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Supplemental Information

Structural Insight into the Assembly

of TRPV Channels

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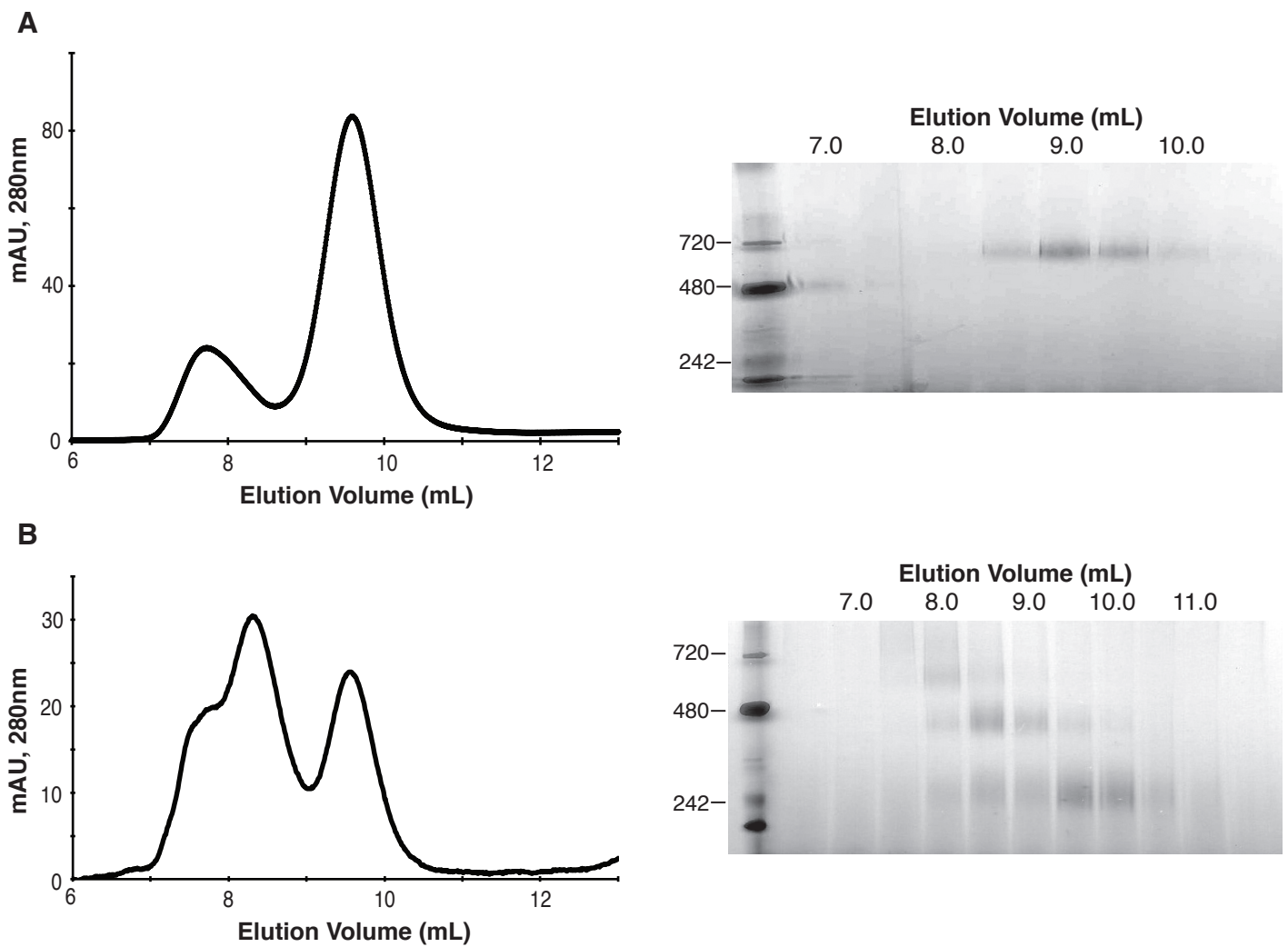


Figure S1, Size-exclusion chromatography (SEC) of purified TRPV2, related to Figure 1 and Figure 2. (A) SEC profile and native gel of MNG purified TRPV2. The native gel represents the elution fractions collected from the SEC column. A single band, corresponding to the molecular weight of pure, detergent-solubilized TRPV2 was detected in fractions from 8.5 to 10 mL. **(B)** SEC profile and native gel analysis of DDM purified TRPV2. The SEC chromatogram shows multiple peaks and the native gel corresponding to the elution fractions shows bands at multiple molecular weights. This heterogeneity in the protein population suggests multiple oligomeric states of TRPV2 in DDM.

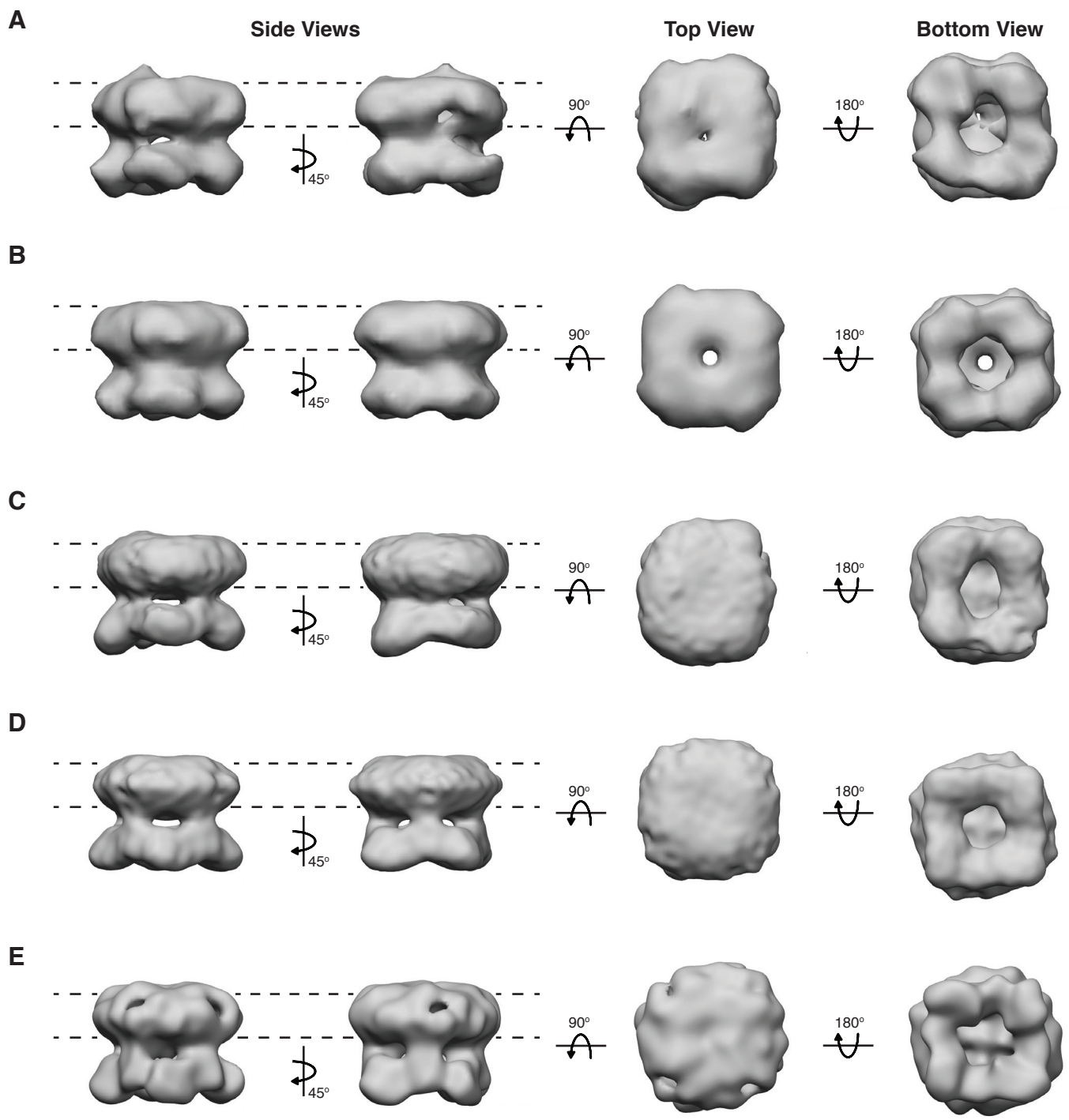


Figure S2, 3D reconstruction of TRPV2 by different software packages, related to Figure 2. (A) Initial models of TRPV2 generated from IMAGIC without imposed symmetry and (B) with imposed four-fold symmetry. (C) 3D maps of TRPV2 generated in FREALIGN without and (D) with four-fold symmetry. (E) The 3D Fourier Space refined map processed without imposed symmetry. The dashed lines indicate the plasma membrane. Scale bars, 25 Å.

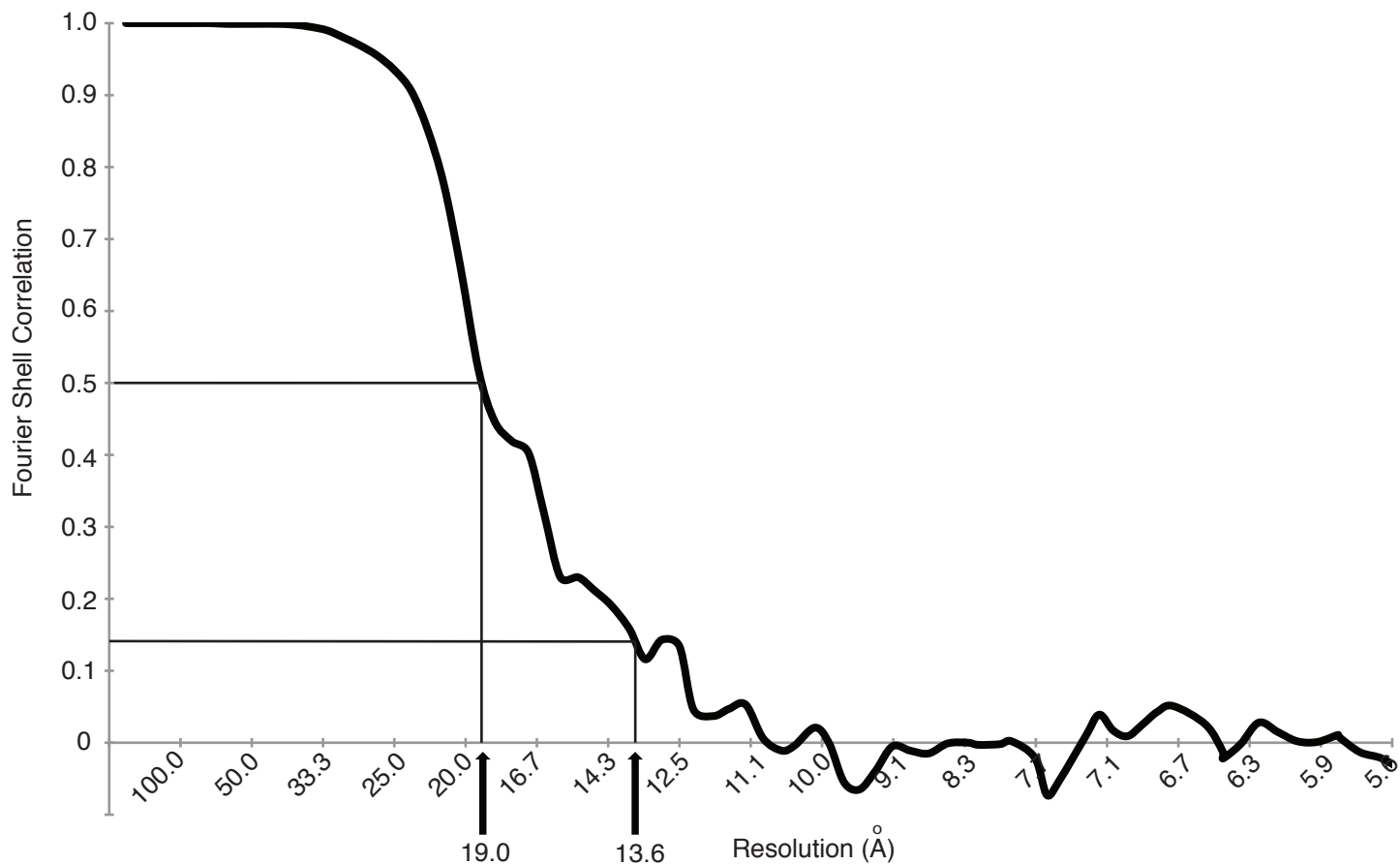


Figure S3, Resolution of the final TRPV2 3D map, related to Figure 2. The gold-standard Fourier shell correlation (FSC) curve gives the resolution of the final 3D Fourier Space refined map with imposed C4 symmetry as 13.6 Å and 19.0 Å using the 0.143 and 0.5 criteria respectively.

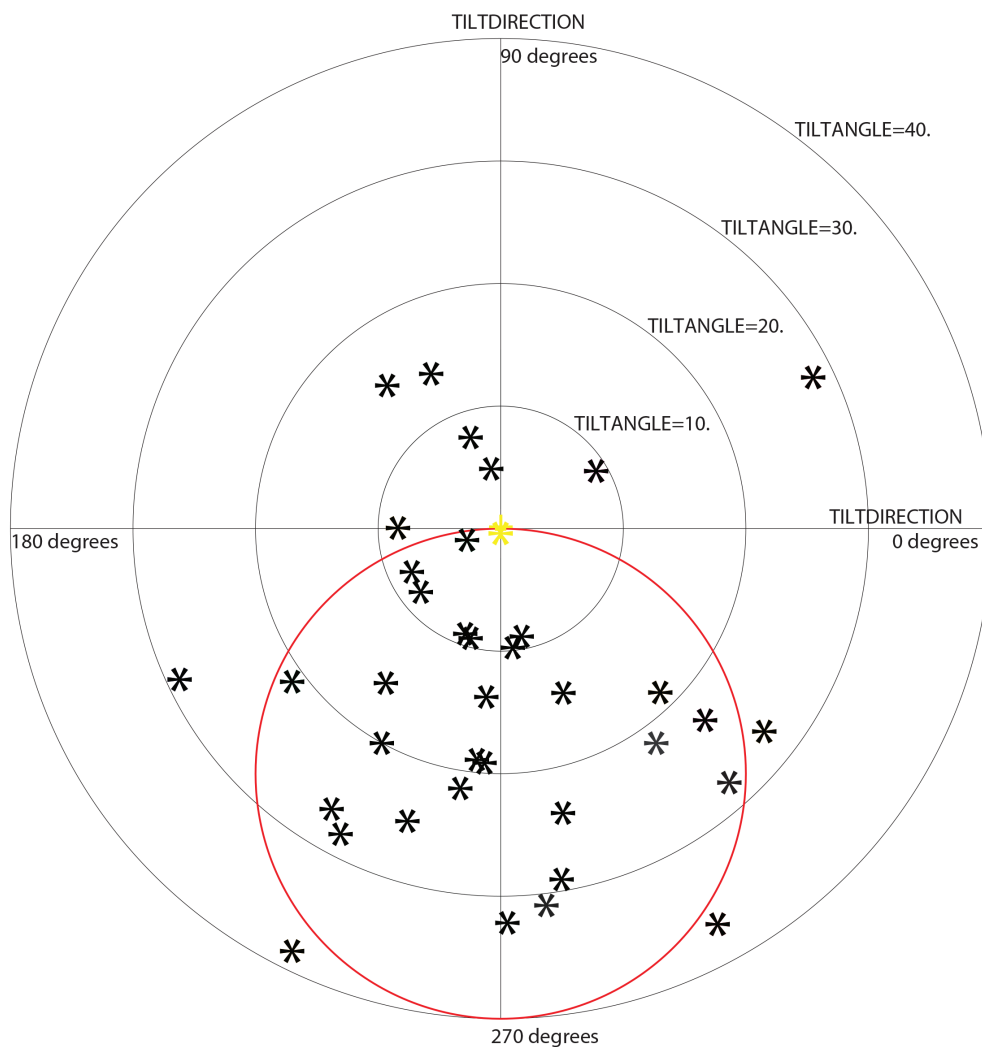


Figure S4, Tilt-Pair Parameter Plot (TPPP) of TRPV2, related to Figure 2.

The red circle has a radius of 20° and is centered at the expected tilt axis of 270° and tilt angle of 20° . The position of the expected tilt axis and tilt angle was determined by processing of a simulated tilt-pair data set for a molecule with a known and distinct handedness. 70% of the plotted TRPV2 tilt-pair points are clustered within the red circle. This TPPP of TRPV2, in comparison with the TPPP of the simulated data set, suggests that the hand of the TRPV2 map is correct.

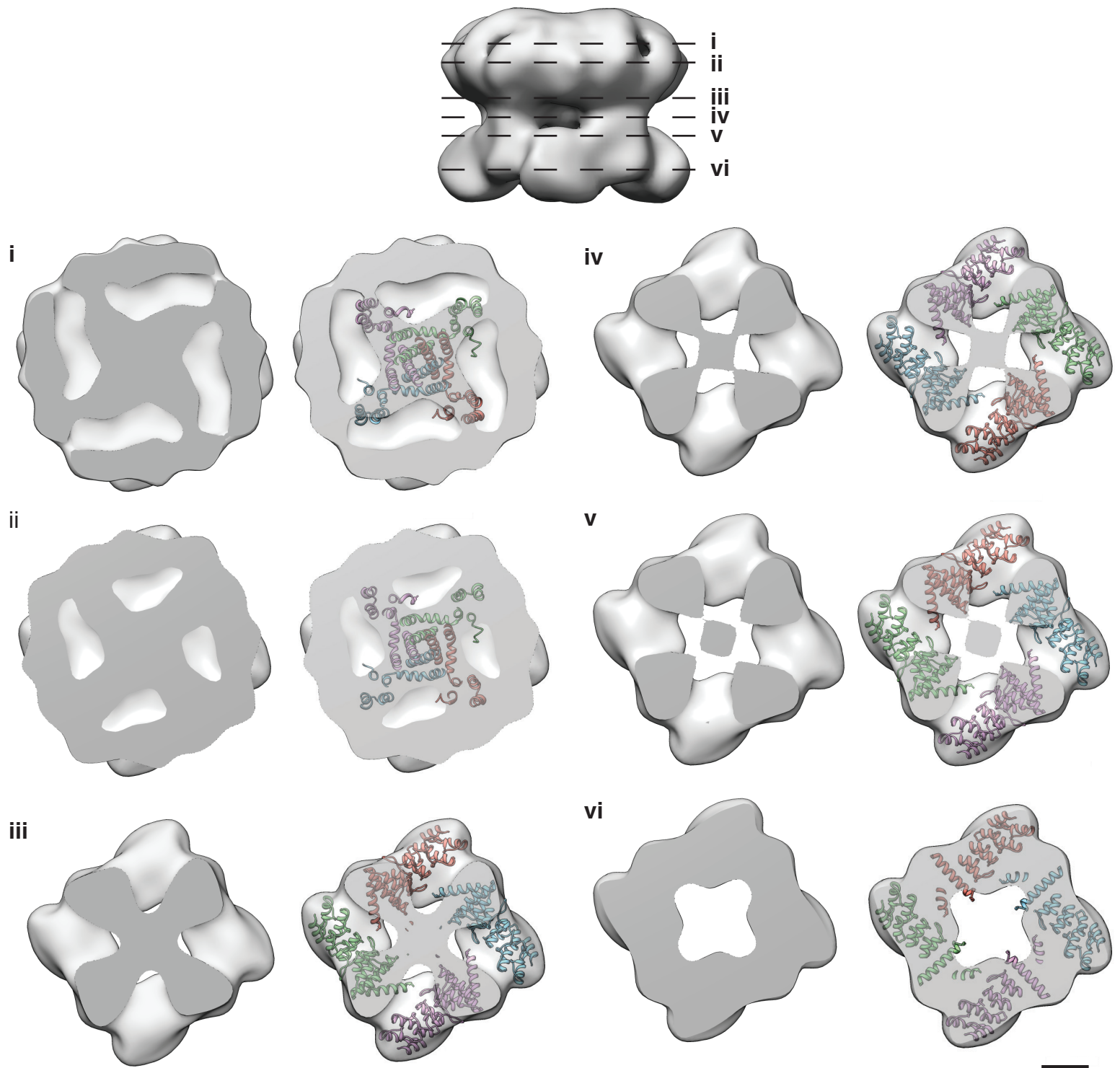


Figure S5, 3-D map of TRPV2 with representative cross-sections through the EM density, related to Figure 4. (i, ii) Cross-sectional views through the TM region of the TRPV2 EM map without and with fitting of the MlotiK1 TM domain (PDB code: 3BEH). (iii, iv, v) Cross-sectional views through the space between the ARD and the TM domain. This is the proposed location of the N-terminus linker region and C-terminus in the TRPV2 EM map. (vi) Cross-sectional views through the region of the TRPV2 EM map where the TRPV2 ARDs were fitted (represented without and with fitting; PDB code: 2ETB). The darker gray densities represent the plane of the cross-section. Scale bar, 25Å.

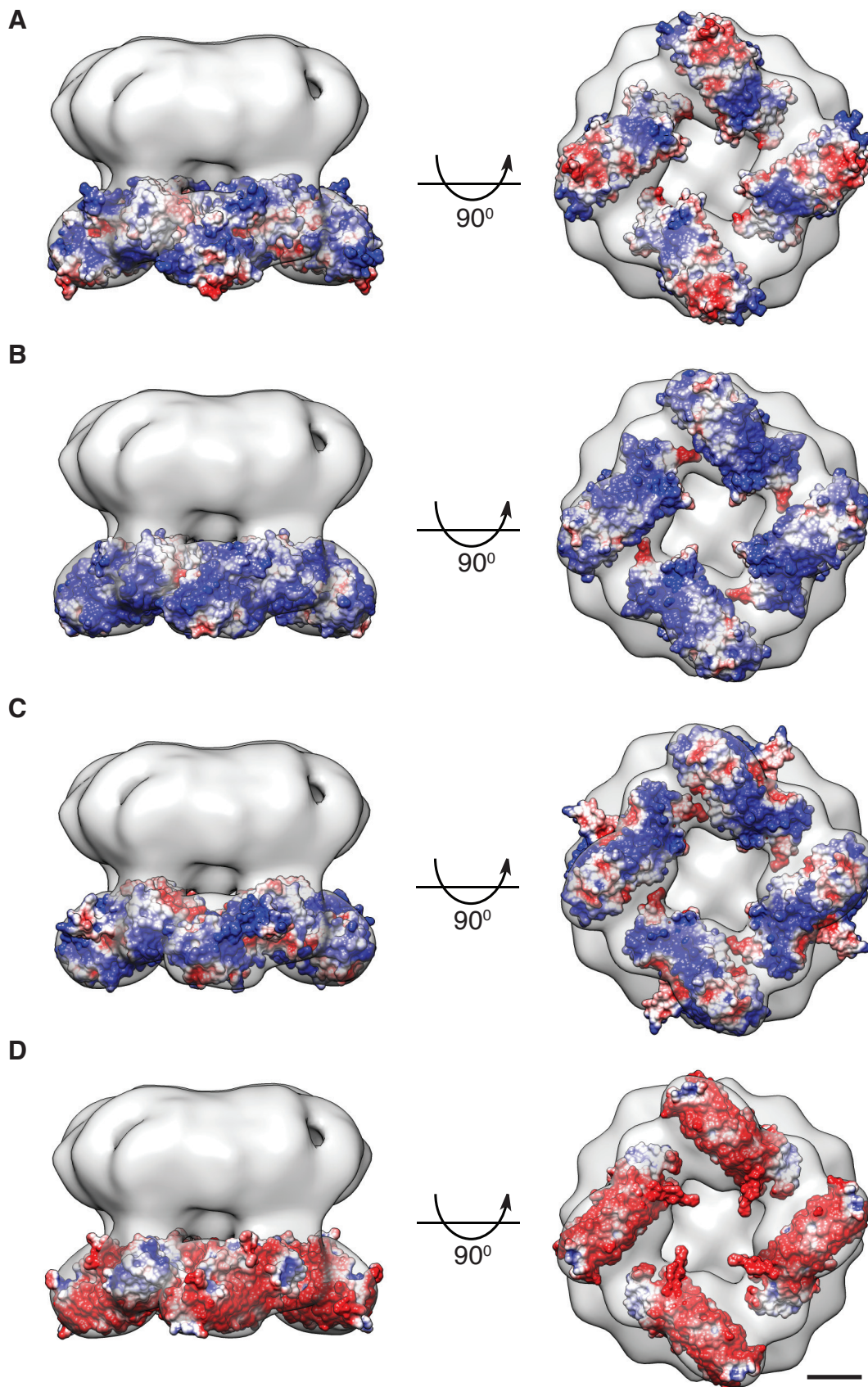


Figure S6, Electrostatic surface potential of TRPV1, TRPV4 and TRPV6 docked into the TRPV2 map, related to Figure 3. Side and bottom views of the calculated electrostatic potential of (A) TRPV1 ARD in the presence of ATP (PDB code: 2PNN), (B) TRPV4 ARD in the presence of ATP (PDB code: 4DX2), (C) TRPV4 ARD in the absence of ATP (PDB code: 4DX1) and (D) TRPV6 ARD (PDB code: 2RFA) docked in the TRPV2 map. Scale bar, 25 Å.

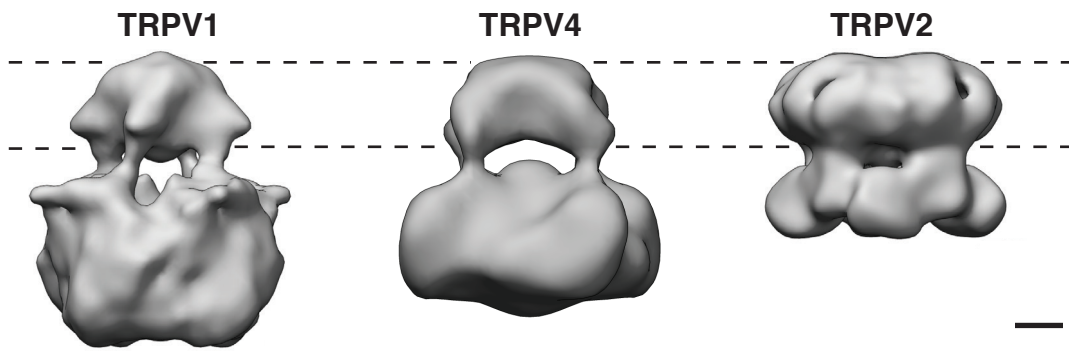


Figure S7, Cryo-EM structures of the TRPV subfamily, related to Figure 2.

Cryo-EM structures of TRPV1 at 19Å (Moiseenkova-Bell et. al., 2008) and TRPV4 at 35Å (Shigematsu et. al., 2010) in comparison to the TRPV2 at 13.6Å. The dashed lines represent the plasma membrane. Scale bar, 25 Å.