

Rosetta Docking Results

Calculated CTD/IQ Interface Energy vs. RMSD

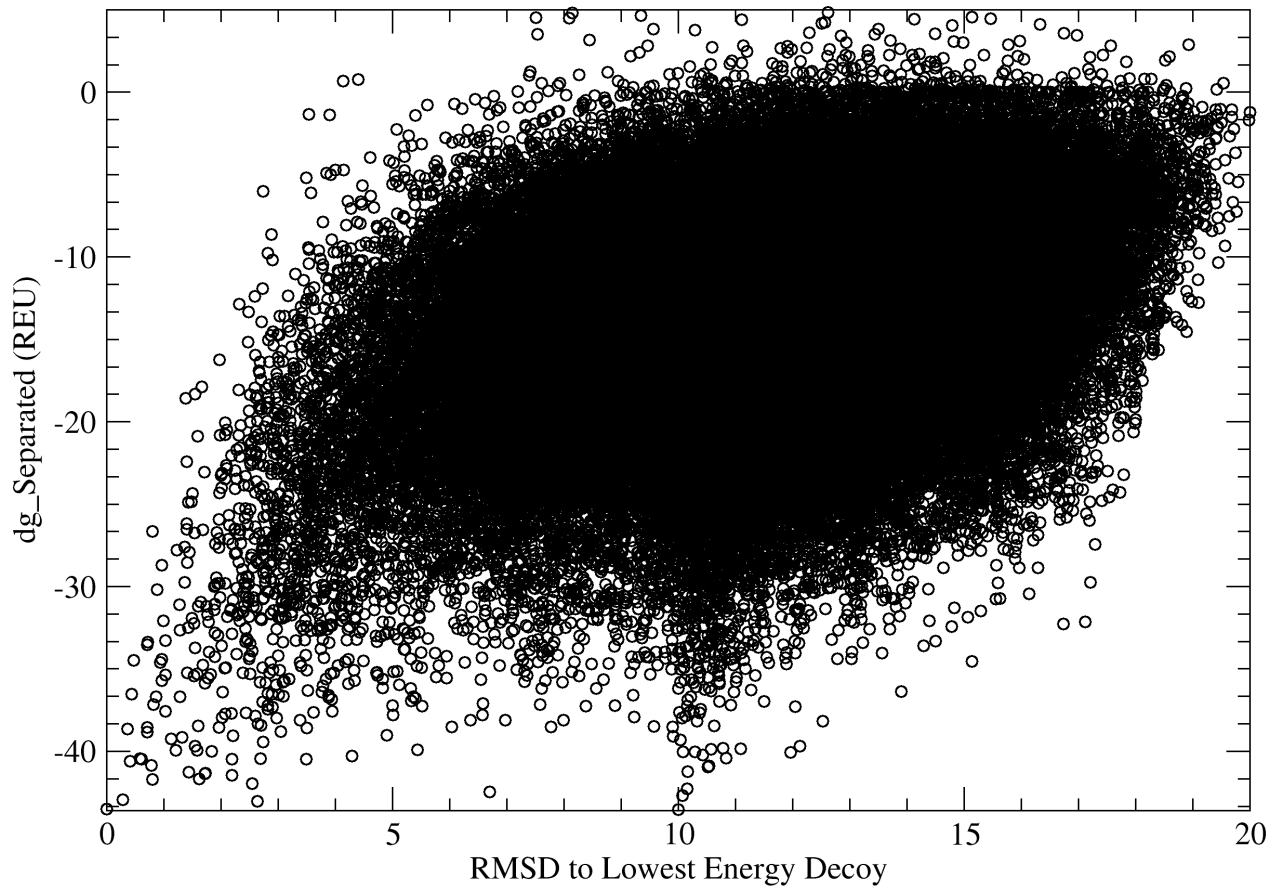
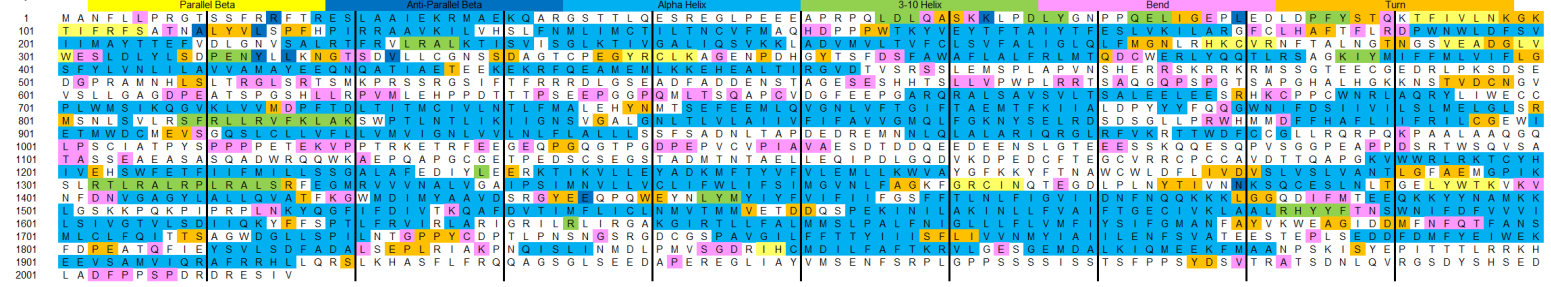


Fig. S1. Plot of Rosetta CTD/IQ motif interface energy (ΔG Separated) vs. RMSD to the lowest energy decoy. The “funnel” shape produced by this data indicated that the calculation adequately sampled the conformational space, and the scoring function successfully distinguished related binding poses from unrelated ones. The small, low-energy cluster around RMSD=10 is related to the preferred conformation by rotating the IQ helix by 180° , in the same binding pocket between helices I and IV.

IQ motif



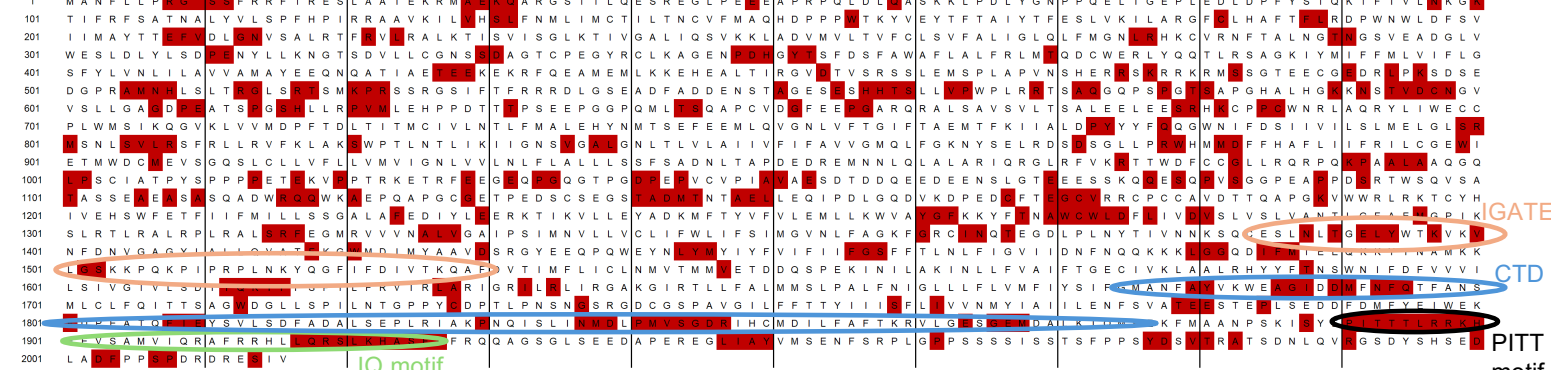
FREE



IGATE



IQ motif vs IGATE



IGATE vs FREE

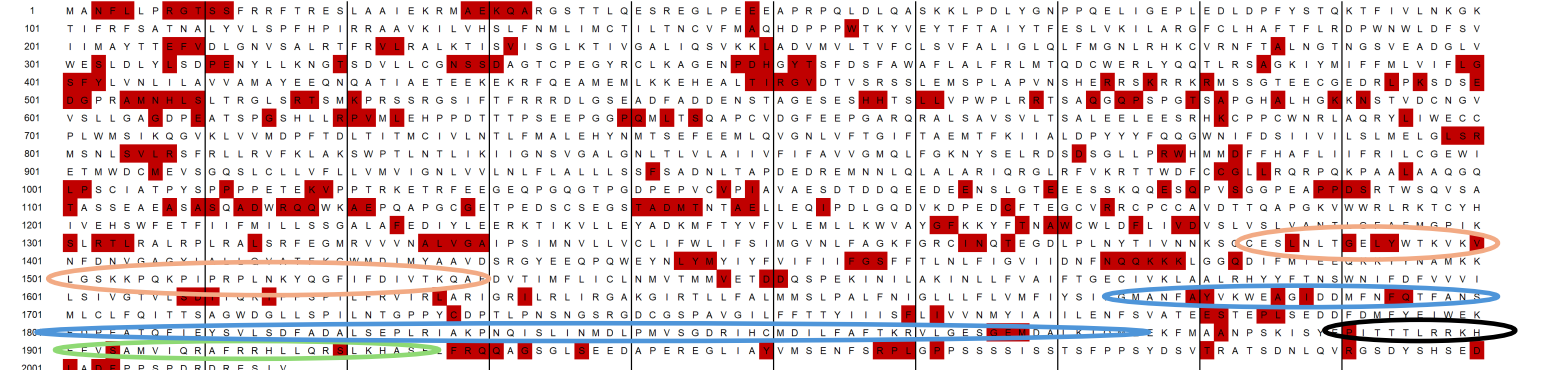


Fig. S2 Secondary structural analysis of Na_v1.5 post-MD models. Lower two panels highlight secondary structural differences arising from translocation of CTD. PITT motif change to accommodate IQ motif interaction.

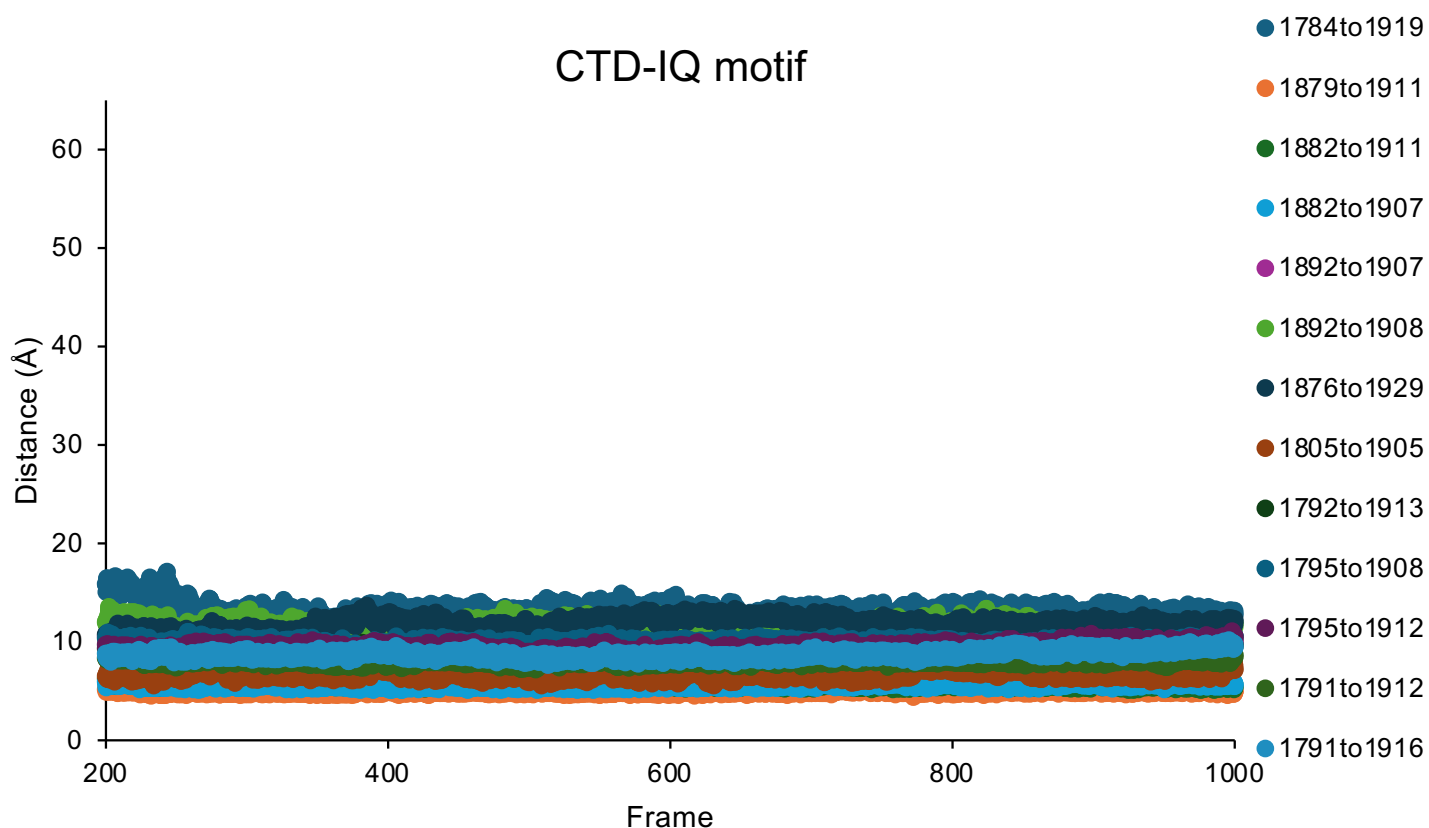
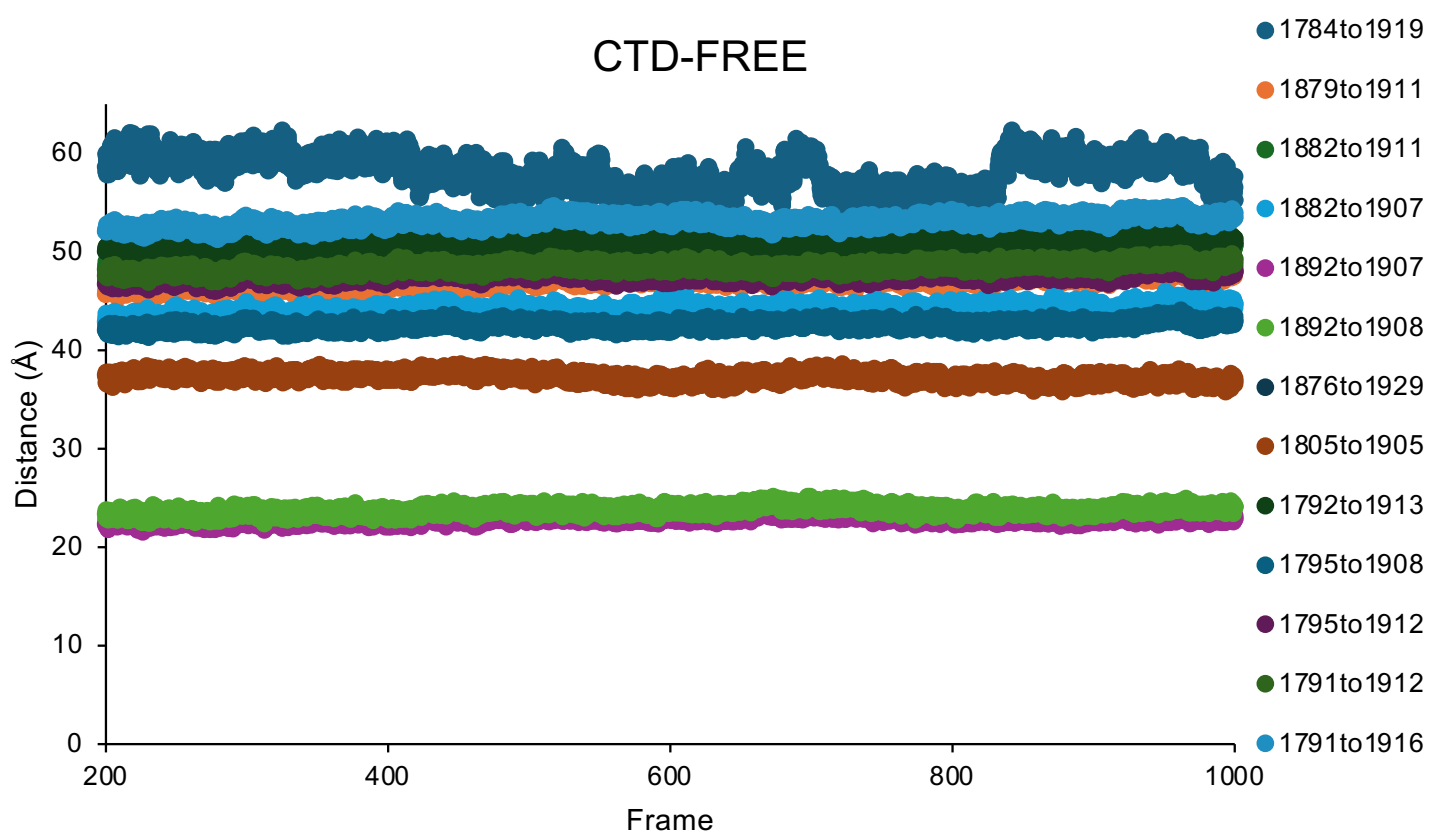


Fig. S3 Binding interface contact distances (alpha carbon) for Na_v1.5 CTD-FREE and CTD-IQ models.

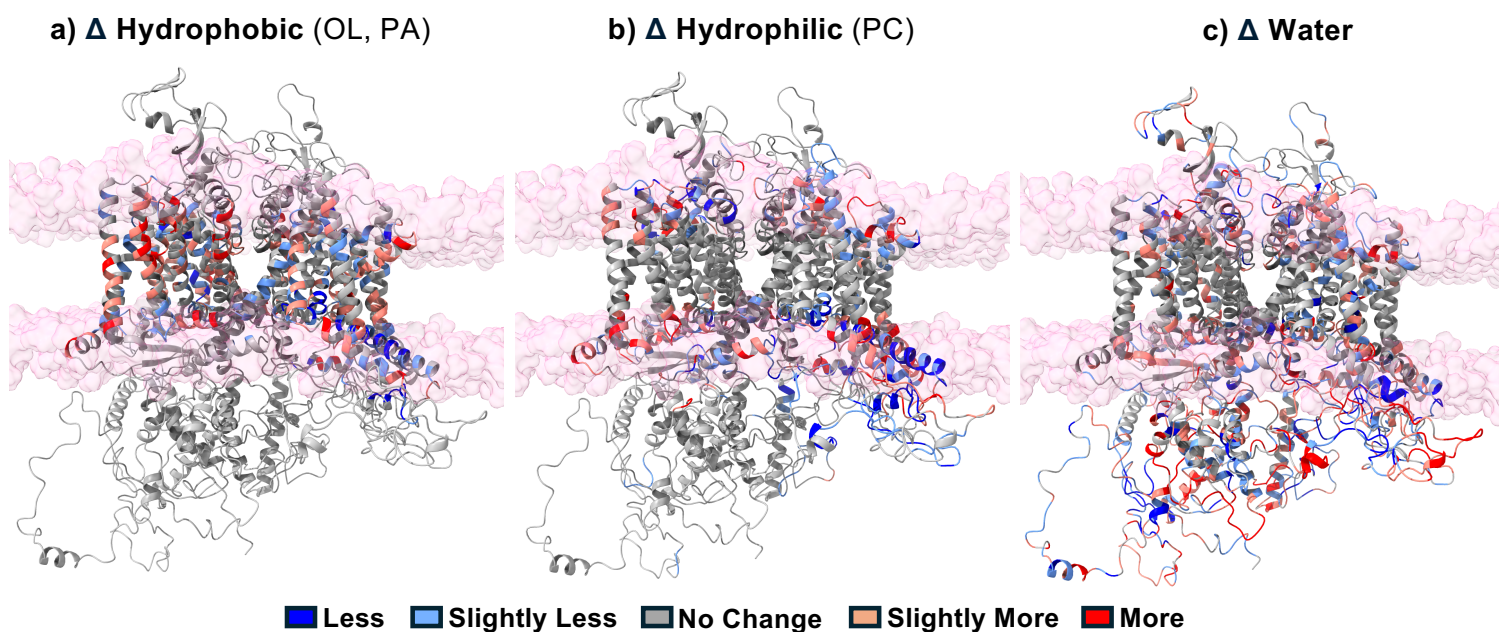


Fig. S4 Post MD model of CTD-IQ motif shown as ribbon diagram with contact differences between CTD-IQ motif and CTD-FREE MD simulations for **a)** hydrophobic lipid, **b)** hydrophilic lipid, and **c)** water contacts. Polar head group of lipids (PC) shown in translucent pink surface, hydrophobic lipid tails (OL and PA) omitted for visual clarity.

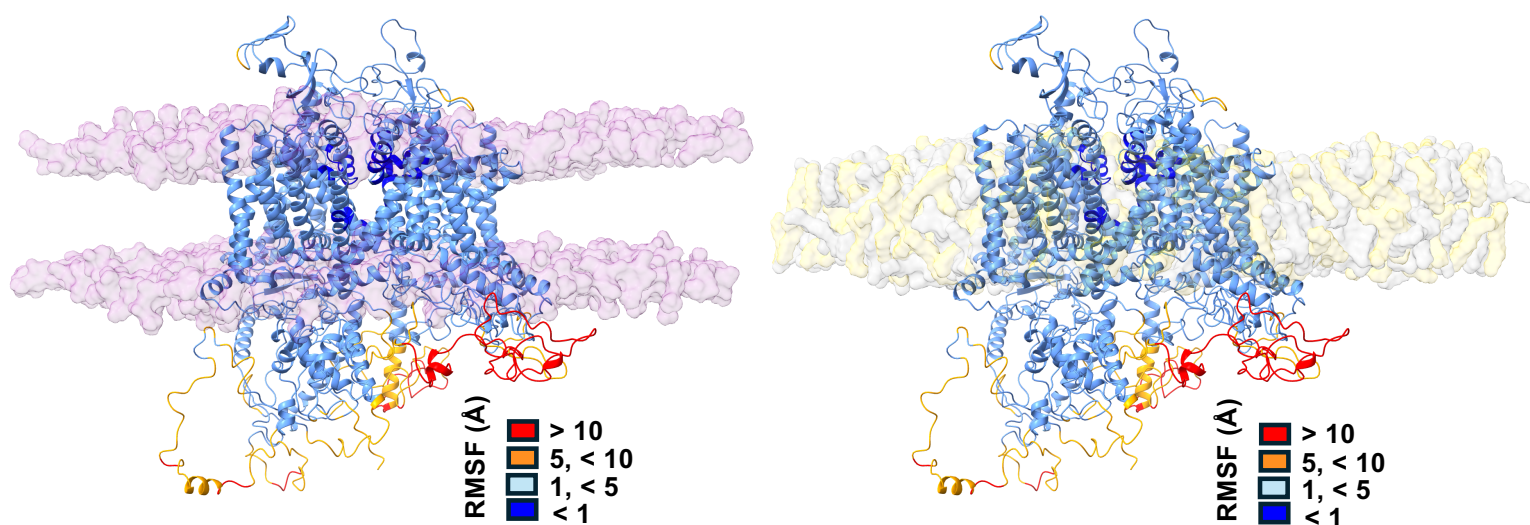


Fig. S5. Post MD CTD-IQ motif model shown as ribbon diagram in lipid bilayer. Polar head group surfaces in pink (left) non polar tails (OL, and PA) in grey and yellow. Average RMSF values for side chains across MD simulations mapped onto of CTD-IQ motif ribbon.

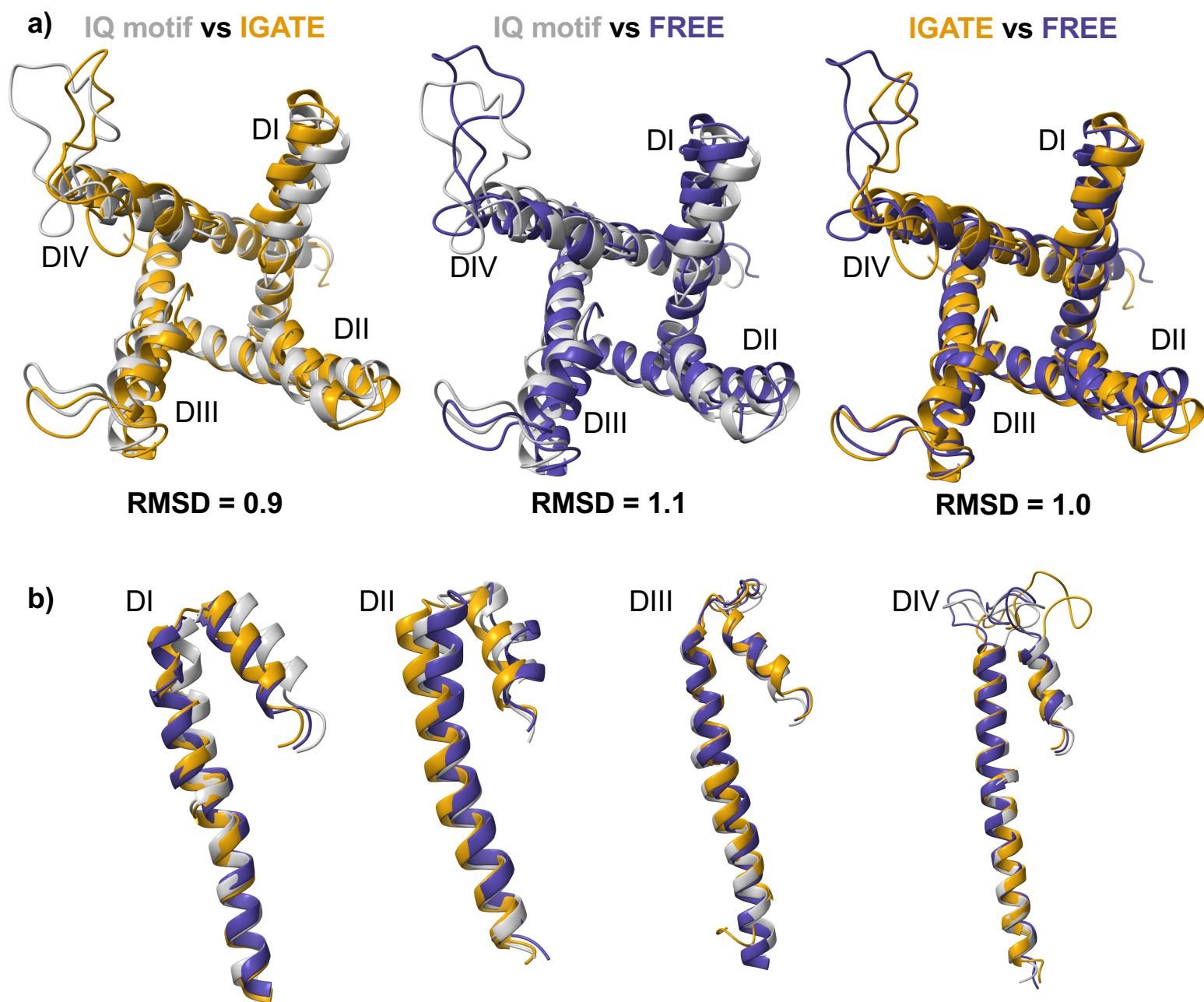


Fig. S6 Structural analysis of pore forming segments (S6). **a)** Post MD models (aligned by transmembrane helices) displaying S6 overlayed from each of the CTD models. RMSD analysis calculated from average ensemble coordinates of amino acids contained within S6. **b)** Side view of each S6 helix aligned by average ensemble coordinates.

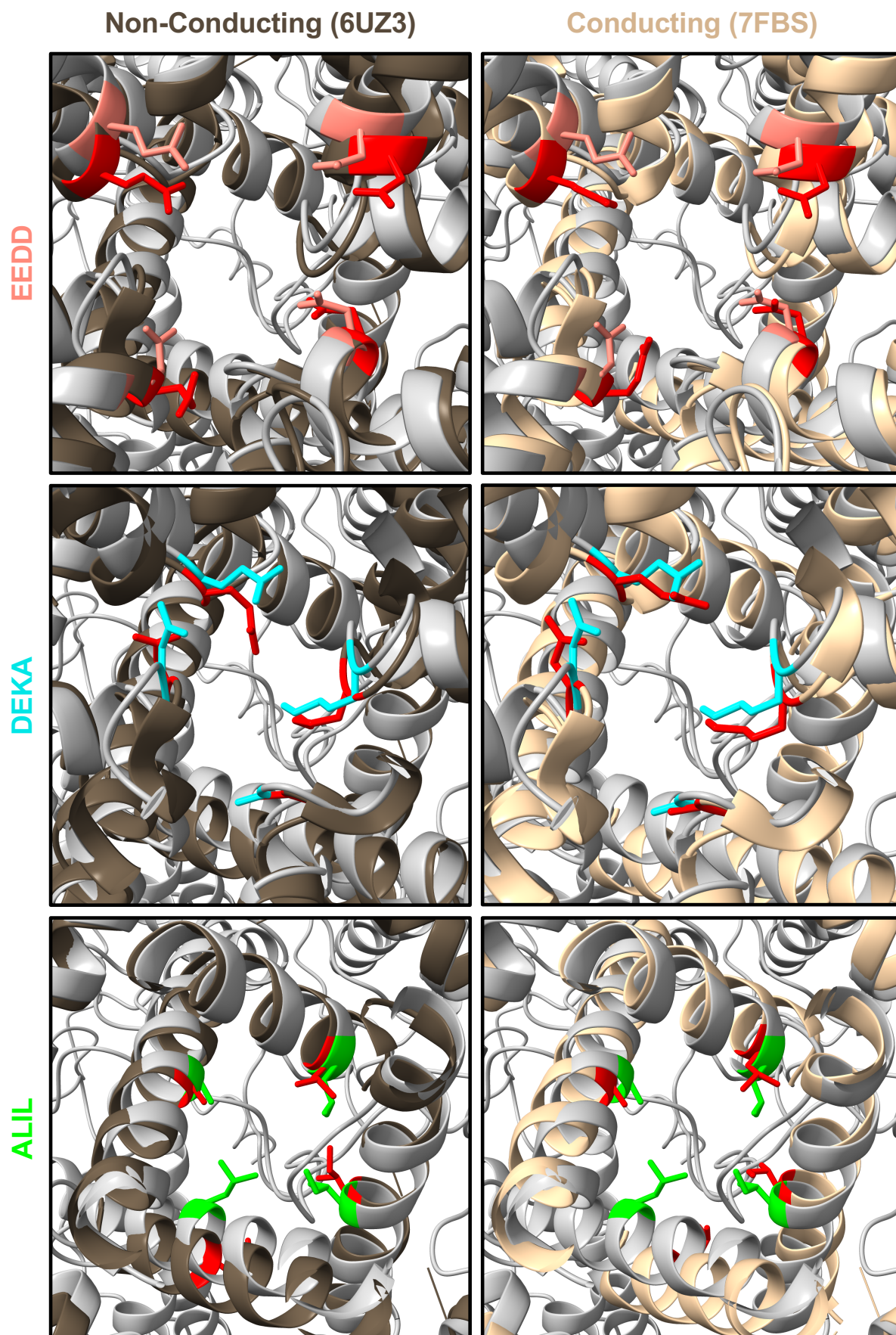


Fig. S7 Overlay of predicted structural features of the CTD-IQ motif channel pore with conducting and non-conducting CryoEM structures. CryoEM side chains (red), post MD model side chains (pink, cyan, and green).

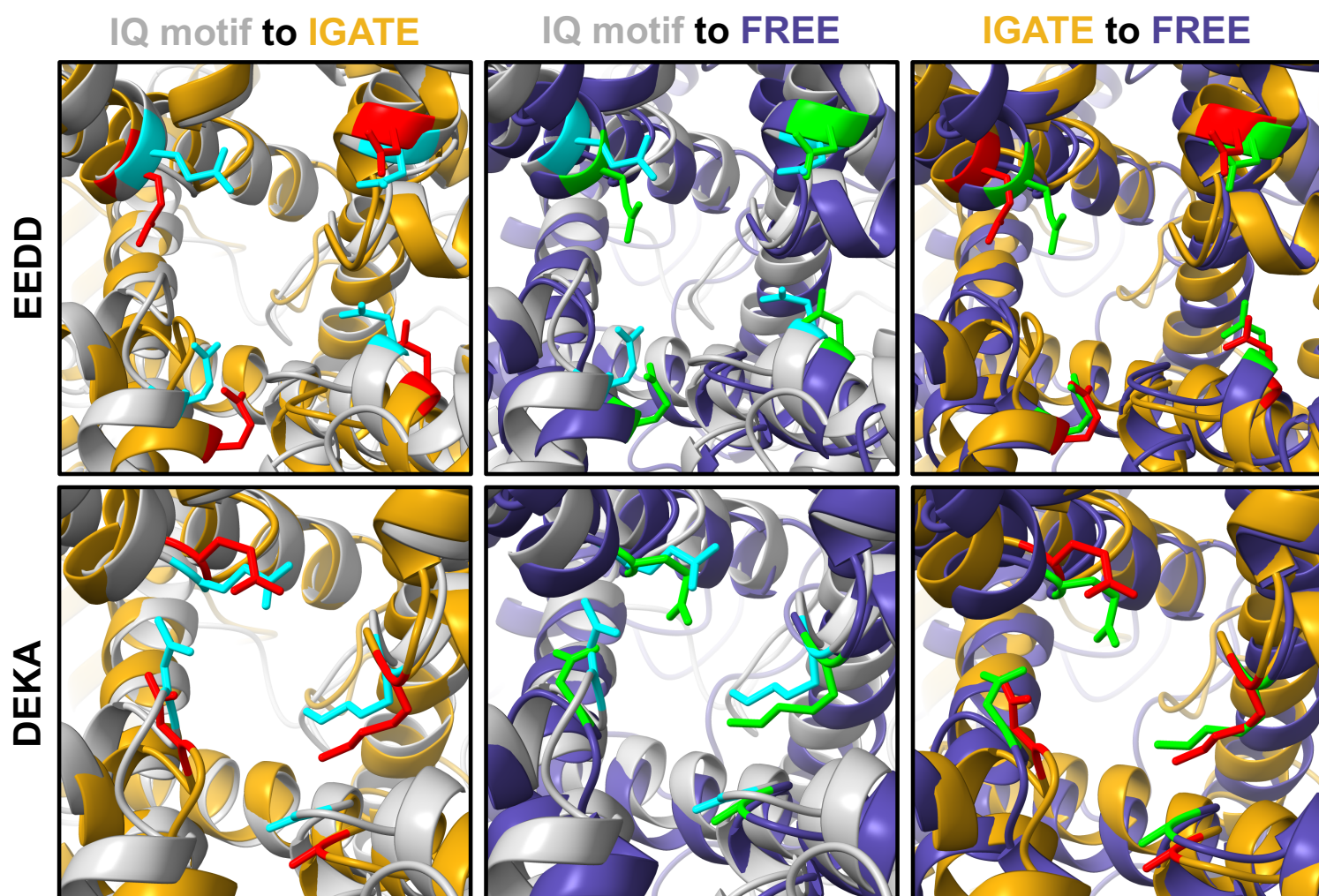


Fig. S8 Overlays of post-MD models comparing amino acid side chain conformations of selectivity filters. Side chains from CTD-IQ motif model **cyan**, CTD-IGATE model **red**, and CTD-FREE model **green**.

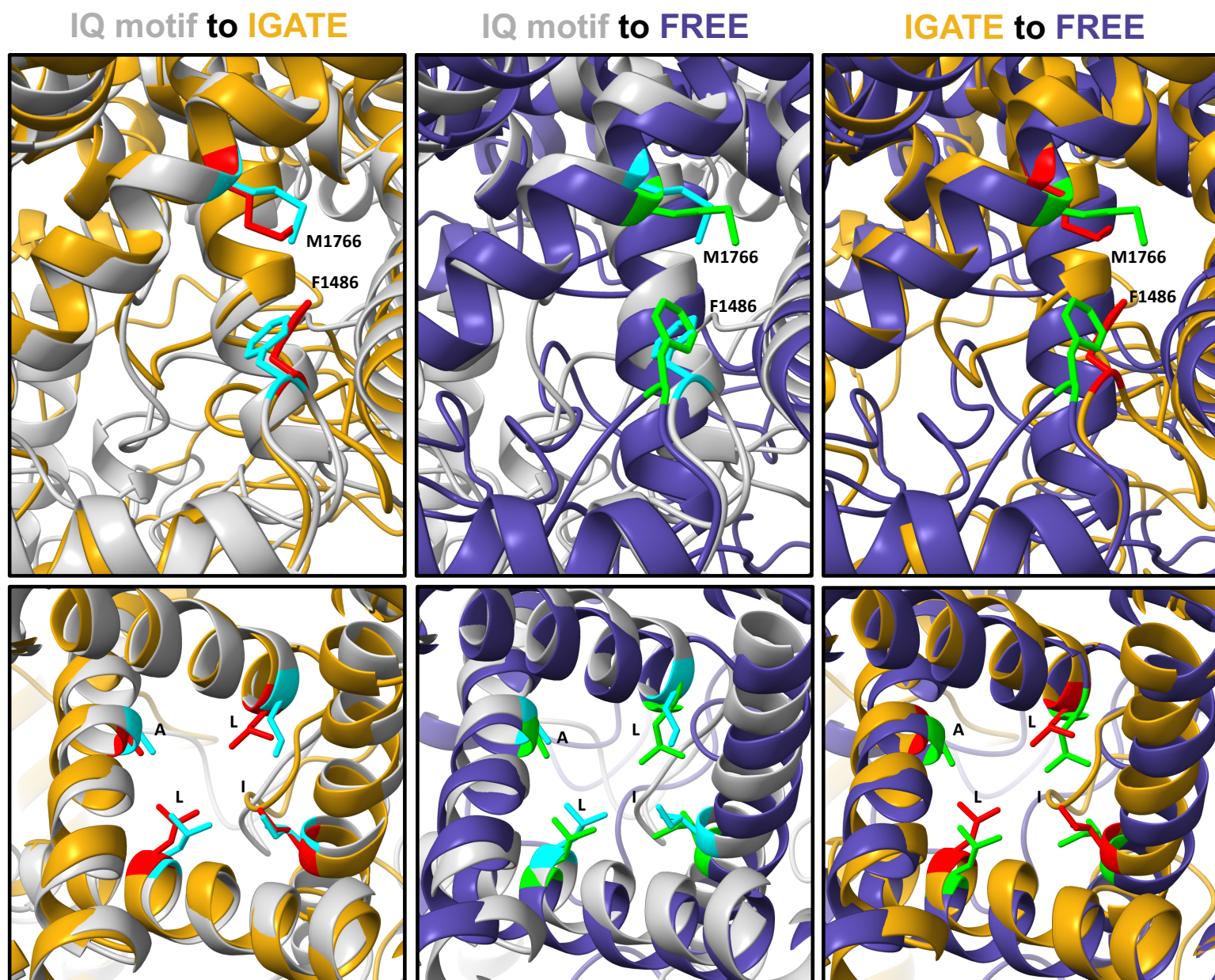


Fig. S9 Overlays of post-MD models highlighting latch receptor interaction and AGATE side chains. Side chains from CTD-IQ motif model **cyan**, CTD-IGATE model **red**, and CTD-FREE model **green**.