



Figures and figure supplements

Electron cryo-microscopy structure of the canonical TRPC4 ion channel

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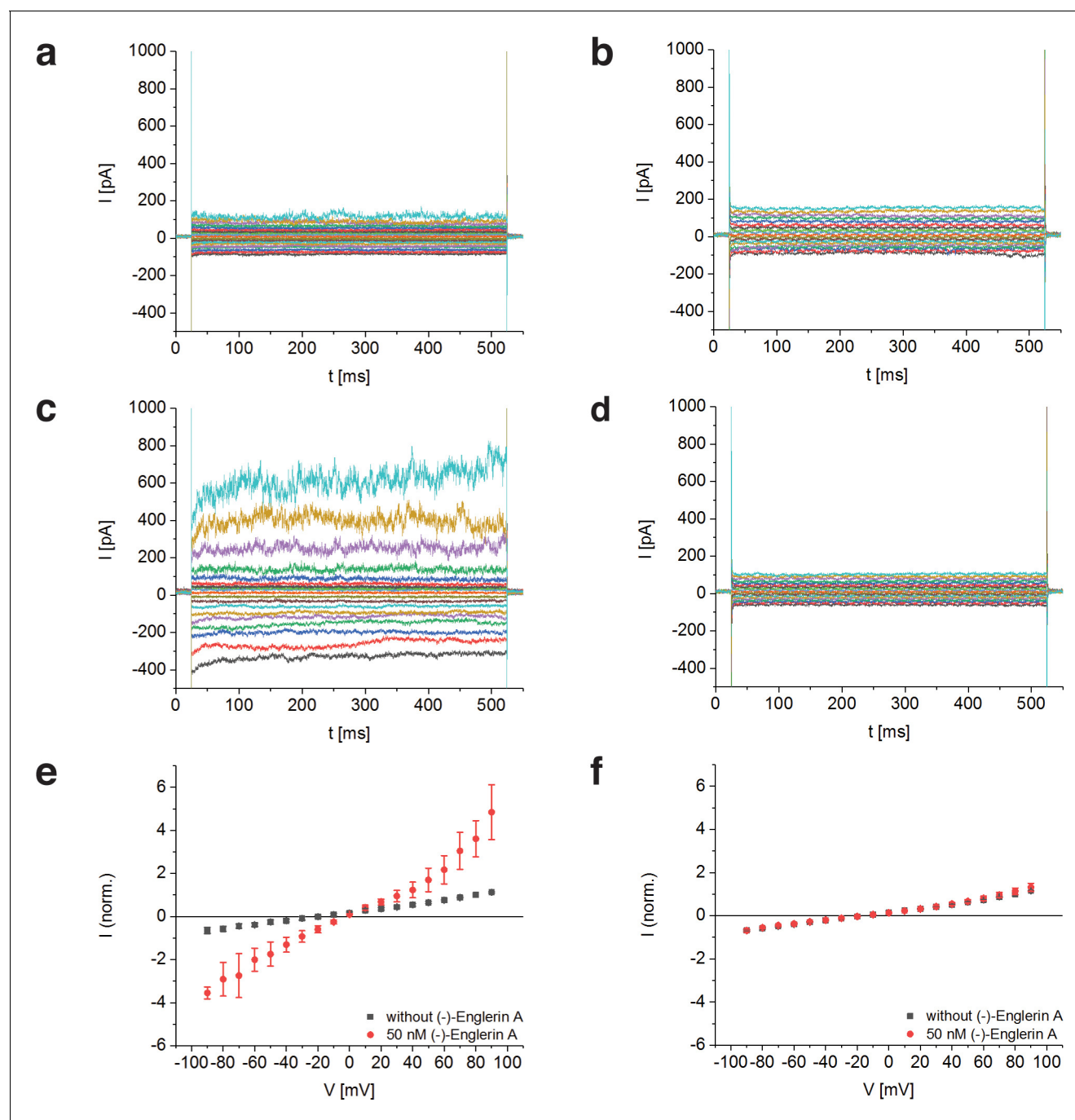


Figure 1. Activation of TRPC4_{DR} by the selective activator (-)-Englerin A. (a–f) HEK293 cells heterologously expressing TRPC4_{DR}-EGFP (a,c,e) and untransfected control cells (b,d,f) were investigated by voltage-clamp experiments in the whole-cell configuration. The membrane potentials were clamped to values ranging from -90 to $+90$ mV in the absence (a,b) and in the presence (c,d) of 50 nM (-)-Englerin A. Upon addition of 50 nM of (-)-Englerin A, the current density at -60 mV increased from -3.1 ± 1.9 pA/pF ($n = 6$) to -16.7 ± 10.7 pA/pF ($n = 6$). In untransfected control cells, the current density in the absence and presence of the activator was virtually the same with values of -2.1 ± 1.2 pA/pF ($V = -60$ mV, $n = 5$) and -1.8 ± 0.9 pA/pF ($V = -60$ mV, $n = 5$) respectively. (e,f) Current-voltage curves in the absence (black squares) and in the presence (red circles) of 50 nM (-)-Englerin A. Currents were normalized to the current value in the absence of (-)-Englerin A at a membrane potential of $+80$ mV. Note that the measurements in the absence and in the presence of (-)-Englerin A were performed on the same cells. Shown are the normalized mean currents of 6 (e) and 5 (f) different cells. Error bars are \pm SEM. The measurements were performed as described in Materials and methods.

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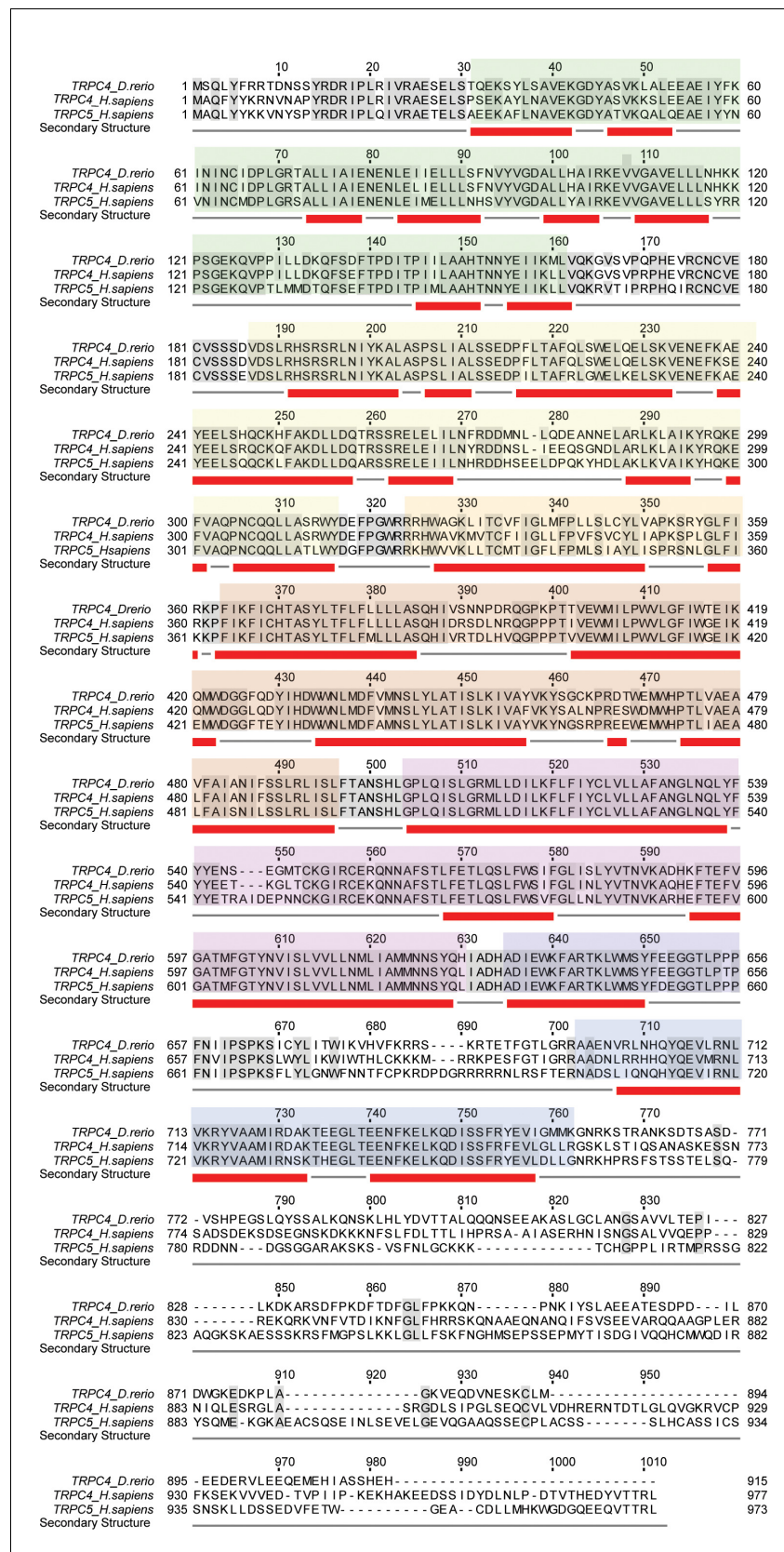


Figure 1—figure supplement 1. Multiple sequence alignment of TRPC4_{DR}, human TRPC4 and human TRPC5. The grey-shaded regions highlight conserved residues. Helices are indicated by red bars. Domains are colored according to **Figure 2b** with high transparency.

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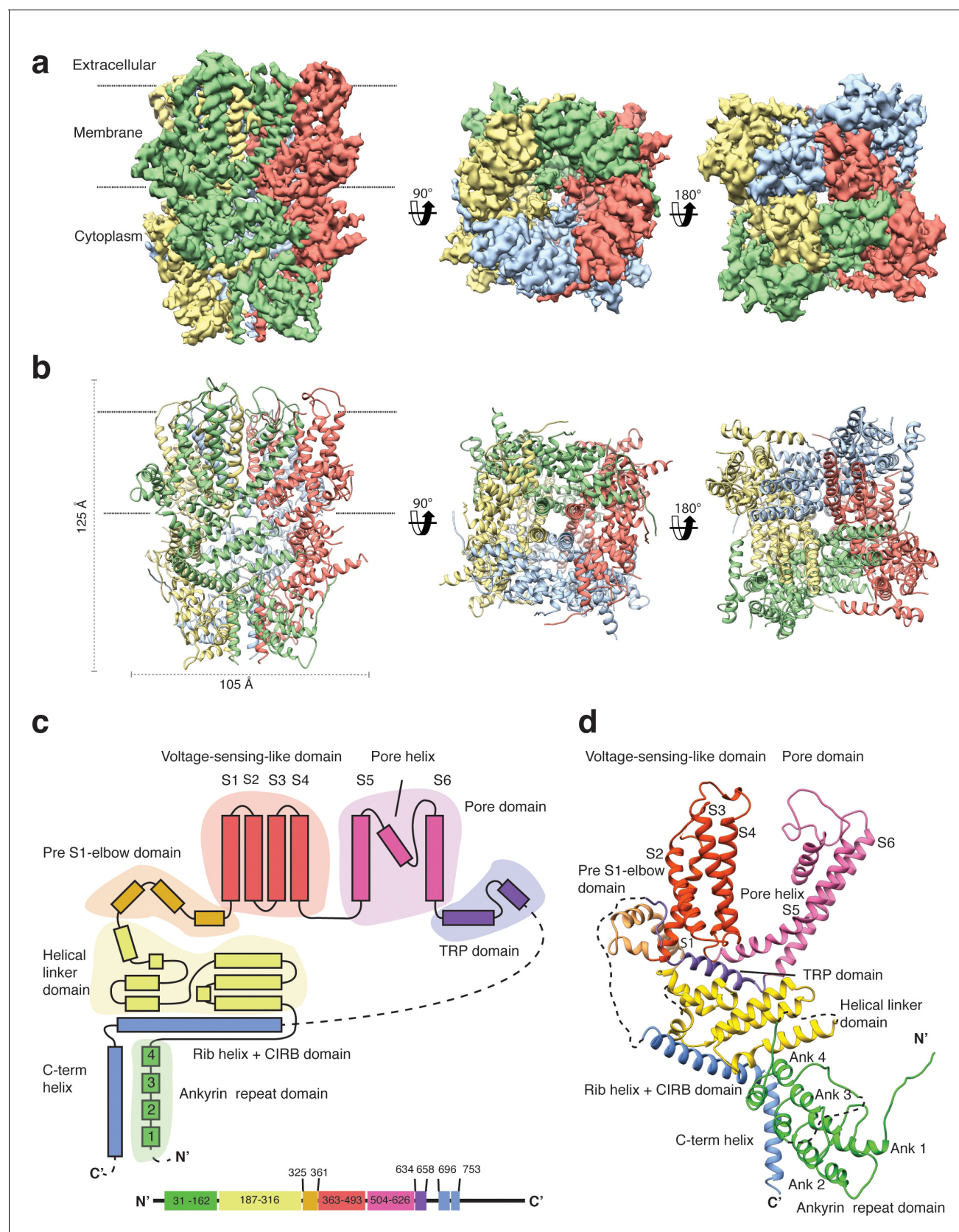


Figure 2. Structure of TRPC4_{DR}. (a) Cryo-EM density map of TRPC4_{DR} with each protomer colored differently and shown as side, bottom and bottom view. (b) Ribbon representation of the atomic model of TRPC4_{DR}. Colors are the same as in (a). (c) Topology diagram depicting the domain structure of TRPC4_{DR}. (d) Detailed ribbon diagram of the TRPC4_{DR} structure.

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Figure 2 continued

organization of a TRPC_{4DR} protomer. (d) Ribbon representation of a TRPC_{4DR} protomer. Each domain is shown in a different color and labeled accordingly.

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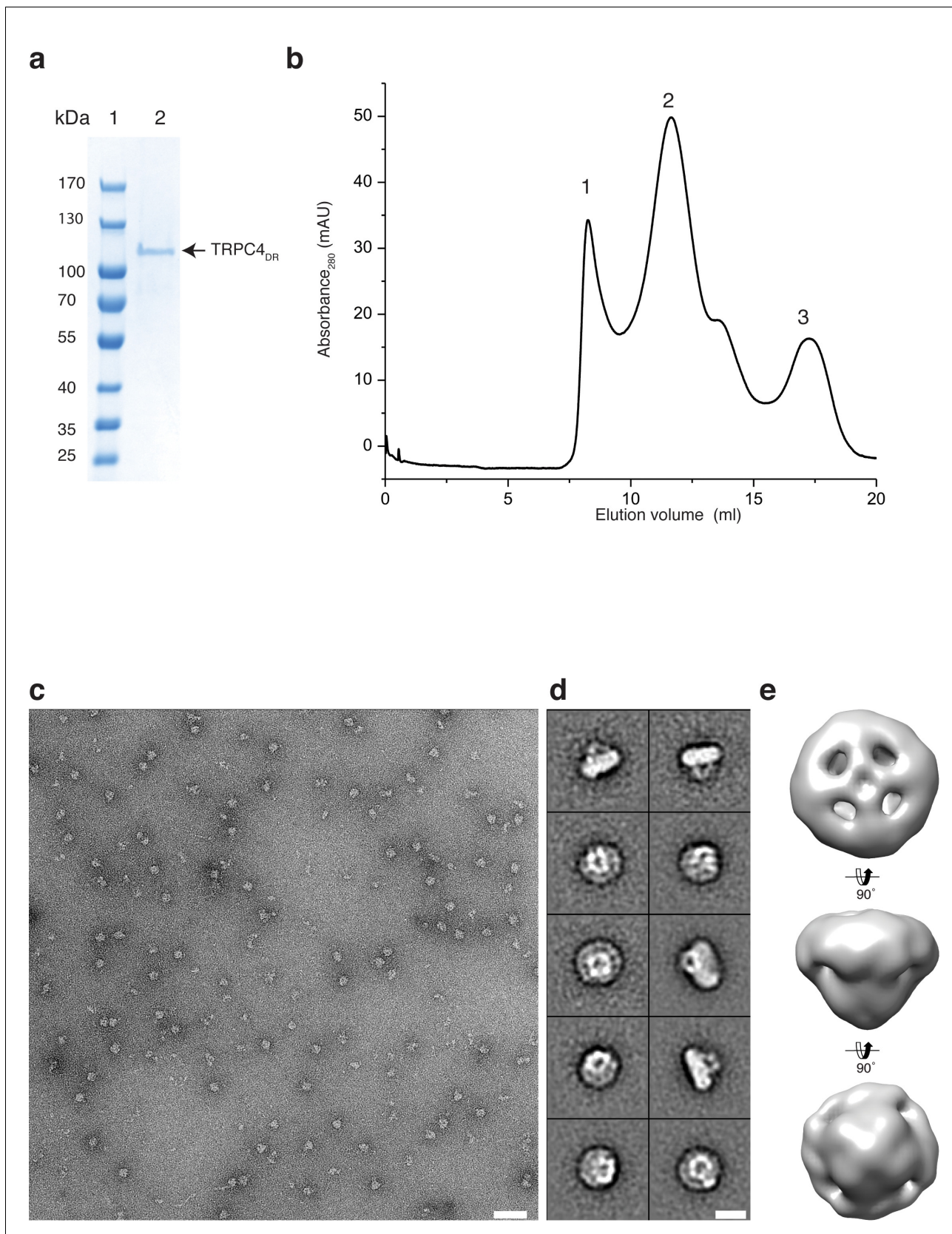


Figure 2—figure supplement 1. Purification of TRPC4_{DR} and negative stain EM of TRPC4_{DR} in amphipols. (a) SDS-PAGE of the peak fraction of TRPC4_{DR} in amphipols after size exclusion chromatography. Lane 1: molecular weight marker, lane 2: protein. (b) Size exclusion chromatography profile

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Figure 2—figure supplement 1 continued

of TRPC4_{DR} in amphipols. Peak 1, 2 and 3 correspond to the void volume, tetrameric TRPC4_{DR} and cleaved GFP, respectively. (c) Representative negative stain electron micrograph of TRPC4_{DR}. Scale bar, 50 nm, (d) Representative 2-D class averages. Scale bar, 10 nm. (e) 3-D reconstruction of negatively stained TRPC4_{DR} shown in different orientations.

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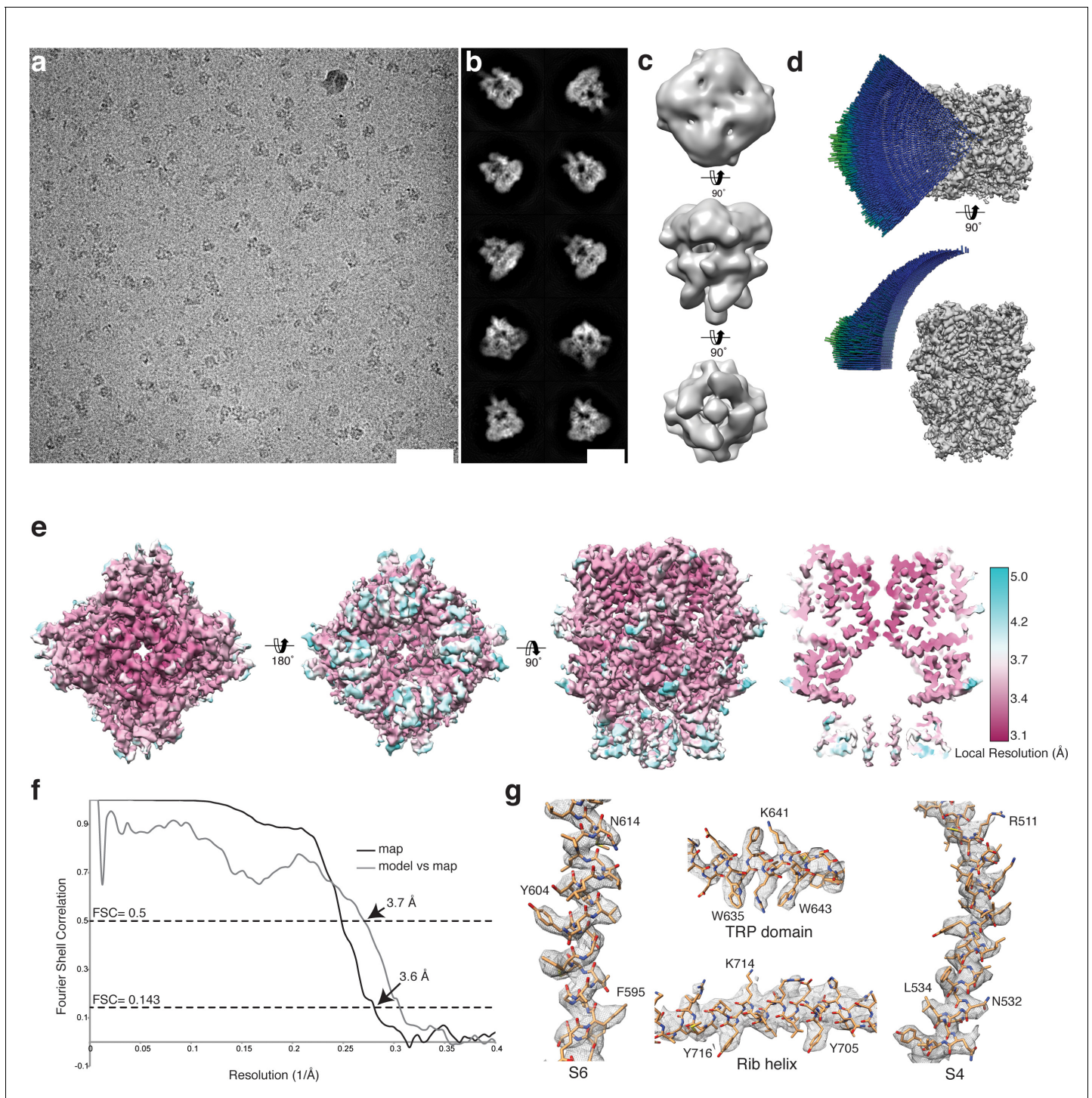


Figure 2—figure supplement 2. Cryo-EM structure of TRPC4_{DR}. (a-b) Representative digital micrograph area (a) and selected 2-D class averages (b) of TRPC4_{DR} embedded in vitrified ice. Scale bars, 50 nm (a), and 10 nm (b). (c) The *ab initio* 3-D reconstruction obtained with RVIPER. (d) Angular distribution of the particles. (e) The cryo-EM density map of TRPC4_{DR} colored according to the local resolution. (f) Fourier Shell Correlation (FSC) curve between maps from two independently refined half data sets (black). The 0.143 criterion indicates an average resolution of 3.6 Å. The grey curve shows the FSC curve between the final map versus the atomic model. (g) Representative regions of the density with fitted atomic model.

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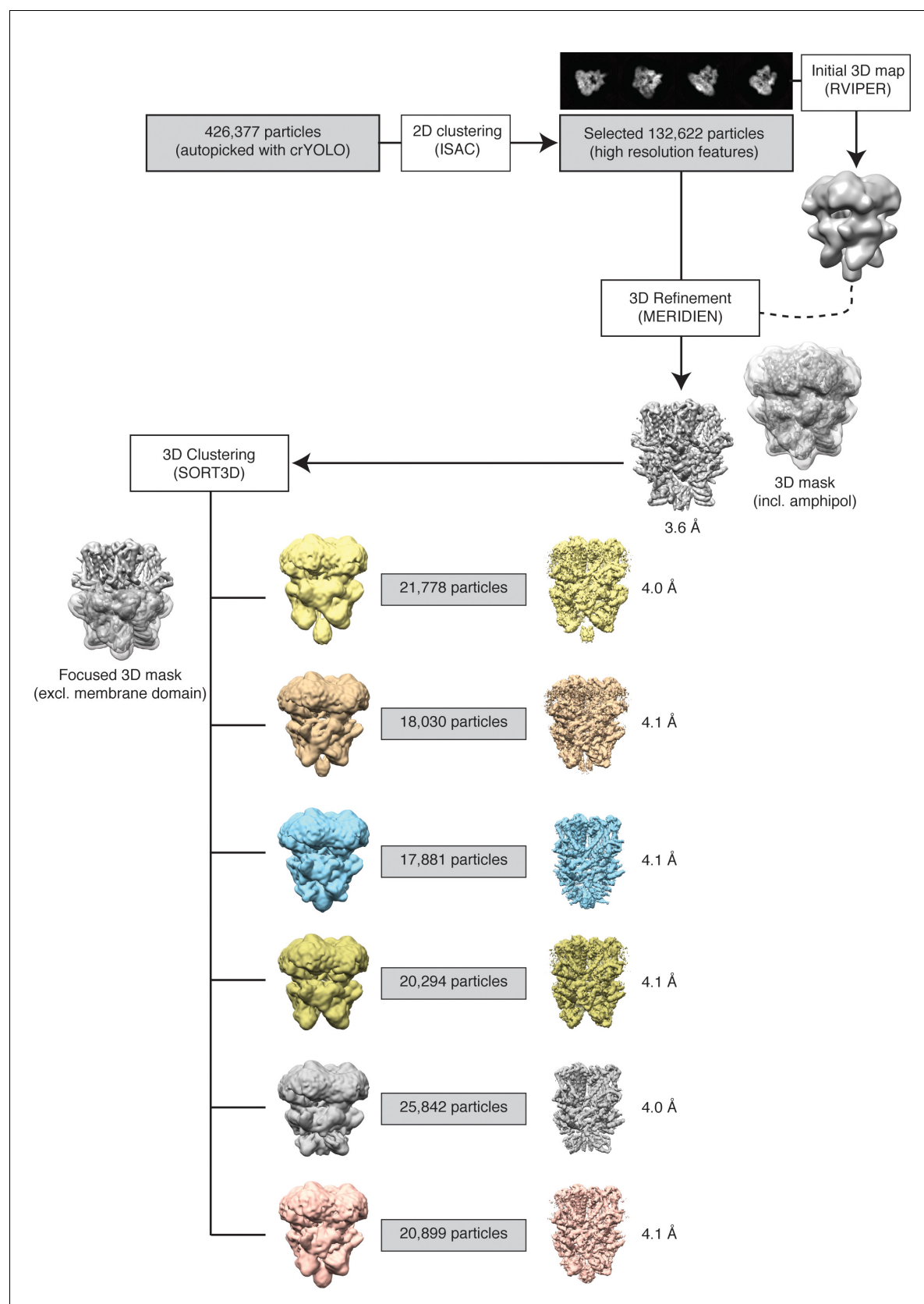


Figure 2—figure supplement 3. Single particle processing workflow for TRPC_{4DR} structure determination.

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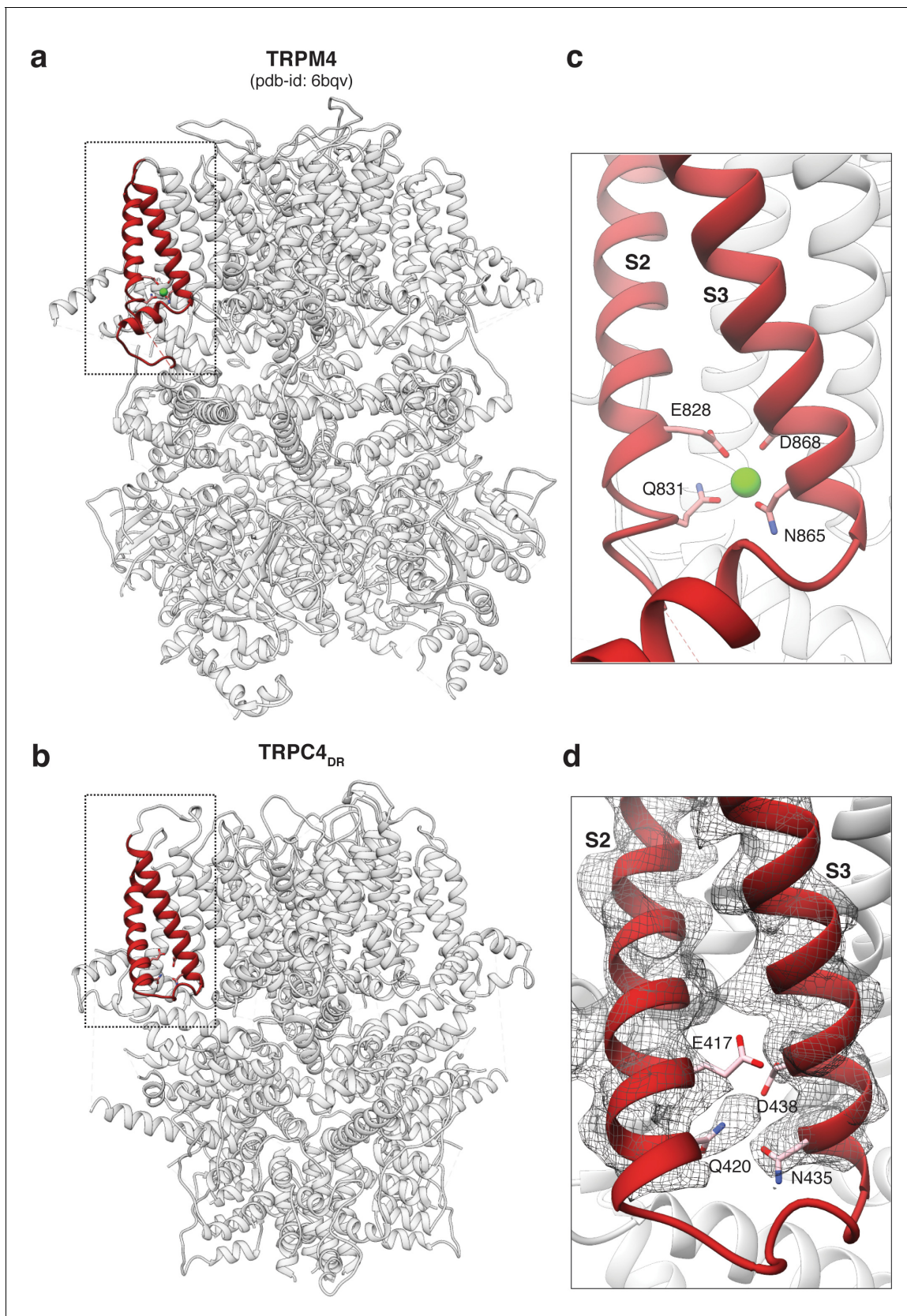


Figure 2—figure supplement 4. Comparison of the Ca^{2+} binding site in TRPM4 and TRPC4_{DR}. (a-b) Side view of TRPM4 (a) and TRPC4_{DR} (b) shown in ribbon representation. Helices involved in Ca^{2+} -binding are shown in red. Zoomed-in view on the Ca^{2+} -binding site in TRPM4 (c) and TRPC4_{DR} (d).

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Residues involved in Ca^{2+} binding in TRPM4 and its topologically equivalent residues in TRPC4_{DR} are shown in stick representation. Ca^{2+} ion is shown as green sphere.

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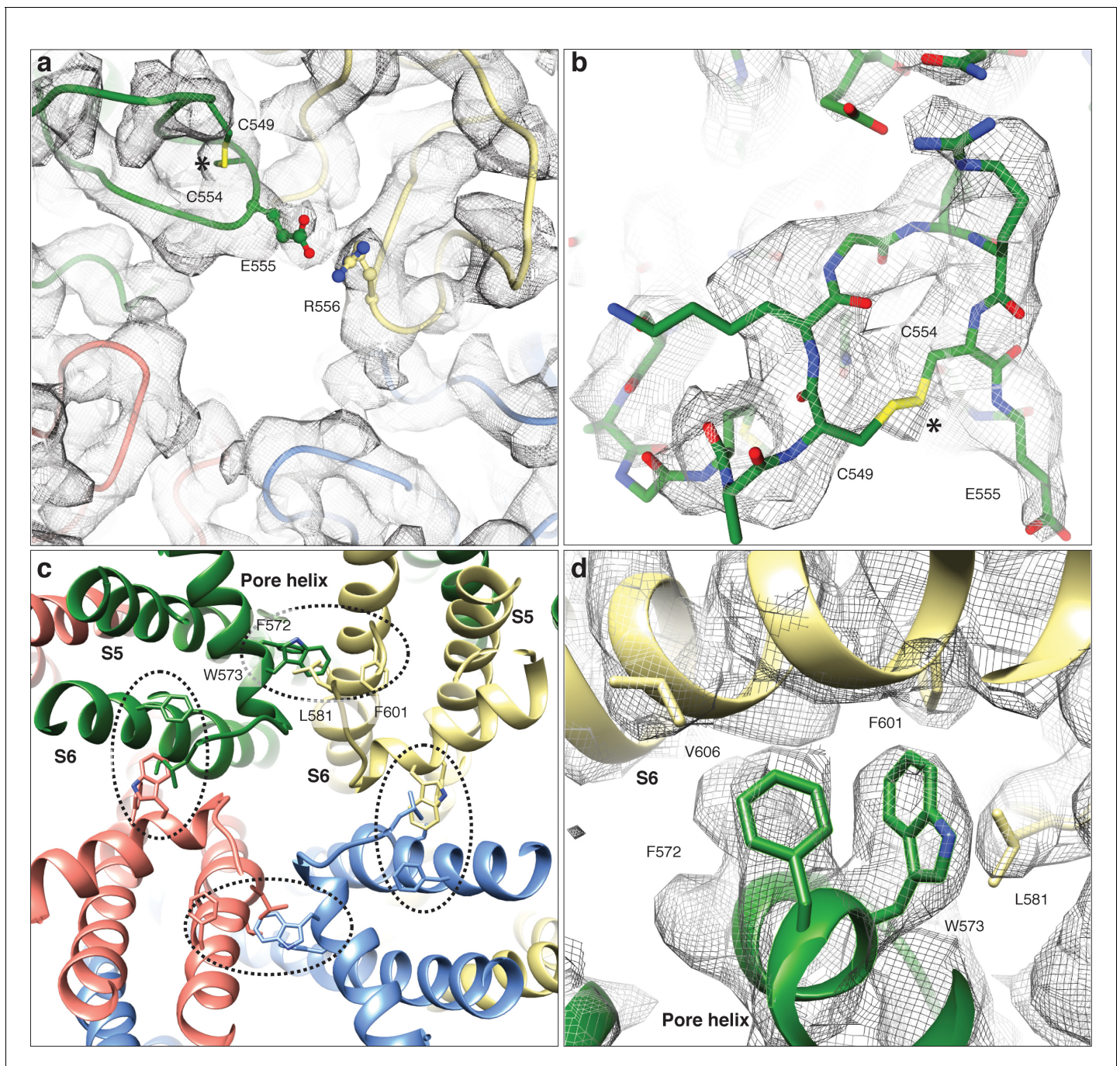


Figure 2—figure supplement 5. LFW motif of the pore region and cysteines involved in disulphide bridges in the TRPC4_{DR} structure. **(a)** Extracellular view near the pore axis showing the loops harboring cysteine residues. The loops are shown in ribbon representation with cysteines shown in stick representation. The neighboring glutamate residue is involved in an electrostatic interaction with an arginine. **(b)** Zoomed-in view on the disulphide bridge region. The chain trace is shown in stick representation including the side chains. The disulphide bridge is indicated by an asterisk. Electron density in (a) and (b) is shown in wired grey mesh. **(c)** LFW motif region viewed along the pore axis from the extracellular side. Each protomer is colored differently. The LFW motif and interacting residues are shown in stick representation. The interface at the LFW motif is highlighted with a black dotted ellipse. **(d)** Zoomed-in view on the interface at the LFW motif. The density is shown in wired grey mesh with key residues highlighted.

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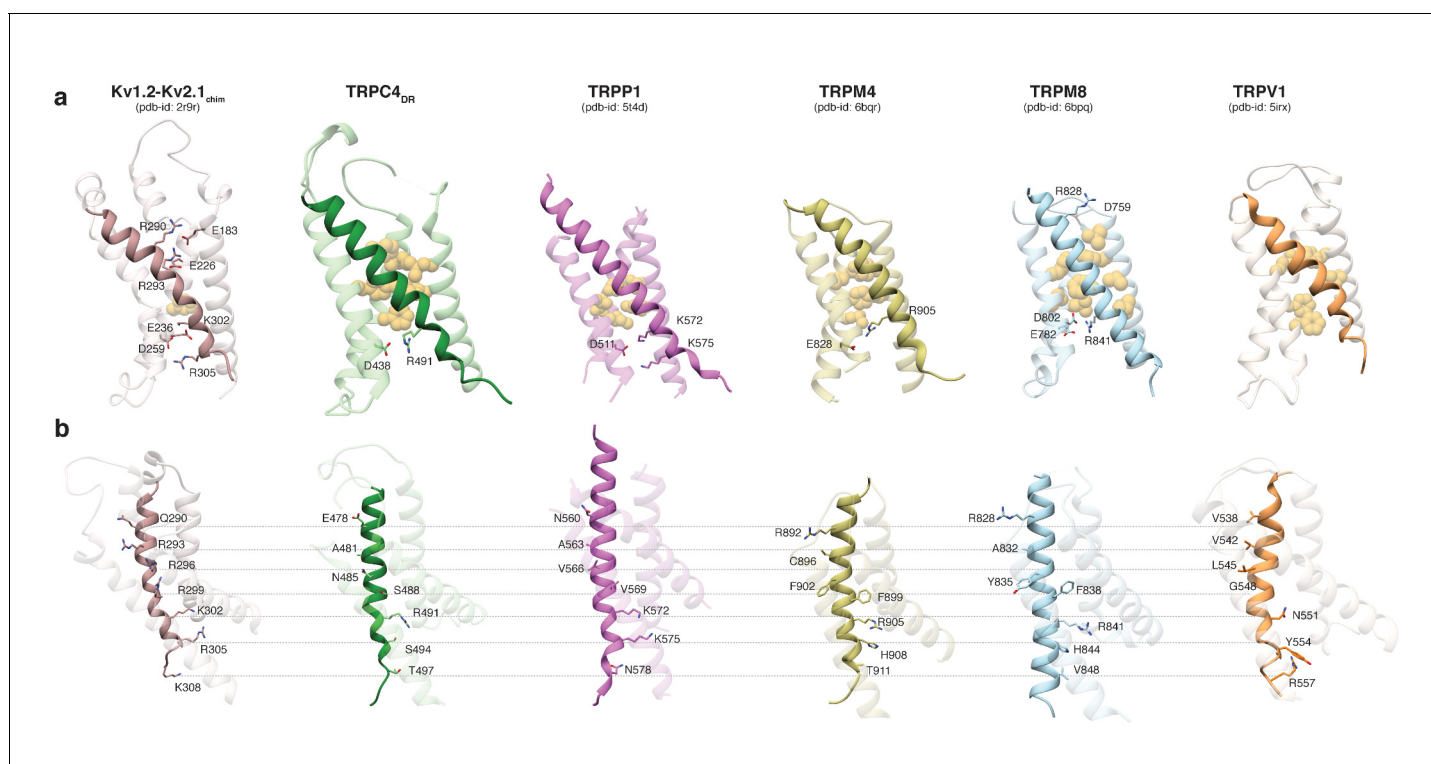


Figure 3. Comparison of the VSL domain of selected TRP family members with the voltage-sensing domain of the chimeric Kv1.2-Kv2.1 channel. **(a)** The voltage-sensing domain of the chimeric Kv1.2-Kv2.1 channel and the VSL domains of TRPC4_{DR}, TRPP1, TRPM4, TRPM8 and TRPV1 are shown in ribbon representation. The S4 helix in each case is highlighted with dark shaded color. The rest of the domains are shown in light transparent color. The residues which form the hydrophobic patch in the middle of the domain are shown in golden yellow sphere representation with light transparency. The residues involved in ion-pair interactions are shown in stick representation. **(b)** The structures in **(a)** are rotated to better view the residues important for voltage sensing in the S4 helix of the chimeric Kv1.2-Kv2.1 channel and topological equivalent residues of TRP channels are shown in stick representation and labeled.

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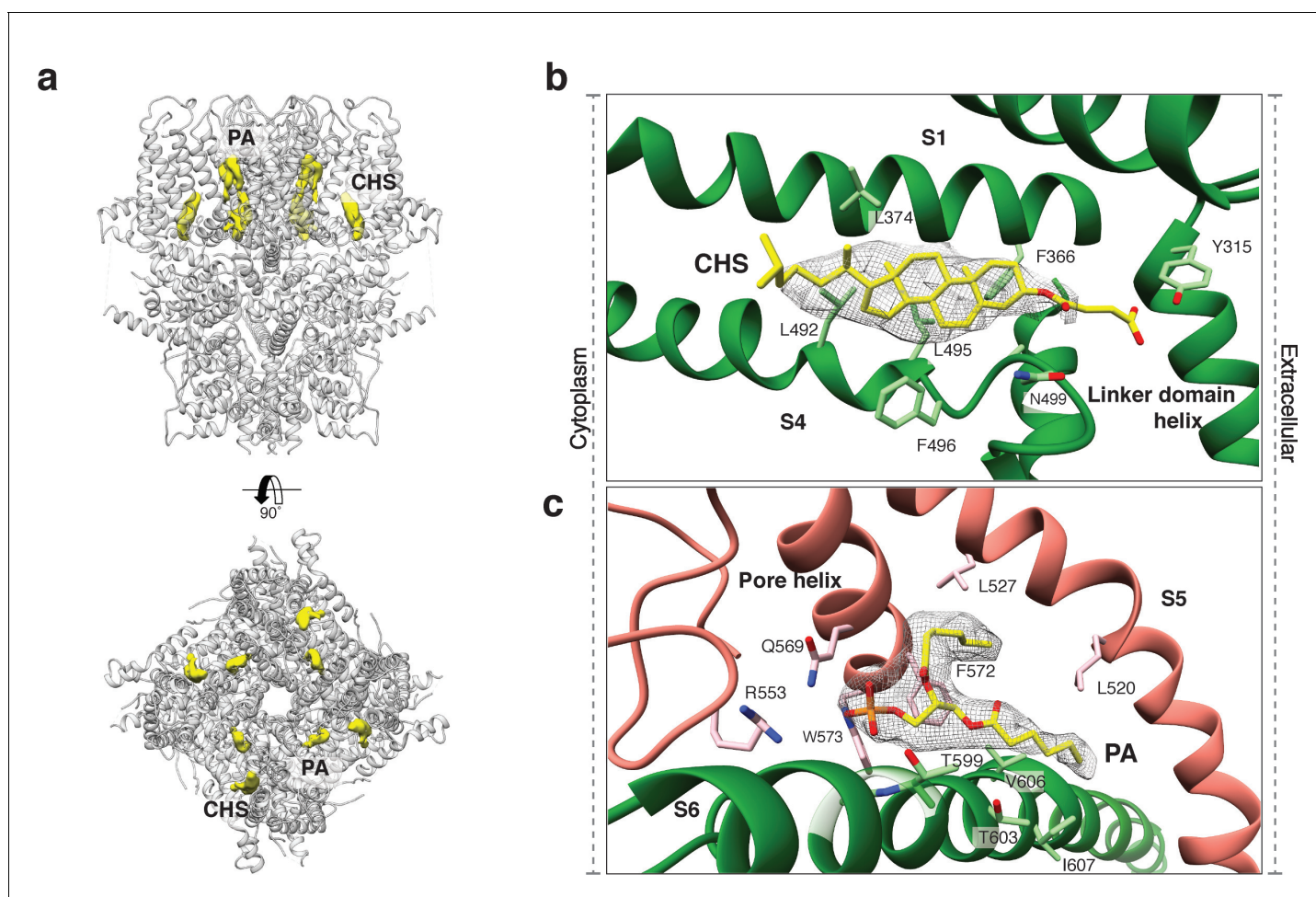


Figure 4. Lipid binding sites in the TRPC4_{DR} structure. (a) Side and top view of the TRPC4_{DR} structure with lipid densities highlighted in yellow against the model shown in ribbon representation. (b–c) Zoomed-in view on the cholesterol hemisuccinate (CHS) and phosphatidic acid (PA) binding sites, respectively. CHS and PA molecules are shown in yellow stick representation.

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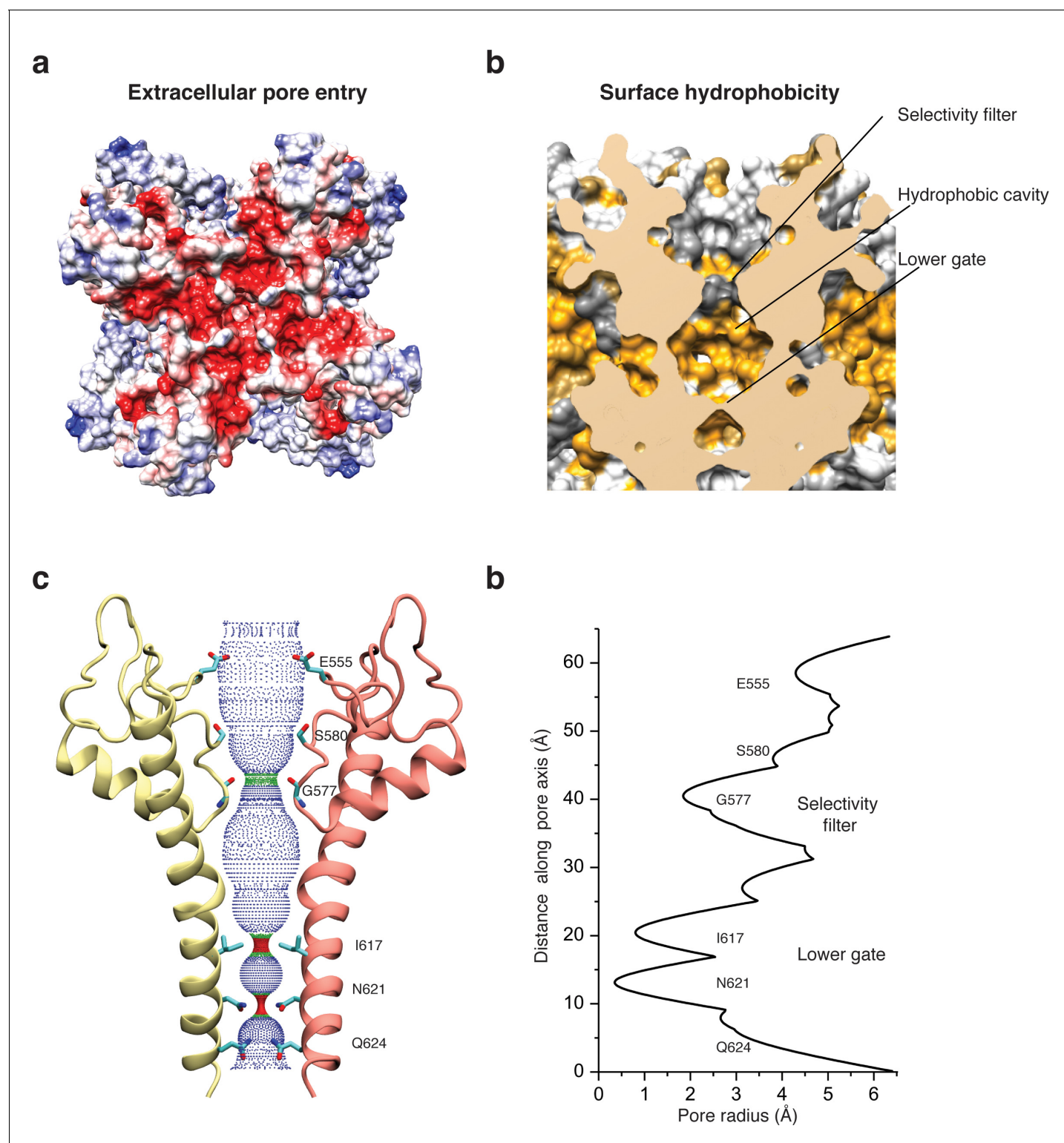


Figure 5. Architecture of the pore domain. (a) Surface electrostatic Coulomb potential at the extracellular mouth of TRPC4_{DR}. (b) Hydrophobic surface of the pore shown in vertical cross section. Hydrophobic patches are colored orange. (c) Ion conduction pore of TRPC4_{DR} shown with diagonally facing protomers shown in ribbon representation. Critical residues important for gating and selection are shown in stick representation. (d) Pore radius determined along the pore axis using HOLE (Smart et al., 1996).

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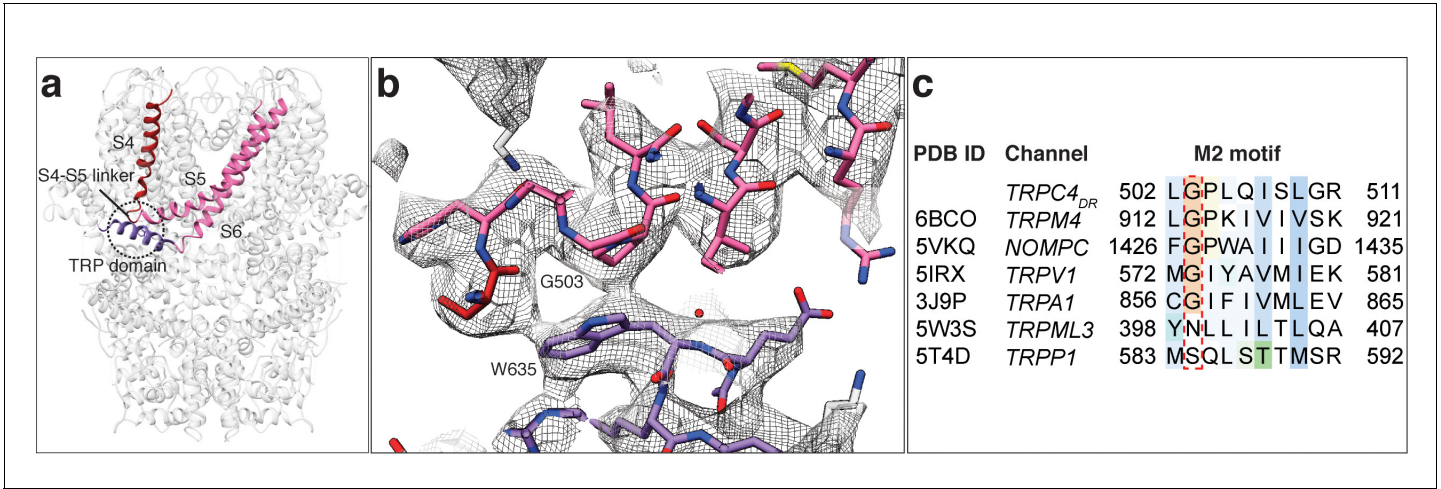


Figure 6. Interaction of the TRP domain with a conserved glycine in the S4-S5 linker. (a) Ribbon overview indicating the interaction site between the TRP domain and the S4-S5 linker. Key helices are shown in different colors. The rest of the protein is shown in light grey. (b) Zoomed-in view on the interaction site with key residues labeled. The chain trace is shown in stick representation. (c) Structure-based sequence alignment of TRP family members. The conserved glycine in the M2 motif is highlighted by a red dotted box.

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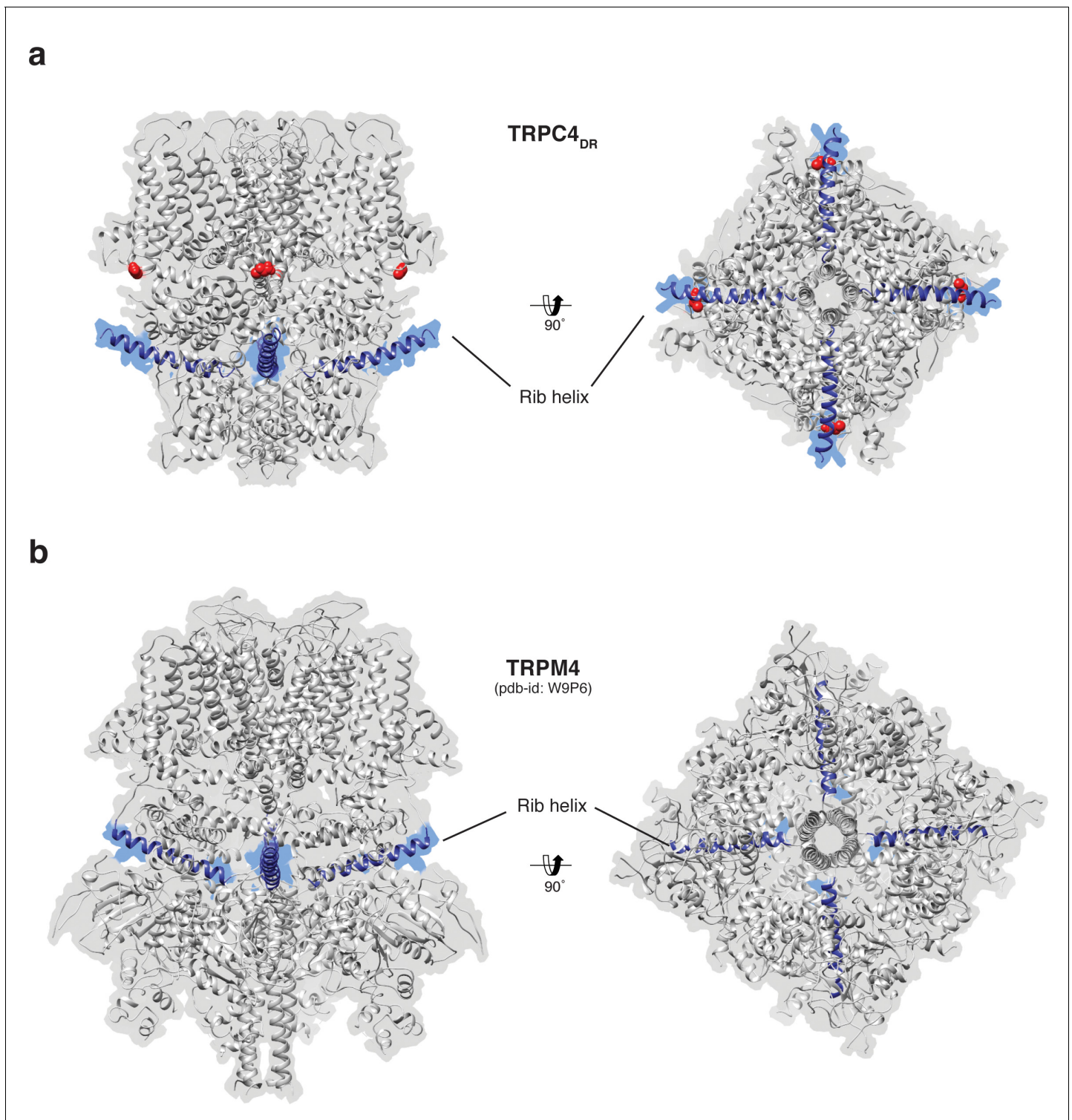


Figure 7. Position of the Rib helix in TRPC4_{DR} and TRPM4. (a-b) Side and bottom view of the atomic models of TRPC4_{DR} (a), and TRPM4 (b). The Rib helix is highlighted in blue color and the density of the map is shown in the background with high transparency. E648 in TRPC4_{DR} is shown in sphere representation and colored red.

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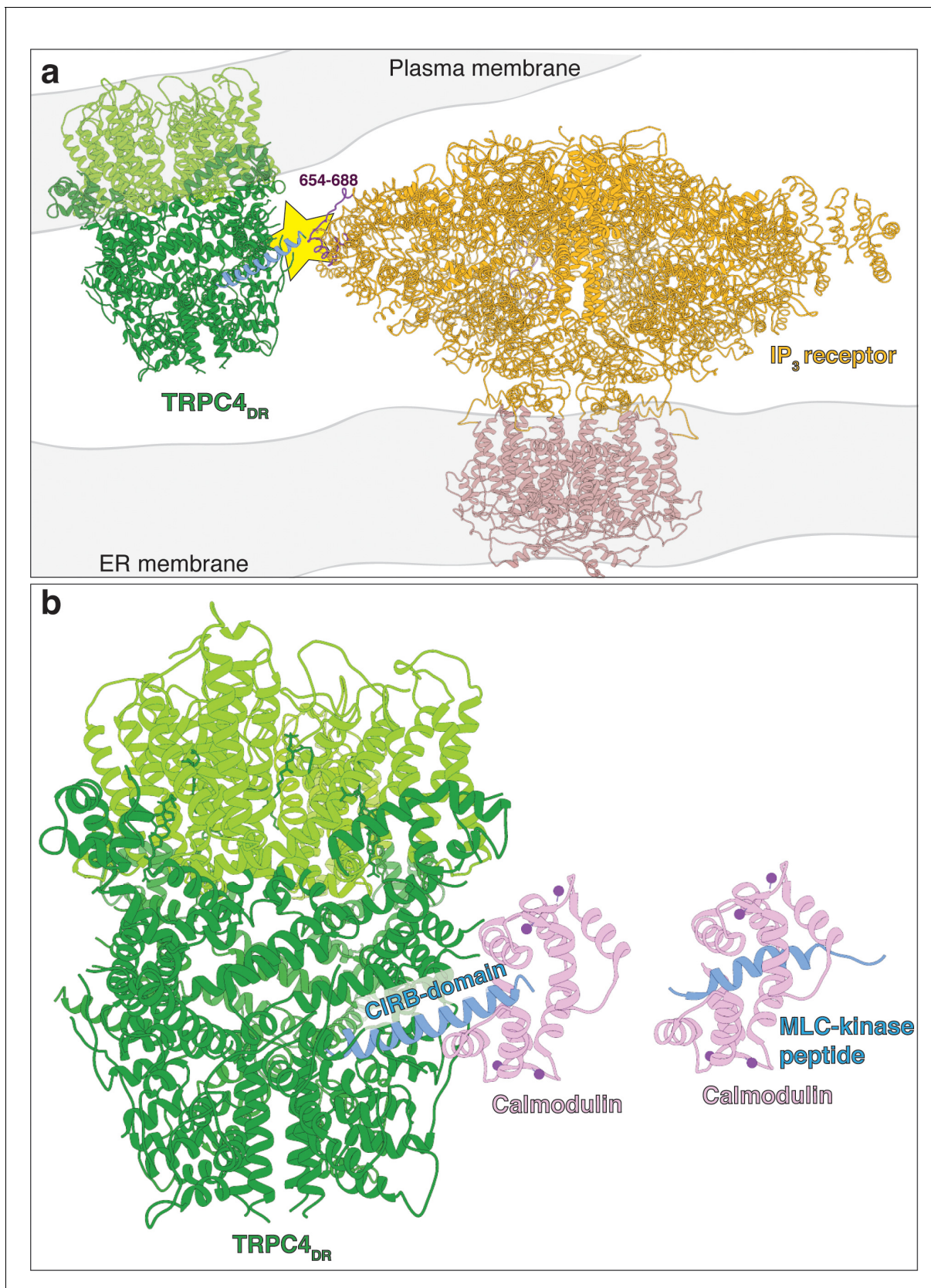


Figure 7—figure supplement 1. Predicted model of TRPC4_{DR} interaction with IP₃ receptors and calmodulin. (a) Possible interaction of an IP₃ receptor with TRPC4_{DR}. The putative site of interaction (Boulay et al., 1999; Tang et al., 2001) is highlighted with a yellow star. The CIRB domain of TRPC4_{DR} is Figure 7—figure supplement 1 continued on next page

Figure 7—figure supplement 1 continued

shown in blue. The predicted interacting region of the IP₃ receptor is highlighted in dark color. **(b)** Interaction of calmodulin with the CIRB domain of TRPC4_{DR}. Calmodulin is shown in pink. Calcium is shown as spheres. Calmodulin has been fitted to the structure of TRPC4_{DR} in analogy to the MLC-kinase peptide bound to calmodulin in the crystal structure (PDB-ID: 2LV6).

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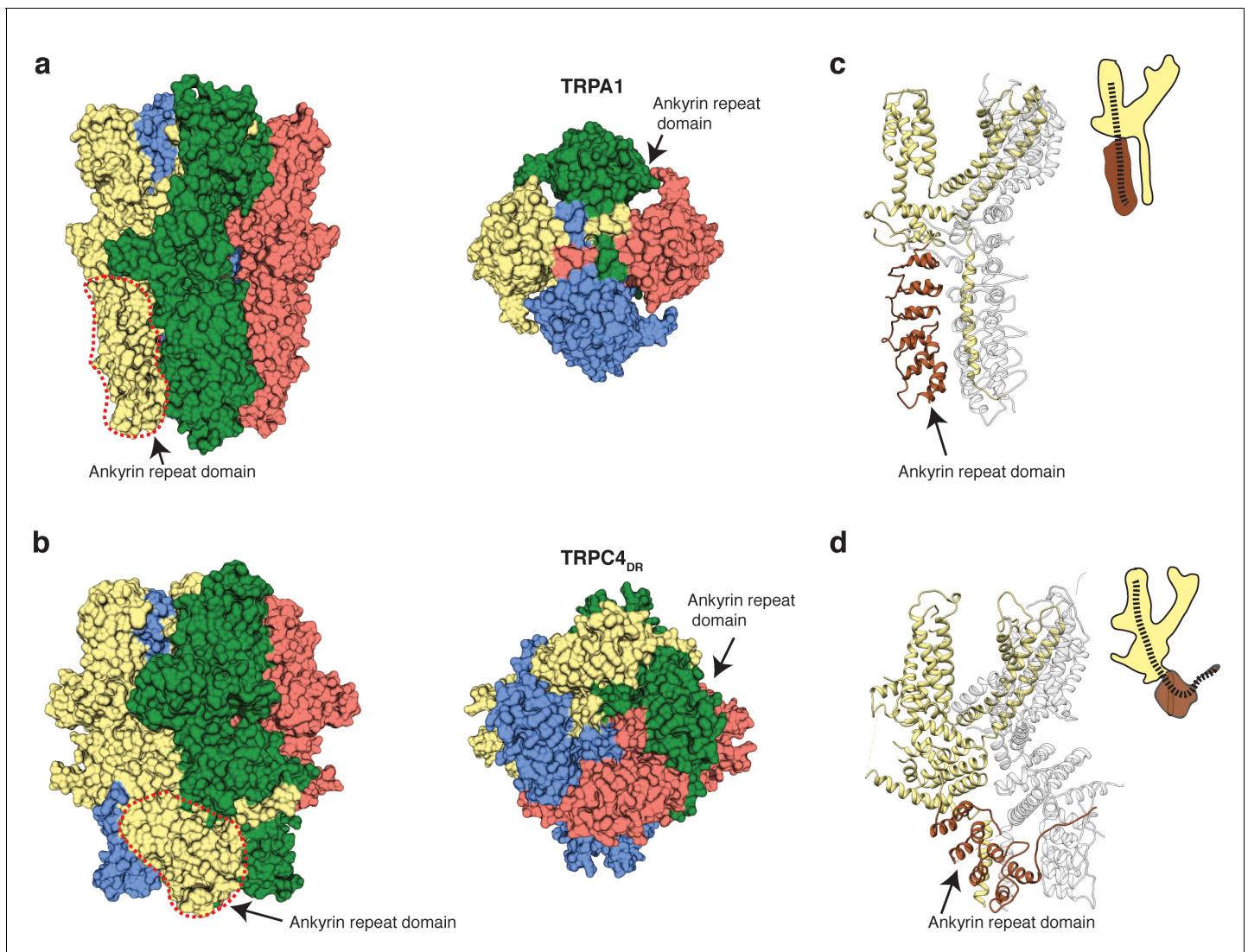


Figure 8. Comparison of the ankyrin domain arrangement of TRPC4_{DR} and TRPA1. (a–b) Side and bottom view of the structures of TRPA1 (PDB-ID: 3J9P) (a) and TRPC4_{DR} (b) in surface representation. Each subunit is colored with unique colors. (c–d) Ribbon and cartoon representation of TRPA1 (c) and TRPC4_{DR} (d) showing two protomers in side view. The ankyrin repeats of one protomer are highlighted in brown.

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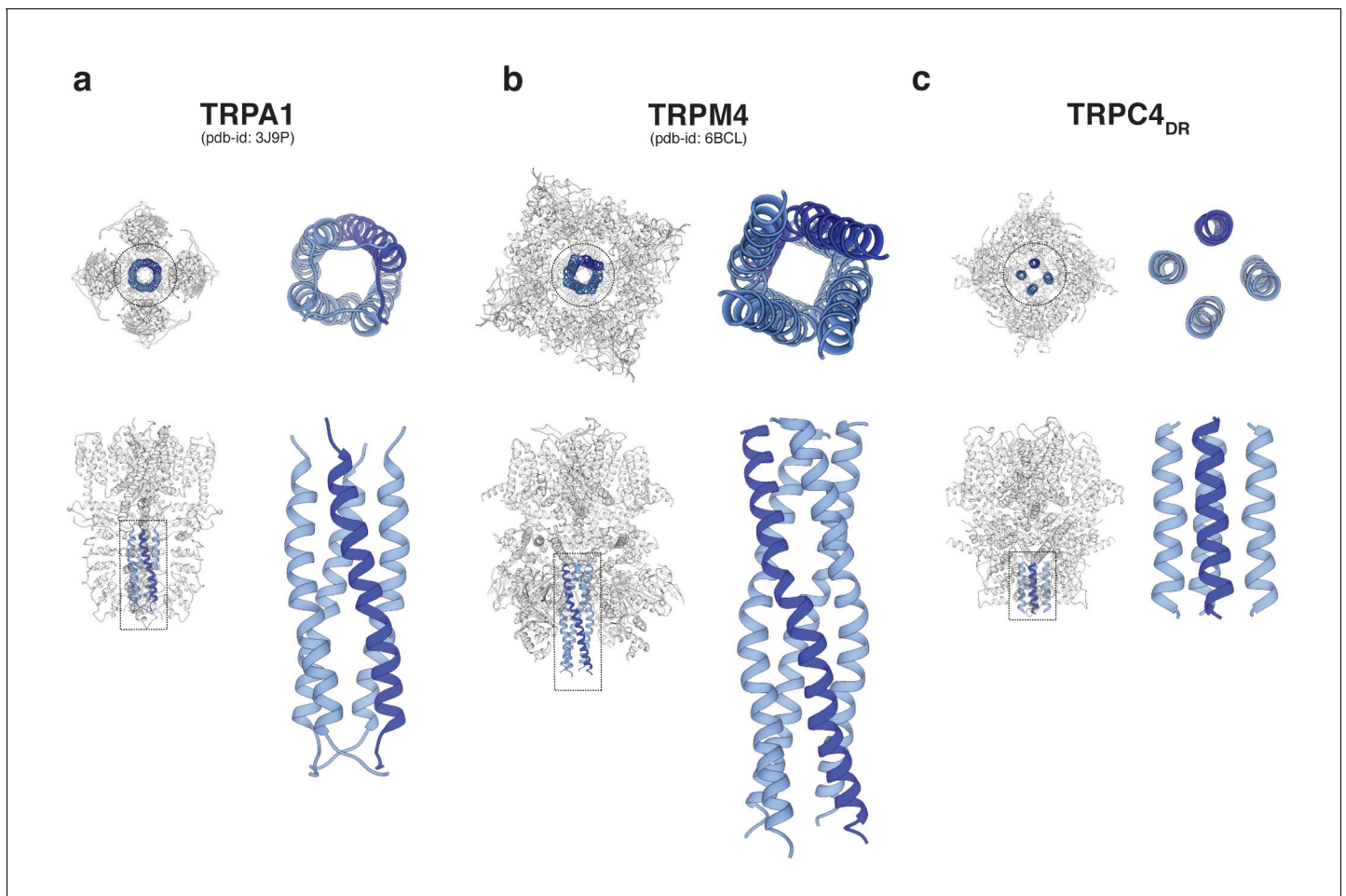


Figure 9. Comparison of the C-terminal helix architecture in TRPA1, TRPM4 and TRPC4_{DR}. (a-c) Each panel shows the complete tetramer in bottom and side view on the left and the zoomed-in view of the C-terminal helix alone in bottom and side view on the right. The C-terminal helix is shown in blue with one helix highlighted in shaded dark blue.

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