Supplementary Figures

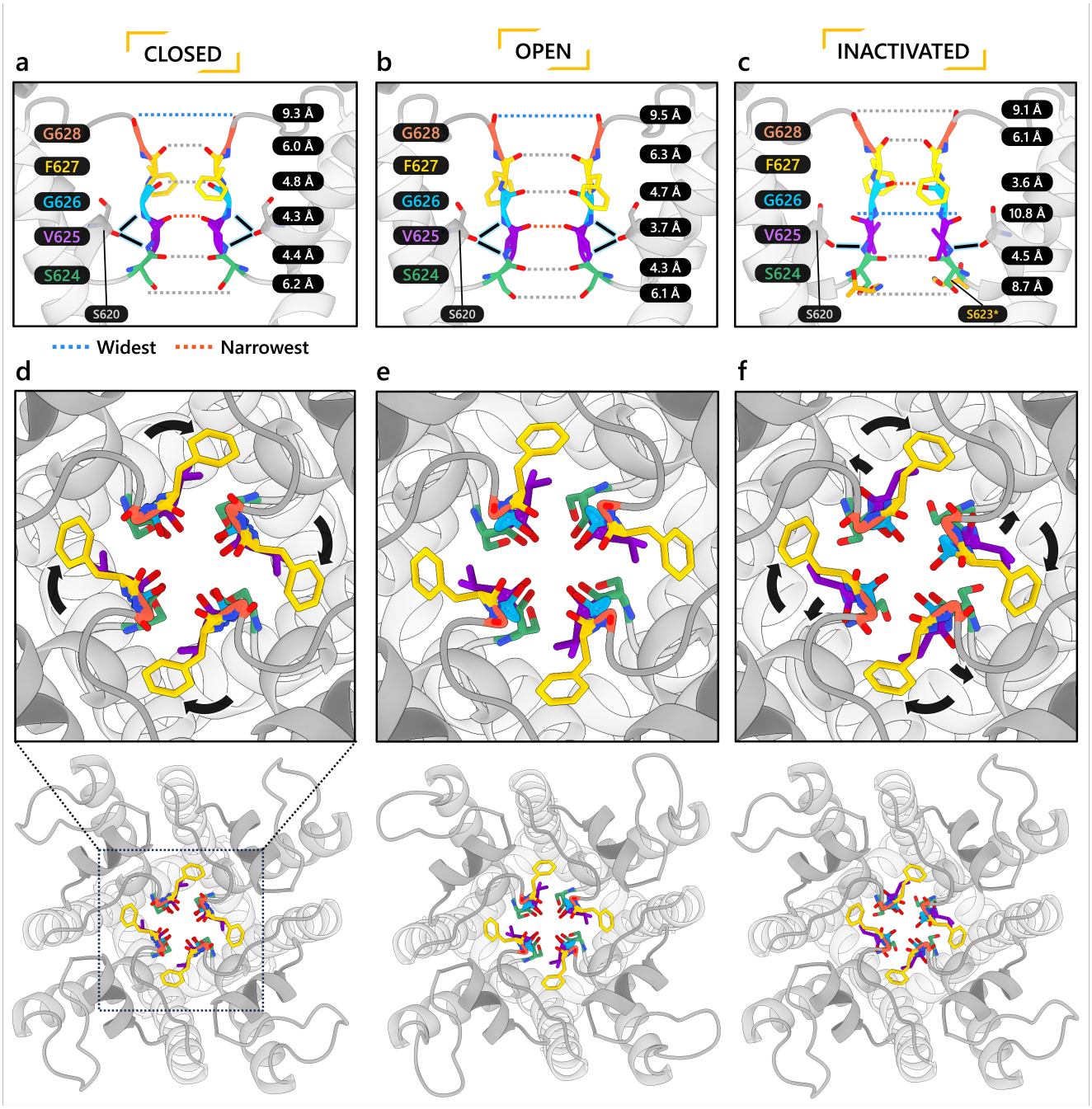

Figure S1. Comparison of the SF in hERG closed- (a, d), open- (b, e), and inactivated-state (c, f) models. a, b, c) Measurement of the distances between each carbonyl oxygen lining the conduction pathway in the SF. In the open- and closed-state models, S620 backbone carbonyl interacts with G626 and S624 backbone amide NH groups. In the inactivated-state model, the hydrogen bond between S620 and G626 is absent due to a reorientation of V625 backbone. However, at the bottom of the SF, S624 sidechain interacts with S623 backbone carbonyl from an adjacent subunit (denoted by *). **d, e, f)** View of the SF from the extracellular side. Large arrows indicate the rotation of the F627 side chains, while small arrows show the rotation of the loops that connect the upper SF to the S6 helix, all relative to the equivalent structural elements in the open-state model.

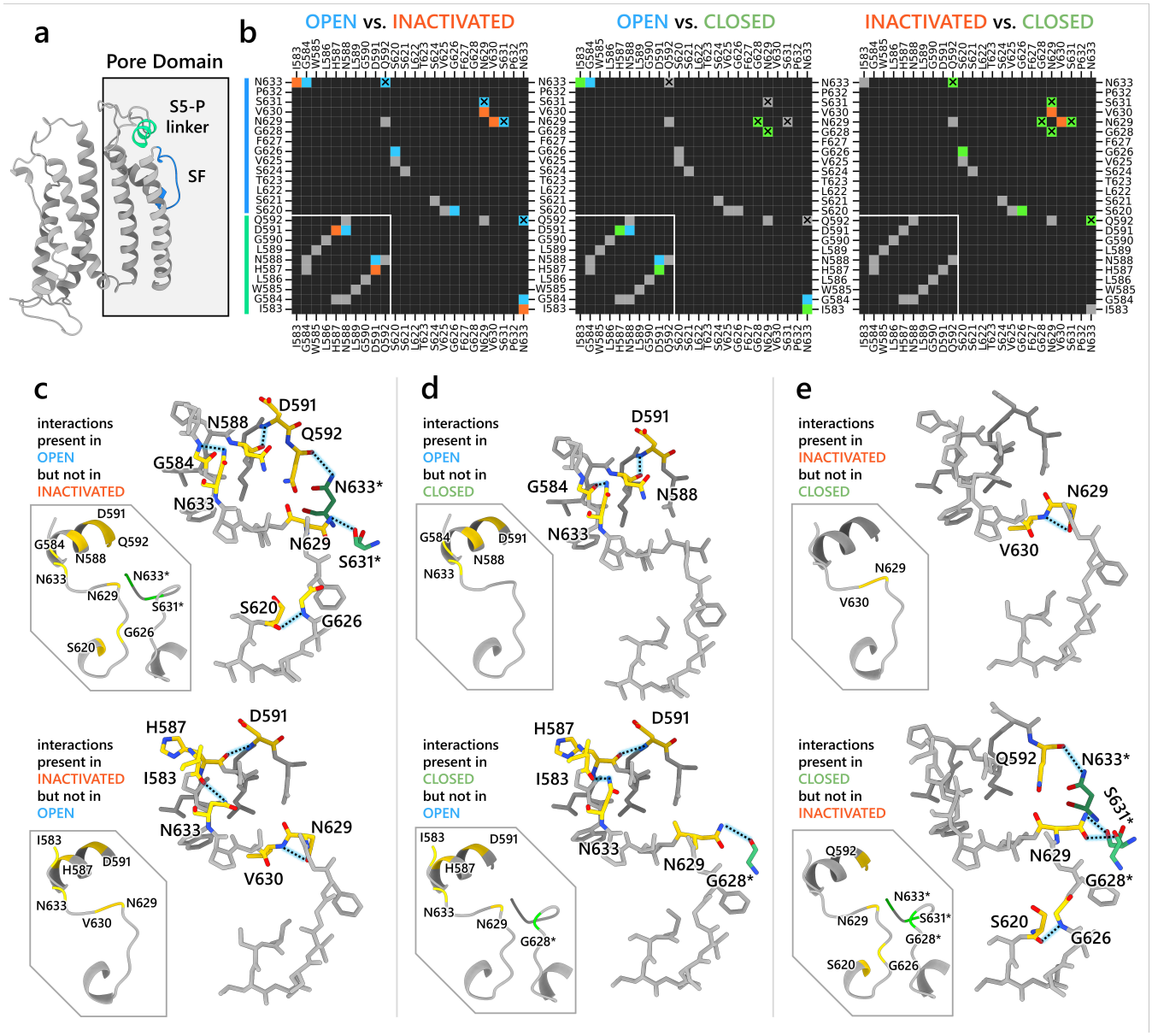

Figure S2. Interaction network analysis showcasing residue-residue interactions in the S5-P linker (I583 – Q592) and region surrounding the SF (S620 – N633). a) An image of a hERG channel subunit with the analyzed S5-P linker and SF regions colored in light green and light blue, respectively. **b)** Heatmaps showing intrasubunit and intersubunit (marked by X) interactions between each residue in the analyzed regions. The interactions analyzed are hydrogen bonding, π stacking, cation-π, and salt bridges. Black cells indicate no interactions. Gray cells indicate an interaction is present in both states. Blue, orange, and green colored cells indicate the interaction is present only in the open, inactivated, or closed state, respectively, but not in the other state being compared in the map. White lines are added to separate S5-P linker residues from the SF region residues. **c, d, e)** Visualization of the interactions being present in one state but not the other. Gold-colored residues are involved in the interactions. Green-colored residues, named with an asterisk at the end, are from an adjacent chain but are interacting with gold-colored residues. Dashed lines represent hydrogen bonds.

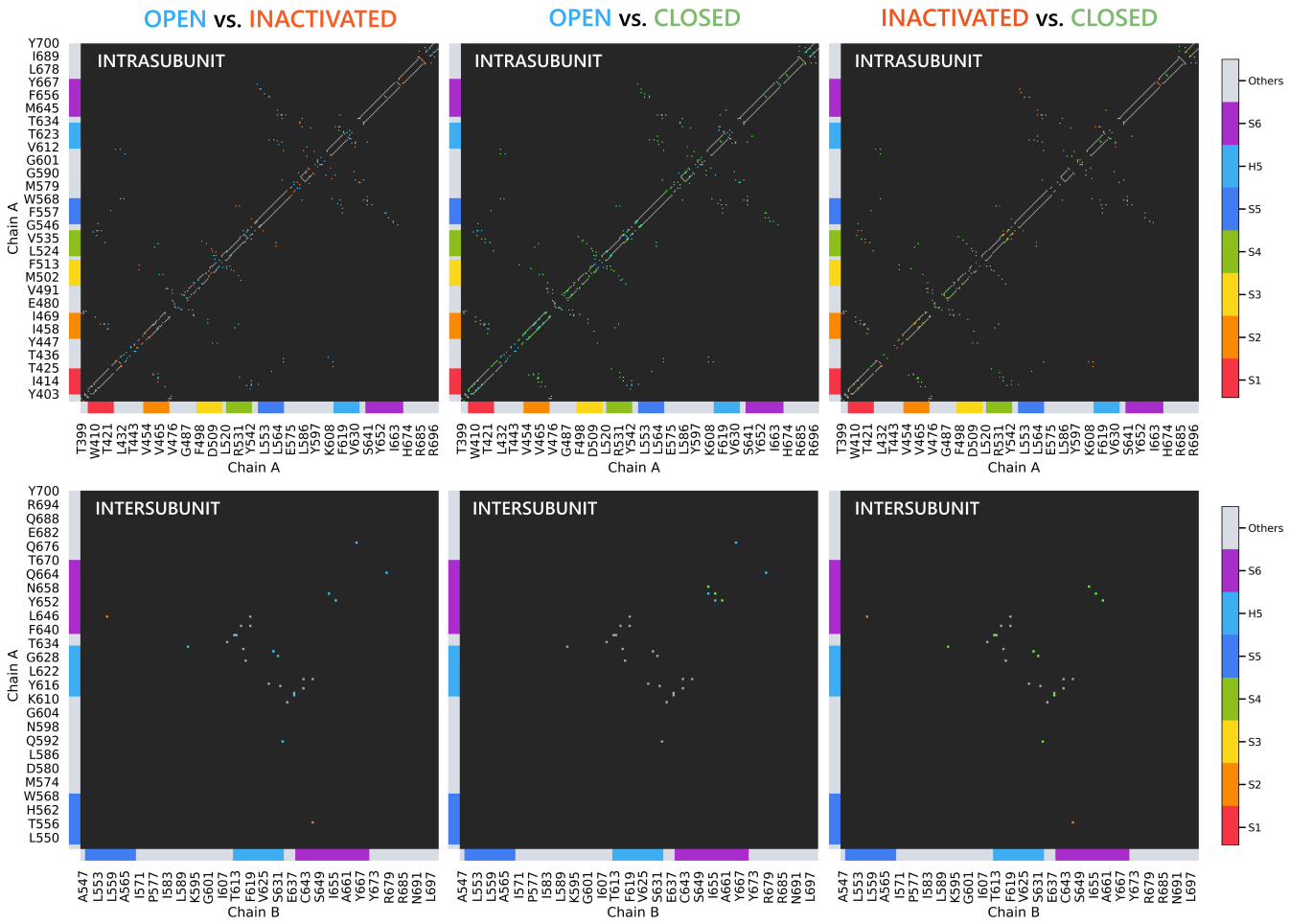

Figure S3. Distance-based contact maps comparing intra- and intersubunit contacts between each model. Two residues whose C_α atoms are within 6 Å of each other are considered to be in contact, provided there are no C_α atoms belonging to a third residue in between. Black cells indicate no contacts. Gray cells indicate a contact is present in both states being compared. Blue, orange, and green colored cells indicate the interaction is present only in the open, inactivated, or closed state, respectively, but not in the other state being compared in the map. Colored topology labels are included along the left and bottom edges of the maps showing the specific segments of the hERG channel to which the residues correspond.

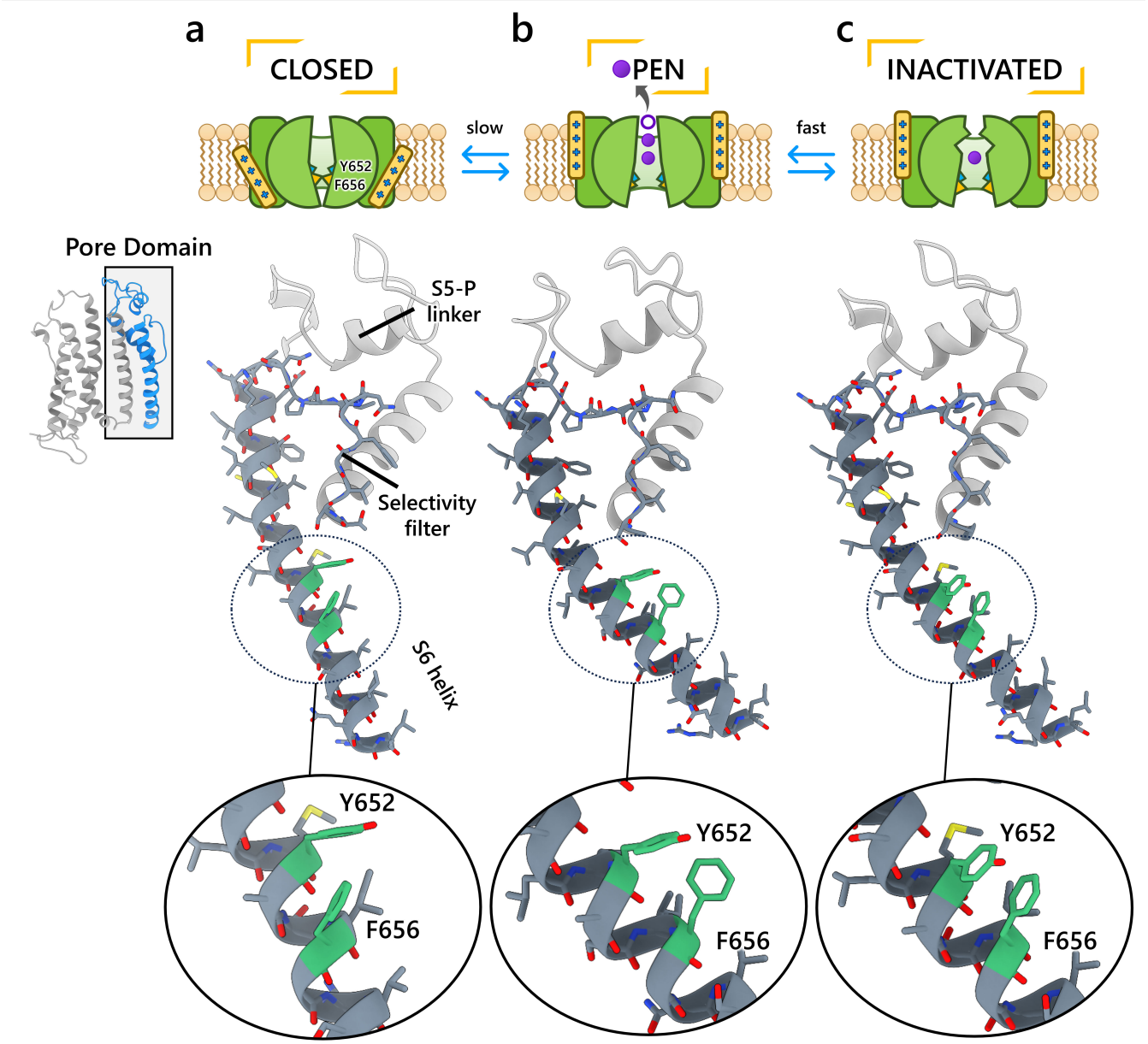

Figure S4. Comparison of the S6 helix conformation for the hERG closed- (a), open- (b), and inactivated-state (c) models. Residues E575 – L666 from the pore domain are visualized as dark gray ribbons. Selectivity filter (SF) residues and those on the S6 helix are shown with their backbone and side chains displayed as colored sticks. C atoms are gray, O are red, N are blue, S are yellow, H are not shown. The drug binding residues Y652 and F656 are highlighted in green.

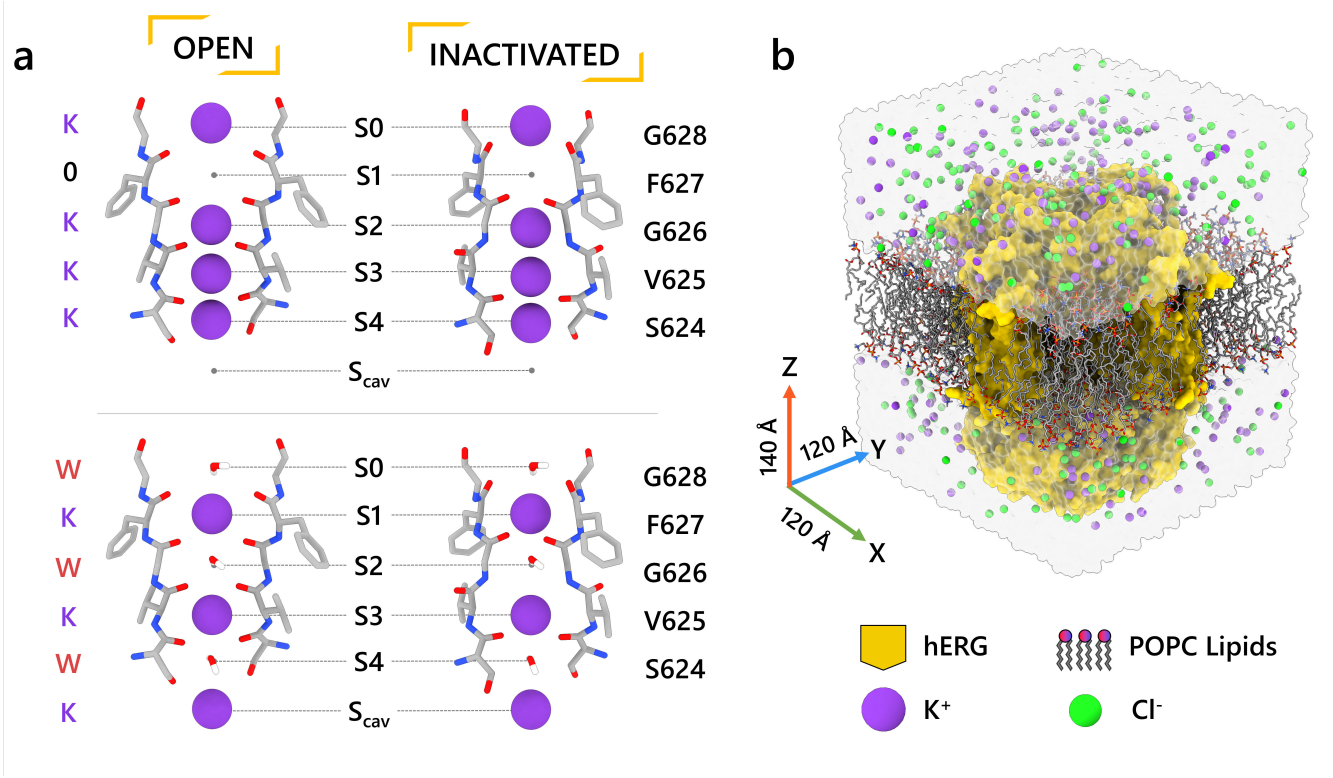

Figure S5. Setup of MD simulations to assess ion conduction in the open and inactivated hERG channel models. a) Initial configuration of the SF, set to fill with either all K^+ ions (top), or alternating K^+ and water molecules (bottom). **b)** An example MD simulation box showing a hERG channel model (shown in yellow surface representation) embedded in POPC lipid bilayer (shown as sticks) and solvated by an aqueous 0.3 M KCl solution (shown as a transparent surface with K^+ and Cl^- ions shown as purple and green balls, respectively).

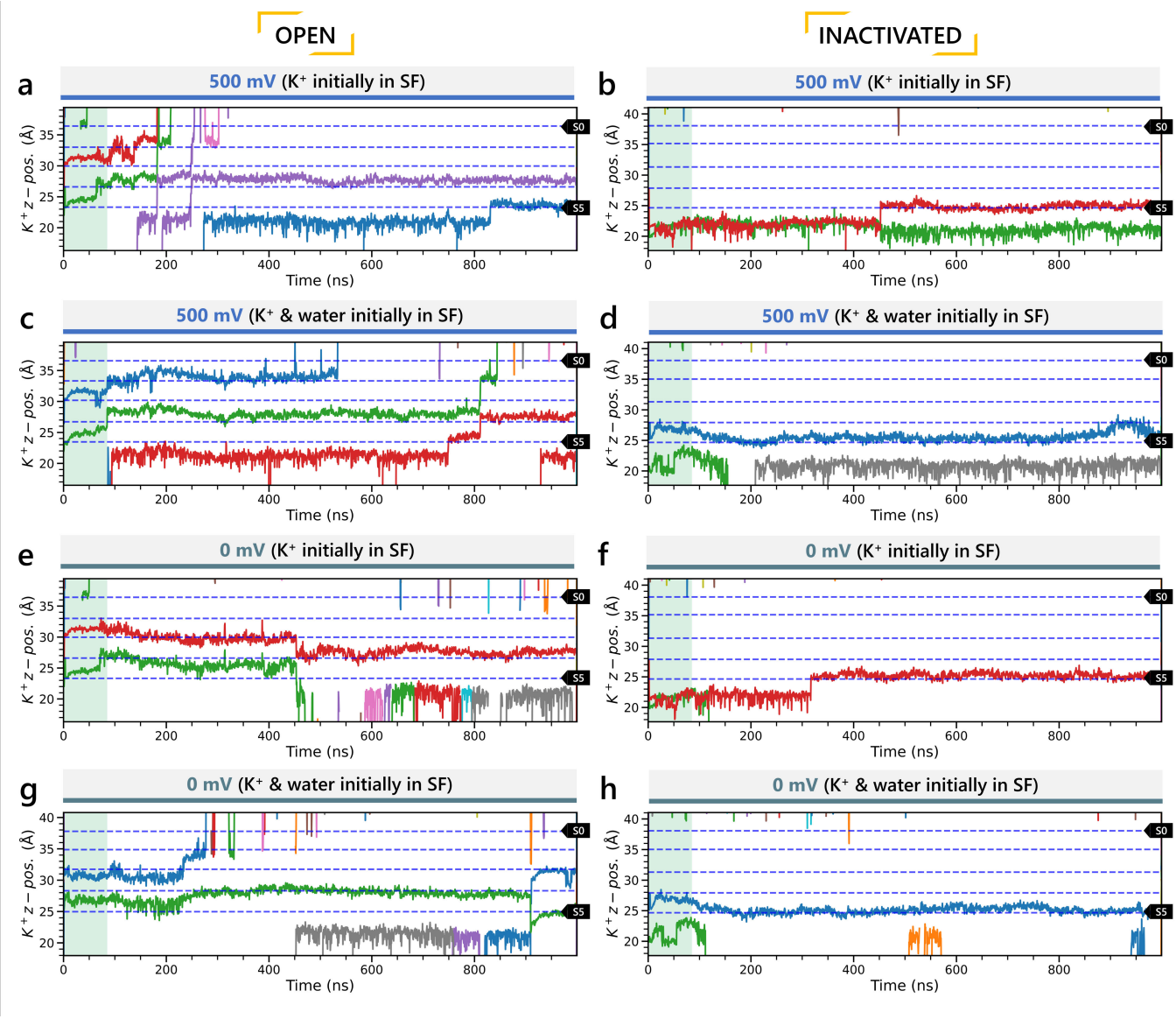

Figure S6. Movement of K^+ ions through hERG selectivity filter (SF). The *z* coordinates of K^+ ions are tracked as they traverse through the pore of the channel from the intracellular gate (lower *y*-axis limit) to the extracellular space (upper *y*-axis limit). Putative K^+ binding sites in the SF (S0 – S5) are marked using blue dashed lines in the plots. **a, c)** Molecular dynamics (MD) simulations with the applied 500 mV membrane voltage of the open-state model with the SF initially configured to have only K^+ ions (panel **a**) or alternating K^+ / water molecules (panel **c**), respectively. **b, d)** MD simulations with the applied 500 mV membrane voltage of the inactivated-state model with the SF initially configured to have only K^+ ions (panel **b**) or alternating K+ / water molecules (panel **d**), respectively. **e, g)** MD simulations without applied membrane voltage of the open-state model with the SF initially configured to have only K^+ ions (panel **e**) or alternating K^+ / water molecules (panel **g**), respectively. **f, h)** MD simulations without applied membrane voltage of the inactivated-state model with the SF initially configured to have only K^+ ions (panel **f**) or alternating K^+ / water molecules (panel **h**), respectively.

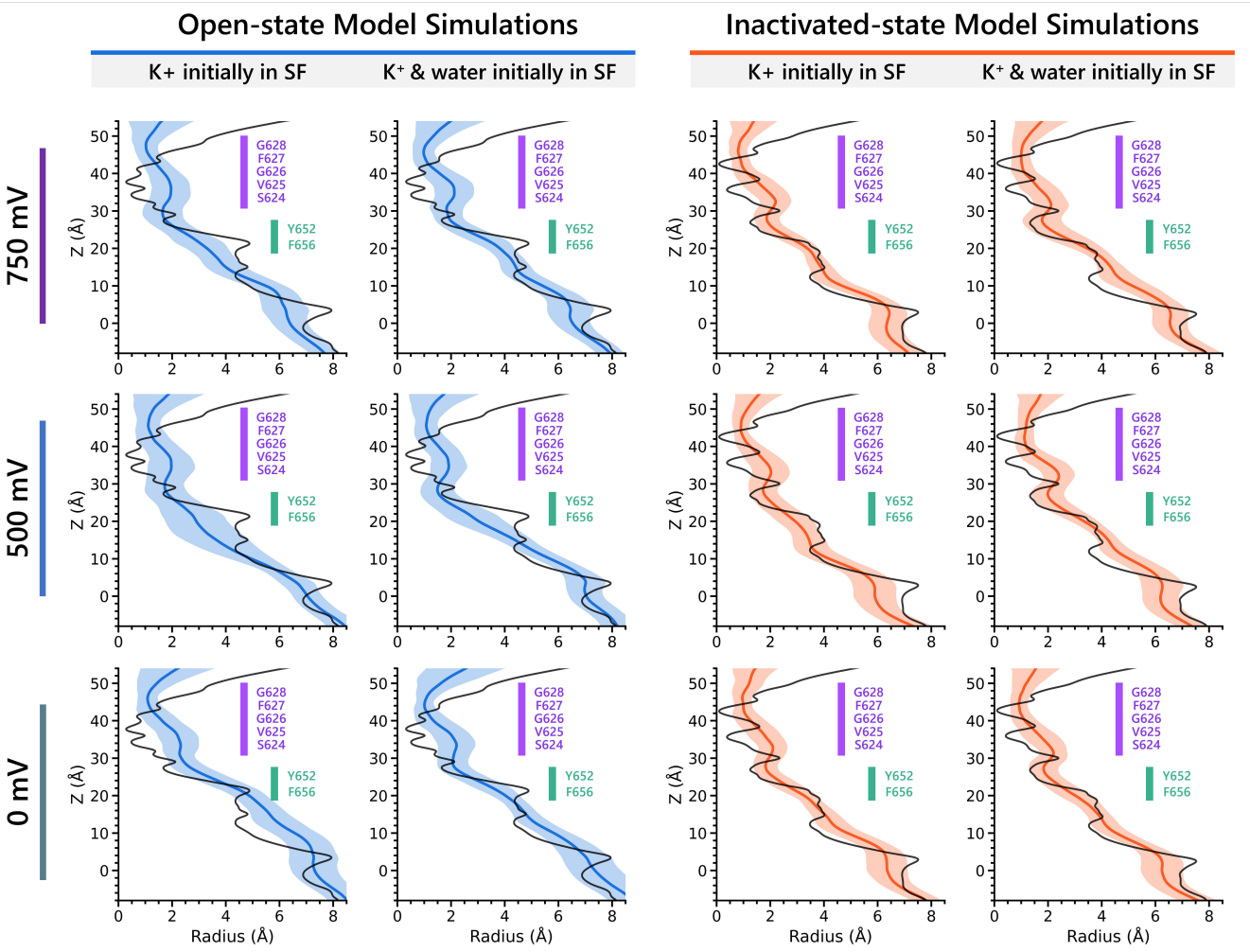

Figure S7. Analysis of modulations of the selectivity filter (SF) conformations and pore radii over the course of the 1 μs long molecular dynamics (MD) simulations. The blue/orange-colored lines represent the average pore radii, and the shaded regions represent the standard deviation measured in MD simulations for a given *Z* value. The black lines represent the initial pore radii. The label on the left indicates the voltage of the MD simulations in each row.

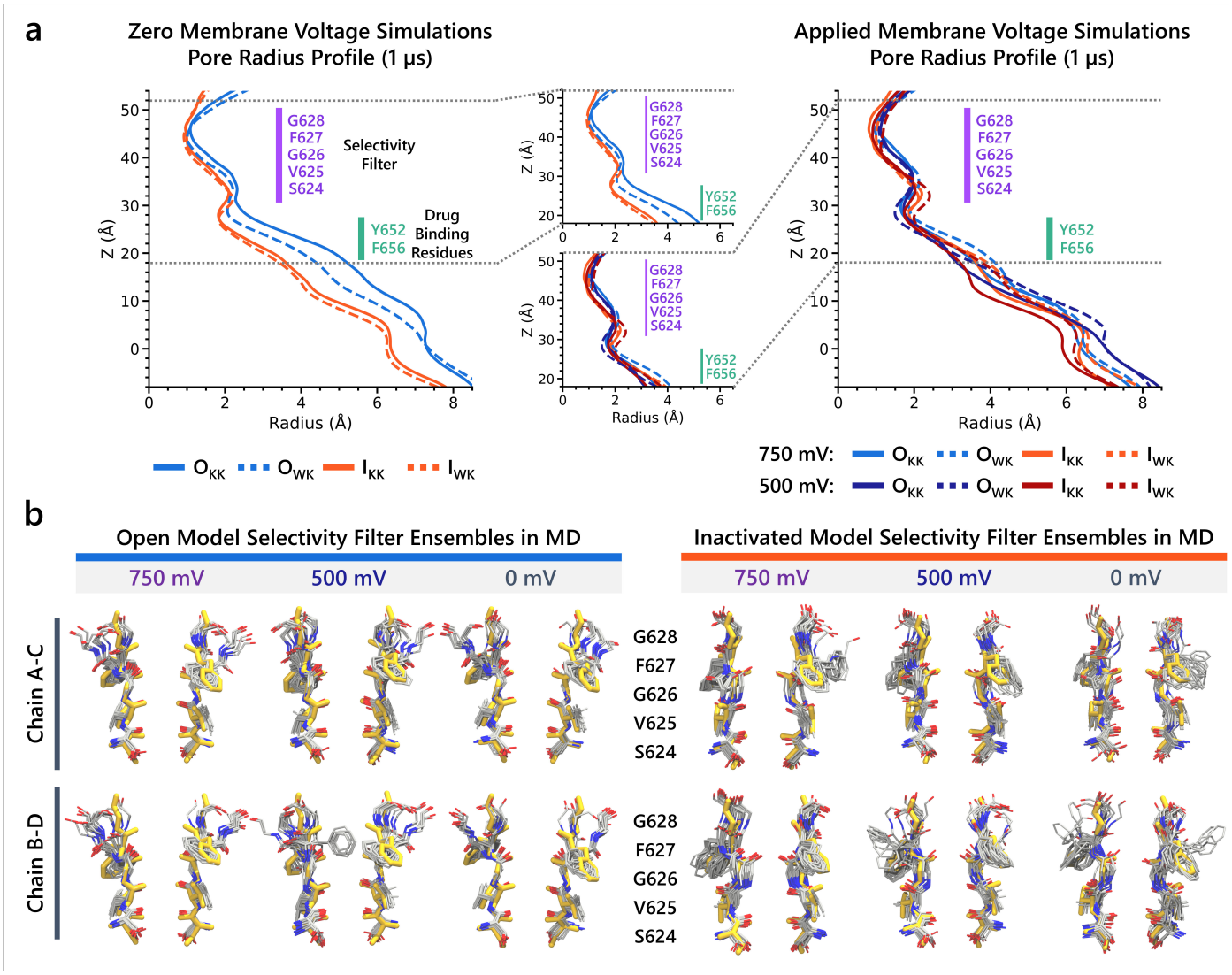

Figure S8. Analysis of dynamics of the SF and pore conformations over the course of the 1 μs MD simulations. a) Pore radius averaged over each 1 μs long MD simulations with (right) or without (left) applied membrane voltage. Open- and inactivated-state model MD simulations are notated as O and I, respectively, with the subscripts KK and WK denoting whether the SF initially configured to have only K^+ ions or alternating K^+ / water molecules, respectively. **b)** Ensembles of SF conformation over the course of each MD simulation superimposed. The golden-colored conformation indicates the initial conformation.

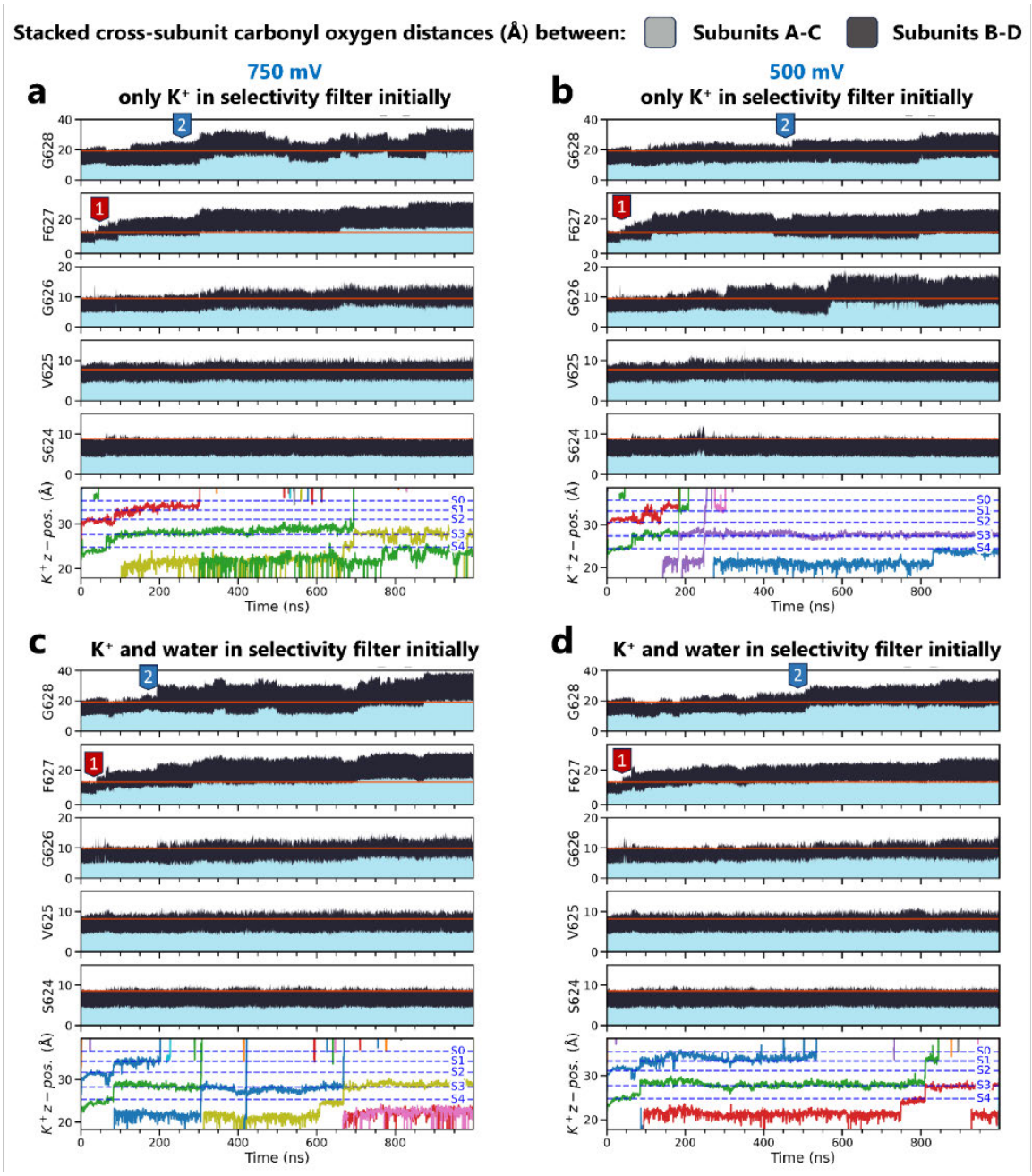

Figure S9. Cross-subunit distances between carbonyl oxygens of open-state hERG selectivity filter residues during MD simulations under different applied voltage and initial K^+ ion position conditions. Movement of potassium ions (denoted by differently colored lines) is shown at the bottom for reference. Red lines indicate initial distances. Labels 1 and 2 in red and blue, respectively, indicate a sequential dilation process exhibited by the hERG channel: the SF near residues F627 dilates first, followed by that around G628.

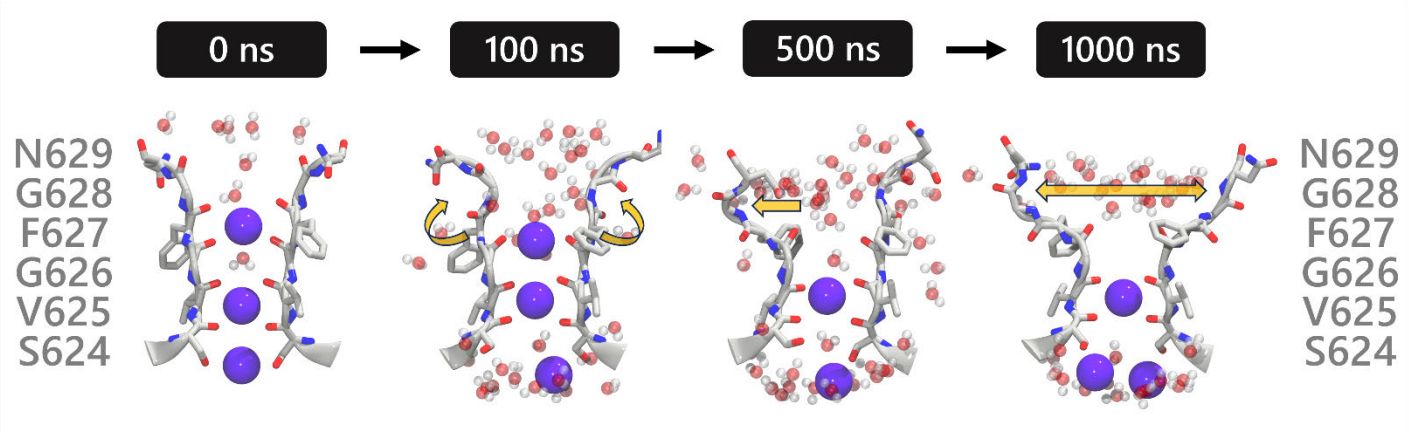

Figure S10. Sequential dilation steps of hERG upper selectivity filter (SF). SF residues are shown as gray sticks, water molecules as red and white spheres, and K^+ as purple spheres. The first step, occurring around 100 ns, involves the flipping of F627 carbonyl oxygen, creating a small dilation at this level. At 500 ns, further dilation can be seen at the level of residues F627 and G628 in one subunit. At 1000 ns, the entire upper region of the SF dilates further. Frames were taken from an MD simulation of the open-state hERG channel with K^+ and water initially in the SF prior to transmembrane voltage of 750 mV being applied.

Figure S11. GALigandDock drug docking results to different hERG channel models. Each bar plot represents the estimated free energy of binding in Rosetta energy units (R.E.U.) for the named drug to the open-, inactivated, and closed-state hERG channel models. Lower values mean more favorable binding. 25,000 docking poses were generated for each drug/channel model pairing. The top 50 poses were clustered, and the plotted energy represents the average free energy of the top cluster along with the standard deviation. The first suffixes (0), (+), and (±) indicate whether the drug is in the neutral, cationic, or zwitterionic form, respectively. The second suffixes * and † indicate validation from experimental studies (Alexandrou et al., 2006; Numaguchi et al., 2000; Perrin et al., 2008; Wang et al., 1997) showing whether the drug prefers binding to hERG inactivated state (*) or does not (†), respectively.

Movie S1 (separate file). Animation depicting the hERG channel transitioning through various states, beginning in the open state and ending in the closed state using the structural models developed in this study.