GPCR-Cholesterol Interactions Highlighting Cholesterol Dynamics at Receptor Occupancy Sites

Site Dynamics
Fast Exchange
Hopping
Receptor Dynamics
Slow Exchange

BIOPHYSICS, BIOMATERIALS, LIQUIDS, SOFT MATTER
Explores GPCR—Lipid Interactions by Molecular Dynamics Simulations: Excitement, Challenges, and the Way Forward

Durba Sengupta, Xavier Prasanna, Madhura Mohole, and Amitabha Chattopadhyay

ABSTRACT: G-protein-coupled receptors (GPCRs) are seven transmembrane receptors that mediate a large number of cellular responses and are important drug targets. One of the current challenges in GPCR biology is to analyze the molecular signatures of receptor–lipid interactions and their subsequent effects on GPCR structure, organization, and function. Molecular dynamics simulation studies have been successful in predicting molecular determinants of receptor–lipid interactions. In particular, predicted cholesterol interaction sites appear to correspond well with experimentally determined binding sites and estimated time scales of association. In spite of several success stories, the methodologies in molecular dynamics simulations are still emerging.

In this Feature Article, we provide a comprehensive overview of coarse-grain and atomistic molecular dynamics simulations of GPCR—lipid interaction in the context of experimental observations. In addition, we discuss the effect of secondary and tertiary structural constraints in coarse-grain simulations in the context of functional dynamics and structural plasticity of GPCRs. We envision that this comprehensive overview will help resolve differences in computational studies and provide a way forward.
specific cholesterol binding sites in GPCR crystal structures have been reported by X-ray studies, while NMR studies suggest that these interactions occur at nanosecond and microsecond time scales. Importantly, it has been suggested that GPCRs exhibit cholesterol-dependent conformational dynamics, although molecular details of the conformational dynamics are yet to be resolved. Additional membrane components, such as phospholipids and sphingolipids, have been reported to modulate ligand binding in GPCRs. In fact, phospholipids have been suggested to represent a special class of allosteric modulators for GPCRs. To gain insight into GPCR–membrane interactions, it would therefore be important to take into account the functional dynamics of GPCRs.

In spite of the considerable progress made toward understanding GPCR structure and dynamics by experimental methods in the last few years, the information obtained is still limited in terms of spatial and temporal resolution. Recent molecular dynamics simulations have made large strides toward complementing experimental data by providing a comprehensive molecular picture of membrane proteins in general and GPCRs in particular. Receptor activation represents an important aspect where atomistic simulations have helped uncover molecular details in close collaboration with experimental observations. We suggest a recent review by Dror and co-workers to interested readers for an overview of the activation mechanisms. However, studies on the interaction of GPCRs with membrane lipids are relatively sparse. In this Feature Article, we focus on coarse-grain simulations of GPCR–lipid interactions and how these interactions modulate GPCR structure and organization. In the process, we provide a comprehensive overview of the major success stories in addressing GPCR–membrane interactions using computational approaches.

Figure 1. The β₂-adrenergic receptor and the CCM cholesterol interaction site. (a) A schematic representation of the β₂-adrenergic receptor with each helix colored individually. The high occupancy cholesterol site on transmembrane helix IV corresponding to the CCM site (b) resolved crystallographically and (c) predicted from coarse-grain simulations. (d–g) Dynamics and translation of the cholesterol molecule at transmembrane helix IV sampled during simulations. The transmembrane helix is colored blue, the highlighted side chains are colored white, and the bound cholesterol molecule is shown in magenta with the polar head group (−OH) shown in cyan. Reproduced with permission from ref 40. Copyright 2014 Elsevier.
the CCM site during the course of the simulation. Interestingly, a large dynamics of cholesterol was observed at a high predictive power of the simulation methodology used. The experimentally resolved site (see Figure 1) is noteworthy and points to a high predictive power of the receptor superimposed on the plot for clarity.

The β2-adrenergic receptor is an important member of the GPCR family and serves as an excellent prototype for monitoring GPCR structure and function. The receptor is involved in muscle relaxation following activation, and its dysfunction is associated with cardiac diseases and asthma. The structural characterization of the β2-adrenergic receptor by high resolution crystallography provided an important step toward understanding molecular level details of GPCR function. Subsequently, Kobilka and co-workers were able to resolve two cholesterol molecules at the groove formed by transmembrane helices I, II, III, and IV. This site in the crystal structure of the β2-adrenergic receptor was termed the cholesterol consensus motif (CCM). The main residues comprising this site are shown in Figure 1. The hydroxyl moiety of the cholesterol is found to interact with an Arg residue, while the sterol ring interacts with Trp and Ile residues on transmembrane helix IV. Although the CCM site was resolved experimentally, GPCRs may behave differently in cubic and lamellar lipidic mesophases and the CCM could be specific to membrane lipid environment.

To analyze cholesterol sites for GPCRs in membrane bilayers, we previously performed coarse-grain simulations of the β2-adrenergic receptor embedded in 1-palmitoyl-2-oleoylsn-glycerol-3-phosphocholine (POPC) bilayers at varying cholesterol concentration using the MARTINI coarse-grain force-field. The simulations were able to identify the CCM site as a high occupancy site. A representative snapshot from the simulations is shown in Figure 1b. The CCM site was identified by calculating the maximum occupancy of cholesterol, i.e., the maximum time a cholesterol molecule is bound to each site of the receptor, during the course of the simulations. A high cholesterol occupancy was observed at transmembrane helix IV in multiple simulations at three different cholesterol concentrations, thereby indicating that a cholesterol molecule consistently interacted with the receptor at the CCM site. The close comparison between the simulations and the experimentally resolved site (see Figure 1) is noteworthy and points to a high predictive power of the simulation methodology used. Interestingly, a large dynamics of cholesterol was observed at the CCM site during the course of the simulation. The cholesterol molecule at the CCM site interacted with several adjacent sites in transmembrane helix IV. These sites are represented in Figure 1c–f. In particular, a site closer to the center of the helix, that displayed prominent ring stacking interactions with the Trp residue of the CCM, was observed to be favorable for cholesterol interaction. The high entropy exhibited by the cholesterol at this site would contribute to the total free energy of interaction at this site.

An important aspect of β2-adrenergic receptor–cholesterol interaction is the presence of multiple occupancy sites. The spatial density of cholesterol, averaged over the trajectory, is shown in Figure 2 with the top view of the receptor superimposed. For infinitely long sampling, the total occupancy of cholesterol corresponds to the average spatial density. Several cholesterol sites could be distinguished, with those in the upper leaflet marked e1–3, those in the lower leaflet i2–5, and a high density site at the center of the bilayer marked m1. The site i3 corresponds to the CCM site discussed above. However, an inter-site hopping between sites i3 and m1 was also observed. The interaction sites were found to be consistent with the study of Cang and co-workers who used atomistic simulations to identify cholesterol interaction sites in the β2-adrenergic receptor. Importantly, coarse-grain simulations were able to reproduce most sites identified by atomistic simulations, although the site marked i1 was not observed. Recent work from the Vattulainen group has confirmed the sites i2, i4/5, and e2, although the remaining sites were not identified as high density sites. In spite of these differences, the main occupancy sites determined from two independent simulation techniques are similar. These observations point toward force-field-independent results and the high accuracy of the coarse-grain methodology.

The time scales of cholesterol interaction from simulations were observed to be microseconds for the high occupancy sites and nanoseconds for the low occupancy sites. The coarse-grain time scale dynamics of high interaction sites, although consistent with non-annular sites, appeared to be faster than previously suggested. Interestingly, an NMR study analyzing the interaction of membrane cholesterol with the β2-adrenergic receptor was able to identify two distinct time scales of interactions that the authors assigned to the nanosecond and microsecond time scales. The time scales reported in the simulations should be considered as a qualitative description due to the nature of the coarse-grain force-field. Nonetheless,
these results emphasize high predictive power of the simulation methodology.

COMPARISON OF CHOLESTEROL INTERACTION SITES IN THE SEROTONIN1A RECEPTOR AND THE β2-ADRENERGIC RECEPTOR

The serotonin1A receptor is one of the most comprehensively studied seven transmembrane domain GPCRs in the serotonin receptor family.44,45 As a consequence of its indispensable role in neurological functions, the serotonin1A receptor has emerged as a major drug target in the development of therapeutics against neuropsychiatric disorders such as anxiety, depression, schizophrenia, and Parkinson’s disease.46 The cholesterol occupancy sites of the serotonin1A receptor have been calculated at varying cholesterol concentration, using a similar coarse-grain methodology.47 The serotonin1A receptor shares a high sequence similarity with the β2-adrenergic receptor.48 In fact, the serotonin1A receptor was initially discovered as an “orphan” β-adrenergic receptor and was later identified as a serotonin receptor (“deorphanized”).49 It was not a priori clear whether the GPCR–lipid interactions of the two receptors would differ or not. Coarse-grain simulations were able to identify a large number of cholesterol interaction sites in the serotonin1A receptor.47 Interestingly, the CCM site at transmembrane helix IV could not be distinguished as a high-occupancy cholesterol site. Multiple sites were observed at transmembrane helices I, II, V, VI, and VII. Of these, the site at transmembrane helix V corresponds to a putative cholesterol recognition motif, the CRAC motif.50 The site is comprised of -L/V-(X)1−5-Y-(X)1−5-R/K- residues, that has also been identified in other cholesterol binding proteins. Subsequently, simulations confirmed the stochasticity of cholesterol interactions.51

To analyze the spatial distribution of cholesterol, we reanalyzed our simulations51 and plotted the spatial distribution function of cholesterol around the receptor for the upper and lower leaflets separately (see Figure 3). As expected, a high cholesterol density site was observed at the lower leaflet of transmembrane helix V near the CRAC site of the serotonin1A receptor. Similarly, transmembrane helix I was found to have high cholesterol density in its vicinity in the lower leaflet. In the upper leaflet, transmembrane helix VI exhibited a high cholesterol density. Surprisingly, we observed high cholesterol density near transmembrane helix IV, but this was not
identified as a high occupancy site. It is possible that, due to the high dynamics at this region, it has a lower maximum occupancy for cholesterol and longer sampling could help resolve the site. Of these sites, transmembrane helix V was identified as a cholesterol hot-spot by all-atom simulations. The close comparison of cholesterol sites from atomistic and coarse-grain simulations clearly demonstrates that these cholesterol interaction sites are force-field independent.

In the next step, we analyzed the effect of the cholesterol association sites on the dimerization of the receptor. Receptor association has been suggested to be modulated by direct effects via cholesterol interaction sites or indirect effects of membrane bilayer properties. Simulations indicate that a combination of these effects could modulate the association of the receptors. In general, the main sites of receptor association correspond to those with high membrane perturbations, but these are modulated by specific cholesterol interactions. For example, in the case of the β2-adrenergic receptor, high cholesterol occupancy at transmembrane helix IV (from the CCM sites i3 and m1) of the receptor blocks it from the dimer interface at high cholesterol concentrations. On the other hand, the cholesterol interaction sites of comparable energetics in the serotonin1A receptor appear to induce greater plasticity in receptor association and, as a consequence, several dimer interfaces were identified for the serotonin1A receptor. Interestingly, the flexibility of the dimer itself was observed to be correlated with the presence of cholesterol at the dimer interface. With increasing computational resources, the next step would be to analyze the molecular details of the role of membrane cholesterol in the oligomerization of these receptors.

PHOSPHOLIPID INTERACTION SITES IN THE SEROTONIN1A RECEPTOR MATCH EXPERIMENTAL PHOSPHOLIPID SITES IN RELATED GPCRS

A related question that arises is whether there are any phospholipid interaction sites. A recent crystal structure of the adenosine2A receptor revealed multiple electron density sites corresponding to phospholipid interaction sites. Prominent among them is the site at the cleft formed by transmembrane helices I and VII (Figure 4). In a series of coarse-grain simulations, we were able to identify the same site as a phospholipid binding site in both the β2-adrenergic receptor and the serotonin1A receptor. In the absence of cholesterol, or at low cholesterol concentrations, this site is the highest occupancy site, but another high occupancy site was observed at transmembrane helix V with increasing cholesterol concentration. Although further work is needed to
understand the role of these phospholipid sites, it is encouraging that simulations are able to accurately predict important phospholipid sites.

THE SEROTONIN<sub>1A</sub> RECEPTOR BINDS GM1 GANGLIOSIDE VIA A SPHINGOLIPID BINDING DOMAIN

Sphingolipids are essential components of the cell membrane and are recognized as diverse and dynamic regulators of a multitude of cellular processes. Sphingolipids have been shown to regulate the function of GPCRs, such as the serotonin<sub>1A</sub> receptor. In particular, ligand binding was shown to be sphingolipid-dependent, but the underlying molecular mechanism was not clear. Similar to phospholipids and cholesterol, the modulatory effect of sphingolipids on GPCR function could be a result of direct or indirect interaction, or a combination of both. Direct interactions are supported by the fact that several sphingolipid-dependent membrane proteins appear to have a consensus “sphingolipid binding domain” (SBD) that consists of a characteristic combination of aromatic, basic, and turn-inducing residues. The SBD motif has been reported in serotonin receptors and shown to be evolutionarily conserved. Interestingly, the motif is present at one of the extracellular loops, and not in the transmembrane region of the serotonin<sub>1A</sub> receptor. However, experiments with isolated SBD peptide did not show significant binding in model membranes, thereby highlighting the relevance of the overall “context”. In addition, it has been recently reported that representative GPCRs such as the serotonin<sub>1A</sub> receptor contain a putative sphingolipid-binding motif (SBM), which is an intrinsic feature of the receptor and is conserved throughout natural evolution.

We investigated the interaction of GM1 ganglioside with the serotonin<sub>1A</sub> receptor using coarse-grain molecular dynamics simulations. Our results showed that GM1 binds to the predicted SBD in the extracellular loop 1 region of the serotonin<sub>1A</sub> receptor (see Figure 5). The sugar moiety of GM1 ganglioside was found to interact with the aromatic residue W102 and flanking residues K101 and T103. An interesting aspect of the interaction site is that, though it is independent of cholesterol, the presence of cholesterol allows a closer interaction of GM1 with the receptor. The importance of cholesterol has been previously reported for the interactions of the HIV-1 gp120 glycoprotein with a related glycosphingolipid (Gb3). In addition, we observed that the interaction of the serotonin<sub>1A</sub> receptor with GM1 stabilizes a “flip-out” conformation of W120 such that it points away from the central lumen of the receptor. This conformation is dependent on the cholesterol-modulated GM1 distribution around the receptor. A similar orientation of aromatic residues has been reported in the binding subunit of Shiga-like toxin.

CURRENT METHODOLOGIES: ADVANTAGES AND LIMITATIONS

Molecular dynamics simulations have made enormous strides in the past few years in terms of both accuracy and computational power. In this Feature Article, we have highlighted coarse-grain simulation studies on GPCR–lipid interactions that closely match current experimental data and have high predictive power. Despite these successes, several strategies have been used by various groups, and the choice of the methodology lacks consensus. One of the main questions that arises is whether a coarse-grain force-field can have the accuracy of atomistic force-fields despite having a lower chemical resolution. Even when a coarse-grain force-field is chosen, parameter sets used for different applications vary. We discuss below the advantages and limitations of each of these methods in order to compare their suitability for exploring multiple aspects of GPCR–lipid interactions.

DISTINGUISHING BETWEEN ATOMISTIC AND COARSE-GRAIN SIMULATIONS

In conjunction with coarse-grain simulation studies, several atomistic simulations of GPCR–lipid interactions have been reported. Atomistic simulations have been able to leapfrog the nanosecond time scale toward the microsecond regime with increasing computational power. In particular, they have been able to identify important interactions of GPCRs with lipids, and a few have been successful in mapping out multiple cholesterol interaction sites. Interestingly, a competition between cholesterol and the ligand at the orthosteric ligand binding site has been reported. Interactions with membrane lipids have been shown to result in differences in the overall conformational dynamics of transmembrane domains and loop regions of GPCRs. Several atomistic force-fields, including CHARMM, OPLS, and Desmond force-fields, have been used for these studies. Detailed comparisons between these force-fields have not been reported, although most cholesterol sites that have been identified appear to be force-field-independent. In addition, a rigorous comparison between GPCR–lipid interactions predicted using atomistic simulations and coarse-grain simulations is still missing. We have highlighted a few cases in this Feature Article where comparisons can be directly made. In general, atomistic simulations inherently have an increased chemical resolution and as a result a higher predictive power, but the sampling of these interaction sites is limited. The majority of atomistic simulations are able to sample at the microsecond time regime, and it remains difficult for these simulations to comprehensively sample multi-microsecond time scale events.

Coarse-grain force-fields, especially the MARTINI force-field, are being increasingly used to analyze GPCR–lipid interactions. The main advantage of coarse-grain simulations is the increased sampling efficiency. Simulation trajectories of multiple microsecond time scales are easily achievable with current computational resources. The MARTINI force-field is one of the most widely used coarse-grain force-fields mainly due to its transferability and predictive power. The force-field was parametrized on the basis of solvation and partitioning free energies and has been successful in analyzing facets of several membrane proteins. In particular, the protein–lipid interactions in model systems have been reported to be accurately represented. The main limitations of the MARTINI force-field are simplistic electrostatics and reduced structural flexibility due to the use of positional restraints on the secondary structural elements. This is of critical disadvantage in systems where large secondary structural dynamics, such as helix folding/unfolding, takes place. The way forward could perhaps be an efficient combination of coarse-grain and atomistic approaches. Overall, the MARTINI force-field appears to be well suited to study GPCR–lipid interactions and has been reported to match previous atomistic simulations, as well as experimental observations.
TO USE OR NOT TO USE ARTIFICIAL TERTIARY STRUCTURAL CONSTRAINTS IN COARSE-GRAIN SIMULATIONS

An important methodological difference within the MARTINI force-field is the use of the elastic network model, that is a set of constraints to hold the protein tertiary structure constant. The elastic network model was parametrized by Periole and co-workers, and allows only the harmonic protein dynamics.\textsuperscript{40,44} Anharmonic modes as well as large-scale deviations from the starting structure (such as helix repositioning) are not permitted by the elastic network. Originally, the MARTINI force-field was parametrized without the elastic network,\textsuperscript{41,42} and several membrane protein studies have been reported without the use of the elastic network.\textsuperscript{43,45–47} A large number of GPCR simulations have been reported without the underlying elastic network\textsuperscript{48–50} or with only an early version of the elastic network.\textsuperscript{60} However, large conformational variations have also been reported in the absence of the elastic network in certain GPCRs.\textsuperscript{59} Interestingly, several coarse-grain studies described above that are shown to reproduce binding sites determined from experiments or atomistic simulations were performed without the elastic network. In this regard, it is important to note that the flexibility of GPCRs has been reported to be critical for its function,\textsuperscript{5,10,11,90,91} and as a first estimate, a more flexible structure is probably a better model for GPCRs. Additionally, the MARTINI coarse-grain force-field has been shown to reproduce transmembrane helix packing\textsuperscript{92} and association energetics\textsuperscript{93–95} in single transmembrane helical proteins. Helix packing in the larger GPCRs has not been critically tested, but helix–helix interactions predicted by the MARTINI force-field without considering tertiary structural restraints was shown to reproduce experimental structural data.\textsuperscript{76} Taken together, the MARTINI force-field appears to have high predictive power and is uniquely positioned to analyze molecular details of GPCR–lipid interactions and its effect on GPCR organization.

THE WAY FORWARD

Although simulations have been used to analyze several facets of GPCR–lipid interactions, standardized methodologies are still emerging for different systems. Detailed thermodynamic and kinetic analysis of lipid binding sites that can be achieved by simulations with improved computational resources would provide an unparalleled insight into GPCR–lipid interactions. Currently, it is important to compare results obtained from computational approaches with experimental data and test the robustness of the results. As a cautionary note, each receptor should perhaps be tested individually for correct use of the simulation parameters. Another important step could be to match aspects of atomistic and coarse-grain simulations, and a close comparison with experimental data, whenever possible.

An emerging concept from molecular dynamics simulations is the large dynamics in GPCR–lipid interactions. For example, several cholesterol interaction sites have been proposed, such as the CRAC\textsuperscript{50} site, the CCM\textsuperscript{28} site, and more recently an aromatic signature binding mode.\textsuperscript{37} The plasticity of the binding sites and the dynamics within a site or between neighboring sites increases the repertoire of cholesterol interaction sites. As suggested before,\textsuperscript{47} stochasticity at the cholesterol sites, as well as competition with other lipid molecules, could provide diversity in cholesterol occupancy sites. This diversity plays a critical role in fine-tuning GPCR function in varying membrane microenvironments. However, the link between cholesterol binding and receptor activation has not yet been fully understood. The multiple interaction sites identified point toward a conformational modulation in both the ligand-bound and unbound states of the receptor. Increased receptor flexibility in the absence of cholesterol was previously reported by us by experimental\textsuperscript{31} and computational\textsuperscript{52} approaches and also by Vattulainen and co-workers.\textsuperscript{42} In addition, Weinstein/Khelashvili and co-workers earlier reported a link between conformational dynamics upon ligand binding and cholesterol dynamics associated with the serotonin\textsubscript{2A} receptor.\textsuperscript{76} Clearly, further studies are necessary to unravel the detailed molecular mechanism underlying the link between activation of GPCRs and cholesterol interaction sites in GPCRs.

In conclusion, the computational frameworks described here match several aspects of current experimental data and make testable predictions. As the spatial and temporal resolution of experimental methods improves, it would be interesting to test the computational predictions to novel experimental data. In particular, mass spectrometric methods are being increasingly used to analyze protein–lipid interactions and could provide an improved resolution of these interactions.\textsuperscript{98} A closer match between experimental and computational studies would help uncover new aspects of GPCR–lipid interactions and provide a comprehensive understanding of receptor function.

AUTHOR INFORMATION

Corresponding Authors
*Phone: +91-20-2590-2408. E-mail: d.sengupta@ncl.res.in.
*Phone: +91-40-2719-2578. E-mail: amit@ccmb.res.in.

ORCID
Durba Sengupta: 0000-0002-3138-1024
Amitabha Chattopadhyay: 0000-0002-2618-2565

Notes
The authors declare no competing financial interest.

Biographies

Dr. Durba Sengupta is a faculty member at the National Chemical Laboratory in Pune, India. She received her B.Sc. degree in Chemistry from St. Stephen’s College, University of Delhi, and M.Sc. in Biotechnology from Indian Institute of Technology-Bombay, Mumbai. Subsequently, she received her Ph.D. from University of Heidelberg and was a Postdoctoral Fellow at University of Groningen. The central objective of her research is to understand self-organization and self-assembly in cell membranes using multiscale simulation approaches. Currently, the main focus of her research group is to analyze functional dynamics of membrane receptors, in particular GPCRs.
Dr. Xavier Prasanna received his B.Sc. and M.Sc. (Microbiology) from St. Joseph’s College, Bangalore. He received his Ph.D. from National Chemical Laboratory, Pune. His research interests are in using multiscale simulations to examine the effect of membrane components on higher order organization of G-protein-coupled receptors.

Madhura Mohole received her B.Sc. from Department of Biotechnology, M.E.S. Abasaheb Garware College, Pune, and M.Sc in Bioinformatics from Bioinformatics Centre, University of Pune. She is currently pursuing her Ph.D. at National Chemical Laboratory, Pune. Her research includes understanding GPCR structure, organization, and function using computational methods.

Prof. Amitabha Chattopadhyay received his B.Sc. with Honors in Chemistry from St. Xavier’s College (Calcutta) and M.Sc. in Chemistry from Indian Institute of Technology, Kanpur. He obtained his Ph.D. from the State University of New York at Stony Brook and was a Postdoctoral Fellow at the University of California, Davis. He subsequently joined the Centre for Cellular and Molecular Biology in Hyderabad, where he is currently a SERB Distinguished Fellow. Prof. Chattopadhyay’s work is focused on the role of membrane lipids on the function of G-protein-coupled receptors and its implications in health and disease using experimental and simulation approaches. A translational extension of this work has been on the role of host membrane lipids on the entry of intracellular pathogens into host cells.

In addition, his group pioneered the development and application of the wavelength-selective fluorescence approach as a novel tool to monitor organization and dynamics of probes and proteins in membranes and micelles. Prof. Chattopadhyay was awarded the prestigious TWAS (The World Academy of Sciences) Prize, Shanti Swarup Bhatnagar Award, and Ranbaxy Research Award. He is an elected Fellow of TWAS, the Royal Society of Biology, Royal Society of Chemistry, and all Indian Academies of Science. He has served on the editorial board of a large number of reputed journals.

■ ACKNOWLEDGMENTS

D.S. and A.C. gratefully acknowledge the project (EMR/2016/002294) from the Science and Engineering Research Board (Govt. of India). A.C. gratefully acknowledges support from SERB Distinguished Fellowship (Department of Science and Technology, Govt. of India). A.C. is a Distinguished Visiting Professor at Indian Institute of Technology (Bombay) and Adjunct Professor at Tata Institute of Fundamental Research (Mumbai), RMIT University (Melbourne, Australia), and Indian Institute of Science Education and Research (Kolkata). We thank members of our research groups for critically reading the manuscript.

■ REFERENCES

(13) Rosenbaum, D. M.; Cherezov, V.; Hansson, M. A.; Rasmussen, S. G. F.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Yao, X.-J.; Weis, W. I.; Stevens, R. C.; et al. GPCR Engineering Yields High-resolution...


the Serotonin$_{1A}$ Receptor in Membrane Bilayers of Varying Cholesterol Content Revealed by All Atom Molecular Dynamics Simulation. *Mol. Membr. Biol.* 2015, 32, 127−137.


(64) Pala, Y. D.; Ganguly, S.; Chattopadhyay, A. Metabolic Depletion of Sphingolipids Impairs Ligand Binding and Signaling of Human Serotonin$_{1A}$ Receptors. *Biochemistry* 2010, 49, 2389−2397.


