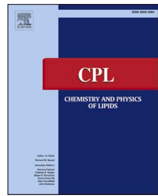
Chemistry and Physics of Lipids 239 (2021) 105120

Contents lists available at ScienceDirect

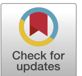
Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip



Perspectives

Cholesterol footprint in high-resolution structures of serotonin receptors: Where are we now and what does it mean?



Parijat Sarkar, Amitabha Chattopadhyay *

CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, 500 007, India

ARTICLE INFO

Keywords:
Serotonin receptors
Serotonin$_{1A}$ receptor
Crystal structure
cryo-EM
Cholesterol binding site(s)
Cholesterol sensitivity

ABSTRACT

An emerging feature of several high-resolution GPCR structures is the presence of closely bound cholesterol molecules. In this Perspective, we share the excitement of the recent advancements in GPCR structural biology. We further highlight our laboratory's journey in comprehensively elucidating functional sensitivity of GPCRs (using the serotonin$_{1A}$ receptor as a representative neurotransmitter GPCR) to membrane cholesterol and validation using a variety of assays and molecular dynamics simulations. Although high-resolution structures of many GPCRs have been reported in the last few years, the structure of the serotoin$_{1A}$ receptor proved to be elusive for a long time. Very recently the cryo-EM structure of the serotoin$_{1A}$ receptor displaying 10 bound cholesterol molecules has been reported. We conclude by providing a critical analysis of caveats involved in GPCR structure determination.

Serotonin (5-hydroxytryptamine, 5-HT) receptors are a large family of receptors involved in regulating a diverse array of physiological signaling pathways in organisms that span a wide evolutionary range. Except the members of the serotonin$_3$ receptor subfamily which act as ligand-gated ion channels, all other serotonin receptors belong to the family of seven transmembrane domain G protein-coupled receptors (GPCRs) (Nichols and Nichols, 2008; Sarkar et al., 2018, 2021; Barnes et al., 2021). Serotonin receptors constitute the largest class of non-odorant GPCRs (Nichols and Nichols, 2008) and predate the emergence of other biogenic amine receptors (Peroutka and Howell, 1994). Among the members of serotonin receptors belonging to the GPCR superfamily, the serotonin$_{1A}$ receptor occupies a central position due to a number of reasons (Pucadyil et al., 2005a; Lacivita et al., 2008; Fiorino et al., 2014; Sarkar et al., 2018). For example, it was the first serotonin receptor to be cloned, sequenced and antibodies were raised against it (Fargin et al., 1988). Another factor that helped is the early availability of a selective agonist, 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT), that allowed biochemical, physiological and pharmacological analysis of the serotonin$_{1A}$ receptor (Gozlan et al., 1983). Importantly, the serotonin$_{1A}$ receptor also represents a popular drug target (Lacivita et al., 2008; Fiorino et al., 2014).

In the late 1990′s, one of us (A.C.) became interested in lipid interactions of GPCRs. Till then, there were very few reports on this topic in the literature. However, it was well known by that time that integral membrane proteins such as Ca^{2+}/Mg^{2+}-ATPase (London and Feigenson, 1981; Simmonds et al., 1982) and ion channels such as the nicotinic acetylcholine receptor (Criado et al., 1984; Fong and McNamee, 1986) exhibit lipid-dependent function due to lipid-protein interactions. The GPCR of interest was a recently 'deorphanized' GPCR (Fargin et al., 1988), namely, the serotonin$_{1A}$ receptor. As mentioned above, this receptor attained appreciable popularity in a few years after its discovery due to its key role in serotonergic signaling, anxiety and depression, and as a drug target (Müller et al., 2007; Lacivita et al., 2008; Fiorino et al., 2014). For example, knockout mice lacking the serotonin$_{1A}$ receptor and displaying increased anxiety-related behavior were simultaneously generated by several groups and the resulting publications appeared in the same year, even in the same journal (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Importantly, these results showed how a single gene knockout could alter complex behavior and offered relevant animal models for conditions such as anxiety disorders and aggression (Gingrich and Hen, 2001). The choice of this receptor therefore appeared to be appropriate from a functional perspective.

Although the serotonin$_{1A}$ receptor enjoyed a functional advantage, literature on its structure and lipid interaction was missing at this point (the first GPCR structure, that of rhodopsin, appeared in 2000 (Palczewski et al., 2000)). Our initial hunch of exploring cholesterol sensitivity of the serotonin$_{1A}$ receptor had its origin in two (unconnected at that point) facts: (i) localization of the serotonin$_{1A}$ receptor in several

* Corresponding author.
E-mail address: amit@ccmb.res.in (A. Chattopadhyay).

https://doi.org/10.1016/j.chemphyslip.2021.105120
Received 1 June 2021; Received in revised form 24 July 2021; Accepted 24 July 2021
Available online 29 July 2021
0009-3084/© 2021 Elsevier B.V. All rights reserved.

regions of the brain detected by mRNA (Kobilka et al., 1987; Albert et al., 1990; Chalmers and Watson, 1991), and (ii) the important physiological role of brain cholesterol (Dietschy and Turley, 2001; Chattopadhyay and Paila, 2007). Our early experiments showed that depletion of cholesterol from membrane preparations from hippocampal tissue, an excellent source of the serotonin$_{1A}$ receptor, using methyl-β-cyclodextrin (MβCD), a soluble sterol carrier that can be effectively used to physically deplete membrane cholesterol (Zidovetzki and Levitan, 2007; Mahammad and Parmryd, 2015; Jafurulla et al., 2019), resulted in a concentration-dependent loss in ligand binding and G-protein coupling of the serotonin$_{1A}$ receptor (Pucadyil and Chattopadhyay, 2004, 2005). The change in ligand binding could be reversed upon replenishment of cholesterol using MβCD-cholesterol complex, thereby implying that the changes were indeed due to cholesterol depletion. Our initial hypothesis was that membrane cholesterol at certain sites in the receptor was instrumental in maintaining an active conformation (capable of ligand binding). To validate that this was indeed the underlying mechanism behind cholesterol-dependent activity of the serotonin$_{1A}$ receptor, in the next step, we carried out a series of experiments to test this hypothesis. These included experiments using (i) cholesterol oxidase which oxidizes cholesterol to cholestenone (Pucadyil et al., 2005b), (ii) sterol-complexing agents such as nystatin (Pucadyil et al., 2004) and sterol-binding detergents like digitonin (Paila et al., 2005), and (iii) eventually metabolic inhibition of cholesterol biosynthesis using proximal and distal inhibitors (Paila et al., 2008; Shrivastava et al., 2010). The common underlying theme behind all these experiments was to prevent receptor-cholesterol interaction by oxidizing, sequestering and binding of cholesterol, or inhibiting its biosynthesis (Pucadyil and Chattopadhyay, 2006; Paila and Chattopadhyay, 2010). Since all these diverse treatments resulted in loss of receptor activity, we concluded that membrane cholesterol is necessary for the function of the serotonin$_{1A}$ receptor, irrespective of the actual experimental strategy by which cholesterol-receptor interaction was prevented. Another group around this time reported that cholesterol depletion using MβCD lead to attenuation of signaling of the serotonin$_{1A}$ receptor (Sjögren et al., 2008).

In general, lipid sensitivity of membrane proteins and receptors is believed to originate either due to direct interaction of cholesterol with the membrane protein (specific effect) or due to lipid-induced modulations of membrane physical properties (general effect) (Lee, 2005; Paila and Chattopadhyay, 2009). Our interpretation of the above results made us propose that the cholesterol sensitivity of the serotonin$_{1A}$ receptor was due to closely interacting cholesterol molecules (Paila et al., 2009). How does one 'see' such closely located cholesterol molecules in the receptor structure? This became possible with the availability of increasingly high-resolution crystal structures (subsequently, cryo-EM structures) of GPCRs. Although individual lipid molecules could be observed in high-resolution crystal structures of membrane proteins since late 1990′s (Luecke et al., 1999; Gonen et al., 2005), generating high-resolution structures of GPCRs turned out to be more challenging. This is due to the inherent conformational plasticity (flexibility) exhibited by GPCRs. Whereas this structural plasticity is vital for the functional diversity exhibited by GPCRs, it poses considerable challenge for structural biology of GPCRs. A number of approaches have been utilized to tackle this problem (Ghosh et al., 2015). These include stabilizing the flexible regions of GPCRs using monoclonal antibody (Day et al., 2007), replacing the dynamic third intracellular loop with lysozyme (Cherezov et al., 2007; Rosenbaum et al., 2007) or more recently, a nanobody (Manglik et al., 2017). With these advancements, more high-resolution structures of GPCRs started emerging from 2007 onward (Cherezov et al., 2007; Rosenbaum et al., 2007; Hanson et al., 2008). A novel feature of many of these GPCR structures was the presence of closely bound cholesterol molecules (Jafurulla and Chattopadhyay, 2013; Chattopadhyay, 2014). For example, structures of the β_2-adrenergic receptor (Cherezov et al., 2007; Hanson et al., 2008), A_{2A} adenosine receptor (Liu et al., 2012) and metabotropic glutamate receptor 1 (Wu et al., 2014) displayed bound cholesterol molecules. These observations gave rise to the question: how important are these bound cholesterol molecules for cholesterol-sensitive function of these GPCRs? As we write this Perspective, the list of GPCRs displaying bound cholesterol molecules has gone up significantly (~40 % of published GPCR structures display bound cholesterol molecule(s) (Sarkar and Chattopadhyay, unpublished observations)).

In spite of the fact that the serotonin$_{1A}$ receptor was one of the first GPCRs whose function was shown to be modulated by cholesterol (Pucadyil and Chattopadhyay, 2004, 2005), the structure of the serotonin$_{1A}$ receptor became available only recently (Xu et al., 2021). Interestingly, the high-resolution cryo-EM structure of the *apo*-form of the serotonin$_{1A}$ receptor shows the maximum number of bound cholesterol molecules (10 per receptor monomer) in its structure (see Fig. 1). The other structures reported in this paper include the serotonin-bound and aripiprazole-bound forms with 4 and 3 molecules of bound cholesterol, respectively. Whereas these are interesting observations, the biological relevance of this diversity in bound cholesterol molecules is not apparent at this point. Importantly, in a recent work from our laboratory (Kumar et al., 2021), we showed using all-atom molecular dynamics simulations that a cholesterol molecule is found near a cholesterol recognition/interaction amino acid consensus (CRAC) motif (see later) in a position almost identical to the one reported in the cryo-EM structure of the serotonin$_{1A}$ receptor (PDB ID: 7E2X). It is envisioned that with more reports of well-resolved GPCR structures with bound cholesterol molecule(s), accompanied with more information on their function, could help generate certain pattern in this large, diverse and emerging structural database.

Although the structure of the serotonin$_{1A}$ receptor took a relatively long time to be solved, high-resolution structures of other members of the serotonin family of GPCRs have emerged since 2013. At present, there are 25 solved structures of 7 serotonin receptor subtypes (belonging to the serotonin GPCR family) are reported. Table 1 shows a

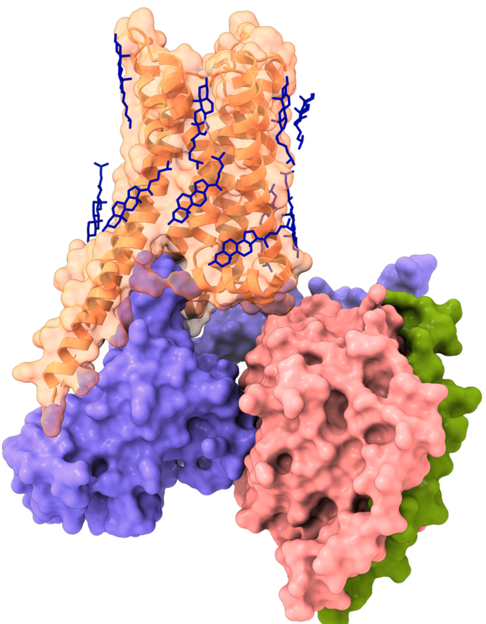


Fig. 1. Bound cholesterol molecules in the crystal structure of the human serotonin$_{1A}$ receptor. Model of the *apo*-serotonin$_{1A}$ receptor-G_i complex showing multiple (a total of 10) cholesterol molecules (represented as blue sticks) bound to the surface of the serotonin$_{1A}$ receptor (represented as orange semi-transparent surface). The G_i complex is shown as surface ($G\alpha_{i1}$ in light blue, $G\beta_1$ in pink and $G\gamma_2$ in green). The snapshot of cholesterol-bound structure of the serotonin$_{1A}$ receptor was generated from its cryo-EM structure (Xu et al., 2021) (PDB: 7E2X) using UCSF ChimeraX (https://www.rbvi.ucsf.edu/chimerax/) (Pettersen et al., 2021).

Table 1
Current Available Structures of Serotonin Receptors[a].

Receptor	#[b]	Method	PDB	Resolution (Å)	Bound ligand/effector	Missing structural region(s)	Refs.
		cryo-EM	7E2X	3.0	(apo)/$G\alpha_{i1}\beta_1\gamma_2$	N	Xu et al., 2021
Serotonin$_{1A}$	3	cryo-EM	7E2Y	3.0	Serotonin/$G\alpha_{i1}\beta_1\gamma_2$	N	Xu et al., 2021
		cryo-EM	7E2Z	3.1	Aripiprazole/$G\alpha_{i1}\beta_1\gamma_2$	N	Xu et al., 2021
		x-ray	4IAQ	2.8	Dihydroergotamine	N, ICL3	Wang et al., 2013
		x-ray	4IAR	2.7	Ergotamine	N, ICL3	Wang et al., 2013
Serotonin$_{1B}$	5	x-ray	5V54	3.9	Methiothepin	N, ICL3	Yin et al., 2018
		cryo-EM	6G79	3.78	Donitriptan/$G_{o1}\beta_1\gamma_2$	N	García-Nafría et al., 2018
		x-ray	7C61	3.0	Ergotamine	N, ICL3	Miyagi et al., 2020
Serotonin$_{1D}$	1	cryo-EM	7E32	2.9	Serotonin/$G\alpha_{i1}\beta_1\gamma_2$	N, ICL3	Xu et al., 2021
Serotonin$_{1E}$	1	cryo-EM	7E33	2.9	BRL-54443/$G\alpha_{i1}\beta_1\gamma_2$	N, ICL3	Xu et al., 2021
		x-ray	6A93	3.0	Risperidone	N, C, ICL3	Kimura et al., 2019
		x-ray	6A94	2.9	Zotepine	N, C, ICL3	Kimura et al., 2019
Serotonin$_{2A}$	5	x-ray	6WGT	3.4	LSD	N, C, ICL3	Kim et al., 2020
		x-ray	6WH4	3.4	Methiothepin	N, C, ICL3	Kim et al., 2020
		cryo-EM	6WHA	3.36	25-CN-NBOH/$G\alpha_q\beta_1\gamma_2$	N, C	Kim et al., 2020
		x-ray	4IB4	2.7	Ergotamine	N, C, ICL3	Wacker et al., 2013
		x-ray	4NC3	2.8	Ergotamine	N, C, ICL3	Liu et al., 2013
		x-ray	5TUD	3.0	Ergotamine	N, C, ICL3	Ishchenko et al., 2017
Serotonin$_{2B}$	8	x-ray	5TVN	2.9	LSD	N, C, ICL3	Wacker et al., 2017
		x-ray	6DRX	3.1	Lisuride	N, C, ICL3	McCorvy et al., 2018
		x-ray	6DRY	2.92	Methylergonovine	N, C, ICL3	McCorvy et al., 2018
		x-ray	6DRZ	3.1	Methysergide	N, C, ICL3	McCorvy et al., 2018
		x-ray	6DS0	3.19	LY266097	N, C, ICL3	McCorvy et al., 2018
Serotonin$_{2C}$	2	x-ray	6BQG	3.0	Ergotamine	N, C, ICL3	Peng et al., 2018
		x-ray	6BQH	2.7	Ritanserin	N, C, ICL3	Peng et al., 2018

[a] Abbreviations: N, N-terminus; C, C-terminus; ICL3, intracellular loop 3; cryo-EM, cryo-electron microscopy; x-ray, x-ray diffraction; BRL-54443, 5-hydroxy-3-(1-methylpiperidin-4-yl)-1*H*-indole; LSD, lysergic acid diethylamide; 25-CN-NBOH, 2-([2-(4-cyano-2,5-dimethoxyphenyl)ethylamino]methyl)phenol; LY266097, 1-[(2-Chloro-3,4-dimethoxyphenyl)methyl]-2,3,4,9-tetrahydro-6-methyl-1*H*-pyrido[3,4-*b*]indole hydrochloride.
[b] Number of available structures.

detailed breakdown of this data. Table 2 shows available serotonin receptor structures with bound cholesterol molecule(s) as representative snapshots. The table shows that the number of bound cholesterol molecules vary between 1 (serotonin$_{1D}$ and serotonin$_{2B}$ receptors) and 10 (serotonin$_{1A}$ receptor, the *apo*-form). As individual GPCRs are characterized with multiple structures (bound to various ligands, depending on their functional state), we observe multiple available cholesterol-bound structures for serotonin$_{1A}$, serotonin$_{2A}$ and serotonin$_{2B}$ receptors, with the serotonin$_{2B}$ receptor displaying maximum number (a total of 7) structures.

Lipid molecules which are co-crystallized with membrane proteins are often localized in protein-protein interfaces in oligomeric proteins and have been termed as 'nonannular' lipids (Lee, 2003). This prompted us to speculate that bound cholesterol molecules in GPCR structures could be occupying nonannular sites (Paila et al., 2009). Nonannular lipid sites typically display lack of accessibility to the annular lipids, *i.e.*, these sites cannot be displaced in a competition with annular lipids. Subsequently, coarse-grain molecular dynamics simulations showed that cholesterol binding sites in GPCRs could be located deep in the hydrophobic interior of the receptor (Genheden et al., 2017). If GPCRs show bound cholesterol in their structures, what are the specific sites where cholesterol gets bound? This question has been around even before the arrival of a relatively large number of high-resolution GPCR structures. One of the first GPCR structure that addressed this issue was of the human β_2-adrenergic receptor (Hanson et al., 2008). In this work, two cholesterol molecules were observed in a specific cholesterol binding site, defined as the *strict* cholesterol consensus motif (CCM), formed by transmembrane helices 1-4 (TM1–4) which consists of four critical amino acids in two transmembrane helices (TM2 and 4) (Hanson et al., 2008). On the other hand, CRAC motifs were discovered much before as a linear sequence motif implicated in the interaction of cholesterol with membrane proteins such as the peripheral-type benzodiazepine receptor (Li and Papadopoulos, 1998) and caveolin-1 (Epand et al., 2005; Epand, 2006). The CRAC motif consists of a linear sequence of amino acids from the N-terminal to C-terminal direction. The sequence of amino acids follows the order: a branched nonpolar leucine (or valine), followed by 1–5 amino acids (no preference), an aromatic tyrosine residue, another segment of 1–5 amino acids (no preference), and lastly, a basic lysine (or arginine) residue $[(L/V)\text{-}(X)_{1-5}\text{-}Y\text{-}(X)_{1-5}\text{-}(R/K)]$. The concept of CRAC motifs was further refined by invoking another type of CRAC motif, termed the CARC motif, that is oriented in an opposite direction of the polypeptide chain (Baier et al., 2011; Fantini and Barrantes, 2013; Fantini et al., 2016, 2019; Jafurulla et al., 2019). We reported the presence of CRAC motifs in several GPCRs (the serotonin$_{1A}$ receptor, the β_2-adrenergic receptor and rhodopsin) that exhibit cholesterol-sensitive function (Jafurulla et al., 2011). In case of the serotonin$_{1A}$ receptor, our analysis showed the presence of CRAC motifs in TM2, TM5 and TM7. Subsequently, presence of CRAC motif was reported for type-1 cannabinoid (CB_1) receptor (Oddi et al., 2011). Interestingly, coarse-grain molecular dynamics simulations of the serotonin$_{1A}$ receptor showed preferential (dynamic) occupancy of membrane cholesterol in some of the CRAC sites in the serotonin$_{1A}$ receptor (Sengupta and Chattopadhyay, 2012). For a comprehensive account of cholesterol binding motifs in GPCRs, see Sarkar and Chattopadhyay, 2020.

To provide mechanistic insights into cholesterol sensitivity for the serotonin$_{1A}$ receptor, we recently examined the molecular basis of cholesterol sensitivity of the receptor function by mutating various key residues in the CRAC motifs of the receptor and monitoring corresponding functional readout (cAMP signaling) (Kumar et al., 2021). These results were complemented by all-atom molecular dynamics simulations. The take-home of our work is that the functional sensitivity of the serotonin$_{1A}$ receptor to membrane cholesterol is lost when the residue K101 in a CRAC motif in TM2 is mutated, indicating the role of K101 as a molecular sensor of membrane cholesterol. To the best of our knowledge, our results constitute one of the first reports that comprehensively demonstrated that cholesterol sensitivity could be knocked out by a single point mutation in a specific cholesterol binding site. We believe that future work on cholesterol-sensitive GPCR function using a combination of experimental (such as site-directed mutagenesis followed by measurement of cellular signaling) and computational approaches could be helpful in identifying structural features in receptor

Table 2
Serotonin Receptor Structures with Bound Cholesterol[a].

Receptor	PDB	Snapshot[b]	#Chol[c]	Refs.	Refs. for cholesterol-sensitive function
$Serotonin_{1A}$	7E2X		10	Xu et al., 2021	Pucadyil and Chattopadhyay, 2004, 2005, 2007 Pucadyil et al., 2005b Paila et al., 2008 Sjögren et al., 2008 Shrivastava et al., 2010 Gutierrez et al., 2016 Jafurulla et al., 2017 Kumar and Chattopadhyay, 2020, 2021 Sarkar et al., 2020
	7E2Y		4		
	7E2Z		3		
$Serotonin_{1D}$	7E32		1	Xu et al., 2021	Not available
$Serotonin_{2A}$	6A93 6A94		2 2 (1/monomer)	Kimura et al., 2019	Sommer et al., 2009 Ludka et al., 2014
	6WGT		2	Kim et al., 2020	
	6WH4		2 (1/monomer)		
$Serotonin_{2B}$	4IB4		1	Wacker et al., 2013	Not available
	4NC3		1	Liu et al., 2013	
	5TVN		1	Wacker et al., 2017	
	6DRX 6DRY 6DRZ 6DS0		1 1 1 1	McCorvy et al., 2018	

[a] Data generated by searching the PDB database for serotonin receptor structures with cholesterol as a small molecule ligand.
[b] Snapshots of cholesterol-bound structure of serotonin receptors (cholesterol in orange and receptor in blue) were generated using UCSF ChimeraX (Pettersen et al., 2021).
[c] Number of cholesterol molecules per PDB structure.

that are responsible for cholesterol sensitivity.

These recent developments on the presence of closely bound cholesterol molecules in high-resolution GPCR structures are exciting, yet its biological relevance is still emerging (Chattopadhyay, 2014). A major reason for this is that data on cholesterol-sensitivity of many GPCRs are simply not available yet (*e.g.*, see Table 2 for cholesterol-sensitive members of the serotonin receptor family). In addition, there are certain factors involving heavy protein engineering to aid GPCR crystallization needs to be sorted out in this context (Ghosh et al., 2015). In spite of the fact that the extramembranous regions (loops) of GPCRs are critical in GPCR function and signaling (Turner et al., 2004; Wheatley et al., 2012; Pal and Chattopadhyay, 2019; Kharche et al., 2021), the flexible loops corresponding to these regions are usually stabilized using monoclonal antibody (Day et al., 2007), or replaced with lysozyme (Cherezov et al., 2007; Rosenbaum et al., 2007), or a nanobody (Manglik et al., 2017) in the available structures. Additionally, structure determination of GPCRs is commonly carried out using a heavily engineered (mutated for thermal stability) receptor. Further, structure determinations are often carried out in detergent dispersions (micelles) or lipidic cubic phases (not in membrane bilayers). Although micelles could mimic many properties of lipid bilayers, the conformations of GPCRs in the micellar environment could differ from that in the membrane bilayer due to intrinsic difference in the radius of curvature (the micellar surface has a much larger curvature than the bilayer) (Mukherjee and Chattopadhyay, 1994). In addition, the membrane interface (a crucial part of the bilayer in terms of membrane organization, dynamics and function (Haldar et al., 2011; Pal and Chattopadhyay, 2017)) differs from the micellar interface which is much

thinner. On the other hand, although lipidic cubic phase membranes are popular for GPCR crystallization (Caffrey, 2015), the physiological significance of bound cholesterol molecules in GPCR crystal structures in lipidic cubic phases is still emerging (Khelashvili et al., 2012). It is possible that the bound cholesterol molecules observed in GPCRs in lipidic cubic phases could represent aspects of membrane lipid environment specifically in lipidic cubic phases (different from lamellar bilayer phases). Another caveat is that GPCR structures are often determined by crystallization (or cryo-EM) in lipidic cubic phases (or micelles) containing cholesterol hemisuccinate (CHS), which is used to replace cholesterol in the receptor. A number of studies addressing the ability of CHS to mimic cholesterol (Kulig et al., 2014, 2015; Augustyn et al., 2019) suggests that it could depend on actual experimental conditions and it appears that the jury is still out on this topic.

Taken together, it appears that whereas bound cholesterol molecules in emerging high-resolution GPCR structures are indeed exciting, it would perhaps be prudent to exercise sufficient caution in extrapolating bound cholesterol in GPCR structures to their cholesterol-sensitive function. The road ahead could lie in mutating specific residues of GPCRs and actually monitoring their function in a cholesterol-dependent fashion. However, this does not rule out early pointers from bound cholesterol molecules in GPCRs as an indication of functionally relevant cholesterol molecules. In any event, a comprehensive understanding of cholesterol-sensitive function of GPCRs, using a judicious combination of experimental and computational approaches, could lead to a better understanding of GPCR function in health and disease, and better therapeutics since GPCRs represent major drug targets (Sriram and Insel, 2018; Chan et al., 2019). So far as the serotonin$_{1A}$ receptor is concerned, the recent availability of high-resolution structures (Table 2) displaying bound cholesterol molecules provides a novel platform to set up experiments addressing the role of cholesterol in its diverse cholesterol-sensitive function, including recently reported receptor endocytosis and trafficking (Kumar and Chattopadhyay, 2020, 2021). Viewed from this perspective, the next few years could be exciting in this research area.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

Work in A.C.'s laboratory was generously funded by Department of Biotechnology, Govt. of India, Council of Scientific and Industrial Research, Indo-French Centre for the Promotion of Advanced Research, Life Sciences Research Board (India), University of Melbourne (IRRTF grant), and Science & Engineering Research Board, Department of Science and Technology, Govt. of India. A.C. gratefully acknowledges SERB Distinguished Fellowship (SERB, DST, Govt. of India). P.S. was supported as a Senior Project Associate by a CSIR FBR grant to A.C. We thank members of the Chattopadhyay laboratory for critically reading the manuscript.

References

Albert, P.R., Zhou, Q.-Y., Van Tol, H.H.M., Bunzow, J.R., Civelli, O., 1990. Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine$_{1A}$ receptor gene. J. Biol. Chem. 265, 5825–5832.

Augustyn, B., Stepien, P., Poojari, C., Mobarak, E., Polit, A., Wisniewska-Becker, A., Róg, T., 2019. Cholesteryl hemisuccinate is not a good replacement for cholesterol in lipid nanodiscs. J. Phys. Chem. B 123, 9839–9845.

Baier, C.J., Fantini, J., Barrantes, F.J., 2011. Disclosure of cholesterol recognition motifs in transmembrane domains of the human nicotinic acetylcholine receptor. Sci. Rep. 1, 69.

Barnes, et al., 2021. International Union of Basic and Clinical Pharmacology. CX. Classification of receptors for 5-hydroxytryptamine; pharmacology and function. Pharmacol. Rev. 73, 310–520.

Caffrey, M., 2015. A comprehensive review of the lipid cubic phase or *in meso* method for crystallizing membrane and soluble proteins and complexes. Acta Crystallogr. F Struct. Biol. Commun. 71, 3–18.

Chalmers, D.T., Watson, S.J., 1991. Comparative anatomical distribution of 5-HT$_{1A}$ receptor mRNA and 5-HT$_{1A}$ binding in rat brain – a combined in situ hybridization/in vitro receptor autoradiographic study. Brain Res. 561, 51–60.

Chan, H.C.S., Li, Y., Dahoun, T., Vogel, H., Yuan, S., 2019. New binding sites, new opportunities for GPCR drug discovery. Trends Biochem. Sci. 44, 312–330.

Chattopadhyay, A., 2014. GPCRs: lipid-dependent membrane receptors that act as drug targets. Adv. Biol. 2014, 143023.

Chattopadhyay, A., Paila, Y.D., 2007. Lipid-protein interactions, regulation and dysfunction of brain cholesterol. Biochem. Biophys. Res. Commun. 354, 627–633.

Cherezov, V., Rosenbaum, D.M., Hanson, M.A., Rasmussen, S.G.F., Thian, F.S., Kobilka, T.S., Choi, H.-J., Kuhn, P., Weis, W.I., Kobilka, B.K., Stevens, R.C., 2007. High-resolution crystal structure of an engineered human β_2-adrenergic G protein-coupled receptor. Science 318, 1258–1265.

Criado, M., Eibl, H., Barrantes, F.J., 1984. Functional properties of the acetylcholine receptor incorporated in model lipid membranes. Differential effects of chain length and head group of phospholipids on receptor affinity states and receptor-mediated ion translocation. J. Biol. Chem. 259, 9188–9198.

Day, P.W., Rasmussen, S.G.F., Parnot, C., Fung, J.J., Masood, A., Kobilka, T.S., Yao, X.J., Choi, H.-J., Weis, W.I., Rohrer, D.K., Kobilka, B.K., 2007. A monoclonal antibody for G protein-coupled receptor crystallography. Nat. Methods 4, 927–929.

Dietschy, J.M., Turley, S.D., 2001. Cholesterol metabolism in the brain. Curr. Opin. Lipidol. 12, 105–112.

Epand, R.M., 2006. Cholesterol and the interaction of proteins with membrane domains. Prog. Lipid Res. 45, 279–294.

Epand, R.M., Sayer, B.G., Epand, R.F., 2005. Caveolin scaffolding region and cholesterol-rich domains in membranes. J. Mol. Biol. 345, 339–350.

Fantini, J., Barrantes, F.J., 2013. How cholesterol interacts with membrane proteins: an exploration of cholesterol-binding sites including CRAC, CARC, and tilted domains. Front. Physiol. 4, 31.

Fantini, J., Di Scala, C., Baier, C.J., Barrantes, F.J., 2016. Molecular mechanisms of protein-cholesterol interactions in plasma membranes: functional distinction between topological (tilted) and consensus (CARC/CRAC) domains. Chem. Phys. Lipids 199, 52–60.

Fantini, J., Epand, R.M., Barrantes, F.J., 2019. Cholesterol-recognition motifs in membrane proteins. Adv. Exp. Med. Biol. 1135, 3–25.

Fargin, A., Raymond, J.R., Lohse, M.J., Kobilka, B.K., Caron, M.G., Lefkowitz, R.J., 1988. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-HT$_{1A}$ receptor. Nature 335, 358–360.

Fiorino, F., Severino, B., Magli, E., Ciano, A., Caliendo, G., Santagada, V., Frecentese, F., Perissutti, E., 2014. 5-HT$_{1A}$ receptor: an old target as a new attractive tool in drug discovery from central nervous system to cancer. J. Med. Chem. 57, 4407–4426.

Fong, T.M., McNamee, M.G., 1986. Correlation between acetylcholine receptor function and structural properties of membranes. Biochemistry 25, 830–840.

García-Nafría, J., Nehmé, R., Edwards, P.C., Tate, C.G., 2018. Cryo-EM structure of the serotonin 5-HT$_{1B}$ receptor coupled to heterotrimeric G_o. Nature 558, 620–623.

Genheden, S., Essex, J.W., Lee, A.G., 2017. G protein coupled receptor interactions with cholesterol deep in the membrane. Biochim. Biophys. Acta 1859, 268–281.

Ghosh, E., Kumari, P., Jaiman, D., Shukla, A.K., 2015. Methodological advances: the unsung heroes of the GPCR structural revolution. Nat. Rev. Mol. Cell Biol. 16, 69–81.

Gingrich, J.A., Hen, R., 2001. Dissecting the role of the serotonin system in neuropsychiatric disorders using knockout mice. Psychopharmacology 155, 1–10.

Gonen, T., Cheng, Y., Sliz, P., Hiroaki, Y., Fujiyoshi, Y., Harrison, S.C., Walz, T., 2005. Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. Nature 438, 633–638.

Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski, J., Hamon, M., 1983. Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature 305, 140–142.

Gutierrez, M.G., Mansfield, K.S., Malmstadt, N., 2016. The functional activity of the human serotonin 5-HT$_{1A}$ receptor is controlled by lipid bilayer composition. Biophys. J. 110, 2486–2495.

Haldar, S., Chaudhuri, A., Chattopadhyay, A., 2011. Organization and dynamics of membrane probes and proteins utilizing the red edge excitation shift. J. Phys. Chem. B 115, 5693–5706.

Hanson, M.A., Cherezov, V., Griffith, M.T., Roth, C.B., Jaakola, V.-P., Chien, E.Y.T., Velasquez, J., Kuhn, P., Stevens, R.C., 2008. A specific cholesterol binding site is established by the 2.8 Å structure of the human β_2-adrenergic receptor. Structure 16, 897–905.

Heisler, L.K., Chu, H.-M., Brennan, T.J., Danao, J.A., Bajwa, P., Parsons, L.H., Tecott, L. H., 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT$_{1A}$ receptor mutant mice. Proc. Natl. Acad. Sci. U.S.A. 95, 15049–15054.

Ishchenko, A., Wacker, D., Kapoor, M., Zhang, A., Han, G.W., Basu, S., Patel, N., Messerschmidt, M., Weierstall, U., Liu, W., Katritch, V., Roth, B.L., Stevens, R.C., Cherezov, V., 2017. Structural insights into the extracellular recognition of the human serotonin 2B receptor by an antibody. Proc. Natl. Acad. Sci. U.S.A. 114, 8223–8228.

Jafurulla, M., Chattopadhyay, A., 2013. Membrane lipids in the function of serotonin and adrenergic receptors. Curr. Med. Chem. 20, 47–55.

Jafurulla, M., Tiwari, S., Chattopadhyay, A., 2011. Identification of cholesterol recognition amino acid consensus (CRAC) motif in G-protein coupled receptors. Biochem. Biophys. Res. Commun. 404, 569–573.

Jafurulla, M., Nalli, A., Chattopadhyay, A., 2017. Membrane cholesterol oxidation in live cells enhances the function of serotonin$_{1A}$ receptors. Chem. Phys. Lipids 203, 71–77.

Jafurulla, M., Kumar, G.A., Rao, B.D., Chattopadhyay, A., 2019. A critical analysis of molecular mechanisms underlying membrane cholesterol sensitivity of GPCRs. Adv. Exp. Med. Biol. 1115, 21–52.

Kharche, S., Joshi, M., Chattopadhyay, A., Sengupta, D., 2021. Conformational plasticity and dynamic interactions of the N-terminal domain of chemokine receptor CXCR1. PLoS Comput. Biol. 17, e1008593.

Khelashvili, G., Albornoz, P.B., Johner, N., Mondal, S., Caffrey, M., Weinstein, H., 2012. Why GPCRs behave differently in cubic and lamellar lipidic mesophases. J. Am. Chem. Soc. 134, 15858–15868.

Kim, K., Che, T., Panova, O., DiBerto, J.F., Lyu, J., Krumm, B.E., Wacker, D., Robertson, M.J., Seven, A.B., Nichols, D.E., Shoichet, B.K., Skiniotis, G., Roth, B.L., 2020. Structure of a hallucinogen-activated Gq-coupled 5-HT_{2A} serotonin receptor. Cell 182, 1574–1588.

Kimura, K.T., Asada, H., Inoue, A., Kadji, F.M.N., Im, D., Mori, C., Arakawa, T., Hirata, K., Nomura, Y., Nomura, N., Aoki, J., Iwata, S., Shimamura, T., 2019. Structures of the 5-HT_{2A} receptor in complex with the antipsychotics risperidone and zotepine. Nat. Struct. Mol. Biol. 26, 121–128.

Kobilka, B.K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T.S., Francke, U., Lefkowitz, R.J., Caron, M.G., 1987. An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. Nature 329, 75–79.

Kulig, W., Tynkkynen, J., Javanainen, M., Manna, M., Rog, T., Vattulainen, I., Jungwirth, P., 2014. How well does cholesteryl hemisuccinate mimic cholesterol in saturated phospholipid bilayers? J. Mol. Model. 20, 2121.

Kulig, W., Jurkiewicz, P., Olzyńska, A., Tynkkynen, J., Javanainen, M., Manna, M., Rog, T., Hof, M., Vattulainen, I., Jungwirth, P., 2015. Experimental determination and computational interpretation of biophysical properties of lipid bilayers enriched by cholesteryl hemisuccinate. Biochim. Biophys. Acta 1848, 422–432.

Kumar, G.A., Chattopadhyay, A., 2020. Statin-induced chronic cholesterol depletion switches GPCR endocytosis and trafficking: insights from the $serotonin_{1A}$ receptor. ACS Chem. Neurosci. 11, 453–465.

Kumar, G.A., Chattopadhyay, A., 2021. Membrane cholesterol regulates endocytosis and trafficking of the $serotonin_{1A}$ receptor: insights from acute cholesterol depletion. Biochim. Biophys. Acta 1866, 158882.

Kumar, G.A., Sarkar, P., Stepniewski, T.M., Jafurulla, M., Singh, S.P., Selent, J., Chattopadhyay, A., 2021. A molecular sensor for cholesterol in the human $serotonin_{1A}$receptor. Sci. Adv. 7, eabh2922.

Lacivita, E., Leopoldo, M., Berardi, F., Perrone, R., 2008. 5-HT_{1A} receptor, an old target for new therapeutic agents. Curr. Top. Med. Chem. 8, 1024–1034.

Lee, A.G., 2003. Lipid-protein interactions in biological membranes: a structural perspective. Biochim. Biophys. Acta 1612, 1–40.

Lee, A.G., 2005. How lipids affect the activities of integral membrane proteins. Biochim. Biophys. Acta 1666, 62–87.

Li, H., Papadopoulos, V., 1998. Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern. Endocrinology 139, 4991–4997.

Liu, W., Chun, E., Thompson, A.A., Chubukov, P., Xu, F., Katritch, V., Han, G.W., Roth, C. B., Heitman, L.H., IJzerman, A.P., Cherezov, V., Stevens, R.C., 2012. Structural basis for allosteric regulation of GPCRs by sodium ions. Science 337, 232–236.

Liu, et al., 2013. Serial femtosecond crystallography of G protein-coupled receptors. Science 342, 1521–1524.

London, E., Feigenson, G.W., 1981. Fluorescence quenching in model membranes. 2. Determination of local lipid environment of the calcium adenosine triphosphatase from sarcoplasmic reticulum. Biochemistry 20, 1939–1948.

Ludka, F.K., Constantino, L.C., Kuminek, G., Binder, L.B., Zomkowski, A.D.E., Cunha, M. P., Dal-Cim, T., Rodrigues, A.L., Tasca, C.I., 2014. Atorvastatin evokes a serotonergic system-dependent antidepressant-like effect in mice. Pharmacol. Biochem. Behav. 122, 253–260.

Luecke, H., Schobert, B., Richter, H.-T., Cartailler, J.-P., Lanyi, J.K., 1999. Structure of bacteriorhodopsin at 1.55 Å resolution. J. Mol. Biol. 291, 899–911.

Mahammad, S., Parmryd, I., 2015. Cholesterol depletion using methyl-β-cyclodextrin. Methods Mol. Biol. 1232, 91–102.

Manglik, A., Kobilka, B.K., Steyaert, J., 2017. Nanobodies to study G protein-coupled receptor structure and function. Annu. Rev. Pharmacol. Toxicol. 57, 19–37.

McCorvy, J.D., Wacker, D., Wang, S., Agegnehu, B., Liu, J., Lansu, K., Tribo, A.R., Olsen, R.H.J., Che, T., Jin, J., Roth, B.L., 2018. Structural determinants of 5-HT_{2B} receptor activation and biased agonism. Nat. Struct. Mol. Biol. 25, 787–796.

Miyagi, H., Asada, H., Suzuki, M., Takahashi, Y., Yasunaga, M., Suno, C., Iwata, S., Saito, J.-I., 2020. The discovery of a new antibody for BRIL-fused GPCR structure determination. Sci. Rep. 10, 11669.

Mukherjee, S., Chattopadhyay, A., 1994. Motionally restricted tryptophan environments at the peptide lipid interface of gramicidin channels. Biochemistry 33, 5089–5097.

Müller, C.P., Carey, R.J., Huston, J.P., De Souza Silva, M.A., 2007. Serotonin and psychostimulant addiction: focus on 5-HT_{1A}-receptors. Prog. Neurobiol. 81, 133–178.

Nichols, D.E., Nichols, C.D., 2008. Serotonin receptors. Chem. Rev. 108, 1614–1641.

Oddi, S., Dainese, E., Fezza, F., Lanuti, M., Barcaroli, D., De Laurenzi, V., Centonze, D., Maccarrone, M., 2011. Functional characterization of putative cholesterol binding sequence (CRAC) in human type-1 cannabinoid receptor. J. Neurochem. 116, 858–865.

Paila, Y.D., Chattopadhyay, A., 2009. The function of G-protein coupled receptors and membrane cholesterol: specific or general interaction? Glycoconj. J. 26, 711–720.

Paila, Y.D., Chattopadhyay, A., 2010. Membrane cholesterol in the function and organization of G-protein coupled receptors. Subcell. Biochem. 51, 439–466.

Paila, Y.D., Pucadyil, T.J., Chattopadhyay, A., 2005. The cholesterol-complexing agent digitonin modulates ligand binding of the bovine hippocampal $serotonin_{1A}$ receptor. Mol. Membr. Biol. 22, 241–249.

Paila, Y.D., Murty, M.R.V.S., Vairamani, M., Chattopadhyay, A., 2008. Signaling by the human $serotonin_{1A}$ receptor is impaired in cellular model of Smith-Lemli-Opitz syndrome. Biochim. Biophys. Acta 1778, 1508–1516.

Paila, Y.D., Tiwari, S., Chattopadhyay, A., 2009. Are specific nonannular cholesterol binding sites present in G-protein coupled receptors? Biochim. Biophys. Acta 1788, 295–302.

Pal, S., Chattopadhyay, A., 2017. What is so unique about biomembrane organization and dynamics? In: Chattopadhyay, A. (Ed.), Membrane Organization and Dynamics. Springer, Heidelberg, pp. 1–9.

Pal, S., Chattopadhyay, A., 2019. Extramembranous regions in G protein-coupled receptors: cinderella in receptor biology? J. Membr. Biol. 252, 483–497.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M., Miyano, M., 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289, 739–745.

Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T., Tóth, M., 1998. Increased anxiety of mice lacking the $serotonin_{1A}$ receptor. Proc. Natl. Acad. Sci. U.S.A. 95, 10734–10739.

Peng, et al., 2018. 5-HT_{2C} receptor structures reveal the structural basis of GPCR polypharmacology. Cell 172, 719–730.

Peroutka, S.J., Howell, T.A., 1994. The molecular evolution of G protein-coupled receptors: focus on 5-hydroxytryptamine receptors. Neuropharmacology 33, 319–324.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Meng, E.C., Couch, G.S., Croll, T.I., Morris, J. H., Ferrin, T.E., 2021. UCSF ChimeraX: structure visualization for researchers, educators, and developers. Protein Sci. 30, 70–82.

Pucadyil, T.J., Chattopadhyay, A., 2004. Cholesterol modulates ligand binding and G-protein coupling to $serotonin_{1A}$ receptors from bovine hippocampus. Biochim. Biophys. Acta 1663, 188–200.

Pucadyil, T.J., Chattopadhyay, A., 2005. Cholesterol modulates the antagonist-binding function of hippocampal $serotonin_{1A}$ receptors. Biochim. Biophys. Acta 1714, 35–42.

Pucadyil, T.J., Chattopadhyay, A., 2006. Role of cholesterol in the function and organization of G-protein coupled receptors. Prog. Lipid Res. 45, 295–333.

Pucadyil, T.J., Chattopadhyay, A., 2007. Cholesterol depletion induces dynamic confinement of the G-protein coupled $serotonin_{1A}$ receptor in the plasma membrane of living cells. Biochim. Biophys. Acta 1768, 655–668.

Pucadyil, T.J., Shrivastava, S., Chattopadhyay, A., 2004. The sterol-binding antibiotic nystatin differentially modulates agonist and antagonist binding of the bovine hippocampal $serotonin_{1A}$ receptor. Biochem. Biophys. Res. Commun. 320, 557–562.

Pucadyil, T.J., Kalipatnapu, S., Chattopadhyay, A., 2005a. The $serotonin_{1A}$ receptor: a representative member of the serotonin receptor family. Cell. Mol. Neurobiol. 25, 553–580.

Pucadyil, T.J., Shrivastava, S., Chattopadhyay, A., 2005b. Membrane cholesterol oxidation inhibits ligand binding function of hippocampal $serotonin_{1A}$ receptors. Biochem. Biophys. Res. Commun. 331, 422–427.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R., 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc. Natl. Acad. Sci. U.S.A. 95, 14476–14481.

Rosenbaum, D.M., Cherezov, V., Hanson, M.A., Rasmussen, S.G., Thian, F.S., Kobilka, T. S., Choi, H.J., Yao, X.J., Weis, W.I., Stevens, R.C., Kobilka, B.K., 2007. GPCR engineering yields high-resolution structural insights into β_2-adrenergic receptor function. Science 318, 1266–1273.

Sarkar, P., Chattopadhyay, A., 2020. Cholesterol interaction motifs in G protein-coupled receptors: slippery hot spots? Wiley Interdiscip. Rev. Syst. Biol. Med. 12, e1481.

Sarkar, P., Kumar, G.A., Pal, S., Chattopadhyay, A., 2018. Biophysics of serotonin and the $serotonin_{1A}$ receptor: fluorescence and dynamics. In: Pilowsky, P. (Ed.), Serotonin: The Mediator That Spans Evolution. Elsevier, Amsterdam, pp. 3–22.

Sarkar, P., Jafurulla, M., Bhowmick, S., Chattopadhyay, A., 2020. Structural stringency and optimal nature of cholesterol requirement in the function of the $serotonin_{1A}$ receptor. J. Membr. Biol. 253, 445–457.

Sarkar, P., Mozumder, S., Bej, A., Mukherjee, S., Sengupta, J., Chattopadhyay, A., 2021. Structure, dynamics and lipid interactions of serotonin receptors: excitements and challenges. Biophys. Rev. 13, 101–122.

Sengupta, D., Chattopadhyay, A., 2012. Identification of cholesterol binding sites in the $serotonin_{1A}$ receptor. J. Phys. Chem. B 116, 12991–12996.

Shrivastava, S., Pucadyil, T.J., Paila, Y.D., Ganguly, S., Chattopadhyay, A., 2010. Chronic cholesterol depletion using statin impairs the function and dynamics of human $serotonin_{1A}$ receptors. Biochemistry 49, 5426–5435.

Simmonds, A.C., East, J.M., Jones, O.T., Rooney, E.K., McWhirter, J., Lee, A.G., 1982. Annular and non-annular binding sites on the (Ca^{2+}+Mg^{2+})-ATPase. Biochim. Biophys. Acta 693, 398–406.

Sjögren, B., Csöregh, L., Svenningsson, P., 2008. Cholesterol reduction attenuates 5-HT_{1A} receptor-mediated signaling in human primary neuronal cultures. Naunyn Schmiedebergs Arch. Pharmacol. 378, 441–446.

Sommer, B., Montaño, L.M., Carbajal, V., Flores-Soto, E., Ortega, A., Ramírez-Oseguera, R., Irles, C., El-Yazbi, A.F., Cho, W.J., Daniel, E.E., 2009. Extraction of membrane cholesterol disrupts caveolae and impairs serotonergic (5-HT_{2A}) and histaminergic (H_1) responses in bovine airway smooth muscle: role of Rho-kinase. Can. J. Physiol. Pharmacol. 87, 180–195.

Sriram, K., Insel, P.A., 2018. G protein-coupled receptors as targets for approved drugs: how many targets and how many drugs? Mol. Pharmacol. 93, 251–258.

Turner, J.H., Gelasco, A.K., Raymond, J.R., 2004. Calmodulin interacts with the third intracellular loop of the serotonin 5-$hydroxytryptamine_{1A}$ receptor at two distinct

sites. Putative role in receptor phosphorylation by protein kinase C. J. Biol. Chem. 279, 17027–17037.

Wacker, D., Wang, C., Katritch, V., Han, G.W., Huang, X.-P., Vardy, E., McCorvy, J.D., Jiang, Y., Chu, M., Siu, F.Y., Liu, W., Xu, H.E., Cherezov, V., Roth, B.L., Stevens, R.C., 2013. Structural features for functional selectivity at serotonin receptors. Science 340, 615–619.

Wacker, D., Wang, S., McCorvy, J.D., Betz, R.M., Venkatakrishnan, A.J., Levit, A., Lansu, K., Schools, Z.L., Che, T., Nichols, D.E., Shoichet, B.K., Dror, R.O., Roth, B.L., 2017. Crystal structure of an LSD-bound human serotonin receptor. Cell 168, 377–389.

Wang, C., Jiang, Y., Ma, J., Wu, H., Wacker, D., Katritch, V., Han, G.W., Liu, W., Huang, X.-P., Vardy, E., McCorvy, J.D., Gao, X., Zhou, X.E., Melcher, K., Zhang, C., Bai, F., Yang, H., Yang, L., Jiang, H., Roth, B.L., Cherezov, V., Stevens, R.C., Xu, H.E., 2013. Structural basis for molecular recognition at serotonin receptors. Science 340, 610–614.

Wheatley, M., Wootten, D., Conner, M.T., Simms, J., Kendrick, R., Logan, R.T., Poyner, D.R., Barwell, J., 2012. Lifting the lid on GPCRs: the role of extracellular loops. Br. J. Pharmacol. 165, 1688–1703.

Wu, H., Wang, C., Gregory, K.J., Han, G.W., Cho, H.P., Xia, Y., Niswender, C.M., Katritch, V., Meiler, J., Cherezov, V., Conn, P.J., Stevens, R.C., 2014. Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. Science 344, 58–64.

Xu, P., Huang, S., Zhang, H., Mao, C., Zhou, X.E., Cheng, X., Simon, I.A., Shen, D.D., Yen, H.Y., Robinson, C.V., Harpsøe, K., Svensson, B., Guo, J., Jiang, H., Gloriam, D. E., Melcher, K., Jiang, Y., Zhang, Y., Xu, H.E., 2021. Structural insights into the lipid and ligand regulation of serotonin receptors. Nature 592, 469–473.

Yin, W., Zhou, X.E., Yang, D., de Waal, P.W., Wang, M., Dai, A., Cai, X., Huang, C.Y., Liu, P., Wang, X., Yin, Y., Liu, B., Zhou, Y., Wang, J., Liu, H., Caffrey, M., Melcher, K., Xu, Y., Wang, M.W., Xu, H.E., Jiang, Y., 2018. Crystal structure of the human 5-HT_{1B} serotonin receptor bound to an inverse agonist. Cell Discov. 4, 12.

Zidovetzki, R., Levitan, I., 2007. Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies. Biochim. Biophys. Acta 1768, 1311–1324.