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The ganglioside GM1 interacts with the serotonin$_{1A}$ receptor via the sphingolipid binding domain

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ABSTRACT

Glycosphingolipids are minor yet essential components of eukaryotic cell membranes and are involved in a variety of cellular processes. Although glycosphingolipids such as GM1 have been previously reported to influence the function of G protein-coupled receptors (GPCRs), the molecular mechanism remains elusive. In this paper, we have explored the interaction of GM1 with the serotonin$_{1A}$ receptor, an important neurotransmitter receptor that belongs to the GPCR family. To examine the molecular basis of the interaction of GM1 with the serotonin$_{1A}$ receptor, we performed a series of coarse-grain molecular dynamics simulations of the receptor embedded in membrane bilayers containing GM1. Our results show that GM1 interacts with the serotonin$_{1A}$ receptor predominantly at the extracellular loop 1 and specifically at the sphingolipid binding domain (SBD). The SBD motif consists of a characteristic combination of aromatic, basic and turn-inducing residues, and is evolutionarily conserved in case of the serotonin$_{1A}$ receptor. The interaction of the SBD site with GM1 appears to stabilize a 'flip-out' conformation in which W102 of the extracellular loop 1 flips out from the central lumen of the receptor toward the membrane. The population of the 'flip-out' conformation is increased in the presence of cholesterol. Our data strongly suggest that a direct interaction between GM1 and the SBD site of the serotonin$_{1A}$ receptor may occur in vivo. In view of the reported role of GM1 and the serotonin$_{1A}$ receptor in neurodegenerative diseases, GM1-receptor interaction assumes significance in the context of malfunctioning of neuronal GPCRs under such conditions.

1. Introduction

G protein-coupled receptors (GPCRs) represent a diverse class of transmembrane proteins that respond to a variety of physical, chemical and biological stimuli [1–3]. They are primarily involved in transducing signals from outside to inside of the cell across the plasma membrane. As a result, they play a central role in regulating a variety of physiological processes and form a crucial target for drug research [4–7]. In fact, more than 50% of the currently marketed clinical drugs directly or indirectly modulate GPCR activity [8]. The structural core of GPCRs is composed of seven transmembrane helices, which are connected by alternating extracellular and intracellular loops (see Fig. S1). Ligands bind GPCRs at either the central core or at the extracellular loop regions. Key structural and dynamic features of the central core of GPCRs have been elucidated and shown to be critical in receptor function [9]. The intracellular loops, especially the intracellular loop 3 (ICL 3) has been associated with G-protein and β-arrestin coupling [10]. In addition, the extracellular loops (ECLs) play an important role in GPCR function, particularly ligand binding and regulation, but the molecular details are less clear [11].

An interesting and emerging feature of GPCRs is the modulation of GPCR function by membrane lipids such as cholesterol and phospholipids [12–15]. This effect is mediated by both specific interactions with the receptor transmembrane domain and by indirect effects that alter membrane physical properties [16,17]. Bound cholesterol and phospholipid molecules reported in recent crystal structures of GPCRs suggest specific receptor-lipid interactions [18–21]. Computational studies have been successful in reproducing specific interactions and predicting several lipid interaction sites at or between transmembrane helices [22–27]. Indirect effects such as membrane fluidity have been shown to be correlated to receptor function [28]. In addition, experimental [29,30] and simulation [31–33] studies have shown that difference in local membrane thickness (hydrophobic mismatch) could affect receptor organization and function.

Abbreviations: GM1, monosialotetrahexosylganglioside; GPCR, G protein-coupled receptor; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SBD, sphingolipid binding domain.

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Sphingolipids constitute ~10–20% of total membrane lipids and have been implicated in cell signaling, growth, differentiation and neoplastic transformation [34,35]. The distribution of sphingolipids in the bilayer has been extensively studied and it is postulated that they coalesce with cholesterol to form ordered lipid domains that laterally segregate from the bulk membrane [36–38]. However, this view has been recently questioned [39–41]. The function of several membrane proteins, including GPCRs, has been reported to be dependent on sphingolipids [42,43]. One of the best studied GPCRs in the context of sphingolipid-dependent effects, is the serotonin$_{1A}$ receptor [43], a representative GPCR involved in behavior, development and cognition [44].

Previous work by us and others has demonstrated that metabolic depletion of glycosphingolipids affects receptor function [43,45,46]. Similar to phospholipids and cholesterol, the regulatory effect of glycosphingolipids on GPCR function could be a result of direct or indirect interaction, or a combination of both. Direct interactions are implicated by the fact that several sphingolipid-dependent membrane proteins appear to have a consensus 'sphingolipid binding domain' (SBD). SBD was initially identified and characterized in HIV-1 surface envelope glycoprotein gp120 and amyloid proteins that were known to exhibit sphingolipid-dependent conformational transitions, and was later identified in a wide range of proteins including receptors, toxins, and viral proteins [47–49]. The SBD motif consists of a characteristic combination of aromatic, basic and turn-inducing residues. The aromatic residues in this domain have been predicted to be crucial in interaction with the sugar moiety of glycosphingolipids, with charged residues forming electrostatic bonds with sphingomyelin [48,49]. We have previously shown that this motif is present in serotonin receptors and appears to be evolutionarily conserved in case of the serotonin$_{1A}$ receptor [50]. Interestingly, the motif is present at the extracellular loop, and not at the transmembrane domain of the receptor. It has been recently reported that the SBD motif of the serotonin$_{1A}$ receptor binds preferentially to gangliosides relative to other sphingolipids [51]. However, the nature of the interaction between GPCRs and sphingolipids in general, and glycosphingolipids in particular, remains poorly explored. To the best of our knowledge, no reports exist delineating direct or indirect interaction of glycosphingolipids with GPCRs.

In this work, we have explored the interaction of the ganglioside GM1, the most common glycosphingolipid type (typically ~2–5% of total membrane lipids), with the serotonin$_{1A}$ receptor. Toward this goal, we performed a series of coarse-grain molecular dynamics simulations, totaling 400 μs, of the serotonin$_{1A}$ receptor embedded in membrane bilayers containing GM1. Our results show that GM1 binds to the serotonin$_{1A}$ receptor predominantly at the extracellular loop 1 and specifically at the SBD site. The interaction of the receptor with GM1 appears to stabilize a 'flip-out' conformation in which W102 of the extracellular loop 1 flips out from the central lumen of the receptor toward the membrane. These results demonstrate that GM1 directly modulates conformational dynamics of the extracellular loop 1 of the serotonin$_{1A}$ receptor, and could have important consequence in ligand binding and function of the receptor.

2. Methods

2.1. System setup

Molecular dynamics simulations of the serotonin$_{1A}$ receptor were performed in the presence of GM1 to investigate GPCR-glycosphingolipid interaction. Simulations were performed in bilayers with different lipid compositions: POPC, POPC/cholesterol (POPC/chol), POPC/GM1 and POPC/GM1/cholesterol (POPC/GM1/chol) (see Table S1 for system composition). The composition of the POPC/GM1/chol bilayer was chosen so as to realistically represent the physiological cell membrane. POPC/GM1, POPC/chol and POPC bilayers were simulated as controls. All bilayer compositions including those with GM1 were self assembled from a random starting conformation and equilibrated for 50 ns. The bilayer was aligned such that the GM1 cluster was on the upper leaflet, corresponding to the outer leaflet of the cell membrane. A coarse-grain representation of the homology model of serotonin$_{1A}$ receptor [52], obtained from our earlier work [23,53], was inserted into the equilibrated bilayer such that the initial minimum distance between the receptor and any GM1 lipid was at least 2 nm. Ten independent simulations, each of 10 μs, were performed with different starting receptor orientation and initial velocity. Lipid bilayers without the receptor were simulated as control for same time.

2.2. Simulation parameters

All simulations were carried out using GROMACS version 4.5.5 [54], with MARTINI force-field version 2.2 [55–59]. Energy minimization was carried out using steepest descent algorithm. Shift potential were used for non-bonded interactions with electrostatic interactions shifted to zero in the range of 0.0–1.2 nm and van der Waals interaction shifted to zero in the range of 0.9–1.2 nm. Temperature of each molecular group in the system was weakly coupled to a thermostat at 300 K using the v-rescale algorithm [60] with a coupling constant of 0.1 ps. Semi-isotropic pressure coupling was maintained at 1 bar independently in the plane of the bilayer and perpendicular to the bilayer using Berendsen's barostat algorithm [61] with a coupling constant of 0.5 ps and a compressibility of 3×10^{-5} bar^{-1}. Initial velocities for the simulations were chosen randomly from a Maxwell distribution at 300 K. Bond lengths were kept constant using the LINCS algorithm [62]. A time step of 5 fs was used for the simulations with neighbor list updated every 10 steps. Periodic boundary conditions were maintained along x, y and z direction. Simulations were rendered using VMD software [63] and MARTINI secondary structure rendering scripts. Plots were generated using Grace.

2.3. Analysis

2.3.1. Residue-wise maximum occupancy of GM1

The maximum occupancy of GM1 was calculated at each amino acid residue of the serotonin$_{1A}$ receptor. We define maximum occupancy as the maximum time of the simulation for which GM1 remains associated with the residue, based on a cut-off distance of 0.55 nm [23]. The value was averaged over all simulations for each bilayer composition and normalized. A value of 1 indicates that GM1 remains associated with the given residue for the longest time during the simulation while a value of zero indicates that GM1 never interacted with the residue. In the plots corresponding to the occupancy of the individual GM1 beads, the MARTINI bead names (GM1-17) were replaced by the headgroup bead number (HG1-17), to avoid confusion. The mapping remains the same.

2.3.2. Spatial distribution of GM1 around the serotonin$_{1A}$ receptor

Spatial density distribution of GM1 around the serotonin$_{1A}$ receptor was calculated using the g_spatial routine in GROMACS package. The receptor was centered in the bilayer with its translational and rotational motion removed. The voxel element was set to 0.07 nm in each direction. The calculated 3D spatial distribution function was averaged over the extracellular leaflet.

2.3.3. Tryptophan orientation

The orientation of W102 residue in the extracellular loop 1 of the serotonin$_{1A}$ receptor was calculated by measuring the angle made by the vector connecting coarse-grain beads SC1 and SC4 of W102 with the bilayer normal. The values were calculated for the entire simulation time in POPC and POPC/chol bilayers and subsequent to GM1 association in POPC/GM1 and POPC/GM1/chol bilayers.

2.3.4. Preferential partitioning of lipid species

Preferential partitioning of the membrane lipids (or receptor) is calculated as the relative number of contacts of a particular component with each of the other components, normalized for the total number of lipids (and receptor) in the system [59,64]:

$$p_A = \frac{(c_A/n_A)}{\sum_x (c_x/n_x)}$$

where, p_A is the preferential partitioning with membrane component A, c_A the number of contacts with component A, n_A the number of molecules of component A. Contacts were defined with respect to GL1 and GL2 beads for POPC, AM1 and AM2 beads for GM1 and ROH bead for cholesterol. In case of the receptor, we chose CG beads of the receptor at the height of phosphate headgroup region of POPC as reference. Two molecules were considered to be in 'contact' if they were within 0.55 nm. The preferential partitioning was calculated for the last 1 μs of the simulation time from each of the sets and averaged across the sets.

2.3.5. Cholesterol 'flip-flop' rate

To calculate the rate of transbilayer diffusion ('flip-flop') of cholesterol, we calculated the number of transitions for each cholesterol molecule between the extracellular and intracellular leaflets during the entire simulation time. The value was averaged over the number of cholesterol molecules and the total simulation time.

3. Results

3.1. GM1 clusters interact with the serotonin$_{1A}$ receptor

In order to probe the interaction of GM1 and the serotonin$_{1A}$ receptor, coarse-grain simulations were performed with the receptor embedded in lipid bilayers of varying composition. In total, ten simulations of 10 μs each were carried out in POPC/GM1/chol, POPC/GM1, POPC/chol and POPC bilayers. The total simulation time was 400 μs, corresponding to 1.6 ms of effective simulation time. During the initial equilibration of the bilayer, GM1 molecules rapidly clustered in the outer leaflet, both in the presence and absence of cholesterol. A representative snapshot of the initial system in POPC/GM1/chol bilayers is shown in Fig. 1a. In the initial state, the receptor was placed at a distance of at least 2 nm from the GM1 cluster. The time evolution of minimum distance between the receptor and GM1 during the simulations is shown in Fig. 1(b,c). The dark blue stretches in the plot correspond to stable GM1-receptor interactions and the multiple colored bands indicate binding and unbinding events. As evident from the figure, GM1 clusters diffused in the bilayer and subsequently interacted with the receptor at a sub-microsecond timescale. Representative time courses of the simulations containing GM1 are shown in Fig. S2. It was observed that the initial contact sites did not always result in a continued stable association at that site. In most cases, after the initial contact with the receptor, GM1 clusters did not dissociate completely, but diffused around the receptor interacting with it at several non-overlapping sites. In a few cases, GM1 dissociated completely from the receptor and subsequently interacted at the same or different site. After the initial binding/unbinding events, the stable interaction sites were sampled and these did not alter significantly during the simulation. Interactions of the receptor with cholesterol and POPC were observed in all bilayers, as previously reported [23].

3.2. GM1 interacts with the extracellular loop 1 of the serotonin$_{1A}$ receptor

To analyze the interacting sites of GM1, we characterized its spatial distribution with respect to the receptor averaged over ten sets of simulations (Fig. 2a,b). The receptor is superimposed on the density plots for clarity. In POPC/GM1/chol bilayers, high GM1 density was observed around the cleft formed by transmembrane helices II and III, followed by relatively low density at helices I, V and VI (Fig. 2a). In contrast, in POPC/GM1 bilayer, GM1 density was highest at transmembrane helices VI and VII (Fig. 2b). The GM1 density at the site of the cleft formed by transmembrane helices II and III reduced drastically and was close to zero.

To characterize the molecular determinants of this interaction, we calculated the maximum occupancy time of GM1, that is the maximum time it interacts with each amino acid residue (Fig. 2c,d). Surprisingly, high GM1 occupancy was observed mainly at the extracellular loops and the N-terminal region of transmembrane helix I. A low or negligible occupancy of GM1 was observed around the transmembrane helices (as opposed to cholesterol or POPC [23]). A visual observation confirmed that the bulky headgroup of GM1 made stable contact with the extracellular loops while the acyl chains were dynamic and did not appear to interact directly with the transmembrane helices. In both POPC/GM1/chol and POPC/GM1 bilayers, the highest occupancy of GM1 was observed around the extracellular loop 1 that connects transmembrane helices II and III (Fig. 2). In addition, in POPC/GM1 bilayers, an increased GM1 occupancy was observed at the extracellular loop 3. The high occupancy of GM1 at extracellular loop 1 is consistent with high density at the adjacent transmembrane helices II and III in POPC/GM1/chol bilayers (Fig. 2a). Surprisingly, GM1 density around transmembrane helices II and III was reduced in POPC/GM1 bilayers, despite its high occupancy at extracellular loop 1. This implies that the bulky headgroup of GM1 interacts with the extracellular loop 1 from sites further away over the top of the receptor. Taken together, these results suggest that GM1 interacts with multiple sites on the receptor, with the highest occupancy at the extracellular loop 1.

3.3. Characterizing the sphingolipid binding domain at the extracellular loop 1

To identify the specific sites of GM1 interaction, we calculated the maximum occupancy of each headgroup bead of GM1 at the residues (residues 97–109) comprising the extracellular loop 1 (Fig. 3). The highest occupancy of the GM1 headgroup beads was at the residues in the central segment of the extracellular loop 1, in particular residues 100–104. In POPC/GM1/chol bilayers, the highest occupancy was at residues W102 and K101 with GM1 headgroup beads HG4 and HG7 (representing sugar moieties 2 and 3 [58]), respectively (Fig. 3a). In addition, other flanking residues, particularly N100 and T103 displayed a high occupancy. Representative snapshots of the GM1 distribution around the receptor confirmed direct interaction of W102 and the flanking residues with GM1 (Fig. S3). The same pattern was observed in POPC/GM1 bilayers. The highest occupancy was at the residue W102, followed by T103 (Fig. 3b). The corresponding interaction sites on GM1 headgroup were at HG2 and HG7 (representing sugar moieties 1 and 3 [58,59]) respectively. Interestingly, high GM1 interaction sites on the extracellular loop 1 comprises the putative sphingolipid binding domain (SBD) identified earlier [49].

An important difference between GM1 binding mode in the presence and absence of cholesterol was in the headgroup beads of GM1 that interacted with the receptor. In POPC/GM1/chol bilayers, several headgroup beads, even those further away from the bilayer interacted with residues in the extracellular loop 1. Since the GM1 molecules clustered around transmembrane helices II and III (Fig. 2a), i.e., close to the extracellular loop 1, it allows an interaction with both the proximal and the distal headgroup beads (see Fig. S3a). However, in POPC/GM1, GM1 density was highest around transmembrane helices VI and VII (Fig. 2b) and the GM1 was further away from the extracellular loop 1. In this arrangement, the extracellular loop 1 would bend over the top of the receptor to interact with GM1 (Fig. S3b). As a result, the extracellular loop 1 could interact mainly with the GM1 headgroup beads close to the bilayer surface. Headgroup beads further away from the bilayer surface displayed a reduced interaction with residues in

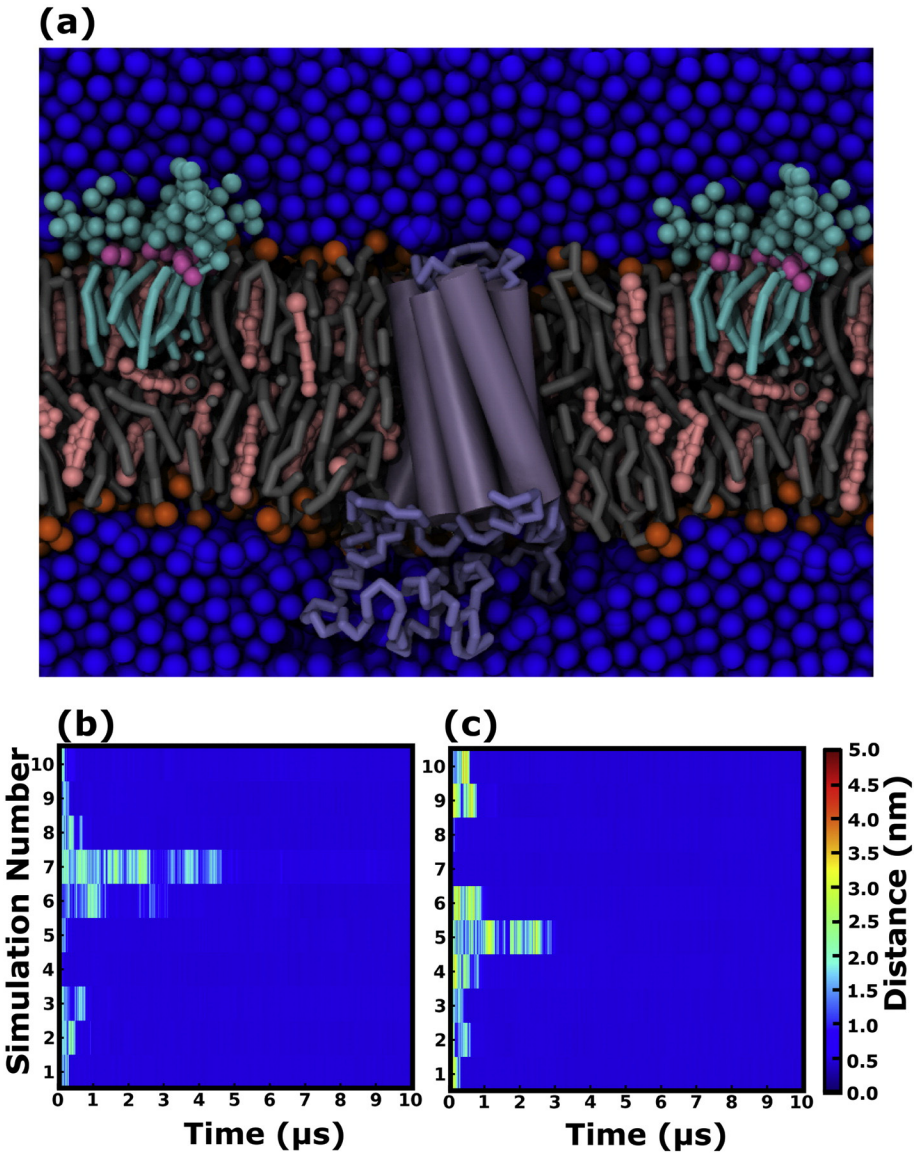

Fig. 1. Interaction of GM1 with the serotonin$_{1A}$ receptor. (a) A representative snapshot of the serotonin$_{1A}$ receptor in POPC/GM1/chol bilayer at the start of the simulations. The receptor is shown as gray cylinders, with the loops in licorice representation. GM1 is shown in cyan, the phospholipids in gray (choline headgroup bead in orange), cholesterol in salmon and the water beads in blue. The minimum distance between the receptor and GM1 during the course of the simulation in (b) POPC/GM1/chol and (c) POPC/GM1 bilayers is shown. The color-coded scale bar shows the range of distance between the receptor and GM1. The interacting state, in which GM1 and the receptor interacts, is characterized by distances less than 0.55 nm, corresponding to the dark blue stretches in the plot. The non-interacting state is characterized by distances greater than 0.55 nm represented by yellow, green and light blue regions in the plot. Each row in the panels represents an independent simulation (numbered along the ordinate) while time is shown along the abscissa.

extracellular loop 1, relative to POPC/GM1/chol bilayers. These interaction sites identified were averaged over ten simulations and appeared to be consistent. Taken together, these results suggest that there exists a 'specific binding site' for GM1 on the serotonin$_{1A}$ receptor, but sites on GM1 itself do not show any specificity in their association with the receptor.

3.4. GM1 stabilizes a 'flip-out' conformation of extracellular loop 1

A distinctive feature of GM1-receptor interaction was its association with the residue W102. To examine its role in GM1 interaction, we analyzed the orientational dynamics of W102 in the presence and absence of GM1. The orientation was calculated as the angle of the indole side chain to the membrane normal (Fig. 4a; see Methods for details). In the absence of GM1, i.e., in POPC and POPC/chol bilayers, the side chain of W102 was oriented ~45° to the membrane normal (Fig. 4b). A visual inspection of these conformations showed that the residue was oriented toward the central lumen of the serotonin$_{1A}$ receptor (Fig. 4c,d). In POPC/GM1 bilayers, W102 adopts an angle of ~60° with the membrane normal (Fig. 4b). In this conformation, the side chain was directed upward largely over the central lumen (Fig. 4e). Interestingly, a bimodal distribution was observed in the orientation of W102 in POPC/GM1/chol bilayers, with peaks at ~60° and 90° (Fig. 4b), possibly indicating conformational plasticity in this complex mixture. In the first conformation (corresponding to the peak at ~60°), the residue projects upward from the central lumen, similar to POPC/GM1 bilayers. Interestingly, in the second orientation (corresponding to the peak at ~90°), W102 orients itself parallel to the plane of lipid headgroup (Fig. 4f) and points away from the receptor. As a consequence, the extracellular loop 1 itself points outward from the receptor lumen. The 'flip-out' conformation results from the interaction of W102 with GM1, leading to an outward orientation of the tryptophan side chain, in a cholesterol-dependent manner.

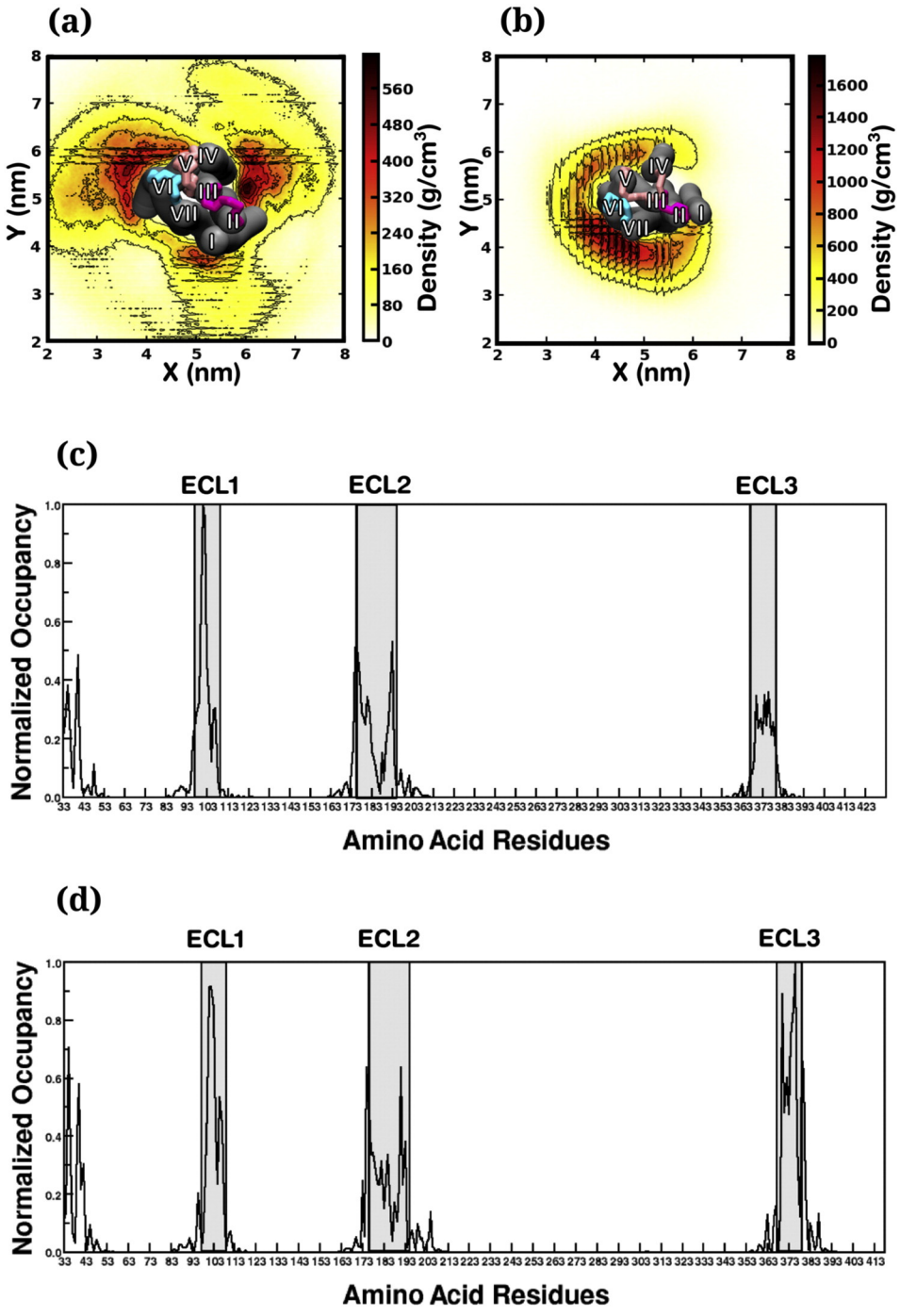

Fig. 2. GM1 occupancy around the serotonin$_{1A}$ receptor. Spatial density distribution of GM1 around the serotonin$_{1A}$ receptor in (a) POPC/GM1/chol and (b) POPC/GM1 bilayers. The spatial distribution corresponds to the *xy* membrane plane and is averaged over the *z*-axis along the extracellular leaflet. A top view of the receptor is superimposed on the density plot. Transmembrane helices are shown in gray and numbered accordingly. The extracellular loop 1 is shown in magenta, loop 2 in pink and loop 3 in cyan. The normalized maximum occupancy times of GM1 around each amino acid residue of the serotonin$_{1A}$ receptor in (c) POPC/GM1/chol and (d) POPC/GM1 bilayers are shown. The shaded regions correspond to the extracellular loops and are labeled. Data shown are averages over 10 sets of simulations and normalized for each system.

3.5. Identifying the effect of serotonin$_{1A}$ receptor on GM1 clusters

Sphingolipids (including GM1) and cholesterol have been implicated to form lipid 'microdomains', in which they demonstrate a preferential partitioning of certain lipid components [36–38]. To identify the effect of the receptor on the mixing of different lipid components, we calculated the 'preferential partitioning' of membrane components. Preferential partitioning has been previously used to characterize clustering of lipid species in multi-component bilayers [59,64]. We calculated the preferential partitioning for each molecule in POPC/GM1/chol bilayers in the presence and absence of the receptor (Table 1; see Methods for details). The values were calculated over the last microsecond of the simulation and averaged. As expected, GM1 exhibited the highest preference for interaction with itself ($p_A = 0.952$) resulting in the formation of large clusters. The preferential partitioning of GM1 with cholesterol was much lower ($p_A = 0.038$) and it exhibited the lowest preference for POPC. Interestingly, the self association of POPC appears to be more

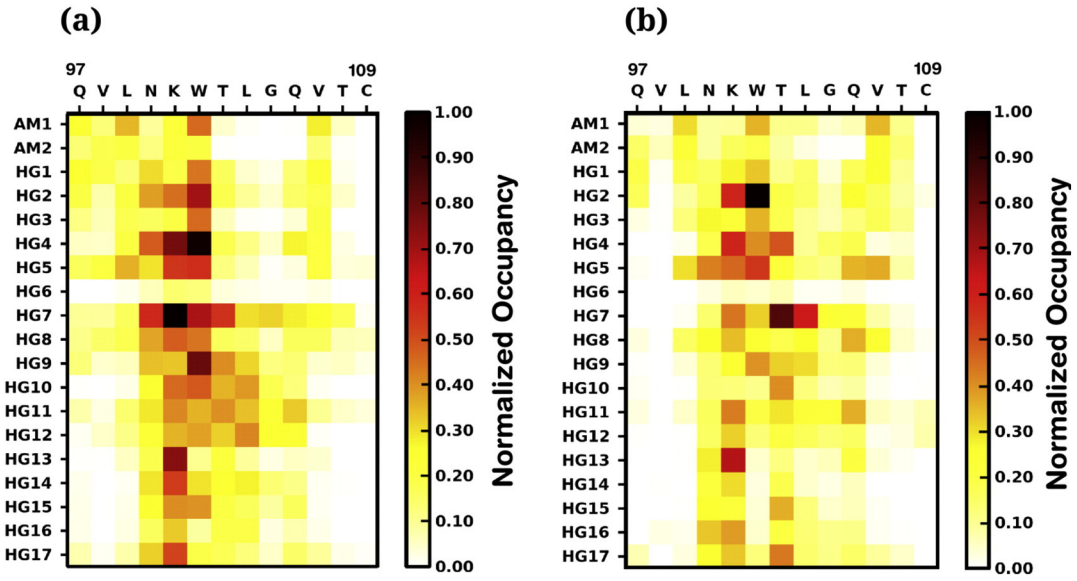

Fig. 3. Interaction sites of GM1 on the extracellular loop 1. The maximum occupancy of GM1 headgroup beads at amino acid residues 97–109 of the extracellular loop 1 in (a) POPC/GM1/chol and (b) POPC/GM1 bilayers. The maximum occupancy was calculated for the headgroup beads (HG1-HG17) and the sphingosine backbone beads (AM1 and AM2).

favorable ($p_A = 0.545$) than its association with other lipids. Cholesterol shows least preference for self association among the lipids ($p_A = 0.116$), suggesting that it seldom forms 'clusters' at these concentrations. In the presence of the receptor, the 'preferential partitioning' of the membrane components remained qualitatively similar. The preferential partition of the receptor (protein) was the highest with GM1 ($p_A = 0.557$), followed by cholesterol ($p_A = 0.294$), and relatively low preference for POPC ($p_A = 0.149$). The reduced interaction of POPC and cholesterol with the receptor, compared to GM1 is consistent with the radial distribution function of lipid species (Fig. S4). The first peaks for cholesterol and POPC are at the same distance (~0.5 nm) from the receptor surface as in GM1, but are much smaller in magnitude. Increased partitioning of the receptor with GM1 suggests that serotonin$_{1A}$ receptor preferentially localizes in GM1-rich regions. This effect could arise mainly from specific interaction between the receptor and GM1, since model peptides do not co-localize in sphingolipid-rich regions [59].

At the time scales of the simulations, cholesterol molecules exhibit both lateral as well as transbilayer diffusion in the membrane. Since GM1 and serotonin$_{1A}$ receptor demonstrated an increased association with cholesterol compared to POPC, we quantitated the effect of these interactions on the rate of transbilayer diffusion ('flip-flop') of cholesterol (Table S2). In POPC/chol bilayers, the average flip-flop rate of cholesterol was 0.06 flips/μs which increased to 0.09 flips/μs in the presence of receptor. In POPC/GM1/chol bilayers, cholesterol molecules showed more frequent transitions across the two leaflets. The average flip-flop rate increased to 0.11 flips/μs in absence of the receptor. In the presence of the serotonin$_{1A}$ receptor, cholesterol flip-flop rate did not exhibit appreciable variation (0.10 flips/μs).

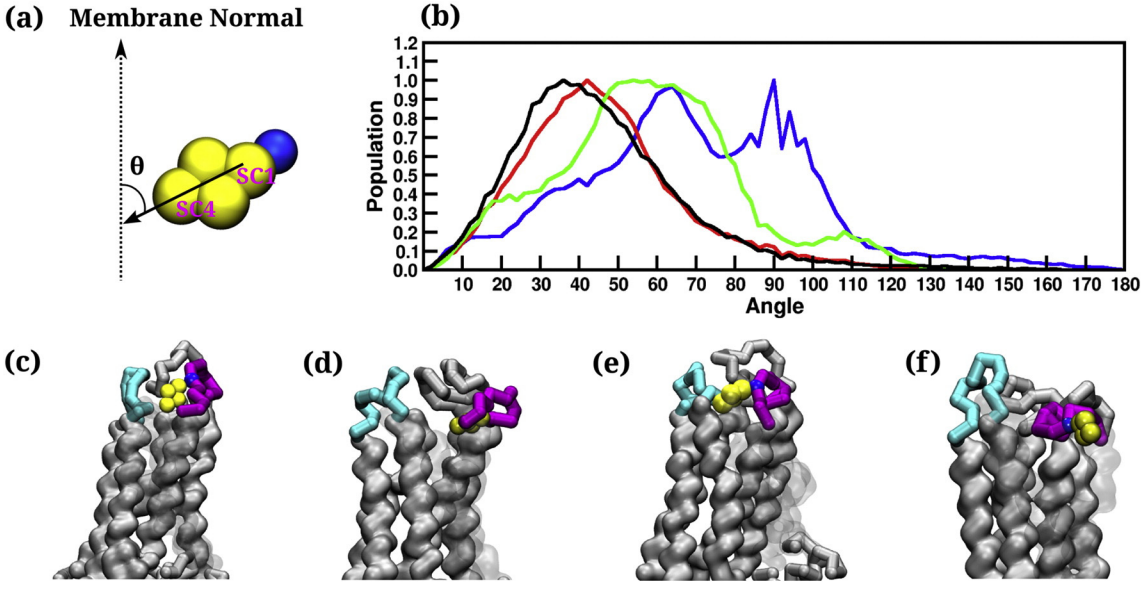

Fig. 4. Interaction of W102 with GM1. (a) A schematic representation of the orientation of W102 with respect to the bilayer normal. (b) The population distribution of W102 orientation in POPC (black), POPC/chol (red), POPC/GM1 (green) and POPC/GM1/chol (blue) bilayers. The schematic representation of the orientations of W102 in (c) POPC (d) POPC/chol (e) POPC/GM1 and (f) POPC/GM1/chol bilayers. Residue W102 is shown in van der Waals representation with its backbone in blue and side chain in yellow. The receptor is shown in gray. Extracellular loop 1 is highlighted in magenta and extracellular loop 3 is shown in cyan.

Table 1
Preferential partitioning of membrane components (p_A) in POPC/GM1/chol bilayers in the presence and absence of the serotonin$_{1A}$ receptor[a].

	Cholesterol	POPC	GM1	Receptor
With receptor:				
Cholesterol	0.194	0.348	0.345	0.113
POPC	0.375	0.420	0.131	0.074
GM1	0.047	0.011	0.911	0.032
Receptor	0.294	0.149	0.557	–
Without receptor:				
Cholesterol	0.116	0.404	0.480	
POPC	0.342	0.545	0.113	
GM1	0.038	0.010	0.952	

[a] See Methods for details.

4. Discussion

The interaction of GPCRs with membrane components assumes relevance in the context of GPCR biology. Membrane cholesterol has been shown to be crucial for the organization and function of a variety of GPCRs [12–15]. In this context, a number of structural features of membrane proteins have been suggested to be involved in preferential association with cholesterol [65,66]. One of the most studied motifs in membrane proteins, that exhibit sensitivity to cholesterol content, is the CRAC motif [65,67]. The CRAC motif was initially identified in peripheral-type benzodiazepine receptors [67], and was later identified in GPCRs such as rhodopsin, the β_2-adrenergic receptor, the serotonin$_{1A}$ receptor [68] and the human type I cannabinoid receptor [69]. Similarly, proteins that interact with (glyco)sphingolipids often appear to have a characteristic amino acid sequence, termed the 'sphingolipid-binding domain' (SBD) [47–50]. SBD has been identified in a number of proteins such as HIV-1 gp120, Alzheimer's beta amyloid peptide and the prion protein [47]. We have previously identified the SBD motif (LNKWTLGQVTC) in the serotonin$_{1A}$ receptor corresponding to amino acids 99 to 109 (see Fig. S1). This specific sequence contains the characteristic combination of basic (K101), aromatic (W102) and turn-inducing residues (G105), usually found in SBDs [48,70]. Interestingly, the SBD motif in the serotonin$_{1A}$ receptor overlaps with the CRAC motif in transmembrane helix II.

Sphingolipids constitute a small but essential constituent of the eukaryotic cell membrane that regulate several physiological processes [34,35]. They are reported to be required for endocytosis, protein sorting, ion conductance and GPCR function [42,43]. Several membrane proteins involved in these physiological processes have been reported to interact directly with sphingolipids [70]. For example, the nerve growth factor receptor tyrosine kinase has been shown to interact directly with gangliosides [71]. Structural motifs for sphingolipid interactions, such as the SBD [50] and the VXXTLXXIY signature sequence [72] have been identified. In this work, we have examined the interaction of GM1 with the serotonin$_{1A}$ receptor using coarse-grain molecular dynamics simulations. Our results demonstrate that GM1 binds to the predicted SBD motif in the extracellular loop 1 of the serotonin$_{1A}$ receptor. The sugar moiety of GM1 interacts with the aromatic residue W102, and flanking residues, K101 and T103. These results are in overall agreement with previous work which reported copatching of a fraction (~30%) of the serotonin$_{1A}$ receptor with GM1 [73].

We have previously shown that the serotonin$_{1A}$ receptor possesses a characteristic SBD motif that is conserved over natural evolution across various phyla among serotonin receptors [50]. However, experiments with SBD peptide derived from the receptor did not exhibit significant binding in model membranes, thereby pointing to the importance of the overall *context* of the receptor architecture. Since the extracellular loop 1 interacts with the sugar moieties of GM1, located above the membrane, it is possible that such an interaction mode is not feasible with the truncated peptide alone. An interesting aspect of the interaction site is that though it is independent of cholesterol, the presence of cholesterol allows a closer and more extended interaction of GM1 with the receptor (Figs. 2 and 3). Previous studies suggested that cholesterol increases interaction affinity between HIV-1 gp120 glycoprotein and the glycosphingolipid, globotriaosylceramide (Gb3) [74].

Our work suggests that interaction of the receptor with GM1 stabilizes a 'flip-out' conformation, in which the aromatic residue (W102) in SBD points away from the central lumen of the receptor and is exposed to the solvent. This conformation is dependent on the cholesterol-modulated GM1 distribution around the receptor (Fig. 4b). A similar outward-facing orientation of tryptophan residue has been reported in the crystal structure of Shiga-like toxin bound to an analogue of the Gb3 trisaccharide [75]. In a previous study, it was shown that the Trp residue is directed toward the central pore of the Shiga-like toxin pentamer, but on interaction with the carbohydrate domain, a conformational change occurs leading to increased solvent exposure [76]. It may be noted here that the Trp residue is structurally analogous to serotonin (5-hydroxytryptamine) [77], the natural ligand of serotonin$_{1A}$ receptor. It would be interesting to speculate whether the 'flip-out' conformation of the tryptophan could facilitate the entry of the endogenous ligand (serotonin). This is based on our earlier observation that metabolic depletion of sphingolipids or removal of sphingomyelin headgroup modulates ligand binding and downstream signaling of the serotonin$_{1A}$ receptor [78–80].

It is important to note here that the regulation of neuronal GM1 levels have been shown to be crucial, with any change in GM1 levels resulting in severe neurodegenerative disorders. For example, accumulation of GM1 due to the deficiency or malfunctioning of GM1-β-galactosidase (that catalyzes the hydrolysis of GM1) results in a neurodegenerative disorder called GM1-gangliosidosis, a class of sphingolipidoses (i.e., lysosomal sphingolipid storage diseases). GM1-gangliosidosis is an autosomal recessive disorder and to date, no successful treatment is available for this disease [81]. On the other hand, deficiency of GM1 has been shown to be implicated in Parkinson's disease [82,83], a neurodegenerative disorder primarily characterized by defective motor symptoms. However, it is often accompanied by depression, anxiety and dementia, which have been shown to be tightly correlated with modulation of neuronal serotonergic system [84]. In particular, the role of serotonin$_{1A}$ receptors in pharmacotherapy of Parkinson's disease has been reported [85]. In this context, our results showing specific interaction of GM1 with the serotonin$_{1A}$ receptor, an important neurotransmitter receptor, assume relevance and could contribute to our overall understanding of the molecular mechanism of such diseases.

Our results highlight the significance of the extracellular loop 1 in receptor function. Although the effect of membrane lipids on the transmembrane domain is beginning to be established, the interaction of loop regions with membrane lipids and its effect on regulating GPCR function is less understood. The importance of extracellular loops in regulating GPCR function, especially ligand access has been previously reported [11]. In adenosine A_{2B} receptor, specific amino acid residues in the extracellular loop 1 were found to be crucial for ligand binding [86]. Similarly, presence of specific residues at critical positions in the extracellular regions have been reported for other GPCRs and shown to be crucial for ligand binding and receptor activation [87–91]. In addition, lipid-mediated regulation of membrane protein function has been observed in dopamine transporter, where the N-terminus is involved in regulating substrate efflux [92,93]. These findings further strengthen the idea that extracellular regions regulate membrane protein function that could possibly be influenced by their interaction with membrane lipids.

In conclusion, we show here that GM1 binds to the serotonin$_{1A}$ receptor, specifically at the proposed SBD site, by performing multiple coarse-grain molecular dynamics simulations of the receptor in membrane bilayers with varying compositions of GM1 and cholesterol. Interaction of GM1 with the receptor at the SBD results in a conformational change of the tryptophan (W102) residue away from the central

lumen of the receptor, in a cholesterol-dependent manner. Our results suggest a direct role of GM1-GPCR interaction in modulating ligand binding and receptor function, and could provide novel insight in malfunctioning of neuronal GPCRs in neurodegenerative disorders involving GM1.

Conflict of interest

The authors declare no conflict of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2016.08.009.

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