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The influence of lipids and biological membranes on the conformational equilibria of GPCRs: Insights from NMR spectroscopy

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G protein-coupled receptors (GPCRs) function within cellular membranes, complex and dynamic environments. Rather than serving as a passive background, lipid membranes actively influence GPCR drug responses and signaling. Studies utilizing nuclear magnetic resonance (NMR) spectroscopy have revealed key insights into receptor–lipid interactions, enabled by the compatibility of NMR experiments with many different membrane systems and physiological temperature, conditions more closely reflecting the native cellular environment. NMR data have revealed new mechanistic insights that explain how specific lipids regulate GPCR activation, how bulk membrane properties influence receptor dynamics, and how different membrane mimetics affect GPCR behavior. These findings establish a framework for bridging *in vitro* structural studies with *in vivo* biological and pharmacological data.

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Introduction

G protein-coupled receptors (GPCRs) are crucial sensory integral membrane proteins. Earlier structural and biophysical studies focused on elucidating mechanisms of receptor–ligand and receptor–protein molecular recognition, specifically how extracellular ligands and intracellular signaling partners engaged the receptor [1–3]. More recently, attention has shifted toward the integral role of the membrane environment itself, particularly how lipid-receptor interactions modulate ligand binding and agonist-induced signaling [4]. A growing body of structural and functional evidence supports the view that lipids can profoundly influence GPCR activity, not only as allosteric or orthosteric modulators, but also through bulk membrane properties such as curvature [5] and fluidity. Insights into GPCR-lipid interactions provide a critical framework for bridging *in vitro* structural and pharmacological observations with the receptor's physiological functions *in vivo*.

NMR spectroscopy has a long-standing history studying biological membranes and lipid–protein interactions, with pioneering investigations of lipid bilayers dating back over five decades [6–8]. More recently, NMR has emerged as a powerful tool for elucidating the mechanisms of G protein-coupled receptor (GPCR) signaling by enabling the observation of multiple conformational states in equilibrium and revealing how small-molecule ligands modulate these conformational landscapes, as has been reviewed [9–13]. Important advantages of NMR include the flexibility to study membrane proteins in a wide range of membrane or membrane-mimetic systems and at physiologically relevant temperature. GPCR studies have increasingly leveraged these strengths to uncover new mechanistic insights into how lipids and biological membranes influence receptor conformational equilibria.

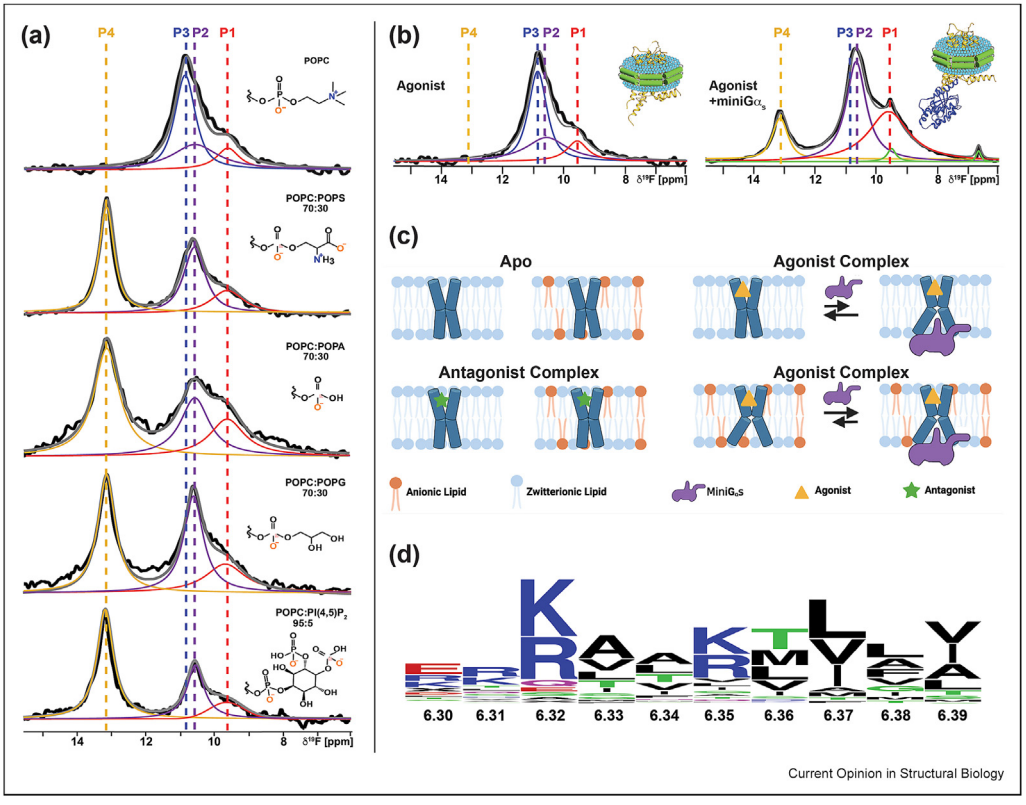
This review highlights recent contributions from NMR spectroscopy to our current understanding of how biological membranes and lipids influence GPCR signaling. We discuss NMR studies that directly probe GPCRs as well as investigations into the effects of membrane environments on GPCR interactions with their partner signaling proteins. Examples from the literature illustrate the application of diverse NMR methodologies, including ^{19}F NMR and multi-dimensional experiments enabled by various stable-isotope labeling strategies. Insights derived from both solid-state and solution NMR approaches are discussed, with an emphasis on unique strengths of each method. Collectively, these studies underscore how NMR has advanced our molecular understanding of GPCR function by revealing how specific lipid species and bulk membrane properties influence receptor activation, modulate interactions with effector proteins, and affect the interpretation of GPCR activity in membrane mimetic systems.

Defining mechanisms underlying the impact of specific lipids on GPCR signaling

Data from structural and biophysical literature increasingly highlight the important roles of specific lipids, including tissue-specific lipids and lipids with distinct chemical properties, as modulators of GPCR activity [14,15]. A compelling example involves the fatty acids docosahexaenoic acid (DHA) and arachidonic acid (ARA), which modulate the activity of the human A_{2A} adenosine receptor ($A_{2A}AR$), a class A GPCR that regulates dopamine release. DHA and ARA together constitute ~14 % of the total lipid content in the mammalian brain striatum, a region where $A_{2A}AR$ is extensively expressed [16]. Shimada and coworkers reported distinct changes in 2D heteronuclear correlation spectra of [[α,β,β-^{2}H,methyl-^{13}C] Met,u-^{2}H] $A_{2A}AR$ reconstituted in lipid nanodiscs with and without DHA [17]. Spectral changes were most pronounced near the receptor intracellular surface, particularly at labeled sites in transmembrane (TM) helices 3 and 6. Based on these and additional NMR data from the same study, the authors proposed that DHA shifts the $A_{2A}AR$ conformational equilibrium to populate active conformational states promoting signaling complex formation, offering a mechanistic rationale for DHA-enhanced $A_{2A}AR$ activity [17].

In a separate study utilizing ^{19}F NMR spectroscopy of $A_{2A}AR$ in lipid nanodiscs, Eddy and colleagues investigated the mechanisms by which anionic lipids influence the activity of human $A_{2A}AR$ (Figure 1) [18]. One key finding was the shift in the conformational ensemble of agonist-bound $A_{2A}AR$ to favor a larger population of an active state in lipid nanodiscs containing a binary mixture of zwitterionic and anionic lipids. In contrast, $A_{2A}AR$ in lipid nanodiscs without anionic lipids showed significantly reduced population of the active conformation, consistent with a decrease in nucleotide exchange in the same conditions [18]. By integrating NMR spectroscopy, functional assays, and computational modeling and simulations, the authors proposed a mechanism in which anionic phospholipids interact with

Figure 1



Insights from NMR into the impact of anionic lipids on the conformational equilibria of the human A_{2A} adenosine receptor ($A_{2A}AR$). **(a)** The 1D ^{19}F NMR spectra of agonist-bound human $A_{2A}AR$ in lipid nanodiscs containing different defined mixtures of lipids. The yellow peak labeled P4 is an active conformational state observed in the presence of anionic lipids but missing in spectra of the agonist-bound receptor in nanodiscs containing only zwitterionic lipids. **(b)** ^{19}F NMR spectra of agonist-bound $A_{2A}AR$ in nanodiscs containing POPC. The active conformational state P4 is observed upon addition of an engineered $G\alpha_S$ protein, however the population of this state is lower than when in the presence of anionic lipids. **(c)** Schematic of the impact of anionic lipids on the conformational equilibria of apo, antagonist-bound, and agonist-bound $A_{2A}AR$, showing that anionic lipids populate an active-like conformation of the receptor that recognizes the G protein. **(d)** Conservation of residues at the intracellular end of TM VI among 290 class A GPCRs. The size of each amino acid corresponds to its frequency of occurrence at that position. Numbers indicate the residue positions in the Ballesteros-Weinstein nomenclature. The figures in all panels were adapted from reference 18, with permission.

a cluster of charged amino acids near the intracellular surface of the receptor [18]. This cluster also engages with the C-terminal region of G proteins, thereby promoting G protein recognition and the formation of ternary signaling complexes. These charged residues, located at positions 6.32 and 6.35 (numbers refer to the Ballesteros-Weinstein nomenclature [19]) were found to be highly conserved not only among class A GPCRs, but across all GPCR classes (Figure 1), suggesting a potentially general mechanism for receptor–lipid interactions that regulate signaling activity [18].

Ziarek and co-workers used $^{13}C^{\varepsilon}H_3$-methionine NMR to investigate the impact of the anionic lipid phosphatidylinositol-4,5-bisphosphate (PIP_2), a key modulator of GPCR signaling, on the conformational dynamics of the neurotensin receptor 1 (NTS1) [20]. They found that PIP_2 interactions with the intracellular surface of NTS1 allosterically modulated conformational dynamics at the receptor orthosteric binding pocket and conserved activation hot spots [20]. A subsequent NMR study of NTSR1 showed that zwitterionic lipids stabilized the secondary structure of the amphipathic helix 8 but detergents did not [21].

Huster and colleagues have conducted solid-state NMR studies of the neuropeptide Y2 receptor (NPY2R), including comparative analyses of receptor conformational dynamics in membrane environments composed of saturated DMPC lipids [22] and monounsaturated POPC lipids [23]. A key finding was that NPY2R exhibited greater rigidity when reconstituted in POPC membranes compared to DMPC membranes, suggesting increased receptor order correlated with either longer lipid acyl chains or lipid saturation and may be associated with enhanced receptor helical content [23].

Mechanistic insights into cholesterol-mediated regulation of GPCR signaling

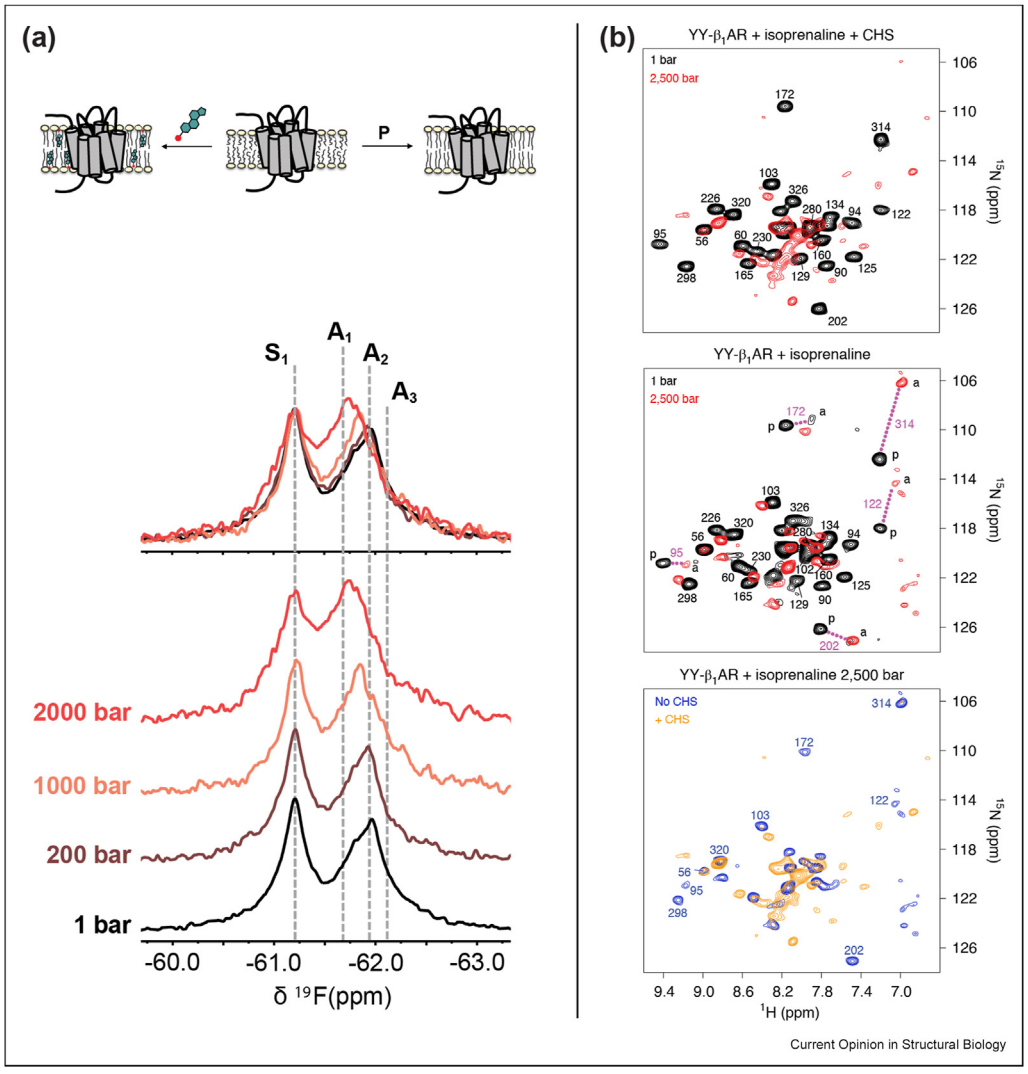
Because of its well-documented role in regulating membrane fluidity and its frequent association with GPCRs in crystal and cryo-EM structures, cholesterol has been a special focus in lipid-receptor NMR studies. Cholesterol–receptor interactions are complex, with cholesterol and its analogs thought to act as either agonists or antagonists. These multifaceted roles have also been investigated through computational simulations [24–27]. A key unresolved question remains whether cholesterol influences GPCR function through direct receptor–cholesterol interactions or indirectly by altering the bulk physical properties of the membrane. Utilizing ^{19}F NMR spectroscopy with the human $A_{2A}AR$, Prosser and co-workers observed that cholesterol behaved as a weak allosteric modulator, enhancing $A_{2A}AR$ signaling activity [28] (Figure 2a). These findings were further supported by ^{19}F NMR experiments recorded with increasing applied pressure, which mimicked the ordering effects of cholesterol on the lipid bilayer. With increasing pressure, ^{19}F NMR data showed a shift in the $A_{2A}AR$ conformational ensemble that favored an increased population of an active state [28], consistent with observations that increased membrane order enhanced $A_{2A}AR$ nucleotide exchange [28].

In a subsequent ^{19}F NMR study of $A_{2A}AR$ in lipid nanodiscs, Eddy and colleagues proposed a model of cholesterol's influence on $A_{2A}AR$ activity through both indirect influence on membrane properties and direct receptor–cholesterol interactions [29]. In the absence of anionic lipids, cholesterol and cholesterol analogs significantly increased the population of the $A_{2A}AR$ active conformational state. Cholesterol-driven activation occurred even for nanodiscs containing only 1–2 molecules of cholesterol per nanodisc, supporting the idea that specific receptor–cholesterol interactions influenced receptor activation [29]. However, in the presence of anionic lipids, the impact of cholesterol on $A_{2A}AR$ activation was marginal, suggesting that in these conditions, cholesterol's impact may be more indirect [29], aligning with similar conclusions by Prosser and colleagues [28]. In a separate study of the β_2-adrenergic receptor (β_2AR), saturation-transfer NMR experiments by Milon and colleagues indicated that β_2AR interacted with cholesterol but not with the structurally similar molecule ergosterol [30], suggesting that GPCR-cholesterol interactions were specific for β_2AR.

The influence of the cholesterol analog cholesterol hemisuccinate (CHS), which is frequently used in GPCR structural and biophysical studies, was investigated in NMR experiments with ^{15}N-Val labeled β_1-adrenergic receptor (β_1AR) [31] (Figure 2b). Two-dimensional TROSY NMR spectra of β_1AR showed that without CHS, increasing pressure shifted the β_1AR conformational equilibrium toward an active conformation. Addition of CHS blocked pressure-induced β_1AR activation, supporting the idea that CHS acted as a negative allosteric modulator of β_1AR signaling. This result suggested that even among class A receptors, different GPCRs may be influenced by cholesterol and cholesterol analogs in surprisingly different ways.

The influence of cholesterol on the chemokine receptor CCR3 has also been explored. Functional studies by Wylie and colleagues demonstrated that increasing cholesterol concentrations in SMALPs and lipid vesicles enhanced both GTP hydrolysis and binding of the chemokine CCL11 [32]. Follow-up modeling and multi-dimensional NMR studies using uniformly ^{15}N,^{13}C-labeled CCR3 revealed cholesterol-induced alterations in the conformation and dynamics of extracellular loop 2 (ECL2), ECL3, and the transmembrane helices [33], offering mechanistic insights into how cholesterol modulates CCR3 function through conformational changes.

Figure 2



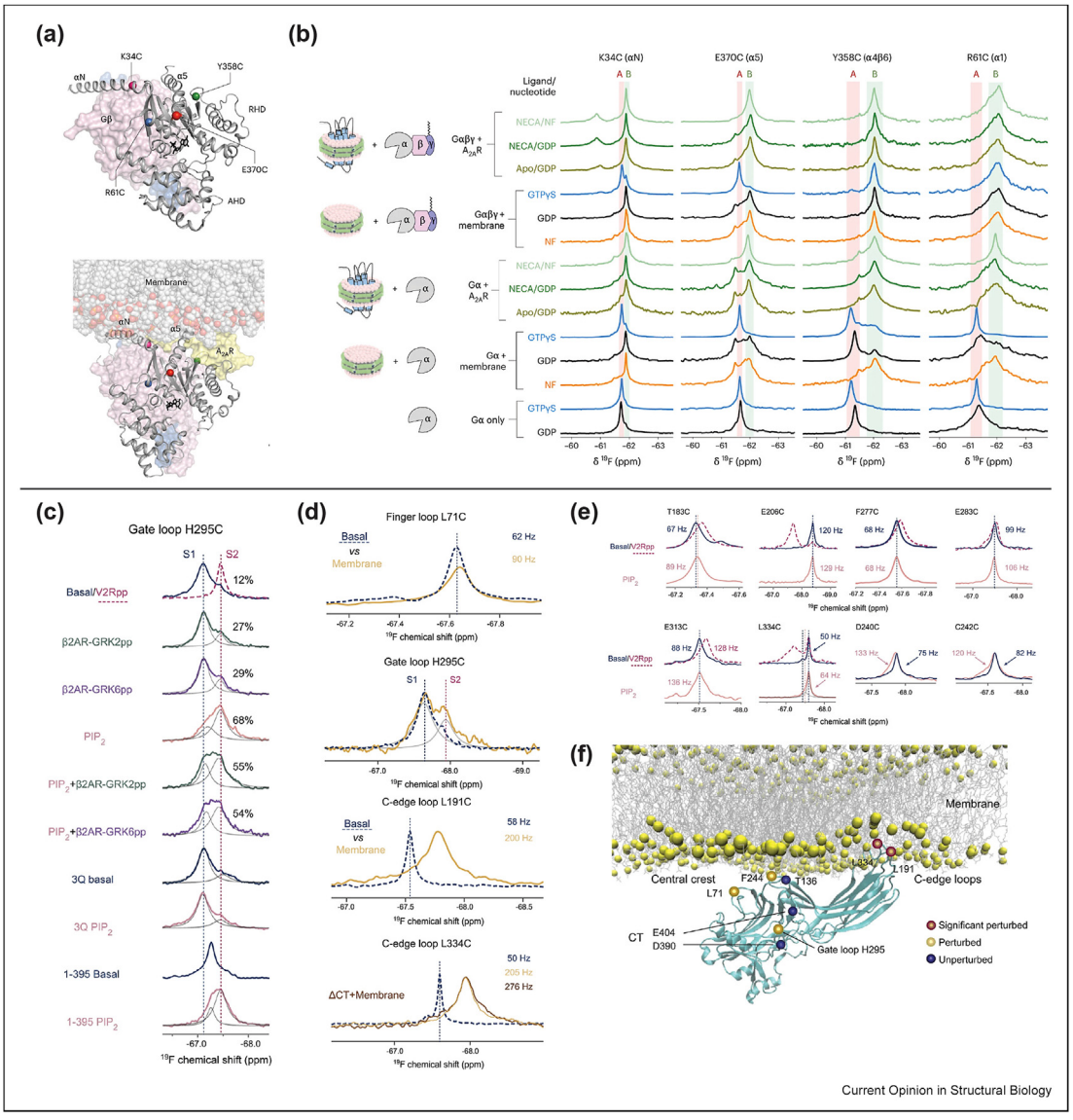
Changes in lipid bilayer properties, driven by the presence of cholesterol or applied external pressure, manifested as NMR-observed changes in GPCR conformational equilibria. **(a)** ^{19}F NMR observations of the impact of applied external pressure, which is used to modulate bulk properties of lipid membranes. One-dimensional ^{19}F NMR spectra of A_{2A}AR, labeled at position V229C, in lipid nanodiscs recorded over a range of increasing applied pressures. Functional states are annotated and indicated by the grey dashed lines. Increasing pressure increased the population of active conformational ensembles, as indicated by the increase in signal intensity for the state A1. **(b)** NMR observations of the impact of pressure and CHS on the conformation and structural plasticity of β_1AR, as observed in superimposed 2D [^{15}N,^{1}H]-TROSY spectra. Resonance assignments are annotated on the spectra. The dashed lines connect residues with two resonances observed for a preactive state, 'p', and active conformational state, 'a'. Panels **a** and **b** adapted from references 24 and 27, respectively, with permission.

Elucidating the effects of lipid membranes on GPCR recognition and interactions with partner signaling proteins

Evidence from structural, biophysical, and functional studies [34–37] support the idea that lipid membranes play an active role facilitating and controlling interactions of receptors with partner signaling proteins, especially with G proteins and β-arrestins. Using ^{19}F NMR spectroscopy, Prosser and co-workers investigated conformational changes with the stimulatory G protein α subunit, $G\alpha_S$, upon interacting with phospholipid membranes and A_{2A}AR (Figure 3a and b) [38]. One of the key results from this work showed that the N-terminus of $G\alpha_S$ interacted with the lipid bilayer even in the absence of a palmitoylated $G\alpha_S$ N-terminus, driving conformational changes at additional locations in $G\alpha_S$ that facilitated complex formation with A_{2A}AR [38].

NMR studies have also investigated the role of phospholipid membranes on the interactions between receptors and β-arrestins and especially arrestin-2, also

Figure 3



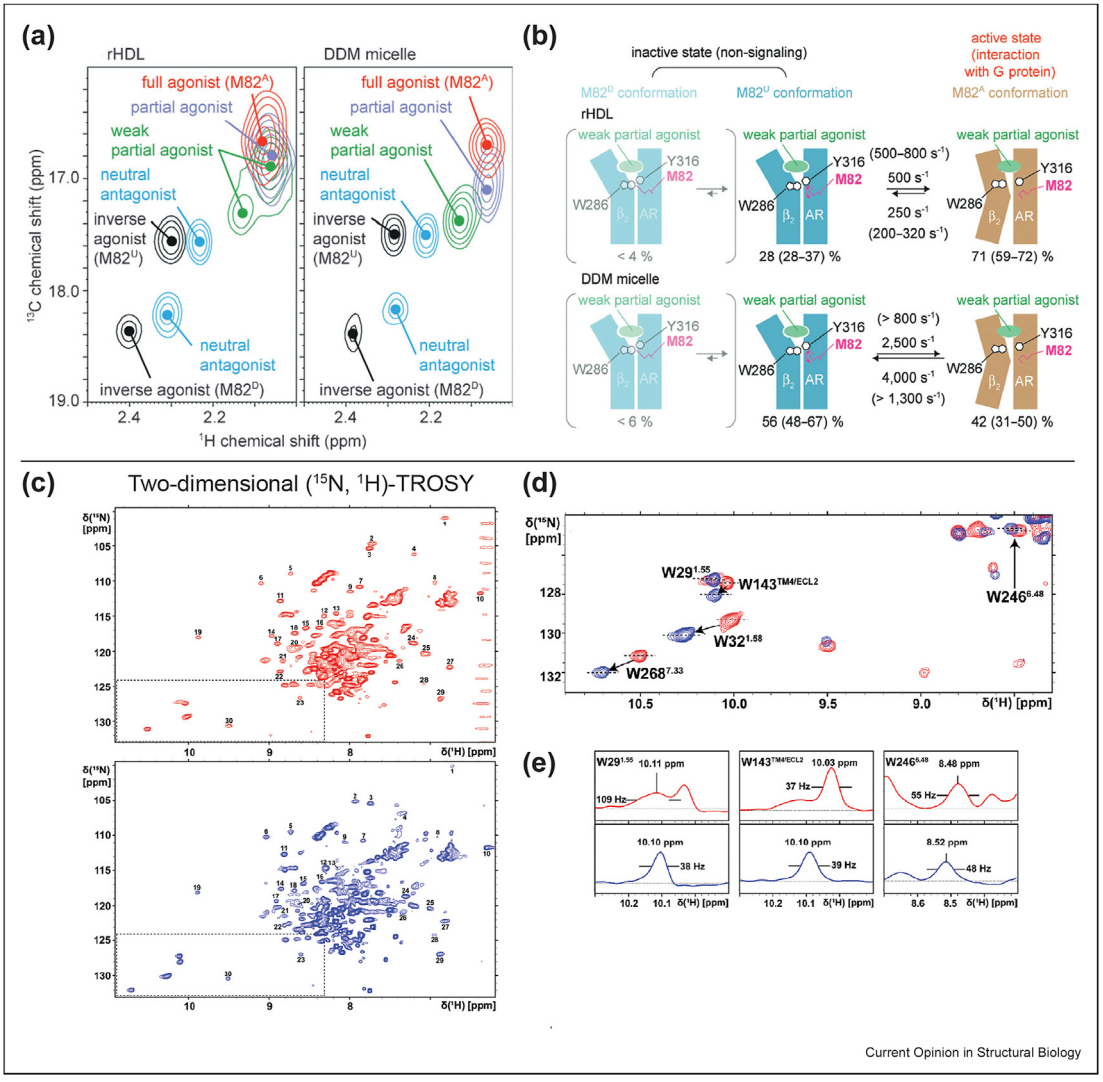
The influence of membrane lipids on GPCR interactions with partner proteins as viewed by ^{19}F NMR. **(a)** Crystal structure of the trimeric G_S protein in complex with GDP. $G\alpha_S$ is shown in ribbon representation with several sites selected for ^{19}F NMR labeling shown as colored spheres and annotated with the residue numbers. Gβ and Gγ are shown in pink and blue surface representations, respectively. **(b)** The conformational landscape of $G\alpha_S$ viewed by ^{19}F NMR for $G\alpha_S$ labeled at several distinct single cysteine positions. Experimental conditions included $G\alpha_S$ in the presence of lipid nanodiscs, denoted as "membrane", and lipid nanodiscs containing A_{2A}AR. Horizontal lines indicate two global conformational states observed in all spectra. **(c)** NMR spectra of ^{19}F-labeled at position H295C in the gate loop of βarr1. Spectra were recorded in different conditions, including in the presence of the lipid PIP_2. **(d)** NMR spectral comparison of several ^{19}F-labeled sites of βarr1 in the presence and absence of lipid nanodiscs, labeled as "Membrane". **(e)** ^{19}F NMR spectra showing the impact of PIP_2 interactions with βarr1. **(f)** Crystal structure of βarr1 with positions viewed by ^{19}F NMR shown as circles, which are colored according to the extent of perturbation to the NMR signals due to the presence of lipid nanodiscs. Panels **a** and **b** adapted from reference 34, and **c**–**f** adapted from reference 35, with permission.

known as βarr1. A ^{19}F NMR study by Hu and coworkers observed distinct changes in the conformation of βarr1 upon interaction with PIP_2 and the formation of an intermediate conformational state, which was proposed to be an activation mechanism distinct from interactions with the phosphorylated receptor C-terminus (Figure 3c–f) [39]. Shimada and coworkers utilized 2D HMQC NMR to observe deuterated and selectively isoleucine-methylated βarr1 in the presence of lipid nanodiscs and a chimeric version of the β_2AR, finding that changes in NMR spectra were not observed for arrestin in the presence of lipids in nanodiscs without receptor, but that lipids were necessary for facilitating complex formation with the chimeric receptor [40].

Delineating the influence of membrane mimetic systems on GPCR structure and conformational dynamics

The practical challenges associated with purifying membrane proteins for structural and biophysical studies have driven the development of a variety of membrane and membrane-mimetic systems, including detergents, nanodiscs, and lipid vesicles. These systems aim to provide a stable environment that preserves the native structure and function of membrane proteins, but it is increasingly appreciated that these systems can unintentionally impact the structures and conformational dynamics of membrane proteins. An earlier study by Prosser and coworkers compared the conformational equilibria of β_2AR between two different detergent systems, concluding that the choice of detergent exhibited a significant influence on the rates of exchange among different β_2AR conformational states in ^{19}F NMR spectra [41]. Shimada and colleagues compared the conformational equilibria of β_2AR between DDM micelles and lipid nanodiscs (Figure 4a) [42]. In two-dimensional [^{13}C,^{1}H]-HMQC spectra of selectively

Figure 4



NMR comparison of the impact of different membrane mimetic environments on GPCR conformation and structural plasticity. **(a)** Comparison of 2D [^{13}C,^{1}H]-HMQC spectra of selectively deuterated and methyl-^{13}C-Met β_2AR complexes with ligands of different efficacies in reconstituted high-density lipoproteins (rHDL), i.e., lipid nanodiscs, and DDM detergent micelles. NMR resonances from M82 of different β_2AR–ligand complexes are superimposed. **(b)** Schematic comparing the β_2AR conformational states and rates of conformational exchange observed by NMR between rHDL and detergent micelle preparations. Conformations with populations less than 10 % are shown in fainter colors. **(c)** Two-dimensional [^{15}N,^{1}H]-TROSY spectra of an A_{2A}AR complex with an antagonist in LMNG detergent micelles (red) and lipid nanodiscs (blue). **(d)** Superimposed expanded 2D sections from the dashed boxes shown in (c). Assigned tryptophan residues are annotated. **(e)** One-dimensional cross sections taken from the plots shown in (d) comparing the chemical shifts and line widths of highlighted tryptophan residues between detergent and lipid nanodisc preparations. Panels **a** and **b** adapted from reference 38, and panels **c**–**e** adapted from reference 40, with permission.

deuterated and methyl-^{13}C-Met labeled β_2AR, they observed changes in the chemical shifts and relative intensities of methionine signals between detergent and nanodisc preparations for complexes of β_2AR with different ligands. These differences reflected variations in the rates of exchange among conformational states that depended on the surrounding environment (Figure 4a) [42]. Lefkowitz, Prosser and colleagues observed differences in the conformational equilibria of β_2AR in ^{19}F NMR spectra comparing detergent and lipid nanodisc preparations [43], which were attributed to differences in the constitutive activity levels of the receptor between different environments.

The conformation of antagonist-bound A_{2A}AR was compared between LMNG/CHS and lipid nanodisc preparations in a study by the Wüthrich group (Figure 4b) [44]. Two-dimensional TROSY spectra of [u-^{15}N, ~70 % ^{2}H]-A_{2A}AR in complex with ZM241385 were similar between LMNG/CHS and nanodisc preparations, suggesting that the receptor adopted comparable conformations in these two environments (Figure 4b). The Wüthrich group further assessed the conformational equilibria of A_{2A}AR between detergent and lipid nanodisc preparations using ^{19}F NMR, concluding that the conformational ensemble of A_{2A}AR measured at 25 °C in lipid nanodiscs more closely resembled that observed in LMNG/CHS than in DDM/CHS micelles [45].

Eddy and colleagues used ^{19}F NMR to compare the conformational equilibria of A_{2A}AR between detergent micelle and lipid nanodisc preparations, including a systematic comparison of the conformational equilibria measured over a range of temperatures [46]. An important result from this study was that the active state population of A_{2A}AR observed in ^{19}F NMR spectra quantitatively correlated with measured cAMP data across a range of ligands with different efficacies for A_{2A}AR in lipid nanodiscs measured at physiological temperature but not for A_{2A}AR in DDM/CHS detergent. In a subsequent study, Eddy and coworkers used magic angle spinning (MAS) solid-state NMR to observe the conformational equilibria of A_{2A}AR in lipid vesicles of defined compositions [47]. Comparison of the conformational equilibria of A_{2A}AR between lipid nanodiscs, detergent micelles, and vesicles showed similar conformational equilibria for antagonist-bound A_{2A}AR but different relative populations for the agonist-bound conformational ensemble in the different membrane systems [47], suggesting that different bulk membrane properties may impact GPCR conformational equilibria.

Conclusions and outlook

NMR studies have provided compelling evidence that lipids and lipid membranes significantly influence the conformational equilibria of GPCRs and their interactions with partner proteins. To date, NMR studies of GPCR-lipid interactions have mostly focused on class A receptors, and exciting opportunities will arise by studying receptors from additional classes. NMR experiments are capable of more precisely characterizing lipid-interacting sites on receptors and quantitatively measuring the lifetimes of lipid-receptor interactions, following related work with channel proteins [48–50]. Future studies leveraging these capabilities could provide insights that support the intriguing possibility of targeting lipid-interacting sites with small molecule allosteric modulators.

Improved understanding of how lipids and lipid membranes affect receptor signaling will likely drive improvement of pharmacological applications, for example by clarifying how cellular membrane environments influence the measurement of drug efficacy or enabling identification of novel small molecules. A recent application of high-resolution magic angle spinning (HRMAS) NMR with A_{2A}AR in unpurified cell membranes demonstrated that native cell membrane environments can improve sample stability and small molecule ligand screening in more physiologically relevant conditions [51]. The NMR studies of lipid-receptor interactions reviewed here lay the foundation for the long-term goal of investigating GPCR structure and dynamics directly within the native cellular environment. Achieving this goal will likely benefit from breakthroughs that improve signal-to-noise in NMR experiments, including through dynamic nuclear polarization (DNP) NMR, which has been utilized to demonstrate the feasibility of probing structure and dynamics of bacterial membrane proteins within their native contexts [52–54].

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

No data was used for the research described in the article.

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** of outstanding interest

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