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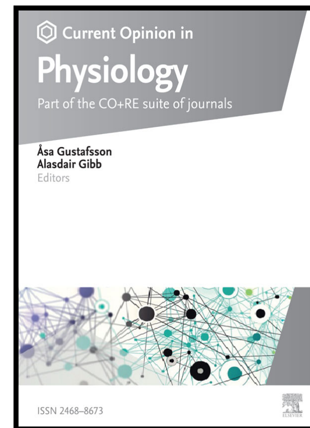

Membrane force reception: mechanosensation in GPCRs and tools to address itShort title: GPCR force reception

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Membrane force reception: mechanosensation in GPCRs and tools to address it

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Abstract

To survive, all organisms must detect and respond to mechanical cues in their environment. Cells are subjected to a plethora of mechanical forces, such as hydrostatic pressure, cell-cell contact, stretch, compression, and shear stress. Mechanosensitive membrane proteins have evolved across all life kingdoms to sense and respond to forces in the membrane. Bacterial mechanosensitive ion channels provide a blueprint for understanding the fundamental mechanisms that underpin cellular responses to mechanical signals. Recently, the identification of eukaryotic force transducers, which includes membrane proteins other than channels, has led to the recognition of common structural hallmarks and unified biophysical mechanisms that could potentially link these diverse proteins. Accumulating evidence suggests G protein-coupled receptors (GPCRs) are candidates for pressure sensing in mammals. This review summarises the current knowledge on mechanosensitive GPCRs, describes the tools used to assess their mechanosensitivity, and aims to highlight the key characteristics that link these receptors to established mechanosensors.

Introduction

Mechanotransduction is the process by which mechanical force is converted into intracellular biochemical signals. At the most basic level, mechanotransduction protects prokaryotes from osmotic shock under extreme turgor pressure [1-4]. In animals, it permits not only the sense of touch but also proprioception, hearing, pain and vascular regulation [4]. Because mechanotransduction is conserved across all life, it is thought to be one of the most primitive sensory transmission pathways [3].

The primary target for mechanical force in cells is the plasma membrane. Membranes are highly dynamic structures, composed mostly of phospholipids, cholesterol, and proteins, which form a protective barrier around the cell. Mechanotransduction, therefore, relies on the presence of membrane-embedded MS macromolecules. Most MS proteins identified to date are ion channels, biomolecules that contain a central pore through which ions and water can cross the semi-impermeable lipid bilayer. Patch clamp electrophysiology studies on the bacterial MS channels of large (MscL) and small (MscS) conductance laid the foundations for our understanding of the biophysical principles underlying mechanotransduction [5,6] and were crucial in establishing a model for mechanosensing in membranes.

Mechanisms for force sensing

It is widely accepted that MS channels in bacteria gate in response to bilayer stretch alone. This observation led to the proposal of the "force-from-lipids" model, which states that membrane protein conformation and function are governed by protein interactions with surrounding lipids [5,7] [Figure 1]. These interactions are influenced by both tension-induced global stretching of the membrane and local distortions caused by membrane curvature [8]. Membrane stretch thins the bilayer and alters the equilibrium of pressure known as the transbilayer pressure profile [9]. Hydrophobic mismatch ensues between the membrane-facing domains of intrinsic proteins and the bilayer, inducing a shift in the protein conformation [8].

In the case of MscS [10,11], MscL [12-14], and the mammalian two-pore domain potassium (K_2P) channels TRAAK [15,16], TREK-1 [16,17] and TREK-2 [18], dissociation of lipids from hydrophobic pockets under membrane tension appears to drive a conformational change consistent with channel gating [19]. These findings prompted the development of a novel mechanism. Derived from the force-from-lipids principle, the "lipid-moves-first" model posits that lipids behave as non-specific, low-affinity negative allosteric modulators for integral MS channels [10,12,13,20] [Figure 1].

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Membrane proteins are so intimately associated with annular lipids that the acyl chains penetrate into the TM cavities. In this model, when membrane stretch increases the total planar area of the bilayer, hydrophobic forces "pull" lipids out of the pockets and into the bilayer to satisfy the equilibrium position [10,20]. In response, the protein adjusts its conformation to protect the newly exposed hydrophobic residues. When tension decreases, the process is reversed. Indeed, several high-resolution structures of MscS in detergent and membrane mimetics (termed nanodiscs), solved by X-ray crystallography [10] and cryogenic electron microscopy (cryo-EM) [11,21-23], report lipid-like density within the TM pockets. In comparison to the open-state channel, it is clear that these lipid-binding regions significantly reduce in volume when the channel opens [10,24]. Of course, it could be argued that the reverse is true; that when integral membrane proteins rearrange, their associated lipids must reorganise too [7]. However, this model would necessitate strongly bound lipids to act as force transducers, which is not consistent with the requirement for the entropy-driven free exchange of lipids between proteins and the bilayer [10,20]. Further, it is known that MS channels are sensitive to changes in membrane thickness and asymmetry induced by the addition of amphipathic molecules, suggesting that force is transmitted from rather than to the bilayer [5,10,25].

It is not yet clear if these lipid-centric models extend to mechanotransduction in the cells of higher organisms. In the alternate "tethered" model, force is relayed to channels *via* additional structures such as the extracellular matrix (ECM), intracellular cytoskeleton, or a combination of both [4] [Figure 1]. It was recently suggested that the human MS channels PIEZO1 and PIEZO2 are functionally tethered to the actin cytoskeleton [26]. Although this study is the first to suggest the force-from-tether model in mammalian cells, molecular dynamics simulations and patch clamp electrophysiology experiments performed on the Drosophila MS channel NompC suggest that tethers to microtubules allow force to be transmitted to the channel [27]. There is also evidence to advocate for the force-from-lipids model for mammalian mechanotransduction. PIEZO1 in membrane blebs and lipid droplets, which lack auxiliary elements, retains its MS [28,29]. Human TRAAK and TREK-1 respond exquisitely to applied force in excised patches following reconstitution into liposomes [16]. Given the complexity of these systems, a hybrid model with relative contributions from both the membrane and tethers seems to be the most appropriate.

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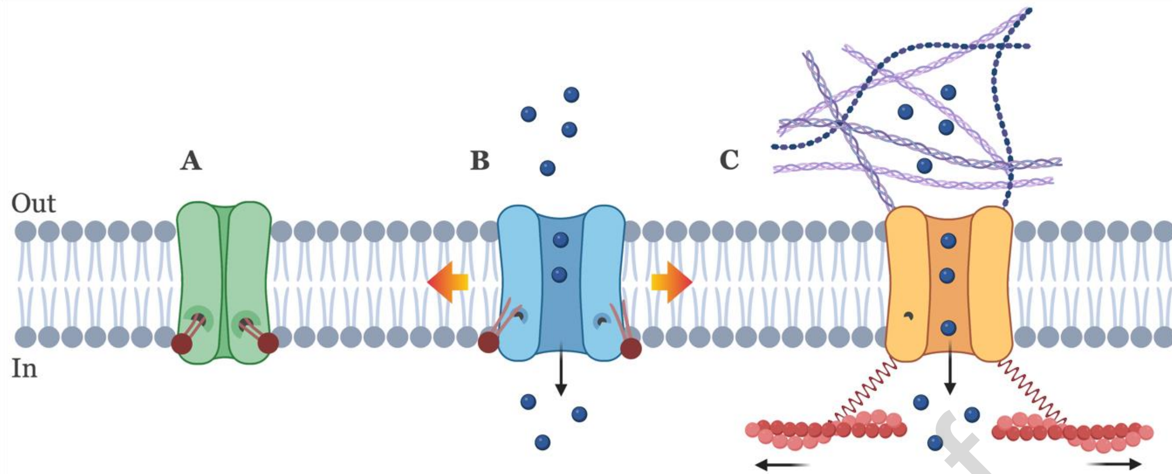

Figure 1. Schematic representation of current hypotheses for mechanosensing in membranes. A. According to the "lipid-moves-first" model, closed-state integral membrane proteins, such as ion channels and receptors, are stabilised by lipids occupying cytoplasmic-facing pockets. **B.** The "force-from-lipids" model encompasses all lipid membrane-driven mechanisms resulting in protein conformational changes in response to pressure in the bilayer. **C.** The "force-from-tether" model describes how attachments to auxiliary cellular components, namely the ECM and the cytoskeleton, are involved in conformational changes that lead to ion influx. Created using BioRender.com, adapted from [30].

In the last 30 or so years, new classes of human MS proteins have been identified, and advances in structural and functional studies have revealed common characteristics that may point towards a universal mechanism for force sensing. An increasing number of G protein-coupled receptors (GPCRs) have been shown to respond to mechanical stimuli in a ligand-independent manner. This review aims to highlight the role of GPCRs in mechanotransduction in humans and to evaluate their position within the mechanosensing paradigm. We will also discuss existing tools and propose new approaches to address mechanosensitivity in GPCRs.

G protein-coupled receptors

GPCRs make up the largest family of membrane proteins in humans, with some 800 members. They recognise a vast array of chemical signals, including most hormones and neurotransmitters, to mediate important physiological processes, including vision, olfaction, and taste [31,32]. Despite huge variability in function, all GPCRs share two defining characteristics. Firstly, their structure is comprised of an extracellular N-terminus, seven transmembrane (TM) α-helices (TM1-TM7), connected by three intracellular loops and three extracellular loops, and an intracellular C-terminus [31].

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Secondly, they couple to heterotrimeric guanine nucleotide-binding proteins (G proteins), formed from Gα, Gβ and Gγ subunits [31].

One subfamily of receptors, the adhesion GPCRs (aGPCRs), also includes an extended N-terminal extracellular region composed of cell-adhesive protein motifs and a GPCR autoproteolysis-inducing domain (GAIN) [33]. Autoproteolysis dissects the receptor into an extracellular N-terminal fragment (NTF) and a C-terminal fragment (CTF), the latter of which includes the 7TM region. However, not all aGPCRs are thought to be cleavable at the GAIN domain due to the absence of a highly conserved sequence motif within the GPCR proteolysis site (GPS) [33]. Interestingly, aGPCRs are implicated in mechanotransduction *via* a tether-like mechanism due to enhanced cell-cell or cell-ECM interactions [34,35]. Examples of putative MS aGPCRs include EMR2 (ADGRE2), CD97 (ADGRE5), GPR56 (ADGRG1), GPR114 (ADGRG5), GPR126 (ADGRG6), latrophilin-1 (ADGRL1) and VLGR1 (ADGRV1) [34,35]. For this subset of receptors, activation and signalling is triggered by the interaction of a tethered low-affinity peptide agonist (termed the *Stachel)* with the 7TM region [34-36]. Whilst the exact mechanism of MS activation is yet to be elucidated, it's hypothesised that mechanical force causes separation of the NTF and CTF, exposing the *Stachel* to the 7TM binding site, and/or that mechanical perturbation of the N terminus induces a conformational reorientation of the *Stachel* to an active position [34,35].

For rhodopsin-like class A GPCRs, initiation of signalling is canonically achieved by ligands interacting with a binding site typically found within the TM helices on the extracellular side of the receptor. Ligand-induced structural rearrangement of the TM region allows the signal to be transmitted across the cell membrane. Agonists shift the receptor energy landscape towards the "active" receptor conformation, so that the receptor spends more of its time in a conformation that can bind transducer proteins, such as G proteins, GPCR kinases (GRKs), and arrestins, intracellularly [31,32,37]. It is thought that mechanical forces acting on cells can also elicit intracellular signals by inducing a shift in the conformational equilibrium of GPCRs embedded in the plasma membrane.

Mechanosensitive GPCRs

The first reported putative MS GPCR was the angiotensin II type-1 receptor (AT_1R), a mediator of cardiac hypertrophy and myogenic vasoconstriction. Mechanical stretch of cardiomyocytes expressing AT_1R in the absence of its endogenous peptide angiotensin II (Ang II) was shown to activate downstream extracellular signal-regulated

kinases (ERKs), Jak2, and inositol phosphates [38,39]. Importantly, stretch-induced receptor activation was inhibited by an AT_1R-selective inverse agonist [38]. This work was supported by Mederos y Schnitzler et al. (2008) [40], who co-expressed AT_1R with transient receptor potential channel-6 (TRPC6), a mechano-insensitive ion channel activated by diacylglycerol (DAG). Osmotic membrane stretch activated TRPC6-dependent cation currents *via* AT_1R-mediated phospholipase C (PLC) activation [40]. The authors carried out similar patch clamp experiments with the histamine H1 receptor (H_1R), type-5 muscarinic acetylcholine receptor (M_5R) and vasopressin 1a receptor (V1aR) to establish a broader role for Gαq/11-coupled GPCRs as MS proteins [40]. The role of Gαq/11-coupling was later challenged by Rakesh et al. (2010) [41], who demonstrated *via* a DAG reporter assay that mechanical activation of AT_1R operates through β-arrestin recruitment, independently of G proteins or Ang II.

Most MS GPCRs identified to date have important roles in vascular function. Smooth muscle cells of small resistance arteries are inherently sensitive to changes in intraluminal pressure and constrict in response to elevated tension [42]. This phenomenon is known as the Bayliss effect or the myogenic response. In vivo, blood flow exerts a frictional force known as shear stress on the endothelial cells (ECs) that line the vessel walls. The identity of specific mechanosensors located in vascular ECs has been a topic of debate for decades. GPCRs are among the list of candidates, which also includes ion channels, junctional complexes, integrins, and the cytoskeleton, all of which are thought to influence the myogenic response [43].

It is possible that the endothelial response to shear stress requires synergistic cooperation between two or more candidates. For example, it has been shown that vasoconstriction mediated by the MS ion channel transient receptor potential vanilloid 4 (TRPV4) is potentiated by several GPCRs, including the type-3 muscarinic acetylcholine receptor (M_3R) in rat arterioles [44] and AT_1R and proteinase-activated receptors (PAR1 and PAR2) in mouse aorta [45]. In another study, it was shown that shear stress in bovine aortic ECs activates PIEZO1, resulting in the release of adrenomedullin, which subsequently activates its Gs-coupled receptor, calcitonin receptor-like receptor CALCRL [46]. Importantly, Xu et al. (2018) [47] demonstrated that shear stress-induced calcium transients mediated by the MS receptor G protein-coupled receptor 68 (GPR68) were present even when PIEZO1 and PIEZO2 were knocked down. The same study showed that PIEZO1-dependent calcium transients were activated by turbulent shear stress but not by smooth laminar flow in HEK293T cells transfected with mouse Piezo1 [47]. Taken together, these data suggest that both ion channels and GPCRs are important for shear force sensing. Indeed, it is likely that

ECs integrate different types and intensities of mechanical input to generate the appropriate biological response. Ion channels, which respond within milliseconds, are likely required in instances of acute or rapid mechanical stress. Conversely, metabotropic receptors function more slowly and may result in long-term cellular and tissue modifications in response to mechanical signals. Other examples of reported shear stress sensors include the receptors bradykinin B2 (B_2R) [48], dopamine D2 [49] and D5 [50], H_1R [49], adenosine A2A [49], sphingosine 1-phosphate [51], apelin [52] and parathyroid hormone type-1 [53].

Mechanistic insights into mechanosensing GPCRs

The principal technique for studying MS ion channels is single channel patch clamp electrophysiology. Since ion transport is not a characteristic feature of receptor function, most studies on MS GPCRs monitor indirect readouts, such as downstream effectors, to infer protein conformational changes. However, a few studies have linked mechanical activation to conformational transitions directly using fluorescence resonance energy transfer (FRET) [48,49,53]. Recently, the structural basis of receptor activation by mechanical force was attributed to helix 8 (H8), a short α-helix found in most receptors located immediately after TM7 [49]. Erdogmus et al. (2019) [49] observed a reduction in FRET signals between fluorophores inserted at the C-terminus and proximal end of H8 in H_1R following shear stress, which the authors suggest was caused by H8 stretch. The removal of H8 from H_1R by C-terminal truncation resulted in insensitivity to mechanical stress, and insertion of the motif into one isoform of the gonadotropin-releasing hormone receptor (GnRHR), which intrinsically lacks H8, was sufficient to confer mechanosensitivity on the previously insensitive receptor [49]. On the other hand, Ozkan et al. (2021) [54] did not observe suppression of shear stress-induced fluorescent signals upon deletion of H8 from GPR68. However, the force applied in this study (2.6 dyn/cm^2) was much lower and is at the lower end of the physiological range. Arteries are exposed to shear stress of up to 10 dyn/cm2 and in arterioles, pressure can reach up to 70 dyn/cm^2 [55]. Involvement of the H8 region in mechanosensing is partially supported by an earlier mutagenesis-based study, which demonstrated that mechanical stress causes TM7 in AT_1R to rotate anticlockwise, away from H8, towards the ligand-binding pocket [56].

Structural similarities between GPCRs and MS ion channels

Recognition of common structural features among mechanosensitive proteins has led to consensus on a potential common underlying mechanism for pressure sensing. Despite vast differences in their global structures, most known mechanosensitive ion

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channels have an amphipathic helix positioned approximately parallel to the cytoplasmic membrane [57] [Figure 2]. This helix has no sequence conservation or defined length, yet it has been resolved in the structures of MscS [10,21,22], MscL [58,59], PIEZO1 [60,61], PIEZO2 [62], TRPV4 [63], TRAAK [15], TREK-1 [17], and TREK-2 [18]. In all cases, the orientation of this helix in relation to the adjacent TM helices allows for the formation of pockets that are responsible for mechanical sensing and response in these channels [10,12,13] [Figure 2]. First identified in MscS [64], hydrophobic pockets located close to the protein-bilayer interface have since been found in many MS ion channels [10-12,16-18,21,22,65,66]. It is interesting that H8 in GPCRs closely resembles the horizonal helices found in MS channels and is also inherently amphipathic [67]. A new study on the so-called "pocketome" of 557 GPCRs revealed nine newly identified pockets, most of which were located around the TM7 bundle, in close proximity to H8 [68]. Mutagenesis of key residues within these pockets in M_3R and B_2AR significantly affected G-protein activation and β-arrestin recruitment [68]. We hypothesise that the positioning of H8 in GPCRs gives rise to the formation of hydrophobic pockets analogous to those first identified in MS channels [10,12,64, 69,75], and that lipid contacts in this region directly affect receptor function, as exemplified in MS ion channels [11-13,19,23].

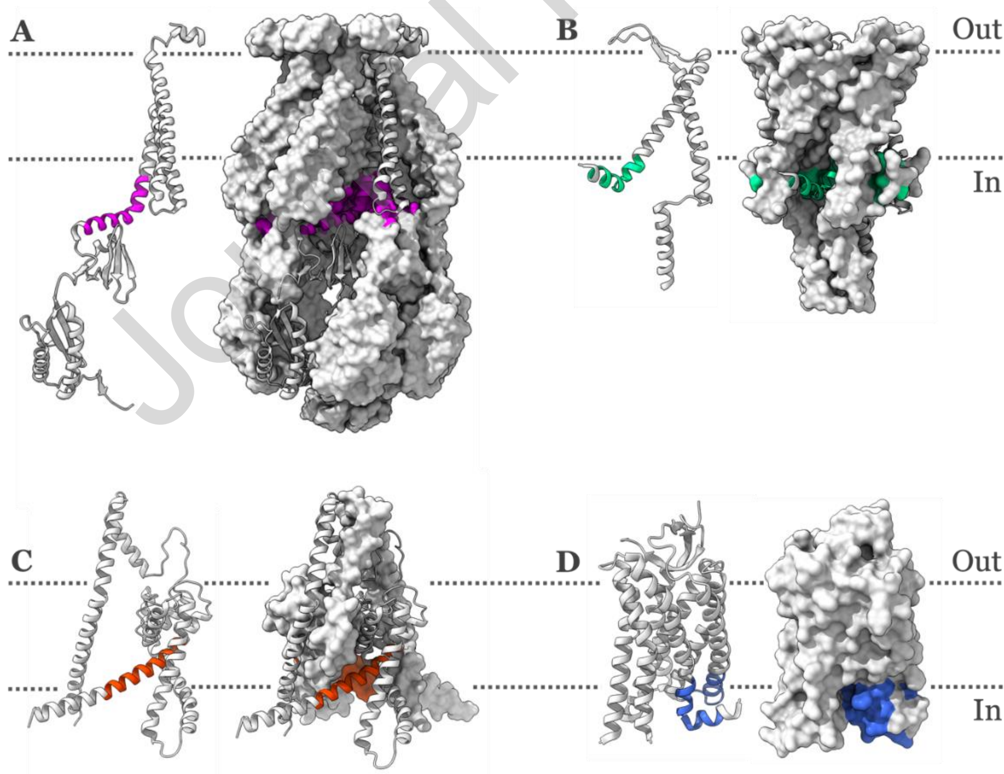

Figure 2. Mechanosensitive membrane proteins feature an amphipathic helix positioned horizontally and in close proximity to the intracellular protein-bilayer interface. The orientation of this helix permits the formation of lipid-binding pockets. A.

Homoheptameric ion channel MscS (PDB 6PWP). One subunit is shown in cartoon form, with remainder in surface view to highlight the various cavities formed between subunits and around the amphipathic helix (magenta). **B.** Homopentameric channel MscL (PDB 2OAR). The pocket-forming region is shown in green. **C.** Pseudotetrametic dimer TREK-1 (PDB 6W84). A single subunit is shown as a cartoon, with the helix of interest highlighted in orange. **D.** Monomeric GPCR AT_1R (PDB 6OS0) is depicted in both cartoon and surface view. The terminal end of TM7 and TM1, and the proximal end of helix 8 (H8), are coloured blue.

Tools for studying MS GPCRs

From a structural perspective, monitoring MS ion channels by electrophysiology may also be regarded as an indirect method. Recently, advanced electron paramagnetic resonance (EPR) spectroscopic techniques, such as pulsed electron-electron double resonance (PELDOR) spectroscopy, also known as double electron-electron resonance (DEER) spectroscopy, have been used to study integral membrane protein conformation and oligomerisation by enabling accurate distances to be measured between engineered protein spins [12,64,69-73]. PELDOR spectroscopy in combination with site-directed spin-labelling of residues at the entrance to hydrophobic pockets in MscL [12] revealed an expanded state of the channel, comparable to the reported sub-conducting state of *Methanosarcina acetivorans* MscL (MaMscL) [74]. PELDOR spectroscopy was first applied to the structurally-disparate MscS channel and successfully assigned its conformation in native conditions [64,75-78]. Whilst EPR has been useful to monitor MS channel conformation in response to changes in protein-lipid contacts [12,69], spectroscopic techniques have not yet been used to study mechanically-activated proteins by membrane tension application directly. This is because both NMR and EPR require measurements to be performed on purified (and/or reconstituted) protein samples in solution, and it has been challenging to mimic membrane tension outside of an electrophysiology or a cell-based assay set-up. Recently, a new method for simulating membrane tension was reported in the form of membrane-fluidising agents named cyclodextrins (CDs) [11,79] [Figure 3]. CDs are truncated cone-shaped molecules formed from cyclic oligosaccharides, which have hydrophobic interiors and hydrophilic exteriors [80] [Figure 3]. Their chemical structure means CDs are able to extract lipids from bilayers, altering the hydrophobic force equilibrium, akin to membrane stretch. Following treatment with β-CD, cryo-EM analysis suggested that MscL adopts a flatter, more open-like structure [79], and that MscS alters its conformation [11]. Future studies on MS GPCRs could combine several of the above techniques in order to address mechanical force sensing at a molecular level [Figure 3]. GPCRs are typically less than 50 kDa in weight, meaning they are relatively small for most cryo-EM applications, despite recent advances [81,82], and they are notoriously difficult to crystallise [83]. However, PELDOR studies on AT_1R [84]

and B_2AR [85] have successfully determined distinct conformations stabilised by "biased" ligands [84] and resolved previously unknown intermediate conformations formed along the activation pathway [85]. More recently, PELDOR was used to assess the effect of cholesterol analogue binding on B_1AR conformation and dimerisation [86]. PELDOR is not limited by protein size, and it is therefore reasonable to suggest this method can be applied to MS GPCRs. The molecular mechanisms behind GPCR force transmission are not yet well understood. CD-treatment of GPCRs reconstituted into lipid bilayers, monitored by pulsed-EPR, could be a powerful approach for determining if MS GPCRs operate analogously to other MS proteins and channels.

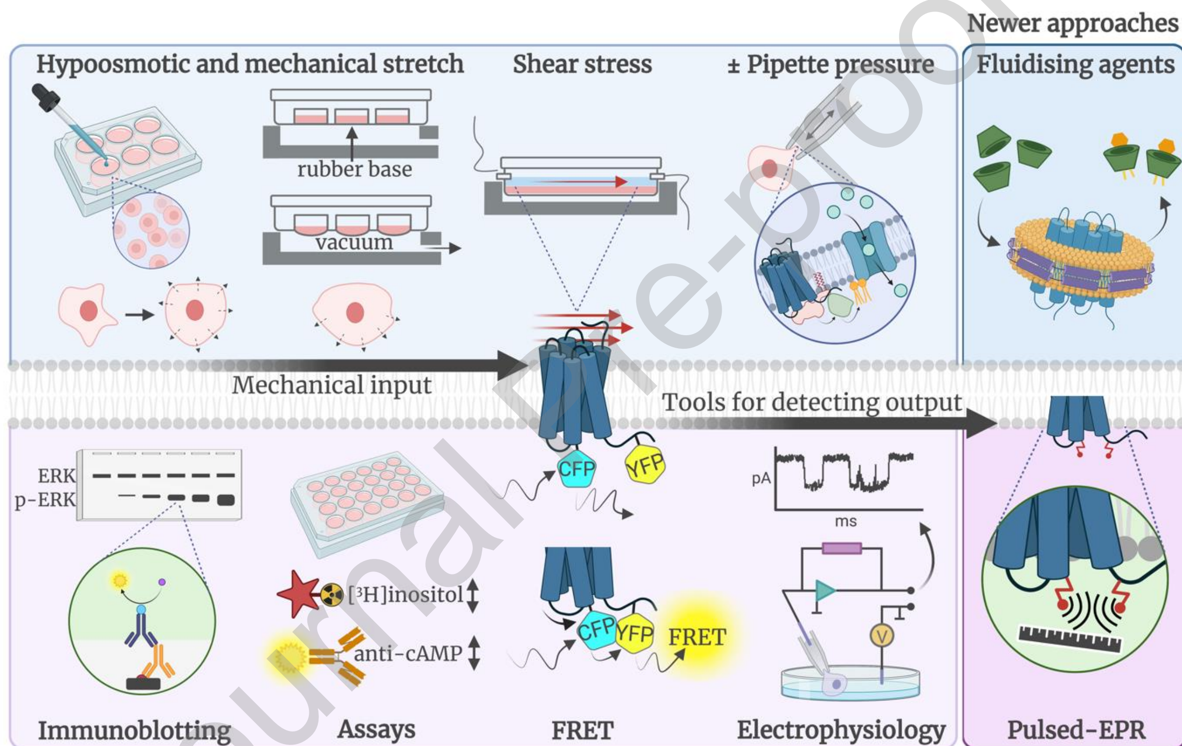

Figure 3. Tools for simulating membrane force in cells or lipid nanodiscs, and methods for detecting mechanical responses. Traditionally, studies on MS GPCRs use hypoosmotic stress, mechanical stretch or shear stress to mimic mechanical forces in whole-cell assays. The biological readout for such assays typically focuses on detecting changes in downstream effectors, such as phosphorylated ERKs, inositol phosphates or cyclic AMP. Electrophysiology may be used if the receptor is functionally tethered to an ion channel. A new approach for simulating membrane tension includes the use of cyclodextrins (CDs) to remove lipids from the bilayer. Detecting structural changes, rather than biological responses, is possible using bioluminescence (e.g., FRET) and pulsed electron paramagnetic resonance (EPR) spectroscopy, also known as PELDOR or DEER spectroscopy. Created using BioRender.com.

Conclusions and Future Perspectives

There is convincing evidence to suggest class A GPCRs are capable of sensing membrane tension and relaying force signals across the bilayer to trigger intracellular

pathways. However, very little is known about the potential mechanisms required for force transduction in GPCRs. Many studies involving both prokaryotic and eukaryotic MS molecules, such as MscS, MscL, PIEZOs, and K_2P ion channels, suggest these proteins conform to the force-from-lipids paradigm, meaning their conformation and thus function is at least partially membrane-lipid driven. Here, we have highlighted the importance of hydrophobic pockets and their lipid availability for force sensing, inspired by recent studies on bacterial mechanosensitive channels. Given that GPCRs, unlike ion-conducting channels, cannot be studied by patch clamp electrophysiology, a platform consisting of pulsed-EPR spectroscopic tools could enable the acquisition of interspin distances with Angstrom resolution and reliably reveal the conformational state of GPCRs in a native-like membrane environment under certain conditions, such as tension. H8 in GPCRs was previously identified as an important structural motif for MS, and we here hypothesise that the lipid-moves-first model, which stems from the general force-from-lipids principle, may apply to GPCRs. By applying the methods and techniques used to study established MS molecules, we may delineate the mechanisms of MS GPCRs in a comparable manner.

Acknowledgements
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References

1. Levina, N., Tötemeyer, S., Stokes, N.R., Louis, P., Jones, M.A. and Booth, I.R. (1999). Protection of Escherichia coli cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. *The EMBO Journal*, 18(7), pp.1730–1737.
2. Naismith, J.H. and Booth, I.R. (2012). Bacterial Mechanosensitive Channels—MscS: Evolution's Solution to Creating Sensitivity in Function. *Annual Review of Biophysics*, 41(1), pp.157–177.
3. Martinac, B. (2012). Mechanosensitive ion channels. *Channels*, 6(4), pp.211–213.
4. Ranade, Sanjeev S., Syeda, R. and Patapoutian, A. (2015). Mechanically Activated Ion Channels. *Neuron*, 87(6), pp.1162–1179.
5. ****Martinac, B., Adler, J. and Kung, C. (1990). Mechanosensitive ion channels of E. coli activated by amphipaths. *Nature*, 348(6298), pp.261–263.**

   Using *E. coli* spheroplasts and doping lipid bilayers with amphipathic compounds, the authors were the first to show that bacterial mechanosensitive ion channels sense and respond to membrane tension.

6. Sukharev, S. (2002). Purification of the Small Mechanosensitive Channel of Escherichia coli (MscS): the Subunit Structure, Conduction, and Gating Characteristics in Liposomes. *Biophysical Journal*, 83(1), pp.290–298.
7. Teng, J., Loukin, S., Anishkin, A. and Kung, C. (2014). The force-from-lipid (FFL) principle of mechanosensitivity, at large and in elements. *Pflügers Archiv - European Journal of Physiology*, 467(1), pp.27–37.
8. Martinac, B., Bavi, N., Ridone, P., Nikolaev, Y.A., Martinac, A.D., Nakayama, Y., Rohde, P.R. and Bavi, O. (2018). Tuning ion channel mechanosensitivity by asymmetry of the transbilayer pressure profile. *Biophysical Reviews*, 10(5), pp.1377–1384.
9. Cantor, R.S. (1999). Lipid Composition and the Lateral Pressure Profile in Bilayers. *Biophysical Journal*, 76(5), pp.2625–2639.
10. ****Pliotas, C., Dahl, A., Rasmussen, T., Mahendran, K., Smith, T., Marius, P., Gault, J., Banda, T., Rasmussen, A., Miller, S., Robinson, C., Bayley, H., Sansom, M., Booth, I. and Naismith, J. (2015). The role of lipids in mechanosensation. Nature Structural & Molecular Biology, 22(12), pp.991-998.**

    Pliotas et al. proposed a novel mechanism for mechanosensing, known as the "lipid-moves-first" model. Structural, biophysical and computational experiments on MscS confirmed the presence of lipids in 'pockets' near the cytoplasmic membrane leaflet, which reversibly exchange with bilayer lipids.
11. Zhang, Y., Daday, C., Gu, R.-X., Cox, C.D., Martinac, B., de Groot, B.L. and Walz, T. (2021). Visualization of the mechanosensitive ion channel MscS under membrane tension. *Nature*, 590(7846), pp.509–514.
12. ***Kapsalis, C., Wang, B., El Mkami, H., Pitt, S.J., Schnell, J.R., Smith, T.K., Lippiat, J.D., Bode, B.E. and Pliotas, C. (2019). Allosteric activation of an ion channel triggered by modification of mechanosensitive nano-pockets. Nature Communications, 10(4619), p.4619.**

    The authors used PELDOR (DEER) spectroscopy and Molecular dynamics to show that the high-pressure threshold channel MscL senses and responds to membrane forces through an allosteric mechanism. Lipids act as negative allosteric modulators moving out from the pockets as a consequence of increased tension.

13. Wang, B., Lane, B.J., Kapsalis, C., Ault, J.R., Sobott, F., El Mkami, H., Calabrese, A.N., Kalli, A.C. and Pliotas, C. (2022). Pocket delipidation induced by membrane tension or modification leads to a structurally analogous mechanosensitive channel state. *Structure*, 30(4), pp.608-622.e5.

14. Lane, B.J. and Christos Pliotas (2023). Approaches for the modulation of mechanosensitive MscL channel pores. *Frontiers in Chemistry*, 11:1162412
15. ***Brohawn, S.G., Campbell, E.B. and MacKinnon, R. (2014). Physical mechanism for gating and mechanosensitivity of the human TRAAK K+ channel. *Nature*, 516(7529), pp.126–130.**

    This manuscript provides the first experimental evidence that mammalian mechanosensitive ion channels sense membrane tension in the absence of cellular accessories, by showing that TRAAK reconstituted into liposomes gates in response to applied pressure.
16. Brohawn, S., Su, Z. and MacKinnon, R. (2014). Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K+ channels. *Proceedings of the National Academy of Sciences*, 111(9), pp.3614-3619.
17. Lolicato, M., Arrigoni, C., Mori, T., Sekioka, Y., Bryant, C., Clark, K.A. and Minor, D.L. (2017). K2P2.1 (TREK-1)–activator complexes reveal a cryptic selectivity filter binding site. *Nature*, 547(7663), pp.364–368.
18. Dong, Y., Pike, A., Mackenzie, A., McClenaghan, C., Aryal, P., Dong, L., Quigley, A., Grieben, M., Goubin, S., Mukhopadhyay, S., Ruda, G., Clausen, M., Cao, L., Brennan, P., Burgess-Brown, N., Sansom, M., Tucker, S. and Carpenter, E. (2015). K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac. *Science*, 347(6227), pp.1256-1259.
19. ***Kefauver, J.M., Ward, A.B. and Patapoutian, A. (2020). Discoveries in structure and physiology of mechanically activated ion channels. *Nature*, 587(7835), pp.567–576.**

    This review provides an overview of mechanosensitive proteins reported to-date, including mechanisms and hypotheses for mechanosensation.
20. Pliotas, C. and Naismith, J.H. (2017). Spectator no more, the role of the membrane in regulating ion channel function. *Current Opinion in Structural Biology*, 45, pp.59–66.
21. Rasmussen, T., Flegler, V., Rasmussen, A. and Böttcher, B. (2019). Structure of the mechanosensitive channel MscS embedded in the membrane bilayer. *Journal of Molecular Biology*, 431(17), pp.3081-3090.
22. Reddy, B., Bavi, N., Lu, A., Park, Y. and Perozo, E. (2019). Molecular basis of force-from-lipids gating in the mechanosensitive channel MscS. *eLife*, 8(e50486).
23. Flegler, V.J., Rasmussen, A., Borbil, K., Boten, L., Chen, H.-A., Deinlein, H., Halang, J., Hellmanzik, K., Löffler, J., Schmidt, V., Makbul, C., Kraft, C., Hedrich, R., Rasmussen, T. and Böttcher, B. (2021). Mechanosensitive channel gating by delipidation. *Proceedings of the National Academy of Sciences*, 118(33).

Journal Pre-proof

24. Lai, J., Poon, Y., Kaiser, J. and Rees, D. (2013). Open and shut: crystal structures of the dodecylmaltoside solubilized mechanosensitive channel of small conductance from Escherichia coli and Helicobacter pylori at 4.4 Å and 4.1 Å resolutions. *Protein Science*, 22(4), pp.502-509.
25. Perozo, E., Cortes, D.M., Sompornpisut, P., Kloda, A. and Martinac, B. (2002). Open channel structure of MscL and the gating mechanism of mechanosensitive channels. *Nature*, 418(6901), pp.942–948.
26. Wang, J., Jiang, J., Yang, X., Zhou, G., Wang, L. and Xiao, B. (2022). Tethering Piezo channels to the actin cytoskeleton for mechanogating *via* the cadherin-β-catenin mechanotransduction complex. *Cell Reports*, 38(6), p.110342.
27. Wang, Y., Guo, Y., Li, G., Liu, C., Wang, L., Zhang, A., Yan, Z. and Song, C. (2021). The push-to-open mechanism of the tethered mechanosensitive ion channel NompC. *eLife*, 10(e58388).
28. Cox, C.D., Bae, C., Ziegler, L., Hartley, S., Nikolova-Krstevski, V., Rohde, P.R., Ng, C.-A., Sachs, F., Gottlieb, P.A. and Martinac, B. (2016). Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension. Nature Communications, 7(10366).
29. Syeda, R., Florendo, Maria N., Cox, Charles D., Kefauver, J.M., Santos, Jose S., Martinac, B. and Patapoutian, A. (2016). Piezo1 Channels Are Inherently Mechanosensitive. *Cell Reports*, 17(7), pp.1739–1746.
30. Ridone, P., Vassalli, M. and Martinac, B. (2019). Piezo1 mechanosensitive channels: what are they and why are they important. *Biophysical Reviews*, 11(5), pp.795–805.
31. Rosenbaum, D.M., Rasmussen, S.G.F. and Kobilka, B.K. (2009). The structure and function of G-protein-coupled receptors. *Nature*, 459(7245), pp.356–363.
32. Hilger, D., Masureel, M. and Kobilka, B.K. (2018). Structure and dynamics of GPCR signaling complexes. *Nature Structural & Molecular Biology*, 25(1), pp.4–12.
33. Hamann, J., Aust, G., Demet Araç, Engel, F.B., Formstone, C.J., Fredriksson, R., Hall, R.A., Harty, B.L., Kirchhoff, C., Knapp, B., Krishnan, A., Liebscher, I., Lin, H.-H., Martinelli, D.R., Monk, K.R., Peeters, M.C., Piao, X., Prömel, S., Torsten Schöneberg and Schwartz, T.W. (2015). International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G Protein–Coupled Receptors. *Pharmacological Reviews*, 67(2), pp.338–367.
34. Lin, H.-H., Ng, K.-F., Chen, T.-C. and Tseng, W.-Y. (2022). Ligands and Beyond: Mechanosensitive Adhesion GPCRs. *Pharmaceuticals*, 15(2), p.219.
35. Wilde, C., Mitgau, J., Suchý, T., Schöneberg, T. and Liebscher, I. (2022). Translating the force—mechano-sensing GPCRs. *American Journal of Physiology-Cell Physiology*, 322(6), pp.C1047–C1060.

Journal Pre-proof

36. Demet Araç, Boucard, A.A., Bolliger, M., Nguyen, J., S. Michael Soltis, Südhof, T.C. and Brunger, A.T. (2012). A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *The EMBO Journal*, 31(6), pp.1364–1378.
37. Wootten, D., Christopoulos, A., Marti-Solano, M., Babu, M.M. and Sexton, P.M. (2018). Mechanisms of signalling and biased agonism in G protein-coupled receptors. *Nature Reviews Molecular Cell Biology*, 19(10), pp.638–653.
38. Zou, Y., Akazawa, H., Qin, Y., Sano, M., Takano, H., Minamino, T., Makita, N., Iwanaga, K., Zhu, W., Kudoh, S., Toko, H., Tamura, K., Kihara, M., Nagai, T., Fukamizu, A., Umemura, S., Iiri, T., Fujita, T. and Komuro, I. (2004). Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nature Cell Biology*, 6(6), pp.499-506.
39. Yatabe, J., Sanada, H., Yatabe, M.S., Hashimoto, S., Yoneda, M., Felder, R.A., Jose, P.A. and Watanabe, T. (2009). Angiotensin II type 1 receptor blocker attenuates the activation of ERK and NADPH oxidase by mechanical strain in mesangial cells in the absence of angiotensin II. *American Journal of Physiology-Renal Physiology*, 296(5), pp.F1052–F1060.
40. Mederos y Schnitzler, M., Storch, U., Meibers, S., Nurwakagari, P., Breit, A., Essin, K., Gollasch, M. and Gudermann, T. (2008). Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction. *The EMBO Journal*, 27(23), pp.3092-3103.
41. Rakesh, K., Yoo, B., Kim, I.-M., Salazar, N., Kim, K.-S. and Rockman, H.A. (2010). β-Arrestin–Biased Agonism of the Angiotensin Receptor Induced by Mechanical Stress. *Science Signaling*, 3(125).
42. Davis, M. and Hill, M. (1999). Signaling mechanisms underlying the vascular myogenic response. *Physiological Reviews*, 79(2), pp.387-423.
43. Li, H., Zhou, W.-Y., Xia, Y.-Y. and Zhang, J.-X. (2022). Endothelial Mechanosensors for Atheroprone and Atheroprotective Shear Stress Signals. *Journal of Inflammation Research*, 15(1), pp.1771–1783.
44. Darby, W.G., Potocnik, S., Ramachandran, R., Hollenberg, M.D., Woodman, O.L. and McIntyre, P. (2018). Shear stress sensitizes TRPV4 in endothelium-dependent vasodilatation. *Pharmacological Research*, 133, pp.152–159.
45. Saifeddine, M., El-Daly, M., Mihara, K., Bunnett, N.W., McIntyre, P., Altier, C., Hollenberg, M.D. and Ramachandran, R. (2015). GPCR-mediated EGF receptor transactivation regulates TRPV4 action in the vasculature. *British Journal of Pharmacology*, 172(10), pp.2493–2506.
46. Iring, A., Jin, Y.-J., Albarrán-Juárez, J., Siragusa, M., Wang, S., Dancs, P.T., Nakayama, A., Tonack, S., Chen, M., Künne, C., Sokol, A.M., Günther, S., Martínez,

Journal Pre-proof

A., Fleming, I., Wettschureck, N., Graumann, J., Weinstein, L.S. and Offermanns, S. (2019). Shear stress–induced endothelial adrenomedullin signaling regulates vascular tone and blood pressure. *Journal of Clinical Investigation*, 129(7), pp.2775–2791.

47. ***Xu, J., Mathur, J., Vessieres, E., Hammack, S., Nonomura, K., Favre, J., Grimaud, L., Petrus, M., Francisco, A., Li, J., Lee, V., Xiang, F., Mainquist, J., Cahalan, S., Orth, A., Walker, J., Ma, S., Lukacs, V., Bordone, L., Bandell, M., Laffitte, B., Xu, Y., Chien, S., Henrion, D. and Patapoutian, A. (2018). GPR68 senses blood flow and is essential for vascular physiology. *Cell*, 173(3), pp.762-775.**

    GPR68, an orphan receptor, was identified as a candidate for pressure sensing in vascular endothelial cells. GPR68 responds to turbulent shear stress but not to smooth laminar flow, highlighting the complex and nuanced response of mammalian force detectors.

48. Chachisvilis, M., Zhang, Y. and Frangos, J. (2006). G protein-coupled receptors sense fluid shear stress in endothelial cells. *Proceedings of the National Academy of Sciences*, 103(42), pp.15463-15468.
49. ***Erdogmus, S., Storch, U., Danner, L., Becker, J., Winter, M., Ziegler, N., Wirth, A., Offermanns, S., Hoffmann, C., Gudermann, T. and Mederos y Schnitzler, M. (2019). Helix 8 is the essential structural motif of mechanosensitive GPCRs. *Nature Communications*, 10(1).**

    The authors investigated the molecular mechanisms of mechanosensation in class A GPCRs. Helix 8, a common structural motif found in most GPCRs, was found to be critical for the force response in G_{q11}- $G_{i/o}$- and G_s-coupled receptors, and even conferred mechanosensitivity to a previously-insensitive GPCR.

50. Abdul-Majeed, S. and Nauli, S. (2011). Dopamine receptor type 5 in the primary cilia has dual chemo- and mechano-sensory roles. *Hypertension*, 58(2), pp.325-331.
51. Jung, B., Obinata, H., Galvani, S., Mendelson, K., Ding, B., Skoura, A., Kinzel, B., Brinkmann, V., Rafii, S., Evans, T. and Hla, T. (2012). Flow-regulated endothelial S1P receptor-1 signaling sustains vascular development. *Developmental Cell*, 23(3), pp.600-610.
52. Kwon, H., Wang, S., Helker, C., Rasouli, S., Maischein, H., Offermanns, S., Herzog, W. and Stainier, D. (2016). In vivo modulation of endothelial polarization by Apelin receptor signalling. *Nature Communications*, 7(1).
53. Zhang, Y., Frangos, J. and Chachisvilis, M. (2009). Mechanical stimulus alters conformation of type 1 parathyroid hormone receptor in bone cells. *American Journal of Physiology-Cell Physiology*, 296(6), pp.C1391-C1399.

Journal Pre-proof

54. Ozkan, A.D., Gettas, T., Sogata, A., Phaychanpheng, W., Zhou, M. and Lacroix, J.J. (2021). Mechanical and chemical activation of GPR68 probed with a genetically encoded fluorescent reporter. *Journal of Cell Science*, 134(16).
55. Papaioannou, T.G., Stefanadis, C. (2005). Vascular Wall Shear Stress: Basic Principles and Methods. *Hellenic Journal of Cardiology*, 46(1), pp.9-15.
56. Yasuda, N., Miura, S., Akazawa, H., Tanaka, T., Qin, Y., Kiya, Y., Imaizumi, S., Fujino, M., Ito, K., Zou, Y., Fukuhara, S., Kunimoto, S., Fukuzaki, K., Sato, T., Ge, J., Mochizuki, N., Nakaya, H., Saku, K. and Komuro, I. (2008). Conformational switch of angiotensin II type 1 receptor underlying mechanical stress-induced activation. *EMBO reports*, 9(2), pp.179–186.
57. Bavi, N., Cortes, D.M., Cox, C.D., Rohde, P.R., Liu, W., Deitmer, J.W., Bavi, O., Strop, P., Hill, A.P., Rees, D., Corry, B., Perozo, E. and Martinac, B. (2016). The role of MscL amphipathic N terminus indicates a blueprint for bilayer-mediated gating of mechanosensitive channels. *Nature Communications*, 7(1).
58. Chang, G., Spencer, R.H., Lee, A.T., Barclay, M.T., Rees, D.C. (1998). Structure of the MscL Homolog from Mycobacterium tuberculosis: A Gated Mechanosensitive Ion Channel. *Science*, 282(5397), pp.2220-2226.
59. Li, J., Guo, J., Ou, X., Zhang, M., Li, Y. and Liu, Z. (2015). Mechanical coupling of the multiple structural elements of the large-conductance mechanosensitive channel during expansion. *Proceedings of the National Academy of Sciences*, 112(34), pp.10726-10731.
60. Saotome, K., Murthy, S., Kefauver, J., Whitwam, T., Patapoutian, A. and Ward, A., (2018). Structure of the mechanically activated ion channel Piezo1. *Nature*, 554(7693), pp.481-486.
61. Zhao, Q., Zhou, H., Chi, S., Wang, Y., Wang, J., Geng, J., Wu, K., Liu, W., Zhang, T., Dong, M., Wang, J., Li, X. and Xiao, B., (2018). Structure and mechanogating mechanism of the Piezo1 channel. *Nature*, 554(7693), pp.487-492.
62. Wang, L., Zhou, H., Zhang, M., Liu, W., Deng, T., Zhao, Q., Li, Y., Lei, J. Li, X, Xiao, B. (2019). Structure and mechanogating of the mammalian tactile channel PIEZO2. *Nature*, 573(7773), pp.225-229.
63. Deng, Z., Paknejad, N., Maksaev, G., Sala-Rabanal, M., Nichols, C.G., Hite, R.K. and Yuan, P. (2018). Cryo-EM and X-ray structures of TRPV4 reveal insight into ion permeation and gating mechanisms. *Nature Structural & Molecular Biology*, 25(3), pp.252–260.
64. Ward, R., Pliotas, C., Branigan, E., Hacker, C., Rasmussen, A., Hagelueken, G., Booth, Ian R., Miller, S., Lucocq, J., Naismith, James H. and Schiemann, O. (2014). Probing the Structure of the Mechanosensitive Channel of Small Conductance in

Journal Pre-proof

Lipid Bilayers with Pulsed Electron-Electron Double Resonance. *Biophysical Journal*, 106(4), pp.834–842.

65. Yang, X., Lin, C., Chen, X., Li, S., Li, X. and Xiao, B. (2022). Structure deformation and curvature sensing of PIEZO1 in lipid membranes. *Nature*, 604(7905), pp.377–383.
66. Dionysopoulou, M., Yan, N., Wang, B., Pliotas, C. and Diallinas, G. (2022). Genetic and cellular characterization of MscS-like putative channels in the filamentous fungus Aspergillus nidulans. *Channels*, 16(1), pp.148–158.
67. Dijkman, P.M., Muñoz-García, J.C., Lavington, S.R., Kumagai, P.S., dos Reis, R.I., Yin, D., Stansfeld, P.J., Costa-Filho, A.J. and Watts, A. (2020). Conformational dynamics of a G protein–coupled receptor helix 8 in lipid membranes. *Science Advances*, 6(33).
68. Hedderich, J.B., Persechino, M., Becker, K., Heydenreich, F.M., Gutermuth, T., Bouvier, M., Bünemann, M. and Kolb, P. (2022). The pocketome of G-protein-coupled receptors reveals previously untargeted allosteric sites. *Nature Communications*, 13(1).
69. Kapsalis, C., Ma, Y., Bode, B.E. and Pliotas, C. (2020). In-Lipid Structure of Pressure-Sensitive Domains Hints Mechanosensitive Channel Functional Diversity. *Biophysical Journal*, 119(2), pp.448–459.
70. Hartley, A.M., Ma, Y., Lane, B.J., Wang, B. and Pliotas, C. (2020). Using pulsed EPR in the structural analysis of integral membrane proteins. *Electron Paramagnetic Resonance*, pp.74–108.
71. Lane, B.J., Wang, B., Ma, Y., Calabrese, A.N., El Mkami, H. and Pliotas, C. (2022). HDX-guided EPR spectroscopy to interrogate membrane protein dynamics. *STAR Protocols*, 30(4), pp.608-622.e5
72. Michou, M., Kapsalis, C., Pliotas, C. and Skretas, G. (2019). Optimization of Recombinant Membrane Protein Production in the Engineered Escherichia coli Strains SuptoxD and SuptoxR. *ACS Synthetic Biology*, 8(7), pp.1631–1641.
73. Haysom, S.F., Machin, J., Whitehouse, J.M., Horne, J.E., Fenn, K., Ma, Y., Hassane El Mkami, Nils Böhringer, Schäberle, T.F., Ranson, N.A., Radford, S.E. and Christos Pliotas (2023). Darobactin B Stabilises a Lateral-Closed Conformation of the BAM Complex in E. coli Cells. *Angewandte Chemie International Edition*, p.e202218783.
74. Li, J., Guo, J., Ou, X., Zhang, M., Li, Y. and Liu, Z. (2015). Mechanical coupling of the multiple structural elements of the large-conductance mechanosensitive channel during expansion. *Proceedings of the National Academy of Sciences*, 112(34), pp.10726-10731.
75. Pliotas, C., Ward, R., Branigan, E., Rasmussen, A., Hagelueken, G., Huang, H., Black, S.S., Booth, I.R., Schiemann, O. and Naismith, J.H. (2012). Conformational state of

Journal Pre-proof

the MscS mechanosensitive channel in solution revealed by pulsed electron–electron double resonance (PELDOR) spectroscopy. *Proceedings of the National Academy of Sciences*, 109(40).

76. Valera, S., Ackermann, K., Pliotas, C., Huang, H., Naismith, J.H. and Bode, B.E. (2016). Accurate Extraction of Nanometer Distances in Multimers by Pulse EPR. *Chemistry - A European Journal*, 22(14), pp.4700–4703.
77. Ackermann, K., Pliotas, C., Valera, S., Naismith, J.H. and Bode, B.E. (2017). Sparse Labeling PELDOR Spectroscopy on Multimeric Mechanosensitive Membrane Channels. *Biophysical Journal*, 113(9), pp.1968–1978.
78. Pliotas, C. (2017). Ion Channel Conformation and Oligomerization Assessment by Site-Directed Spin Labeling and Pulsed-EPR. *Methods in Enzymology*, pp.203–242.
79. Cox, C.D., Zhang, Y., Zhou, Z., Walz, T. and Martinac, B. (2021). Cyclodextrins increase membrane tension and are universal activators of mechanosensitive channels. *Proceedings of the National Academy of Sciences*, 118(36).
80. Hammoud, Z., Khreich, N., Auezova, L., Fourmentin, S., Elaissari, A. and Greige-Gerges, H. (2019). Cyclodextrin-membrane interaction in drug delivery and membrane structure maintenance. *International Journal of Pharmaceutics*, 564, pp.59–76.
81. Danev, R., Belousoff, M., Liang, Y.-L., Zhang, X., Eisenstein, F., Wootten, D. and Sexton, P.M. (2021). Routine sub-2.5 Å cryo-EM structure determination of GPCRs. *Nature Communications*, 12(1).
82. Zhang, X., Johnson, R.M., Drulyte, I., Yu, L., Kotecha, A., Danev, R., Wootten, D., Sexton, P.M. and Belousoff, M.J. (2021). Evolving cryo-EM structural approaches for GPCR drug discovery. *Structure*, 29(9), pp.963-974.e6.
83. Rasmussen, S.G.F., Choi, H.-J., Rosenbaum, D.M., Kobilka, T.S., Thian, F.S., Edwards, P.C., Burghammer, M., Ratnala, V.R.P., Sanishvili, R., Fischetti, R.F., Schertler, G.F.X., Weis, W.I. and Kobilka, B.K. (2007). Crystal structure of the human β2 adrenergic G-protein-coupled receptor. *Nature*, 450(7168), pp.383–387.
84. Wingler, L.M., Elgeti, M., Hilger, D., Latorraca, N.R., Lerch, M.T., Staus, D.P., Dror, R.O., Kobilka, B.K., Hubbell, W.L. and Lefkowitz, R.J. (2019). Angiotensin Analogs with Divergent Bias Stabilize Distinct Receptor Conformations. *Cell*, 176(3), pp.468-478.e11.
85. ****Manglik, A., Kim, T., Masureel, M., Altenbach, C., Yang, Z., Hilger, D., Lerch, Michael T., Kobilka, T., Thian, F., Hubbell, Wayne L., Prosser, R. Scott and Kobilka, Brian K. (2015). Structural Insights into the Dynamic Process of β2 -Adrenergic Receptor Signaling. *Cell*, 161(5), pp.1101–1111.**

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The authors used PELDOR (DEER) and NMR spectroscopies, to assign distinct conformations of B_2AR and estimated the relative populations of each conformation in response to ligands.

86. Kubatova, N., Schmidt, T., Schwieters, C.D. and Clore, G.M. (2023). Quantitative analysis of sterol-modulated monomer–dimer equilibrium of the β 1 -adrenergic receptor by DEER spectroscopy. *Proceedings of the National Academy of Sciences*, 120(7).

Declaration of Competing Interest

The authors declare no conflict of interest

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