**Molecular insights to lipid-assisted Ca²⁺-regulation
in the TRP channel Polycystin-2**

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22 **Abstract**

23 Polycystin-2 (PC2), a calcium-activated cation TRP channel is involved in diverse
24 Ca²⁺-signaling pathways. Malfunctioning Ca²⁺-regulation in PC2 triggers autosomal
25 dominant polycystic kidney disease. Here we report two cryoEM structures in distinct
26 channel states obtained from the full-length human PC2 in complex with lipids and
27 cations. The structures reveal conformational differences in the selectivity filter and in
28 the large exoplasmic domain (TOP domain) displaying differing *N*-glycosylation. The
29 more open structure complexes one cation located below the selectivity filter (single-
30 ion mode, PC2_{SI}), while multiple cations are strung along the translocation pathway in
31 the second structure (multi-ion mode, PC2_{MI}). Ca²⁺-binding at the entrance of the
32 selectivity filter suggests Ca²⁺-blockage in PC2_{MI} and we observe density for the
33 Ca²⁺-sensing C-terminal EF-hand in the unblocked PC2_{SI} state. The states show an
34 altered interaction of lipids with pore loop and TOP domain reflecting the functional
35 diversity of PC2 at different locations due different membrane compositions.

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42 Key words: Ca²⁺ signaling, cryoEM, membrane protein structure, Polycystin-2, TRP
43 channel

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45 **Introduction**

46
47 The cytoplasmic Ca^{2+} concentration of Ca^{2+} is spatially and temporally regulated by a
48 finely balanced interplay between active Ca^{2+} export and Ca^{2+} influx through Ca^{2+}
49 sensitive channels¹. The Transient Receptor Potential (TRP) channel Polycystin-2
50 (PC2)² is a nonselective cation channel that participates in a variety of different Ca^{2+} -
51 dependent signaling pathways including the Wnt pathway^{2,3}. PC2 is found in the
52 endoplasmic reticulum (ER)^{4,5}, in the primary cilium^{6,7}, in the plasma membrane^{6,8}
53 and its subcellular distribution varies greatly in different cell types. Single-channel
54 studies indicate that PC2 conducts monovalent and divalent ions including Ca^{2+} ⁹⁻¹¹.
55 In the ER, PC2 was also reported to act as a Ca^{2+} -activated Ca^{2+} release channel^{11,12}.
56 Different experimental approaches have suggested that PC2 also modulates the
57 cytosolic calcium concentration indirectly by interacting with key proteins in Ca^{2+}
58 import¹³ including TRPV4, TRPC1¹⁴ and TRPC4 at the ciliary or somatic
59 membrane¹⁵⁻¹⁷. In the ER, PC2 modulates calcium release through interaction with the
60 inositol 1,4,5-triphosphate receptor (IP₃R)¹³ or with the ryanodine receptor RyR¹².
61 The most prominent interaction partner of PC2 is Polycystin-1 (PC1) is^{18,19}. It is a
62 channel-like receptor that competitively mediates PC2 complex formation¹⁸⁻²⁰.
63 Mutations in PC2 or PC1 that affect their mutual interaction are amongst the triggers
64 of Autosomal Dominant Polycystic Kidney Disease (ADPKD)^{2,21}.
65
66 Trafficking of PC2 is controlled through a set of specific N- and C-terminal
67 sequences, and is dependent on the availability of a variety of interaction
68 partners^{12,13,16}. An N-terminal R₆VXP motif is required for relocation of PC2 to the
69 primary cilium²². *N*-glycosylation in the exoplasmic domain of loop1 between the
70 first and second transmembrane helices is essential for trafficking of PC2 to the Golgi
71 apparatus and beyond²³. Retention of PC2 in the early secretory pathway involves an
72 ER retention signal⁵ in the C-terminal domain⁵, which is adjacent to a downstream
73 Ca^{2+} binding EF-hand motif²⁴ and coiled-coil helices. While the coiled-coil regions
74 are instrumental for complex formation and hetero-oligomerization^{4,18,19,25,26}, the
75 conformation of the EF-hand motif is essential for the ability to open the full-length
76 PC2, and thus for Ca^{2+} activation.
77 The open probability of PC2 is bell-shaped between ~10 nM and ~1 mM free Ca^{2+} ²⁷.
78 Although the activation at low Ca^{2+} concentrations can be assigned to the Ca^{2+} -

sensing EF-hand, the reason for inactivation at high Ca^{2+} concentration is largely unknown²⁸.

Until recently there were no structures available for the membrane-embedded part of the channel. The accompanying paper²⁹, reports a cryoEM structure of a terminal truncated construct solved in combination with X-ray crystallography data that reveal a closed state, which we will refer to as PC2_{CL}. We report two structures of full-length human PC2 in different conformational states at 4.2Å and 4.3Å, determined by cryoEM, using the PC2_{CL} structure as an initial model. The two states are open at the selectivity filter and in complex with lipids and cations, which are not observed in PC2_{CL}. The conformational differences between the two states provide first insights into a blocking mechanism in PC2 inactivation at high Ca^{2+} concentrations and highlight the impact of the membrane composition on PC2 function. While this manuscript was under review Shen et al. reported another single-particle electron cryo-microscopy (cryoEM) structure of a N- and C-terminal truncated PC2 construct reconstituted into nanodiscs³⁰.

Results

Single-particle cryoEM of two PC2 forms — Full-length PC2 was overexpressed in HEK293 GnT I⁻ cells³¹ (Supplementary Fig 1a, b). Two to three days after induction we observed the formation of massively enlarged and highly vesiculated ER membranes, known as crystalloid ER, by electron microscopy of thin plastic sections (Supplementary Fig 1c). Focused ion beam milling (FIB-SEM)³² revealed that the crystalloid ER is a network of elongated tubes (Supplementary Fig 1d). Immunogold-labeling of thin plastic sections indicated that the majority of PC2 was in the ER, although some PC2 was also present in the plasma membrane (Supplementary Fig 1e). PC2 was purified by Strep-II tag affinity chromatography and reconstituted in amphipols A8-35. Western Blot analysis, MS-MS mass spectrometry and treatment with PNGase F confirmed the presence of full-length, N-glycosylated PC2, which was investigated by single particle cryoEM (Supplementary Fig 2). 3D classification of the cryoEM data set revealed two distinct conformational states of PC2 that were present in roughly equal proportions (Supplementary Fig 3). We performed homology-model guided *de novo* building of the trans-membrane region. Unlike the structures of the TRPV and TRPA channels,³³⁻³⁵ PC2 has a large exoplasmic

113 insertion in loop1 between S1 and S2, the TOP domain. Initial attempts to resolve the
114 structure of the TOP domain were not successful until the electron density was
115 compared with the PC2_{CL} structure²⁹, at which point it became clear that the two 3D
116 classes represented two different channel conformations. When data sets from both
117 3D classes were merged, the resulting map was ambiguous and could not be modeled
118 with an acceptable number of backbone dihedral angle outliers, even though, due to
119 the twofold larger number of particles, the nominal resolution was 3.9Å. Individual
120 processing of both forms resulted in two maps at overall resolutions of 4.2Å and
121 4.3Å, respectively (Fig 1a-d; Supplementary Fig 4). Differences between the two
122 maps were clearly visible at a high sigma level of 4 (Fig 1f,g). The models were
123 refined using phenix.real_space_refine³⁶ and the Rosetta³⁷ toolbox for structure
124 determination in cryoEM densities. The structures show the six transmembrane (TM)
125 helices, the TOP domain and the pore (Fig 1c-e, Table 1). Although both structures
126 were obtained from the full-length construct, the N- and C-terminal domain were not
127 resolved at atomic level. Cytoplasmic densities were only observed 4.3Å map. The
128 well resolved map regions corresponded to those of the truncated PC2_{CL} structure²⁹.
129 Different to PC2_{CL} several spherical non-protein densities were observed in the ion
130 translocation pathway of both maps at intensities comparable to those of the protein
131 densities, suggesting the presence of cations in the pore. The difference map revealed
132 densities in the 4.2Å map above and below the selectivity filter, whereas only one
133 such density was observed, below the selectivity filter in the 4.3Å map (Fig 1g).
134 Additional densities were found in both maps at the cytoplasmic exit of the channel.
135 In the following discussion we refer to the two PC2 structures reported here as the
136 multi-ion state, or PC2_{MI}, which was obtained from the 4.2Å map and the single-ion
137 state or PC2_{SI}, which was obtained from the 4.3Å map.

138
139 The two full-length PC2 structures as well as PC2_{CL} exhibit the characteristic TRP
140 channel architecture of six trans-membrane helices (S1-S6) and the hairpin loop (P-
141 loop) between S5 and S6 (Fig 1 c,d). In the PC2 tetramer the selectivity filter is lined
142 by Asp643, Gly642, Leu641 and together with residues in S6 they form the ion
143 translocation pathway of PC2 (Fig 2a-c). Its unique fold of the TOP domain
144 comprises three α -helices, five β -sheets and their intervening loops EL1, EL2, and
145 EL3 (Fig 1c,d). Its detailed structure is described in detail in the accompanying paper
146 ²⁹.

147 PC2_{MI} and PC2_{SI} deviate from each other in several regions of the TOP domain; in
 148 loop2 between S2 and S3; in the linker between S4 and S5; in the cytoplasmic ends of
 149 S5 and S6 and in the region of the P-loop that connects PH1 and PH2 to S5 and S6 (P-
 150 loop junctions) (Fig 1e). The PC2_{MI} and PC2_{SI} states differ from the closed state
 151 PC2_{CL} reported in the accompanying paper²⁹ in the orientation of the TOP domain
 152 and the S1-S2 and S5-S6 regions (Supplementary Fig 5c,d).
 153
 154 *Cation binding sites in PC2* – PC2_{MI} and PC2_{SI} are significantly more open at the
 155 selectivity filter and the lower gates (Fig 2c) compared to PC2_{CL} and several spherical
 156 densities are observed in the ion pathway. However, PC2_{MI} shows a restriction at
 157 Asn681, which is less pronounced in PC2_{SI} and PC2_{CL}.
 158
 159 On the basis of coordination geometry and the comparison to the X-ray structures of
 160 Ca_vAB³⁸ (pdb entry code 4MVO) (Supplementary Fig 5a) and TRPV6³⁵ (pdb entry
 161 code 5IWK), the non-protein density at the entrance of the selectivity filter of the
 162 PC2_{MI} tetramer (Fig 3a,b) may represent a hydrated Ca²⁺ ion (Ca1) as 2 mM Ca²⁺ was
 163 present throughout purification. Ca1 is coordinated by a planar arrangement of four
 164 Asp643 carboxyls (Fig 3c,d). However, given that PC2 is believed to be non-selective
 165 for cations the remaining spherical densities (Cat2-Cat4), which are missing clear
 166 coordination, might represent monovalent ions as sodium, which was present at a
 167 concentration of 75 mM throughout the purification and cryo-preservation.
 168 Nevertheless, Cat2 and Cat3 are found at positions analog to Ca²⁺ binding sites in
 169 Ca_vAB³⁸, which also shows a restriction at an analog position to Asn681. Considering
 170 the higher concentration and similar permeability of Na⁺ ions, it is possible that Na⁺,
 171 rather than Ca²⁺ ions are bound in some of the sites on the ion pathway. Potassium
 172 ions were not included in the buffer, so it is unlikely that there are K⁺ ions bound in
 173 these sites in the structure, but it is possible that K⁺ ions could occupy these sites in
 174 the cell. The four backbone carbonyl oxygens of Leu641 can coordinate a cation, such
 175 as Ca²⁺ or Na⁺, in the Ca2 site just below the selectivity filter.
 176 The selectivity filter is more open in PC2_{SI} with a pore radius of 1.7 Å at Leu641
 177 determined by the program HOLE compared to 1.4 Å in PC2_{MI} and 1.0 Å in PC2_{CL}.
 178 We observe changes in the conformation of Asp643, a residue previously discussed as
 179 a water stripper in PC2²⁸. Its interaction with Arg638, which contributes to the
 180 architecture of the P-loop, is altered and affects the pore radius (Fig 1g). Moreover,

181 the Ca1 site, which in Ca_vAB³⁸ and TRPV6³⁵ (Supplementary Fig 5a) is thought to be
 182 a high-affinity site, is not formed in PC2_{SI}. Comparing both open structures the most
 183 likely interpretation is that only PC2_{MI} represents a state in which Ca²⁺ is bound to the
 184 Ca1 site. The environments of the Cat3 and Cat4 sites in PC2_{MI} are hydrophobic and
 185 cannot be unambiguously assigned as Ca²⁺ binding sites. Similar, the elongated
 186 density below the Cat2* site in PC2_{SI} (Fig 3b) can accommodate two cations, one of
 187 which would be coordinated in the selectivity filter by the four backbone carbonyl
 188 oxygens of Leu641.
 189 Phe669 between the Cat3 and Cat4 site restricts the channel diameter. In Ca_vAB, fatty
 190 acid chains participate in an even tighter channel constriction at this point
 191 (Supplementary Fig 5b). At the channel exit, Asn681 and Ser685 coordinate a
 192 spherical density at the Cat5 site (Fig 3 inset) in PC2_{MI} and PC2_{SI}.
 193 α1, EL1, α2 and β5 in the TOP domain form a spacious, negatively charged
 194 vestibule. PC2_{MI} shows a confined entrance gate from the exoplasmic side of the
 195 membrane at Thr265 and Lys267 (Cat0, Fig 2c and Fig 3). At its narrowest point the
 196 vestibule entrance has a radius of 3.0 Å in PC2_{MI}, while it is open in PC2_{SI} and
 197 PC2_{CL}, with radii of 4.7 Å and 4.4 Å, respectively. Thus, in PC2_{MI} the access would
 198 be restricted for hydrated ions such as Ca²⁺, Na⁺ and K⁺, which have radii of 4.1 Å,
 199 3.6 Å and 3.3 Å, but would permit the passage of dehydrated ions, and thus acting
 200 like a pre-selectivity filter.
 201
 202 *Differing N-glycosylation in PC2_{MI} and PC2_{SI}* – The five potential N-glycosylation
 203 sites²³ Asn299, Asn305, Asn328, Asn362, and Asn375 are all located in EL2 or EL3.
 204 Four of five asparagine residues are N-glycosylated in PC2_{MI} (Asn299 in EL2;
 205 Asn328, Asn362, and Asn375 in EL3) and contribute to the well-ordered loop regions
 206 (Fig 1c,d, Fig 4). A disulfide bridge between Cys331 and Cys344 plus a helix
 207 segment at Gln335-Glu340 stabilize EL3 to enable its interaction with the hairpin in
 208 loop3 of the neighboring protomer (Fig 4a). This disulfide bridge is also not observed
 209 in PC2_{CL} and in PC2_{SI}, although in general PC2_{CL} is more similar to PC2_{MI} than
 210 PC2_{SI}. In PC2_{SI}, Asn305, Asn362 and Asn375 are N-glycosylated, but Asn328 is not
 211 (Fig 4b). The non-glycosylated asparagine interacts with Glu343. From the
 212 comparison with PC2_{SI} it can be inferred that a bulky glycan group at Asn305
 213 prevents the intra-tetramer interactions of loop3 as observed in PC2_{MI}. Loop3 in

214 PC2_{SI} instead interacts with EL2 in the same protomer accounting for the partly
 215 disordered TOP domain.
 216
 217 The conformational changes in the TOP domain of the two structures are associated
 218 with differing patterns of *N*-glycosylation. *N*-glycosylation of Asn305 and Asn328 are
 219 mutually exclusive; if one is glycosylated, the other is not. One explanation is that *N*-
 220 glycosylation of one site renders the other site inaccessible to glycosylation. It is an
 221 intriguing question as to whether *N*-glycosylation triggers the conformational changes
 222 between PC2_{MI} and PC2_{SI} or if the differing states might dictate the differing *N*-
 223 glycosylation patterns. Differences in *N*-glycosylation affect the conformation of β 5
 224 and the position of α 1 (Fig 4c,d). Most interactions of the TOP domain with the P-
 225 loop of the neighboring protomer are significantly different in PC2_{MI} and PC2_{SI} (Fig
 226 4c,d). In PC2_{MI}, Glu651 is in contact with Trp455 in β 5 and Gln456 with Gln622 of
 227 PH1. In PC2_{SI}, Gln622 interacts with Ser254 in α 1 and Glu651 in PH2 interacts with
 228 the TOP domain via Lys271. Gln622, Glu651 and Asn653 in the P-loop joints act as
 229 anchor points. PH1 and PH2 move closer in PC2_{MI} (Fig 4c) compared to PC2_{SI} (Fig
 230 4d), which would switch the interaction between Asp643 and Arg638 in the
 231 selectivity filter (Fig 1f,g).
 232
 233 *Lipid coordination in PC2* – Both maps show a number of elongated densities in the
 234 hydrophobic cleft between S4 of one protomer and S5-S6 of the next protomer (Fig
 235 5a). We attribute three of these densities to single-chain fatty acids. Two fatty acid
 236 chains (FA1 and 2) belong to the outer membrane leaflet and a third (FA3) to the
 237 cytoplasmic leaflet. Cholesteryl hemisuccinate (CHS), which was added during
 238 purification, fits two more compact densities. A continuous density large enough to
 239 accommodate a phospholipid molecule was found between S4, and EL3, β 5 and S6 of
 240 the adjacent protomer. In both maps, the space and head-group coordination by
 241 surrounding positive residues supports a phosphatidic acid (PA) binding. Other
 242 negatively charged head groups, e.g., PI or PG, would not fit. The observed relative
 243 positions of the lipids and fatty acids are similar to those observed in the recent
 244 structure of TRPV1 in lipid nanodiscs³⁹ (Fig 5b).
 245
 246 The PA lipid interacts extensively with the TOP domain in PC2_{SI}. His379 and Trp380
 247 in EL3 and Gln456 and Arg440 in the partly unfolded β 5 and Asn653 in the P-loop

248 interact with the phosphate group of PA (Fig 5d). This binding site is specific to
 249 PC2_{SI}, while in PC2_{MI} the PA is less tightly coordinated (Fig 5c). The extended
 250 conformation of EL3 in this form shifts His379 out of range for tight head group
 251 coordination and only Gln557 in S4 interacts with the PA glycerol backbone. The
 252 TOP domain and P-loop are not involved in lipid interactions in PC2_{MI}.
 253
 254 Phe634 in PH1 and Phe666 in S6 coordinate FA1 in the two PC2 structures. Gln630
 255 interacts with FA2 and FA1 in both states, although at slightly different position. In
 256 PC2_{MI} Phe605 interacts with FA2, while in PC2_{SI} Phe605 re-orientates and FA2 is
 257 deflected (Supplementary Fig 6a,b). FA3 is located in the cytoplasmic leaflet
 258 coordinated by Phe567, Ile571 and Phe574 in S4 and Phe667 in S6 of the adjacent
 259 protomer (Fig 5f, Supplementary Fig 6a,b).
 260
 261 Changes in the fatty acid coordination between the two open states are related to a
 262 bulky density close to S1 and S4 at the cytoplasmic side of PC2_{SI} (Fig 5e,
 263 Supplementary Fig 6c,d). This density, which is absent in PC2_{MI}, would
 264 accommodate the EF-hand motif of the C-terminal domain that harbors the Ca²⁺
 265 binding site (Fig 5f, Supplementary Fig 6d). Arg581 in the loop between S4 and the
 266 S4-S5 linker helix might provide an interaction partner for negatively charged
 267 residues in α 4 of the EF-hand. The S4-S5 linker loop has a different conformation in
 268 PC2_{MI}, accounting for changes in the coordination of Asp511. The Asp511Val
 269 mutation has been shown to cause loss of channel function in PC2 and is a well-
 270 characterized mutation found in patients with ADPKD^{2,11,21}. In PC2_{SI} Asp511 is
 271 coordinated by Lys575, while it interacts with Lys572 upon conformational changes
 272 of S4 in PC2_{MI}. A more detailed discussion on the structural impact of this and other
 273 ADPKD sequence variants can be found in the accompanying paper²⁹.
 274
 275

276 Discussion

277 *Ca²⁺ blocking in PC2* –PC2 is described as Ca²⁺-dependent Ca²⁺-permeable
 278 nonselective cation channel⁸. Ca²⁺ binding to the C-terminal EF-hand is thought to
 279 gate PC2 activation occurring at cytosolic Ca²⁺ concentrations in the nM- μ M range.
 280 Cations were only observed in the ion translocation pathway of the full-length PC2
 281 structures with the EF-hand motif, while PC2_{CL} is missing the Ca²⁺ sensing EF-hand

motif is not in complex with ions. We assume that the different channel conformations in the three structures (see Supplementary Movies 1 & 2) might reflect different activation states.

PC2 is also suggested to be a Ca^{2+} leak channel and as such conductance would depend on the Ca^{2+} concentration gradient and the permeability and/or selectivity for Ca^{2+} over other cations. A ‘multi-ion mode’ similar to PC2_{MI} was observed recently in TRPV6^{35} , which is exclusively selective for Ca^{2+} . Therefore, we propose that PC2_{MI} is a Ca^{2+} -blocked state. Ca^{2+} -binding to the highly coordinated Ca1 site (Fig 3c) might block monovalent cation currents^{40,41}, which is in good agreement with the inactivation of the channel at high Ca^{2+} concentrations³⁰. The downstream cation sites (Cat2-Cat4) would allow stepwise binding with relatively low chemical potential energy barriers between them, thus increasing conductance by a knock-off mechanism. At higher Ca^{2+} concentrations the stronger charge of the divalent Ca^{2+} ions might modulate conductance and selectivity for Ca^{2+} over monovalent ions. Ca^{2+} might block the pre-selectivity filter as well, which could be part of a Ca^{2+} regulation mechanism. In PC2_{SI} , where the Ca^{2+} sensing C-terminal EF-hand motif is observed, the pre-selectivity and the selectivity filters are significantly more open.

Lateral and apical entries in the TOP domain – In PC2_{MI} the vestibule entrance is restricted at the Cat0 site (Figs 2a,d, 3c) and would prevent the efflux of stripped water on this path. An amphipathic funnel extends from a position above the selectivity filter to the hydrophobic cavity that accommodates the PA lipid and the fatty acids (Fig 2d). This funnel might serve as a water pathway that affects the hydration states of the vestibule. It appears that the selectivity filter remains blocked as long as the excess of water is not removed from the ion translocation pore. Gln630 and FA1, which is located at the similar relative position of a phosphatidylcholine lipid in TRPV1^{39} , restrict the funnel exit suggesting a role for lipids in this potential conductance regulation (Fig 6). In PC2_{SI} the entrance gate of the vestibule is more open and a fatty acid entirely block the funnel exit (Fig 2e).

Role of lipid binding in activation – Lipids were also found in TRPV1^{39} contributing to its activation mechanism. However, lipid specificity and coordination in the two TRP channels is very different. In TRPV1 , a phosphatidylinositide (PI) binds in the same position in the cytoplasmic membrane leaflet as FA3 in PC2. While FA3 mainly

interacts with S4, PI is a competitive vanilloid antagonist of TRPV1, affecting the orientation the S4-S5 linker and stabilizes the resting state³⁹. Both open PC2 structures differ from each other and from the closed state at the S4-S5 linker (Fig 1e, Supplementary Fig 5c,d). In PC2_{SI} an interaction of the S4-S5 linker likely interacts with residues in α 4 in the EF-hand (Fig 5e,f), causing a conformational change at the lower gates reminiscent of the vanilloid-agonist binding in TRPV1³⁹. A salt-bridge between an arginine in S4 and a glutamate residue in S5 is formed in the open state of TRPV1. We do not observe a similar interaction at this exact position in PC2, but the interaction between Asp511 in S3 with Lys572 or Lys575 in S4 might stabilize the open conformation of the lower gates (Fig 5f).

In the exoplasmic leaflet, the phosphatidylcholine lipids bound to TRPV1 are loosely coordinated, while PA at the same position in PC2 forms a tight tripartite complex with the P-loop and the TOP domain (Fig 5c,d). In TRPV1, docking of the DkTx toxin is supported by lipid-interactions and its binding opens the upper gates. In PC2, a stretch of EL3 from the fifth *N*-glycosylation site Asn375, which is only present in human PC2²³ up to Gly390, enters the hydrophobic cavity. This region of the TOP domain, together with the PA lipid might be involved in stabilizing the open PC2_{SI} state. The PA-TOP domain complex establishes the more extended P-loop conformation in PC2_{SI} (Fig 4d) and ultimately triggers an opening of the selectivity filter. PA was not added during purification. Being the main precursor for lipid biosynthesis, PA is present only at low steady-state levels in the ER. Its removal from the biosynthetic equilibrium by high expression levels of PC2 would therefore affect the ER lipid pool in many ways. PA is also known to affect membrane curvature. Depletion of PA plus a potential subsequent up-regulation of PA biosynthesis would be expected to impact on the shape and extent of the ER resulting the crystalloid formation.

The role of lipids in PC2 activation is not confirmed by functional data, but our maps indicate that the type of lipid could be crucial for the conformation of the selectivity filter due to the lipid-mediated interaction between the P-loop and TOP domain. The positively charged groove of the PA binding site could also accommodate other negatively charged lipids, if the orientation of the TOP domain orientation changes slightly. A bulkier lipid such as PI would push the TOP domain away from the

350 membrane, and hence might change the selectivity or activation mode in PC2. The
351 preferred locations of PC2 outside the ER such as the ciliary membrane or the plasma
352 membrane differ in the content of negatively charged lipids. For example the ciliary
353 membrane contains elevated levels of phosphoinositides and sterols. The differences
354 in lipid composition could contribute directly to the functional diversity of PC2 at
355 different locations.

356 The two open states of PC2 provide insights into a regulatory action of Ca^{2+} to
357 modulate cation currents. The conserved lipid binding sites in PC2 provide the first
358 glimpse of a potential lipid-mediated activation mechanism that involves also the
359 TOP and C-terminal domains. The binding of different membrane lipids would help
360 to explain the location-dependent functional diversity reported for PC2 to some
361 extent. Taken together, the structures of PC2 in open and closed conformations
362 provide a wealth of new information not only on the structural impact of ADPKD
363 mutations, but also on how PC2 interacts with ions and lipids, how these interactions
364 affect the conformations of the channel, and potentially providing mechanisms for
365 regulation of the channel.

366

367 **Author contributions**

368 C.Z. directed research; M.W. performed cloning, established the expression systems,
369 collected and processed cryoEM data; M.W., L.K., R.R. and S.R. expressed and
370 purified PC2; D.R. performed FIBS-SEM tomography; F.J. carried out freeze-
371 fracture, thin-sectioning and immuno-gold labeling; M.W. and V.H. performed
372 negative stain SP analysis; M.G.M. and S.S. carried out homology modeling, M.G.,
373 A.C.W.P, J.H.T. and E.P.C. contributed the structure of the closed conformation of
374 PC2; M.G.M. and C.Z. performed structure determination and refinement and
375 analyzed the data; M.G.M., W.K., M.W., E.P.C., and C.Z. wrote the manuscript.

376

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Figure legends

Figure 1: Segmented map volumes showing PC2 protomers of the 4.2Å structure (PC2_{MI}) and the 4.3Å structure (PC2_{SI}) **(a, b)** Transmembrane helices S1 (orange), S2 (red), S3 (yellow) and S4 (mauve), the pore helices S5 (magenta), S6 (purple) and P-loop with two segments of a helix hairpin (PH1 and PH2, green). **(c)** Secondary structure model of PC2_{MI} and **(d)** of PC2_{SI}. Trans-membrane helices are colored as in (a); the three α -helices and five β -sheets of the TOP domain (Tetragonal Opening for Polycystin) are shown in different shades of green and blue; the loops are grey. Neighboring protomers are indicated as blank silhouettes. **(e)** Structural differences between PC2_{MI} and PC2_{SI}. PC2_{MI} is shown as a tube model; the C α r.m.s.d. is indicated by the color and radius of the tubes (blue > 0Å, white ~1.5Å and red >3Å) in the superposition with PC2_{SI} (black wire). The tetrameric PC2 with densities in the translocation pathway are shown in Figure 3. **(f)** Top and **(g)** side view of a difference map between PC2_{MI} and PC2_{SI} (mesh at the 4 σ contour level; blue indicates positive difference density and red indicates negative difference density). Structure models for PC2_{MI} (left) and PC2_{SI} (right) are shown in complex with cations (green spheres in PC2_{MI} or grey sphere in PC2_{SI}).

Figure 2: Ion translocation pathway in PC2_{MI} and PC2_{SI}. **(a)** Profile of the PC2_{MI} translocation pathway and **(b)** profile of the PC2_{SI} translocation pathway as the radius along the pathway generated with the script HOLE. Parts of PC2_{MI} and PC2_{SI} C α traces are shown as black wire for orientation. The conformations of T265, K267, D643, L641, F669, L677 and N681 along the pathway constrain the ion translocation pathway, which is less restricted in PC2_{SI} than in PC2_{MI} **(c)** Radius of the translocation pathway for both PC2 forms in comparison to the closed form (PC2_{CL}, see accompanying paper ²⁹). Constricting residues are indicated. Sagittal slice through the electrostatic surface representation of PC2_{MI} **(d)** and PC2_{SI} **(e)**. The vestibule formed by the TOP domains is highly negatively charged (red). Exit funnels in both structures are located at different positions (yellow arrows). Ca²⁺ and other cations are shown as green and blue spheres (compare Figure 3c,d).

Figure 3: Cation binding sites in PC2. **(a)** Top view on the PC2_{MI} tetramer. Each protomer is in a different color; cations are shown as spheres. Broken lines indicate the approximate viewing direction in (c). **(b)** Cytoplasmic view of the PC2_{MI} tetramer; color-coded as in (a). **(c)** Cations along the translocation pathway in PC2_{MI}. Isolated densities (2 σ) along the translocation pathway are shown as black mesh. Positions overlapping with Ca²⁺ binding sites in Ca_vAB and TRPV6 are indicated with green spheres (compare Fig. S5a). **(d)** Isolated densities (2 σ) along the translocation pathway in PC2_{SI} are shown as black mesh. The protein is color-coded as in (a) and few prominent side chains are shown.

Figure 4: N-glycosylation and tetramer interactions of PC2_{MI} and PC2_{SI}. **(a)** N-glycosylation pattern and disulfide bridge in PC2_{MI}; **(b)** same location in PC2_{SI}. Key residues are shown as sticks with map densities (mesh at 2.5 σ). **(c)** Conformational changes in the extracellular domain switching interactions with residues in the P-loop of PC2_{MI} and in PC2_{SI} **(d)**. Key residues are shown as sticks with map densities (mesh at 2.5 σ). Interactions are shown as broken black lines. Left: overview of the PC2_{MI} protomer indicating the positions of detailed views in (a)-(d).

Figure 5: Lipid and fatty acid coordination in PC2. **(a)** Non-protein density (blue) in PC2_{MI} and in PC2_{SI} is assigned to phosphatidic acid (PA, light green stick models) in the hydrophobic cleft formed by S3-S6 of one protomer and S5-S6 of the adjacent protomer. Three elongated densities (green mesh in (a)) are assigned to single-chain saturated 16:0 palmitic acids; two compact densities (purple mesh in (a)) are assigned to cholesteryl hemisuccinate (CHS, purple). **(b)** Surroundings of PA/FA1-3 superposed on lipid positions in the structure of TRPV1 (pdbID: 5IRZ). Lipids and phosphatidylinositol (L1-3 and PI) bound to TRPV1 are shown as purple sticks, with PI in the cytoplasmic leaflet. **(c)** Coordination of PA in PC2_{MI} and **(d)** PC2_{SI}. **(e)** Density attributed to the C-terminal domains of the respective protomer are shown as volumes. Fitted structure of an EF-hand (orange ribbon, 2Y4Q) is indicated. FA2 bends around this density. Total density is shown as gray mesh. **(f)** Close-up view of the proposed interaction of the EF-hand and Arg581 in the S4-S5 linker of PC2_{SI} (magenta). The interaction between Asp511 with Lys575 and Lys572 in PC2_{SI} (magenta) and PC2_{MI} (grey) differs significantly, and the conformation of Arg581 is different.

Figure 6: Schematic representation of the multi-ion mode of PC2_{MI} and single-ion mode of PC2_{SI}. **(a)** Conductance regulation of cations (green spheres) might be achieved by Ca²⁺ (circled red) blocking in the selectivity and pre-selectivity filter (TOP) leading to water accumulation in the amphipathic funnel (blue arrow). **(b)** In PC2_{SI}, lipid-mediated interactions of C-terminal and TOP domains induce further opening of the upper and lower gates through the formation of a tri-partite complex, FA1 (magenta) blocks the amphipathic funnel. Red arrows indicate the relative conformational differences between PC2_{SI} and PC2_{MI}.

602 **Table 1** | Cryo-EM data, refinement and model statistics.

Cryo-EM data	PC2 _{SI} , 5MKE, EMD-3523	PC2 _{MI} , 5MKF, EMD-3524
Data Collection:		
Microscope	JEOL 3200FSC	
Voltage (kV)	300	
Defocus range (μm)	0.5 – 4.0	
Pixel size (Å)	1.14	
Average e ⁻ dose per image (e ⁻ /Å ²)	1.8	
Particles (initial)	267010	
Particles (final, %)	42268 (16)	35318 (13)
Reconstruction:		
Symmetry	C4	C4
Resolution (unmasked, Å)	4.5	4.5
Resolution (masked, Å)	4.3	4.2
Map sharpening <i>B</i> -factor (Å ²)	-200	-200
Model composition:		
Non-hydrogen atoms	15704	15848
Protein residues	1900	1924
Non-protein residues	36	36
Refinement:		
Resolution (Å)	4.3	4.2
Map sharpening factor (Å ²)	-105	-95
Model to map fit (CC)	0.6924	0.7203
Rms deviations:		
Bonds (Å)	0.009	0.009
Angles (°)	1.155	1.091
Validation:		
Clashscore	48.28	31.05
Rotamer outliers (%)	0.23	0.23
Ramachandran Plot (% favoured)	91.30	93.32
Ramachandran Plot (% outliers)	0.21	0

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Online Methods

Molecular insights to lipid-assisted Ca^{2+} -regulation in the TRP channel Polycystin-2

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24 **Extended Methods**

25

26 *Cloning, expression and protein production* – The human *PKD2* gene with a StrepII-
27 tag fused to the C-terminus was cloned into the pACMV-tetO vector^{31, 42} via the
28 restriction sites KpnI and XhoI. Transfection of eukaryotic cells and selection of a
29 stable cell line – HEK293S GnT I⁻ was performed in a 6 well tissue culture plate in
30 DMEM/HAM F12 media with 10 % FBS, 50 U/ml Pen/Strep and 5 µg/ml blasticidin
31 at 37°C and 5 % CO₂. HEK293S GnT I⁻ were tested on mycoplasma contamination.
32 For selection of a stable HEK293S GnT I⁻ cell line 350 µg gentamycin was added into
33 DMEM/HAM F12 media with 10 % FBS, 50 U/ml Pen/Strep and 5 µg/ml blasticidin.
34 Single cells were transferred into 96 well tissue culture plates. Test expressions were
35 performed in 6 well plates, whereas expression for protein purification was performed
36 in suspension with volumes up to 4 l. Expression was induced with the final addition
37 of media containing tetracycline and sodium butyrate to a final concentration of 3
38 µg/ml and 5 mM, respectively. 48 h after induction cells were harvest by
39 centrifugation (25 min, 3.600 g at 4°C). Cells were re-suspended in PBS buffer and
40 centrifuged again for 10 min at 4.500 g and 4°C. The cell pellet was re-suspended in
41 buffer (25 mM HEPES pH 7.5, 10 % glycerol, 150 mM NaCl, 2 mM CaCl₂, complete
42 protease inhibitor) for cell disruption. Cells were lysed using a cell disruptor
43 (Constant Systems) at 2.0 kbar and 4°C. Cell debris was removed by centrifugation
44 for 15 min at 10.000 g and 4°C and membranes were sedimented at 150,000 g, 4°C
45 for 60 min, re-suspended 10 - 50 ml buffer (25 mM HEPES pH 7.5, 10 % glycerol,
46 150 mM NaCl, 2.5 mM CaCl₂) and homogenized with a Dounce homogenizer. The
47 total protein concentration was adjusted to 2.5 mg/ml and stirred while detergent was
48 slowly added to a final detergent concentration of 1 % / 0.4 % LMNG/CHS (w/w).
49 PC2 was purified using strep-tactin sepharose from (IBA). Proteins were eluted with 5
50 mM desthiobiotin. Amphipol A8-35 was added to the purified PC2 at a 3-fold excess
51 (w/w) and the mixture was incubated at 4°C for 4 h. 15 mg of activated biobeads were
52 added per 1 ml protein-detergent-amphipol mixture and incubated over night at 4°C.
53 Biobeads were removed using a plastic column. PC2 was concentrated using Vivaspin
54 4 (Sartorius) centrifugal filter units with a molecular weight cut-off of 100 kDa to a
55 final volume of 50 µl. Size-exclusion chromatography was performed on an Ettan
56 system (GE Healthcare) using a Superose 6 3.2/300 and absorption was measured at
57 280 nm.

58 *Electron cryomicroscopy (cryo-EM)* – For cryo-EM PC2 in amphipol A8-35 was
59 concentrated to 1 mg/ml and aliquots of 3 μ l were placed on glow-discharged holey
60 carbon grids (Quantifoil R2/2) and plunge frozen using a Vitrobot IV (FEI) at 100 %
61 humidity, 10 °C with 9 s blotting time. CryoEM images were collected at liquid
62 nitrogen temperature on a JEOL 3200 FSC electron microscope operating at 300 kV
63 with an in-column energy filter and equipped with a K2 Summit direct electron
64 detector camera (Gatan). The slit width was adjusted to 20 eV and images were
65 recorded in counting mode at a nominal magnification of 30,000x, corresponding to a
66 pixel size of 1.14Å on the specimen. The total exposure time was 7 s leading to an
67 accumulated dose of 55 e/Å². Each image was fractionated into 35 sub frames with an
68 accumulation time of 0.2 s. Defocus values in the final datasets range from 0.5 - 4.0
69 μ m.

70
71 *Image-processing* – Global beam induced motion of images was corrected using first
72 MOTIONCORR 2.0⁴³ and second Unblur⁴⁴⁻⁴⁶. The contrast transfer function for each
73 image was determined using CTFFIND3⁴⁷. An initial set of 25,000 particles were
74 picked manually and subjected to 2D class averaging. The six most representative 2D
75 class averages were filtered to 20Å and used as templates for reference based particle
76 picking⁴⁸. Each micrograph was manually inspected, false positive particles are
77 deleted and false negative particles were selected, yielding in total 267,010 particles
78 from 1,450 micrographs. Subsequent 2D and 3D classifications were performed using
79 RELION-1.4⁴⁹. A first 3D classification without symmetry imposed using the TRPV1
80 channel (pdb entry code 3J5P)³³ filtered to 60Å as reference and a sampling of 15 °
81 resulted in a subset of 162,074 particles. In subsequent 3D classification the best 3D
82 class-average of the first classification was used as a starting model, the sampling was
83 set to 7.5 ° and C4 symmetry was imposed. This gave a subset of 108,240 particles,
84 which were analyzed by 2D class averaging using ‘*ignore first peak*’ in the RELION-
85 1.4 GUI⁴⁹. The same particle subset was used for a 3D gold-standard refinement⁵⁰
86 resulting in a resolution of 4.2 Å. Particle based beam-induced movement correction
87 and polishing were performed^{51,52} using a running average of seven movie frames and
88 a standard deviation of one pixel for the translational alignments which yielded a
89 reconstruction with a resolution of 3.9Å. A subsequent 3D classification with a
90 sampling rate of 7.5 ° and imposing C4 symmetry, separated two different
91 conformations of PC2. The two conformations were refined separately, resulting in

92 two different cryoEM structures with resolutions of 4.2Å and 4.3Å, respectively.
 93 Densities were sharpened by applying *B*-factors⁵³ estimated using RELION-1.4⁴⁹.
 94 Local resolutions in different parts of the maps were estimated using RESMAP⁵⁴.
 95 Densities were visualized and atomic models were fitted in cryoEM densities using
 96 UCSF Chimera⁵⁵.
 97
 98 *Model building for the S1-S4 and pore domains* – Template structures, homologous to
 99 the target sequence, were initially detected by iterative HMM-HMM comparison
 100 (HHblits) and chosen in accordance to their amino acid sequence identity and
 101 similarity. Initial alignment was obtained in an automated way by the AlignMe
 102 program⁵⁶. MODELLER⁵⁷ was then used for comparative structure modeling to
 103 obtain an initial model. TRPA1 (pdb entry code 3J9P) served as template for S1 and
 104 S3-S5, TRPV1 (pdb entry code 3J5Q) for S5-S6, and the TRPV2 (pdb entry code
 105 5AN8) fitted best for S2-S3. After manually correcting the sequence-to-sequence
 106 alignment the final model was obtained from FLEX-EM (<http://salilab.org/Flex-EM>).
 107 The FLEX-EM model was investigated by EMRinger⁵⁸ and subjected to iterative runs
 108 of refinement using the Rosetta tool^{46, 59} for structure determination in cryoEM
 109 density and Phenix-real-space-refinement^{45, 60}. Regions that that could not be modeled
 110 based on homology, were either manually corrected or build *de novo* with COOT⁴⁴.
 111
 112 *Purification of crystalloid ER membranes* – The HEK293S GnT I⁻ cell line stably
 113 expressing PKD2 was cultured in T175 tissue culture plates until 70 % confluence
 114 and protein production was induced by the addition of 3 µg/ml tetracycline and 5 mM
 115 sodium butyrate. Cells were harvest by centrifugation for 10 min at 4,500 g and 4°C
 116 and resuspended in 3 ml of ice cold 40 mM HEPES pH 7.2, 5 mM MgCl₂, 5 mM
 117 EGTA, complete protease inhibitor and DNase. Cells were passaged 35 times
 118 through a 27-gauge needle in 1 ml batches on ice. Cell debris was sedimented at 1,000
 119 g, 4 °C for 10 min. Cell pellets were resuspended in buffer without DNase and
 120 centrifuged again. The cell pellet was then resuspended in buffer containing 2.2 M
 121 sucrose and a continuous sucrose gradient (1.5 M - 0.7 M sucrose in 40 mM HEPES
 122 pH 7.2, 5 mM MgCl₂, 5 mM EGTA and complete protease inhibitor) was added on
 123 top. Membranes were separated by centrifugation for 20 h at 40,000 g at 4 °C. The
 124 gradient was separated in 1 ml fractions and the fraction that contained the highest
 125 amount of PC2 (determined by SDS-PAGE) was used for freeze-fracture labeling.

126

127 *Immunology of thin sections* – The stable cell line was cultured in 6 well tissue culture
128 plates until 70 % confluence and protein production was induced by the addition of 3
129 µg/ml tetracycline and 5 mM sodium butyrate. Cells or purified crystalloid ER were
130 fixed for 2 h at room temperature with 2 % (v/v) paraformaldehyde in PBS buffer.
131 The samples were washed three times with PBS buffer for 10 min and incubated with
132 2 % glycine in PBS buffer for 2.5 h at room temperature before they were washed
133 again three times with PBS buffer. The sample was embedded in 4 % Bacto Agar
134 (BD), dehydrated using an ethanol gradient series (1x 20 min 30 %, 2x 20 min 50 %,
135 2x 30 min 70 %, 2x 30 min 90 %, 2x 30 min 100 %) and infiltrated with LR White
136 Resin, medium grade Acrylic Resin (Resin Company). Polymerization was carried
137 out at 55 °C for 36 h and 80 - 120 nm thin sections were prepared with an Ultracut S
138 Microtome (Leica). Thin sections were incubated with saturated sodium periodate for
139 1 h at room temperature. The sample was washed five times (1x 10 min with 2 %
140 glycine in PBS buffer, 1x 10 min with PBS buffer, 1x 10 min with 1 % BSA in PBS
141 buffer containing 0.1 % Tween 20, 2x 10 min with 0.1 % BSA in PBS buffer and 0.1
142 % Tween 20) before the anti-PC2 antibody (YCE2, Santa Cruz Biotechnology) in
143 PBS buffer with 0.1 % BSA was added for 1 h. The first antibody was removed,
144 followed by four washing steps with PBS for 5 min each, before the second antibody
145 (anti-mouse coupled to 18 nm gold, Thermo Fisher Scientific) in PBS buffer with 0.1
146 % BSA was added for 1 h. Free antibody was removed by washing the sample three
147 times for 5 min with PBS buffer. 1 % (v/v) glutaraldehyde in PBS was added for 10
148 min before the sample was washed three times with ddH₂O and stained with 1 %
149 (w/v) uranyl acetate and lead citrate. Samples were analyzed using an EM208S
150 electron microscope (FEI).

151

152 *FIB SEM* – The stable cell line was cultured in 6 well tissue culture plates until 70 %
153 confluence and protein production was induced by the addition of 3 µg/ml tetracycline
154 and 5 mM sodium butyrate. Medium was removed and 2.5 % (v/v) glutaraldehyde in
155 100 mM sodium cacodylate pH 7.4 was used to prefix cells or purified crystalloid ER
156 for 2 h at room temperature. Prefixed samples were washed three times with 100 mM
157 sodium cacodylate pH 7.2 for 15 min, scraped out and subsequently embedded in 4 %
158 Bacto Agar (BD). Samples were dehydrated using an ethanol gradient series (1x20
159 min 30 %, 2x20 min 50 %, 2x30 min 70 %, 2x30 min 90 %, 2x 30 min 100 %) and

160 infiltrated with Low Viscosity Premix Kit-Medium (2x 20 min 30 %, 2x 30 min 50 %,
161 2x 30 min 75 %, overnight 100 %, 2x 2 h 100 %) (Agar Scientific). Polymerization
162 was performed for 16 h at 60°C. For FIBS SEM resin blocks were trimmed with a
163 glass knife to expose the cells, and the blocks were glued to a SEM stub using silver
164 paint. Gold was sputtered onto the sample to minimize electrostatic charging. Samples
165 were analyzed in a Zeiss Auriga FIB/SEM at an acceleration voltage of 10 kV to
166 localize embedded cells. An additional platinum layer was deposited using the gas
167 injection system of the system. A 30 kV gallium beam was used to prepare a block
168 face, which was subsequently analyzed by FIB-SEM tomography at an acceleration
169 voltage of 1.8 kV. A 600 pA Ga⁺ beam was used to abrade the block face and to
170 collect a 3D data set. The slice thickness was 10 nm. Images were recorded using the
171 EsB detector. Image stacks were aligned and segmented using the Amira package.

172
173

174 **Data availability**

175 EM density maps have been deposited at the EMDB with accession codes
176 EMD-3524 (PC2_{MI}) and EMD-3523 (PC2_{SI}). Atomic models have been deposited at
177 the Protein Data Bank with accession codes PDB 5MKF (PC2_{MI}) and 5MKE (PC2_{SI}).
178 Source data for figures 1a, 1b, 1f, 1g, 3c, 3d, 4, 5a, 5e are available with in the
179 respective repository protein data banks. Other supporting data are available from
180 authors upon reasonable request.

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Supplementary Figure 1

PC2 expression in HEK 293 GnT I⁻ cells.

(a) Laser confocal microscopy after 48h of PC2 expression (b) Western blot analysis against the C-terminal domain of PC2 of Triton-solubilized HEK 293 GnT I⁻ cells (1) before induction, (2) 6 h, (3) 12 h, (4) 24 h, (5) 48 h, and (6) 72 h after induction (c) Crystalloid formation in HEK 293 GnT I⁻ cells in thin plastic sections overexpressing PC2 12 h to 72 h after induction. (d) Ion ablation tomography and 3D reconstruction reveals that crystalloids after 72 h are a honeycomb of tubular ER vesicles. (e) Immuno-gold labeling of the PC2 C-terminal domain (C-term) in thin plastic sections of whole cells after 48 h. Gold labels are visible as small black dots.

Supplementary Figure 2

Purification and single-particle cryoEM of human PC2.

(a) Gel filtration profile (Superose-6) of PC2 in amphipol A8-35 after purification on streptactin resin. The PC2 tetramer elutes at a retention volume of about 1.35 ml. Absorption was measured at 280 nm. **(b)** SDS-PAGE of amphipol reconstituted PC2. **(c)** Cryo-micrograph of PC2 tetramers recorded at a defocus of 2.5 μm . **(d)** Four representative 2D class averages.

Supplementary Figure 3

Single-particle processing.

267 010 particles were semi-automatically selected and subjected to 3D classification without symmetry imposed. The best class containing 162 074 particles was further classified with C4 symmetry, resulting in two good classes. Particles of both classes were combined and subjected to a 3D refinement, followed by particle polishing. Two distinct PC2 conformations were obtained by further 3D classification with C4 symmetry, followed by two independent 3D refinements of the two best 3D class averages.

Supplementary Figure 4

Local resolution and FSC curves of the 4.2 Å structure (PC2_{MI}) and 4.3 Å structure (PC2_{SI})

Local map resolution (rainbow bar, in Å) was determined with RESMAP. **(a)** Exoplasmic, **(b)** side and **(c)** cytoplasmic view of PC2_{MI} and respective views **(h-g)** of PC2_{SI}. **(d)** Histogram of the local resolution indicates a mean resolution of ~4.0 Å for PC2_{MI} and 4.0 - 4.5 Å PC2_{SI}. **(e)** Correlation of the masked and unmasked map with the Phenix-refined model for the 4.2 Å and **(k)** for the 4.3 Å dataset. **(f)** Correlation of individual half-maps (blue and magenta) and summed map of the the 4.2 Å structure refined with Phenix (red) or REFMAC (green), compared to the respective correlation of the 4.3 Å structure **(l)**.

Supplementary Figure 5

Cation binding in PC2 and comparison of the presumed open states to the closed PC2_{CL}

(a) Ca^{2+} (green spheres) and potential other cations (blue spheres) in PC2_{MI} aligned with Ca^{2+} in CavAB (yellow spheres, pdb entry code 4MVO) and TRPV6 (magenta, pdb entry code 5IWP). Gray spheres indicate the position of cations in PC2_{SI}. (b) Spatial constraints in the translocation pathway of PC2 states with CavAB (magenta, pdb entry code 4MVO). Radii along the translocation pathway of PC2_{MI} (green) and PC2_{SI} (blue) were calculated using the script HOLE. Residues forming narrow constrictions are indicated. Structural difference between PC2_{MI}, PC2_{SI} and the closed state PC2_{CL} (Grieben *et al.*, 2016): (c) PC2_{MI} and (d) PC2_{SI} are shown as worm models; colors and channel diameters indicate C_{α} r.m.s.d. relative to PC2_{CL} (blue 0Å, white 1.5Å and red >3Å, larger diameter stands for larger deviation; C_{α} of PC2_{CL} is shown as black wire).

Supplementary Figure 6

Fatty acid binding affects C-terminal conformation.

Coordination of fatty acids (FA1-3) in the upper membrane leaflet and one to the lower leaflet in PC2_{ML} (a) and PC2_{SI} (b). The phosphatidic acid (PA) and the adjacent helices with prominent residues are indicated. (c) Cytoplasmic view on the PC2_{SI} (4.3Å) model. The PC2_{SI} (4.3Å) map contains continuous density (black mesh) that can be traced (orange pearl chain) to an additional globular density attributed to the EF-hand (transparent volume). (d) EF-hand causes a change in the coordination of the fatty acid network shown for one protomer (orange) shown as side-view of panel (c). (e) The structure of the EF-hand (pdbID: 2Y4Q) fitted into the additional density.