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A tale of detergent tails: GPCR activation beyond ligands

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https://doi.org/10.1016/j.str.2025.03.005

In this issue of *Structure*, Banerjee et al.[1] use single-molecule FRET to explore how various detergents and cholesterol influence the conformational dynamics of the extracellular domain of the metabotropic glutamate receptor 2 (mGluR2). They show that the local membrane environment modulates the receptor's active-inactive state equilibrium and identifies specific cholesterol-binding sites, offering insights into potential drug-targeting strategies for mGluR2.

G protein-coupled receptors (GPCRs) are dynamic membrane proteins that interact with one another, soluble signaling proteins, and structural components of the plasma membrane to form functional complexes. As the largest family of membrane proteins in the human genome, with over 800 identified,[2] GPCRs play a critical role in regulating various human physiological processes involved in behavior, immune response, vision, and taste. With more than 45% of FDA-approved drugs targeting GPCRs, understanding their structure and function is a priority in biologically relevant research. Human GPCRs are categorized into four major classes based on sequence homology and functional similarity. Class A GPCRs (rhodopsin-like receptors) constitute the largest and most extensively studied class of GPCRs. Other classes include class B (secretin and adhesion family), class C (glutamate family), and class F (frizzled family), each playing distinct roles in various physical processes.[3,4] All GPCRs share a conserved seven-transmembrane (7-TM) domain, connected by three intracellular and three extracellular loops. Class C GPCRs, which include metabotropic glutamate receptors (mGlu receptors), γ-aminobutyric acid receptors ($GABA_B$ receptors), Ca^{2+}-sensing receptors (CaS receptors), sweet and amino acid taste receptors, and several orphan receptors, are multidomain proteins that function as constitutive dimers.[5] These receptors are distinguished by a large extracellular region that contains a Venus flytrap (VFT) module, where ligand recognition and binding occur, and a cysteine-rich domain (CRD) that separates the 7-TM domain from the VFT module. The CRD is present in all class C GPCRs except for $GABA_B$ receptors.[6] mGlu, $GABA_B$, and CaS receptors are key therapeutic targets that play vital roles in disorders affecting the central nervous system (CNS) and calcium homeostasis.[6]

For the structural and functional properties of GPCRs to be studied, GPCRs are often solubilized in detergent micelles and other membrane mimetics, which help maintain their structural integrity.[3] This approach has facilitated the determination of many GPCR structures across different classes. However, the effect of detergents on GPCR structure and stability remains a subject of ongoing debate. Additionally, it is well established that upon ligand binding, GPCRs undergo conformational changes within their TM region, resulting in the activation of diverse signaling networks. These conformational changes suggest that receptor activation dynamics can be influenced by the lipid environment surrounding the receptor.[7] While the impact of lipid bilayer properties on class A GPCRs has been extensively studied, this area remains underexplored for class C GPCRs. In a recent study published in *Structure*, Banerjee et al.[1] provide new insights into how the local lipid environment around metabotropic glutamate receptor 2 (mGluR2) influences its activation by inducing specific conformational changes in its extracellular domain (Figure 1).

The authors incorporated Förster resonance energy transfer (FRET) sensors into the large extracellular VFT module of mGluR2 and tracked its movements in real-time using state-of-the-art single-molecule FRET (smFRET). They investigated how variations in the chain length and branching of nonionic detergents, commonly used in membrane protein studies, influence receptor conformational states. Across all tested detergents, mGluR2 adopted two primary FRET states corresponding to its active and inactive conformations. When bound to the antagonist LY341495, the receptor consistently favored the inactive high-FRET state, whereas the agonist glutamate shifted the equilibrium toward the active low-FRET state. However, at sub-saturating glutamate concentrations, the choice of detergent influenced the stability of the active state, with branched-chain detergents favoring an active conformation but to a lesser extent than single-chain detergents (Figure 1).

Structural studies of class C GPCRs, such as the $GABA_B$ receptors, have shown that tightly bound lipids at the TM and dimer interface play a functional role in receptor activity.[8] Photo-cross-linking studies and molecular dynamic (MD) simulations have identified at least two interaction sites within the TM domain of mGluR2, with the possibility for additional interactions.[9] Building on these findings, the authors used smFRET to investigate the effects of detergents and cholesterol analogs, specifically cholesteryl hemisuccinate (CHS), on the conformational dynamics of mGluR2. Their results suggest that cholesterol acts as a negative allosteric modulator by reducing the

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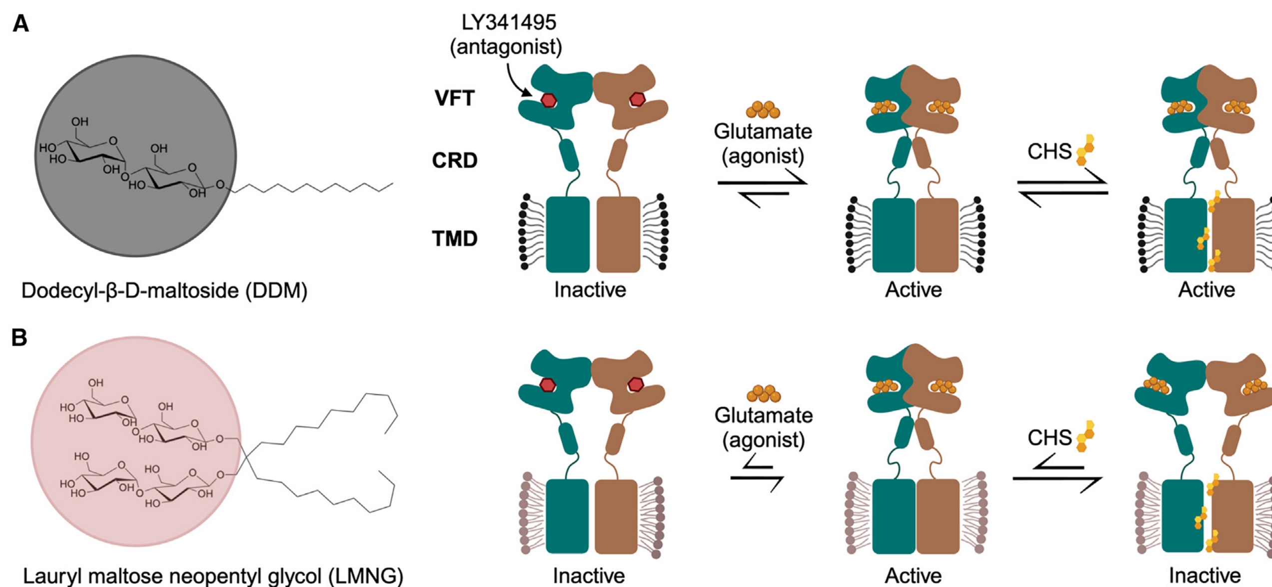

Figure 1. Influence of detergents and cholesterol on mGluR2's extracellular domain dynamics
(A) Schematic representation of the single-chain detergent DDM (left). Glutamate binding shifts the active-inactive equilibrium of DDM-solubilized mGluR2 toward the active state, increasing the occupancy of the active state conformation. This effect is not significantly altered in the presence of cholesterol (right).
(B) Schematic representation of the branched-chain detergent LMNG (left). Glutamate binding also shifts the active-inactive equilibrium of LMNG-mGluR2 toward the active state but to a lesser extent than DDM. Cholesterol, however, reduces the occupancy of the active state conformation (right). The distinct colors represent the two protomers of the mGluR2 homodimer.
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population of the active-state conformation of the VFT module, especially in the presence of long and branched detergents like lauryl maltose neopentyl glycol (LMNG). In contrast, unbranched detergents showed no significant effects (Figure 1A). These results provide further evidence that lipid interactions are crucial for modulating class C GPCR activation, offering insights into how membrane composition shapes receptor function.

Ligand binding to mGluR2 initiates a cascade of conformational changes that propagate from the ligand-binding VFT module through the CRD to the 7-TM domain.[5] Given the loose coupling between the VFT and CRD, the authors hypothesized that the effects of detergents and cholesterol on VFT domain dynamics extend to the CRD. Using a FRET sensor to monitor conformational changes between CRD subunits, they found that, at intermediate and saturating glutamate concentrations, detergents with flexible hydrophobic tails like LMNG and Glyco-diosgenin (GDN) modulate the stability of the CRD active state. Furthermore, the addition of CHS to mGluR2 solubilized in these detergents enhanced cholesterol's negative allosteric effect, acting similarly to a negative allosteric modulator (NAM) (Figure 1B). These findings indicate that CHS's effect extends beyond the VFT domain, influencing the overall conformational dynamics of mGluR2.

Further investigation into cholesterol binding in mGluR2 revealed three putative binding sites based on structural homology with CaSR.[10] These sites, located at the dimer interface, include L744 in helix V and F746 and Y781 in helix VI. Mutagenesis and smFRET experiments in LMNG and LMNG-CHS detergent micelles demonstrated that F746 and Y781 are key cholesterol-binding residues in mGluR2. Under sub-saturating glutamate conditions, mutations at F746 and Y781 significantly reduced the NAM-like effect of CHS observed in the wild-type receptor, suggesting that cholesterol's direct interaction at the dimer interface of mGluR2 is responsible for this effect, shifting the receptor's equilibrium toward a more inactive state.

In conclusion, the study by Banerjee et al.[1] highlights the impact of solubilizing environments on GPCR activation and underscores the need to consider detergent effects when interpreting biochemical and structural data. Notably, in long, branched detergents like LMNG, the presence of CHS reduced the population of the active state conformation, an effect not observed with unbranched detergents such as n-Dodecyl-β-D-Maltoside (DDM). These findings further support the idea that mGluR2 is regulated by its lipid environment, particularly cholesterol, though the exact mechanism is not yet fully understood. More broadly, the results indicate that membrane lipid composition plays a crucial role in modulating mGluR2 activation and dynamics. Given the distinct effects observed with different detergents, future studies should focus on exploring GPCR dynamics in native lipid environments to gain a more comprehensive understanding of their functional properties.

ACKNOWLEDGMENTS

Lamichhane laboratory research is supported by the National Institutes of Health R35GM142946 (R.L). E.A. is supported by a Graduate Advancement & Training Education (GATE) Fellowship from the University of Tennessee–Oak Ridge Innovation Institute.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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