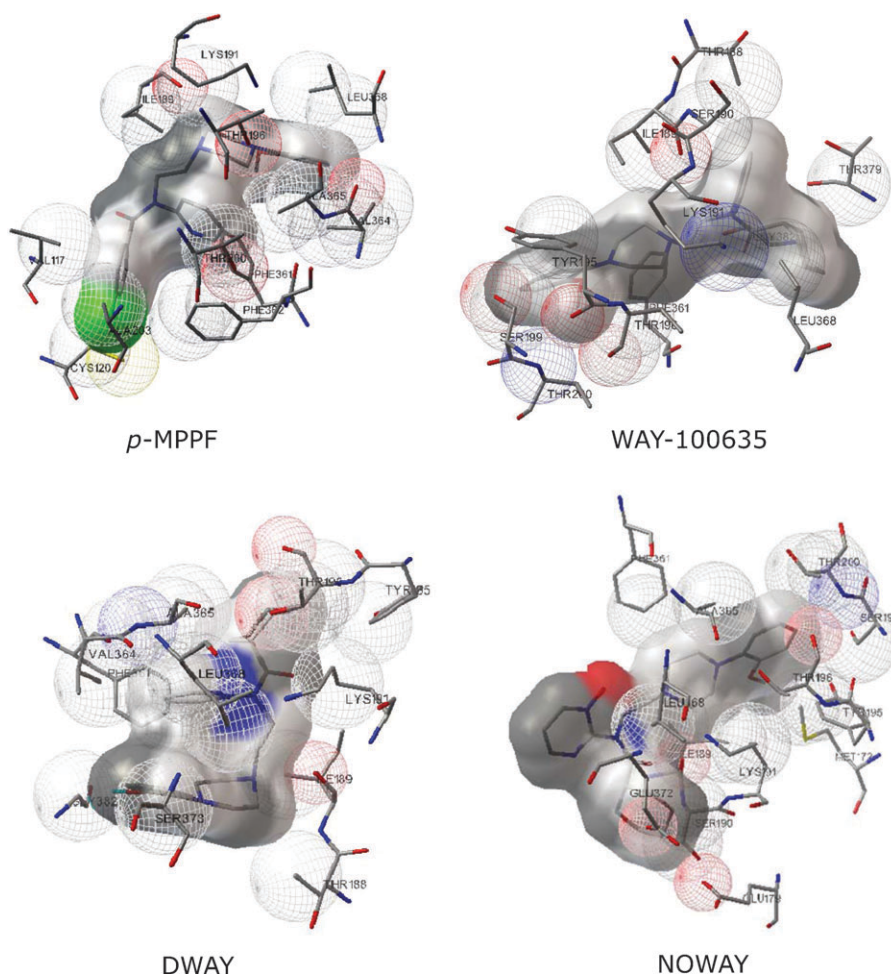


Fig. 10 Representation of docked poses of antagonists to the human serotonin_{1A} receptor. Snapshots of interactions of the serotonin_{1A} receptor with various antagonists from AutoDock are shown. Antagonists include *p*-MPPF, WAY-100635, DWAY and NOWAY.

energies when docked to the receptor in the presence of cholesterol. Our results therefore demonstrate that membrane cholesterol facilitates ligand binding to the serotonin_{1A} receptor.

Interestingly, we have previously shown that the affinity of specific agonist (8-OH-DPAT)⁴⁷ and antagonist (*p*-MPPF)⁴⁸ to the serotonin_{1A} receptor is reduced upon cholesterol depletion.

Table 1 Analysis of the molecular interactions in docked poses^a

Agonist	Interactions involving:		
	Hydrogen bonds	Nonpolar residues	Polar residues
Serotonin	Asp116	Leu78	Asp82
	Cys120	Val85	
	Ser123	Cys119	
	Gly389	Phe354	
	Ser393	Trp358 Tyr390 Asn392	
8-OH-DPAT	Ser190	Cys109	Asp110
		Met172	Asp185
		Trp175	
		Thr177	
		Pro178	
		Pro184	
		Ala186	
		Cys187	
		Thr188	
		Ile189	
5-CT	Asp116	Leu78	Asp82
		Val85	Ser123
		Cys119	Ser393
		Cys120	
		Phe354	
		Trp358	
		Gly389	
		Tyr390	
		Asn392	

^a Residues that are reported to be involved in binding are in bold.

Discussion

Lipid–protein interactions play a crucial role in maintaining the structure and function of integral membrane proteins and receptors.^{49–52} A large portion of any given transmembrane receptor remains in contact with the lipid environment. This raises the obvious possibility that membrane lipids could be important modulators of receptor structure and function. Work from a number of groups has contributed to our understanding of the requirement of specific lipids for maintaining the proper topology, structure and function of membrane proteins.^{49,50,53,54} Cholesterol is an essential and representative lipid in higher eukaryotic cellular membranes.^{55,56} Importantly, cholesterol plays a vital role in the function and organization of GPCRs.^{51,57} These effects of cholesterol on GPCRs have been attributed either to specific interactions of lipids with amino acids in proteins or to bulk properties of membranes.⁵⁸

In addition to the recently reported cholesterol-bound crystal structure of the β_2 -adrenergic receptor,^{17,21} the crystal structure of a photo-stationary state, highly enriched in metarhodopsin I, showed a cholesterol molecule between two rhodopsin monomers.²⁰ These authors also reported that cholesterol could improve the reliability and yield of crystallization. In this structure, cholesterol is shown to be oriented with its tetracyclic ring aligned normal to the membrane bilayer. These authors proposed that some of the tryptophans in transmembrane helices would be able to interact with the tetracyclic ring of cholesterol. Specific cholesterol interaction (*i.e.*, through a direct interaction that could induce a

Table 2 Analysis of the molecular interactions in docked poses

Antagonist	Interactions involving:	
	Nonpolar residues	Polar residues
<i>p</i> -MPPF	Val117	Lys191
	Cys120	
	Ile189	
	Thr196	
	Thr200	
	Ala203	
	Phe361	
	Phe362	
	Val364	
	Ala365	
	Leu368	
WAY-100635	Thr188	Ser190
	Ile189	Lys191
	Tyr195	Ser199
	Thr196	
	Thr200	
	Phe361	
	Leu368	
	Thr379	
	Gly382	
DWAY	Ile189	Lys191
	Thr188	Ser373
	Tyr195	
	Thr196	
	Ala365	
	Leu368	
	Phe361	
	Val364	
	Gly382	
NOWAY	Ile189	Glu372
	Met172	Lys191
	Phe361	Ser199
	Ala365	Ser190
	Thr200	Glu179
	Tyr195	
	Thr196	
	Leu368	

conformational change in the receptor) has been shown for GPCRs such as oxytocin^{57,59,60} and galanin⁶¹ receptors. In addition, new information on lipid-mediated dimerization of cannabinoid receptors has been obtained by homology modeling using the crystal structure of β_2 -adrenergic receptor.⁶² Interestingly, recent molecular dynamics simulations have shown that membrane cholesterol specifically interacts with transmembrane domains of GPCRs such as rhodopsin⁶³ and human A_{2A} adenosine receptor,⁶⁴ thereby stabilizing helix II of the human A_{2A} adenosine receptor. A consequence of this interaction is that the receptor couples to G-proteins only in the presence of cholesterol.

The overall objective of the present work is to generate and validate a reliable homology model of the serotonin_{1A} receptor and use this model to dock well-characterized ligands to the receptor and explore the role of cholesterol in ligand binding. Although homology models of the serotonin_{1A} receptor were previously generated using rhodopsin as a template,^{65,66} our work represents the first comprehensive model of the human serotonin_{1A} receptor generated using the human β_2 -adrenergic receptor as a template. The success of homology modeling

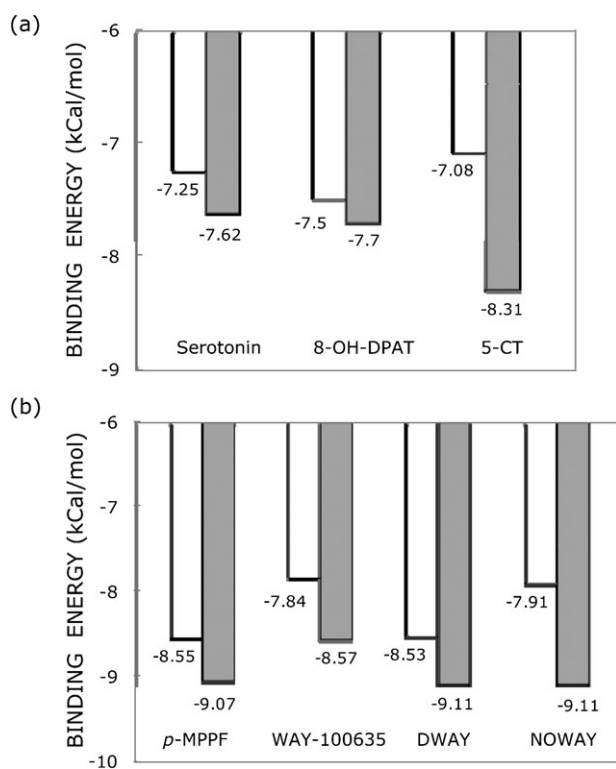


Fig. 11 Predicted binding energies of docked poses of (a) agonists and (b) antagonists to the human serotonin_{1A} receptor in the absence and presence of cholesterol. Binding energies in the absence and presence of cholesterol are shown as empty and filled bars, respectively.

often depends on the similarity of structural and functional relationships between the template and the modeled protein.^{43,67} The quality and refinement of the model would improve with availability of better templates. It is in this context that our present model is more reliable since the sequence similarity (~48%) between the human serotonin_{1A} receptor and human β_2 -adrenergic receptor in the transmembrane region is considerably higher compared to the similarity (~38%) between serotonin_{1A} receptor and rhodopsin sequences.

Previous work from our laboratory has comprehensively demonstrated the requirement of membrane cholesterol in the function and organization of the serotonin_{1A} receptor.^{47,48,51,52,68,69} We have recently proposed that membrane cholesterol could occupy ‘nonannular’ binding site(s) on the receptor.²² Nonannular sites are characterized by lack of accessibility to annular lipids, *i.e.*, these sites cannot be displaced by competition with annular lipids. In the present work, we combined homology modeling with cholesterol-specific conformations of the serotonin_{1A} receptor. Our results constitute an early attempt to incorporate cholesterol specificity of GPCRs in homology modeling. In the context of increasing pharmacological relevance of the serotonin_{1A} receptor, interaction of this transmembrane protein with surrounding lipids (such as cholesterol) assumes greater significance in its structure and function in healthy and diseased states.

GPCRs are involved in a multitude of physiological functions and represent important drug targets. Although

the pharmacological and signaling features of GPCRs have been extensively studied, aspects related to their interaction with membrane lipids have been addressed in very few cases. In this context, the realization that lipids such as cholesterol could influence the structure and function of GPCRs has remarkably transformed our idea regarding the function of this important class of membrane proteins. With progress in deciphering molecular details on the nature of this interaction, our overall understanding of GPCR function in health and disease would improve significantly, thereby enhancing our ability to design better therapeutic strategies to combat diseases related to malfunctioning of these receptors. A comprehensive understanding of GPCR function in relation to the membrane lipid environment is important, in view of the enormous implications of GPCR function in human health,^{7,8} and the observation that several diagnosed diseases are attributed to altered lipid–protein interactions.^{70,71} We conclude that homology modeling,¹² in combination with emerging knowledge of lipid specificities of GPCRs,⁵² represent a promising approach to use in future drug discovery research.

Experimental section

Sequence analysis

All sequences used for the analysis, including the sequence (BBA94488) of the human serotonin_{1A} receptor, were obtained from the NCBI (<http://www.ncbi.nlm.nih.gov/>) database. Sequence alignment of the human serotonin_{1A} receptor and human β_2 -adrenergic receptor was carried out with ClustalX (version 2.0.11). Sequence alignments were performed on sets of receptor sequences to identify conserved residues that could have structural and or functional implications. Sequence alignments were carried out with all subtypes of the serotonin-1 receptor (A, B; D–F) and the serotonin_{1A} receptor from invertebrates and vertebrates. Alignments were constructed using ClustalX and were manually-edited and viewed using Jalview (version 2.4) to retain high equivalence of conserved regions. Residues are numbered according to the human serotonin_{1A} receptor sequence throughout the text.

Homology modeling

The serotonin_{1A} receptor and β_2 -adrenergic receptor are both biogenic amine GPCRs, and enjoy a sequence similarity of ~48%. The human serotonin_{1A} (BBA94488) and human β_2 -adrenergic receptor (2RH1) sequences were aligned using ClustalX and viewed and edited using Jalview. Recently reported crystal structures of the human β_2 -adrenergic receptor were obtained from RCSB (<http://www.rcsb.org/pdb>) and used as templates. Accession numbers of the crystal structures of human β_2 -adrenergic receptors in the absence and presence of cholesterol are 2RH1¹⁷ and 3D4S,²¹ respectively. We chose 3D4S as a template for modeling the human serotonin_{1A} receptor due to the presence of two cholesterol molecules in the structure. The coordinates corresponding to 1–28 and 343–365 segments were not available in 2RH1, and coordinates corresponding to 1–31 and 343–365 segments were not available in 3D4S crystal structures due to poor electron density, and therefore these residues were removed from the query

sequence prior to alignment. The final alignment was used to construct the model using the software Modeller⁷² (version 9.7). A set of 200 models was generated, from which the lowest energy structure was used for further processing. The initial low energy model obtained from Modeller was validated by using PROCHECK⁷³ and WHAT IF⁷⁴ servers. We have also analyzed the side chain orientation of conserved residues (which are conserved at least in all class-A GPCR sequences), by superposing our model against the available crystal structures (2RH1 and 3D4S) in order to analyze the goodness of fit with other structures. We focused mostly on the modeling of the transmembrane helical bundle.

Docking study

We carried out blind docking using AutoDock (version 4.0). Blind docking is often used assuming that the ligand binding site is unknown and the structures of the ligand and the receptor are known. In our case, it would be necessary to perform dockings to search the entire surface of the receptor. This was achieved using AutoGrid to create grid maps, with 40 points in each dimension, and creating sets of adjacent grid map volumes that cover the entire transmembrane region of the receptor. Rigid protein-flexible ligand docking of seven different ligands, including agonists and antagonists, was carried out with AutoDock. The agonists include serotonin (natural ligand) and 8-OH-DPAT, and antagonists are *p*-MPPF and WAY-100635. The AutoDock graphical interface AutoDockTools was used to keep polar hydrogen atoms and add partial charges for protein using the Gasteiger charges. The grid maps were calculated using the auxiliary program Autogrid 4.0. Blind docking was performed using a grid box of 40 × 40 × 40 cells (each cell has dimensions 0.375 × 0.375 × 0.375 Å) by moving it around to cover the entire transmembrane region. The Lamarckian genetic algorithm (LGA) was selected for ligand conformational searching. Docking was carried out with a population size of 150 with 25 × 10⁵ evaluations and a maximum of 27000 generations. Conformations were analyzed using the MGL AutoDock tools (version 1.5.2). Chemical structures of ligands were obtained from PubChem and three dimensional structures of ligands were generated using Open Babel (version 2.2.3).

Molecular dynamics simulations of receptor models

Molecular dynamics simulations were performed to validate homology models of the serotonin_{1A} receptor in the absence and presence of cholesterol. Systems were represented using the MARTINI coarse-grain force-field (version 2.1).^{75,76} Models were mapped to their coarse-grain representations. The receptor model in the absence of cholesterol was simulated in a pure POPC membrane containing 200 lipid molecules and the receptor model in the presence of cholesterol was simulated in a POPC-cholesterol mixed bilayer containing 80 cholesterol and 80 POPC molecules. Simulations were performed using the GROMACS program package (version 4.0.2),⁷⁷ with the scheme developed for coarse-grain simulations, under periodic boundary conditions. Temperature was weakly coupled (coupling time 0.1 ps) to a thermostat at 300 K using the Berendsen

algorithm.⁷⁸ Pressure was weakly coupled (coupling time 1.0 ps, compressibility 5 × 10⁻⁵ bar) using a semi-isotropic coupling scheme, in which the lateral and perpendicular pressures are coupled independently at 1 bar. Non-bonded interactions were treated with a switch function from 0–1.2 nm for Coulomb interactions and 0.9–1.2 nm for LJ interactions (pair-list update frequency of once per 10 steps). A time step of 25 fs was used.

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