Lipid Interactions of a Ciliary Membrane TRP Channel: Simulation and Structural Studies of Polycystin-2

Graphical Abstract

Highlights

- Lipid interactions of PC2 channels have been explored by MD simulation and cryo-EM
- PIP₂ binds to a site corresponding to the vanilloid/lipid binding site of TRPV1
- Cholesterol binds between the S3 and S4 helices and S6 of the adjacent subunit
- PC2, in common with other channels, may be modulated by PIPs and cholesterol

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In Brief

Wang et al. use molecular dynamics simulations and cryoelectron microscopy to explore interactions of the PC2 channel with lipids. Phosphatidylinositol phosphates (PIPs) bind to a site corresponding to the vanilloid/lipid binding site of TRPV1, whereas cholesterol binds to a different site. This suggests PC2 may be modulated by PIPs and cholesterol.
Lipid Interactions of a Ciliary Membrane TRP Channel: Simulation and Structural Studies of Polycystin-2

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SUMMARY

Polycystin-2 (PC2) is a transient receptor potential (TRP) channel present in ciliary membranes of the kidney. PC2 shares a transmembrane fold with other TRP channels, in addition to an extracellular domain found in TRPP and TRPML channels. Using molecular dynamics (MD) simulations and cryoelectron microscopy we identify and characterize PIP2 and cholesterol interactions with PC2. PC2 is revealed to have a PIP binding site close to the equivalent vanilloid/lipid binding site in the TRPV1 channel. A 3.0-Å structure reveals a binding site for cholesterol on PC2. Cholesterol interactions with the channel at this site are characterized by MD simulations. The two classes of lipid binding sites are compared with sites observed in other TRPs and in Kv channels. These findings suggest PC2, in common with other ion channels, may be modulated by both PIPs and cholesterol, and position PC2 within an emerging model of the roles of lipids in the regulation and organization of ciliary membranes.

INTRODUCTION

Ion channels are of considerable importance in numerous aspects of cell physiology (Hille, 2001), and mutations in channels cause many human diseases (Bagal et al., 2013). The transient receptor potential (TRP) superfamily of non-selective cation channels is a major class of ion channels found in all eukaryotes. They are involved in many aspects of cellular function, including thermosensation, osmotic pressure regulation, mechanosensation, and detection of noxious substances (Vetter and Lewis, 2011). TRP channels are activated and inhibited by a range of mechanisms, in response to thermal or chemical stimuli and/or mechanical forces (Venkatachalam and Montell, 2007). TRP channel mutations have been implicated in a number of different diseases (Nilius and Owsianik, 2010) and consequently are of interest as potential drug targets (Moran, 2018). Several TRP channels are modulated by membrane lipids (e.g., Fine et al., 2018; Wilkes et al., 2017; Yin et al., 2019), suggesting the possibility of lipid-based therapies (Ciardo and Ferrer-Montiel, 2017).

Mammalian TRP channels can be divided into five families, the TRPC (classical or canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin), and TRPA (Ankyrin) subfamilies (Rohacs, 2009). Structurally, all TRP channels have a tetrameric architecture assembled from identical or similar subunits. Each of the four subunits is composed of six transmembrane (TM) helices (S1–S6) with a pore loop region between S5 and S6 (Cao et al., 2013; Gao et al., 2016; Grieben et al., 2017; Huynh et al., 2016; Jin et al., 2017; Liao et al., 2013; Paulsen et al., 2015; Saotome et al., 2016; Shen et al., 2016; Su et al., 2018b; Wilkes et al., 2017; Zubcevic et al., 2016). TM helices S1 to S4 form a voltage sensor-like domain (VSLD), which is packed against the pore domain (S5-Pore-S6) of the adjacent chain, as is also seen in Kv channels, where structural data reveal that lipids mediate interactions of the voltage sensor domain with the pore (Long et al., 2007).

As a member of the TRPP subfamily, the polycystin-2 (PC2, also known as PKD2 or TRPP1) homo-tetramer has the same fold as other TRP channels. An extracellular domain (referred to as the TOP domain) is formed from a 218-residue insertion between S1 and S2 plus a 20-residue insertion between S3 and S4 of the VSLD (Grieben et al., 2017; Shen et al., 2016; Wilkes et al., 2017). These structures have a remarkably similar fold, despite the fact that they were solved in a range of environments (detergent, nanodiscs, and amphipols), with both truncated and full-length protein, and it is interesting that even with full-length protein, the structures observed in the maps were of very similar length. Structures of the closely related TRPP2L1 protein (Hulse et al., 2018; Su et al., 2018b), and also of the PC1/PC2 1:3 hetero-tetrameric complex (Su et al., 2018a) have also recently been determined.

Mutations in PC2 are responsible for approximately 15% of autosomal dominant polycystic kidney disease (ADPKD), which is one of the most prevalent genetic disorders in humans, affecting 4 to 6 million people worldwide (Wilson, 2004). Most
other cases of ADPKD (70%) are caused by mutations in PC1, which forms a 1:3 complex with PC2 by replacing one PC2 subunit in the channel region (Su et al., 2018a). ADPKD is characterized by formation and progressive enlargement of fluid-filled renal cysts in both kidneys, which ultimately causes kidney failure (Pavel et al., 2016). Cysts or diverticula also frequently develop in intestines, liver, and pancreas (Wilson, 2004). In addition, ADPKD is associated with increased risk of cardiovascular dysfunction including aortic aneurysms, hypertension, and heart-valve defects (Boucher and Sandford, 2004). Genetic data analysis has shown that there are at least 278 mutations in PC2 associated with ADPKD (see http://pkdb.pkdcure.org) (Gout et al., 2007). However, the underlying mechanisms by which these mutations lead to ADPKD are still poorly understood.

PC2 is widely distributed with relatively high expression in tubules within kidney cells (Chauvet et al., 2002), and was originally proposed to contribute to the transduction of extracellular mechanical stimuli caused by bending of the cilia into intracellular Ca\textsuperscript{2+} signals in the primary cilia of kidney epithelium (Nauli et al., 2003). In addition to its role in primary cilia, it has been proposed that PC2 could function as an intracellular Ca\textsuperscript{2+} release channel in the endoplasmic reticulum membrane (Koulen et al., 2002). However, more recent studies have suggested that Ca\textsuperscript{2+} signaling may not be involved in ciliary mechanosensation (Delling et al., 2016), and that ciliary PC2 is a non-selective Na\textsuperscript{+},K\textsuperscript{+} channel, with low levels of Ca\textsuperscript{2+} currents, rather than a Ca\textsuperscript{2+} release channel in the endoplasmic reticulum membrane (Koulen et al., 2002). Recently, cryo-EM and molecular dynamics (MD) simulation studies have provided an important tool for defining lipid interactions with membrane proteins (Corradi et al., 2018; Hedger and Sansom, 2016). For example, they have been used to predict PIP\textsubscript{2} binding sites on Kir channels (Schmidt et al., 2013; Stansfeld et al., 2009) and GPCRs (Yen et al., 2018), and to explore allosteric modulation of GPCRs by cholesterol (Manna et al., 2016). Given the growing number of structures of ion channels, binding affinities and specificity of interactions with lipids can be studied in silico via MD simulations (Domanski et al., 2017; Hedger et al., 2016, 2019) to provide an indication of possible mechanisms of activation and allosteric modulation of channels by lipids.

Here we use a combination of MD simulations and cryo-EM to identify and characterize PIP\textsubscript{2} and cholesterol interactions with PC2. Simulations predict a phospholipid binding site corresponding to lipid-like density observed in cryo-EM maps, and free energy calculations suggest that this binding site is selective for PIP\textsubscript{2} molecules over other phospholipids. The proposed PIP\textsubscript{2} binding site is close to the equivalent vanilloid/lipid binding site in the TRPV1 channel (Gao et al., 2016). We also identify a binding site for cholesterol in PC2 located between the VSLD and pore domain. This binding site may be compared with cholesterol sites observed in other TRP channels and in Kv channels. Together, these results suggest that PC2, in common with other ion channels, may be modulated by both PIPs and cholesterol, and thus locate PC2 within an emerging model of the complex roles of lipids in the regulation and organization of ciliary membranes (Weiss et al., 2019).

RESULTS AND DISCUSSION

A Possible Phospholipid Interaction Site Suggested by Simulations

An initial exploration of possible phospholipid interaction sites on the TM domain of PC2 was made using atomistic MD
Simulations in which the PDB: 5K47 PC2 structure (a representative of several PC2 structures, see below) was embedded in a lipid bilayer made up of a single species of phospholipid (palmitoyl-oleyl-phosphatidylcholine [POPC]; Figure 1A). This process was repeated for all three molecular structures of wild-type PC2 (PDB: 5K47, 5MKF, 5T4D; see Figure S1) and also for a gain-of-function mutant (F604P) of PC2 (PDB: 6D1W) (Zheng et al., 2018), yielding a total of more than 2 ms of atomistic simulations of PC2 in a phosphatidylcholine (PC) bilayer (Table S1). The simulations were examined in terms of regions of high probability density of occurrence of phospholipid molecules on the protein surface. In all 12 simulations (i.e., three repeats for each of the four structures, PDB: 5K47, 5MKF, 5T4D, 6D1W) (Zheng et al., 2018), yielding a total of more than 2 ms of atomistic simulations of PC2 in a phosphatidylcholine (PC) bilayer (Table S1). The simulations were examined in terms of regions of high probability density of occurrence of phospholipid molecules on the protein surface. In all 12 simulations (i.e., three repeats for each of the four structures, PDB: 5K47, 5MKF, 5T4D, 6D1W), high lipid occurrence densities (Figure 1B) were observed in a pocket exposed to the intracellular leaflet of the lipid bilayer, between TM helices S3, S4, and S5 (Figure 1C), corresponding to one POPC lipid molecule bound to each subunit of the PC2 tetramer. These results are illustrated for PDB: 5K47 in Figures 1B and 1C, and similar results for PDB: 5MKF and 5T4D are shown in Figure S1. Side chains of residues in S3, S4, and S5 create a hydrophobic pocket, within which the acyl tails of the bound lipid molecules reside. The phosphate oxygens of the bound lipid formed hydrogen bonds to the indole ring of Trp507 in S3 and to the hydroxyl group of Ser591 in the S4-S5 linker.

Thus, in a simple model lipid bilayer, we observe a phospholipid binding site on PC2 that is close to the proposed lipid and vanilloid (e.g., capsaicin) binding sites observed in cryo-EM studies of the related TRPV1 and TRPV2 channels (Gao et al., 2016). Lipid or detergent binding has been observed at a similar location in cryo-EM structures of TRPV6 (McGoldrick et al., 2018; Singh et al., 2018), TRPV5 (Hughes et al., 2018a), TRPC3 (Fan et al., 2018), TRPC4 (Vinayagam et al., 2018), TRPM4 (Auten et al., 2018; Duan et al., 2018b), TRPML3 (Hirschi et al., 2017), and TRPM7 (Duan et al., 2018a). This site has also been suggested to be the binding site for the TRPV5 inhibitor econazole (Hughes et al., 2018a) and for PI(4,5)P2 (Hughes et al., 2018b). Together with our simulation results, this comparison with other TRP channels suggested that a combined experimental and computational approach to lipid interactions with PC2 was needed.

**Cryo-EM Reveals Lipid-like Density**

To investigate possible binding of anionic phospholipids to PC2, we determined two cryo-EM structures of PC2: in the presence of PI(4,5)P2 to 3 Å resolution (Figures 2A, 3A, and 3B), and of PI(3,5)P2 to 3.4 Å (Figure 2B). (See Figures S2 and S3, and Table S2, for details of cryo-EM data and processing, and Figure S4 for further examples of density plus structure from the 3 Å resolution map.) We used similar conditions to our original 4.2-Å PC2 structure (PDB: 5K47), with a truncated construct (residues Pro185 to Asp723), purified in the detergent n-undecyl-D-maltopyranoside (UDM).

The overall conformation of the protein is the same as our original structure, i.e., a closed state at both the selectivity filter and the lower gate, although we did in this case observe a disulfide bond in the TLC1 region of the TOP domain (between...
residues Cys331 and Cys344; see Figure S4C). In our earlier structure (PDB: 5K47), Cys331 and Cys344 appeared to be in their reduced form with free thiol groups. In our current maps, there is clear density showing that a disulfide bond is formed between these two residues (Figure S4C), which is consistent with the 3-A˚ PC2 structure PDB: 5T4D (Shen et al., 2016). The two structures reported here also differ from our previous structure in that they both display a spherical density below the selectivity filter consistent with a bound ion (see Figure S4B). These densities are visible at several thresholds, and the peaks are prominent even in the un-averaged (C1) maps, which indicates that they are unlikely to be noise peaks on the symmetry axis but rather may correspond to bound cations. The identity of the bound cation is unclear as both calcium and sodium ions were present in the buffer used to prepare the EM grids. In contrast, there is only a region of weak elongated density present in the equivalent location in the 3-A˚ PC2 structure (EMD-8354/PDB: 5T4D).

Having observed these new features in both of the new maps, we inspected all lipid-like non-protein densities present in the TM region. In particular, we examined the region of the maps where phospholipid was found in the simulations to see whether there was any evidence for density possibly corresponding to tightly bound lipids at these sites. This revealed clear non-protein densities located between S3, S4, and S5 in both the PI(4,5)P₂ and PI(3,5)P₂ maps, suggestive of lipid and/or detergent occupancy (Figures 2C, 3C, and S5). The density observed in the PI(3,5)P₂ complex (Figure 2B) may correspond to a lipid molecule, although the presumed head group region of the density is not sufficiently well-defined to unequivocally interpret it as a PIP molecule. In the higher-resolution PI(4,5)P₂ structure, although there is density at the same site (Figure 2A), it differs in shape and could be interpreted as two UDM detergent molecules. Although PI(3,5)P₂ maps suggest that a lipid molecule may be bound at this site, there are few interactions between the region where the head group lies and the potential lipid. Overall, although the overall map quality is high and reveals the larger amino acid side chains (see Figures 3 and S4), for the most part the resolution in the non-protein portion is not sufficient to unequivocally assign a given lipid molecule species to this site in the final cryo-EM models, and so no lipid was placed at this site in the final PDB file. In addition to this possible lipid binding site, a region of density within the upper segment of S1–S4 helices was found in both cryo-EM maps, which exhibits clear characteristics of a UDM detergent molecule (see Figure S5A), and four other densities in the upper leaflet between S4 of one subunit and S5 and S6 of adjacent subunit were also attributed to UDM molecules. Their positions are close to the lipids/single-chain fatty acids included in the previously published structure of PC2 in amphipol (PDB: 5MKE/5MKF; Wilkes et al., 2017) (see Figure S5A). Density corresponding to a cholesterol molecule is clearly defined adjacent to S4 (Figures 2A, 2B, 3C, and S5A). This is discussed in more detail below.

In other TRP channel structures with lipid molecules built into similar locations to the potential PIP site we observed (e.g., Gao et al., 2016; Hughes et al., 2018b), the lipid head groups are coordinated not only by residues on the S3, S4, and S5 TM helices, but also by residues in the cytosolic pre-S1 or post-S6 domains. The lack of cytosolic domains in our constructs may contribute to the flexibility of the head group of the bound lipid, helping to explain why the densities observed for these structures are not well resolved in the head group region. Similarly, there is undefined density in this region in structures obtained using soy extract polar lipids (which are likely to
contain some PIs (Shen et al., 2016). We inspected the cryo-EM density map of the latter structure (https://www.emdataresource.org/EMD-8354), which was determined with PC2 in nanodiscs. In the region of interest, there is density for “well resolved” (Shen et al., 2016) lipids in the same site as in our structures, which could represent a bound phospholipid molecule.

**PC2 Interactions with PIPs**

Taking together the lipid-like cryo-EM density alongside results of our initial simulations, and given the lipid interactions of other TRP channels (see above), it seemed possible that the hydrophobic pocket identified could be a binding site for anionic phospholipids. Because unambiguous experimental identification of the bound species was not possible, we returned to simulations to extend the interpretation of the experimental density.

We used coarse-grained (CG) simulations of PC2 in a lipid bilayer containing multiple lipid species (Ingolfsson et al., 2014; Koldsø et al., 2014) to explore the possible specificity of the phospholipid binding site. We embedded PC2 in an in vivo mimetic bilayer (Figure 4A), which contained the anionic lipids phosphatidylserine (PS) and PIP2 in the inner leaflet, a glycolipid (GM3) in the outer leaflet, and cholesterol (CHOL) in both leaflets of the bilayer. The lipid composition of the in vivo mimetic bilayer membrane provided an approximation to the major lipid species likely to be present within a mammalian cell membrane (Koldsø et al., 2014; Sampaio et al., 2011). Thus, the outer (i.e., extracellular or organelle luminal) leaflet contained PC:PE:SM:GM3:CHOL = 40:10:15:10:25; and the inner (i.e., intracellular) leaflet contained PC:PE:PS:PIP2:CHOL = 10:40:15:10:25. This provides an overall PIP2 concentration of 5%, which is within the physiological range for a mammalian cell membrane (Sampaio et al., 2011; van Meer et al., 2008).

Three independent simulations (each of 5 μs duration) of a single PC2 channel (structure PDB: 5K47) inserted in an in vivo mimetic lipid bilayer with different random distributions of lipid molecules were performed. Simulations were also performed for the constitutively active F604P PC2 mutant (PDB: 6D1W; see Table S1). In simulations, PIP2 molecules diffused in the bilayer on a timescale of microseconds (Figures 4B and S6; Movie S1) resulting in random encounters with the channel molecule followed by binding to the previously identified sites on PC2, as demonstrated by tracking the distance versus time of the PIP2 molecules from their eventual binding sites (Figure 4B). From a final snapshot of one such simulation (Figure 4C), it can be seen that a PIP2 molecule has bound to each of the four sites on PC2. The head groups of the PIP2 molecules each interact with up to five basic residues in the S3/S4/S5 region: Arg504, Lys572, Lys575, Arg592, and Lys595. These residues are highly conserved in PC2 from different species (Figure S7), and this interaction is persistent in both wild-type and mutant PC2. Given the presence of other negatively charged lipid species (PS) in the bilayer and of positively charged patches on the protein surface, the observation that PIP2 molecules were able...
to bind to the intracellular binding site suggests that this site may be specific for PIP2 and related lipids.

**Free Energy Landscapes for PC2/Phospholipid Interactions**

To explore the possible selectivity of the PIP2/intracellular site interaction in more detail, we calculated potentials of mean force (PMFs) based on CG simulations. A PMF provides a one-dimensional free energy landscape for lipid/PC2 interactions, and allows us to explore the specificity of this interaction (Figure 5A). This approach has been used to explore interactions of anionic lipids (e.g., cardiolipin and PIP2) with a number of transporters (e.g., ANT1 [Hedger et al., 2016]), ion channels (e.g., Kir channels [Domanski et al., 2017]), and receptors (e.g., class A GPCRs [Song et al., 2019; Yen et al., 2018]).

To estimate and compare the free energy landscapes for phosphatidylinositol monophosphate (PIP), PIP2, phosphatidylinositol trisphosphate (PIP3), PC, and for PS, each interacting with PC2 at the intracellular site defined by S3/S4/S5, CG models of a PC2 channel molecule embedded in a PC bilayer with a PIP, PIP2, PIP3, or PS molecule inserted into each of the four binding sites were used. For computational efficiency, the PC2 structure was truncated, removing the TOP domain (Ser244–Leu462). The initial configurations were lipid bound states. One of the bound lipid molecules was pulled away from the binding site to assess the free energy of interaction. A one-dimensional reaction coordinate was defined as the distance, in the plane of the bilayer, between the centers of mass of the lipid head group and two serines (Ser505 and Ser591) within the lipid binding site of PC2. The free energy profile (or PMF) for PIP2 shows a clear minimum close to the initial position of the PIP2 molecule in the binding site, with a well depth of –37 kJ/mol (Figure 5A). This is comparable with the well depth for PIP2 binding to the Kir2.2 channel (Domanski et al., 2017).

Comparison of PMF profiles as a function of window simulation duration suggested that convergence had been achieved by 1.5 μs per window (see Figure S8) and that errors on the well depths of the PMFs are of the order of ±3 kJ/mol (see Figure S8C). PIP and PIP3 exhibited weaker binding to PC2 with free energies of –20 and –28 kJ/mol, respectively. To confirm this, we performed CG free energy perturbation simulations (Corey et al., 2019), which suggested ΔΔG of +21 and +4 kJ/mol for the conversion of bound PIP2 to PIP and PIP3 respectively, which is consistent with the pattern of selectivity derived from the PMFs (from which the corresponding ΔΔGs are +17 and +9 kJ/mol). We note that, due to the limitation of the CG model, we were unable to distinguish between PIP(4,5)P2 and PI(3,5)P2 interactions by calculation of CG PMFs. However, the simple anionic lipid PS showed significantly weaker binding to PC2 with a well depth of approximately –9 kJ/mol, indicating a clear selectivity of the binding site for PIP species over PS. PC shows even weaker interactions, as would be anticipated given the PMF is evaluated for a single lipid molecule in an environment of a PC bilayer. The two minima in the PMF for the PC are likely to reflect annular shells of relatively immobilized lipid molecules around the PC2 channel, as have been seen for a number of the binding site to assess the free energy of interaction. A one-dimensional reaction coordinate was defined as the distance, in the plane of the bilayer, between the centers of mass of the lipid head group and two serines (Ser505 and Ser591) within the lipid binding site of PC2. The free energy profile (or PMF) for PIP2 shows a clear minimum close to the initial position of the PIP2 molecule in the binding site, with a well depth of –37 kJ/mol (Figure 5A). This is comparable with the well depth for PIP2 binding to the Kir2.2 channel (Domanski et al., 2017).
channels and other membrane proteins in simulations (Goose and Sansom, 2013; Niemela et al., 2010).

Testing the Predicted Lipid Specificity
The predicted binding of PIP$_2$ to PC2 was tested biochemically using PIP strips (Shirey et al., 2017), nitrocellulose membranes with lipids including phosphoinositides and other phospholipids spotted onto their surface. Protein binding to relevant lipids can be detected with antibodies to the tagged protein. This method has been used, for example, to confirm binding of PIP$_2$ to the GluA1 ionotropic glutamate receptor (Seebohm et al., 2014). The results from this assay (Figure S9) suggest that truncated PC2 in UDM micelles can bind a range of PIPs, including PIP$_2$ species. However, variation between the biological repeats (see Figure S9) is not unique to PC2; for example, they have also been reported for PIP$_2$ molecules bound to Kir channels (e.g., Lacin et al., 2017).

A PIP$_2$ Binding Site
Having established that PC2 binds PIP$_2$ selectively, we examined the interactions of the protein with the lipid in more detail, based on a CG simulation snapshot structure of the PC2-PIP$_2$ complex corresponding to the energy minimum in the PMF. As noted above, the head group of PIP$_2$ interacts with five basic residues: Arg504, Lys572, Lys575, Arg592, and Lys595. In particular, the 1$^\text{st}$-phosphate interacts closely with Arg592, and the 4$^\text{th}$- and 5$^\text{th}$-phosphates interact with Lys572 and Lys575, respectively. Comparable binding sites for PIP$_2$ were formed by clusters of basic residues as seen in other ion channels, e.g., Kir channels (Hansen et al., 2011; Lacin et al., 2017), and GPCRs (Song et al., 2019; Yen et al., 2018).

To evaluate the proposed PIP$_2$ binding site in more detail, a CG structure corresponding to the free energy minimum was converted into an atomistic representation. A two-stage atomistic simulation was then performed. Firstly, a short (30 ns) simulation was performed in which harmonic restraints were applied to the distances between the 1$^\text{st}$-, 4$^\text{th}$-, and 5$^\text{th}$-phosphates of PIP$_2$ and the side chains of Arg592, Lys575, and Lys572, respectively, in order to relax the atomistic model while maintaining the interactions seen in the CG PMF calculations. The distance restraints were then removed and three replicates of an unrestrained simulation (durations 200–250 ns) were run to allow the PIP$_2$ to explore more fully the binding site on PC2. Final snapshots from the restrained and unrestrained simulations are shown (Figure 6A). The interactions between the lipid head group and the interacting residues are seen to be dynamic and to vary stochastically between replicate simulations. Thus, for residues Arg504, Lys575, Arg592, and Lys595, fluctuating numbers of hydrogen bonds were formed with PIP$_2$ throughout the simulations (Figure 6B; Movie S2). Such dynamic fluctuations in the interactions of bound PIP$_2$ are not unique to PC2; for example, they have also been reported for PIP$_2$ molecules bound to Kir channels (e.g., Lacin et al., 2017).

PIP Interactions in TRP Channels
The proposed PIP interaction site on the TM domain of PC2 may be compared with a lipid binding site, close to the vanilloid ligand-binding site, which has been seen in the structure of TRPV1 and has been interpreted as corresponding to a bound phosphatidylinositol molecule (Gao et al., 2016). Comparison of the two sites (Figures 7A and 7C) reveals strong similarities, especially the location of the anionic phosphate-containing lipid head group at the N-terminal region of the S3 helix dipole (Hol et al., 1978). Furthermore, the PIP$_2$ interaction site on PC2 and the PI site on TRPV1 both agree well with the lipid-like density in our 3.4-Å cryo-EM map (Figure 7B). To explore this possible common binding site further we extended our CG simulations in a mixed lipid in vivo mimetic to 12 different TRP channel structures (see Table S1). For each of the TRP channel structures we then analyzed the mean contact duration of each residue with the head group of a PIP$_2$ molecule over the course of the simulation. These contact durations ranged up to >1 μs (e.g., Figures 4B and S6). The results (Figure 7D) revealed a degree of conservation of the proposed PIP channel interaction between different families of TRP channels. In particular, an aromatic contact in S3 and basic contacts in S4 and S5 are
also seen for TRPML2, TRPML1, TRPML3, and, to a lesser extent, in TRPV5 and TRPV6.

Returning to the cryo-EM data, we noted that with the more soluble version of PIP2 (i.e., PI(3,5)P2), we observed in the map a small outward movement (\( \sim 2 \) Å; see Figure S5A) of the VSLD at the S2–S3 linker region and an inward movement (\( \sim 2 \) Å) toward the central axis of the cytosolic extension of S6 helix in the PI(3,5)P2 structure compared with the PI(4,5)P2 structure (which may correlate with the apparently stronger binding of PI(3,5)P2 seen in the PIP strip assay). This is suggestive of an interplay between occupancy of the PIP2 site and the interactions between the VSLD and pore domains. However, we note that our structures are of a truncated construct of PC2, and it is possible that the missing intracellular domain(s) may be needed to observe a more extensive change in conformation.

**Cholesterol Interactions**

Further examination of the cryo-EM density also revealed a possible site for (co-purified) cholesterol (Figure 8A; see also Figures 3C and S5A) located between the S3 and S4 helices of the VSLD and pore domain S6 helix of the adjacent subunit. The cholesterol must have remained bound to PC2 throughout the extraction and purification process, since neither cholesterol, nor the cholesterol mimic, cholesteryl hemi-succinate (CHS), was used in the PC2 purifications. Interestingly, comparable density is also visible in the cryo-EM maps for the PDB: 5T4D structure, consistent with the presence of cholesterol in this structure (Shen et al., 2016).

Given the importance of cholesterol in ciliary membranes (Garcia et al., 2018), we analyzed our CG-MD simulations of PC2 in an in vivo mimetic (i.e., mixed lipid, see above and Table S1) bilayer to identify possible interactions of cholesterol with the channel (Figures 8B, 8C, and S10A). These simulations indicated a cholesterol interaction site corresponding to that revealed by the cryo-EM density. This indicates that this site is likely to interact with cholesterol present in the bilayer. Having identified a cholesterol binding site, we performed CG PMF calculations to estimate the free energy landscape for the PC2-cholesterol interaction (Figure 8D). These calculations showed a weaker interaction than for PIPs (above), with a free energy well depth comparable with that for binding of cholesterol to other membrane proteins (e.g., Hedger et al., 2019).

To further evaluate the cholesterol binding site, we performed atomistic simulations (3 × 250 ns) starting from coordinates for cholesterol built into the cryo-EM density. From the simulations it is evident that cholesterol interacts dynamically...
at this site, which is located at the interface between the VSLD and S6, with the long axis of cholesterol running approximately parallel to helices S3 and S4. Thus, for the four symmetry-related binding sites on the PC2 channel, one site exhibits stably bound cholesterol, one site has a cholesterol that transiently dissociates then rebinds, and the two other sites show intermediate behavior (noting that these differences are simply the stochastic dynamics of a single molecule simulation; Figures 9A and S10B). This is consistent with, e.g., simulation studies of cholesterol on GPCRs (see Hedger et al., 2019 for a detailed discussion), which indicate relatively dynamic, loose binding as is also seen for PC2 (Figure 9B). The hydroxyl group of cholesterol mainly hydrogen bonded to Gln557 and Asn560. The steroid nucleus of cholesterol sits within a shallow hydrophobic pocket formed by a group of isoleucine (Ile561 and Ile659), leucine (Leu517 and Leu656), and valine (Val564 and Val655) residues (Figure 9C). The hydrocarbon chain of cholesterol showed considerable mobility throughout the simulation, which may explain the lack of density around this region in our lower resolution cryo-EM map.

Conclusions
Using an approach combining multiscale MD simulations and cryo-EM, we identified a phospholipid binding pocket in the human TRP channel PC2 between the S3 and S4 TM helices

![Figure 7. Comparison of PI Lipids Bound to PC2 and to TRPV1](image)

Comparison of PI lipids bound to PC2 and to TRPV1 with cryo-EM density. (A–C) (A) PIP2 bound to PC2 (as revealed by the current simulation study); (B) lipid-like density in the cryo-EM maps of PC2 obtained in the presence of PI(3,5)P2 (3.4 Å resolution; see Figure 5); (C) PI bound to TRPV1 (as revealed by cryoelectron microscopy, PDB ID 5IRZ). In each case the lipid molecule or density is located between the S3, S4, and S5 helices of the VSLD. (D) A sequence alignment colored on contacts with PIP2 in the mixed lipid simulations. Residues of the region around the binding pocket between the S3, S4, and S5 helices are colored (on a white to red scale) based on the mean duration of the interactions of PIP2 head groups with each residue. The five basic residues of PC2 which form interactions with the head group of PIP2 at the binding site (i.e., R504, K572, K575, R592, and K595) are boxed. See also Figure S5.
gestive of PIP2 and cholesterol binding at the two sites identified on PC2. However, the cryo-EM data only allowed unambiguous confirmation of these predictions in the case of cholesterol, not PIP2. PIP strip assays were used to identify possible phospholipid species interacting with PC2, but quantification of relative binding affinities for different lipids could not be established. In our PMF calculations, coarse graining of the lipid models does not allow distinction between different PIP species with the same charge. In addition, the PC2 construct used for PIP strips is truncated (as is the construct in the structural studies and the simulations). Therefore, it is uncertain whether full-length PC2 protein would exhibit exactly the same lipid binding behavior, especially for the PIP binding site which is close to the termini of the truncation.

Comparison with other ion channels and their lipid interactions suggest that the PIP2-binding site on PC2 may be of functional importance. A number of TRP and related channels show anionic lipid interactions in this region, close to the S4-S5 helix which links the VSLD/VSD to the pore domain. For example, TRPV1-PI, TPRVS-PI2, and Kv1.2/2.1-anionic lipid interactions all occur at similar sites to the PC2-PIP2 interaction (Figure 10A). PIP2 regulates a number of TRP channels (as discussed above) and also a number of Kv channels (Kruse et al., 2012) via interactions with the S4-S5 linker. This suggests that the PC2 PIP2-binding site near the S4-S5 linker may be a regulatory/allosteric site. Interestingly, some pathogenic missense variants (see http://pkdb.mayo.edu/) in PC2 occur in the vicinity of the PIP2 site, e.g., L517R, D511V, and N580K (the latter located at the start of the S4-S5 linker), and might be expected to perturb interactions with PIP2.

Figure 8. Cholesterol Interactions with PC2
(A) Cryo-EM density (from the 3.0 Å map, contoured at 2.2σ; see Figure 2A) corresponding to a binding site for cholesterol located between the S3 and S4 helices and helix S6 of the adjacent subunit.
(B) Cholesterol observed to bind to the same site in CG simulations of PC2 in an in vivo mimetic mixed lipid bilayer (see Figure 3).
(C) Distance from the binding site as a function of time for four cholesterol molecules which bind to PC2 during a simulation in a mixed lipid bilayer.
(D) Potential of mean force for the interaction of cholesterol with the binding site on PC2. See also Figures S5 and S10.

We should consider possible limitations of this study. The resolution currently achievable in cryo-EM studies of membrane proteins means that it is sometimes difficult to unambiguously identify bound lipids (however, see Laverty et al., 2019, for a counterexample where lipid identification proved possible). In particular, certain regions of cryo-EM have lower resolution than other regions of the maps. The regions containing the lipids, usually on the outer surface of the protein, are often of lower resolution, making it more challenging to determine the nature of the bound lipids. Molecular simulations thus provide a valuable tool in assessing possible identities of bound lipids. In our studies, MD simulations are strongly suggestive of PIP2 and cholesterol binding at the two sites identified on PC2. However, the cryo-EM data only allowed unambiguous confirmation of these predictions in the case of cholesterol, not PIP2. PIP strip assays were used to identify possible phospholipid species interacting with PC2, but quantification of relative binding affinities for different lipids could not be established. In our PMF calculations, coarse graining of the lipid models does not allow distinction between different PIP species with the same charge. In addition, the PC2 construct used for PIP strips is truncated (as is the construct in the structural studies and the simulations). Therefore, it is uncertain whether full-length PC2 protein would exhibit exactly the same lipid binding behavior, especially for the PIP binding site which is close to the termini of the truncation.

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Structural and simulation studies have shown that the movement of the S4-S5 linker is important for gating in voltage-gated cation channels. For example, in the voltage-gated potassium channel Kv1.2, the constriction or dilation of the pore domain is controlled by the voltage sensor domain through the S4-S5 linker (Long et al., 2005). The TM helices of TRP channels and voltage-gated cation channels share high structural similarity, indicating a possible common functional role of the S4-S5 linker. Indeed, it has been shown that TRPV1 (Gao et al., 2016; Yang et al., 2015), TRPV2 (Yang et al., 2016), and TRPV4 (Teng et al., 2015) use the same mechanism to gate the channels, and disruption of the interaction configurations at the linker region by ligands/lipids could change the gating probability.

It has been suggested that the basal regions of primary cilia membranes contain PIP2 while the upper regions of the ciliary membrane have an elevated level of P(4)P (Garcia et al., 2018; Nakatsu, 2015). Thus, it is reasonable to suppose that PC2 binds to PIP2 in vivo. In this context it is of interest that OCLR1, a lipid phosphatase that converts P(4,5)P2 to P(4)P, modulates the length of cilia in renal epithelial cells, and that loss of its function in Lowe syndrome is associated with progressive renal malfunction (Rbaibi et al., 2012).
Our structural and simulation data also reveal a cholesterol binding site on the outer-leaflet-facing surface of the PC2 molecule. This seems to be close to the site suggested to interact with a phospholipid (modeled as phosphatidic acid; see PDB: 2MKF and 2MKE) in the study of Wilkes et al. (2017). It is distinct from but adjacent to the sites interpreted as corresponding to CHS in the latter study. Examination of structures (Figure 10B) of a number of other TRP channels (e.g., TRPM2 and TRPML3) and more distantly related channels (e.g., Kv10.1) reveals multiple cholesterol interaction sites between the VSLD/VSD and the pore domain. Although interactions of TRP channels with cholesterol have not been studied in detail, some physiological data are available (Morales-Lazaro and Rosenbaum, 2017). For PC2, mutations of two of the residues forming the cholesterol binding site (Leu517Arg and Leu656Trp) are classified as “likely pathogenic” and “likely hypomorphic,” respectively (see http://pkdb.mayo.edu). It is tempting to suggest the likely biological importance of cholesterol interactions with PC2. For example, cholesterol plays a key role in signaling via the ciliary GPCR Smoothened, and it is possible that different regions of cilia differ in the cholesterol content of their membranes (Luchetti et al., 2016). However, further biochemical studies are needed to establish a functional role of cholesterol in regulation of PC2 in ciliary membranes. It is also interesting to note that pregnenolone sulfate has been identified as an activator of PC2, in the presence of other TRP channels (Kleene et al., 2019). Given the similarity between the structures of cholesterol and pregnenolone, we speculate that the two molecules could occupy the same binding site, placing the sulfate on the extracellular surface, near the pore, thus activating the channel. Given the emerging importance of cholesterol interactions with other ion channels and with receptors (Bukiya and Rosenhouse-Dantsker, 2017; De Jesus-Perez et al., 2018; Duncan et al., 2019; Lee, 2018) it would not be surprising if cholesterol interactions with TRP and related channels were of functional importance.

Overall, the structural and simulation results presented here suggest that further studies of possible functional effects of PIPs and/or of cholesterol on PC2 channels are merited. From a broader perspective, this study demonstrates that cryo-EM and MD together provide a powerful combination for revealing lipid interactions of ion channels, enabling identification of the molecular identity and interactions of lipid-like densities observed in structures. This is of particular importance when the resolution of cryo-EM maps is such that possible lipid molecules are difficult to identify unambiguously. Our results help to establish that the interactions of TRP channels with lipids will enable definition of novel druggable sites on this physiologically important class of channel molecules.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.str.2019.11.005.

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Figure 10. Comparison of Lipid Binding Sites of TRP and Kv Channels

(A) Anionic lipid (blue acyl tails and red phosphate oxygens) binding sites close to the S4 linker: TRPV1 and PI (PDB: 5IRZ), TRPV5 and PIP2 (PDB: 6DMU), Kv1.2/Kv2.1 chimera and PG (PDB: 2R9R), and PC2 and PIP2 (this study).

(B) Cholesterol (green carbons and red oxygens) binding sites between the VSD/VSLD and the central pore domains of: TRPM2 (PDB: 6C07), TRPML3 (PDB: 5W3S), Kv10.1 (PDB: 5K7L), and PC2 (this study). In each case the transmembrane region of the channel structure is shown, with the location of the lipid bilayer shown via the broken gray lines.
AUTHOR CONTRIBUTIONS

Q.W., M.G., C.N., A.B., and A.C.W.P. conducted the experiments and analyzed the data. R.A.C., G.H., and P.A. provided and advised on simulation methods. Q.W., J.S., E.P.C., and M.S.P.S. designed the project. E.P.C. and M.S.P.S. wrote the paper, and all authors contributed to revisions of the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Abraham, M.J., Murtola, T., Schulz, R., Páll, S., Smith, J.C., Hess, B., and Lindahl, E. (2015). GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1–2, 19–25.

Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W., et al. (2010). PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221.

Autzen, H.E., Myasnikov, A.G., Campbell, M.G., Asarnow, D., Julius, D., and Cheng, Y.F. (2018). Structure of the human TRPM4 ion channel in a lipid nanodisc. Science 359, 228–232.

Bagal, S., Brown, A.D., Cox, P.J., Omoto, K., Owen, R.M., Pryde, D.C., Sidders, B., Skerratt, S.E., Stevens, E.B., Storer, R.L., et al. (2013). Ion channels as therapeutic targets: a drug discovery perspective. J. Med. Chem. 56, 593–624.

Basak, S., Schmandt, N., Gicheru, Y., and Chakrapani, S. (2017). Crystal structure and dynamics of a lipid induced potential desensitized-state of a pentameric ligand-gated channel. Elife 6, https://doi.org/10.7554/eLife.23886.

Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., DiNola, A., and Haak, J.R. (1984). Molecular dynamics with coupling to an external bath. J. Chem. Phys. 81, 3684–3690.

Best, R.B., Zhu, X., Shim, J., Lopes, P.E.M., Mittal, J., Feig, M., and Mackerell, A.D. (2012). Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone phi, psi and side-chain chi(1) and chi(2) dihedral angles. J. Chem. Theory Comput. 8, 3257–3273.

Boucher, C., and Sandford, R. (2004). Autosomal dominant polycystic kidney disease (ADPKD, MIM 173900, PKD1 and PKD2 genes, protein products PIP2 activation and thermosensation in TRP channels. Proc. Natl. Acad. Sci. U S A 101, 1233–1241.

Brauchi, S., Orta, G., Mascayano, C., Salazar, M., Raddatz, N., Urbina, H., Rosenmann, E., Gonzalez-Nilo, F., and Latore, R. (2007). Dissection of the components for PIP2 activation and thermosensation in TRP channels. Proc. Natl. Acad. Sci. U S A 104, 10246–10251.

Bukiya, A.N., and Rosenhouse-Dantsker, A. (2017). Synergistic activation of G protein-gated inwardly rectifying potassium channels by phospholipid fatty acids modulate the calcium-activated chloride channel TMEM16A (ANO1). Biochim. Biophys. Acta 1863, 299–312.

Daniels, R.L., Takashima, Y., and McKney, D.D. (2009). Activity of the neuronal cold sensor TRPM8 is regulated by phospholipase C via the phospholipid phosphoinositide 4,5-bisphosphate. J. Biol. Chem. 284, 1570–1582.

Dawaliby, R., Trubbia, C., Delporte, C., Masureeri, M., Van Antwerpen, P., Koblika, B.K., and Govaerts, C. (2016). Allosteric regulation of G protein-coupled receptor activity by phospholipids. Nat. Chem. Biol. 12, 35–39.

De Jesus-Perez, J.J., Cruz-Rangel, S., Espino-Saldana, A.E., Martinez-Torres, A., Qu, Z.Q., Hartzell, H.C., Corral-Fernandez, N.E., Perez-Cornejo, P., and Arreola, J. (2018). Phosphatidylinositol 4,5-bisphosphate, cholesterol, and fatty acids modulate the calcium-activated chloride channel TMEM16A (ANO1). Biochim. Biophys. Acta 1853, 299–312.

de Jong, D.H., Singh, G., Bennett, W.F.D., Amarez, C., Wassenaar, T.A., Schafer, L.V., Periolo, X., Tieleman, D.P., and Marrink, S.J. (2013). Improved parameters for the Martini coarse-grained protein force field. J. Chem. Theory Comput. 9, 687–697.

Dellinger, M., Indzhylukian, A.A., Liu, X., Li, Y., Xie, T., Corey, D.P., and Clapham, D.E. (2016). Primary cilia are not calcium-responsive mechanosensors. Nature 537, 656–660.

Domanjki, J., Hediger, G., Best, R., Stansfeld, P.J., and Sansom, M.S.P. (2017). Convergence and sampling in determining free energy landscapes for membrane protein association. J. Phys. Chem. B 121, 3364–3375.

Duan, J., Li, Z., Li, J., Hulse, R.E., Santa-Cruz, A., Valinsky, W.C., Abria, S.A., Krapivinsky, G., Zhang, J., and Clapham, D.E. (2016a). Structure of the mammalian TRPM7, a magnesium channel required during embryonic development. Proc. Natl. Acad. Sci. U S A 113, EB201–EB210.

Duan, J.J., Li, Z.L., Li, J., Santa-Cruz, A., Sanchez-Martinez, S., Zhang, J., and Clapham, D.E. (2018b). Structure of full-length human TRPM4. Proc. Natl. Acad. Sci. U S A 115, 2377–2382.

Duncan, A.L., Song, W., and Sansom, M.S.P. (2019). Lipid-dependent regulation of ion channels and GPCRs: insights from structures and simulations. Annu. Rev. Pharmacol. Toxicol. 60, https://doi.org/10.1146/annurev-pharmaco-010919-023411.

Dunning, J., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132.

Emsley, P., and Cowtan, K. (2010). Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132.

Essmann, U., Perera, L., Berkowitz, M.L., Darden, T., Lee, H., and Pedersen, L.G. (1995). A smooth particle mesh Ewald method. J. Chem. Phys. 103, 8577–8593.

Fan, C., Choi, W., Sun, W.N., Du, J., and Lu, W. (2018). Structure of the human lipid-gated cation channel TRPC3. Elife 7, https://doi.org/10.7554/eLife.36852.

Fine, M., Schmiege, L., and Li, X.C. (2018). Structural basis for PtdIns(2)-mediated TRPM1 regulation. Nat. Commun. 9, 4192.

Fiser, A., and Sali, A. (2003). Modeller: generation and refinement of homology-based protein structure models. Methods Enzymol. 374, 461–491.

Gao, Y., Cao, E.H., Julius, D., and Cheng, Y.F. (2016). TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. Nature 534, 347–351.

Garcia, G., Ralegh, D.R., and Reiter, J.F. (2018). How the ciliary membrane is organized inside-out to communicate outside-in. Curr. Biol. 28, R421–R434.

Gosse, J.E., and Sansom, M.S.P. (2013). Reduced lateral mobility of lipids and proteins in crowded membranes. PLoS Comput. Biol. 9, e1003033.

Gout, A.M., Martin, N.C., Brown, A.F., and Ravine, D. (2007). PKDb: polycystic kidney disease mutation database—a gene variant database for autosomal dominant polycystic kidney disease. Hum. Mutat. 28, 654–659.
Griepen, M., Pike, A.C.W., Shintre, C.A., Venturi, E., El-Ajouz, S., Tessitore, A., Shrestha, L., Mukhopadhyay, S., Mahajan, P., Chulk, R., et al. (2017). Structure of the polycystic kidney disease TRP channel Polycystin-2 (PC2). Nat. Struct. Mol. Biol. 24, 114–122.

Hansen, S.B. (2016). Lipid agonism: the PI(4,5)P_2 paradigm of ligand-gated ion channels. Biochim. Biophys. Acta 1851, 620–628.

Hansen, S.B., Tao, X., and MacKinnon, R. (2011). Structural basis of PI(3)P_2 activation of the classical inward rectifier K+ channel Kir2.2. Nature 477, 495–498.

Hediger, G., Koldso, H., Chavent, M., Siebold, C., Rohatgi, R., and 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.

Hediger, G., Rouse, S.L., Domański, J., Chavent, H., Koldso, H., and 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.

Hediger, G., and Sansom, M.S.P. (2018). Lipid interaction sites on channels, transporters and receptors: recent insights from molecular dynamics simulations. Biochim. Biophys. Acta 1858, 2390–2400.

Hess, B., Bekker, H., Berendsen, H.J.C., and Fraaije, J.G.E.M. (1997). LINCS: a linear constraint solver for molecular simulations. J. Comput. Chem. 18, 1465–1472.

Hille, B. (2001). Ionic Channels of Excitable Membranes (Sinauer Associates Inc), p. 814.

Hirsch, M., Herzik, M.A., Wie, J.H., Suo, Y., Borschel, W.F., Ren, D.J., Lander, G.C., and Lee, S.Y. (2017). Cryo-electron microscopy structure of the lysosomal calcium-permeable channel TRPML3. Nature 550, 411–414.

Hol, W.G.J., van Duijnen, P.T., and Berendsen, H.J.C. (1997). LINCS: a linear constraint solver for molecular simulations. J. Comput. Chem. 18, 1465–1472.

Hille, B. (2001). Ionic Channels of Excitable Membranes (Sinauer Associates Inc), p. 814.

Hirsch, M., Herzik, M.A., Wie, J.H., Suo, Y., Borschel, W.F., Ren, D.J., Lander, G.C., and Lee, S.Y. (2017). Cryo-electron microscopy structure of the lysosomal calcium-permeable channel TRPML3. Nature 550, 411–414.

Hol, W.G.J., van Duijnen, P.T., and Berendsen, H.J.C. (1997). LINCS: a linear constraint solver for molecular simulations. J. Comput. Chem. 18, 1465–1472.

Huang, J., Rauscher, S., Nawrocki, G., Ren, D.J., Melo, M.N., van Eerden, F.J., Arnarez, C., Lopez, C.A., Ingolfsson, H.I., Melo, M.N., van Eerden, F.J., Amare, C., Lopez, C.A., Wassenaar, T.A., Perie, X., de Vries, A.H., Telemans, D.P., and Marrink, S.J. (2014). Lipid organization of the plasma membrane. J. Am. Chem. Soc. 136, 14554–14559.

Jin, P., Bulykey, D., Guo, Y.M., Zhang, W., Guo, Z.H., Huyhn, W., Wu, S.P., Meltzer, S., Cheng, T., Jan, L.Y., et al. (2017). Electron cryo-microscopy structure of the mechanotransduction channel NOMPC. Nature 547, 118–122.

Jorgensen, W.L., Chandresekhar, J., Madura, J.D., Impey, R.W., and Klein, M.L. (1983). Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935.

Kimanius, D., Forsberg, B.O., Scheres, S.H.W., and Lindahl, E. (2016). Accelerated cryo-EM structure determination with parallelisation using GPUs in RELION-2. Elife 5, e18722.

Kleene, S.J., Siroky, B.J., Ladero-Figueroa, J.A., Dixon, B.P., Pachciarz, N.W., Lu, L., and Kleene, N.K. (2019). The TRP2P2-dependent channel of renal primary cilia also requires TRPM3. PLoS One 14, e0214053.

Klein, R.M., Ufret-Vincenty, C.A., Hua, L., and Gordon, S.E. (2008). Determinants of molecular specificity in phosphoinositide regulation. J. Biol. Chem. 283, 26208–26216.

Klimovich, P.V., Shirts, M.R., and Mobley, D.L. (2015). Guidelines for the analysis of free energy calculations. J. Comput. Aided Mol. Des. 29, 397–411.

Koldse, H., Shorthouse, D., Hélène, J., and Sansom, M.S.P. (2014). Lipid clustering correlates with membrane curvature as revealed by molecular simulations of complex lipid bilayers. PLoS Comput. Biol. 10, e1003911.

Koulen, P., Cai, Y.Q., Geng, L., Maeda, Y., Nishimura, S., Witzgall, R., Ehrlich, B.E., and Somlo, S. (2002). Polycystin-2 is an intracellular calcium release channel. Nat. Cell Biol. 4, 191–197.

Kruze, M., Hammond, G.R.V., and Hille, B. (2012). Regulation of voltage-gated potassium channels by PI(4,5)P_2. J. Gen. Physiol. 140, 189–205.

Kucukelbir, A., Sigworth, F.J., and Tagare, H.D. (2014). Quantifying the local resolution of cryo-EM density maps. Nat. Methods 11, 63–65.

Lacín, E., Aryan, P., Glaaser, I., Bodhinathan, K., Tasi, E., Marsh, N., Tucker, S.J., Sansom, M.S.P., and Slesinger, P.A. (2017). Dynamic role of the tether helix in PI(4,5)P_2-dependent gating of a neuronal GIRK potassium channel. J. Gen. Physiol. 149, 799–811.

Laverty, D., Desai, R., Uchański, T., Masulis, S., Stec, W.J., Malinauskas, T., Zivanov, J., Pardon, E., Steyaert, J., Miller, K.W., et al. (2019). Cryo-EM structure of the human α1(3)-3 GABA_A receptor in a lipid bilayer. Nature 565, 516–520.

Lee, A.G. (2018). A database of predicted binding sites for cholesterol on membrane proteins, deep in the membrane. Biophys. J. 115, 522–532.

Lee, J., Cha, S.K., Sun, T.J., and Huang, C.L. (2005). PI(3)P activates TRPV5 and releases its inhibition by intracellular Mg_2+. J. Gen. Physiol. 126, 439–451.

Levitan, I., Singh, D.K., and Rosenhouse-Dantsker, A. (2014). Cholesterol binding to ion channels. Front. Physiol. 5, 65.

Liao, M.F., Cao, E.H., Julius, D., and Cheng, Y.F. (2013). Structure of the TRPV1 ion channel determined by electron cryo-microscopy. Nature 504, 107–112.

Liu, D., and Liman, E.R. (2003). Intracellular Ca_2+ and the phospholipid PI(4,5)P_2 regulate the taste transduction ion channel TRPM5. Proc. Natl. Acad. Sci. U S A 100, 15160–15165.

Liu, X.W., Vien, T., Duan, J.J., Sheu, S.H., DeCaen, P.G., and Clapham, D.E. (2018). Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium. Elife 7, e33183.

Long, S.B., Campbell, E.B., and MacKinnon, R. (2005). Voltage sensor of Kv1.2: structural basis of electromechanical coupling. Science 309, 903–908.

Long, S.B., Tao, X., Campbell, E.B., and MacKinnon, R. (2007). Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. Nature 450, 376–382.

Lucchini, G., Sircar, R., Kong, J.H., Nachtergaele, S., Sagner, A., Byrne, E.F.X., Covey, D.F., Siebold, C., and Rohatgi, R. (2016). Cholesterol activates the G-protein coupled receptor Smoothened to promote Hedgehog signaling. Elife 5, e20304.

Manna, M., Niemela, M., Tynkkynen, J., Javanainen, M., Kulig, W., Muller, D.J., Rog, T., and Vattulainen, I. (2016). Mechanism of allosteric regulation of beta2-adrenergic receptor by cholesterol. Elife 5, https://doi.org/10.7554/eLife.18432.

McGorlick, D.L., Green, B.K.O., Stotz, C., Shire, M., and Volkman, B.M. (2018). Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935.

Marrink, S.J. (2008). The MARTINI coarse grained force field: extension to proteins. J. Chem. Theory Comput. 4, 516–520.

Moran, M.M. (2018). TRP channels as potential drug targets. Annu. Rev. Pharmacol. Toxicol. 58, 309–329.
Aphiphosoinositide code for primary cilia. Dev. Cell 34, 379–380.
Nauli, S.M., Alenghat, F.J., Luo, Y., Williams, E., Vassilev, P., Lil, X.G., Elia, A.E.H., Lu, W.N., Brown, E.M., Quinn, S.J., et al. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat. Genet. 33, 129–137.
Niemelä, P.S., Miettinen, M.S., Montecell, L., Hammaren, H., Bjelkmar, P., Murtoa, T., Lindahl, E., and Vattulainen, I. (2010). Membrane proteins diffuse as dynamic complexes with lipids. J. Am. Chem. Soc. 132, 7574–7575.
Nilius, B., and Owsianik, G. (2010). Transient receptor potential channelopas.
Pflugers Arch. 460, 437–450.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Oates, J., and Watts, A. (2011). Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr. Opin. Struct. Biol.
Yang, F., Vu, S., Yarov-Yarovoy, V., and Zheng, J. (2016). Rational design and validation of a vanilloid-sensitive TRPV2 ion channel. Proc. Natl. Acad. Sci. U S A 113, E3657–E3666.

Yang, F., Xiao, X., Cheng, W., Yang, W., Yu, P.L., Song, Z.Z., Yarov-Yarovoy, V., and Zheng, J. (2015). Structural mechanism underlying capsaicin binding and activation of the TRPV1 ion channel. Nat. Chem. Biol. 11, 518–524.

Yen, H.Y., Hoi, K.K., Liko, I., Hedger, G., Horrell, M.R., Song, W.L., Wu, D., Heine, P., Warne, T., Lee, Y., et al. (2018). PtdIns(4,5)P-2 stabilizes active states of GPCRs and enhances selectivity of G-protein coupling. Nature 559, 424–427.

Yin, Y., Le, S.C., Hsu, A.L., Borgnia, M.J., Yang, H., and Lee, S.-Y. (2019). Structural basis of cooling agent and lipid sensing by the cold-activated TRPM8 channel. Science 363, 945.

Zhang, Z., Okawa, H., Wang, Y.Y., and Liman, E.R. (2005). Phosphatidylinositol 4,5-bisphosphate rescues TRPM4 channels from desensitization. J. Biol. Chem. 280, 39185–39192.

Zhang, Z., Toth, B., Szollosi, A., Chen, J., and Csanady, L. (2018). Structure of a TRPM2 channel in complex with Ca2+ explains unique gating regulation. Elife 7, e36409.

Zheng, S.Q., Palovcak, E., Armache, J.P., Verba, K.A., Cheng, Y.F., and Agard, D.A. (2017). MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 331–332.

Zheng, W., Yang, X.Y., Hu, R.K., Cai, R.Q., Hofmann, L., Wang, Z.F., Hu, Q.L., Liu, X., Bulkey, D., Yu, Y., et al. (2018). Hydrophobic pore gates regulate ion permeation in polycystic kidney disease 2 and 2L1 channels. Nat. Commun. 9, 2302.

Zubcevic, L., Herzik, M.A., Chung, B.C., Liu, Z.R., Lander, G.C., and Lee, S.Y. (2016). Cryo-electron microscopy structure of the TRPV2 ion channel. Nat. Struct. Mol. Biol. 23, 180–186.
## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Chemicals, Peptides and Recombinant Proteins** | | |
| n-Dodecyl-β-D-Maltopyranoside (DDM) | Anatrace | Cat# D310S |
| n-Undecyl-β-D-Maltopyranoside (UDM) | Anatrace | Cat# U300LA |
| SF-900™ II SFM | Thermo Fisher Scientific | Cat# 10902088 |
| Insect-XPRESSSTM Protein-free Insect Cell Medium | Lonza | Cat# BE12-730Q |
| α-L-Fucosidase from bovine kidney | Sigma-Aldrich | Cat# F5884 |
| Bovine Serum Albumin | Sigma-Aldrich | Cat# A7030 |
| DYKDDDK Tag Monoclonal Antibody (FG4R), HRP | Thermo Fisher Scientific | Cat# MA1-91878-HRP; RRID: AB_2537626 |
| 18:0-20:4 PI(4,5)P₂ | Avanti Polar Lipids, Inc. | Cat# 850165 |
| 18:1 PI(3,5)P₂ | Avanti Polar Lipids, Inc. | Cat# 850154 |
| **Critical Commercial Assays** | | |
| Superose 6 Increase 10/300 GL | GE Healthcare | Cat# 29-0915-96 |
| PIP Strips™ Membranes | Thermo Fisher Scientific | Cat# P23751 |
| TALON® Metal Affinity Resin | Clontech Laboratories, Inc. | Cat# 635504 |
| **Bacterial and Virus Strains** | | |
| MAX Efficiency DH10Bac™ | Thermo Fisher Scientific | Cat# 10361012 |
| **Deposited Data** | | |
| TRPV1 structure | (Gao et al., 2016) | PDB: 5IRZ |
| TRPV5 structure | (Hughes et al., 2018b) | PDB: 6DMU |
| Kv1.2/Kv2.1 structure | (Long et al., 2007) | PDB: 2R9R |
| TRPM2 structure | (Zhang et al., 2018) | PDB: 6C07 |
| TRPML3 structure | (Hirschi et al., 2017) | PDB: 5WGS |
| Kv1.0.1 structure | (Whicher and MacKinnon, 2016) | PDB: 5K7L |
| Coordinates of human PC2 with 18:0-20:4 PI(4,5)P₂ | This study | PDB: 6T9N |
| Coordinates of human PC2 with 18:1 PI(3,5)P₂ | This study | PDB: 6T9O |
| Cryo-EM map of human PC2 with 18:0-20:4 PI(4,5)P₂ | This study | EMDB: 10418 |
| Cryo-EM map of human PC2 with 18:1 PI(3,5)P₂ | This study | EMDB: 10419 |
| **Experimental Models: Cell Lines** | | |
| Spodoptera frugiperda (Sf9) insect cells | Thermo Fisher Scientific | Cat# 11496015 |
| **Recombinant DNA** | | |
| Human PC2: 185-723 cloned into the expression vector pFBCT10HF-LIC | Addgene | Plasmid #98226 |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Primer:hPC2-185 Forward: | Eurofin Genomics | N/A |
| | TTAAGAAGGAGATATACATATGCCCCGAGTG GCTGGGCG | |
| Primer:hPC2-723 Reverse: | Eurofin Genomics | N/A |
| | GATTGGAAGTAGAGGTTCTCTGCATCCACG GTATTTTTTTCAGT | |
| **Software and Algorithms** | | |
| GROMACS | Abraham et al., 2015 | www.gromacs.org |
| VMD | Humphrey et al., 1996 | https://www.ks.uiuc.edu/Research/vmd/ |
| PyMOL | DeLano, 2002 | https://pymol.org/2/ |
| MotionCor2 | Zheng et al., 2017 | https://emcore.ucsf.edu/cryo-EM-software |

(Continued on next page)
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contacts, Mark Sansom (mark.sansom@bioch.ox.ac.uk). All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell Culture for Protein Expression
PC2 proteins (a truncated stable construct, hPC2: Pro185 - Asp723) was expressed and purified from baculovirus infected Spodoperta frugiperda (Sf9) insect cells, grown in Sf900II serum free, protein-free insect cell medium with L-glutamine (Thermo Fisher Scientific).

METHOD DETAILS

Simulation Model Preparation
N-acetyl-D-glucosamines were removed from all protein structures, and missing side chains and loops were modelled using MODELLER version 9v10 (Fiser and Sali, 2003). Models were visually inspected. Structurally realistic models with the lowest value of the MODELLER objective function were chosen for subsequent simulations.

CG Simulations
CGMD simulations were performed using GROMACS version 4.6 (www.gromacs.org) (Pronk et al., 2013) with the MARTINI version 2.1 force field (Monticelli et al., 2008). CG simulations within PMF calculations were done with GROMACS version 5.1 (www.gromacs.org) (Abraham et al., 2015) with the MARTINI version 2.2 force field (de Jong et al., 2013). An elastic network (Periole et al., 2009) was applied with elastic bond force constant of 500 kJ/mol/nm² and an upper elastic bond cut-off of 0.9 nm. The standard MARTINI water beads with van der Waals radii of 0.21 nm were used to solvate all systems, which were then neutralised with NaCl at a concentration of 0.15 M. CG lipids and ions were described by MARTINI version 2.0 lipids and ions respectively.

Each simulation system contains only one copy of a protein. Initially, PC lipids were randomly placed around the proteins. Correct positioning of protein in a lipid bilayer was achieved by a 100 ns self-assembly simulation (Scott et al., 2008). The CG system for PDB ID: 5K47 was equilibrated for 400 ns after the initial self-assembly. Simulation systems for the other PC2 structures (i.e. PDB ID: 5MKF and 5T4D) were set up by aligning and replacing the protein in the PDB 5K47 POPC-only system, and then equilibrating for 1 μs. Systems used for mixed-lipid simulations were set up by exchanging PC molecules for other lipids using a locally developed script.

All CGMD simulations were performed at a temperature of 310 K and a pressure of 1 bar. V-rescale thermostat (Bussi et al., 2007) was used to maintain the temperature using a coupling time constant of τ_v = 1 ps. Protein, lipids, and solvent (water + ions) were coupled separately to the temperature bath. For self-assembly and equilibration simulations, pressure was controlled by semi-isotropic pressure coupling with the Berendsen barostat (Berendsen et al., 1984) with a compressibility of 5 × 10⁻⁹/bar and a time constant of τ_p = 4 ps. Production runs used a Parrinello-Rahman barostat with coupling constant of 12 ps and compressibility of 3 × 10⁻⁴/bar for pressure control.
**Atomistic Simulations**

These were setup and run using a two-step multiscale procedure (Stansfeld et al., 2015; Stansfeld and Sansom, 2011), starting with a CG simulation to stably insert the protein in a lipid bilayer (see above), followed by conversion to an atomistic representation and subsequent atomistic simulations. A fragment based protocol (Stansfeld and Sansom, 2011) was used for the CG to atomistic conversion. For the PDB 5K47 system in a PC-only bilayer, the final frame of the 400 ns equilibration was converted to atomistic representation. Three repeats of 200 ns atomistic simulations with different initial random seeds were performed after a 6 ns unrestrained equilibration. Simulations were carried out using GROMACS version 4.6 with the CHARMM36 force field (Best et al., 2012). The water model used was TIP3P (Jorgensen et al., 1983). Atomistic systems for PDBs 5MKF and 5T4D were converted from the final frame of the 1 μs CG equilibration runs. Three repeats of 250 ns atomistic simulations were done for both structures after 1ns fully restrained equilibration.

Simulation systems of PC2 structures with bound cholesterol were setup using the CHARMM-GUI (www.charmm-gui.org). The protein with bound cholesterols were embedded in POPC-only bilayer. Simulations were performed using GROMACS version 5.1 with the CHARMM36m (Huang et al., 2017) force field and TIP3P water model (Jorgensen et al., 1983). Equilibration of the system was done in six steps with gradually decreasing restraint force constants on the protein and cholesterol molecules. Three repeats of 250 ns unrestrained atomistic simulations were then performed.

For all atomistic MD simulations, the long-range electrostatics (< 1 nm) was modelled with the Particle Mesh Ewald (PMF) method (Essmann et al., 1995). Temperature coupling was done with V-scale thermostat at 310 K. The Parrinello-Rahman barostat (Parrinello and Rahman, 1981) with a reference pressure of 1 bar and a compressibility of 4.5 × 10^{-5}/bar was applied for pressure control. Covalent bonds are constrained to their equilibrium length by the LINCS algorithm (Hess et al., 1997). The integration steps of all simulations were set to 2 fs.

**PMF Calculations**

For PC2 system, protein truncation was done on the equilibrated PIP2 and PC systems. Equilibrations were performed with unbiased MD simulations for 100 ns. Position restraints with a force constant of 1000 kJ/mol/nm² were used to prevent protein translation during equilibration.

For PC2, the reaction coordinates were defined as the distance between the COM of Ser505 and Ser591 of PC2 and the whole head group of PC, PS (N*, PO* and GL* beads), PIP, PIP2 and PIP3 (RP*, PO* and GL* beads). 38 umbrella sampling windows were evenly spaced on the reaction coordinates between -0.7 and 3 nm with a force constant of 500 kJ/mol/nm². Position restraints (400 kJ/mol/nm² in the xy plane) were applied to the backbone beads of Ser505 and Ser591 in each subunit to prevent rotation and translation of the protein. The reaction coordinates are approximately parallel to the X axis of the simulation box and a weak positional restraint of 100 kJ/mol/nm² was applied to the corresponding pulling groups of the lipids to limit its movements along the Y axis.

The PLUMED2 package (Tribello et al., 2014) was used to define the reaction coordinates and apply biasing to pull the lipids. WHAM was used to unbias all umbrella sampling simulations. For systems of all PIPs, the dissociation study was done on one of the four bound lipid molecules. For PC system, the protein was placed in pure PC bilayers, and one PC molecule situated in one of the binding sites was chosen to be pulled away from the proteins. For the PS system, the pulled PC in the PC system was replaced with a PS molecule, and a similar pulling protocol was applied. Simulations were run for 3 μs per window for all PIP systems and for 2 μs for the PC and PS systems. To calculate the energies, the first 800 ns of each window was discarded for all PIP systems, and the first 500 ns for the PC and PS systems.

**Free Energy Perturbation Calculations**

CG FEP calculations were performed as described in (Corey et al., 2019), using 21 x 250 ns windows evenly spaced along the reaction coordinate, with 3 repeats run per system. Energies were computed on the final 225 ns of each window using multistate Bennett acceptance ratio, as implemented with alchemical analysis (Klimovich et al., 2015).

**Simulation Visualisation and Analysis**

Protein structures were visualised with VMD (Humphrey et al., 1996) or PyMOL (DeLano, 2002). Simulation trajectories were processed using GROMACS. Averaged lipid density maps were generated using the VMD Volmap plugin tool with three-dimensional grids every 0.1 nm.

**Protein Expression and Purification**

The human PKD2 gene which encodes the PC2 (polycystin-2, PC2 or TRPP1) protein was purchased from the Mammalian Gene Collection (MGD, 138446; IMAGE, 8327731, BC112263). A truncated stable construct (hPC2: Pro185 - Asp723) was used, with a C-terminal purification tag containing a TEV cleavage site, a His₉ sequence, and a FLAG tag, was cloned into the expression vector pFB-CT10HF-LIC (available from The Addgene Nonprofit Plasmid Repository). DH10Bac competent cells were used for the production of baculovirus. Recombinant baculoviruses were used to infect Spodoptera frugiperda (Sf9) insect cells, grown in 250 mL suspension in SF9001 serum free, protein-free insect cell medium with L-glutamine (Thermo Fisher Scientific) at 27 °C, when cell density reached ~2 × 10^6/ml for virus amplification at 27 °C in 1 L shaker flasks. 1 L of Sf9 insect cells in Insect-XPRESS Protein-free Insect Cell Medium with L-glutamine (Lonza) in a 3 L flask was infected with 5 ml of the harvested P2 (second passage) viruses for 65 h at 27 °C. Cells were harvested 65 h post-transduction by centrifugation for 15 min at 1500 g and 4 °C.

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Extraction buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 20 mM CaCl$_2$, 5% glycerol and Roche protease-inhibitor cocktail was used to re-suspend the cell culture pellets to a volume of 50mL/L. Cells were lysed on ice with VCX 750 sonicator and 13mm probe (PRO Scientific Inc.) for 5 min, 3 sec on, 12 sec off and 35% amplitude. 1% (w/v) DDM was added to the cell lysis and incubated for 1 h at 4°C. Cell debris was removed by centrifugation for 1h at 35,000g and 4°C. To purify by immobilised metal affinity chromatography, the detergent solubilised protein was batch bound to Co$^{2+}$-charged Talon resin (Clontech) by gentle rotation at 4°C for 1 h. The resin was washed with 15 column volumes of extraction buffer supplemented with 0.01% DDM and 30 mM imidazole, pH 8.0 and, to exchange the detergent, with another 15 column volumes of the same buffer replacing DDM with 0.035% UDM. For SEC, the protein was eluted from Talon resin with extraction buffer supplemented with 0.035% UDM and 400 mM imidazole. The eluted protein was divided into two batches. One was further purified via size-exclusion chromatography (SEC) with a Superose 6 increase 10/300GL column (GE Healthcare) pre-equilibrated with SEC buffer (0.035% UDM, 20 mM HEPES, pH 7.5, 200 mM NaCl and 20 mM CaCl$_2$) for PIP strip experiments. The other was buffer exchanged into SEC buffer using a PD-10 column (GE Healthcare). The protein was then treated with 0.4 units of bovine kidney α-L-fucosidase (Sigma) at 18°C, overnight. The pH was adjusted to 7 before further enzymatic treatment at a ratio of 0.75:0.5:1 (w/w/w) TEV protease, PNGase F, PC2 for another 24 h at 18°C. Reverse His-tag purification was performed to clear out the His$_6$-tagged TEV protease and uncleaved PC2. The protein was concentrated to 0.5 ml with a 100-kDa-cutoff concentrator (Vivaspin 20, Sartorius), and further purified by SEC as above.

**PIP Strip Assay**

PIP strip membranes (Thermo Fisher Scientific P23751) were blocked in 3% (w/v) fatty acid-free BSA (Sigma-Aldrich) in TBST (50 mM Tris-HCl, pH 7.5, 150 mM NaCl and 0.1% (v/v) Tween 20) for 1 h. The membranes were then incubated in the same solution with 0.5 μg/ml of detergent solubilised His$_{16}$/FLAG-tagged PC2 overnight at 4°C with gentle agitation. The membranes were washed 3 times over 30 min in fatty acid-free BSA-TBST. One more 3h-incubation with 0.5 μg/ml PC2 and subsequent wash steps were performed at 4°C. The membranes were incubated for 1h with 1:2000 dilution of HRP conjugated anti-FLAG monoclonal antibody (Thermo Fisher Scientific MA1-91878-HRP) at room temperature. Finally, the membranes were washed 6 times over 1 h in TBST, and the protein that was bound to the membrane by virtue of its interaction with phospholipid was detected by enhanced chemiluminescence.

**Cryo-EM Grid Preparation and Data Acquisition**

18:0-20:4 PI(4,5)P$_2$ and 18:1 PI(3,5)P$_2$ lipid extract (Avanti) dissolved in chloroform was dried under an argon stream. 18:0-20:4 PI(4,5)P$_2$ (0.5 mg/ml) and 18:1 PI(3,5)P$_2$ (~1 mg/ml) stock was prepared by resolubilising dried lipids in a buffer containing 20 mM HEPES (pH 7.5), and 150 mM NaCl via bath sonication for ~1h. Purified PC2 was incubated with 18:0-20:4 PI(4,5)P$_2$ at a molar ratio of 1:20 (PC2 tetramer: 18:0-20:4 PI(4,5)P$_2$) and 18:1 PI(3,5)P$_2$ at a molar ratio of 1:40 (PC2 tetramer: 18:1 PI(3,5)P$_2$) overnight at 4°C. The sample was cleared by centrifugation at 21,000g for 30 min. For cryo-EM, 3 μl of PC2 with PI(4,5)P$_2$ sample at a protein concentration of ~4 mg/ml or PC2 with PI(3,5)P$_2$ sample at a protein concentration of ~3.5 mg/ml was applied to glow-discharged Quantifoil 1.2/1.3 holey carbon 300 mesh copper grids. Grids were plunge frozen in liquid ethane using a Vitrobot Mark IV (FEI, Thermo Fisher Scientific) set to 5°C, 100% relative humidity, 3.5 s blotting time and -15 blotting force. Data for PC2 with PI(4,5)P$_2$ were collected on a Titan Krios 300-kV transmission electron microscope equipped with a post-column Gatan image filter (GIF; 200eV slit width) operating in zero-loss mode and a Gatan K2 Summit direct electron detector camera at Central Oxford Structural Molecular Imaging Centre (COSMIC). Movies were captured for 0.4 s per frame over 8 s. The calibrated pixel size and dose rate were 0.822 Å/pix and ~6.55 electrons/Å$^2$/s, respectively (total dose 52.4e/Å$^2$). Images were collected in a defocus range between -1.0 and -3.0 μm under focus. Data for PC2 with PI(3,5)P$_2$ were collected on a Titan Krios 300-kV transmission electron microscope at the Electron Bio-Imaging Centre (eBic, Diamond Light Source) equipped with a Gatan K2 Summit direct electron detector camera mounted behind a GIF and operated in zero-loss mode (0-20eV). Movies were captured for 0.2 s per frame over 7 s. The calibrated pixel size and dose rate were 0.816 Å/pix and ~6.0 electrons/Å$^2$/s, respectively (total dose 42e/Å$^2$). Images were collected in a defocus range between -1.0 and -3.1 μm under focus in 0.3 μm steps.

**Image Processing**

The beam-induced motion in the movies and frames were dose-weighted using MotionCor2 (Zheng et al., 2017). Aligned frames in each movie were averaged to produce a micrograph for further processing. The contrast transfer function (CTF) parameters were estimated using CTFIND-4.1 (Rohou and Grigorieff, 2015). Micrographs with ice contamination or poor CTF cross correlation scores were discarded and the remaining micrographs were processed using RELION 3 (Kimanius et al., 2016). Particle picking was performed using Gautomatch (URL: http://www.mrc-lmb.cam.ac.uk/kzhang/).

For the dataset of PC2 with PI(4,5)P$_2$, a set of 77,399 particles were picked from 1,597 micrographs and sorted into 2D classes. Representative 2D classes were used as templates for autopicking after low-pass filtering to 30 Å. A total of 147,001 particles were automatically picked. Three rounds of iterative 2D classification were performed in RELION to remove bad particles. A low-resolution reference model was generated ab initio. The initial model was used for the first round of 3D classification without symmetry imposed. A subset of 73,883 particles was used for subsequent 3D classification with C4 symmetry imposed. The data was then used for the first round of 3D ‘gold-standard’ refinement, which resulted in and initial reconstruction with a nominal unmasked resolution of 3.5 Å. Subsequent 3D classification was performed without further image alignments followed by iterative CTF refinement and Bayesian宁
polishing. The final subset of particles was subjected to further auto-refinement in RELION, which converged at an unmasked resolution of 3.12 Å. RELION post-processing using unfiltered half maps and a soft-edged mask to exclude the region occupied by the detergent micelle yielded a final B-factor sharpened map (-84.56Å²) with a nominal resolution of 2.96 Å (FSC=0.143).

For the dataset of PC2 with PI(3,5)P2, a set of 323,538 particles were initially picked from 3,353 micrographs and classified into 2D classes. Representative well resolved class averages were used as templates for reference-based particle picking after low-pass filtering to 30 Å. A total of 224,402 particles were automatically picked using GAUTOMATCH. Three rounds of iterative 2D classification were performed in RELION to remove bad particles. A low-resolution reference model was generated ab initio. The initial model was used for the first round of 3D classification without symmetry imposed. One further round of 3D classification with C1 symmetry was performed on a set of 97,892 particles from classes with the best estimated resolutions. A subset of 91,844 particles was used for subsequent 3D classification with C4 symmetry imposed. The data was then used for the first round of 3D ‘gold-standard’ refinement, which resulted in and initial reconstruction at a resolution of 4.1 Å. A final set of 37,297 particles was selected from 3D classification performed without further image alignment. Iterative CTF refinement and Bayesian polishing were performed prior to a final auto-refinement procedure in RELION, which converged at an unmasked resolution of 3.66 Å. Subsequent post-processing, using a soft-edged mask that excluded the detergent micelle, produced a 3.4 Å resolution map. Reported resolutions were based on a FSC threshold of 0.143. Local resolutions across the whole map were estimated using RESMAP (Figure S14) (Kucukelbir et al., 2014).

Model Building
The previously published model of apo PC2 (PDB 5K47) was used as an initial model and fitted into the cryo-EM map of lipid-bound PC2. The model was manually adjusted in Coot (Emsley and Cowtan, 2004). In the original PDB 5K47 structure, the disulphide bond between Cys311 and Cys334 appeared to be reduced. In both PIP complexes, there is clear density showing that a disulphide bond is formed between these cysteines and has been built in both structures. One spherical density corresponding to the size of a dehydrated sodium or calcium ion is clearly present below the selectivity filter in each of the PIP complexes maps with the density deeper in the central pore in PI(3,5)P2 map compared to PI(4,5)P2 map. No such density is present in the equivalent location in map EMD-8354 (corresponding to PDB id ST4D; (Shen et al., 2016)). This difference in density is likely to originate from the presence of calcium ions in our buffer which were not present in that for 5T4D/EMD-8354. Therefore, calcium ions were into the ion density in both of our structures. A series of B-factor sharpened maps were used to guide model building. Final models of both structures were globally refined and minimised in real space against the RELION3 C4 post-processed, automatically B-factor sharpened maps with NCS constraints, secondary structure and rotamer restraints imposed and no Ramachandran restraints applied using the phenix.real_space_refine module in PHENIX (Adams et al., 2010). The refinement protocol was validated by taking the final refined models, applying a random shift of up to 0.3Å to the atomic coordinates and then refining the resultant shifted model against the halfmap1. Model-to-map FSCs were calculated using phenix.mtriage against halfmap1 (FSCwork), halfmap2 (FSCfree) and the full map (FSCsum).

QUANTIFICATION AND STATISTICAL ANALYSIS
Statistical analysis and software used can be found in the relevant sections of the methods and the figure legends.

DATA AND CODE AVAILABILITY
This study did not generate new software. The simulation trajectory datasets supporting the current study have not been deposited as a public repository for MD simulation data does not yet exist. Coordinates of the models generated by this study (as representative frames from simulations revealing the interactions of PC2 with PIP2 and with cholesterol) are available from the corresponding author on request. Details of deposited coordinates and density are provided in the Key Resources Table. The accession numbers for the deposited coordinates reported in this paper are PDB: 6T9N, 6T9O.