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Review

Molecular dynamics simulations of GPCR–cholesterol interaction: An emerging paradigm☆

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ABSTRACT

G protein-coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across cell membranes and represent major targets in the development of novel drug candidates. Membrane cholesterol plays an important role in GPCR structure and function. Molecular dynamics simulations have been successful in exploring the effect of cholesterol on the receptor and a general consensus molecular view is emerging. We review here recent molecular dynamics studies at multiple resolutions highlighting the main features of cholesterol-GPCR interaction. Several cholesterol interaction sites have been identified on the receptor that are reminiscent of nonannular sites. These cholesterol hot-spots are highly dynamic and have a microsecond time scale of exchange with the bulk lipids. A few consensus sites (such as the CRAC site) have been identified that correspond to higher cholesterol interaction. Interestingly, high plasticity is observed in the modes of cholesterol interaction and several sites have been suggested to have high cholesterol occupancy. We therefore believe that these cholesterol hot-spots are indicative of 'high occupancy sites' rather than 'binding sites'. The results suggest that the energy landscape of cholesterol association with GPCRs corresponds to a series of shallow minima interconnected by low barriers. These specific interactions, along with general membrane effects, have been observed to modulate GPCR organization. Membrane cholesterol effects on receptor structure and organization, that in turn influences receptor cross-talk and drug efficacy, represent a new frontier in GPCR research. This article is part of a Special Issue entitled: Lipid-protein interactions. Guest Editors: Amitabha Chattopadhyay and Jean-Marie Ruysschaert.

Contents

Abbreviations: CCM, cholesterol consensus motif; CRAC, cholesterol recognition amino acid consensus; GPCR, G protein-coupled receptor; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine

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1. Introduction

G protein-coupled receptors (GPCRs) are a superfamily of membrane-embedded receptors that mediate diverse cellular responses [1,2]. As a result of their central role in several cellular signaling networks, GPCRs constitute the largest family of clinical drug targets [3]. The importance of GPCRs in health and disease has led to sustained research in understanding various aspects of receptor function, such as ligand binding and G-protein coupling [4,5]. An emerging aspect of GPCR research is the recent advance in high resolution structural characterization of several GPCRs [6–10]. However, the role of membrane lipids in GPCR structure and function still represents a relatively less explored area of research [11].

GPCRs consist of seven transmembrane helices that traverse the membrane and an eighth helix that lies beneath the surface of the membrane [12]. These receptors require cellular membranes or membrane-mimetic environments for their function. Consequently, the receptors have been shown to be sensitive to the membrane lipid composition [11,13,14]. An important lipid in this context is cholesterol which has been demonstrated to modulate function of several GPCRs in a receptor-dependent manner, including the serotonin$_{1A}$ receptor [15–17], the β_2-adrenergic receptor [17,18] and rhodopsin [19]. In case of the cholecystokinin receptor, ligand binding and signaling were reported to decrease upon cholesterol depletion, but internalization and tracking were unaltered [20]. In contrast, it has been shown that cholesterol is not essential for the function of the neurotensin receptor 1 [21].

Membrane cholesterol has been reported to stabilize the serotonin$_{1A}$ receptor [22] and modeling studies suggest that the receptor is more compact in the presence of cholesterol [23]. In the case of the β_2-adrenergic receptor, cholesterol has been shown to improve receptor stability and appears to be necessary for crystallization [6,8,24,25]. In addition, the organization of the serotonin$_{1A}$ receptor has been demonstrated to be cholesterol-dependent [26,27]. Interestingly, several GPCR crystal structures have reported close interaction of the receptor with cholesterol [6,8,10,28]. The physiological significance of this close interaction is still emerging in the context of these receptors being crystallized in the lipidic cubic phase [29]. As discussed above, the effect of membrane cholesterol on GPCR organization and function appears to be receptor-dependent, although the data set for this analysis is still modest. A careful analysis therefore would be prudent to address the effect of membrane cholesterol on various GPCRs.

1.1. GPCR-cholesterol interaction: direct and indirect effects

The effects of cholesterol on GPCR function and organization have been suggested to arise due to either direct or indirect effects [30]. Direct effects are those that arise from bound lipid molecules, and affect receptor structure and dynamics by directly interacting with it [31–33]. Indirect effects are caused due to the changes in physical properties of the membrane that indirectly modulate receptor structure and dynamics [13,34,35]. Lipid molecules involved in a direct effect could be distinguished as annular and nonannular lipids [31]. Annular lipids refer to a shell or annulus of lipid molecules surrounding the protein periphery that interact with the receptor surface, and are exchangeable with bulk lipids (a similar shell of lipids has been postulated to be responsible for targeting proteins to specific membrane domains [36]). On the other hand, nonannular lipids are postulated to be not accessible to annular lipids, *i.e.*, they cannot be exchanged with annular lipids. The location of nonannular sites correspond to inter or intramolecular (interhelical) protein surfaces. Lipids bound to nonannular sites are considered to possess higher specificity relative to annular lipids. It has been suggested that cholesterol binding sites in GPCRs could be nonannular [37].

As stated above, indirect effects could arise from changes in the physical properties of the membrane due to the presence or absence of cholesterol. For example, cholesterol modulates membrane bilayer thickness [38] and order [39], and could indirectly affect receptor function by influencing the dimension and rigidity of the membrane [35]. Modulation of GPCR structure and function by hydrophobic mismatch has been previously reported [40–42]. In addition, it has been shown that capsaicin, which increases membrane elasticity [43], modulates ligand binding activity of the hippocampal serotonin$_{1A}$ receptor [44]. Recent crystal structures of GPCRs that have resolved closely spaced cholesterol molecules [6,8,10,28] appear to support a direct effect, which could be related to the crystallization conditions [29]. The specificity of GPCR-cholesterol interaction has been further validated from studies in which close structural analogs of cholesterol were not able to maintain receptor activity [45–47].

1.2. Multi-scale simulations

Molecular dynamics simulations have emerged as an important approach to analyze the molecular details of GPCR structure and function. Simulations help us address questions that are difficult to access with state-of-the-art experimental approaches due to technical complexities and challenges. Atomistic molecular dynamics simulations have been extensively used to probe different aspects of GPCR dynamics [48]. With increasing computational power, unbiased atomistic simulations have been used to study the activation mechanism of GPCRs at the microsecond time scale [49–51]. Using these approaches, a substantial conformational heterogeneity has been reported to be essential for the function of the β_2-adrenergic receptor [52]. In parallel, coarse-grain simulations are being increasingly used to explore microsecond time scale dynamics and organization [53]. In particular, coarse-grain simulations are proving to be useful to analyze the energetics of GPCR association [54]. However, these studies did not explore the effect of multicomponent bilayers and different bilayer constituents, especially cholesterol.

To distinguish between direct and indirect cholesterol interactions, a few molecular dynamics simulations have been performed with GPCRs embedded in cholesterol-rich bilayers. The nanosecond time scale regime sampled in early atomistic molecular dynamics simulations was not able to probe lateral organization in the membrane [55–57]. Although cholesterol was suggested to be predominantly excluded from the receptor surface [55,56], a few studies were able to identify sites where strong contacts were observed during the simulation [57]. With increased computational resources and improved multi-scale simulations, longer time regimes have been simulated. Recent microsecond time scale atomistic and coarse-grain simulations have been able to sample the lateral diffusion of cholesterol. An example of a representative GPCR, the serotonin$_{1A}$ receptor [58], embedded in a multi-component bilayer containing cholesterol is shown in Fig. 1. The serotonin$_{1A}$ receptor is shown in the figure with the coarse-grain force-field, MARTINI [59,60], that has been shown to reproduce lipid-protein interactions well [61–63]. In these simulations, the cholesterol molecules undergo free rotation and translation, and are able to interact with the receptor in an unbiased manner. The simulations allow identification of the cholesterol interaction sites without an external bias, but sampling issues could be a concern, and adequate care is needed to ensure sampling of sufficient number of binding/unbinding events. In this review, we highlight the current molecular view of GPCR-cholesterol interaction utilizing molecular dynamics simulation and how it affects GPCR structure and dynamics by direct and indirect effects.

2. Cholesterol interaction sites: annular and nonannular

One of the longest time scale simulations was performed with the serotonin$_{1A}$ receptor in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) membrane bilayers with varying concentrations of cholesterol [64]. Multiple simulations adding up to sub-millisecond time scale regimes were performed with the receptor in the membrane environment represented by the MARTINI coarse-grain force-field. During the course of the simulations, cholesterol molecules

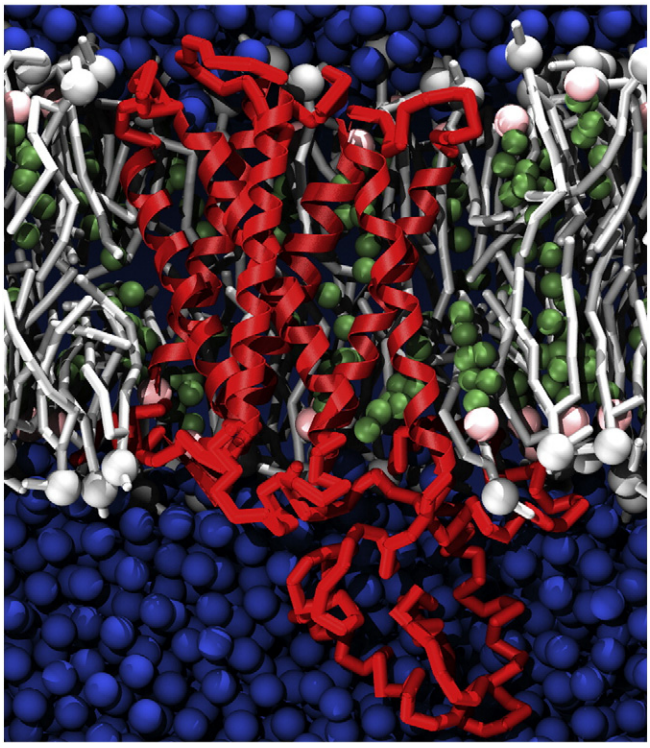

Fig. 1. A representative snapshot of the serotonin$_{1A}$ receptor in a membrane bilayer of POPC and 30% cholesterol. The backbone of the receptor is shown in red, cholesterol molecules are shown in green, and the hydroxyl group in pink. POPC molecules are shown in gray (with its phosphate group in white) and the surrounding water molecules in blue. The figure represents one of the systems studied in ref. [64]. Adapted with permission from Ref. [64] (copyright 2014 American Chemical Society).

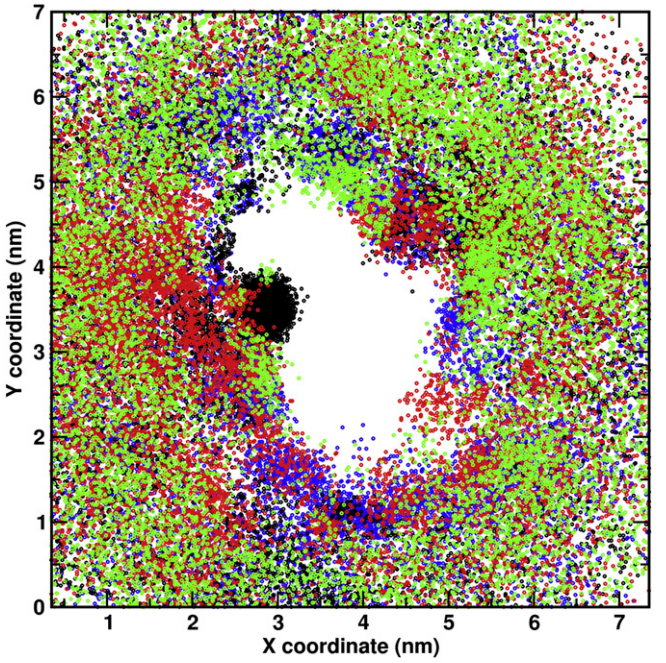

Fig. 2. Diffusion of cholesterol molecules around the serotonin$_{1A}$ receptor in a membrane bilayer of POPC and 30% cholesterol. The x and y coordinates of four representative cholesterol molecules during the course of the simulation are shown in different colors. The trajectories were fitted using the receptor as the reference. The figure shows that several cholesterol molecules (red, green and blue) sample a large part of the simulation box (*i.e.*, sample the bulk membrane), while a few (shown in black) bound to specific sites show constrained dynamics.

diffused freely and multiple cholesterol binding/unbinding events were sampled. Fig. 2 depicts the sampling of a few randomly chosen cholesterol molecules. The figure shows that one of the cholesterol molecules (shown in green) diffuses freely throughout the simulation box, approaching the receptor several times but not interacting with it for long periods of time. In contrast, the cholesterol molecule represented in black interacts with the receptor for a substantial period of time. The representative green cholesterol molecule, which undergoes fast exchange with bulk lipids, could be indicative of annular lipids when interacting with the receptor surface. The black cholesterol molecule, on the other hand, could be representative of a nonannular lipid. From such a cholesterol population distribution, we can identify regions of high cholesterol density, often used as an indicator of cholesterol interaction sites. Sites of high cholesterol density are analogous to sites with high total occupancy time, *i.e.*, the time any cholesterol molecule was bound at a site. The total occupancy time accounts for non-specific cholesterol binding, and multiple binding/unbinding events with low residency times, especially at high cholesterol concentrations. For this reason, a second measure (the maximum occupancy time) was proposed. The maximum occupancy time is defined as the time a given cholesterol molecule was bound to a particular site. Only specific binding is accounted for the maximum occupancy time, especially at high cholesterol concentrations. This measure could be viewed as a distinguishing feature between the annular (high rate of exchange with bulk lipids) and nonannular sites (low rate of exchange with the bulk). With substantial sampling, both measures of high density and maximum occupancy will identify the same sites, but could differ if sampling is not adequate. It was shown in a related study [65] that indeed the site with the highest density corresponds to the site of high maximum occupancy.

By analyzing the maximum occupancy of cholesterol molecules at different sites on the serotonin$_{1A}$ receptor, several cholesterol 'hot-spots' could be identified [64]. These hot-spots correspond to the sites at which the maximum occupancy was, on an average, higher than at the remaining regions of the receptor. The cholesterol occupancy sites were observed to be present on both the extracellular and the intracellular side of the receptor. In another study, sub-microsecond time scale simulation of the A_{2A} adenosine receptor revealed a large number of sites with increased cholesterol interaction [66]. Although the simulations sampled shorter time scales, the atomistic force-field used in the study allowed a more detailed view of the cholesterol interaction. In case of the β_2-adrenergic receptor, several cholesterol interaction sites were identified by two independent studies using atomistic and coarse-grain approaches [65,67]. Interestingly, these sites exhibited close agreement. It is therefore becoming clear that state-of-the-art molecular dynamics simulations probing the microsecond time scale regime are able to identify cholesterol hot-spots on GPCRs. Taken together, these studies indicate the presence of a large number of sites of high cholesterol occupancy in GPCRs, indicative of nonannular sites.

3. Cholesterol hot-spots are dynamic in nature

The cholesterol hot-spots identified in GPCRs such as the serotonin$_{1A}$ receptor [64], the A_{2A} adenosine receptor [66] and the β_2-adrenergic receptor [65,67] appear to be highly dynamic. Most cholesterol molecules exchange with bulk lipids within nanoseconds. Cholesterol molecules were observed to be associated with the receptor in microsecond time scale at the cholesterol hot-spots, *i.e.*, the average lifetimes of the interacting cholesterol molecules were of the order of microseconds [64]. In a few shorter atomistic simulations, some cholesterol molecules were observed to be stably bound throughout the simulation period [67]. In general, the time scales of binding/unbinding at the hot-spots are in the microsecond regime, while the association/dissociation occurs at the nanosecond time scale at most other sites. Fig. 3 shows a

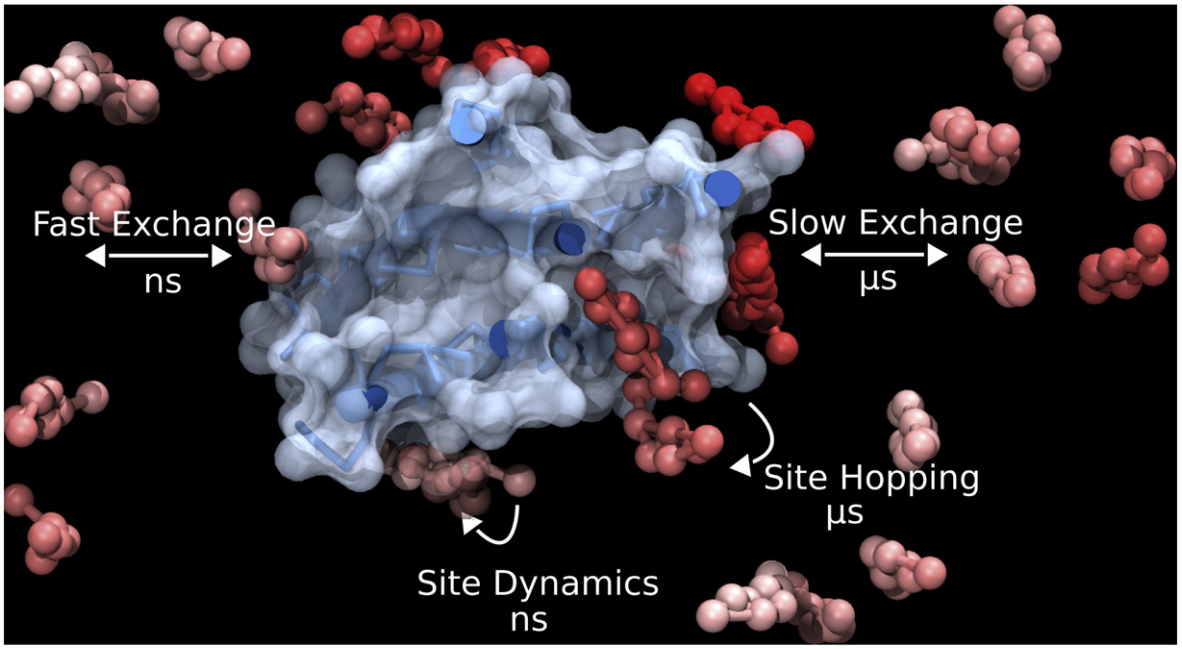

Fig. 3. Dynamics and diversity of cholesterol occupancy sites in GPCRs. A few sites show fast exchange with bulk membrane lipids and low occupancy at the receptor surface. Other sites characteristically exhibit slow exchange with bulk lipids and high occupancy at the GPCR surface. Fast nanosecond time scale dynamics is observed in several sites. Site hopping in microsecond time scale between proximal sites is also observed.

schematic representation of the fast and slow exchange sites in the serotonin$_{1A}$ receptor.

Even when 'bound' to the receptor, these sites are associated with considerable cholesterol dynamics. The cholesterol molecules are quite mobile when associated with the receptor and interact with several residues in the vicinity. As a result, the pattern of the contact made at each interaction site shows high variability [66]. For example, the cholesterol hydroxyl group could interact with several nearby polar and charged groups [64]. A few of the contacts could also be broken for short time periods [68]. Interestingly, site hopping has also been observed between sites that are close together in space [65]. The flexibility at the interaction site is also observed between different binding events, and different association modes of the cholesterol molecule may be sampled. Although limited sampling of the microsecond time scale simulations could lead to differences between the occupancy at different sites, similarities are observed in the nature of these sites. As a result, we believe that these cholesterol hot-spots are indicative of 'high occupancy sites' rather than 'binding sites'.

4. Consensus sites of cholesterol interaction

The location of the cholesterol occupancy sites appears to be receptor-dependent, although a few consensus sites are beginning to emerge. Some of these sites are in deep protein pockets, and located at the interface of transmembrane helices. One of the first putative cholesterol interaction sites was identified from crystallographic studies of the β_2-adrenergic receptor [8] and termed as the cholesterol consensus motif (CCM). This site is located at the groove of the transmembrane helices II and IV. The site is observed to correspond to high cholesterol occupancy in coarse-grain simulations [65], but suggested to be not as frequently occupied in the atomistic simulations [67] of the β_2-adrenergic receptor. Cang et al. [67] showed using atomistic simulations that only a single cholesterol molecule was associated with the receptor at that site and the second molecule drifted away and was replaced by a phospholipid molecule. Further, high dynamics was observed in the coarse-grain simulations at the CCM site and a microsecond time scale interconversion was observed between this site and other nearby cholesterol high-occupancy sites [65]. The CCM site was identified from crystal structures and could be specific to the crystallization conditions. Interestingly, since this binding mode correlates well to that observed in the simulation, it could indicate that cholesterol occupancy at this site is independent of the lipid packing arrangement, although more dynamic than suggested from crystallographic studies. In total, four sites were identified at the extracellular side of the receptor and five toward the intracellular side [65,67]. A good agreement was found between most high density sites identified in the coarse-grain [65] and atomistic [67] simulations. Interestingly, high cholesterol density was observed at the CCM site in atomistic simulations of the β_1- adrenergic receptor [69]. However, differences were observed in the cholesterol density at the other cholesterol 'hot-spots'.

The serotonin$_{1A}$ receptor displayed several cholesterol interaction sites on the receptor surface [64]. Low cholesterol interaction was observed at the corresponding CCM site at the cleft of transmembrane helices II and IV. Another site suggested to be a putative cholesterol interaction site is the cholesterol recognition amino acid consensus (CRAC) site that has been identified in several GPCR sequences [70]. The cholesterol occupancy was on an average high at a CRAC site on transmembrane helix V of the serotonin$_{1A}$ receptor in coarse-grain simulations [64]. However, cholesterol occupancy was high at several non-CRAC sites including transmembrane helices I, II, VI and VII. Similar to the adrenergic receptor family, the serotonin receptor family displays differences in the cholesterol hot-spots. For example, high cholesterol density was reported in the serotonin$_{2A}$ receptor at the intracellular end of transmembrane helices I, II and IV, the extracellular side of transmembrane helices II and III, and at both termini of transmembrane helices VI and VII [71].

Rhodopsin has been studied in several computational studies and early molecular dynamics studies suggested that cholesterol was mainly excluded from the receptor surface [55,56]. More recent data suggest the presence of high cholesterol density sites [57,72]. Khelashvili et al. [57] and Horn et al. [72] identified different sites using atomistic and coarse-grain approaches, but with an agreement in the cholesterol high density site at transmembrane helix VII. The regions of high cholesterol density identified in rhodopsin using coarse-grain approaches appear to closely match with those in opsin [72]. One of these sites, located at the cytoplasmic face of transmembrane helices III, IV and V, has also

been identified in the A_{2A} adenosine receptor [66]. Atomistic simulations were able to identify two additional sites at the extracellular side of the receptor [66]. One of these sites was substantiated by high cholesterol density in a recent crystal structure of the A_{2A} adenosine receptor [10]. Interestingly, none of the above studies were able to discern high cholesterol density at the CCM site.

5. Plasticity of cholesterol interaction

Several cholesterol interaction sites have been identified in GPCRs by different computational studies. A comparison of these sites shows variability between different sets and approaches, although some common features emerge. The interaction of cholesterol with the cholesterol occupancy sites identified appears to be stochastic. Although on an average the same sites are sampled, the deviation between the actual number of events sampled in the same time scales [66] and the time of occupancy between different simulation sets is high [64]. Additionally, as discussed earlier, a few contacts could also be broken for short time periods, resulting in reduced occupancy at those sites [68]. Even the 'specific interaction' of GPCRs with membrane cholesterol shows high flexibility in the probability of association. Although protein clefts, charged or aromatic amino acids are involved in the interaction of cholesterol with GPCRs, none of these are indispensable, and different combinations of these factors could provide diversity in cholesterol occupancy sites [69]. It appears therefore that the interaction of cholesterol with GPCRs is highly plastic.

The plasticity of GPCR-cholesterol interaction arises due to multiple factors. The first factor is the dynamics at the sites and between sites, such that the interaction of the cholesterol molecules with individual residues varies. A second factor is that the high occupancy sites could have equal or comparable probability of cholesterol interaction, such that there is a competition between these sites. The probability of occupancy at these sites therefore could vary. To be able to discern these small differences in the probabilities, a complete sampling of all possible sites at the millisecond to second time scale is required. Yet another factor contributing to plasticity of cholesterol interaction sites is that some of these could represent sites with competition with other lipid components. A competition with phospholipids has been suggested for the CCM site from atomistic simulations [67]. In addition, high occupancy for both phospholipids and cholesterol at several sites has been reported [64]. Interestingly, the presence or absence of other bilayer components, such as sphingolipids, could additionally fine-tune the plasticity of cholesterol occupancy. The modulation of GPCR function by sphingolipids has been demonstrated for the $serotonin_{1A}$ receptor [73,74]. The plasticity of the cholesterol hot-spots could be dependent on ligand binding as well, and interestingly the high occupancy sites were suggested to be ligand-dependent in the case of the $adenosine_{2A}$ receptor [68] but not for the $serotonin_{1A}$ receptor [64].

The emerging model regarding the energy landscape of cholesterol association with GPCRs is that it corresponds to a series of shallow minima interconnected by low barriers. A schematic view of the relative energy landscape of the cholesterol occupancy sites is shown in Fig. 4. The cholesterol occupancy sites could be represented by individual residues or by a sub-space at the receptor surface (such as the CCM site). Although the occupancy sites are shown as interconnected, they are usually accessed via an exchange with the annular lipids and less often by direct site hopping. The free energy of these sites, calculated from the relative populations, is of the order of kT at room temperature [66]. The interaction energy between cholesterol and the receptor is relatively low, although high cholesterol dynamics reduces the entropic penalty of binding, thereby increasing the free energy of association at these sites. Both the minima and the barriers could be modulated by other lipid components. Ligand binding could further tune the energetics of cholesterol interaction. Taken together, it is becoming clear that cholesterol occupancy sites in GPCRs are weak, dynamic yet essential.

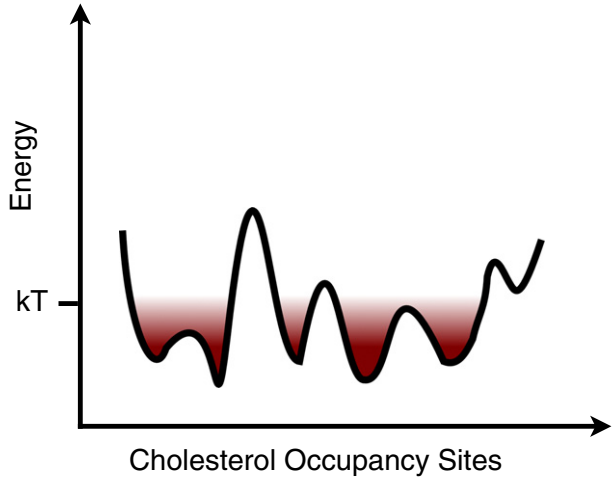

Fig. 4. A schematic representation of the energy landscape illustrating cholesterol interaction sites in GPCRs. The landscape corresponds to a series of shallow minima interconnected by low energy barriers. The abscissa can be thought to correspond to individual occupancy sites represented by each residue or by a sub-space at the receptor surface (such as the CCM site). Although shown as interconnected, the sites are most likely to be accessed via an exchange with the annular lipids and less often by direct site hopping. Note that the energy barriers and the minima could be modulated by other membrane components such as sphingolipids.

6. Modulating GPCR structure and association

Although cholesterol interaction sites have been reported in GPCRs, its effect on the structure and dynamics of GPCRs is still not clear. One of the few studies focusing on protein structural integrity [57] reported structural differences in the kink regions in transmembrane helices I, II and VII. However, later work by Lyman and coworkers [68] analyzed the effect of cholesterol on the A_{2A} adenosine receptor and reported a force-field dependent dynamics of the kink regions. Another study [71] correlated the functionally relevant conformational flexibility of the $serotonin_{2A}$ receptor with the interaction of cholesterol, but it was not clear whether this conformational flexibility was directly due to the interaction of cholesterol. The differences in receptor structure arising from indirect effects, such as membrane order, are less tractable and have not been studied in a systematic manner. It appears that the effect of cholesterol on receptor structure and dynamics is subtle and would require more detailed analysis.

6.1. Interplay between direct and indirect effects

Long time scale simulations, especially using coarse-grain force-fields, have allowed us to study GPCR association at a molecular level. A pioneering study, focusing on rhodopsin organization in bilayers of varying thickness, suggested that receptor association is influenced by bilayer perturbations around the receptor [53]. The energy penalty from mismatch between the hydrophobic length of the transmembrane helices and the bilayer appears to be a driving factor for receptor oligomerization. A similar trend was reported for β_1- and β_2-adrenergic receptors, in which the mismatch was observed to be higher in the presence of cholesterol [75]. The authors suggested that the increased mismatch is related to the increased oligomerization in the presence of cholesterol [75]. In both studies, a correlation was reported between the helices with mismatch and their occurrence at oligomer interfaces [53,75]. To address the direct and indirect effects in a more tractable system, the dimerization of the β_2-adrenergic receptor was studied at different cholesterol concentrations [65,76]. Modulation of the dimer interface was observed by increasing concentrations of cholesterol (see Fig. 5). The presence of transmembrane helix IV at the dimer interface was observed to be inversely correlated with the occupancy of cholesterol at this site [65]. This was one of the first studies that correlated a

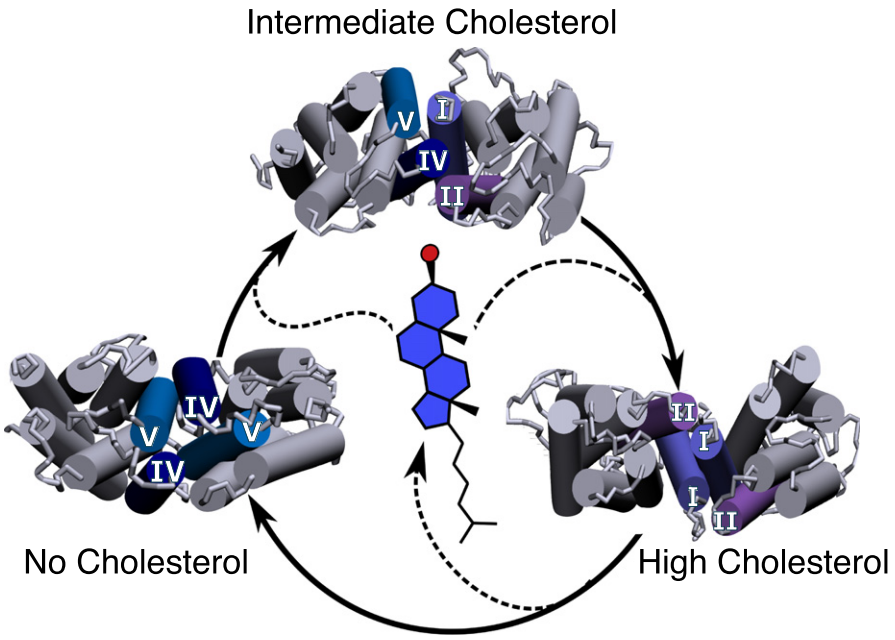

Fig. 5. Modulation of the dimer interface of the β_2-adrenergic receptor by membrane cholesterol content. Previous work using coarse-grain molecular dynamics simulations showed that the dimerization of the receptor is modulated by membrane cholesterol content [65]. The receptor dimer interface changes from predominantly transmembrane helices IV and V (shown in blue) at low membrane cholesterol to an interface consisting of transmembrane helices I and II (purple) at high membrane cholesterol. Cholesterol is shown at the center in blue with the polar hydroxyl group in red. Such conformational plasticity at the dimer interface with varying cholesterol content gives rise to interesting possibilities in future drug development (see Ref. [65] for more details). Data taken from Ref. [65].

direct interaction of cholesterol with GPCR association. Similar to previous reports [53,75], the helices with a mismatch were observed at the dimer interface. However, the population analysis did not completely match the extent of the mismatch, *i.e.*, the transmembrane helices with the maximum mismatch were not necessarily those that were maximally observed at the dimer interface [76]. These studies appear to suggest that the driving forces for GPCR association are much more complex than just hydrophobic mismatch or cholesterol occupancy. Both direct and indirect membrane effects contribute toward the dimerization of the receptor. An examination of the energetics of several dimer interfaces revealed similar energetics and several interfaces were calculated to be favorable [54]. The comparable energetics of the dimer interfaces, coupled with the plasticity of the cholesterol occupancy sites, could modulate dimer interfaces in a cholesterol-dependent manner.

7. Conclusions and future perspectives

Multi-scale simulations have shown that membrane cholesterol preferentially interacts with certain sites on GPCRs. Nonetheless, the association of cholesterol to membrane proteins in general, and GPCRs in particular, is currently being extensively explored. A general picture of cholesterol interaction sites is emerging, although no consensus model has been reached. The cholesterol interaction sites represent hot-spots that are reminiscent of high occupancy sites, instead of binding sites. Several such high occupancy sites are present at the surface of GPCRs. Cholesterol molecules at these hot-spots exhibit microsecond time scale lifetimes and exhibit fast exchange with bulk lipids. At the high occupancy sites, cholesterol molecules exhibit fast dynamics and stochasticity is observed in the characteristic interaction patterns. The plasticity in the cholesterol occupancy sites appears to be related to the low but comparable energetics of the different sites, and competition with other bilayer components. Although cholesterol-rich bilayers are observed to directly affect receptor organization, the effects on the structure and dynamics of the receptor are less clear. The driving force of receptor organization is complex and both hydrophobic mismatch and cholesterol sites contribute to GPCR oligomerization. Membrane cholesterol effects on receptor structure and organization, that could modulate drug efficacy, represent a new frontier in GPCR research.

Conflict of interest

The authors declare no conflict of interest

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