Review Article

The Influence of Phosphoinositide Lipids in the Molecular Biology of Membrane Proteins: Recent Insights from Simulations

George Hedger [1,*], **and Hsin-Yung Yen** [2,3]

*1 - **Department of Life Sciences,** Sir Ernst Chain Building, Imperial College London, London, SW7 2AZ, UK*
*2 - **Institute of Biological Chemistry,** Academia Sinica, Taipei 115, Taiwan*
*3 - **Department of Chemistry,** University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK*

***Correspondence to George Hedger:** g.hedger@imperial.ac.uk (G. Hedger)*
https://doi.org/10.1016/j.jmb.2025.168937
Editor: Gerhard Hummer

Abstract

The phosphoinositide family of membrane lipids play diverse and critical roles in eukaryotic molecular biology. Much of this biological activity derives from interactions of phosphoinositide lipids with integral and peripheral membrane proteins, leading to modulation of protein structure, function, and cellular distribution. Since the discovery of phosphoinositides in the 1940s, combined molecular biology, biophysical, and structural approaches have made enormous progress in untangling this vast and diverse cellular network of interactions. More recently, *in silico* approaches such as molecular dynamics simulations have proven to be an asset in prospectively identifying, characterising, explaining the structural basis of these interactions, and in the best cases providing atomic level testable hypotheses on how such interactions control the function of a given membrane protein. This review details a number of recent seminal discoveries in phosphoinositide biology, enabled by advanced biomolecular simulation, and its integration with molecular biology, biophysical, and structural biology approaches. The results of the simulation studies agree well with experimental work, and in a number of notable cases have arrived at the key conclusion several years in advance of the experimental structures.

Summary: Hedger and Yen review developments in simulations of phosphoinositides and membrane proteins.

© 2025 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Phosphoinositides are a family of membrane lipids with far reaching influences on cellular function.[1] The study of phosphoinositides has a rich history dating back many decades.[2–7] This work has revealed roles in cell signalling,[8] trafficking,[9] cytoskeletal organisation,[10] and regulation of ion passage across membranes.[11] Pathogenic variance in the kinases and phosphatases which control the relative cellular abundance of phosphoinositides are associated with a number of human diseases including growth disorders, cancers, and neurological conditions.[12–16] Underlying these cellular functions are molecular level interactions of phosphoinositides with integral and peripheral membrane proteins. Experimental methods such as X-ray crystallography have made significant early contributions to revealing the detailed architecture of membrane proteins in complex with phosphoinositides.[17] The advent of technologies including atomic resolution cryo-EM[18] and native mass spectrometry[19] have accelerated that understanding.[20–22] Such structural studies have been coupled to intricate biochemical work to show how specific phosphoinositides control the function of certain membrane proteins. Despite progress, resolving bound phosphoinositides in structures[23]

0022-2836/© 2025 The Author(s). Published by Elsevier Ltd.This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

and relating this information to function and dynamics at the molecular-level using experimental techniques remains non-trivial from static structures alone. *In silico* approaches such as molecular dynamics (MD) simulations have experienced rapid technological development in the area of membranes and membrane proteins.[24,25] This has led to a surge in simulation studies, and a number of notable contributions to uncovering new aspects of phosphoinositide biology. Many of these simulations have been performed in an integrated fashion with experiments. The simulations serve to computationally reunite experimentally determined membrane protein structures with their complex lipid bilayer environment, and then capture the atomic level dynamics and structural aspects of how the membrane protein interacts with phosphoinositides. In a number of cases, simulations have prospectively predicted phosphoinositide binding sites on integral membrane proteins several years in advance of experimental structures being determined. In the very best examples the simulations have provided a detailed mechanistic view into how the structure and dynamics of the interaction then lead to the phosphoinositide altering the function of a given membrane protein.

Within this review we examine how simulations and complementary experiments have revealed new and exciting molecular-level aspects of phosphoinositide biology. We begin with a brief survey of cell membranes, phosphoinositides, and biomolecular simulation approaches.

Cell membranes

Cells and subcellular compartments are defined by membranes. Membranes are composed of a lipid bilayer and embedded membrane proteins. This bilayer is compositionally complex, containing hundreds of lipid species asymmetrically distributed between leaflets.[26] Advances in structural biology of membrane proteins[18] and in lipidomics[27,28] have led to enormous progress in uncovering the compositional complexity and molecular level architecture of membranes. The structures of ca. 1700 unique membrane proteins have been determined (https://blanco.biomol.uci.edu/mpstruc/) alongside ca. 49,000 lipid structures (https://www.lipidmaps.org/databases/lmsd/overview). The composition of a membrane and its organisation varies substantially by membrane type, stage in the life cycle, metabolic state, signalling state, and health and disease.[29,30] Lipids interact with membrane proteins and alter their structure, function, localisation, and oligomeric states.[31] These interactions may take a variety of forms, including binding to specific well-defined sites on the membrane-exposed surface of a protein with complementary geometry, binding to sites deep within a protein, or more dynamic interactions with disordered patches of complementary charge or grease. They may also occur indirectly by influences on the local biophysical properties of the membrane. Lipids may even represent the native ligand for a membrane protein, as has been proposed for activation of Smoothened by cholesterol.[32,33] Examples of regulation of membrane protein function by phosphoinositides include receptor tyrosine kinases (RTKs),[34] inward rectifying potassium (Kir) channels,[35] G-protein-coupled receptors (GPCRs),[21] neurotransmitter transporters[36,37] and syntaxins.[38] In the case of phosphoinositides, another major type of lipid-protein interaction is the recruitment and orientation of soluble proteins, so called 'peripheral membrane proteins' (PMPs) to the membrane surface by direct association of the PMP with the phosphoinositide headgroup.[39,40]

The phosphoinositide family

Phosphoinositides are phosphorylated derivates of phosphatidylinositol (PI) (Figure 1). Differential decoration of the *myo*-inositol headgroup with varying numbers and positions of phosphate groups give rise to seven derivates in humans. The inositol group adopts a chair conformation with inorganic ions complexed to the negative phosphoryl groups.[41] The acyl tails show unusual uniformity compared to other phospholipids, and are usually of the 1-stearoyl-2-arachidonoyl variety.[42] The overall abundance of phosphoinositides is estimated at less than 1% of cellular phospholipids, with species such as $PI(3,4,5)P_3$ being even rarer.[43,44] This seeming dichotomy led to the famous adage 'Tiny lipids with giant impact on cell regulation'.[1] Precise measurements of abundance remain challenging, not least as it is a moving target. The relative abundance of different phosphoinositide species is under constant dynamic spatiotemporal control, and varies by cell type, signalling state, and myriad other cellular conditions. The distribution of phosphoinositide species also varies by subcellular location (Figure 1C),[14,44,45] which confers unique biochemical properties to the host membranes. Localisation to specific membranes, as well as localisation to particular regions within the same membrane,[38] can lead to substantially higher local concentrations than the total cellular fraction would imply. Phosphoinositides also exhibit leaflet asymmetry, with e.g. $PI(4,5)P_2$ being found within the inner leaflet of the plasma membrane, with reports of it constituting up to 5% of total lipids.[8,46] While the outer leaflet of human plasma membranes is abundant in enormous lipids with complex glycosylation patterns,[47,48] within the inner leaflet it is phosphoinositides which bear one of the largest headgroups relative to other phospholipids, which protrude beyond the membrane surface into solution. They are also the most negatively charged of the major plasma membrane inner leaflet lipids. These two properties in particular set them apart and confer a suite of biochemical interactions not available to other phospholipids. During apoptosis

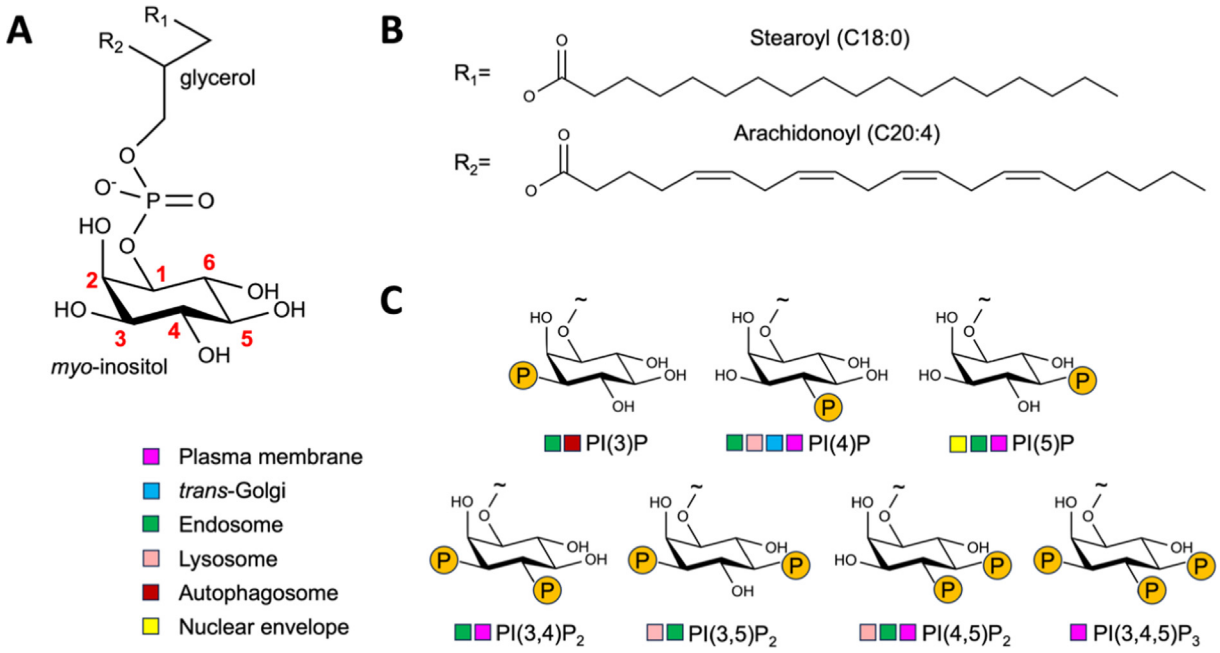

Figure 1. The phosphoinositide family. (A) Structure of the phosphatidylinositol headgroup depicting the chair conformation and numbering scheme of the myo-inositol moiety. (B) The predominant fatty acid tails. (C) Headgroup structures of the seven eukaryotic phosphoinositides, derived from phosphatidylinositol. The average cellular distributions are indicated. Note that this varies with cell type and myriad other variables. See Refs. 14,44 for further detailed discussion of cellular distribution.

this plasma membrane asymmetry is disrupted, and phosphoinositides have been reported to be present in elevated levels within the outer leaflet where they may act as 'eat-me' signals in cell death.[49] Although not canonically referred to as phosphoinositides, membrane lipids containing the core phosphatidylinositol structure have been seen in other domains of life, for example in mycobacteria.[50,51]

Computational approaches for studying phosphoinositides

A variety of computational approaches are available to learn something about the interactions and dynamics of biomolecules. These include docking,[52,53] artificial intelligence (AI) methods,[54–57] quantum mechanics/molecular mechanics (QM/MM) approaches,[58,59] and molecular dynamics (MD) simulations.[60,61] Amongst these, MD simulation has shown particular utility in the study of lipid–protein interactions.[62–65] At its core, MD simulation is a physics-based method to predict how a group of particles (representing the biomolecule(s) of interest) propagate through time and space, using classical mechanics and an underlying potential energy function which describes particle interactions. The functional form of this potential energy function and the set of parameters associated with it are termed the 'force field'. Parameters for a given biomolecule are obtained from experiments and quantum mechanical calculations. The successful application of the simulation relies on having a well-parameterised model and sufficient sampling of conformational space, coupled to appropriate simulation design and analysis. Common force field choices for membrane protein systems include CHARMM,[66,67] AMBER,[68–70] and MARTINI.[24,71] Simulations may be performed at all-atom resolution, or at 'coarse-grained' (CG) resolution, in which groups of atoms are represented as single particles. This is the case for the popular MARTINI force field,[24] which has shown particular utility in simulation of membrane systems and lipid-protein interactions.[25,64,72,73] CG resolutions decrease the computational load of the calculations and allow greater sampling of time and space. The compromise on atomic detail may be tempered by conversion[74,75] of the end-point of a CG simulation back to all-atom detail followed by subsequent all-atom simulation, i.e. multiscale simulation.[76] Other approaches to addressing the sampling challenge include the use of special purpose supercomputers such as ANTON,[77] and a group of techniques collectively termed 'enhanced sampling',[78] which are essentially statistical mechanical and algorithmic tricks to enforce sampling of certain regions of space or 'collective variables'. This review will share examples of all three of these strategies as it pertains to simulations of phosphoinositides.

Amongst phospholipids, phosphoinositides represent one of the more challenging lipid groups for MD simulation due to their highly charged headgroups. For example, while the net headgroup charge of $PI(4,5)P_2$ lipids has been

estimated as ca. −4 by ^{31}P NMR and QM methods in simplified systems,[41,79] the magnitude and charge distribution may vary substantially *in vivo* depending on physiological microenvironment.[80,81] This last part is key, as a PI(4,5)P_2 lipid in the vicinity of a cluster of basic residues may be induced toward the −5 state, while in other cellular scenarios may adopt forms with reduced net charge. The vast majority of simulation approaches employ a fixed charge model in which charge states are set at the beginning of the simulation and remain thus. The choice of charge state is therefore non-trivial. While this is a general challenge in MD simulations,[82] it is perhaps more acute for certain phosphoinositides. Though -4 variants of PI(4,5)P_2 are most common,[83] -5 variants have been seen.[84] What is most appropriate depends on the system in question and testing of multiple variants may be necessary. Despite these challenges, significant effort has been put into the continued improvement of phosphoinositide parameters, and upgraded versions continue to be released.[83,85,86] This even extends beyond the core group of mammalian phosphoinositides to more exotic forms with additional modifications, such as those found in *Mycobacterium tuberculosis*.[87]

A theme which will become apparent in the course of this review is that amongst the various phosphoinositide species, PI(4,5)P_2 has received particularly high levels of attention in simulation and structural studies. This may in part correlate with its inherent biological importance and its relative abundance under a range of cellular states. It may also reflect its localisation to (the inner leaflet of) the plasma membrane, where much membrane protein research has historically tended to focus, especially from a pharmacological perspective. Pragmatic factors such as the early availability of parameters and seminal simulation works on PI(4,5)P_2 may also have contributed.[38,84,88,89] We note that simulation parameters are now available for all phosphoinositides species in both all-atom and CG resolutions, and continue to be improved.[83,85,90,91]

More generally, parameters now exist for many thousands of different membrane lipid species.[86,92,93] This explosion in parameter space has been coupled to knowledge from experimental lipidomics[27,29] to allow simulations in complex asymmetric membranes which mimic e.g. cell-type specific plasma membranes[94,95] and other subcellular membranes.[25,96–98] The mutual lipid-lipid interplay between species within complex biologically relevant membranes can be critical to the biological activity of phosphoinositides[99] and the potential to capture this in simulations is a powerful ability. In addition to improvements in parameter quality, new tools for the setup and analysis of simulations continue to be developed.[93,100–105] In particular, specialised tools for the analysis of lipid-protein interaction have emerged, including PyLipID[106] and ProLint,[107] as well as refinement of more advanced simulation methodologies such as calculating $\Delta\Delta$Gs for lipid binding.[108]

These specific advances have occurred to the continual background march of hardware improvement[109] and general force field quality.[24] Notably, the widespread use of GPU computing and the corresponding updates to simulation code have dramatically increased the timescales accessible to research groups.[110] CG simulations of small membrane proteins such as GPCRs may now reach hundreds of microseconds without breaking too much of a sweat, while length scales of hundreds of nanometres have also been seen.[111–116] On commodity compute devices atomistic simulations are comfortably of the order of several microseconds for most groups and system sizes, while special purpose devices may routinely reach hundreds of microseconds as a matter of course.[117] The 'dawn' of exascale computing[118] will further increase accessible time and length scales.[119]

Coupled with new experimental capabilities in structural biology[18] and biophysics,[120] these improvements in simulation approaches have been applied alongside experiments by hundreds of scientists to unlock the molecular basis for many exciting new facets of phosphoinositide biology. In the following section we highlight a range of case studies. We then draw it together to summarise an emerging picture of simulation-based analysis of phosphoinositide influence on membrane protein structure, function, and dynamics. We end by taking a critical look at challenges and imminent advances.

Recent Case Studies

G-protein-coupled receptors

GPCRs constitute the largest family of human membrane proteins[121] and play key roles in signal transduction at the plasma membrane,[122] and at subcellular membranes.[123–125] The core unit of these receptors consists of a seven transmembrane helical bundle, which may be differentially decorated with extracellular and intracellular domains.[126] GPCRs are activated by a diverse array of stimuli including binding of small molecule odorants,[127] peptide hormones,[128] neurotransmitters,[129] and by photons of light.[130] This leads to a conformational change in the helical bundle, coupling to intracellular interaction partners such as G-proteins and arrestins, and signal propagation to downstream cascades ultimately leading to a cellular outcome.[131]

Membrane lipids bind and control GPCR function via diverse modes. The role of cholesterol has been the subject of particularly extensive study by experiment and simulation.[32,132–134] Phospholipids have been known to bind and modulate GPCR structure and function for some time.[135–138] However, it is only relatively recently that phosphoinosi-

tide modulation of GPCRs has moved into the spotlight.[22]

An integrated simulation and native mass spectrometry study in 2018 discovered that PI(4,5)P_2 lipids were able to bind directly to Class A GPCRs and enhance G-protein coupling.[21] In this work, the interactions of PI(4,5)P_2 with the Neurotensin receptor 1 (NTSR1), the Adenosine A_{2A} receptor (A_{2A}R), and the β_1 adrenergic receptor (β_1AR) were first detected by structural native mass spectrometry. CG simulations of up to 100 μs duration were then employed to predict the locations of these interactions on the membrane exposed surface of the GPCR. Key residues seen to bind PI(4,5)P_2 in the simulations were used to inform a mass spectrometry mutagenesis strategy which identified TM4 and TM1 as the major PI(4,5)P_2 binding region on NTSR1, alongside several lower affinity sites (Figure 2A). Interestingly, in both simulations and mass spectrometry experiments, PI(4,5)P_2 outcompeted all other phosphoinositide subtypes, including the more negatively charged PI(3,4,5)P_3. This is indicative of specific complementary geometries and not only simple correlation with net headgroup charge. High-throughput CG simulations then expanded this analysis to nine other Class A GPCRs, demonstrating conserved PI(4,5)P_2 interaction, albeit with variability in the precise interaction pattern. The functional consequences of this interaction were demonstrated by employing potential of mean force (PMF) simulations[139] in which a mini-G-protein was pulled away from the A_{2A}R under ±PI(4,5)P_2 conditions. This showed a ca. 50% enhancement in the stability of GPCR-G-protein complex in the presence of PI(4,5)P_2, which acted as a molecular glue bridging between the GPCR and G-protein. This agreed well with complementary mass spectrometry G-protein coupling experiments which showed selective enhancement of complex formation for mini-Gα_s over mini-Gα_i and mini-Gα_{12}, and with fluorescence-based GTPase functional assays in the presence and absence of PI(4,5)P_2.[21] Intriguingly, two years after this discovery, a cryo-EM structure was published of NTSR1 bound to β-arrestin 1.[140] This structure showed a short-chain dioctyl PI(4,5)P_2 lipid bound at the same high affinity TM4 site previously identified in the simulations and mass spectrometry (Figure 2B). These combined works uncovered the structural and functional basis for a new biological role for PI(4,5)P_2 in GPCR signalling, with broad consequences for cell biology.[8,141] Of particular note, while most known roles of phosphoinositides have their origins in the culture plates and test tubes of cell biology and molecular biology approaches, the present story began with simulations and chemistry at the molecular level within computers and mass spectrometers.

Structural coverage of GPCRs has increased exponentially in recent years.[142] The number of experimentally determined structures in the PDB and GPCRdb is now well over one thousand.[143] The advent of predictive AI models such as the AlphaFold suite,[54] Chai-1,[57] and RoseTTAFold[56] provide another means of obtaining initial structural coordinates for simulation,[144–146] alongside tradi-

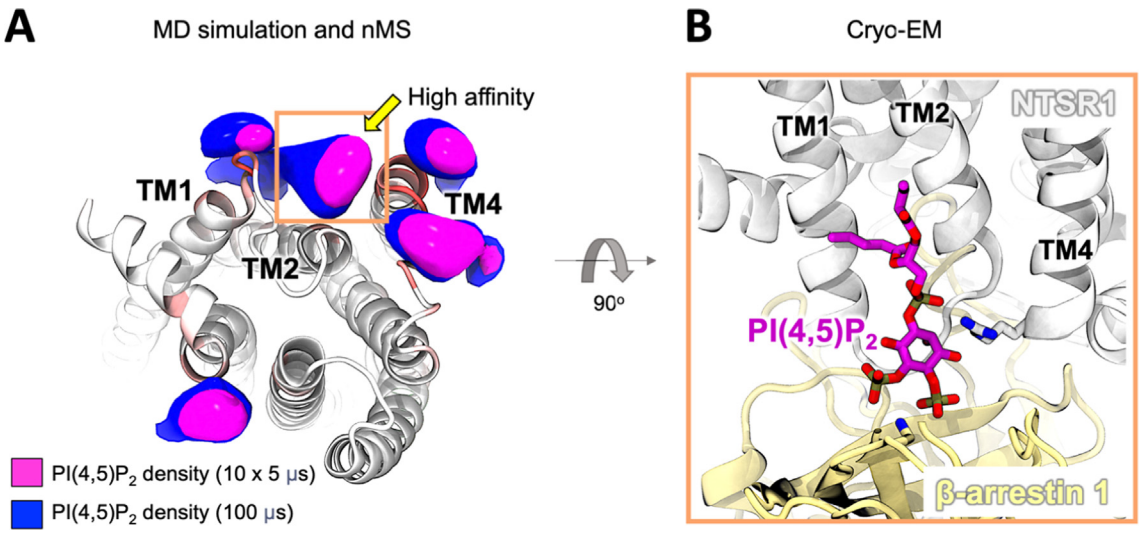

Figure 2. PI(4,5)P_2 binding sites on the NTSR1 GPCR identified by simulation and experiment. (A) View from the cytosol onto the base on the NTSR1 showing simulation derived densities for PI(4,5)P_2 around the TM helices.[21] Densities are shown for an ensemble of short simulations (magenta), and one long simulation (blue) to demonstrate convergence of the simulation over these timescales. The site which showed the highest affinity for PI(4,5)P_2 in the simulations and nMS is labelled. Helices with red patches indicate residues with high levels of direct phosphoinositide interaction. (B) Side view of a Cryo-EM structure of NTSR1 in complex with β-arrestin 1 showing short-chain dioctyl PI(4,5)P_2 resolved at the same high affinity site (PDB ID: 6UP7).[140]

tional homology modelling.[147] This has presented new opportunities for molecular simulations. A number of studies have leveraged the increased availability of structures to perform simulations of GPCRs in both active and inactive states to assess the conformational dependence of phosphoinositide interactions.[148–154] Song et al., identified differences in the free energies of interaction for PI(4,5)P_2 with binding sites on the A_{2A}R between inactive, active, and active-miniG states.[148] Ma et al., subsequently showed the stiffening effects of PI(4,5)P_2 on both the inactive and active states of A_{2A}R in limiting the accessible conformational landscape.[151] Later experiments employing ^{19}F NMR in nanodiscs suggested that in addition to the role of PI(4,5)P_2 in enhancing G-protein coupling,[21] it may also increase population of the A_{2A}R active state in the absence of G-protein.[155] CG simulations combined with experimental measurements have reported similar PI(4,5)P_2 induced stabilisation of the active state of the Ghrelin receptor,[154] as well as an influence on its coupling to β-arrestin 1.[156] Most recently PI(4,5)P_2 interaction with ICL3 has been suggested to stabilise the active state of β_2AR.[153]

Schiøtt and colleagues performed detailed work on the Class B Glucagon receptor (GCGR), finding that in contrast to Class A GPCRs, PI(4,5)P_2 preferentially bound the inactive state of the receptor.[150] Interestingly, the authors also found that binding was modulated by acyl tail saturation pattern, supported by native mass spectrometry measurements. This is a novel observation as phosphoinositides *in vivo* have particularly uniform acyl tail composition of the stearoyl/arachidonoyl form,[42] and most interactions seen in simulations and structures tend to be dominated by headgroup electrostatics, with tails being dynamic and/or unresolved due to inherent flexibility and the use of short-chain derivatives.[17] An influence of acyl tail saturation pattern on PI(4,5)P_2 clustering propensity has also been reported in simulations in the absence of protein.[157]

Amongst Class F GPCRs, CG simulations of the hedgehog signal transducer Smoothened (SMO) predicted PI(4,5)P_2 encounter complexes.[158] This is intriguing as SMO signalling is intricately linked to primary cilia, a region which contains special zones of phosphoinositide enrichment.[159] Joubert's syndrome, a human ciliopathy characterized by impaired Hh signaling and human birth defects, can be caused from mutations in a 5-positon phosphatase (Inpp5e) which lead to alterations in the distribution of ciliary PI(4,5)P_2.[12,159–161]

As well as focussed simulations of different conformational states of particular GPCRs, simulations may also assess trends across larger groups. Sejdiu and Tieleman performed simulations of 23 unique GPCRs and characterised their 'lipid fingerprint', that is, the unique lipid interaction profile of each GPCR.[149] While phosphoinositide species interacted with all 23 GPCRs, the location and strength of interaction showed substantial variation. Most recently, CG simulations of 15 human Class B1 GPCRs were performed in both active and inactive states.[162] A conserved state-dependent pattern of PI(4,5)P_2 interaction was seen across the subfamily at the TM6/7 – H8 interface, in agreement with earlier CG simulations of GCGR.[150,152] This observation is particularly intriguing as two antagonists of GCGR have been structurally resolved bound at this same interaction site (PDB ids: 5EE7 and 5XEZ).[163,164] A positive allosteric modulator (PAM) used to stabilise structures of glucose-dependent insulinotropic polypeptide receptor (GIPR) also binds adjacent to this site, and has been used to stabilise the receptor in complex with the antidiabetic therapeutic tirzepatide (Mounjaro) (PDB id: 7RBT).[165] This raises the intriguing prospect of possible direct phosphoinositide / drug competition and synergistic binding effects at this region.

Phosphoinositide interactions with GPCRs have also been modelled in large membrane patches measuring up to ca. 135 nm across and containing 144 GPCR proteins embedded in plasma membrane like lipid compositions[166–168] (Figure 3A). As well as providing excellent statistics on phosphoinositide behaviour, these simulations allow assessment of *in vivo* like factors including e.g. competition effects between different phosphoinositide species such PI(4,5)P_2 and PI(3,4,5)P_3, the involvement of membrane curvature, and of lateral protein–protein interactions. In this spirit, clustering of PI(4,5)P_2 around the S1P receptor 1 (S1PR1)[166] and NTSR1[167] have been observed. Similarly, work on the A_{2A}R embedded in complex membranes employed simulations coupled to Markov state modelling to predict PI(4,5)P_2 mediated enhancement of A_{2A}R oligomerisation[168] (Figure 3B).

A common thread in all simulation studies of GPCRs in multicomponent membranes, is that despite comprising a small percentage of the simulated lipid composition, phosphoinositides routinely outcompete all other inner leaflet phospholipids for interaction with the GPCR. This is especially pertinent for lipid species which, like phosphoinositides, have net anionic headgroup charges such as phosphatidylserine (PS) and phosphatidylglycerol (PG) and which have previously been reported to modulate GPCRs.[135,169–171] This highlights the importance of including the biologically relevant phosphoinositide species in experiments and simulations investigating interactions of lipids with GPCRs. It also raises the question of how many anionic phospholipid interaction sites and functional effects previously reported from *in vitro* and *in silico* studies of GPCRs in simple membranes without phosphoinositides may be abrogated or enhanced by phosphoinositides *in vivo.*

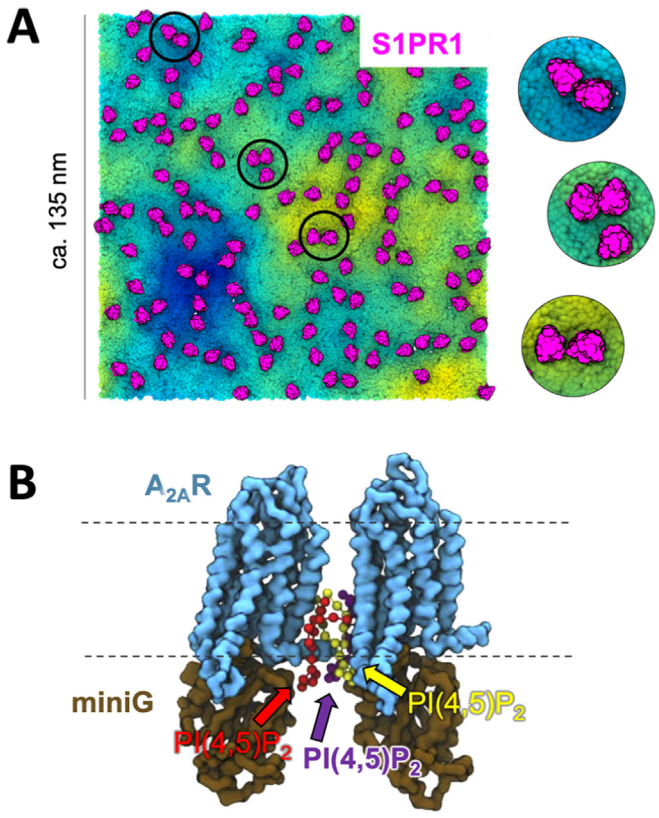

Figure 3. Phosphoinositides can mediate lateral GPCR interactions. (A) 144 S1PR1 GPCRs (pink) in a membrane with complex asymmetric lipid distribution mimicking that of a mammalian plasma membrane. The image shown is from the end of a 10 μs CG simulation. (B) An $A_{2A}R$ dimer with interfacial $PI(4,5)P_2$ lipids mediating the interaction. We thank Dr. Heidi Koldsø and Dr. Wanling Song for the figure. Figure (adapted) reprinted with permission from 166 and 168. Copyright (2015) American Chemical Society.

Ion channels

Ion channels have a particularly close relationship with phosphoinositides.[35] This relationship has been subject to extensive structural, functional, and computational study.[172–174] We focus on transient receptor potential (TRP) channels, for which phosphoinositide modulation is diverse and emerging, and on inward-rectifying potassium (Kir) channels, which require $PI(4,5)P_2$ for activation.

Transient receptor potential channels. Transient receptor potential (TRP) channels are a major class of tetrameric ion channel,[175] and their dysfunction has been implicated in a range of diseases.[176–178] TRP channels have been shown to be functionally modulated by lipids including sterols and phospholipids.[179] The binding of phosphoinositides to TRP channels has been explored in a range of structural and simulation studies.[20,180–186] In 2020, a combined simulation, biochemical, and structural study explored the binding of a number of phosphoinositide species to polycystin-2[181] (PC2; also known as TRPP2 or PKD2). Cryo-EM structures of PC2 in the presence of $PI(3,5)P_2$ (to 3.4 Å), and of $PI(4,5)P_2$ (to 3 Å) revealed non-protein density between the transmembrane helices S3, S4 and S5 (Figure 4). As is often the case in structural studies, lipid identity could not be unambiguously assigned from the experimental electron density alone. The authors addressed this challenge by performing CG simulations of PC2 in lipid bilayers, in which they observed spontaneous binding of $PI(4,5)P_2$ lipids to the same S3, S4, S5 region seen in the PC2 cryo-EM structures (Figure 4A,B). This region had also previously been seen to bind PI, $PI(4,5)P_2$, and vanilloids (e.g. capsaicin) in TRPV1/5 cryo-EM structures and MD simulations of TRPV6.[20,180] Gao et al., noted in their phosphoinositide bound structure the possibility of the pocket being suited to bind a range of phosphoinositide species.[20] Simulations designed to calculate free energies of lipid association with this site on PC2 from the PMF[139] well-depth obtained values of ca. −9 kJ/mol (PS), −20 kJ/mol (PI), −28 kJ/mol ($PI(3,4,5)P_3$), and −37 kJ/mol ($PI(4,5)P_2$). While this suggested a specific interaction of PIP_2 over PS, PI, and $PI(3,4,5)P_3$, the authors noted the limitations of the CG simulation model in distinguishing between the $PI(4,5)P_2$ and $PI(3,5)P_2$ isoforms. A biochemical assay in the form of 'PIP strips' provides one route to experimental interrogation of lipid

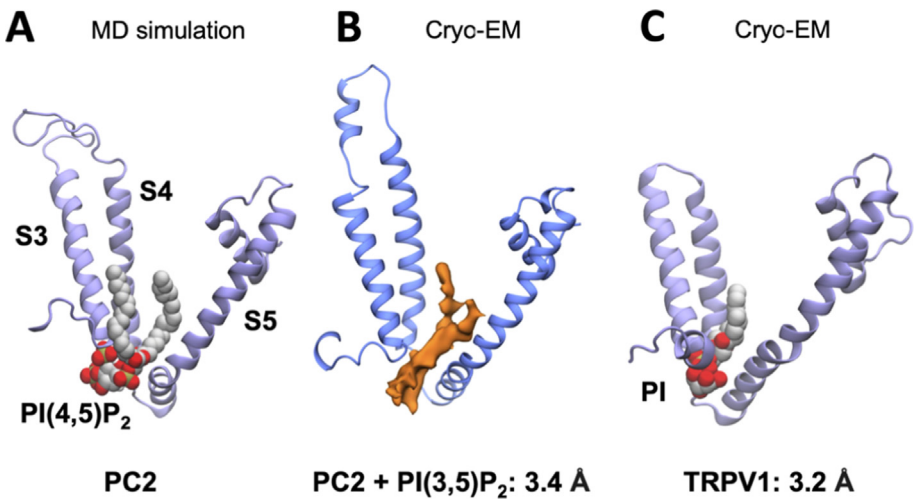

Figure 4. A phosphoinositide binding site on PC2. (A) CG simulation-based identification of a $PI(4,5)P_2$ binding site between helices S3/4/5 of PC2.[181] (B) Lipid density seen at the same site in a cryo-EM structure of PC2 solved in the presence of $PI(3,5P)_2$ (PDB ID: 6T9O, EMDB: 10419).[181] (C) Phosphatidylinositol bound to the same site in a cryo-EM structure of TRPV1 (PDB ID: 5IRZ).[20] Figure modified from 181.

selectivity.[187,188] This assay consists of a nitrocellulose membrane dotted with different lipid species. Binding of the protein of interest to these lipid spots can then be detected using antibodies. Wang et al., employed this approach for PC2 in detergent micelles to demonstrate selective binding of phosphoinositide species over other lipids (PA, PS, PE, S1P), in agreement with the simulations.[181] However high variation between biological repeats circumscribed further dissection of selectivity between phosphoinositide species.

Delling and colleagues recently applied detailed electrophysiology and simulation work to demonstrate binding of a ciliary-enriched oxysterol (7β,27-DHC) to PC2 at a site which included the S4-5 linker, and functional modulation of channel current.[182] This oxysterol site overlapped with the putative phosphoinositide binding pocket previously identified from the structures and simulations.[20,181] Remarkably, inclusion of either $PI(3,5)P_2$ or $PI(4,5)P_2$ decreased the modulatory effects of the oxysterol on PC1/2 current density, while PI(4)P completely abolished channel activation.[182] Taken together, the high affinity competition indicated both the veracity of the previously identified phosphoinositide binding pocket, and suggests PI(4)P may be the key biologically relevant phosphoinositide species in controlling PC2 channel function at this site. This finding aligns with the cellular localisation of PC2 channels to cilia membranes, a region enriched in PI(4)P lipids.[159]

These studies highlight both the technical challenges of interrogating phosphoinositide interactions with membrane proteins, and how these challenges may be met by an integrated approach combining simulation and experiment. The paradigm of using CG simulation to assign molecular identity to putative lipid density in cryo-EM structures was recently developed into the LipIDens tool.[23]

In addition to phosphoinositide interaction sites with defined tertiary structure, interactions with intrinsically disordered regions (IDRs) of membrane proteins have been observed[189,190]. In the context of ion channels a fascinating example is provided by the ca. 150 residue N-terminal IDR of TRPV4.[191] Goretzki et al., integrated a suite of experimental approaches and multiscale simulations to map the molecular level detail of $PI(4,5)P_2$ interaction with the IDR ensemble, and its relationship to channel activity. CG simulations combined with NMR experiments delineated a mechanism whereby interaction of $PI(4,5)P_2$ with a particular region of the IDR led to a mechanical pull-force on the ankyrin repeat domain (ARD), which ultimately led to force transduction to the TRPV4 core. The extent and structural basis for phosphoinositide selectivity when it comes to membrane-proximal IDR ensembles is intriguing to consider, and simulations combined with machine learning technologies are increasingly well positioned to further address this.[192,193,66]

Inward rectifying potassium channels. Potassium channels facilitate the selective flow of K^+ ions across cellular membranes. These tetrameric ion channels are one of the best characterised and most ubiquitously expressed integral membrane proteins with wide ranging roles in physiology and disease.[194] The three main groups of potassium channels are inward rectifying (Kir), voltage-gated (Kv), and two-pore (K2P) potassium channels. A variety of structural[17,195] and functional[196,197] studies have established central roles

for lipids in potassium channel biology. A particularly intimate relationship is seen between Kir channels and $PI(4,5)P_2$ lipids, which act as channel activators.[198] This section highlights some of the key ways in which simulations have enriched our understanding of various aspects of phosphoinositide biology as it relates to Kir channels, as well as remaining challenges.

At the macroscale, it is now possible to simulate large patches of membrane containing hundreds of proteins embedded in complex membrane compositions.[94,111] Thus Duncan et al., performed CG simulations of membrane patches containing >100 Kir2.2 channels in a plasma membrane like lipid composition[112] (Figure 5A). Simulations under a range of defined lipid regimes and protein concentrations revealed a role for $PI(4,5)P_2$ in influencing channel crowding and bilayer fluctuations. Follow up work assessed the nanoscale interactions of $PI(4,5)P_2$ with Kir2.2[199] by leveraging the large complex membranes to gather sufficient statistics to assess competition effects between different lipids. $PI(4,5)P_2$ was seen to bind at the experimentally determined primary site on Kir2.2[17] (Figure 5). Interactions at a secondary[200,201] anionic lipid site were also observed. Though $PI(4,5)P_2$ interaction was most favourable over other lipid species at both sites, anionic PS lipids (net headgroup charge -1) were able to bind if the $PI(4,5)P_2$ concentration was low enough. The molecular basis of $PI(4,5)P_2$ selectivity over other phospholipids resulted from protrusion of the comparatively larger inositol headgroups into solution, with the 4′ and 5′ phosphoryl moieties able to interact with key basic residues of the cytoplasmic domains which were beyond the reach of phospholipids with smaller headgroups. Thus in this case selectivity resulted both from structure and from charge. Free energy perturbation (FEP) simulations were applied to estimate the $\Delta\Delta$ G for transforming $PI(4,5)P_2$ to PS at both lipid binding sites. The calculations showed the selectivity for $PI(4,5)P_2$ over PS was significantly greater at the primary interaction site ($\Delta\Delta G$ = 43 k J/mol) than at the secondary interaction site ($\Delta\Delta G$ = 29 kJ/mol). Notably, the magnitude of the unfavourable $\Delta\Delta G$ for the secondary site was reduced from 29 kJ/mol to 17 kJ/mol if a $PI(4,5)P_2$ lipid was also present within the primary site. That is, PS interaction at the secondary site became more favourable if a $PI(4,5)P_2$ lipid was also present within the primary site. This delicate interplay between the two sites may provide the cell with an additional means of differentially modulating channel function by controlling the relative local abundance of competing lipid species.[199]

The location of the primary $PI(4,5)P_2$ interaction site characterised by Duncan et al., was first predicted in simulations of KirBac1.1, a Kir3.1-KirBac1.3 chimera, and a homology model of Kir6.2 performed in 2009.[84] This early work employed CG simulations of $PI(4,5)P_2$ in simple membranes containing phosphatidylcholine (PC) and $PI(4,5)P_2$. Simulations of 5 μs duration were sufficient to observe spontaneous and sustained binding of $PI(4,5)P_2$. Key clusters of basic residues which formed the binding site for the inositol headgroup had previously been suggested to be involved in Kir2.1 interactions with $PI(4,5)P_2$.[203–205] A subsequently solved Kir2.2 crystal structure showed short-chain (dioctanoyl) $PI(4,5)P_2$ density at the same site (Figure 5). Interestingly, in this case lipid binding seemed robust both to the acyl tail composition/absence, and the use of non-eukaryotic structures and homology models. Further simulation studies of Kir2.2 have continued to support the veracity of the $PI(4,5)P_2$ site.[206–208]

Interestingly, this site shows excellent agreement with an AI model we predicted for this review using the Chai-1 web interface[57] (https://lab.chaidiscovery.com/), and only the primary sequence of the Kir2.2 monomer and the SMILES string for $PI(4,5)P_2$ as input (Figure 5D, G). The authors of this model report similar capabilities to AlphaFold3, and the web tool places no limitations on small molecule types which may be assessed. The emerging ability of AI models to predict protein structures in complex with small molecules, including lipids, is likely to be a major accelerator for phosphoinositide research, providing initial coordinate sets from which to launch dynamical physics-based simulation and experimental investigations.

The functional consequences of $PI(4,5)P_2$ binding for gating and Kir channel conductance have been explored in a range of simulations works.[209–213] Tour de force simulations from the D. E. Shaw Research group recently further defined the mechanism of $PI(4,5)P_2$ activation of Kir2.2 in atomic detail.[214] Starting from a closed-state structure,[17] Jogini et al., introduced a previously characterised mutation at the bundle crossing to obtain an open conducting state, capture key conformational changes at the main activation gate and cytoplasmic domain, and demonstrate the dynamical basis for $PI(4,5)P_2$ stabilisation of the open-state in all-atom detail (Figure 6). A particularly notable feature of this work is the degree of sampling achieved, with timescales of up to 200 μs reached for an individual simulation, and on aggregate over a millisecond across all simulations. Despite this immense sampling, the authors note that the presence of $PI(4,5)P_2$ and bulk PS failed to evoke pore-opening of the WT channel, as would be expected in the event of a sufficiently accurate force field coupled to sufficient sampling. This suggests that while substantial progress continues to be made, there remains scope for development of all-atom MD in capturing certain mechanistic aspects of phosphoinositide biology occurring on long timescales.

Further studies have leveraged MD simulations to assess the molecular detail of phosphoinositide interaction in the context of Kv channels,[215–221] and K2P channels.[222,223] Other examples of phos-

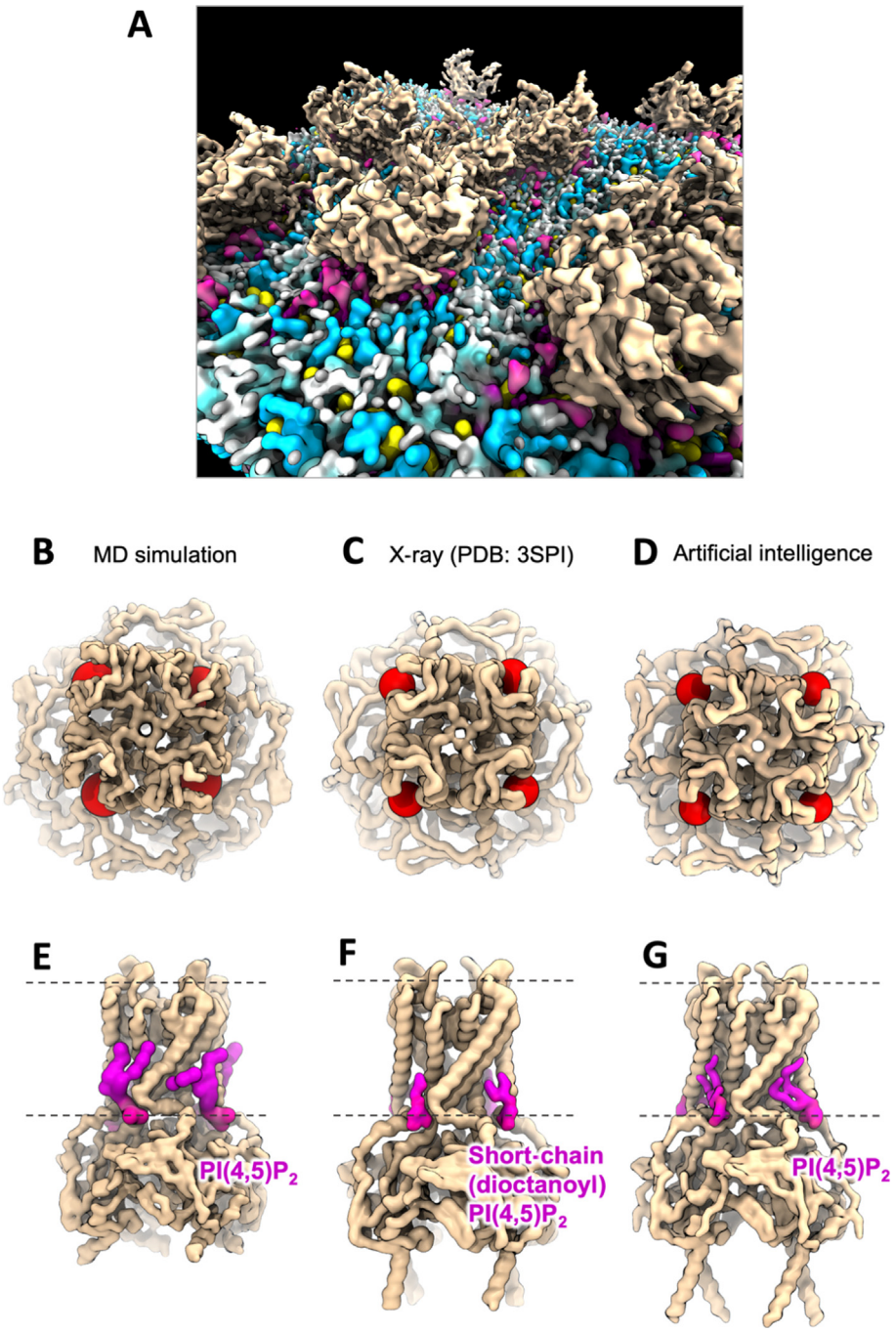

Figure 5. Primary PI(4,5)P_2 binding site on Kir2.2 from simulation, experiment, and AI. (A) View of the inner leaflet surface from a simulation containing multiple Kir2.2 channels embedded in a complex and crowded membrane environment.[199] PI(4,5)P_2 (magenta) is clustered around the channels (beige). (B) The primary PI(4,5)P_2 sites identified on homotetrameric Kir2.2 by CG MD simulation.[84,199] (C) the same site identified by X-ray crystallography (PDB id: 3SPI),[17] and (D) by AI. The AI coordinates shown were predicted using Chai-1[57] (https://lab.chaidiscovery.com/), the monomeric FASTA sequence from PDB id: 3SPI, and the PubChem[202] SMILES string for PI(4,5)P_2. The red spheres denote the position of the phosphate group bridging the glycerol and inositol moieties of PI(4,5)P_2. The perspective shown is from the extracellular side of the bilayer. (E–G) Lateral view of the same structures, with the PI(4,5)P_2 lipids shown in magenta. Dotted lines denote the approximate position of the bilayer – solvent interface. We thank Dr. Anna Duncan for providing simulation coordinates used to produce the figure.

phoinositides controlling non-K^+ ion channel structure and function include the calcium-dependent chloride ion channel TMEM16A/ANO1,[224–226] mechanosensitive channels such as Piezo1,[227] and voltage-gated sodium channels.[228] A particularly notable example is the inositol trisphosphate

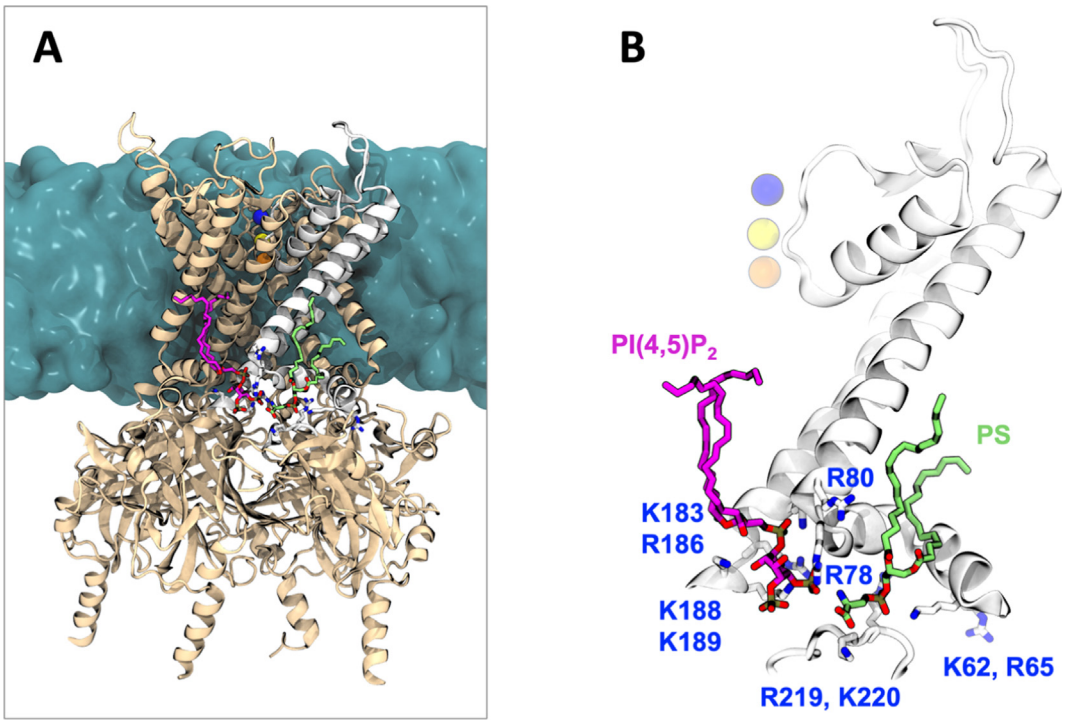

Figure 6. Atomic architecture of a $PI(4,5)P_2$ binding site on Kir2.2.[214] (A) Cross-section of an ANTON simulation snapshot showing a $PI(4,5)P_2$ lipid (magenta) binding site on tetrameric Kir2.2 (beige) embedded in a lipid bilayer (blue surface). An adjacent PS lipid is shown in green. (B) Zoomed in view on the $PI(4,5)P_2$ site showing how the headgroup is cradled by basic residues during the simulation. K^+ ions within the selectivity filter are shown in the background as blue, yellow, and orange spheres. We thank Dr. Vishwanath Jogini, Dr. Morten Ø. Jensen, and D. E. Shaw Research for providing coordinates used to produce the figure.

receptor (IP_3R), where $PI(4,5)P_2$ primes the receptor by partially occupying the orthosteric IP_3-binding site.[229]

Peripheral membrane proteins

Peripheral membrane proteins (PMPs) exist largely in aqueous solution and interact with the hydrophilic surface of membranes. This is a particularly important group of proteins in relation for phosphoinositide biology, as it encompasses the kinases[230] and phosphatases[231] which synthesise and degrade the various phosphoinositide species and thereby regulate their relative abundance and localisation in membranes.[232] The interactions of PMPs with phosphoinositides is also intimately involved in intracellular signalling cascades, for instance in the enzymatic cleavage of $PI(4,5)P_2$ into the second messengers diacylglycerol and inositol-trisphosphate (IP_3).[233]

Interactions of PMPs with membranes may take a variety of forms. These include binding of a specific lipid headgroup to a specific site on the PMP, more dynamic interactions between patches of complementary charge on the protein surface and the net anionic charge of the inner leaflet, and by insertions of hydrophobic loops or lipidic post translational modifications, e.g. myristoylation.[234]

Structure determination of PMPs in solution or indeed bound to individual lipids is now relatively routine. However, the main biological function of PMPs takes place on the surface of membranes. Stabilising PMPs on membranes to the level required for atomic level structure determination remains challenging.[235] Simulations have played a critical role in reuniting experimentally determined structures of PMPs with phosphoinositide-containing lipid membranes *in silico* to determine their lipid interactions, binding orientation and dynamics. This is particularly important as the biologically relevant membrane context can influence the conformation of the PMP, and reveal new lipid binding sites.[236]

Extensive all-atom simulations have revealed the molecular basis for how $PI(3,4,5)P_3$ drives binding of Bruton's tyrosine kinase (Btk) to membranes and allosterically stabilises the experimentally determined dimeric form of the protein.[237] Starting from two copies of the monomeric form of Btk in solution, this study was able to simulate spontaneous binding to $PI(3,4,5)P_3$ membranes and Btk dimerization. This impressive feat was achieved through use of the ANTON supercomputer[77] coupled to a flavour of enhanced sampling simulation termed 'tempered binding'.[238]

Simulations may also be employed alongside complementary biophysical data to pre-orientate a given PMP on the membrane. Thus in early works in this area Lai et al., used electron paramagnetic resonance (EPR) spectroscopy data to obtain approximate geometries for the C2 domain of Protein kinase Cα relative to $PI(4,5)P_2$ containing membranes.[239] These geometries were used as starting points for simulations, thereby decreasing the level of sampling required compared to *de novo* binding from solution. This approach enabled refinement of C2 domain orientation, determination of lipid:protein stoichiometry, and a molecular level view of key $PI(4,5)P_2$ interactions. This same approach was subsequently applied to reveal the molecular basis of stereospecific binding of the GRP1 PH domain to $PI(3,4,5)P_3$.[240]

All-atom simulations of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ binding to PMPs have also been performed[241–244] using the highly mobile membrane mimetic (HMMM) model.[92] This approach replaces the hydrophobic core of the lipid bilayer with organic solvent, atop which phosphoinositide headgroups with shortened acyl tails diffuse. The net effect is to increase the rates of lipid diffusion and therefore enhance the rate at which they explore possible interaction modes with the PMP.[245] In a recent example of the HMMM approach, Pant and colleagues addressed the orientational dynamics of Arf1 GTPase on $PI(4,5)P_2$ membranes[241] (Figure 7). This particular PMP is localised to the membrane *via* a myristoylated amphipathic N-terminal helix. The increased diffusion rates provided by the HMMM model were leveraged to identify three principle orientational states of myristoylated Arf1 on membranes with different lipid compositions, which were subsequently refined with microsecond all-atom simulations with full lipid representation. Interactions with $PI(4,5)P_2$ were key for driving full membrane association and population of these three states, in qualitative agreement with neutron reflectometry (NR) and NMR experiments.[241]

High-throughput CG simulations have been used to assess patterns of phosphoinositide interaction across whole families of pH domain-containing PMPs.[236,246] Yamamoto and colleagues examined the spontaneous binding of the apo forms of 13 different PH domains to $PI(3,4,5)P_3$ and $PI(4,5)P_2$ membranes.[236] Overlay of the simulation derived phosphoinositide interactions showed good agreement with available experimental X-ray crystallographic and NMR structures of the phosphoinositide bound forms of these proteins. In addition to the experimentally known interactions, the simulations also revealed a number of new non-canonical phosphoinositide binding sites for select PH domains. Mutation of key residues within these sites allowed delineation of the main contributors to phosphoinositide binding. For all PH domains, a degree of less specific phosphoinositide clustering around the protein was also indicated to contribute to membrane association. This was consistent with observations from large scale biochemical assessment of 91 PH domains,[247] and was later supported by free energy calculations for the GRP1 PH domain interacting with $PI(3,4,5)P_3$ clusters of varying size,[248] and by a herculean body of work to extend analysis to 100 different mammalian PH domains.[249]

Further simulation works delineating phosphoinositide interaction with PMPs include on Vinculin,[250] the Dok7 PH domain,[251] the K-Ras G

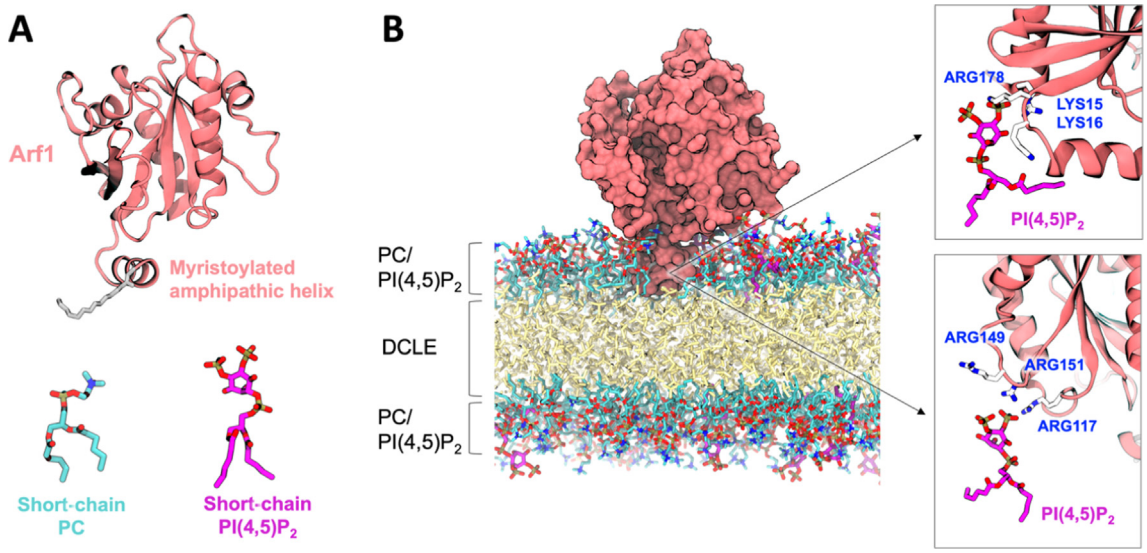

Figure 7. Arf1 membrane interactions.[241] (A) Structure of myristoylated Arf1 and short-chain PC/$PI(4,5)P_2$. (B) Simulation snapshot showing a cross-section of Arf1 bound to a PC/$PI(4,5)P_2$ highly mobile membrane mimetic (HMMM) containing a DCLE organic solvent core. Interactions of Arf1 with $PI(4,5)P_2$. We thank Dr. Shashank Pant and Prof. Emad Tajkhorshid for providing coordinates used to render the figure.

domain,[252] KRas4b,[244] KRas4b and RAF1,[253] PLD2,[254] focal adhesion kinase,[255,256] PTEN and SHIP2 C2 domains,[257] PTEN and voltage sensitive phosphatase,[258] Arf/GEF,[259] PLCγ1,[260] Gelsolin,[261] ORP/Osh proteins,[262–264] GRP1 PH domain,[265] murine kindlin-3 PH domain,[266] BIN1/M-Amphiphysin2,[267] lymphocyte specific kinase (LCK),[268] Smurf1 C2 domain,[269] StarD4,[270,271] SNX11,[272] WD repeat domain phosphoinositide-interacting protein 2 (WIPI2),[273] tubby domains,[274] and phosphatidylinositol-transfer proteins.[275] The number of different research groups turning to simulations to interrogate the molecular basis of how their favourite PMP interacts with phosphoinositides is indicative of both the need in this area, and the power of molecular simulation in meeting that need. Notably, while most works to date have dealt with mammalian PMP interaction with PI(4,5)P_2 and PI(3,4,5)P_3 lipids, simulations have also shown utility in exploring other phosphoinositide species in non-mammalian contexts, such as PI(4)P interactions with *Plasmodium vivax* Perforin-Like Proteins (PLPs).[276] Future CG simulations of PMPs are likely to benefit from the GōMartini model of protein structure, which has recently been reported to further enhance the accuracy of conformational modelling of PLCδ1 interacting with PI(4,5)P_2 bilayers.[277] For a recent excellent review on the generalized application of molecular dynamics simulations to PMPs broadly.[278]

Receptor tyrosine kinases

Many fundamental cellular signals are transduced across the plasma membrane by human receptor tyrosine kinases (RTKs).[279] This family of 58 cell surface receptors share a common molecular architecture consisting of an extracellular ligand binding ectodomain, a single-pass transmembrane (TM) helix, a flexible juxtamembrane (JM) region, and an intracellular protein kinase domain. A variety of additional soluble domains may decorate different types of RTK leading to substantial variety.[280] Binding of ligands to the ectodomain canonically leads to receptor dimerization, activation of protein kinase activity, and sets in motion a variety of intracellular signalling cascades.[281] Non-canonical forms of signalling by heterodimerisation of RTKs are also possible.[282] Phosphoinositides have been shown experimentally to directly bind a range of RTKs,[283] modulate their function,[34] as well as control their localisation into high order multimers at the cell surface.[284–286]

As major therapeutic targets[287,288] (e.g. Herceptin against the Erbb2 RTK in the treatment of breast cancer) RTKs have been paid particular attention by structural biologists and atomic resolution structure availability is relatively good for select RTKs[289–291]. However intrinsically disordered regions such as the JM region remain more mysterious, despite their known functional importance.[292] This is an area where computational approaches are increasingly making impact.[193] Simulations of the TM-JM constructs of all 58 human RTKs have predicted nanoclustering of PI(4,5)P_2 around the disordered JM[189] (Figure 8A, B). This behaviour was driven *via* direct interaction with a conserved polybasic sequence motif found in the region of the disordered JM immediately adjacent to the TM helix. More detailed work focussing on single RTKs including the epidermal growth factor receptor (EGFR),[293] the tropomyosin receptor kinase A (TrkA),[294] and the ephrin A2 receptor (EphA2R)[295] identified similar JM driven PI(4,5)P_2 interactions. Most recently a combined simulation and NMR study observed this phenomenon for the prolactin receptor JM.[190] Such nanoclustering of PI(4,5)P_2 lipids around individual receptors is especially pertinent when it is considered that PI(4,5)P_2 is a key second messenger substrate in many of the downstream signal cascades which are activated by RTKs. Fluorescence microscopy approaches have indicated that a variety of RTKs are localised into higher order arrays at the cell surface.[284,285,296,297] If each member of this array has its own local pool of PI(4,5)P_2 lipids around it, as predicted in the simulations, this would overall add up to a high local concentration of phosphoinositide lipids, with potential implications for signal transduction efficiency and PMP recruitment. CG simulations of large membranes containing tens of full-length RTKs are now feasible, and are expected to shed further light on the molecular architecture of these clusters (Figure 8C, D).

Simulations have also been used to compute the change in free energy for lipid binding to the EGFR TM-JM dimer within PC bilayers.[298] As expected, PI(4,5)P_2 binding is significantly more favourable than for other plasma membrane phospholipid species. Notably, the $\Delta\Delta G$ values are of a magnitude similar to those obtained for TM helix dimerization for a range of RTKs.[299,300] This would seem to lend credence to the idea that one mechanism of PI(4,5)P_2 modulation of RTK activity may be *via* direct competition with helices and/or modulation of TM dimer crossing angle.[301,302] Barrera and colleagues subsequently tested this concept experimentally for the EphA2R and identified a marked effect of PI(4,5)P_2 in promoting dimerization of a particular crossing angle mode.[303] This is an exciting result which positions helix dimerization as yet another potential microswitch[304] in phosphoinositide biology. Factors which will be important to address experimentally going forward include the influence of soluble domains, competition between different phosphoinositide species, and the universality or otherwise amongst different RTKs and indeed other single-pass membrane proteins.

Other ways in which phosphoinositides influence RTKs include in controlling how the intracellular kinase domains interact with the inner leaflet surface, and recruitment of RTK growth factor ligands to membranes. Chavent et al., used CG simulations to capture the spontaneous binding of

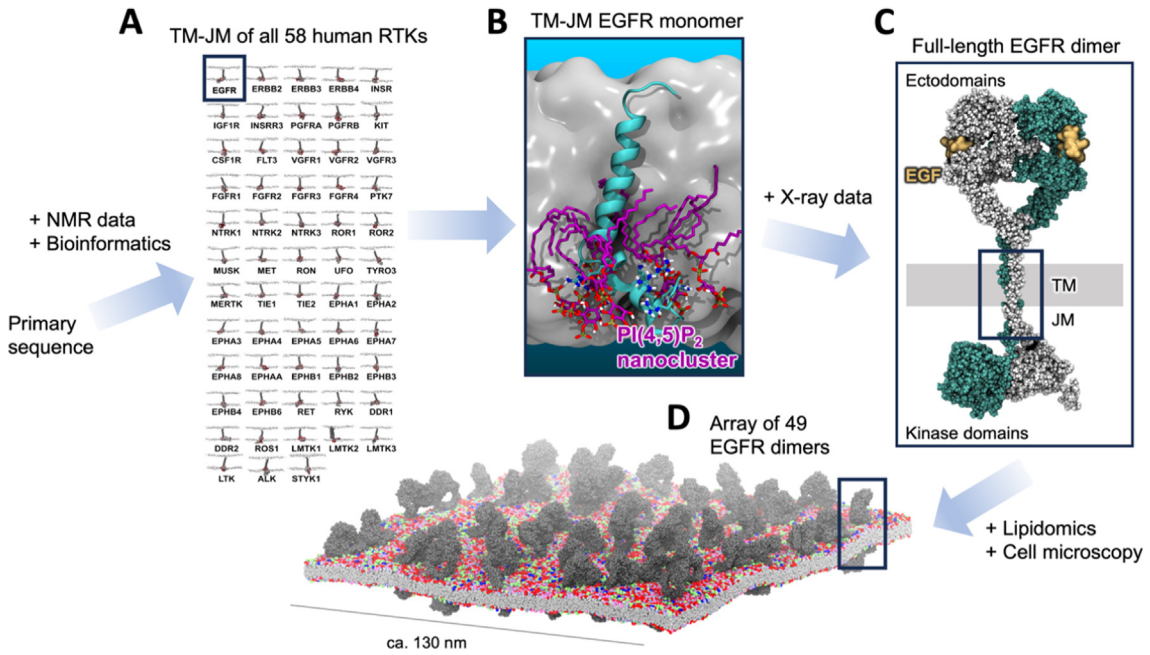

Figure 8. Multiscale simulations of the human RTK superfamily. (A) TM-JM models of all 58 known human RTKs, built from NMR data and secondary structure prediction programs and simulated in 189. (B) A nanocluster of PI(4,5)P_2 lipids around the TM-JM monomer of EGFR/Erbb1. Image is the endpoint of a 1 μs CG simulation followed by 50 ns of all-atom simulation. (C) Full-length model of the EGFR/Erbb1 dimer built from NMR, X-ray and modelling data. (D) 49 full-length EGFR/Erbb1 dimers embedded in a patch of membrane with a lipid composition approximating that of a mammalian plasma membrane, including inner leaflet PI(4,5)P_2 and PI(3,4,5)P_3. The image shown is after 8 μs of simulation. Hedger et al., unpublished.

the kinase domain to the membrane surface.[295] The conceptual framework bears resemblance to the PMP – phosphoinositide simulations described in Section '**Peripheral membrane proteins**', albeit in this case with the kinase domain tethered to the membrane by the flexible JM and TM helix (Figure 9). By coupling the simulations to liposome pull-down assays and prior modelling of EphA2R, a key role for PI(4,5)P_2 was identified in orientating the kinase domains for *trans*-autophosphorylation within EphA2R clusters. Most recently, all-atom simulations have been used to examine heterodimeric EphA2R-EGFR kinase domain interactions with PI(4,5)P_2.[285] This study identified intriguing differences between EphA2R and EGFR, in the context of wider experimental work which demonstrated PI(4,5)P_2 promoted both hetero and homomultimerization of EphA2R and EGFR.

Phosphoinositide species also have key roles in the biology of certain RTK ligands, such as the fibroblast growth factor 2 (FGF2). FGF2 comes into contact with PI(4,5)P_2 lipids in the inner leaflet during its secretion by the type I 'unconventional protein secretion (UPS)' pathway.[305] PI(4,5)P_2 interaction induces a fascinating piece of biology in which FGF2 undergoes oligomerisation to form a membrane pore, before exiting the other side of the membrane to arrive in the extracellular space.[306] Simulations have been applied to capture PI(4,5)P_2 interactions with FGF2 during this process.[99,307] Most recently Lolicato *et al.* combined microsecond atomistic simulations with *in vitro* and *in cellulo* assays to address lipid synergy during the initial membrane recruitment of FGF2.[99] The simulations identified the atomic basis of an intricate lipid interplay in which cholesterol interacted with PI(4,5)P_2 lipids to increase the inositol headgroup visibility to FGF2 in a manner which resulted in faster binding kinetics and more stable FGF2 – PI(4,5)P_2 complexes. This is an important finding for phosphoinositide biology generally, as it highlights the consequences of lipid-lipid interplay and complexity for certain phosphoinositide molecular interactions.

Other membrane proteins

The phosphoinositide interactions of a variety of other membrane proteins have been addressed by simulations. Examples of transporters include PI(4,5)P_2 modulation of the structure and function of the dopamine transporter,[37,308] solute carrier spinster homolog 2 (SPNS2),[309] and SLC4 family transporters.[310–312] PI(3,5)P_2 mediated oligomerisation of the endosomal sodium-proton exchanger NHE9,[313] and phosphatidylinositol stabilisation of purine symporter UapA dimers[314] have also been addressed with simulations. The influence of phosphoinositides on membrane proteins involved in

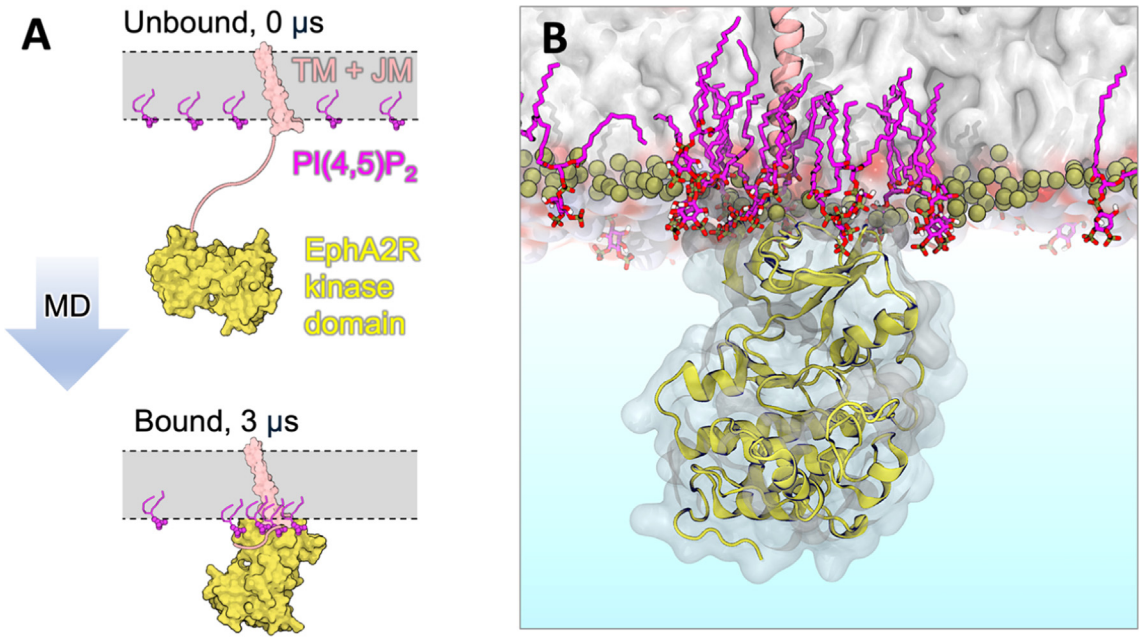

Figure 9. Simulations identify orientations of the EphA2R kinase domain on PI(4,5)P_2 containing membranes. (A) Schematic of start and endpoint of simulations. (B) Representative snapshot of interface 1 identified in 295. The kinase domain (yellow) is shown interacting with a cluster of PI(4,5)P_2 lipids (magenta). The JM is located within the cluster. We thank Dr. Matthieu Chavent for the coordinates used to produce the figure.

viral biology,[315–321] cytoskeleton biology,[322–324] vesicle trafficking,[325] autophagy,[273] and pore formation during programmed cell death,[326] have also been studied.

Phosphoinositide clustering

Beyond specific membrane proteins, simulation studies have also addressed the ability of certain phosphoinositides to self-interact and cluster into areas of enrichment in the absence of membrane proteins.[85,327–330] A key role for divalent calcium has been identified in bridging interactions between the phosphate groups of adjacent PI(4,5)P_2 lipids.[331] This effect did not extend to other common inorganic cations nor to PI(3,5)P_2 lipids,[332] in qualitative agreement with trends seen in fluorescence-based spectroscopy and microscopy experiments.[333] This is particularly interesting in a cell biology context given the role of calcium signalling downstream of many of the signalling cascades in which PI(4,5)P_2 lipids act as key PMP recruitment factors and second messenger substrates.[283] Indeed, direct competition between calcium and PMPs for interaction with PI(4,5)P_2 has been reported[334]. Dynamic modulation of local calcium levels could represent another means of controlling how phosphoinositide species interact with membrane proteins. Acyl tail saturation has also been seen to play a key role in modulating nanocluster formation in CG simulations.[157]

Nanocluster sizes may be increased substantially by the presence of proteins. In a landmark study, van den Bogaart and colleagues addressed the architecture of syntaxin-1A – PI(4,5)P_2 clusters using super-resolution microscopy and CG simulations.[38] Microdomains of up to 73 nm in size were experimentally identified in which PI(4,5)P_2 was the dominant lipid species. Use of phosphatases to knockdown cellular PI(4,5)P_2 levels is a common trick in phosphoinositide biology, and was applied in this case to demonstrate co-clustering of syntaxin-1A was dependent on PI(4,5)P_2. Simulations were run in which 64 copies of the transmembrane helix of syntaxin-1A were embedded into a PI(4,5)P_2:PC:PS membrane. The helices and PI(4,5)P_2 lipids were seen to spontaneously separate into microdomains knitted together by ionic interactions between negative PI(4,5)P_2 phosphate groups and basic residues located at the tips of the syntaxin-1A helices, similar to later observations made for RTKs[189] and the prolactin receptor.[190] These microdomains remained stable on simulation timescales of up to 6 μs, and could be recreated *in vitro* within giant unilamellar vesicle membranes. Notably, in this case the effects were robust to changes in lipid composition including of cholesterol, which has been seen to influence PI(4,5)P_2 interactions in other contexts.[99] Simulations have also played a key role in delineating how phosphoinositide – membrane protein interactions can lead to local remodelling of membranes. BAR domains in particular have been the subject of attention in this area.[267,335–338] Phosphoinositide interactions with a variety of other remodelling proteins including epsin N-terminal homology domain

(ENTH),[339] cavin1,[340] and AP180 N-terminal homology domain (ANTH)[341] have also been studied.

Summary and Concluding Remarks

Simulations have been applied in a variety of ways to address the molecular underpinnings of how phosphoinositides influence membrane proteins (Figure 10). By comparison to experiment it is evident they are capable of accurately identifying phosphoinositide binding sites. This was the case for Class A GPCRs[21] and Kir channels,[84] as confirmed by later X-ray[17] and cryo-EM[140] structures. Simulations may also be used synergistically to interpret ambiguous experimental lipid density in structures.[23] Wang et al., thus used CG-simulations to identify a general phosphoinositide binding site on the PC2 ion channel, in agreement with their cryo-EM structures.[181] This general phosphoinositide site was later refined to a possible PI(4)P site in a subsequent biochemical and cell-based functional study.[182] Characterisation of the energetics of phosphoinositide interaction may be achieved using advanced simulation techniques such as FEP-derived ΔΔGs for lipid binding,[199] and PMF-derived free energy landscapes for PMP association with membranes from solu-

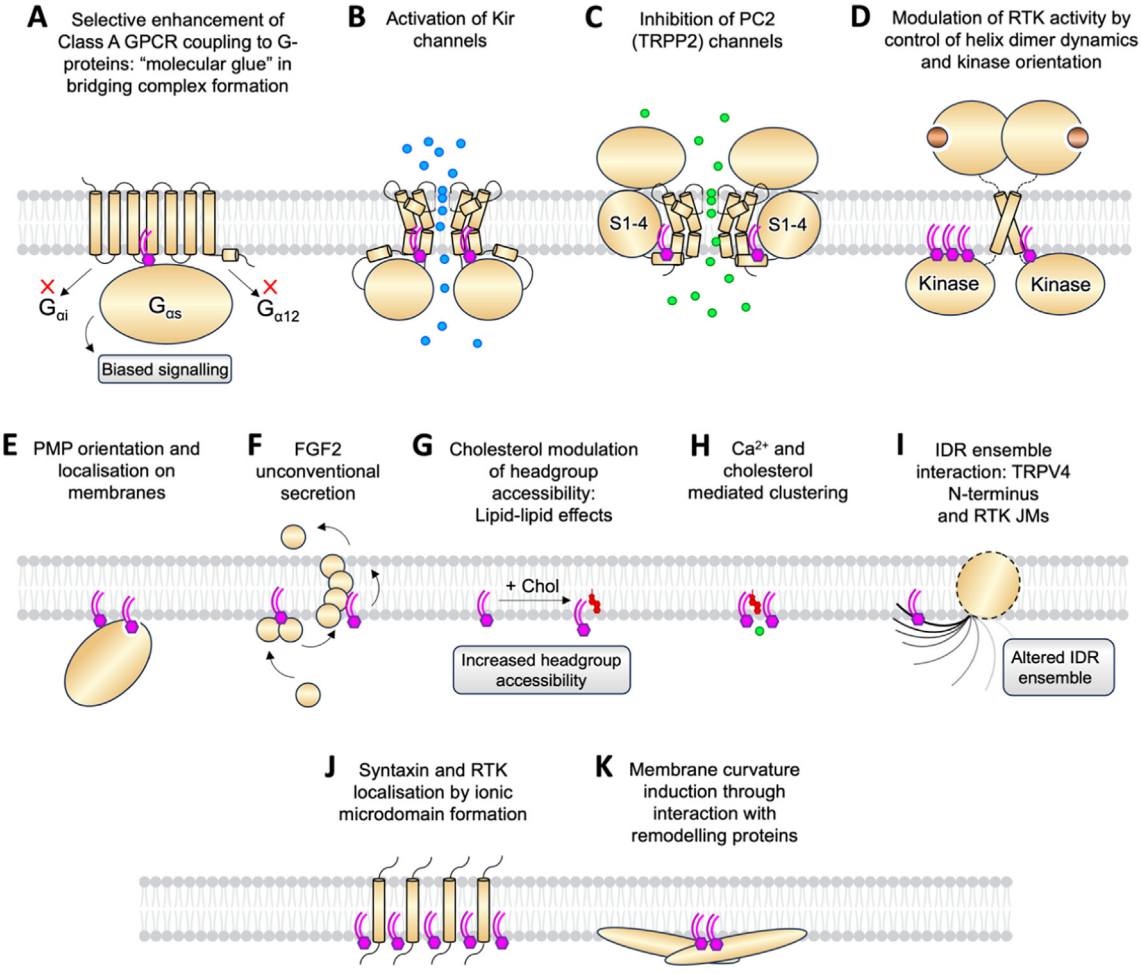

Figure 10. Cellular roles of phosphoinositide lipids studied by simulations. (A) Combined CG MD simulation and nMS based discovery of GPCR binding and functional modulation of G-protein coupling by $PI(4,5)P_2$.[21] (B) Multiscale simulation based identification and characterisation of $PI(4,5)P_2$ binding sites on Kir channels.[84,199,206,214] (C) CG MD simulation identification of a phosphoinositide binding site on PC2 with functional consequence.[181,182] (D) Multiscale simulation of $PI(4,5)P_2$ control of RTK kinase domain orientation, and TM-JM dynamics.[189,293,295,303] (E) CG, all-atom, and HMMM simulation of PI(3)P, PI(4)P, $PI(4,5)P_2$, and $PI(3,4,5)P_3$, interaction with many PMPs: see section 2.3. (F) All-atom simulations of $PI(4,5)P_2$ in FGF2 secretion.[99,307] (G) All-atom simulations of cholesterol – $PI(4,5)P_2$ interplay.[99] (H) Multiscale simulations of phosphoinositide clustering.[331,332,85,99,334] (I) Multiscale simulation of $PI(4,5)P_2$ interaction with IDRs within receptors[189] and channels.[191] (J) Combined CG MD simulation and microscopy of phosphoinositide microdomain formation.[38,189,284] (K) Multiscale and ultra-CG simulations of $PI(4,5)P_2$ interaction with remodelling proteins.[267,335,336,338,339]

tion.[248] Analysis of the interaction patterns in simulations of different conformational states can show in atomic detail how functional effects are conferred by structure, for instance in $PI(4,5)P_2$ stabilisation of the open state of Kir2.2.[199,214] Function may also be prospectively predicted from the structure and dynamics seen in a simulation, for instance in simulation-based prediction that $PI(4,5)P_2$ lipids act as a molecular glue to bridge $A_{2A}R$ – miniG interactions to enhance G-protein coupling, in agreement with nMS and fluorescence-based functional assays.[21]

Increases in computational power[110] and structure availability[18,143] increasingly allow assessment of phosphoinositide interactions at scale across large groups of membrane proteins, for instance RTKs,[189] GPCRs,[149] and PH domains,[249] facilitating assessments of conservation. Expansion of parameter space for phosphoinositides,[83] and membrane lipids generally[69,86,95,342] now allow routine simulation in complex lipid mixtures, which have revealed new aspects of phosphoinositide biology deriving from lipid-lipid interplay.[99,199] The vast majority of simulation studies to date have focussed on $PI(4,5)P_2$, which likely derives from its localisation in the plasma membrane, where membrane protein research has tended to focus. It will be especially welcome to see phosphoinositide research expand further into subcellular membranes containing other phosphoinositide species.

Existing limitations of phosphoinositide simulations, such as fixed charge force fields, and challenges in distinguishing closely related isomers e.g. $PI(3,5)P_2$ and $PI(4,5)P_2$ in CG models[181] have also been seen. Continued developments in multiscale modelling,[74] and constant pH simulations[343,344] will be especially valuable in this area. The CG MARTINI model has shown exceptional utility in *de novo* prediction of phosphoinositide binding sites,[64,72] (converged) free energy calculations,[108,199,345] and modelling of large membrane phenomena.[38,166] At the same time, we would caution the budding explorer of phosphoinositide biology against the risk of overinterpretation of CG models. For instance, in capturing the detailed pattern of phosphoinositide interactions and ensemble of poses within a given binding site, certain ion-induced effects,[85] and differentiation between closely related isomers. Moreover, high-quality (ideally not detergent or chemical probe based) experimental measures of specific lipid interaction energies with membrane proteins are scarce, which makes direct comparison to CG interaction energies challenging.[108] All-atom simulations (including flavours such as HMMM[92,245]) have tended to excel in areas such as refinement of detailed interactions with known binding sites,[206,237] specific ion interactions,[85,329,332] and phosphoinositide-induced membrane protein conformational change.[214,242] Ultra-CG models[346] have also shown utility in modelling of large-scale phenomena such as membrane protrusion formation by I-BAR domains.[335]

In considering the present body of phosphoinositide simulations it is evident that they interact with a huge range of membrane proteins. Unsurprisingly these interactions nearly always feature basic residues on the protein. In the case of integral membrane proteins, these residues tend to be positioned slightly beyond the membrane surface, and are thus optimally positioned to interact with the 3′, 4′, and 5′ phosphoryl groups of phosphoinositides. Such positions are often inaccessible to other phospholipids with smaller headgroups and this is one way specificity is conferred. Given the body of simulation work on phosphoinositides, it should now be possible to derive more detailed heuristics on what characterises a phosphoinositide binding site. Can a suite of pharmacophore and QSAR models for phosphoinositides be developed? Can machine learning technologies be trained on the abundance of simulation and structural data on phosphoinositide interactions with membrane proteins? Leveraging simulation data in this way is a key challenge for computational biochemists. At present simulation data is generally stored individually within the filesystems of research groups, which severely circumscribes large scale meta-analysis of this nature. Upcoming initiatives such as the Molecular Dynamics Data Bank (MDDB)[347] (see https://mddbr.eu/) and MDverse[104] will be welcome advancements and should enable new insights into phosphoinositide biology. Such analysis would be especially welcome from a proof-of-concept therapeutic perspective, as a principal challenge to leveraging lipid binding sites on membrane proteins for structure-based drug design[348,349] is likely to be selectivity.

The advent of AI models for biomolecular structure prediction is expected to further expand the range of questions which can be addressed by physics-based simulations. The widespread availability of increasingly accurate structure predictions of proteins and their conformations will provide new entry points from which to initiate dynamical simulations. This effect will be felt particularly for simulations of membrane proteins, where experimental structure determination remains challenging compared to soluble proteins.[350] Recent developments in AI models which predict small molecules in complex with target proteins[54,56,57] are anticipated to be especially valuable in rapid prediction of phosphoinositide binding sites on membrane proteins, and will serve to provide additional points from which to launch detailed simulation and experimental studies. We also note the evolving ability of cryo-EM to capture time-resolved conformational heterogeneity,[351,352] offering the intriguing possibility for further simulation – experimental synergy in a temporal dimension.

Simulations have grown to occupy a unique place in the toolkit available to the interested connoisseur of phosphoinositide biology. Their synergistic application hand in glove with rigorous structural and functional experiment is likely to continue to reveal new facets of the molecular underpinnings of the cellular effects of phosphoinositides.

CRediT authorship contribution statement

George Hedger: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Investigation, Formal analysis, Conceptualization. **Hsin-Yung Yen:** Writing – review & editing, Writing – original draft, Formal analysis.

DECLARATION OF COMPETING INTEREST

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: "H-Y. Y. was previously an employee and is a founder of OMass Therapeutics.".

Acknowledgements

G. H. is funded by the Wellcome Trust (301619/Z/23/Z). Research in H-Y. Y.'s group is funded by Academia Sinica (AS-GCP-113-L01). Dr. Anna Duncan, Dr. Shashank Pant, Prof. Emad Tajkhorshid, Dr. Matthieu Chavent, Dr. Vishwanath Jogini, Dr. Morten Ø. Jensen, and D. E. Shaw Research are thanked for providing simulation structures used to render figures. Prof. Mark S. P. Sansom is thanked for valuable discussions.

Received 22 October 2024;
Accepted 6 January 2025;
Available online 9 January 2025

Keywords:
phosphoinositide;
PIP_2;
lipid-protein interaction;
MD simulation;
membrane protein

Abbreviations:
MD, molecular dynamics; CG, coarse-grained; cryo-EM, cryogenic electron microscopy; nMS, native mass spectrometry; AI, artificial intelligence; ML, machine learning; NMR, nuclear magnetic resonance; ESR, electron spin resonance; EM, electron microscopy; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PI(4)P, phosphatidylinositol-4-phosphate; $PI(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; $PI(3,4,5)P_3$, phosphatidylinositol-3,4,5-trisphosphate; FEP, free energy perturbation; PMF, potential of mean force; GPCR, G-protein-coupled receptor; NTSR1, neurotensin receptor 1; $A_{2A}R$, Adenosine$_{2A}$ receptor; RTK, receptor tyrosine kinase; JM, juxtamembrane; TRP, transient receptor potential; PC2, polycystin-2; Kir, inward rectifying potassium ion channel; PMP, peripheral membrane protein

References

1. Balla, T., (2013). Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol. Rev.* **93**, 1019–1137. https://doi.org/10.1152/physrev.00028.2012.
2. Agranoff, B.W., (2009). Turtles All the Way: Reflections on myo-Inositol. *J. Biol. Chem.* **284**, 21121–21126. https://doi.org/10.1074/jbc.X109.004747.
3. Irvine, R.F., (2003). 20 years of Ins(1,4,5)P3, and 40 years before. *Nature Rev. Mol. Cell Biol.* **4**, 586–590. https://doi.org/10.1038/nrm1152.
4. Michell, B., (1995). Early steps along the road to inositol-lipid-based signalling. *Trends Biochem. Sci* **20**, 326–329. https://doi.org/10.1016/S0968-0004(00)89061-1.
5. Hokin, L.E., (1987). The road to the phosphoinositide-generated second messengers. *Trends Pharmacol. Sci.* **8**, 53–56. https://doi.org/10.1016/0165-6147(87)90009-5.
6. Jensen, J.B., Falkenburger, B.H., Dickson, E.J., de la Cruz, L., Dai, G., Myeong, J., Jung, S.-R., Kruse, M., Vivas, O., Suh, B.-C., Hille, B., (2022). Biophysical physiology of phosphoinositide rapid dynamics and regulation in living cells. *J. Gen. Physiol.* **154**, https://doi.org/10.1085/jgp.202113074 e202113074.
7. Folch, J., Woolley, D.W., (1942). Inositol, a constituent of a brain phosphatide. *J. Biol. Chem.* **142**, 963–964. https://doi.org/10.1016/S0021-9258(18)45096-X.
8. Wills, R.C., Hammond, G.R.V., (2022). PI(4,5)P2: signaling the plasma membrane. *Biochem. J* **479**, 2311–2325. https://doi.org/10.1042/bcj20220445.
9. Marat, A.L., Haucke, V., (2016). Phosphatidylinositol 3-phosphates—at the interface between cell signalling and membrane traffic. *EMBO J.* **35**, 561–579. https://doi.org/10.15252/embj.201593564.
10. Yin, H.L., Janmey, P.A., (2003). Phosphoinositide regulation of the actin cytoskeleton. *Annu. Rev. Physiol.* **65**, 761–789. https://doi.org/10.1146/annurev.physiol.65.092101.142517.
11. Suh, B.-C., Hille, B., (2005). Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. *Curr. Opin. Neurobiol.* **15**, 370–378. https://doi.org/10.1016/j.conb.2005.05.005.
12. Bielas, S.L., Silhavy, J.L., Brancati, F., Kisseleva, M.V., Al-Gazali, L., Sztriha, L., Bayoumi, R.A., Zaki, M.S., Abdel-Aleem, A., Rosti, R.O., Kayserili, H., Swistun, D., Scott, L.C., Bertini, E., Boltshauser, E., Fazzi, E., Travaglini, L., Field, S.J., Gayral, S., Jacoby, M., Schurmans, S., Dallapiccola, B., Majerus, P.W., Valente, E.M., Gleeson, J.G., (2009). Mutations in INPP5E, encoding inositol polyphosphate-5-phosphatase E, link phosphatidyl inositol signaling to the ciliopathies. *Nature Genet.* **41**, 1032–1036. https://doi.org/10.1038/ng.423.

13. Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S.M., Riggins, G. J., Willson, J.K.V., Markowitz, S., Kinzler, K.W., Vogelstein, B., Velculescu, V.E., (2004). High frequency of mutations of the PIK3CA gene in human cancers. *Science* **304**, 554. https://doi.org/10.1126/science.1096502.
14. Posor, Y., Jang, W., Haucke, V., (2022). Phosphoinositides as membrane organizers. *Nature Rev. Mol. Cell Biol.* **23**, 797–816. https://doi.org/10.1038/s41580-022-00490-x.
15. Hammond, G.R.V., Burke, J.E., (2020). Novel roles of phosphoinositides in signaling, lipid transport, and disease. *Curr. Opin. Cell Biol.* **63**, 57–67. https://doi.org/10.1016/j.ceb.2019.12.007.
16. Burke, J.E., Triscott, J., Emerling, B.M., Hammond, G.R. V., (2023). Beyond PI3Ks: targeting phosphoinositide kinases in disease. *Nature Rev. Drug Discov.* **22**, 357–386. https://doi.org/10.1038/s41573-022-00582-5.
17. Hansen, S.B., Tao, X., MacKinnon, R., (2011). Structural basis of PIP2 activation of the classical inward rectifier K+ channel Kir2.2. *Nature* **477**, 495–498. https://doi.org/10.1038/nature10370.
18. Cheng, Y., (2018). Membrane protein structural biology in the era of single particle cryo-EM. *Curr. Opin. Struct. Biol.* **52**, 58–63. https://doi.org/10.1016/j.sbi.2018.08.008.
19. Laganowsky, A., Reading, E., Allison, T.M., Ulmschneider, M.B., Degiacomi, M.T., Baldwin, A.J., Robinson, C.V., (2014). Membrane proteins bind lipids selectively to modulate their structure and function. *Nature* **510**, 172–175. https://doi.org/10.1038/nature13419.
20. Gao, Y., Cao, E., Julius, D., Cheng, Y., (2016). TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. *Nature* **534**, 347–351. https://doi.org/10.1038/nature17964.
21. Yen, H.-Y., Hoi, K.K., Liko, I., Hedger, G., Horrell, M.R., Song, W., Wu, D., Heine, P., Warne, T., Lee, Y., Carpenter, B., Plückthun, A., Tate, C.G., Sansom, M.S. P., Robinson, C.V., (2018). PtdIns(4,5)P2 stabilizes active states of GPCRs and enhances selectivity of G-protein coupling. *Nature* **559**, 423–427. https://doi.org/10.1038/s41586-018-0325-6.
22. Xu, P., Huang, S., Zhang, H., Mao, C., Zhou, X.E., Cheng, X., Simon, I.A., Shen, D.-D., Yen, H.-Y., Robinson, C.V., Harpsøe, K., Svensson, B., Guo, J., Jiang, H., Gloriam, D. E., Melcher, K., Jiang, Y., Zhang, Y., Xu, H.E., (2021). Structural insights into the lipid and ligand regulation of serotonin receptors. *Nature* **592**, 469–473. https://doi.org/10.1038/s41586-021-03376-8.
23. Ansell, T.B., Song, W., Coupland, C.E., Carrique, L., Corey, R.A., Duncan, A.L., Cassidy, C.K., Geurts, M.M. G., Rasmussen, T., Ward, A.B., Siebold, C., Stansfeld, P. J., Sansom, M.S.P., (2023). LipIDens: simulation assisted interpretation of lipid densities in cryo-EM structures of membrane proteins. *Nature Commun.* **14**, 7774. https://doi.org/10.1038/s41467-023-43392-y.
24. Souza, P.C.T., Alessandri, R., Barnoud, J., Thallmair, S., Faustino, I., Grünewald, F., Patmanidis, I., Abdizadeh, H., Bruininks, B.M.H., Wassenaar, T.A., Kroon, P.C., Melcr, J., Nieto, V., Corradi, V., Khan, H.M., Domański, J., Javanainen, M., Martinez-Seara, H., Reuter, N., Best, R. B., Vattulainen, I., Monticelli, L., Periole, X., Tieleman, D. P., de Vries, A.H., Marrink, S.J., (2021). Martini 3: a general purpose force field for coarse-grained molecular dynamics. *Nature Methods* **18**, 382–388. https://doi.org/10.1038/s41592-021-01098-3.
25. Marrink, S.J., Corradi, V., Souza, P.C.T., Ingólfsson, H.I., Tieleman, D.P., Sansom, M.S.P., (2019). Computational modeling of realistic cell membranes. *Chem. Rev.* **119**, 6184–6226. https://doi.org/10.1021/acs.chemrev.8b00460.
26. Harayama, T., Riezman, H., (2018). Understanding the diversity of membrane lipid composition. *Nature Rev. Mol. Cell Biol.* **19**, 281–296. https://doi.org/10.1038/nrm.2017.138.
27. Han, X., Gross, R.W., (2022). The foundations and development of lipidomics. *J. Lipid Res.* **63** https://doi.org/10.1016/j.jlr.2021.100164.
28. Shevchenko, A., Simons, K., (2010). Lipidomics: coming to grips with lipid diversity. *Nature Rev. Mol. Cell Biol.* **11**, 593–598. https://doi.org/10.1038/nrm2934.
29. van Meer, G., Voelker, D.R., Feigenson, G.W., (2008). Membrane lipids: where they are and how they behave. *Nature Rev. Mol. Cell Biol.* **9**, 112–124. https://doi.org/10.1038/nrm2330.
30. Casares, D., Escribá, P.V., Rosselló, C.A., (2019). Membrane lipid composition: effect on membrane and organelle structure, function and compartmentalization and therapeutic avenues. *Int. J. Mol. Sci.*
31. Levental, I., Lyman, E., (2023). Regulation of membrane protein structure and function by their lipid nano-environment. *Nature Rev. Mol. Cell Biol.* **24**, 107–122. https://doi.org/10.1038/s41580-022-00524-4.
32. Byrne, E.F.X., Sircar, R., Miller, P.S., Hedger, G., Luchetti, G., Nachtergaele, S., Tully, M.D., Mydock-McGrane, L., Covey, D.F., Rambo, R.P., Sansom, M.S. P., Newstead, S., Rohatgi, R., Siebold, C., (2016). Structural basis of Smoothened regulation by its extracellular domains. *Nature* **535**, 517–522. https://doi.org/10.1038/nature18934.
33. Deshpande, I., Liang, J., Hedeen, D., Roberts, K.J., Zhang, Y., Ha, B., Latorraca, N.R., Faust, B., Dror, R. O., Beachy, P.A., Myers, B.R., Manglik, A., (2019). Smoothened stimulation by membrane sterols drives Hedgehog pathway activity. *Nature* **571**, 284–288. https://doi.org/10.1038/s41586-019-1355-4.
34. Michailidis, I.E., Rusinova, R., Georgakopoulos, A., Chen, Y., Iyengar, R., Robakis, N.K., Logothetis, D.E., Baki, L., (2011). Phosphatidylinositol-4,5-bisphosphate regulates epidermal growth factor receptor activation. *Pflügers Archiv – Eur. J. Physiol.* **461**, 387–397. https://doi.org/10.1007/s00424-010-0904-3.
35. Tucker, S.J., Baukrowitz, T., (2008). How highly charged anionic lipids bind and regulate ion channels. *J. Gen. Physiol.* **131**, 431–438. https://doi.org/10.1085/jgp.200709936.
36. Anderluh, A., Hofmaier, T., Klotzsch, E., Kudlacek, O., Stockner, T., Sitte, H.H., Schütz, G.J., (2017). Direct PIP2 binding mediates stable oligomer formation of the serotonin transporter. *Nature Commun.* **8**, 14089. https://doi.org/10.1038/ncomms14089.
37. Hamilton, P.J., Belovich, A.N., Khelashvili, G., Saunders, C., Erreger, K., Javitch, J.A., Sitte, H.H., Weinstein, H., Matthies, H.J.G., Galli, A., (2014). PIP2 regulates psychostimulant behaviors through its interaction with a membrane protein. *Nature Chem. Biol.* **10**, 582–589. https://doi.org/10.1038/nchembio.1545.
38. van den Bogaart, G., Meyenberg, K., Risselada, H.J., Amin, H., Willig, K.I., Hubrich, B.E., Dier, M., Hell, S.W.,

Grubmüller, Diederichsen, U., Jahn, R., (2011). Membrane protein sequestering by ionic protein–lipid interactions. *Nature* **479**, 552–555. https://doi.org/10.1038/nature10545.
39. Cho, W., Stahelin, R.V., (2005). Membrane-protein interactions in cell signaling and membrane trafficking. *Annu. Rev. Biophys.* **34**, 119–151. https://doi.org/10.1146/annurev.biophys.33.110502.133337.
40. Lemmon, M.A., (2008). Membrane recognition by phospholipid-binding domains. *Nature Rev. Mol. Cell Biol.* **9**, 99–111. https://doi.org/10.1038/nrm2328.
41. Slochower, D.R., Huwe, P.J., Radhakrishnan, R., Janmey, P.A., (2013). Quantum and all-atom molecular dynamics simulations of protonation and divalent ion binding to phosphatidylinositol 4,5-bisphosphate (PIP2). *J. Phys. Chem. B* **117**, 8322–8329. https://doi.org/10.1021/jp401414y.
42. Barneda, D., Cosulich, S., Stephens, L., Hawkins, P., (2019). How is the acyl chain composition of phosphoinositides created and does it matter?. *Biochem. Soc. Trans.* **47**, 1291–1305. https://doi.org/10.1042/BST20190205.
43. Hernandez, C.C., Falkenburger, B., Shapiro, M.S., (2009). Affinity for phosphatidylinositol 4,5-bisphosphate determines muscarinic agonist sensitivity of Kv7 K+ channels. *J. Gen. Physiol.* **134**, 437–448. https://doi.org/10.1085/jgp.200910313.
44. Dickson, E.J., Hille, B., (2019). Understanding phosphoinositides: rare, dynamic, and essential membrane phospholipids. *Biochem. J.* **476**, 1–23. https://doi.org/10.1042/BCJ20180022.
45. Viaud, J., Boal, F., Tronchère, H., Gaits-Iacovoni, F., Payrastre, B., (2014). Phosphatidylinositol 5-phosphate: a nuclear stress lipid and a tuner of membranes and cytoskeleton dynamics. *Bioessays* **36**, 260–272. https://doi.org/10.1002/bies.201300132.
46. Zewe, J.P., Miller, A.M., Sangappa, S., Wills, R.C., Goulden, B.D., Hammond, G.R.V., (2020). Probing the subcellular distribution of phosphatidylinositol reveals a surprising lack at the plasma membrane. *J. Cell Biol.* **219** https://doi.org/10.1083/jcb.201906127.
47. Daleke, D.L., (2003). Regulation of transbilayer plasma membrane phospholipid asymmetry. *J. Lipid Res.* **44**, 233–242. https://doi.org/10.1194/jlr.R200019-JLR200.
48. van Meer, G., de Kroon, A.I.P.M., (2011). Lipid map of the mammalian cell. *J. Cell Sci.* **124**, 5–8. https://doi.org/10.1242/jcs.071233.
49. Kim, O.-H., Kang, G.-H., Hur, J., Lee, J., Jung, Y., Hong, I.-S., Lee, H., Seo, S.-Y., Lee, D.H., Lee, C.S., Lee, I.-K., Bonner-Weir, S., Lee, J., Park, Y.J., Kim, H., Shoelson, S. E., Oh, B.-C., (2022). Externalized phosphatidylinositides on apoptotic cells are eat-me signals recognized by CD14. *Cell Death Differ.* **29**, 1423–1432. https://doi.org/10.1038/s41418-022-00931-2.
50. Jackson, M., Crick, D.C., Brennan, P.J., (2000). Phosphatidylinositol is an essential phospholipid of mycobacteria*. *J. Biol. Chem.* **275**, 30092–30099. https://doi.org/10.1074/jbc.M004658200.
51. Ballou, C.E., Lee, Y.C., (1964). The structure of a myoinositol mannoside from *Mycobacterium tuberculosis* glycolipid. *Biochemistry* **3**, 682–685. https://doi.org/10.1021/bi00893a014.
52. Friesner, R.A., Banks, J.L., Murphy, R.B., Halgren, T.A., Klicic, J.J., Mainz, D.T., Repasky, M.P., Knoll, E.H., Shelley, M., Perry, J.K., Shaw, D.E., Francis, P., Shenkin, P.S., (2004). Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **47**, 1739–1749. https://doi.org/10.1021/jm0306430.
53. Brannigan, G., Hénin, J., Law, R., Eckenhoff, R., Klein, M. L., (2008). Embedded cholesterol in the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci.* **105**, 14418–14423. https://doi.org/10.1073/pnas.0803029105.
54. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A.J., Bambrick, J., Bodenstein, S.W., Evans, D.A., Hung, C.-C., O'Neill, M., Reiman, D., Tunyasuvunakool, K., Wu, Z., Žemgulytė, A., Arvaniti, E., Beattie, C., Bertolli, O., Bridgland, A., Cherepanov, A., Congreve, M., Cowen-Rivers, A.I., Cowie, A., Figurnov, M., Fuchs, F.B., Gladman, H., Jain, R., Khan, Y.A., Low, C.M.R., Perlin, K., Potapenko, A., Savy, P., Singh, S., Stecula, A., Thillaisundaram, A., Tong, C., Yakneen, S., Zhong, E. D., Zielinski, M., Žídek, A., Bapst, V., Kohli, P., Jaderberg, M., Hassabis, D., Jumper, J.M., (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500. https://doi.org/10.1038/s41586-024-07487-w.
55. Baek, M., DiMaio, F., Anishchenko, I., Dauparas, J., Ovchinnikov, S., Lee, G.R., Wang, J., Cong, Q., Kinch, L. N., Schaeffer, R.D., Millán, C., Park, H., Adams, C., Glassman, C.R., DeGiovanni, A., Pereira, J.H., Rodrigues, A.V., van Dijk, A.A., Ebrecht, A.C., Opperman, D.J., Sagmeister, T., Buhlheller, C., Pavkov-Keller, T., Rathinaswamy, M.K., Dalwadi, U., Yip, C.K., Burke, J.E., Garcia, K.C., Grishin, N.V., Adams, P.D., Read, R.J., Baker, D., (2021). Accurate prediction of protein structures and interactions using a three-track neural network. *Science* **373**, 871–876. https://doi.org/10.1126/science.abj8754.
56. Krishna, R., Wang, J., Ahern, W., Sturmfels, P., Venkatesh, P., Kalvet, I., Lee, G.R., Morey-Burrows, F. S., Anishchenko, I., Humphreys, I.R., McHugh, R., Vafeados, D., Li, X., Sutherland, G.A., Hitchcock, A., Hunter, C.N., Kang, A., Brackenbrough, E., Bera, A.K., Baek, M., DiMaio, F., Baker, D., (2024). Generalized biomolecular modeling and design with RoseTTAFold All-Atom. *Science* **384**, https://doi.org/10.1126/science.adl2528 eadl2528.
57. Boitreaud, J., Dent, J., McPartlon, M., Meier, J., Reis, V., Rogozhonikov, A., Wu, K., (2024). Chai-1: decoding the molecular interactions of life. *BioRxiv*. https://doi.org/10.1101/2024.10.10.615955.
58. Bannwarth, C., Caldeweyher, E., Ehlert, S., Hansen, A., Pracht, P., Seibert, J., Spicher, S., Grimme, S., (2021). Extended tight-binding quantum chemistry methods. *WIREs Comput. Mol. Sci.* **11**, e1493. https://doi.org/10.1002/wcms.1493.
59. van der Kamp, M.W., Mulholland, A.J., (2013). Combined quantum mechanics/molecular mechanics (QM/MM) methods in computational enzymology. *Biochemistry* **52**, 2708–2728. https://doi.org/10.1021/bi400215w.
60. Hollingsworth, S.A., Dror, R.O., (2018). Molecular dynamics simulation for all. *Neuron* **99**, 1129–1143. https://doi.org/10.1016/j.neuron.2018.08.011.
61. Karplus, M., McCammon, J.A., (2002). Molecular dynamics simulations of biomolecules. *Nature Struct. Biol.* **9**, 646–652. https://doi.org/10.1038/nsb0902-646.

62. Muller, M.P., Jiang, T., Sun, C., Lihan, M., Pant, S., Mahinthichaichan, P., Trifan, A., Tajkhorshid, E., (2019). Characterization of lipid–protein interactions and lipid-mediated modulation of membrane protein function through molecular simulation. *Chem. Rev.* **119**, 6086–6161. https://doi.org/10.1021/acs.chemrev.8b00608.
63. Enkavi, G., Javanainen, M., Kulig, W., Róg, T., Vattulainen, I., (2019). Multiscale simulations of biological membranes: the challenge to understand biological phenomena in a living substance. *Chem. Rev.* **119**, 5607–5774. https://doi.org/10.1021/acs.chemrev.8b00538.
64. Hedger, G., Sansom, M.S.P., (2016). Lipid interaction sites on channels, transporters and receptors: Recent insights from molecular dynamics simulations. *Biochimica et Biophysica Acta (BBA) – B Biomembranes* **1858**, 2390–2400. https://doi.org/10.1016/j.bbamem.2016.02.037.
65. Simons, K., (2016). Cell membranes: a subjective perspective. *Biochimica et Biophysica Acta (BBA) – B Biomembranes* **1858**, 2569–2572. https://doi.org/10.1016/j.bbamem.2016.01.023.
66. Huang, J., Rauscher, S., Nawrocki, G., Ran, T., Feig, M., de Groot, B.L., Grubmüller, H., MacKerell, A.D., (2017). CHARMM36m: an improved force field for folded and intrinsically disordered proteins. *Nature Methods* **14**, 71–73. https://doi.org/10.1038/nmeth.4067.
67. Klauda, J.B., Venable, R.M., Freites, J.A., O'Connor, J. W., Tobias, D.J., Mondragon-Ramirez, C., Vorobyov, I., MacKerell Jr., A.D., Pastor, R.W., (2010). Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types. *J. Phys. Chem. B* **114**, 7830–7843. https://doi.org/10.1021/jp101759q.
68. Dickson, C.J., Madej, B.D., Skjevik, Å.A., Betz, R.M., Teigen, K., Gould, I.R., Walker, R.C., (2014). Lipid14: the amber lipid force field. *J. Chem. Theory Comput.* **10**, 865–879. https://doi.org/10.1021/ct4010307.
69. Dickson, C.J., Walker, R.C., Gould, I.R., (2022). Lipid21: complex lipid membrane simulations with AMBER. *J. Chem. Theory Comput.* **18**, 1726–1736. https://doi.org/10.1021/acs.jctc.1c01217.
70. Tian, C., Kasavajhala, K., Belfon, K.A.A., Raguette, L., Huang, H., Migues, A.N., Bickel, J., Wang, Y., Pincay, J., Wu, Q., Simmerling, C., (2020). ff19SB: amino-acid-specific protein backbone parameters trained against quantum mechanics energy surfaces in solution. *J. Chem. Theory Comput.* **16**, 528–552. https://doi.org/10.1021/acs.jctc.9b00591.
71. Marrink, S.J., Risselada, H.J., Yefimov, S., Tieleman, D. P., de Vries, A.H., (2007). The MARTINI force field: coarse grained model for biomolecular simulations. *J. Phys. Chem. B* **111**, 7812–7824. https://doi.org/10.1021/jp071097f.
72. Corradi, V., Sejdiu, B.I., Mesa-Galloso, H., Abdizadeh, H., Noskov, S.Y., Marrink, S.J., Tieleman, D.P., (2019). Emerging diversity in lipid–protein interactions. *Chem. Rev.* **119**, 5775–5848. https://doi.org/10.1021/acs.chemrev.8b00451.
73. Chavent, M., Duncan, A.L., Sansom, M.S.P., (2016). Molecular dynamics simulations of membrane proteins and their interactions: from nanoscale to mesoscale. *Curr. Opin. Struct. Biol.* **40**, 8–16. https://doi.org/10.1016/j.sbi.2016.06.007.
74. Bennett, W.F.D., Bernardi, A., Ozturk, T.N., Ingólfsson, H. I., Fox, S.J., Sun, D., Maupin, C.M., (2024). ezAlign: a tool for converting coarse-grained molecular dynamics structures to atomistic resolution for multiscale modeling. *Molecules* **29** https://doi.org/10.3390/molecules29153557.
75. Wassenaar, T.A., Pluhackova, K., Böckmann, R.A., Marrink, S.J., Tieleman, D.P., (2014). Going backward: a flexible geometric approach to reverse transformation from coarse grained to atomistic models. *J. Chem. Theory Comput.* **10**, 676–690. https://doi.org/10.1021/ct400617g.
76. Stansfeld, P.J., Sansom, M.S.P., (2011). Molecular simulation approaches to membrane proteins. *Structure* **19**, 1562–1572. https://doi.org/10.1016/j.str.2011.10.002.
77. Shaw, D.E., Deneroff, M.M., Dror, R.O., Kuskin, J.S., Larson, R.H., Salmon, J.K., Young, C., Batson, B., Bowers, K.J., Chao, J.C., Eastwood, M.P., Gagliardo, J., Grossman, J.P., Ho, C.R., Ierardi, D.J., Kolossváry, I., Klepeis, J.L., Layman, T., McLeavey, C., Moraes, M.A., Mueller, R., Priest, E.C., Shan, Y., Spengler, J., Theobald, M., Towles, B., Wang, S.C., (2008). Anton, a special-purpose machine for molecular dynamics simulation. *Commun. ACM* **51**, 91–97. https://doi.org/10.1145/1364782.1364802.
78. Hénin, J., Lelièvre, T., Shirts, M. R., Valsson, O., Delemotte, L. (2022). Enhanced sampling methods for molecular dynamics simulations. arXiv preprint arXiv:2202.04164. https://doi.org/10.48550/arXiv.2202.04164.
79. Kooijman, E.E., King, K.E., Gangoda, M., Gericke, A., (2009). Ionization properties of phosphatidylinositol polyphosphates in mixed model membranes. *Biochemistry* **48**, 9360–9371. https://doi.org/10.1021/bi9008616.
80. McLaughlin, S., Wang, J., Gambhir, A., Murray, D., (2002). PIP2 and proteins: interactions, organization, and information flow. *Annu. Rev. Biophys.* **31**, 151–175. https://doi.org/10.1146/annurev.biophys.31.082901.134259.
81. Borges-Araújo, L., Fernandes, F., (2020). Structure and lateral organization of phosphatidylinositol 4,5-bisphosphate. *Molecules*.
82. Buslaev, P., Aho, N., Jansen, A., Bauer, P., Hess, B., Groenhof, G., (2022). Best practices in constant pH MD simulations: accuracy and sampling. *J. Chem. Theory Comput.* **18**, 6134–6147. https://doi.org/10.1021/acs.jctc.2c00517.
83. Borges-Araújo, L., Souza, P.C.T., Fernandes, F., Melo, M. N., (2022). Improved parameterization of phosphatidylinositide lipid headgroups for the Martini 3 coarse-grain force field. *J. Chem. Theory Comput.* **18**, 357–373. https://doi.org/10.1021/acs.jctc.1c00615.
84. Stansfeld, P.J., Hopkinson, R., Ashcroft, F.M., Sansom, M.S.P., (2009). PIP2-binding site in kir channels: definition by multiscale biomolecular simulations. *Biochemistry* **48**, 10926–10933. https://doi.org/10.1021/bi9013193.
85. Jarin, Z., Venable, R.M., Han, K., Pastor, R.W., (2024). Ion-induced PIP2 clustering with Martini3: modification of phosphate–ion interactions and comparison with CHARMM36. *J. Phys. Chem. B* **128**, 2134–2143. https://doi.org/10.1021/acs.jpcb.3c06523.
86. Pedersen, K.B., Ingólfsson, H.I., P., R.-E.D., Borges-Araujo, L., Andreasen, M.D., Empereur-mot, C., Melcr, J., Ozturk, T.N., Bennett, W.F.D., Kjølbye, L.R., Brasnett, C., Corradi, V., Khan, H.M., Cino, E.A., Crowley, J., Kim, H., Fábián, B., Borges-Araújo, A.C., Pavan, G.M., Lolicato,

F., Wassenaar, T.A., Melo, M.N., Thallmair, S., Carpenter, T.S., Monticelli, L., Tieleman, D.P., Schiøtt, B., Souza, P.C.T., Marrink, S.J., (2024). The Martini 3 lipidome: expanded and refined parameters improve lipid phase behavior. *ChemRxiv*. https://doi.org/10.26434/chemrxiv-2024-8bjrr.

87. Brown, C.M., Corey, R.A., Grélard, A., Gao, Y., Choi, Y. K., Luna, E., Gilleron, M., Destainville, N., Nigou, J., Loquet, A., Fullam, E., Im, W., Stansfeld, P.J., Chavent, M., (2023). Supramolecular organization and dynamics of mannosylated phosphatidylinositol lipids in the mycobacterial plasma membrane. *Proc. Natl. Acad. Sci.* **120**, https://doi.org/10.1073/pnas.2212755120 e2212755120.
88. Lupyan, D., Mezei, M., Logothetis, D.E., Osman, R., (2010). A molecular dynamics investigation of lipid bilayer perturbation by PIP2. *Biophys. J.* **98**, 240–247. https://doi.org/10.1016/j.bpj.2009.09.063.
89. Mallajosyula, S.S., Guvench, O., Hatcher, E., MacKerell Jr., A.D., (2012). CHARMM additive all-atom force field for phosphate and sulfate linked to carbohydrates. *J. Chem. Theory Comput.* **8**, 759–776. https://doi.org/10.1021/ct200792v.
90. Wu, E.L., Qi, Y., Song, K.C., Klauda, J.B., Im, W., (2014). Preferred orientations of phosphoinositides in bilayers and their implications in protein recognition mechanisms. *J. Phys. Chem. B* **118**, 4315–4325. https://doi.org/10.1021/jp500610t.
91. Lee, J., Patel, D.S., Ståhle, J., Park, S.-J., Kern, N.R., Kim, S., Lee, J., Cheng, X., Valvano, M.A., Holst, O., Knirel, Y.A., Qi, Y., Jo, S., Klauda, J.B., Widmalm, G., Im, W., (2019). CHARMM-GUI membrane builder for complex biological membrane simulations with glycolipids and lipoglycans. *J. Chem. Theory Comput.* **15**, 775–786. https://doi.org/10.1021/acs.jctc.8b01066.
92. Qi, Y., Cheng, X., Lee, J., Vermaas, J.V., Pogorelov, T.V., Tajkhorshid, E., Park, S., Klauda, J.B., Im, W., (2015). CHARMM-GUI HMMM builder for membrane simulations with the highly mobile membrane-mimetic model. *Biophys. J.* **109**, 2012–2022. https://doi.org/10.1016/j.bpj.2015.10.008.
93. Wu, E.L., Cheng, X., Jo, S., Rui, H., Song, K.C., Dávila-Contreras, E.M., Qi, Y., Lee, J., Monje-Galvan, V., Venable, R.M., Klauda, J.B., Im, W., (2014). CHARMM-GUI membrane builder toward realistic biological membrane simulations. *J. Comput. Chem.* **35**, 1997–2004. https://doi.org/10.1002/jcc.23702.
94. Ingólfsson, H.I., Melo, M.N., van Eerden, F.J., Arnarez, C., Lopez, C.A., Wassenaar, T.A., Periole, X., de Vries, A. H., Tieleman, D.P., Marrink, S.J., (2014). Lipid organization of the plasma membrane. *J. Am. Chem. Soc.* **136**, 14554–14559. https://doi.org/10.1021/ja507832e.
95. Ingólfsson, H.I., Carpenter, T.S., Bhatia, H., Bremer, P.-T., Marrink, S.J., Lightstone, F.C., (2017). Computational lipidomics of the neuronal plasma membrane. *Biophys. J.* **113**, 2271–2280. https://doi.org/10.1016/j.bpj.2017.10.017.
96. Duncan, A.L., Ruprecht, J.J., Kunji, E.R.S., Robinson, A. J., (2018). Cardiolipin dynamics and binding to conserved residues in the mitochondrial ADP/ATP carrier. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1860**, 1035–1045. https://doi.org/10.1016/j.bbamem.2018.01.017.
97. Hedger, G., Rouse, S.L., Domański, J., Chavent, M., Koldsø, H., Sansom, M.S.P., (2016). Lipid-loving ANTs: molecular simulations of cardiolipin interactions and the organization of the adenine nucleotide translocase in model mitochondrial membranes. *Biochemistry* **55**, 6238–6249. https://doi.org/10.1021/acs.biochem.6b00751.
98. Brown, C.M., Westendorp, M.S.S., Zarmiento-Garcia, R., Stevens, J.A., Rouse, S.L., Marrink, S.J., Wassenaar, T. A., (2024). An integrative modelling approach to the mitochondrial cristae. *BioRxiv*. https://doi.org/10.1101/2024.09.23.613389.
99. Lolicato, F., Saleppico, R., Griffo, A., Meyer, A., Scollo, F., Pokrandt, B., Müller, H.-M., Ewers, H., Hähl, H., Fleury, J.-B., Seemann, R., Hof, M., Brügger, B., Jacobs, K., Vattulainen, I., Nickel, W., (2022). Cholesterol promotes clustering of PI(4,5)P2 driving unconventional secretion of FGF2. *J. Cell Biol.* **221** https://doi.org/10.1083/jcb.202106123.
100. Lee, J., Cheng, X., Jo, S., MacKerell Jr., A.D., Klauda, J. B., Im, W., (2016). CHARMM-GUI input generator for NAMD, gromacs, amber, openmm, and CHARMM/OpenMM simulations using the CHARMM36 additive force field. *Biophys. J.* **110**, 641a. https://doi.org/10.1016/j.bpj.2015.11.3431.
101. Wassenaar, T.A., Ingólfsson, H.I., Böckmann, R.A., Tieleman, D.P., Marrink, S.J., (2015). Computational lipidomics with insane: a versatile tool for generating custom membranes for molecular simulations. *J. Chem. Theory Comput.* **11**, 2144–2155. https://doi.org/10.1021/acs.jctc.5b00209.
102. Kroon, P.C., Grunewald, F., Barnoud, J., van Tilburg, M., Souza, P.C.T., Wassenaar, T.A., Marrink, S.J. (2024). Martinize2 and vermouth: unified framework for topology generation. https://doi.org/10.7554/elife.90627.2.
103. Andreasen, M.D., Souza, P.C.T., Schiøtt, B., Zuzic, L., (2024). Creating coarse-grained systems with COBY: towards higher accuracy in membrane complexity. *BioRxiv*. https://doi.org/10.1101/2024.07.23.604601.
104. Tiemann, J.K.S., Szczuka, M., Bouarroudj, L., Oussaren, M., Garcia, S., Howard, R.J., Delemotte, L., Lindahl, E., Baaden, M., Lindorff-Larsen, K., Chavent, M., Poulain, P., (2024). Mdverse, shedding light on the dark matter of molecular dynamics simulations. *eLife* **12**, https://doi.org/10.7554/eLife.90061 RP90061.
105. Tiemann, J.K.S., Guixà-González, R., Hildebrand, P.W., Rose, A.S., (2017). MDsrv: viewing and sharing molecular dynamics simulations on the web. *Nature Methods* **14**, 1123–1124. https://doi.org/10.1038/nmeth.4497.
106. Song, W., Corey, R.A., Ansell, T.B., Cassidy, C.K., Horrell, M.R., Duncan, A.L., Stansfeld, P.J., Sansom, M. S.P., (2022). PyLipID: a python package for analysis of protein–lipid interactions from molecular dynamics simulations. *J. Chem. Theory Comput.* **18**, 1188–1201. https://doi.org/10.1021/acs.jctc.1c00708.
107. Sejdiu, B.I., Tieleman, D.P., (2021). ProLint: a web-based framework for the automated data analysis and visualization of lipid–protein interactions. *Nucleic Acids Res.* **49**, W544–W550. https://doi.org/10.1093/nar/gkab409.
108. Corey, R.A., Vickery, O.N., Sansom, M.S.P., Stansfeld, P. J., (2019). Insights into membrane protein–lipid interactions from free energy calculations. *J. Chem. Theory Comput.* **15**, 5727–5736. https://doi.org/10.1021/acs.jctc.9b00548.

109. Leiserson, C.E., Thompson, N.C., Emer, J.S., Kuszmaul, B.C., Lampson, B.W., Sanchez, D., Schardl, T.B., (2020). There's plenty of room at the top: what will drive computer performance after Moore's law? *Science* **368**, https://doi.org/10.1126/science.aam9744 eaam9744.
110. Páll, S., Zhmurov, A., Bauer, P., Abraham, M., Lundborg, M., Gray, A., Hess, B., Lindahl, E., (2020). Heterogeneous parallelization and acceleration of molecular dynamics simulations in GROMACS. *J. Chem. Phys.* **153**, https://doi.org/10.1063/5.0018516 134110.
111. Vögele, M., Köfinger, J., Hummer, G., (2018). Hydrodynamics of diffusion in lipid membrane simulations. *Phys. Rev. Lett.* **120**, https://doi.org/10.1103/PhysRevLett.120.268104 268104.
112. Duncan, A.L., Reddy, T., Koldsø, H., Hélie, J., Fowler, P. W., Chavent, M., Sansom, M.S.P., (2017). Protein crowding and lipid complexity influence the nanoscale dynamic organization of ion channels in cell membranes. *Sci. Rep.* **7**, 16647. https://doi.org/10.1038/s41598-017-16865-6.
113. Fowler, P.W., Hélie, J., Duncan, A., Chavent, M., Koldsø, H., Sansom, M.S.P., (2016). Membrane stiffness is modified by integral membrane proteins. *Soft Matter* **12**, 7792–7803. https://doi.org/10.1039/C6SM01186A.
114. Hadden, J.A., Perilla, J.R., (2018). All-atom virus simulations. *Curr. Opin. Virol.* **31**, 82–91. https://doi.org/10.1016/j.coviro.2018.08.007.
115. Gupta, C., Sarkar, D., Tieleman, D.P., Singharoy, A., (2022). The ugly, bad, and good stories of large-scale biomolecular simulations. *Curr. Opin. Struct. Biol.* **73**, https://doi.org/10.1016/j.sbi.2022.102338 102338.
116. Khalid, S., Brandner, A.F., Juraschko, N., Newman, K.E., Pedebos, C., Prakaash, D., Smith, I.P.S., Waller, C., Weerakoon, D., (2023). Computational microbiology of bacteria: advancements in molecular dynamics simulations. *Structure* **31**, 1320–1327. https://doi.org/10.1016/j.str.2023.09.012.
117. Shaw, D.E., Adams, P.J., Azaria, A., Bank, J.A., Batson, B., Bell, A., Bergdorf, M., Bhatt, J., Butts, J.A., Correia, T., Dirks, R.M., Dror, R.O., Eastwood, M.P., Edwards, B., Even, A., Feldmann, P., Fenn, M., Fenton, C.H., Forte, A., Gagliardo, J., Gill, G., Gorlatova, M., Greskamp, B., Grossman, J.P., Gullingsrud, J., Harper, A., Hasenplaugh, W., Heily, M., Heshmat, B.C., Hunt, J., Ierardi, D.J., Iserovich, L., Jackson, B.L., Johnson, N.P., Kirk, M.M., Klepeis, J.L., Kuskin, J.S., Mackenzie, K.M., Mader, R.J., McGowen, R., McLaughlin, A., Moraes, M. A., Nasr, M.H., Nociolo, L.J., O'Donnell, L., Parker, A., Peticolas, J.L., Pocina, G., Predescu, C., Quan, T., Salmon, J.K., Schwink, C., Shim, K.S., Siddique, N., Spengler, J., Szalay, T., Tabladillo, R., Tartler, R., Taube, A.G., Theobald, M., Towles, B., Vick, W., Wang, S.C., Wazlowski, M., Weingarten, M.J., Williams, J.M., Yuh, K. A., (2021). Anton 3: twenty microseconds of molecular dynamics simulation before lunch. *In: Proceedings of the International Conference for High Performance Computing, Networking, Storage and Analysis*. https://doi.org/10.1145/3458817.3487397.
118. Naddaf, M., (2024). The science events to watch for in 2024. *Nature* **625**, 221–223.
119. Gapsys, V., Kopec, W., Matthes, D., de Groot, B.L., (2024). Biomolecular simulations at the exascale: from drug design to organelles and beyond. *Curr. Opin. Struct. Biol.* **88**, https://doi.org/10.1016/j.sbi.2024.102887 102887.
120. Gault, J., Liko, I., Landreh, M., Shutin, D., Bolla, J.R., Jefferies, D., Agasid, M., Yen, H.-Y., Ladds, M.J.G.W., Lane, D.P., Khalid, S., Mullen, C., Remes, P.M., Huguet, R., McAlister, G., Goodwin, M., Viner, R., Syka, J.E.P., Robinson, C.V., (2020). Combining native and 'omics' mass spectrometry to identify endogenous ligands bound to membrane proteins. *Nature Methods* **17**, 505–508. https://doi.org/10.1038/s41592-020-0821-0.
121. Hauser, A.S., Attwood, M.M., Rask-Andersen, M., Schiöth, H.B., Gloriam, D.E., (2017). Trends in GPCR drug discovery: new agents, targets and indications. *Nature Rev. Drug Discov.* **16**, 829–842. https://doi.org/10.1038/nrd.2017.178.
122. Hilger, D., Masureel, M., Kobilka, B.K., (2018). Structure and dynamics of GPCR signaling complexes. *Nature Struct. Mol. Biol.* **25**, 4–12. https://doi.org/10.1038/s41594-017-0011-7.
123. Fasciani, I., Carli, M., Petragnano, F., Colaianni, F., Aloisi, G., Maggio, R., Scarselli, M., Rossi, M., (2022). GPCRs in intracellular compartments: new targets for drug discovery. *Biomolecules* **12**, 1343. https://doi.org/10.3390/biom12101343.
124. Vargas, M.V., Dunlap, L.E., Dong, C., Carter, S.J., Tombari, R.J., Jami, S.A., Cameron, L.P., Patel, S.D., Hennessey, J.J., Saeger, H.N., McCorvy, J.D., Gray, J.A., Tian, L., Olson, D.E., (2023). Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science* **379**, 700–706. https://doi.org/10.1126/science.adf0435.
125. Austin, G., ElEid, L., Oqua, A.I., Manchanda, Y., Poliakova, Y., Bouzakri, K., Montoya, A., Withers, D.J., Jones, B., Millership, S.J., Prokopenko, I., Tomas, A., (2024). An inter-organelle contact between endosomal GLP-1R, ER VAP-B, and the mitochondrial AKAP SPHKAP triggers PKA-dependent MIC19 phosphorylation and β-cell mitochondrial remodelling. *BioRxiv*. https://doi.org/10.1101/2024.04.28.591531.
126. Wootten, D., Christopoulos, A., Marti-Solano, M., Babu, M.M., Sexton, P.M., (2018). Mechanisms of signalling and biased agonism in G protein-coupled receptors. *Nature Rev. Mol. Cell Biol.* **19**, 638–653. https://doi.org/10.1038/s41580-018-0049-3.
127. Billesbølle, C.B., de March, C.A., van der Velden, W.J.C., Ma, N., Tewari, J., del Torrent, C.L., Li, L., Faust, B., Vaidehi, N., Matsunami, H., Manglik, A., (2023). Structural basis of odorant recognition by a human odorant receptor. *Nature* **615**, 742–749. https://doi.org/10.1038/s41586-023-05798-y.
128. Donnelly, D., (2012). The structure and function of the glucagon-like peptide-1 receptor and its ligands. *Br. J. Pharmacol.* **166**, 27–41. https://doi.org/10.1111/j.1476-5381.2011.01687.x.
129. Niswender, C.M., Conn, P.J., (2010). Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* **50**, 295–322. https://doi.org/10.1146/annurev.pharmtox.011008.145533.
130. Palczewski, K., (2006). G Protein–coupled receptor rhodopsin. *Annu. Rev. Biochem* **75**, 743–767. https://doi.org/10.1146/annurev.biochem.75.103004.142743.
131. Liu, S., Anderson, P.J., Rajagopal, S., Lefkowitz, R.J., Rockman, H.A., (2024). G protein-coupled receptors: a

century of research and discovery. *Circ. Res.* **135**, 174–197. https://doi.org/10.1161/CIRCRESAHA.124.323067.
132. Sengupta, D., Chattopadhyay, A., (2015). Molecular dynamics simulations of GPCR–cholesterol interaction: an emerging paradigm. *Biochim. Biophys. Acta Biomembr.* **1848**, 1775–1782. https://doi.org/10.1016/j.bbamem.2015.03.018.
133. Hanson, M.A., Cherezov, V., Griffith, M.T., Roth, C.B., Jaakola, V.-P., Chien, E.Y.T., Velasquez, J., Kuhn, P., Stevens, R.C., (2008). A specific cholesterol binding site is established by the 2.8 Å structure of the human β2-adrenergic receptor. *Structure* **16**, 897–905. https://doi.org/10.1016/j.str.2008.05.001.
134. Oqua, A.I., Chao, K., Eid, L.E., Casteller, L., Miguéns, A., Barg, S., Jones, B., de la Serna, J.B., Rouse, S.L., Tomas, A., (2024). Molecular mapping and functional validation of GLP-1R cholesterol binding sites in pancreatic beta cells. *BioRxiv*. https://doi.org/10.1101/2024.06.22.600087.
135. Dawaliby, R., Trubbia, C., Delporte, C., Masureel, M., Van Antwerpen, P., Kobilka, B.K., Govaerts, C., (2016). Allosteric regulation of G protein–coupled receptor activity by phospholipids. *Nature Chem. Biol.* **12**, 35–39. https://doi.org/10.1038/nchembio.1960.
136. Dijkman, P.M., Watts, A., (2015). Lipid modulation of early G protein-coupled receptor signalling events. *Biochim. Biophys. Acta Biomembr.* **1848**, 2889–2897. https://doi.org/10.1016/j.bbamem.2015.08.004.
137. Inagaki, S., Ghirlando, R., White, J.F., Gvozdenovic-Jeremic, J., Northup, J.K., Grisshammer, R., (2012). Modulation of the interaction between neurotensin receptor NTS1 and Gq protein by lipid. *J. Mol. Biol.* **417**, 95–111. https://doi.org/10.1016/j.jmb.2012.01.023.
138. Velgy, N., Hedger, G., Biggin, P.C., (2018). GPCRs: what can we learn from molecular dynamics simulations? In: Heifetz, A. (Ed.), *Computational Methods for GPCR Drug Discovery*. Springer, New York, New York, NY, pp. 133–158.
139. Roux, B., (1995). The calculation of the potential of mean force using computer simulations. *Comput. Phys. Commun.* **91**, 275–282. https://doi.org/10.1016/0010-4655(95)00053-I.
140. Huang, W., Masureel, M., Qu, Q., Janetzko, J., Inoue, A., Kato, H.E., Robertson, M.J., Nguyen, K.C., Glenn, J.S., Skiniotis, G., Kobilka, B.K., (2020). Structure of the neurotensin receptor 1 in complex with β-arrestin 1. *Nature* **579**, 303–308. https://doi.org/10.1038/s41586-020-1953-1.
141. Janetzko, J., Kise, R., Barsi-Rhyne, B., Siepe, D.H., Heydenreich, F.M., Kawakami, K., Masureel, M., Maeda, S., Garcia, K.C., von Zastrow, M., Inoue, A., Kobilka, B.K., (2022). Membrane phosphoinositides regulate GPCR-beta-arrestin complex assembly and dynamics. *Cell* **185**, 4560–4573.e4519. https://doi.org/10.1016/j.cell.2022.10.018.
142. Zhang, M., Chen, T., Lu, X., Lan, X., Chen, Z., Lu, S., (2024). G protein-coupled receptors (GPCRs): advances in structures, mechanisms, and drug discovery. *Signal Transduct. Target. Ther.* **9**, 88. https://doi.org/10.1038/s41392-024-01803-6.
143. Pándy-Szekeres, G., Munk, C., Tsonkov, T.M., Mordalski, S., Harpsøe, K., Hauser, A.S., Bojarski, A.J., Gloriam, D. E., (2017). GPCRdb in 2018: adding GPCR structure models and ligands. *Nucleic Acids Res.* **46**, D440–D446. https://doi.org/10.1093/nar/gkx1109.
144. Coskun, D., Lihan, M., Rodrigues, J.P.G.L.M., Vass, M., Robinson, D., Friesner, R.A., Miller, E.B., (2024). Using AlphaFold and experimental structures for the prediction of the structure and binding affinities of GPCR complexes via induced fit docking and free energy perturbation. *J. Chem. Theory Comput.* **20**, 477–489. https://doi.org/10.1021/acs.jctc.3c00839.
145. Heo, L., Feig, M., (2022). Multi-state modeling of G-protein coupled receptors at experimental accuracy. *Proteins: Struct. Funct. Bioinf.* **90**, 1873–1885. https://doi.org/10.1002/prot.26382.
146. Miller, E.B., Hwang, H., Shelley, M., Placzek, A., Rodrigues, J.P.G.L.M., Suto, R.K., Wang, L., Akinsanya, K., Abel, R., (2024). Enabling structure-based drug discovery utilizing predicted models. *Cell* **187**, 521–525. https://doi.org/10.1016/j.cell.2023.12.034.
147. Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T., (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* **46**, W296–W303. https://doi.org/10.1093/nar/gky427.
148. Song, W., Yen, H.-Y., Robinson, C.V., Sansom, M.S.P., (2019). State-dependent lipid interactions with the A2a receptor revealed by MD simulations using in vivo-mimetic membranes. *Structure* **27**, 392–403.e393. https://doi.org/10.1016/j.str.2018.10.024.
149. Sejdiu, B.I., Tieleman, D.P., (2020). Lipid-protein interactions are a unique property and defining feature of G protein-coupled receptors. *Biophys. J.* **118**, 1887–1900. https://doi.org/10.1016/j.bpj.2020.03.008.
150. Kjølbye, L.R., Sørensen, L., Yan, J., Berglund, N.A., Ferkinghoff-Borg, J., Robinson, C.V., Schiøtt, B., (2022). Lipid modulation of a Class B GPCR: elucidating the modulatory role of PI(4,5)P2 lipids. *J. Chem. Inf. Model.* **62**, 6788–6802. https://doi.org/10.1021/acs.jcim.2c00635.
151. Ma, N., Lee, S., Vaidehi, N., (2020). Activation microswitches in adenosine receptor A2A function as rheostats in the cell membrane. *Biochemistry* **59**, 4059–4071. https://doi.org/10.1021/acs.biochem.0c00626.
152. Ansell, T.B., Song, W., Sansom, M.S.P., (2020). The glycosphingolipid GM3 modulates conformational dynamics of the glucagon receptor. *Biophys. J.* **119**, 300–313. https://doi.org/10.1016/j.bpj.2020.06.009.
153. Mukhaleva, E., Yang, T., Sadler, F., Sivaramakrishnan, S., Ma, N., Vaidehi, N., (2024). Cellular lipids regulate the conformational ensembles of the disordered intracellular loop 3 in beta2-adrenergic receptor. *iScience* **27** https://doi.org/10.1016/j.isci.2024.110086.
154. Damian, M., Louet, M., Gomes, A.A.S., M'Kadmi, C., Denoyelle, S., Cantel, S., Mary, S., Bisch, P.M., Fehrentz, J.-A., Catoire, L.J., Floquet, N., Banères, J.-L., (2021). Allosteric modulation of ghrelin receptor signaling by lipids. *Nature Commun.* **12**, 3938. https://doi.org/10.1038/s41467-021-23756-y.
155. Thakur, N., Ray, A.P., Sharp, L., Jin, B., Duong, A., Pour, N.G., Obeng, S., Wijesekara, A.V., Gao, Z.-G., McCurdy, C.R., Jacobson, K.A., Lyman, E., Eddy, M.T., (2023). Anionic phospholipids control mechanisms of GPCR-G protein recognition. *Nature Commun.* **14**, 794. https://doi.org/10.1038/s41467-023-36425-z.

156. Gomes, A., Di Michele, M., Roessner, R.A., Damian, M., Bisch, P.M., Sibille, N., Louet, M., Baneres, J.-L., Floquet, N., (2024). Lipids modulate the dynamics of GPCR:β-Arrestin interaction. *BioRxiv*. https://doi.org/10.1101/2024.03.16.585329.
157. Borges-Araújo, L., Domingues, M.M., Fedorov, A., Santos, N.C., Melo, M.N., Fernandes, F., (2021). Acyl-chain saturation regulates the order of phosphatidylinositol 4,5-bisphosphate nanodomains. *Commun. Chem.* **4**, 164. https://doi.org/10.1038/s42004-021-00603-1.
158. Hedger, G., Koldsø, H., Chavent, M., Siebold, C., Rohatgi, R., Sansom, M.S.P., (2019). Cholesterol interaction sites on the transmembrane domain of the hedgehog signal transducer and class F G protein-coupled receptor smoothened. *Structure* **27**, 549–559. e542. https://doi.org/10.1016/j.str.2018.11.003.
159. Garcia-Gonzalo, F.R., Phua, S.C., Roberson, E.C., Garcia III, G., Abedin, M., Schurmans, S., Inoue, T., Reiter, J.F., (2015). Phosphoinositides regulate ciliary protein trafficking to modulate hedgehog signaling. *Dev. Cell* **34**, 400–409. https://doi.org/10.1016/j.devcel.2015.08.001.
160. Chávez, M., Ena, S., Van Sande, J., de Kerchove d'Exaerde, A., Schurmans, S., Schiffmann, S.N., (2015). Modulation of ciliary phosphoinositide content regulates trafficking and sonic hedgehog signaling output. *Dev. Cell* **34**, 338–350. https://doi.org/10.1016/j.devcel.2015.06.016.
161. Nakatsu, F., (2015). A phosphoinositide code for primary cilia. *Dev. Cell* **34**, 379–380. https://doi.org/10.1016/j.devcel.2015.08.008.
162. Chao, K.W., Wong, L., Oqua, A., Kalayan, J., Manchanda, Y., Gebbie-Rayet, J., Hedger, G., Tomas, A., Rouse, S.L., (2024). Human class B1 GPCR modulation by plasma membrane lipids. *BioRxiv*. https://doi.org/10.1101/2024.12.19.629375.
163. Zhang, H., Qiao, A., Yang, D., Yang, L., Dai, A., de Graaf, C., Reedtz-Runge, S., Dharmarajan, V., Zhang, H., Han, G.W., Grant, T.D., Sierra, R.G., Weierstall, U., Nelson, G., Liu, W., Wu, Y., Ma, L., Cai, X., Lin, G., Wu, X., Geng, Z., Dong, Y., Song, G., Griffin, P.R., Lau, J., Cherezov, V., Yang, H., Hanson, M.A., Stevens, R.C., Zhao, Q., Jiang, H., Wang, M.-W., Wu, B., (2017). Structure of the full-length glucagon class B G-protein-coupled receptor. *Nature* **546**, 259–264. https://doi.org/10.1038/nature22363.
164. Jazayeri, A., Doré, A.S., Lamb, D., Krishnamurthy, H., Southall, S.M., Baig, A.H., Bortolato, A., Koglin, M., Robertson, N.J., Errey, J.C., Andrews, S.P., Teobald, I., Brown, A.J.H., Cooke, R.M., Weir, M., Marshall, F.H., (2016). Extra-helical binding site of a glucagon receptor antagonist. *Nature* **533**, 274–277. https://doi.org/10.1038/nature17414.
165. Sun, B., Willard, F.S., Feng, D., Alsina-Fernandez, J., Chen, Q., Vieth, M., Ho, J.D., Showalter, A.D., Stutsman, C., Ding, L., Suter, T.M., Dunbar, J.D., Carpenter, J.W., Mohammed, F.A., Aihara, E., Brown, R.A., Bueno, A.B., Emmerson, P.J., Moyers, J.S., Kobilka, T.S., Coghlan, M. P., Kobilka, B.K., Sloop, K.W., (2022). Structural determinants of dual incretin receptor agonism by tirzepatide. *Proc. Natl. Acad. Sci.* **119**, https://doi.org/10.1073/pnas.2116506119 e2116506119.
166. Koldsø, H., Sansom, M.S.P., (2015). Organization and dynamics of receptor proteins in a plasma membrane. *J. Am. Chem. Soc.* **137**, 14694–14704. https://doi.org/10.1021/jacs.5b08048.
167. Hedger, G., (2017). Characterisation of lipid-protein interactions through computational modelling PhD Thesis. University of Oxford.
168. Song, W., Duncan, A.L., Sansom, M.S.P., (2021). Modulation of adenosine A2a receptor oligomerization by receptor activation and PIP2 interactions. *Structure* **29**, 1312–1325.e1313. https://doi.org/10.1016/j.str.2021.06.015.
169. Bolivar, J.H., Muñoz-García, J.C., Castro-Dopico, T., Dijkman, P.M., Stansfeld, P.J., Watts, A., (2016). Interaction of lipids with the neurotensin receptor 1. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1858**, 1278–1287. https://doi.org/10.1016/j.bbamem.2016.02.032.
170. Eberle, S.A., Gustavsson, M., (2024). Bilayer lipids modulate ligand binding to atypical chemokine receptor 3. *Structure* **32**, 1174–1183.e1175. https://doi.org/10.1016/j.str.2024.04.018.
171. Pluhackova, K., Wilhelm, F.M., Müller, D.J., (2021). Lipids and phosphorylation conjointly modulate complex formation of β2-adrenergic receptor and β-arrestin2. *Front. Cell Dev. Biol.* **9** https://doi.org/10.3389/fcell.2021.807913.
172. Hille, B., Dickson, E.J., Kruse, M., Vivas, O., Suh, B.-C., (2015). Phosphoinositides regulate ion channels. *Biochimica et Biophysica Acta (BBA) – Mol. Cell Biol. Lipids* **1851**, 844–856. https://doi.org/10.1016/j.bbalip.2014.09.010.
173. Rohacs, T., (2024). Phosphoinositide regulation of TRP channels: a functional overview in the structural era. *Annu. Rev. Physiol.* **86**, 329–355. https://doi.org/10.1146/annurev-physiol-042022-013956.
174. Thon, O., Wang, Z., Schmidpeter, P.A.M., Nimigean, C. M., (2024). PIP2 inhibits pore opening of the cyclic nucleotide-gated channel SthK. *Nature Commun.* **15**, 8230. https://doi.org/10.1038/s41467-024-52469-1.
175. Ramsey, I.S., Delling, M., Clapham, D.E., (2006). An introduction to trp channels. *Annu. Rev. Physiol.* **68**, 619–647. https://doi.org/10.1146/annurev.physiol.68.040204.100431.
176. Nilius, B., Voets, T., Peters, J., (2005). TRP channels in disease. *Science's STKE* **2005**, https://doi.org/10.1126/stke.2952005re8 re88.
177. Kaneko, Y., Szallasi, A., (2014). Transient receptor potential (TRP) channels: a clinical perspective. *Br. J. Pharmacol.* **171**, 2474–2507. https://doi.org/10.1111/bph.12414.
178. Bergmann, C., Guay-Woodford, L.M., Harris, P.C., Horie, S., Peters, D.J.M., Torres, V.E., (2018). Polycystic kidney disease. *Nature Rev. Dis. Primers* **4**, 50. https://doi.org/10.1038/s41572-018-0047-y.
179. Taberner, F.J., Fernández-Ballester, G., Fernández-Carvajal, A., Ferrer-Montiel, A., (2015). TRP channels interaction with lipids and its implications in disease. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1848**, 1818–1827. https://doi.org/10.1016/j.bbamem.2015.03.022.
180. Hughes, T.E.T., Pumroy, R.A., Yazici, A.T., Kasimova, M. A., Fluck, E.C., Huynh, K.W., Samanta, A., Molugu, S.K.,

Zhou, Z.H., Carnevale, V., Rohacs, T., Moiseenkova-Bell, V.Y., (2018). Structural insights on TRPV5 gating by endogenous modulators. *Nature Commun.* **9**, 4198. https://doi.org/10.1038/s41467-018-06753-6.

181. Wang, Q., Corey, R.A., Hedger, G., Aryal, P., Grieben, M., Nasrallah, C., Baronina, A., Pike, A.C.W., Shi, J., Carpenter, E.P., Sansom, M.S.P., (2020). Lipid interactions of a ciliary membrane TRP channel: simulation and structural studies of Polycystin-2. *Structure* **28**, 169–184.e165. https://doi.org/10.1016/j.str.2019.11.005.
182. Ha, K., Mundt-Machado, N., Bisignano, P., Pinedo, A., Raleigh, D.R., Loeb, G., Reiter, J.F., Cao, E., Delling, M., (2024). Cilia-enriched oxysterol 7β,27-DHC is required for polycystin ion channel activation. *Nature Commun.* **15**, 6468. https://doi.org/10.1038/s41467-024-50318-9.
183. Ciardo, M.G., Ferrer-Montiel, A., (2017). Lipids as central modulators of sensory TRP channels. *Biochim. Biophys. Acta Biomembr.* **1859**, 1615–1628. https://doi.org/10.1016/j.bbamem.2017.04.012.
184. Clarke, A., Skerjanz, J., Gsell, M.A.F., Wiedner, P., Erkan-Candag, H., Groschner, K., Stockner, T., Tiapko, O., (2024). PIP2 modulates TRPC3 activity via TRP helix and S4-S5 linker. *Nature Commun.* **15**, 5220. https://doi.org/10.1038/s41467-024-49396-6.
185. Wang, L., Cai, R., Chen, X.-Z., Peng, J.-B., (2023). Molecular insights into the structural and dynamical changes of calcium channel TRPV6 induced by its interaction with phosphatidylinositol 4,5-bisphosphate. *J. Biomol. Struct. Dyn.* **41**, 6559–6568. https://doi.org/10.1080/07391102.2022.2109752.
186. Fathizadeh, A., Senning, E., Elber, R., (2021). Impact of the protonation state of phosphatidylinositol 4,5-bisphosphate (PIP2) on the binding kinetics and thermodynamics to transient receptor potential vanilloid (TRPV5): a milestoning study. *J. Phys. Chem. B* **125**, 9547–9556. https://doi.org/10.1021/acs.jpcb.1c04052.
187. Shirey, C.M., Scott, J.L., Stahelin, R.V., (2017). Notes and tips for improving quality of lipid-protein overlay assays. *Anal. Biochem.* **516**, 9–12. https://doi.org/10.1016/j.ab.2016.10.009.
188. Seebohm, G., Wrobel, E., Pusch, M., Dicks, M., Terhag, J., Matschke, V., Rothenberg, I., Ursu, O.N., Hertel, F., Pott, L., Lang, F., Schulze-Bahr, E., Hollmann, M., Stoll, R., Strutz-Seebohm, N., (2014). Structural basis of PI(4,5) P2-dependent regulation of GluA1 by phosphatidylinositol-5-phosphate 4-kinase, type II, alpha (PIP5K2A). *Pflügers Archiv – Eur. J. Physiol.* **466**, 1885–1897. https://doi.org/10.1007/s00424-013-1424-8.
189. Hedger, G., Sansom, M.S.P., Koldsø, H., (2015). The juxtamembrane regions of human receptor tyrosine kinases exhibit conserved interaction sites with anionic lipids. *Sci. Rep.* **5**, 9198. https://doi.org/10.1038/srep09198.
190. Araya-Secchi, R., Bugge, K., Seiffert, P., Petry, A., Haxholm, G.W., Lindorff-Larsen, K., Pedersen, S.F., Arleth, L., Kragelund, B.B., (2023). The prolactin receptor scaffolds janus kinase 2 via co-structure formation with phosphoinositide-4,5-bisphosphate. *eLife* **12**, https://doi.org/10.7554/eLife.84645 e84645.
191. Goretzki, B., Wiedemann, C., McCray, B.A., Schäfer, S. L., Jansen, J., Tebbe, F., Mitrovic, S.-A., Nöth, J., Cabezudo, A.C., Donohue, J.K., Jeffries, C.M., Steinchen, W., Stengel, F., Sumner, C.J., Hummer, G., Hellmich, (2023). Crosstalk between regulatory elements in disordered TRPV4 N-terminus modulates lipid-dependent channel activity. *Nature Commun.* **14**, 4165. https://doi.org/10.1038/s41467-023-39808-4.
192. Thomasen, F.E., Skaalum, T., Kumar, A., Srinivasan, S., Vanni, S., Lindorff-Larsen, K., (2024). Rescaling protein-protein interactions improves Martini 3 for flexible proteins in solution. *Nature Commun.* **15**, 6645. https://doi.org/10.1038/s41467-024-50647-9.
193. Tesei, G., Trolle, A.I., Jonsson, N., Betz, J., Knudsen, F. E., Pesce, F., Johansson, K.E., Lindorff-Larsen, K., (2024). Conformational ensembles of the human intrinsically disordered proteome. *Nature* **626**, 897–904. https://doi.org/10.1038/s41586-023-07004-5.
194. Shieh, C.-C., Coghlan, M., Sullivan, J.P., Gopalakrishnan, M., (2000). Potassium channels: molecular defects, diseases, and therapeutic opportunities. *Pharmacol. Rev.* **52**, 557.
195. Long, S.B., Tao, X., Campbell, E.B., MacKinnon, R., (2007). Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. *Nature* **450**, 376–382. https://doi.org/10.1038/nature06265.
196. Valiyaveetil, F.I., Zhou, Y., MacKinnon, R., (2002). Lipids in the structure, folding, and function of the KcsA K+ channel. *Biochemistry* **41**, 10771–10777. https://doi.org/10.1021/bi026215y.
197. Xu, Y., Ramu, Y., Lu, Z., (2008). Removal of phospho-head groups of membrane lipids immobilizes voltage sensors of K+ channels. *Nature* **451**, 826–829. https://doi.org/10.1038/nature06618.
198. Hansen, S.B., (2015). Lipid agonism: The PIP2 paradigm of ligand-gated ion channels. *Biochimica et Biophysica Acta (BBA) – Mol. Cell Biol. Lipids* **1851**, 620–628. https://doi.org/10.1016/j.bbalip.2015.01.011.
199. Duncan, A.L., Corey, R.A., Sansom, M.S.P., (2020). Defining how multiple lipid species interact with inward rectifier potassium (Kir2) channels. *Proc. Natl. Acad. Sci.* **117**, 7803–7813. https://doi.org/10.1073/pnas.1918387117.
200. Cheng, W.W.L., D'Avanzo, N., Doyle, D.A., Nichols, C.G., (2011). Dual-mode phospholipid regulation of human inward rectifying potassium channels. *Biophys. J.* **100**, 620–628. https://doi.org/10.1016/j.bpj.2010.12.3724.
201. Lee, S.-J., Wang, S., Borschel, W., Heyman, S., Gyore, J., Nichols, C.G., (2013). Secondary anionic phospholipid binding site and gating mechanism in Kir2.1 inward rectifier channels. *Nature Commun.* **4**, 2786. https://doi.org/10.1038/ncomms3786.
202. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E., (2023). PubChem 2023 update. *Nucleic Acids Res.* **51**, D1373–D1380. https://doi.org/10.1093/nar/gkac956.
203. Lopes, C.M.B., Zhang, H., Rohacs, T., Jin, T., Yang, J., Logothetis, D.E., (2002). Alterations in conserved kir channel-PIP2 interactions underlie channelopathies. *Neuron* **34**, 933–944. https://doi.org/10.1016/S0896-6273(02)00725-0.
204. Zhang, H., He, C., Yan, X., Mirshahi, T., Logothetis, D.E., (1999). Activation of inwardly rectifying K+ channels by distinct PtdIns(4,5)P2 interactions. *Nature Cell Biol.* **1**, 183–188. https://doi.org/10.1038/11103.
205. Huang, C.-L., Feng, S., Hilgemann, D.W., (1998). Direct activation of inward rectifier potassium channels by PIP2

and its stabilization by Gβγ. *Nature* **391**, 803–806. https://doi.org/10.1038/35882.
206. Schmidt, M.R., Stansfeld, P.J., Tucker, S.J., Sansom, M. S.P., (2013). Simulation-based prediction of phosphatidylinositol 4,5-bisphosphate binding to an ion channel. *Biochemistry* **52**, 279–281. https://doi.org/10.1021/bi301350s.
207. Pipatpolkai, T., Corey, R.A., Proks, P., Ashcroft, F.M., Stansfeld, P.J., (2020). Evaluating inositol phospholipid interactions with inward rectifier potassium channels and characterising their role in disease. *Commun. Chem.* **3**, 147. https://doi.org/10.1038/s42004-020-00391-0.
208. Pipatpolkai, T., Usher, S.G., Vedovato, N., Ashcroft, F.M., Stansfeld, P.J., (2022). The dynamic interplay of PIP2 and ATP in the regulation of the KATP channel. *J. Physiol.* **600**, 4503–4519. https://doi.org/10.1113/JP283345.
209. Bernsteiner, H., Zangerl-Plessl, E.-M., Chen, X., Stary-Weinzinger, A., (2019). Conduction through a narrow inward-rectifier K+ channel pore. *J. Gen. Physiol.* **151**, 1231–1246. https://doi.org/10.1085/jgp.201912359.
210. Chen, X., Bründl, M., Friesacher, T., Stary-Weinzinger, A., (2020). Computational insights into voltage dependence of polyamine block in a strong inwardly rectifying K+ channel. *Front. Pharmacol.* **11** https://doi.org/10.3389/fphar.2020.00721.
211. Bründl, M., Pellikan, S., Stary-Weinzinger, A., (2021). Simulating PIP2-induced gating transitions in Kir6.2 channels. *Front. Mol. Biosci.* **8** https://doi.org/10.3389/fmolb.2021.711975.
212. Lee, S.-J., Ren, F., Zangerl-Plessl, E.-M., Heyman, S., Stary-Weinzinger, A., Yuan, P., Nichols, C.G., (2016). Structural basis of control of inward rectifier Kir2 channel gating by bulk anionic phospholipids. *J. Gen. Physiol.* **148**, 227–237. https://doi.org/10.1085/jgp.201611616.
213. Lacin, E., Aryal, P., Glaaser, I.W., Bodhinathan, K., Tsai, E., Marsh, N., Tucker, S.J., Sansom, M.S.P., Slesinger, P.A., (2017). Dynamic role of the tether helix in PIP2-dependent gating of a G protein–gated potassium channel. *J. Gen. Physiol.* **149**, 799–811. https://doi.org/10.1085/jgp.201711801.
214. Jogini, V., Jensen, M.Ø., Shaw, D.E., (2022). Gating and modulation of an inward-rectifier potassium channel. *J. Gen. Physiol.* **155** https://doi.org/10.1085/jgp.202213085.
215. Rodriguez-Menchaca, A.A., Adney, S.K., Tang, Q.-Y., Meng, X.-Y., Rosenhouse-Dantsker, A., Cui, M., Logothetis, D.E., (2012). PIP2 controls voltage-sensor movement and pore opening of Kv channels through the S4-S5 linker. *Proc. Natl. Acad. Sci.* **109**, E2399–E2408. https://doi.org/10.1073/pnas.1207901109.
216. Kasimova, M.A., Zaydman, M.A., Cui, J., Tarek, M., (2015). PIP2-dependent coupling is prominent in Kv7.1 due to weakened interactions between S4-S5 and S6. *Sci. Rep.* **5**, 7474. https://doi.org/10.1038/srep07474.
217. Kasimova, M.A., Tarek, M., Shaytan, A.K., Shaitan, K.V., Delemotte, L., (2014). Voltage-gated ion channel modulation by lipids: Insights from molecular dynamics simulations. *Biochimica et Biophysica Acta (BBA) – B Biomembranes* **1838**, 1322–1331. https://doi.org/10.1016/j.bbamem.2014.01.024.
218. Chen, L., Zhang, Q., Qiu, Y., Li, Z., Chen, Z., Jiang, H., Li, Y., Yang, H., (2015). Migration of PIP2 lipids on voltage-gated potassium channel surface influences channel deactivation. *Sci. Rep.* **5**, 15079. https://doi.org/10.1038/srep15079.
219. Zhang, Q., Zhou, P., Chen, Z., Li, M., Jiang, H., Gao, Z., Yang, H., (2013). Dynamic PIP2 interactions with voltage sensor elements contribute to KCNQ2 channel gating. *Proc. Natl. Acad. Sci.* **110**, 20093–20098. https://doi.org/10.1073/pnas.1312483110.
220. Abderemane-Ali, F., Es-Salah-Lamoureux, Z., Delemotte, L., Kasimova, M.A., Labro, A.J., Snyders, D.J., Fedida, D., Tarek, M., Baró, I., Loussouarn, G., (2012). Dual effect of phosphatidyl (4,5)-bisphosphate PIP2 on Shaker K+ channels. *J. Biol. Chem.* **287**, 36158–36167. https://doi.org/10.1074/jbc.M112.382085.
221. Pant, S., Zhang, J., Kim, E.C., Lam, K., Chung, H.J., Tajkhorshid, E., (2021). PIP2-dependent coupling of voltage sensor and pore domains in Kv7.2 channel. *Commun. Biol.* **4**, 1189. https://doi.org/10.1038/s42003-021-02729-3.
222. Kirsch, S.A., Kugemann, A., Carpaneto, A., Böckmann, R. A., Dietrich, P., (2018). Phosphatidylinositol-3,5-bisphosphate lipid-binding-induced activation of the human two-pore channel 2. *Cell. Mol. Life Sci.* **75**, 3803–3815. https://doi.org/10.1007/s00018-018-2829-5.
223. Panasawatwong, A., Pipatpolkai, T., Tucker, S.J., (2022). Transition between conformational states of the TREK-1 K2P channel promoted by interaction with PIP2. *Biophys. J.* **121**, 2380–2388. https://doi.org/10.1016/j.bpj.2022.05.019.
224. Yu, K., Jiang, T., Cui, Y., Tajkhorshid, E., Hartzell, H.C., (2019). A network of phosphatidylinositol 4,5-bisphosphate binding sites regulates gating of the Ca2+ activated Cl- channel ANO1 (TMEM16A). *Proc. Natl. Acad. Sci.* **116**, 19952–19962. https://doi.org/10.1073/pnas.1904012116.
225. Jia, Z., Chen, J., (2021). Specific PIP2 binding promotes calcium activation of TMEM16A chloride channels. *Commun. Biol.* **4**, 259. https://doi.org/10.1038/s42003-021-01782-2.
226. Le, S.C., Jia, Z., Chen, J., Yang, H., (2019). Molecular basis of PIP2-dependent regulation of the Ca2+-activated chloride channel TMEM16A. *Nature Commun.* **10**, 3769. https://doi.org/10.1038/s41467-019-11784-8.
227. Buyan, A., Cox, C.D., Barnoud, J., Li, J., Chan, H.S.M., Martinac, B., Marrink, S.J., Corry, B., (2020). Piezo1 forms specific, functionally important interactions with phosphoinositides and cholesterol. *Biophys. J.* **119**, 1683–1697. https://doi.org/10.1016/j.bpj.2020.07.043.
228. Lin, Y., Tao, E., Champion, J.P., Corry, B., (2024). A binding site for phosphoinositides described by multiscale simulations explains their modulation of voltage-gated sodium channels. *eLife* **12**, https://doi.org/10.7554/eLife.91218 RP91218.
229. Ivanova, A., Atakpa-Adaji, P., Rao, S., Marti-Solano, M., Taylor, C.W., (2024). Dual regulation of IP3 receptors by IP3 and PIP2 controls the transition from local to global Ca^{2+} signals. *Mol. Cell* **84**, 3997–4015.e3997. https://doi.org/10.1016/j.molcel.2024.09.009.
230. Fruman, D.A., Meyers, R.E., Cantley, L.C., (1998). Phosphoinositide kinases. *Annu. Rev. Biochem* **67**, 481–507. https://doi.org/10.1146/annurev.biochem.67.1.481.
231. Hsu, F., Mao, Y., (2015). The structure of phosphoinositide phosphatases: insights into substrate specificity and catalysis. *Biochimica et Biophysica Acta (BBA) – Mol. Cell Biol. Lipids* **1851**, 698–710. https://doi.org/10.1016/j.bbalip.2014.09.015.

232. Burke, J.E., (2018). Structural basis for regulation of phosphoinositide kinases and their involvement in human disease. *Mol. Cell* **71**, 653–673. https://doi.org/10.1016/j.molcel.2018.08.005.
233. Bunney, T.D., Katan, M., (2010). Phosphoinositide signalling in cancer: beyond PI3K and PTEN. *Nature Rev. Cancer* **10**, 342–352. https://doi.org/10.1038/nrc2842.
234. Whited, A.M., Johs, A., (2015). The interactions of peripheral membrane proteins with biological membranes. *Chem. Phys. Lipids* **192**, 51–59. https://doi.org/10.1016/j.chemphyslip.2015.07.015.
235. Moravcevic, K., Oxley, C.L., Lemmon, M.A., (2012). Conditional peripheral membrane proteins: facing up to limited specificity. *Structure* **20**, 15–27. https://doi.org/10.1016/j.str.2011.11.012.
236. Yamamoto, E., Kalli, A.C., Yasuoka, K., Sansom, M.S.P., (2016). Interactions of pleckstrin homology domains with membranes: adding back the bilayer via high-throughput molecular dynamics. *Structure* **24**, 1421–1431. https://doi.org/10.1016/j.str.2016.06.002.
237. Wang, Q., Pechersky, Y., Sagawa, S., Pan, A.C., Shaw, D.E., (2019). Structural mechanism for Bruton's tyrosine kinase activation at the cell membrane. *Proc. Natl. Acad. Sci.* **116**, 9390–9399. https://doi.org/10.1073/pnas.1819301116.
238. Pan, A.C., Jacobson, D., Yatsenko, K., Sritharan, D., Weinreich, T.M., Shaw, D.E., (2019). Atomic-level characterization of protein–protein association. *Proc. Natl. Acad. Sci.* **116**, 4244–4249. https://doi.org/10.1073/pnas.1815431116.
239. Lai, C.-L., Landgraf, K.E., Voth, G.A., Falke, J.J., (2010). Membrane docking geometry and target lipid stoichiometry of membrane-bound PKCα C2 domain: a combined molecular dynamics and experimental study. *J. Mol. Biol.* **402**, 301–310. https://doi.org/10.1016/j.jmb.2010.07.037.
240. Lai, C.-L., Srivastava, A., Pilling, C., Chase, A.R., Falke, J.J., Voth, G.A., (2013). Molecular mechanism of membrane binding of the GRP1 PH domain. *J. Mol. Biol.* **425**, 3073–3090. https://doi.org/10.1016/j.jmb.2013.05.026.
241. Zhang, Y., Soubias, O., Pant, S., Heinrich, F., Vogel, A., Li, J., Li, Y., Clifton, L.A., Daum, S., Bacia, K., Huster, D., Randazzo, P.A., Lösche, M., Tajkhorshid, E., Byrd, R.A., (2023). Myr-Arf1 conformational flexibility at the membrane surface sheds light on the interactions with ArfGAP ASAP1. *Nature Commun.* **14**, 7570. https://doi.org/10.1038/s41467-023-43008-5.
242. Soubias, O., Pant, S., Heinrich, F., Zhang, Y., Roy, N.S., Li, J., Jian, X., Yohe, M.E., Randazzo, P.A., Lösche, M., Tajkhorshid, E., Byrd, R.A., (2020). Membrane surface recognition by the ASAP1 PH domain and consequences for interactions with the small GTPase Arf1. *Sci. Adv.* **6**, https://doi.org/10.1126/sciadv.abd1882 eabd1882.
243. Pant, S., Tajkhorshid, E., (2020). Microscopic characterization of GRP1 PH domain interaction with anionic membranes. *J. Comput. Chem.* **41**, 489–499. https://doi.org/10.1002/jcc.26109.
244. Gregory, M.C., McLean, M.A., Sligar, S.G., (2017). Interaction of KRas4b with anionic membranes: a special role for PIP2. *Biochem. Biophys. Res. Commun.* **487**, 351–355. https://doi.org/10.1016/j.bbrc.2017.04.063.
245. Ohkubo, Y.Z., Pogorelov, T.V., Arcario, M.J., Christensen, G.A., Tajkhorshid, E., (2012). Accelerating membrane insertion of peripheral proteins with a novel membrane mimetic model. *Biophys. J.* **102**, 2130–2139. https://doi.org/10.1016/j.bpj.2012.03.015.
246. Naughton, F.B., Kalli, A.C., Sansom, M.S.P., (2018). Modes of interaction of pleckstrin homology domains with membranes: toward a computational biochemistry of membrane recognition. *J. Mol. Biol.* **430**, 372–388. https://doi.org/10.1016/j.jmb.2017.12.011.
247. Vonkova, I., Saliba, A.-E., Deghou, S., Anand, K., Ceschia, S., Doerks, T., Galih, A., Kugler, K.G., Maeda, K., Rybin, V., van Noort, V., Ellenberg, J., Bork, P., Gavin, A.-C., (2015). Lipid cooperativity as a general membrane-recruitment principle for PH domains. *Cell Rep.* **12**, 1519–1530. https://doi.org/10.1016/j.celrep.2015.07.054.
248. Yamamoto, E., Domański, J., Naughton, F.B., Best, R.B., Kalli, A.C., Stansfeld, P.J., Sansom, M.S.P., (2020). Multiple lipid binding sites determine the affinity of PH domains for phosphoinositide-containing membranes. *Sci. Adv.* **6**, https://doi.org/10.1126/sciadv.aay5736 eaay5736.
249. Le Huray, K.I.P., Wang, H., Sobott, F., Kalli, A.C., (2022). Systematic simulation of the interactions of pleckstrin homology domains with membranes. *Sci. Adv.* **8**, https://doi.org/10.1126/sciadv.abn6992 eabn6992.
250. Braun, L., Schoen, I., Vogel, V., (2021). PIP2-induced membrane binding of the vinculin tail competes with its other binding partners. *Biophys. J.* **120**, 4608–4622. https://doi.org/10.1016/j.bpj.2021.08.018.
251. Buyan, A., Kalli, A.C., Sansom, M.S.P., (2016). Multiscale simulations suggest a mechanism for the association of the Dok7 PH domain with PIP-containing membranes. *PLoS Comput. Biol.* **12**, https://doi.org/10.1371/journal.pcbi.1005028 e1005028.
252. Cao, S., Chung, S., Kim, S., Li, Z., Manor, D., Buck, M., (2019). K-Ras G-domain binding with signaling lipid phosphatidylinositol (4,5)-phosphate (PIP2): membrane association, protein orientation, and function. *J. Biol. Chem.* **294**, 7068–7084. https://doi.org/10.1074/jbc.RA118.004021.
253. Shrestha, R., Carpenter, T.S., Van, Q.N., Agamasu, C., Tonelli, M., Aydin, F., Chen, D., Gulten, G., Glosli, J.N., López, C.A., Oppelstrup, T., Neale, C., Gnanakaran, S., Gillette, W.K., Ingólfsson, H.I., Lightstone, F.C., Stephen, A.G., Streitz, F.H., Nissley, D.V., Turbyville, T.J., (2024). Membrane lipids drive formation of KRAS4b-RAF1 RBDCRD nanoclusters on the membrane. *Commun. Biol.* **7**, 242. https://doi.org/10.1038/s42003-024-05916-0.
254. Han, K., Pastor, R.W., Fenollar-Ferrer, C., (2020). PLD2–PI(4,5)P2 interactions in fluid phase membranes: structural modeling and molecular dynamics simulations. *PLoS One* **15**, https://doi.org/10.1371/journal.pone.0236201 e0236201.
255. Herzog, F.A., Braun, L., Schoen, I., Vogel, V., (2017). Structural insights how PIP2 imposes preferred binding orientations of FAK at lipid membranes. *J. Phys. Chem. B* **121**, 3523–3535. https://doi.org/10.1021/acs.jpcb.6b09349.
256. Zhou, J., Bronowska, A., Le Coq, J., Lietha, D., Gräter, F., (2015). Allosteric regulation of focal adhesion kinase by PIP2 and ATP. *Biophys. J.* **108**, 698–705. https://doi.org/10.1016/j.bpj.2014.11.3454.

257. John, L.H., Naughton, F.B., Sansom, M.S.P., Larsen, A. H., (2023). The role of C2 domains in two different phosphatases: PTEN and SHIP2. *Membranes* **13**, 408. https://doi.org/10.3390/membranes13040408.
258. Kalli, A.C., Devaney, I., Sansom, M.S.P., (2014). Interactions of phosphatase and tensin homologue (PTEN) proteins with phosphatidylinositol phosphates: insights from molecular dynamics simulations of PTEN and voltage sensitive phosphatase. *Biochemistry* **53**, 1724–1732. https://doi.org/10.1021/bi5000299.
259. Karandur, D., Nawrotek, A., Kuriyan, J., Cherfils, J., (2017). Multiple interactions between an Arf/GEF complex and charged lipids determine activation kinetics on the membrane. *Proc. Natl. Acad. Sci.* **114**, 11416–11421. https://doi.org/10.1073/pnas.1707970114.
260. Le Huray, K.I.P., Bunney, T.D., Pinotsis, N., Kalli, A.C., Katan, M., (2022). Characterization of the membrane interactions of phospholipase Cγ reveals key features of the active enzyme. *Sci. Adv.* **8**, https://doi.org/10.1126/sciadv.abp9688 eabp9688.
261. Liepiņa, I., Czaplewski, C., Janmey, P., Liwo, A., (2003). Molecular dynamics study of a gelsolin-derived peptide binding to a lipid bilayer containing phosphatidylinositol 4,5-bisphosphate. *Pept. Sci.* **71**, 49–70. https://doi.org/10.1002/bip.10375.
262. Moser von Filseck, J., Čopič, A., Delfosse, V., Vanni, S., Jackson, C.L., Bourguet, W., Drin, G., (2015). Phosphatidylserine transport by ORP/Osh proteins is driven by phosphatidylinositol 4-phosphate. *Science* **349**, 432–436. https://doi.org/10.1126/science.aab1346.
263. von Filseck, J.M., Vanni, S., Mesmin, B., Antonny, B., Drin, G., (2015). A phosphatidylinositol-4-phosphate powered exchange mechanism to create a lipid gradient between membranes. *Nature Commun.* **6**, 6671. https://doi.org/10.1038/ncomms7671.
264. Allsopp, R.J., Klauda, J.B., (2021). Impact of PIP2 lipids, force field parameters, and mutational analysis on the binding of the Osh4's α6–α7 domain. *J. Phys. Chem. B* **125**, 5296–5308. https://doi.org/10.1021/acs.jpcb.0c10393.
265. Naughton, F.B., Kalli, A.C., Sansom, M.S.P., (2016). Association of peripheral membrane proteins with membranes: free energy of binding of GRP1 PH domain with phosphatidylinositol phosphate-containing model bilayers. *J. Phys. Chem. Lett.* **7**, 1219–1224. https://doi.org/10.1021/acs.jpclett.6b00153.
266. Ni, T., Kalli, A.C., Naughton, F.B., Yates, L.A., Naneh, O., Kozorog, M., Anderluh, G., Sansom, M.S.P., Gilbert, R.J. C., (2017). Structure and lipid-binding properties of the kindlin-3 pleckstrin homology domain. *Biochem. J.* **474**, 539–556. https://doi.org/10.1042/bcj20160791.
267. Picas, L., Viaud, J., Schauer, K., Vanni, S., Hnia, K., Fraisier, V., Roux, A., Bassereau, P., Gaits-Iacovoni, F., Payrastre, B., Laporte, J., Manneville, J.-B., Goud, B., (2014). BIN1/M-Amphiphysin2 induces clustering of phosphoinositides to recruit its downstream partner dynamin. *Nature Commun.* **5**, 5647. https://doi.org/10.1038/ncomms6647.
268. Prakaash, D., Fagnen, C., Cook, G.P., Acuto, O., Kalli, A. C., (2022). Molecular dynamics simulations reveal membrane lipid interactions of the full-length lymphocyte specific kinase (Lck). *Sci. Rep.* **12**, 21121. https://doi.org/10.1038/s41598-022-25603-6.
269. Scott, J.L., Frick, C.T., Johnson, K.A., Liu, H., Yong, S.S., Varney, A.G., Wiest, O., Stahelin, R.V., (2020). Molecular analysis of membrane targeting by the C2 domain of the E3 ubiquitin ligase Smurf1. *Biomolecules* **10**, 229. https://doi.org/10.3390/biom10020229.
270. Xie, H., Weinstein, H., (2024). The selective cargo loading and release functions of the cellular sterol transporter StarD4 are allosterically controlled by the recognition of specific PIP2 subtypes in the targeted membranes. *BioRxiv*. https://doi.org/10.1101/2024.03.26.586881.
271. Talandashti, R., van Ek, L., Gehin, C., Xue, D., Moqadam, M., Gavin, A.-C., Reuter, N., (2024). Membrane specificity of the human cholesterol transfer protein STARD4. *J. Mol. Biol.* **436**, https://doi.org/10.1016/j.jmb.2024.168572 168572.
272. Xu, T., Gan, Q., Wu, B., Yin, M., Xu, J., Shu, X., Liu, J., (2020). Molecular basis for PI(3,5)P2 recognition by SNX11, a protein involved in lysosomal degradation and endosome homeostasis regulation. *J. Mol. Biol.* **432**, 4750–4761. https://doi.org/10.1016/j.jmb.2020.06.010.
273. Rao, S., Skulsuppaisarn, M., Strong, L.M., Ren, X., Lazarou, M., Hurley, J.H., Hummer, G., (2024). Three-step docking by WIPI2, ATG16L1, and ATG3 delivers LC3 to the phagophore. *Sci. Adv.* **10**, https://doi.org/10.1126/sciadv.adj8027 eadj8027.
274. Thallmair, V., Schultz, L., Zhao, W., Marrink, S.J., Oliver, D., Thallmair, S., (2022). Two cooperative binding sites sensitize PI(4,5)P2 recognition by the tubby domain. *Sci. Adv.* **8**, https://doi.org/10.1126/sciadv.abp9471 eabp9471.
275. Grabon, A., Orłowski, A., Tripathi, A., Vuorio, J., Javanainen, M., Róg, T., Lönnfors, M., McDermott, M.I., Siebert, G., Somerharju, P., Vattulainen, I., Bankaitis, V. A., (2017). Dynamics and energetics of the mammalian phosphatidylinositol transfer protein phospholipid exchange cycle. *J. Biol. Chem.* **292**, 14438–14455. https://doi.org/10.1074/jbc.M117.791467.
276. Williams, S.I., Yu, X., Ni, T., Gilbert, R.J.C., Stansfeld, P. J., (2022). Structural, functional and computational studies of membrane recognition by plasmodium perforin-like proteins 1 and 2. *J. Mol. Biol.* **434**, https://doi.org/10.1016/j.jmb.2022.167642 167642.
277. Souza, P.C.T., Borges-Araújo, L., Brasnett, C., Moreira, R.A., Grünewald, F., Park, P., Wang, L., Razmazma, H., Borges-Araújo, A.C., Cofas-Vargas, L.F., Monticelli, L., Mera-Adasme, R., Melo, M.N., Wu, S., Marrink, S.J., Poma, A.B., Thallmair, S., (2024). GōMartini 3: From large conformational changes in proteins to environmental bias corrections. *BioRxiv*. https://doi.org/10.1101/2024.04.15.589479.
278. Larsen, A.H., John, L.H., Sansom, M.S.P., Corey, R.A., (2022). Specific interactions of peripheral membrane proteins with lipids: what can molecular simulations show us?. *Biosci. Rep.* **42** https://doi.org/10.1042/bsr20211406.
279. Schlessinger, J., (2000). Cell signaling by receptor tyrosine kinases. *Cell* **103**, 211–225. https://doi.org/10.1016/S0092-8674(00)00114-8.
280. Lemmon, M.A., Schlessinger, J., (2010). Cell signaling by receptor tyrosine kinases. *Cell* **141**, 1117–1134. https://doi.org/10.1016/j.cell.2010.06.011.
281. Karl, K., Hristova, K., (2021). Pondering the mechanism of receptor tyrosine kinase activation: the case for ligand-

specific dimer microstate ensembles. *Curr. Opin. Struct. Biol.* **71**, 193–199. https://doi.org/10.1016/j.sbi.2021.07.003.
282. Paul, M.D., Hristova, K., (2019). The RTK interactome: overview and perspective on RTK heterointeractions. *Chem. Rev.* **119**, 5881–5921. https://doi.org/10.1021/acs.chemrev.8b00467.
283. McLaughlin, S., Smith, S.O., Hayman, M.J., Murray, D., (2005). An electrostatic engine model for autoinhibition and activation of the epidermal growth factor receptor (EGFR/ErbB) family. *J. Gen. Physiol.* **126**, 41–53. https://doi.org/10.1085/jgp.200509274.
284. Wang, Y., Gao, J., Guo, X., Tong, T., Shi, X., Li, L., Qi, M., Wang, Y., Cai, M., Jiang, J., Xu, C., Ji, H., Wang, H., (2014). Regulation of EGFR nanocluster formation by ionic protein-lipid interaction. *Cell Res.* **24**, 959–976. https://doi.org/10.1038/cr.2014.89.
285. Singh, P.K., Rybak, J.A., Schuck, R.J., Sahoo, A.R., Buck, M., Barrera, F.N., Smith, A.W., (2024). Phosphatidylinositol 4,5-bisphosphate drives the formation of EGFR and EphA2 complexes. *Sci. Adv.* **10**, https://doi.org/10.1126/sciadv.adl0649 eadl0649.
286. Gonzalez-Magaldi, M., Gullapalli, A., Papoulas, O., Liu, C., Leung, A.Y.H., Guo, L., Brilot, A., Marcotte, E.M., Ke, Z., Leahy, D.J., (2024). Structure and organization of full-length epidermal growth factor receptor in extracellular vesicles by cryo-electron tomography. *BioRxiv*. https://doi.org/10.1101/2024.11.25.625301.
287. Kumar, R., Goel, H., Solanki, R., Rawat, L., Tabasum, S., Tanwar, P., Pal, S., Sabarwal, A., (2024). Recent developments in receptor tyrosine kinase inhibitors: a promising mainstay in targeted cancer therapy. *Med. Drug Discovery* **23**, https://doi.org/10.1016/j.medidd.2024.100195 100195.
288. Gschwind, A., Fischer, O.M., Ullrich, A., (2004). The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nature Rev. Cancer* **4**, 361–370. https://doi.org/10.1038/nrc1360.
289. Kovacs, E., Zorn, J.A., Huang, Y., Barros, T., Kuriyan, J., (2015). A structural perspective on the regulation of the epidermal growth factor receptor. *Annu. Rev. Biochem* **84**, 739–764. https://doi.org/10.1146/annurev-biochem-060614-034402.
290. Uchikawa, E., Choi, E., Shang, G., Yu, H., Bai, X.-C., (2019). Activation mechanism of the insulin receptor revealed by Cryo-EM structure of the fully liganded receptor–ligand complex. *eLife* **8**, https://doi.org/10.7554/eLife.48630 e48630.
291. Schlessinger, J., (2014). Receptor tyrosine kinases: legacy of the first two decades. *Cold Spring Harb Perspect Biol.* **6** https://doi.org/10.1101/cshperspect.a008912.
292. Thiel, K.W., Carpenter, G., (2007). Epidermal growth factor receptor juxtamembrane region regulates allosteric tyrosine kinase activation. *Proc. Natl. Acad. Sci.* **104**, 19238–19243. https://doi.org/10.1073/pnas.0703854104.
293. Abd Halim, K.B., Koldsø, H., Sansom, M.S.P., (2015). Interactions of the EGFR juxtamembrane domain with PIP2-containing lipid bilayers: Insights from multiscale molecular dynamics simulations. *Biochimica et Biophysica Acta (BBA) – Gen. Subj.* **1850**, 1017–1025. https://doi.org/10.1016/j.bbagen.2014.09.006.
294. Wang, Z., Fan, H., Hu, X., Khamo, J., Diao, J., Zhang, K., Pogorelov, T.V., (2019). Coaction of electrostatic and hydrophobic interactions: dynamic constraints on disordered TrkA juxtamembrane domain. *J. Phys. Chem. B* **123**, 10709–10717. https://doi.org/10.1021/acs.jpcb.9b09352.
295. Chavent, M., Karia, D., Kalli, A.C., Domański, J., Duncan, A.L., Hedger, G., Stansfeld, P.J., Seiradake, E., Jones, E. Y., Sansom, M.S.P., (2018). Interactions of the EphA2 kinase domain with PIPs in membranes: implications for receptor function. *Structure* **26**, 1025–1034.e1022. https://doi.org/10.1016/j.str.2018.05.003.
296. Singh, P.K., Rybak, J.A., Schuck, R.J., Barrera, F.N., Smith, A.W., (2024). Phosphatidylinositol (4,5)-bisphosphate drives the formation of EGFR and EphA2 complexes. *BioRxiv*. https://doi.org/10.1101/2024.05.03.592400.
297. Abe, M., Yanagawa, M., Hiroshima, M., Kobayashi, T., Sako, Y., (2024). Bilateral regulation of EGFR activity and local PI(4,5)P2 dynamics in mammalian cells observed with superresolution microscopy. *eLife* **13**, https://doi.org/10.7554/eLife.101652 e101652.
298. Hedger, G., Shorthouse, D., Koldsø, H., Sansom, M.S.P., (2016). Free energy landscape of lipid interactions with regulatory binding sites on the transmembrane domain of the EGF receptor. *J. Phys. Chem. B* **120**, 8154–8163. https://doi.org/10.1021/acs.jpcb.6b01387.
299. Chavent, M., Chetwynd, A.P., Stansfeld, P.J., Sansom, M.S.P., (2014). Dimerization of the EphA1 receptor tyrosine kinase transmembrane domain: insights into the mechanism of receptor activation. *Biochemistry* **53**, 6641–6652. https://doi.org/10.1021/bi500800x.
300. Lelimousin, M., Limongelli, V., Sansom, M.S.P., (2016). Conformational changes in the epidermal growth factor receptor: role of the transmembrane domain investigated by coarse-grained metadynamics free energy calculations. *J. Am. Chem. Soc.* **138**, 10611–10622. https://doi.org/10.1021/jacs.6b05602.
301. Bocharov, E.V., Mineev, K.S., Pavlov, K.V., Akimov, S.A., Kuznetsov, A.S., Efremov, R.G., Arseniev, A.S., (2017). Helix-helix interactions in membrane domains of bitopic proteins: Specificity and role of lipid environment. *Biochimica et Biophysica Acta (BBA) – B Biomembranes* **1859**, 561–576. https://doi.org/10.1016/j.bbamem.2016.10.024.
302. Stangl, M., Schneider, D., (2015). Functional competition within a membrane: Lipid recognition vs. transmembrane helix oligomerization. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1848**, 1886–1896. https://doi.org/10.1016/j.bbamem.2015.03.011.
303. Stefanski, K.M., Russell, C.M., Westerfield, J.M., Lamichhane, R., Barrera, F.N., (2021). PIP2 promotes conformation-specific dimerization of the EphA2 membrane region. *J. Biol. Chem.* **296** https://doi.org/10.1074/jbc.RA120.016423.
304. Lolicato, F., Nickel, W., Haucke, V., Ebner, M., (2024). Phosphoinositide switches in cell physiology – from molecular mechanisms to disease. *J. Biol. Chem.* **300** https://doi.org/10.1016/j.jbc.2024.105757.
305. Dimou, E., Nickel, W., (2018). Unconventional mechanisms of eukaryotic protein secretion. *Curr. Biol.* **28**, R406–R410. https://doi.org/10.1016/j.cub.2017.11.074.
306. Pallotta, M.T., Nickel, W., (2020). FGF2 and IL-1β – explorers of unconventional secretory pathways at a glance. *J. Cell Sci.* **133** https://doi.org/10.1242/jcs.250449.

307. Steringer, J.P., Lange, S., Čujová, S., Šachl, R., Poojari, C., Lolicato, F., Beutel, O., Müller, H.-M., Unger, S., Coskun, Ü., Honigmann, A., Vattulainen, I., Hof, M., Freund, C., Nickel, W., (2017). Key steps in unconventional secretion of fibroblast growth factor 2 reconstituted with purified components. *eLife* **6**, https://doi.org/10.7554/eLife.28985 e28985.
308. Khelashvili, G., Stanley, N., Sahai, M.A., Medina, J., LeVine, M.V., Shi, L., De Fabritiis, G., Weinstein, H., (2015). Spontaneous inward opening of the dopamine transporter is triggered by PIP2-regulated dynamics of the N-terminus. *ACS Chem. Nerosci.* **6**, 1825–1837. https://doi.org/10.1021/acschemneuro.5b00179.
309. Tang, H., Li, H., Prakaash, D., Pedebos, C., Qiu, X., Sauer, D.B., Khalid, S., Duerr, K., Robinson, C.V., (2023). The solute carrier SPNS2 recruits PI(4,5)P2 to synergistically regulate transport of sphingosine-1-phosphate. *Mol. Cell* **83**, 2739–2752.e2735. https://doi.org/10.1016/j.molcel.2023.06.033.
310. Zhekova, H.R., Ramirez Echemendía, D.P., Sejdiu, B.I., Pushkin, A., Tieleman, D.P., Kurtz, I., (2024). Molecular dynamics simulations of lipid-protein interactions in SLC4 proteins. *Biophys. J.* **123**, 1705–1721. https://doi.org/10.1016/j.bpj.2024.05.013.
311. Qin, X., Tieleman, D.P., Liang, Q., (2022). Effects of cholesterol and PIP2 on interactions between glycophorin A and Band 3 in lipid bilayers. *Biophys. J.* **121**, 2069–2077. https://doi.org/10.1016/j.bpj.2022.05.001.
312. Chen, T., Vallese, F., Gil-Iturbe, E., Kim, K., Quick, M., Clarke, O., Tajkhorshid, E., (2024). Impact of anionic lipids on the energy landscape of conformational transition in anion exchanger 1 (AE1). *BioRxiv*. https://doi.org/10.1101/2024.10.15.616105.
313. Kokane, S., Meier, P.F., Gulati, A., Matsuoka, R., Pipatpolkai, T., Albano, G., Delemotte, L., Fuster, D., Drew, D., (2024). PI-(3,5)P2-mediated oligomerization of the endosomal sodium/proton exchanger NHE9. *BioRxiv*. https://doi.org/10.1101/2023.09.10.557050.
314. Pyle, E., Kalli, A.C., Amillis, S., Hall, Z., Lau, A.M., Hanyaloglu, A.C., Diallinas, G., Byrne, B., Politis, A., (2018). Structural lipids enable the formation of functional oligomers of the eukaryotic purine symporter UapA. *Cell Chem. Biol.* **25**, 840–848.e844. https://doi.org/10.1016/j.chembiol.2018.03.011.
315. Norris, M.J., Husby, M.L., Kiosses, W.B., Yin, J., Saxena, R., Rennick, L.J., Heiner, A., Harkins, S.S., Pokhrel, R., Schendel, S.L., Hastie, K.M., Landeras-Bueno, S., Salie, Z.L., Lee, B., Chapagain, P.P., Maisner, A., Duprex, W.P., Stahelin, R.V., Saphire, E.O., (2022). Measles and Nipah virus assembly: specific lipid binding drives matrix polymerization. *Sci. Adv.* **8**, https://doi.org/10.1126/sciadv.abn1440 eabn1440.
316. Gc, J.B., Gerstman, B.S., Chapagain, P.P., (2017). Membrane association and localization dynamics of the Ebola virus matrix protein VP40. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1859**, 2012–2020. https://doi.org/10.1016/j.bbamem.2017.07.007.
317. Winski, D., Parent, M.T., Wallace, J.N., Shivanna, K., Aho, B.M., Waters, H., Zimmerberg, J., Sodt, A.J., Hess, S.T., (2024). The cytoplasmic tail domain of influenza hemagglutinin interacts with phosphatidylinositol (4,5)-bisphosphate (PIP2). *Biophys. J.* **123**, 236a–237a. https://doi.org/10.1016/j.bpj.2023.11.1498.
318. Cioffi, M.D., Sharma, T., Motsa, B.B., Bhattarai, N., Gerstman, B.S., Stahelin, R.V., Chapagain, P.P., (2024). Ebola virus matrix protein VP40 single mutations G198R and G201R significantly enhance plasma membrane localization. *J. Phys. Chem. B* **128**, 11335–11344. https://doi.org/10.1021/acs.jpcb.4c02700.
319. Hudait, A., Hurley, J.H., Voth, G.A., (2023). Dynamics of upstream ESCRT organization at the HIV-1 budding site. *Biophys. J.* **122**, 2655–2674. https://doi.org/10.1016/j.bpj.2023.05.020.
320. Banerjee, P., Monje-Galvan, V., Voth, G.A., (2024). Cooperative membrane binding of HIV-1 matrix proteins. *J. Phys. Chem. B* **128**, 2595–2606. https://doi.org/10.1021/acs.jpcb.3c06222.
321. Monje-Galvan, V., Voth, G.A., (2020). Binding mechanism of the matrix domain of HIV-1 gag on lipid membranes. *eLife* **9**, https://doi.org/10.7554/eLife.58621 e58621.
322. Senju, Y., Kalimeri, M., Koskela, E.V., Somerharju, P., Zhao, H., Vattulainen, I., Lappalainen, P., (2017). Mechanistic principles underlying regulation of the actin cytoskeleton by phosphoinositides. *Proc. Natl. Acad. Sci.* **114**, E8977–E8986. https://doi.org/10.1073/pnas.1705032114.
323. Prakash, S., Krishna, A., Sengupta, D., (2023). Cofilin-membrane interactions: electrostatic effects in phosphoinositide lipid binding. *ChemPhysChem* **24**, https://doi.org/10.1002/cphc.202200509 e202200509.
324. Fatunmbi, O., Bradley, R.P., Kandy, S.K., Bucki, R., Janmey, P.A., Radhakrishnan, R., (2020). A multiscale biophysical model for the recruitment of actin nucleating proteins at the membrane interface. *Soft Matter* **16**, 4941–4954. https://doi.org/10.1039/D0SM00267D.
325. Honigmann, A., van den Bogaart, G., Iraheta, E., Risselada, H.J., Milovanovic, D., Mueller, V., Müllar, S., Diederichsen, U., Fasshauer, D., Grubmüller, H., Hell, S. W., Eggeling, C., Kühnel, K., Jahn, R., (2013). Phosphatidylinositol 4,5-bisphosphate clusters act as molecular beacons for vesicle recruitment. *Nature Struct. Mol. Biol.* **20**, 679–686. https://doi.org/10.1038/nsmb.2570.
326. Schaefer, S.L., Hummer, G., (2022). Sublytic Gasdermin-D pores captured in atomistic molecular simulations. *eLife* **11**, https://doi.org/10.7554/eLife.81432 e81432.
327. Koldsø, H., Shorthouse, D., Hélie, J., Sansom, M.S.P., (2014). Lipid clustering correlates with membrane curvature as revealed by molecular simulations of complex lipid bilayers. *PLoS Comput. Biol.* **10**, https://doi.org/10.1371/journal.pcbi.1003911 e1003911.
328. Lee, K.I., Im, W., Pastor, R.W., (2014). Langevin dynamics simulations of charged model phosphatidylinositol lipids in the presence of diffusion barriers: toward an atomic level understanding of corralling of PIP2 by protein fences in biological membranes. *BMC Biophys.* **7**, 13. https://doi.org/10.1186/s13628-014-0013-3.
329. Han, K., Gericke, A., Pastor, R.W., (2020). Characterization of specific ion effects on PI(4,5)P2 clustering: molecular dynamics simulations and graph-theoretic analysis. *J. Phys. Chem. B* **124**, 1183–1196. https://doi.org/10.1021/acs.jpcb.9b10951.
330. Wang, Y.-H., Slochower, D.R., Janmey, P.A., (2014). Counterion-mediated cluster formation by polyphosphoinositides. *Chem. Phys. Lipids* **182**, 38–51. https://doi.org/10.1016/j.chemphyslip.2014.01.001.

331. Han, K., Kim, S.H., Venable, R.M., Pastor, R.W., (2022). Design principles of PI(4,5)P2 clustering under protein-free conditions: specific cation effects and calcium-potassium synergy. *Proc. Natl. Acad. Sci.* **119**, https://doi.org/10.1073/pnas.2202647119 e2202647119.
332. Bradley, R.P., Slochower, D.R., Janmey, P.A., Radhakrishnan, R., (2020). Divalent cations bind to phosphoinositides to induce ion and isomer specific propensities for nano-cluster initiation in bilayer membranes. *R. Soc. Open Sci.* **7**, https://doi.org/10.1098/rsos.192208 192208.
333. Sarmento, M.J., Coutinho, A., Fedorov, A., Prieto, M., Fernandes, F., (2017). Membrane order is a key regulator of divalent cation-induced clustering of PI(3,5)P2 and PI(4,5)P2. *Langmuir* **33**, 12463–12477. https://doi.org/10.1021/acs.langmuir.7b00666.
334. Bilkova, E., Pleskot, R., Rissanen, S., Sun, S., Czogalla, A., Cwiklik, L., Rog, T., Vattulainen, U., CCramer, P.S., Jungwirth, P., Coskun, U., (2017). Calcium Directly Regulates Phosphatidylinositol 4,5-Bisphosphate Headgroup Conformation and Recognition. *J. Am. Chem. Soc.* **139**, 4019–4024. https://doi.org/10.1021/jacs.6b11760.
335. Jarin, Z., Pak, A.J., Bassereau, P., Voth, G.A., (2021). Lipid-composition-mediated forces can stabilize tubular assemblies of I-BAR proteins. *Biophys. J.* **120**, 46–54. https://doi.org/10.1016/j.bpj.2020.11.019.
336. Blood, P.D., Swenson, R.D., Voth, G.A., (2008). Factors influencing local membrane curvature induction by N-BAR domains as revealed by molecular dynamics simulations. *Biophys. J.* **95**, 1866–1876. https://doi.org/10.1529/biophysj.107.121160.
337. Tsai, F.-C., Henderson, J.M., Jarin, Z., Kremneva, E., Senju, Y., Pernier, J., Mikhajlov, O., Manzi, J., Kogan, K., Le Clainche, C., Voth, G.A., Lappalainen, P., Bassereau, P., (2022). Activated I-BAR IRSp53 clustering controls the formation of VASP-actin–based membrane protrusions. *Sci. Adv.* **8**, https://doi.org/10.1126/sciadv.abp8677 eabp8677.
338. Kefauver, J.M., Hakala, M., Zou, L., Alba, J., Espadas, J., Tettamanti, M.G., Gajić, J., Gabus, C., Campomanes, P., Estrozi, L.F., Sen, N.E., Vanni, S., Roux, A., Desfosses, A., Loewith, R., (2024). Cryo-EM architecture of a near-native stretch-sensitive membrane microdomain. *Nature* **632**, 664–671. https://doi.org/10.1038/s41586-024-07720-6.
339. Belessiotis-Richards, A., Higgins, S.G., Sansom, M.S.P., Alexander-Katz, A., Stevens, M.M., (2020). Coarse-grained simulations suggest the epsin N-terminal homology domain can sense membrane curvature without its terminal amphipathic helix. *ACS Nano* **14**, 16919–16928. https://doi.org/10.1021/acsnano.0c05960.
340. Liu, K.-C., Pace, H., Larsson, E., Hossain, S., Kabedev, A., Shukla, A., Jerschabek, V., Mohan, J., Bergström, C. A.S., Bally, M., Schwieger, C., Hubert, M., Lundmark, R., (2022). Membrane insertion mechanism of the caveola coat protein Cavin1. *Proc. Natl. Acad. Sci.* **119**, https://doi.org/10.1073/pnas.2202295119 e2202295119.
341. Belessiotis-Richards, A., Larsen, A.H., Higgins, S.G., Stevens, M.M., Alexander-Katz, A., (2022). Coarse-grained simulations suggest potential competing roles of phosphoinositides and amphipathic helix structures in membrane curvature sensing of the AP180 N-terminal homology domain. *J. Phys. Chem. B* **126**, 2789–2797. https://doi.org/10.1021/acs.jpcb.2c00239.
342. Brown, C.M., Marrink, S.J., (2024). Modeling membranes in situ. *Curr. Opin. Struct. Biol.* **87**, https://doi.org/10.1016/j.sbi.2024.102837 102837.
343. Grünewald, F., Souza, P.C.T., Abdizadeh, H., Barnoud, J., de Vries, A.H., Marrink, S.J., (2020). Titratable Martini model for constant pH simulations. *J. Chem. Phys.* **153**, https://doi.org/10.1063/5.0014258 024118.
344. Bignucolo, O., Chipot, C., Kellenberger, S., Roux, B., (2022). Galvani offset potential and constant-pH simulations of membrane proteins. *J. Phys. Chem. B* **126**, 6868–6877. https://doi.org/10.1021/acs.jpcb.2c04593.
345. Domanski, J., Hedger, G., Best, R.B., Stansfeld, P.J., Sansom, M.S.P, (2017). Convergence and Sampling in Determining Free Energy Landscapes for Membrane Protein Association. *J. Phys. Chem. B* **121**, 3364–3375. https://doi.org/10.1021/acs.jpcb.6b08445.
346. Grime, J., Madsen, J.J. (2019). Efficient simulation of tunable lipid assemblies across scales and resolutions. arXiv preprint arXiv:1910.05362. https://doi.org/10.48550/arXiv.1910.05362.
347. Amaro, R., Åqvist, J., Bahar, I., Battistini, F., Bellaiche, A., Beltran, D., Biggin, P.C., Bonomi, M., Bowman, G.R., Bryce, R. (2024). The need to implement FAIR principles in biomolecular simulations. arXiv preprint arXiv:2407.16584. https://doi.org/10.48550/arXiv.2407.16584.
348. Payandeh, J., Volgraf, M., (2021). Ligand binding at the protein–lipid interface: strategic considerations for drug design. *Nature Rev. Drug Discov.* **20**, 710–722. https://doi.org/10.1038/s41573-021-00240-2.
349. Conflitti, P., Lyman, E., Sansom, M.S.P., Hildebrand, P. W., Gutierrez-de-Teran, H., Carloni, P., Ansell, T.B., Yuan, S., Barth, P., Robinson, A.S., Tate, C.G., Gloriam, D., Grzesiek, S., Eddy, M.T., Prosser, S., Limongelli, V., (2025). Functional dynamics of G protein-coupled receptors reveal new routes for drug discovery. *Nature Rev. Drug Discov*. https://doi.org/10.1038/s41573-024-01083-3.
350. Bai, X.-C., Gonen, T., Gronenborn, A.M., Perrakis, A., Thorn, A., Yang, J., (2024). Challenges and opportunities in macromolecular structure determination. *Nature Rev. Mol. Cell Biol.* **25**, 7–12. https://doi.org/10.1038/s41580-023-00659-y.
351. Papasergi-Scott, M.M., Pérez-Hernández, G., Batebi, H., Gao, Y., Eskici, G., Seven, A.B., Panova, O., Hilger, D., Casiraghi, M., He, F., Maul, L., Gmeiner, P., Kobilka, B.K., Hildebrand, P.W., Skiniotis, G., (2024). Time-resolved cryo-EM of G-protein activation by a GPCR. *Nature* **629**, 1182–1191. https://doi.org/10.1038/s41586-024-07153-1.
352. Amann, S.J., Keihsler, D., Bodrug, T., Brown, N.G., Haselbach, D., (2023). Frozen in time: analyzing molecular dynamics with time-resolved cryo-EM. *Structure* **31**, 4–19. https://doi.org/10.1016/j.str.2022.11.014.