Figures and figure supplements

Cryo-EM structure of the polycystin 2-l1 ion channel

Raymond E Hulse et al.
Figure 1. Architecture of polycystin 2-11 tetrameric ion channel. (a) Side view parallel to the membrane and from the top (extracellular) surface. Distinct subunits in the tetramer are color-coded. (b) 2D topological representation of the polycystin 2-11 monomer; voltage sensor-like domain (VSLD, teal), polycystin mucolipin domain (PMD, orange), and pore domain (PD, violet). (c) Monomer color-coded and matched to Figure 1b: N terminus/VSLD (teal), PMD (orange), C terminus/PD (violet).

DOI: https://doi.org/10.7554/eLife.36931.002
Figure 1—figure supplement 1. Purification, negative staining, 2D class average and function of polycystin 2-L1. (a) SDS-PAGE of amylose purification for supernatant, flow, wash and elution (left). Size exclusion chromatography (Superose 6 Increase) after affinity chromatography and exchange from Hulse et al. eLife 2018;7:e36931. DOI: https://doi.org/10.7554/eLife.36931
detergent to amphipol PMAL-C8 (right). (b) Representative sample of negative-stained polycystin 2-I1 in PMALC8 (scale bar, 200 nm). (c) 8 representative 2D class averages used in data processing. (d) Sample trace of voltage-clamped, liposome-reconstituted recombinant polycystin 2-I1 (top). Amplitude histogram of open and closed events (bottom left) and mean current of open (Level 1) and closed (Level 0) events. Average current = 13.2 pA (±2.8 S.D.) at +125 mV, 1.6 s duration.

DOI: https://doi.org/10.7554/eLife.36931.003
Figure 1—figure supplement 2. Comparison of polycystin 2-11 and polycystin-2. (a) The Cα-RMSD between polycystin 2-11 and polycystin-2 (ST4D) mapped onto the polycystin 2-11 monomer. The average RMSD is 1.5 Å with a range of 0.14 Å (blue) to 9.8 Å (red). (b) Overlay of the polycystin 2-11
Figure 1—figure supplement 2 continued

monomer (green) on the polycystin 2 (gray) monomer; cartoon representation. (c) Polycystin 2-l1 S2 segment (green) and polycystin 2 S2 (gray), showing the relative angle and lateral shift (arrow). S6 relative angle shown at right. (d) Calculation of pore radius (Å) along the pore axis for polycystin-2 (red) and polycystin 2-l1 (black). Residues GS22 and LS21 in the selectivity filter, and IS60 are labeled and are the three narrowest distances.

DOI: https://doi.org/10.7554/eLife.36931.004
Figure 1—figure supplement 3. Single particle data processing. 842,139 particles were automatically selected to generate a 2D class average. Eight classes were selected; a reference from a previous dataset that generated a 3.6 Å map was used to derive four initial classes. The four classes were generated from automatic refinement; one class was selected to generate an additional round of four classes. The subsequent particle was then selected to generate two more classes (two more rounds with a mask to omit contributions from the amphipol). The final, highest resolution structure was selected and then sharpened.

DOI: https://doi.org/10.7554/eLife.36931.005
**Figure 1—figure supplement 4.** 2D and 3D Fourier Shell Correlation (FSC) curve analysis and local resolution map of polycystin 2-11. (a) Angular distribution plot, inner black dashed ring ($\theta = 30^\circ$) and outer white dashed ring ($\theta = 60^\circ$). (b) Corresponding 2D FSC of polycystin 2-11 in C8. (c) Global resolution 3.2 Å, Sphericity 0.871 out of 1. Red line: Global FSC, green line: 1 S.D. of Mean Directional FSC. (d) Local resolution maps at different Z levels: TOP, SIDE, BOTTOM.
(horizontal dashed 0.143 criterion; solid vertical black line is the minimum refinement limit of 8 Å. (c) 3D FSC and preferred orientation analysis of the same dataset with the red line representing the estimated global FSC of 3.2 Å ± 1 SD (green dashed lines). Dashed line 0.143 criterion are at left and the correlated resolution estimations and orientations from the preferred orientation analysis are at the right. Red lines represent global estimated FSC, black lines FSC for the z-axis of the orientation indicated above. Color code (red (poorer) to blue (better) resolution) represents in-plane resolution (x/y axes) and resolution value Z (including graph below) refers to the z-axis directional resolution and corresponding FSC plot. For instance, the side view shows an increased z-axis resolution but a decreased x/y resolution (red) which is consistent with the angular distribution plot. (d) Local resolution map (calculated by ResMAP with a value of box 20 and cutoff 0.5) of the same dataset with top, side, and bottom orientations.

DOI: https://doi.org/10.7554/eLife.36931.006
Figure 1—figure supplement 5. Quality of the EM density map and fit of model. (a) EM density maps are shown for the polycystin 2-l1 monomer in the Voltage Sensor Like Domain for helices S1-S4. (b) The PMD with all secondary structural elements and a general scheme of the three loops that...
Figure 1—figure supplement 5 continued

comprise the Three Leaf Clover (TLC) region (left and below). (c) The Pore Domain for helices S5, S6 and the selectivity filter with pore-helices 1 and 2 (PH1 and PH2).

DOI: https://doi.org/10.7554/eLife.36931.007
Figure 2. The polycystin 2-11 pore domain. (a) Path of permeation depicted with HOLE (left). At right is a close-up view of the selectivity filter (two monomers removed for clarity) with three conserved residues L521, G522, and D523. Pore radius (HOLE) measured along the pore axis (left). (b) Electron density map superimposed on the polycystin 2-11 model; contour level 5.0. (c) TRPP family multiple sequence alignment of conservation projected in color onto the polycystin 2-11 pore domain. Two of four monomers have been removed to increased clarity.

DOI: https://doi.org/10.7554/eLife.36931.008
Figure 3. Structure and conservation of the polycystin mucolipin domain (PMD) of polycystin 2-I1. (a) Sequence conservation projected in color onto the PMD domain for polycystin 2-I1 homologs; Three-leaf clover (TLC) domains labeled. (b) The S3 (green), S3-S4 linker (green, rotated 50°), and TLC1 from the adjacent PMD (red) interact with the PMD of the same domain (gray). (c) Interactions of the PMD (gray) with the pore domain (PD) and PMD of the adjacent monomer (yellow). PMD secondary structural elements helices 1–3, and β-sheets 1 to 4 labeled, along with the PD and Pore Helix (PH1). (d) Disulfide C210-C223 (yellow) in the TLC1 loop (3.4 Å) of the PMD.

DOI: https://doi.org/10.7554/eLife.36931.009
Figure 4. Polycystin mucolipid domain interactions of polycystin 2-11. (a) π-π interaction of residue residing in TLC1 F216 (brown) with the neighboring (green) PMD Y308. (b) Hydrogen bonding interaction (green dash) of PMD residue N311 with the neighboring PMD residue Y224’s amide (blue). (c) Residue W259 in TLC3 of the PMD forms a cation-π stacking interaction with the upper pore domain residue R534. The guanidino group of R534 also forms a hydrogen bond (red dash) with the carbonyl of G260 of the adjacent PMD domain.

DOI: https://doi.org/10.7554/eLife.36931.010
Figure 5. Comparison of charge landscape and local bonding between TRPP and the calcium-selective TRPV5 and TRPV6 channels. (a) The extracellular facing (top) and selectivity filter region (below) of channels with progressively increasing relative permeability to Ca\(^{2+}\); cut-away schematic (far left) for orientation. (b) Stick representation of the selectivity filter region (top) and simplified diagram (below). Local bonding characteristics are highlighted: conserved salt-bridge (red) interaction of polycystin-2 and polycystin 2-I1, the unique hydrogen bond of polycystin 2-I1 (green), and the direct side-chain interaction with Ca\(^{2+}\).
coordination of Ca$^{2+}$ in TRPV5 and TRP6. (c) Alignment with conservation scoring and clustalW coloring scheme of the selectivity filter region shown in (b).
DOI: https://doi.org/10.7554/eLife.36931.011