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Uncovering the intimate relationship between lipids, cholesterol and GPCR activation

Joanne Oates and Anthony Watts

The membrane bilayer has a significant influence over the proteins embedded within it. G protein-coupled receptors (GPCRs) form a large group of membrane proteins with a vast array of critical functions, and direct and indirect interactions with the bilayer are thought to control various essential aspects of receptor function. The presence of cholesterol, in particular, has been the focus of a number of recent studies, with varying receptor-dependent effects reported. However, the possibility of specific cholesterol binding sites on GPCRs remains debatable at present. A deeper structural and mechanistic understanding of the complex and delicately balanced nature of GPCR–bilayer interactions has only been revealed so far in studies with the non-ligand binding, class A GPCR, rhodopsin. Further investigations are essential if we are to appreciate fully the role of the bilayer composition in GPCR activation and signalling; indeed, recent improvements in GPCR expression and purification, along with development of novel reconstitution methods should make these types of biophysical investigations much more accessible. In this review we highlight the latest research on GPCR–membrane interactions and some of the tools available for more detailed studies.

Address
Biomembrane Structure Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

Corresponding author: Watts, Anthony (anthony.watts@bioch.ox.ac.uk)

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Introduction

Our understanding of the structure and function of G protein-coupled receptors (GPCRs) has increased markedly over the past few years due, not least, to innovative purification strategies, which have facilitated structure determination by X-ray crystallography. Of particular note is the work with the β_1 and β_2 adrenergic (β_2AR) and adenosine A2A receptors where high-resolution structures have been reported with a variety of bound ligands, both agonist and antagonist, natural and synthetic [1,2,3•,4–6]. These structures have revealed key features of the ligand binding pocket and the specificity of ligand receptor interactions; critical information in the design of new pharmaceuticals targeted to GPCRs. Crystal structures, in addition to other experimental data, have also begun to inform on the conformational changes that take place during receptor activation. In this case rigid-body movements and rotations of the transmembrane helices are responsible for changes which allow favourable interaction with G proteins [3•,7,8]. Despite this recent explosion in knowledge, understanding the influence of the bilayer environment on the structure, activation, and signalling of a GPCR is somewhat less advanced. A number of crystal structures have revealed tantalising glimpses of specifically bound lipid and other membrane components [1,2,9••,10]. However, care must be taken when interpreting these data since any conclusions about biological relevance must also include *in vivo* and functional data to support crystallographic observations.

Bilayer components have been known to alter the physiological profiles of a vast number of membrane proteins for some while [11–13], either through specific interactions with the protein itself or alteration of membrane physical properties such as curvature, lateral pressure, and bilayer thickness (reviewed in [14]). In the case of GPCRs, the available evidence also points to a clear link between receptor structure and function, and membrane location and composition (Figure 1 summarises the possible mechanisms by which GPCRs are influenced by the membrane). Until recently, the role of the bilayer in GPCR signalling had been, to a certain extent, under-investigated; the exception is early studies with rhodopsin, which showed no lipid-type specificity but rather regulation by cholesterol [13,15–17]. Rhodopsin remains the model GPCR and is largely responsible for our current understanding of GPCR–bilayer interactions. The possibility of a close relationship between cholesterol and GPCRs in general has drawn much attention recently, as new evidence from both functional and structural studies has come to light for a number of receptors, in addition to rhodopsin. One possible role of cholesterol may be to target receptors to specific membrane regions where co-localisation with down-stream signalling components occurs. In fact, the *in vivo* environment should always be considered when investigating membrane–GPCR interactions. In this review we discuss lipid–GPCR interactions focusing specifically on rhodopsin, the association of GPCRs and cholesterol, and the possibility of membrane-domain targeting *in vivo*. We highlight recent progress, areas of controversy, and where more research is needed. The technical challenges and most appropriate methodologies for further investigations will also be considered.

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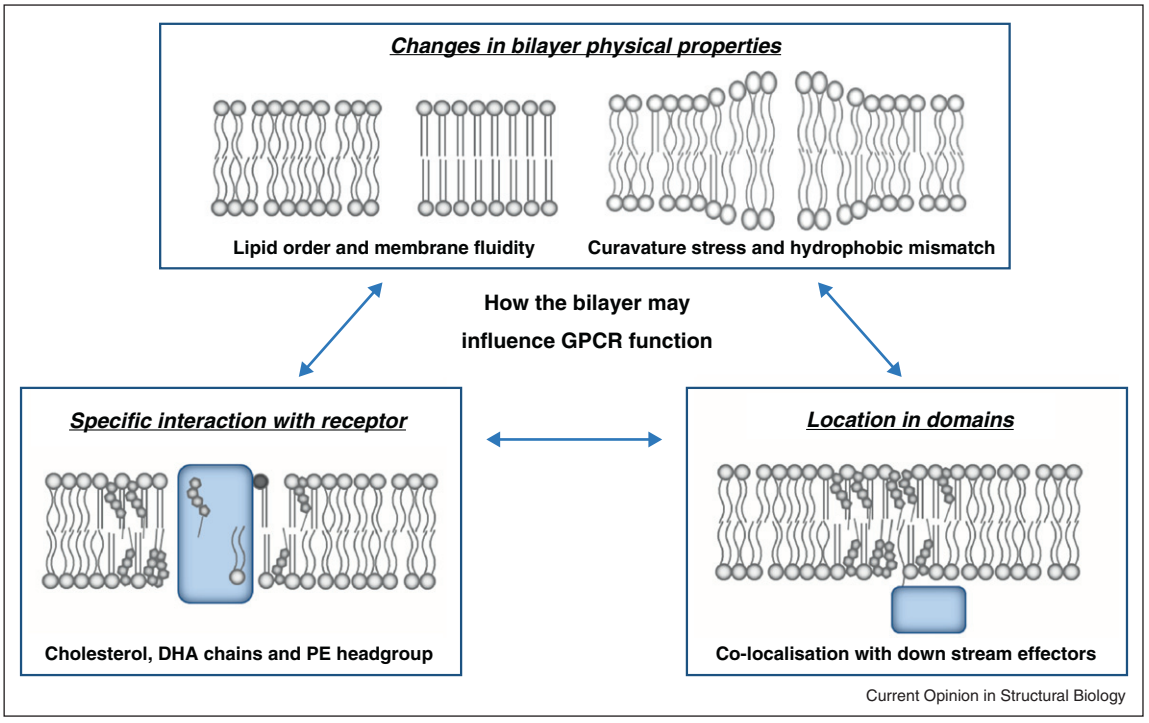
Figure 1



GPCR–bilayer interactions. GPCR activation and signalling can be modulated by the surrounding membrane environment via a number of mechanisms. Alterations in lipid composition can affect physical properties of the bilayer including curvature and fluidity, while specific interactions between bilayer components and a GPCR have also been demonstrated. Receptor targeting to lipid domains may be important in regulation of downstream signalling.

The *in vivo* environment of GPCRs

GPCRs are distributed throughout many tissue types and subcellular compartments. The lipid composition of membranes within these regions can vary considerably and therefore knowledge of the particular location of a GPCR is the first step in understanding the influence of the bilayer (for a review of cellular lipid distribution see [18]). Moreover, alterations in the composition of an individual bilayer are believed to control many aspects of GPCR signalling (see examples in the following sections). With this in mind, *in vitro* investigations of GPCRs, particularly those in detergent, must also include suitable assays to determine biological functions such as ligand binding [19] and G protein coupling in the non-native lipid/detergent environment. Furthermore, differences between detergent and native-like lipid environments have been shown to modify receptor–receptor associations [20••].

One proposed mechanism by which the membrane controls GPCR function is through targeting to specific regions of the bilayer which form highly ordered domains, also known as lipid rafts and caveolae [21–23]. Although the precise nature of such domains remains hotly debated there are several lines of evidence which link GPCR regulation with these structures. Firstly, fatty acid modifications, such as palmitoylation of cysteine residues in the C-terminal helix 8, have been suggested to address receptors to ordered domains [24], influencing receptor activity, G protein coupling, and trafficking; effects well documented for the serotonin and oxytocin receptors [25] (reviewed in [26,27]). Indeed, the non-random distribution of Gα subtypes in the membrane is believed to be a key factor imparting specificity on activation of downstream signals by the oxytocin receptor [27]. Secondly, the intimate association between the lipid-ordering cholesterol and GPCRs, which will be explored further below; and finally, the isolation of GPCRs with detergent-resistant membranes, and other proposed raft components, for example, the association of β_2AR with caveolin 3 in myocyte membranes [28]. The specific influence of receptor targeting and modifications are highly varied with new studies being published at pace. Investigations with the mu and delta opioid receptors showed a differing response to the removal of cholesterol, linked to differential membrane localisation [29•]. The most recent data show palmitoylation of the dopamine D1 receptor is linked to changes in receptor internalisation and turnover [30]; and leads to upregulation of human proteinase-activated receptor 2 [31].

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Whatever emerges on the nature of ordered lipid domains, it is clear that the membrane environment impacts heavily on function; the hypothesis that specific properties of this environment result in co-localisation with downstream signalling components is attractive, and goes a long way to providing a rationale for regulation and specificity in GPCR activation. The mechanisms by which membrane properties control activation are however rather ill-defined, although it seems likely to involve either indirect bilayer effects, specific membrane–GPCR interactions, or a combination of both.

Rhodopsin–membrane interactions

A detailed understanding of the interaction between the membrane environment and GPCR activation is best characterised for rhodopsin (the details of which have been reviewed recently elsewhere [32]). Conformational changes between the MI and MII states in rhodopsin are tightly regulated by physical properties of the membrane in addition to possible direct membrane–receptor interactions. In terms of bilayer properties, current data imply a role for lipids with a phosphatidylethanolamine (PE) head-group, those containing unsaturated acyl chains of docoshexanoyl acid (DHA (22:6)) and cholesterol [16,33,34••]. Flash photolysis studies first showed that the equilibrium between the two states is shifted towards MII due to the presence of lipid with a PE head-group [35]. This lead to the proposal of the flexible surface model [36], in which negative curvature stress between the membrane and protein is matched by the presence of PE lipid. Further experiments using plasmon waveguide resonance (PWR) also demonstrated that an increased PE content correlates with an increase in affinity of the receptor for transducin [37]. A combination of molecular dynamics (MD) simulations and NMR experiments suggest that the highly flexible DHA chains penetrate deeper into the protein and lower the energy barrier for transition to the MII state [33,34••,38–40]. Depletion of DHA has also been shown to affect rhodopsin function in a biological context [41]. Cholesterol inhibits activation probably by reducing acyl chain flexibility and the free volume in the core of the bilayer [15,42] in addition to promoting association with ordered lipid domains.

While it remains clear that bilayer characteristics influence the rhodopsin photocycle, a direct interaction between lipid and protein is less clear. Early work using nitroxide spin label electron spin resonance (ESR), showed no preferential binding between membrane components of differing type (phosphatidylcholine, cardolipin and stearic acid) [13]. However, 'bound' lipid was observed in crystal structures although the type could not be resolved [10]. A specific interaction with DHA chains was evidenced by MD and NMR [33,40], and a direct interaction with the PE head-group has been proposed [34••]. In the latest work it was demonstrated that the PE-dependent MI–MII transition could only partially be explained by curvature stress with an additional component correlating to the hydrogen bonding potential of the head-group. Rhodopsin may also undergo a specific interaction with cholesterol as demonstrated from FRET studies [16] and more recently probed by MD simulations [43].

An appreciation of the biological context of rhodopsin should also be considered in explanation of the data. The rod outer segment (ROS) disk membrane membrane contains a higher proportion of PE and DHA lipids, and less cholesterol than the plasma membrane where the protein remains in an inactive state [15]. The ROS membrane is also asymmetric with respect to lipid distribution, PE being much more abundant in the outer leaflet of the membrane relative to the plasma membrane, and this asymmetry will likely result in different stresses on those parts of the protein residing in the inner and outer leaflet. Asymmetric bilayers are, however, much more difficult to recreate *in vitro*, which means at present any significance of asymmetric lipid distribution cannot be experimentally verified.

Cholesterol and GPCRs

Relative to other membrane components, the influence of cholesterol on GPCR activity is better documented (for a comprehensive review see [44••]). The nature of the interaction however remains ambiguous, whether ascribed to indirect bilayer effects, or specific receptor binding and putative 'non-annular' binding sites [45]. Despite a wealth of evidence for cholesterol modification of GPCR activity the specific response to the presence of cholesterol appears very much receptor-dependent, with reports of both upregulation and downregulation and of direct and indirect action. The data are further confused when one considers that a number of GPCRs can be expressed in the — cholesterol free — *Escherichia coli* cell membrane as stable, ligand-binding [46–48], and G-protein activating receptors [49].

Evidence for a direct interaction between cholesterol and GPCRs has expanded rapidly in recent years, sparked by the discovery of 'bound' cholesterol in the crystal structure of the β_2AR, and subsequent identification of a consensus cholesterol binding motif (CCM) in almost half of all family A GPCRs (Figure 2) [9••]. This motif comprises one residue from helix II, and three from IV. A charged residue at the intracellular face of helix IV is proposed to form the strongest interaction with the cholesterol in terms of binding energy, through an electrostatic interaction with the 3β hydroxyl moiety. Additional interacting residues include a tryptophan and leucine in helix IV, as well as a tyrosine on helix II. A further cholesterol binding motif, found in membrane proteins known to bind cholesterol, has also been reported in GPCRs whose activity is influenced by cholesterol, including rhodopsin and the serotonin 1A receptor [50•]. Direct and indirect interactions have been demonstrated *in vivo* using cholesterol analogues

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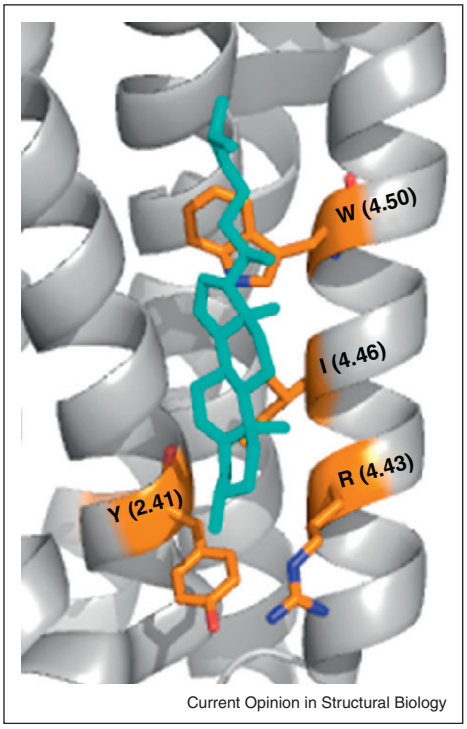
Figure 2



Cholesterol binding motif. The 2.8 Å crystal structure of the β_2 adrenergic receptor revealed 'bound' cholesterol (shown here in light blue) in a cleft between helices II and IV. On the basis of this data a conserved cholesterol-binding site for family A GPCRs was proposed, comprising a tryptophan (or tyrosine), isoleucine (or other bulky aliphatic residue), and a positively charged residue from helix IV, and tyrosine (or tryptophan) on helix II [9••].

in combination with measurements of membrane fluidity and receptor activity. Similar studies carried out on the oxytocin and cholecystokinin (CCK) receptors have even revealed a differing role for cholesterol in related receptors; cholesterol appears to interact directly with the oxytocin receptor, but to effect CCK activity indirectly by modulating bilayer properties [51]. Given that the CCK receptor possesses a CCM this latter observation raises questions about the interpretation of such sequences. Moreover, amongst the receptors possessing a CCM are those which are functionally active in the *E. coli* membrane, such as the neurotensin receptor 1 (NTS1) [47] and β_2AR [48]. Therefore, at present it seems that few general principles, if any, can be drawn with respect to cholesterol–receptor interactions.

Technical challenges

One of the major limiting factors for GPCR studies has been the availability of suitable amounts of stable protein with which to work. Advances in purification methodologies are beginning to overcome this hurdle [47], along with concurrent increases in instrument sensitivity. Critical to the themes covered here is that methods must be chosen which closely mimic the biological context. The development of successful reconstitution strategies for GPCRs, for example with NTS1 [20••], will enable investigation of the effect of bilayer composition on ligand binding and dimerisation. Furthermore, novel reconstitution systems will allow a more faithful *in vivo* replication of the bilayer [52•], and may enable detailed study of aspects difficult to recreate such as bilayer asymmetry. Crystallography, despite revealing exciting new details of GPCR activation, is often undertaken from detergent solution, using mutated or antibody-bound proteins, or in non-native lipid environments any of which experimental peculiarities carries a risk of generating artefacts. Even where these do not occur crystal structures offer little information about the dynamics of receptor–lipid interactions. For these reasons, magnetic resonance techniques provide a complement to crystallographic studies and are far better suited to probing dynamic interactions at the lipid–protein interface [53]. ESR is an ideal technique, as a result of its intrinsic dynamic time scale sensitivity, and protocols first developed in the late 1970s are just as relevant now [54••,55]. NMR is a well-established approach useful for determining bilayer properties as exemplified in rhodopsin studies [33,56]. Dynamic lipid–protein interactions have also been successfully probed with fluorescence-based approaches and have significant potential [57]. The complex nature of the interactions mean MD simulations can be useful to simplify the problem and give a holistic view of process [40,58–60], and PWR has shown potential for studying the lipid-dependence of the association of GPCRs with membrane [37,61•].

Conclusions

Although the membrane bilayer shares a close relationship with the complex signalling pathways of GPCRs, the 'how', 'when' and 'why' of this relationship remains less clear and, if we are to fully understand GPCR activation, must be the focus of future studies. From studies with rhodopsin it is evident that an intricate balance of bilayer–rhodopsin interactions tightly regulates receptor function, with recent data beginning to unravel the mechanistic details and energetic contributions of the different bilayer parameters [34••,62]. Determining whether the applicability of these principles extends to other GPCRs will only be achieved through detailed investigations on the expanding number of GPCRs now amenable to biophysical analysis. The varied influence of cholesterol in terms of mode-of-action and receptor response is striking and its link to putative lipid domains may go some way to explaining the regulatory mechanisms of GPCR activation. The possibility of specific cholesterol binding sites on receptors warrants further investigation; and the exact role of cholesterol with each individual receptor must be studied carefully. Finally, with the caveat that one should remain mindful of receptor functionality and biological context when using model membranes and

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other non-native environments, there are a range of techniques both well-established and novel which will no doubt lead to a much greater understanding of GPCR–bilayer interactions.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, Stevens RC: **The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist**. *Science* 2008, **322**:1211-1217.

2. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK *et al.*: **High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor**. *Science* 2007, **318**:1258-1265.

3. • Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, Leslie AG, Tate CG: **Agonist-bound adenosine A(2A) receptor structures reveal common features of GPCR activation**. *Nature* 2011, **474**:521-525.
The authors make use of innovative purification and crystallisation strategies to obtain ligand-bound GPCR crystal structures, and identify common mechanisms for activation in response to agonist binding.

4. Warne T, Moukhametzianov R, Baker JG, Nehme R, Edwards PC, Leslie AG, Schertler GF, Tate CG: **The structural basis for agonist and partial agonist action on a beta(1)-adrenergic receptor**. *Nature* 2011, **469**:241-244.

5. Xu F, Wu H, Katritch V, Han GW, Jacobson KA, Gao ZG, Cherezov V, Stevens RC: **Structure of an agonist-bound human A2A adenosine receptor**. *Science* 2011, **332**:322-327.

6. Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF *et al.*: **Crystal structure of the human beta2 adrenergic G-protein-coupled receptor**. *Nature* 2007, **450**:383-387.

7. Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, Hofmann KP, Ernst OP: **Crystal structure of opsin in its G-protein-interacting conformation**. *Nature* 2008, **455**:497-502.

8. Choe HW, Park JH, Kim YJ, Ernst OP: **Transmembrane signaling by GPCRs: insight from rhodopsin and opsin structures**. *Neuropharmacology* 2011, **60**:52-57.

9. •• Hanson MA, Cherezov V, Griffith MT, Roth CB, Jaakola VP, Chien EY, Velasquez J, Kuhn P, Stevens RC: **A specific cholesterol binding site is established by the 2.8 Å structure of the human beta2-adrenergic receptor**. *Structure* 2008, **16**:897-905.
This paper provides crystallographic evidence for a cholesterol binding site in the beta 2 adrenergic receptor. Leading to the identification of a conserved cholesterol binding motif throughout family A GPCRs.

10. Li J, Edwards PC, Burghammer M, Villa C, Schertler GF: **Structure of bovine rhodopsin in a trigonal crystal form**. *J Mol Biol* 2004, **343**:1409-1438.

11. Marsh D, Watts A, Pates RD, Uhl R, Knowles PF, Esmann M: **ESR spin-label studies of lipid–protein interactions in membranes**. *Biophys J* 1982, **37**:265-274.

12. Fretten P, Morris SJ, Watts A, Marsh D: **Lipid–lipid and lipid–protein interactions in chromaffin granule membranes. A spin label ESR study**. *Biochim Biophys Acta* 1980, **598**:247-259.

13. Watts A, Volotovski ID, Marsh D: **Rhodopsin–lipid associations in bovine rod outer segment membranes. Identification of immobilized lipid by spin-labels**. *Biochemistry* 1979, **18**:5006-5013.

14. Phillips R, Ursell T, Wiggins P, Sens P: **Emerging roles for lipids in shaping membrane–protein function**. *Nature* 2009, **459**:379-385.

15. Albert AD, Boesze-Battaglia K, Paw Z, Watts A, Epand RM: **Effect of cholesterol on rhodopsin stability in disk membranes**. *Biochim Biophys Acta* 1996, **1297**:77-82.

16. Albert AD, Young JE, Yeagle PL: **Rhodopsin–cholesterol interactions in bovine rod outer segment disk membranes**. *Biochim Biophys Acta* 1996, **1285**:47-55.

17. Albert AD, Yeagle P, Litman BJ: **P NMR investigation of rhodopsin–phospholipid interactions in bovine rod outer segment disk membranes**. *Biophys J* 1982, **37**:34-36.

18. van Meer G, de Kroon AI: **Lipid map of the mammalian cell**. *J Cell Sci* 2011, **124**:5-8.

19. Harding PJ, Attrill H, Ross S, Koeppe JR, Kapanidis AN, Watts A: **Neurotensin receptor type 1: *Escherichia coli* expression, purification, characterization and biophysical study**. *Biochem Soc Trans* 2007, **35**:760-763.

20. •• Harding PJ, Attrill H, Boehringer J, Ross S, Wadhams GH, Smith E, Armitage JP, Watts A: **Constitutive dimerization of the G-protein coupled receptor, neurotensin receptor 1, reconstituted into phospholipid bilayers**. *Biophys J* 2009, **96**:964-973.
The authors describe a strategy for successful reconstitution of a ligand binding GPCR, which can be used as a tool for studies of lipid-dependence. Specific dimerisation is demonstrated in a lipid environment, contrary to the detergent solubilised receptor when assayed at a high detergent:protein ratio.

21. Chini B, Parenti M: **G-protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there?** *J Mol Endocrinol* 2004, **32**:325-338.

22. Ostrom RS, Insel PA: **The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: implications for molecular pharmacology**. *Br J Pharmacol* 2004, **143**:235-245.

23. Barnett-Norris J, Lynch D, Reggio PH: **Lipids, lipid rafts and caveolae: their importance for GPCR signaling and their centrality to the endocannabinoid system**. *Life Sci* 2005, **77**:1625-1639.

24. Chini B, Parenti M: **G-protein-coupled receptors, cholesterol and palmitoylation: facts about fats**. *J Mol Endocrinol* 2009, **42**:371-379.

25. Renner U, Glebov K, Lang T, Papusheva E, Balakrishnan S, Keller B, Richter DW, Jahn R, Ponimaskin E: **Localization of the mouse 5-hydroxytryptamine(1A) receptor in lipid microdomains depends on its palmitoylation and is involved in receptor-mediated signaling**. *Mol Pharmacol* 2007, **72**:502-513.

26. Bjork K, Sjogren B, Svenningsson P: **Regulation of serotonin receptor function in the nervous system by lipid rafts and adaptor proteins**. *Exp Cell Res* 2010, **316**:1351-1356.

27. Rimoldi V, Reversi A, Taverna E, Rosa P, Francolini M, Cassoni P, Parenti M, Chini B: **Oxytocin receptor elicits different EGFR/MAPK activation patterns depending on its localization in caveolin-1 enriched domains**. *Oncogene* 2003, **22**:6054-6060.

28. Xiang Y, Rybin VO, Steinberg SF, Kobilka B: **Caveolar localization dictates physiologic signaling of beta 2-adrenoceptors in neonatal cardiac myocytes**. *J Biol Chem* 2002, **277**:34280-34286.

29. • Levitt ES, Clark MJ, Jenkins PM, Martens JR, Traynor JR: **Differential effect of membrane cholesterol removal on mu- and delta-opioid receptors: a parallel comparison of acute and chronic signaling to adenylyl cyclase**. *J Biol Chem* 2009, **284**:22108-22122.
This paper describes the use of methyl beta cyclodextrin as a tool to study the effect of cholesterol depletion on GPCR signalling. Observed differences within the opioid receptor family are linked to localisation with cholesterol enriched bilayer domains.

30. Kong MM, Verma V, O'Dowd BF, George SR: **The role of palmitoylation in directing dopamine D1 receptor internalization through selective endocytic routes**. *Biochem Biophys Res Commun* 2011, **405**:445-449.

31. Botham A, Guo X, Xiao YP, Morice AH, Compton SJ, Sadofsky LR: **Palmitoylation of human proteinase-activated receptor-2 differentially regulates receptor triggered ERK1/2 activation, calcium signalling, and endocytosis**. *Biochem J* 2011. pii:BJ20101958, doi:10.1042/BJ20101958.

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32. Jastrzebska B, Debinski A, Filipek S, Palczewski K: **Role of membrane integrity on G protein-coupled receptors: rhodopsin stability and function**. *Prog Lipid Res* 2011, **50**:267-277.

33. Soubias O, Teague WE, Gawrisch K: **Evidence for specificity in lipid–rhodopsin interactions**. *J Biol Chem* 2006, **281**:33233-33241.

34. •• Soubias O, Teague WE Jr, Hines KG, Mitchell DC, Gawrisch K: **Contribution of membrane elastic energy to rhodopsin function**. *Biophys J* 2010, **99**:817-824.
This paper provides evidence for a specific interaction between rhodopsin and the headgroup of PE lipids. Furthermore, the authors provide the first evidence for hydrogen bond formation as the main driver of this process.

35. Gibson NJ, Brown MF: **Lipid headgroup and acyl chain composition modulate the MI–MII equilibrium of rhodopsin in recombinant membranes**. *Biochemistry* 1993, **32**:2438-2454.

36. Botelho AV, Gibson NJ, Thurmond RL, Wang Y, Brown MF: **Conformational energetics of rhodopsin modulated by nonlamellar-forming lipids**. *Biochemistry* 2002, **41**:6354-6368.

37. Alves ID, Salgado GF, Salamon Z, Brown MF, Tollin G, Hruby VJ: **Phosphatidylethanolamine enhances rhodopsin photoactivation and transducin binding in a solid supported lipid bilayer as determined using plasmon-waveguide resonance spectroscopy**. *Biophys J* 2005, **88**:198-210.

38. Feller SE, Gawrisch K, Woolf TB: **Rhodopsin exhibits a preference for solvation by polyunsaturated docosohexaenoic acid**. *J Am Chem Soc* 2003, **125**:4434-4435.

39. Mitchell DC, Niu SL, Litman BJ: **Optimization of receptor-G protein coupling by bilayer lipid composition I: kinetics of rhodopsin-transducin binding**. *J Biol Chem* 2001, **276**:42801-42806.

40. Grossfield A, Feller SE, Pitman MC: **A role for direct interactions in the modulation of rhodopsin by omega-3 polyunsaturated lipids**. *Proc Natl Acad Sci U S A* 2006, **103**:4888-4893.

41. Niu SL, Mitchell DC, Lim SY, Wen ZM, Kim HY, Salem N Jr, Litman BJ: **Reduced G protein-coupled signaling efficiency in retinal rod outer segments in response to n-3 fatty acid deficiency**. *J Biol Chem* 2004, **279**:31098-31104.

42. Mitchell DC, Straume M, Miller JL, Litman BJ: **Modulation of metarhodopsin formation by cholesterol-induced ordering of bilayer lipids**. *Biochemistry* 1990, **29**:9143-9149.

43. Khelashvili G, Grossfield A, Feller SE, Pitman MC, Weinstein H: **Structural and dynamic effects of cholesterol at preferred sites of interaction with rhodopsin identified from microsecond length molecular dynamics simulations**. *Proteins* 2009, **76**:403-417.

44. •• Paila YD, Chattopadhyay A: **Membrane cholesterol in the function and organization of G-protein coupled receptors**. *Subcell Biochem* 2010, **51**:439-466.
A comprehensive and up-to-date review of cholesterol–GPCR interactions.

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

46. Weiss HM, Grisshammer R: **Purification and characterization of the human adenosine A(2a) receptor functionally expressed in *Escherichia coli***. *Eur J Biochem* 2002, **269**:82-92.

47. Attrill H, Harding PJ, Smith E, Ross S, Watts A: **Improved yield of a ligand-binding GPCR expressed in *E. coli* for structural studies**. *Protein Exp Purif* 2009, **64**:32-38.

48. Marullo S, Delavier-Klutchko C, Eshdat Y, Strosberg AD, Emorine L: **Human beta 2-adrenergic receptors expressed in *Escherichia coli* membranes retain their pharmacological properties**. *Proc Natl Acad Sci U S A* 1988, **85**:7551-7555.

49. Grisshammer R, Hermans E: **Functional coupling with Galpha(q) and Galpha(i1) protein subunits promotes high-affinity agonist binding to the neurotensin receptor NTS-1 expressed in *Escherichia coli***. *FEBS Lett* 2001, **493**:101-105.

50. • Jafurulla M, Tiwari S, Chattopadhyay A: **Identification of cholesterol recognition amino acid consensus (CRAC) motif in G-protein coupled receptors**. *Biochem Biophys Res Commun* 2011, **404**:569-573.
The authors identify an additional cholesterol binding motif in three representative GPCRs. This motif was originally described for proteins which have been shown to undergo a specific interaction with cholesterol.

51. Gimpl G, Burger K, Fahrenholz F: **Cholesterol as modulator of receptor function**. *Biochemistry* 1997, **36**:10959-10974.

52. • Leptihn S, Thompson JR, Ellory JC, Tucker SJ, Wallace MI: **In vitro reconstitution of eukaryotic ion channels using droplet interface bilayers**. *J Am Chem Soc* 2011, **133**:9370-9375.
The authors describe a novel reconstitution system using droplet interface bilayers, which may be applicable to the study of GPCR–bilayer interactions.

53. Marsh D, Pali T: **The protein–lipid interface: perspectives from magnetic resonance and crystal structures**. *Biochim Biophys Acta* 2004, **1666**:118-141.

54. •• Marsh D: **Electron spin resonance in membrane research: protein–lipid interactions from challenging beginnings to state of the art**. *Eur Biophys J* 2010, **39**:513-525.
An excellent review of EPR to study protein–lipid interactions describing the types of experiments and the information that can be obtained.

55. Watts A: *Protein–Lipid Interactions*. Amsterdam/New York: Elsevier; 1993.

56. Ryba NJ, Dempsey CE, Watts A: **Protein–lipid interactions at membrane surfaces: a deuterium and phosphorus nuclear magnetic resonance study of the interaction between bovine rhodopsin and the bilayer head groups of dimyristoylphosphatidylcholine**. *Biochemistry* 1986, **25**: 4818-4825.

57. Smith AW: **Lipid–protein interactions in biological membranes: a dynamic perspective**. *Biochim Biophys Acta* 2011. pii:S0005-2736(11)00193-3, doi:10.1016/j.bbamem.2011.06.015.

58. Lindahl E, Sansom MS: **Membrane proteins: molecular dynamics simulations**. *Curr Opin Struct Biol* 2008, **18**:425-431.

59. Grossfield A, Pitman MC, Feller SE, Soubias O, Gawrisch K: **Internal hydration increases during activation of the G-protein-coupled receptor rhodopsin**. *J Mol Biol* 2008, **381**:478-486.

60. Paila YD, Tiwari S, Sengupta D, Chattopadhyay A: **Molecular modeling of the human serotonin(1A) receptor: role of membrane cholesterol in ligand binding of the receptor**. *Mol Biosyst* 2011, **7**:224-234.

61. • Hruby VJ, Alves I, Cowell S, Salamon Z, Tollin G: **Use of plasmon waveguide resonance (PWR) spectroscopy for examining binding, signaling and lipid domain partitioning of membrane proteins**. *Life Sci* 2010, **86**:569-574.
The authors describe the development of plasmon waveguide resonance as a tool to study GPCRs in a membrane environment. Evidence is provided for ligand-dependent receptor localisation with membrane domains.

62. Soubias O, Niu SL, Mitchell DC, Gawrisch K: **Lipid–rhodopsin hydrophobic mismatch alters rhodopsin helical content**. *J Am Chem Soc* 2008, **130**:12465-12471.

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