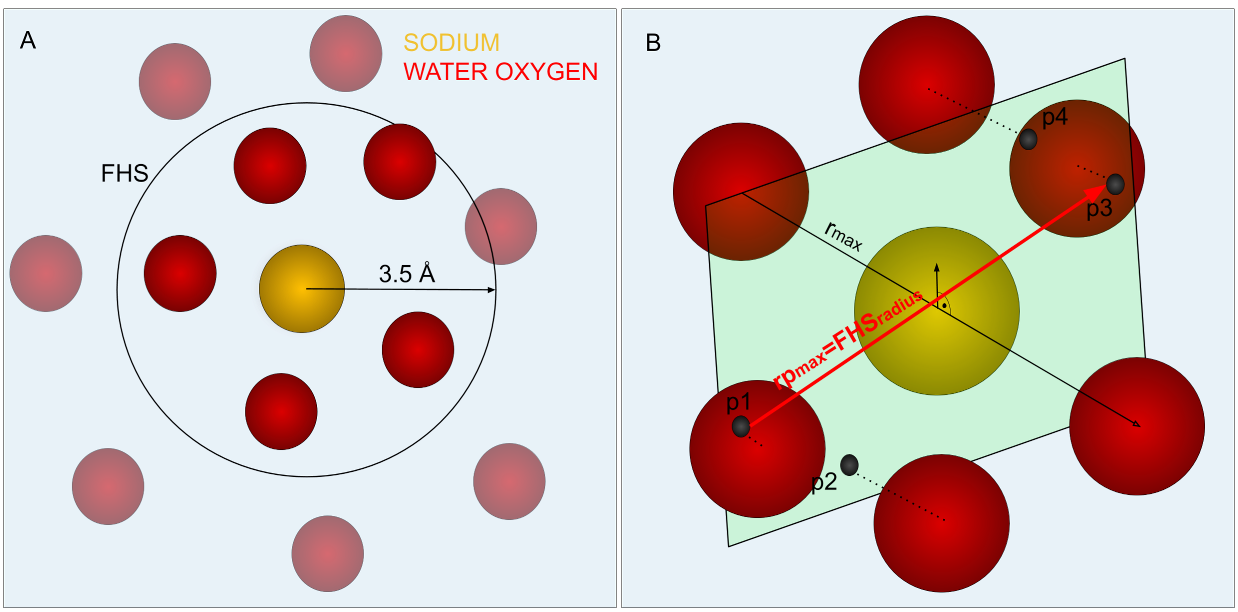
Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

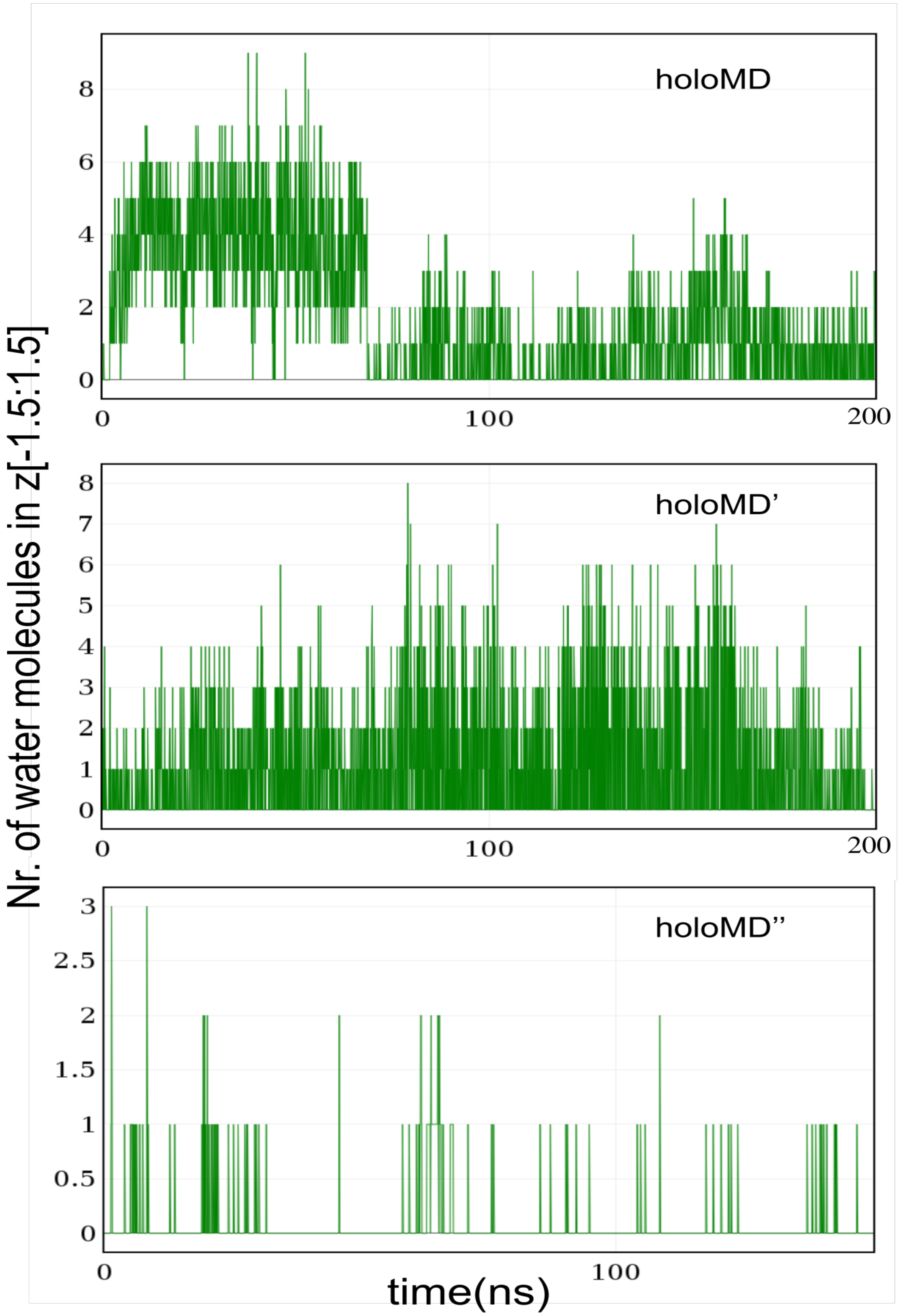
Supporting Information

The mechanism and energetics of a ligand-controlled hydrophobic gate in a mammalian two pore channel

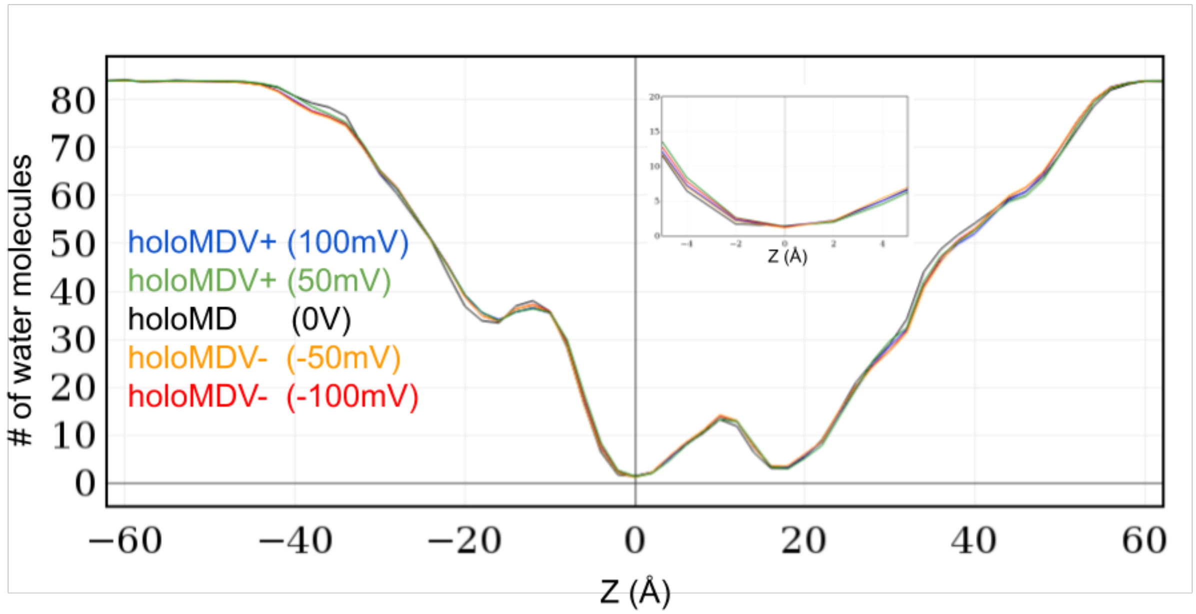
Stefan Milenkovic, Igor V. Bodrenko, Laura Lagostena, Antonella Gradogna, Giovanni Serra, Andrea Bosin, Armando Carpaneto, Matteo Ceccarelli



S1. A) Schematic representation of the FHS calculation criteria where only water oxygens with COMs within 3.5 A range are considered. B) Scheme of FHS radius calculation: once the oxygens within 3.5 A are identified, we select the pair with the maximum distance. Thus we take the projection of all other oxygens in the plane perpendicular to the maximum distance direction. The surface identified by oxygens on that plane is taken as the minimal projection area from which we can identify the minimum steric radius of the FHS.

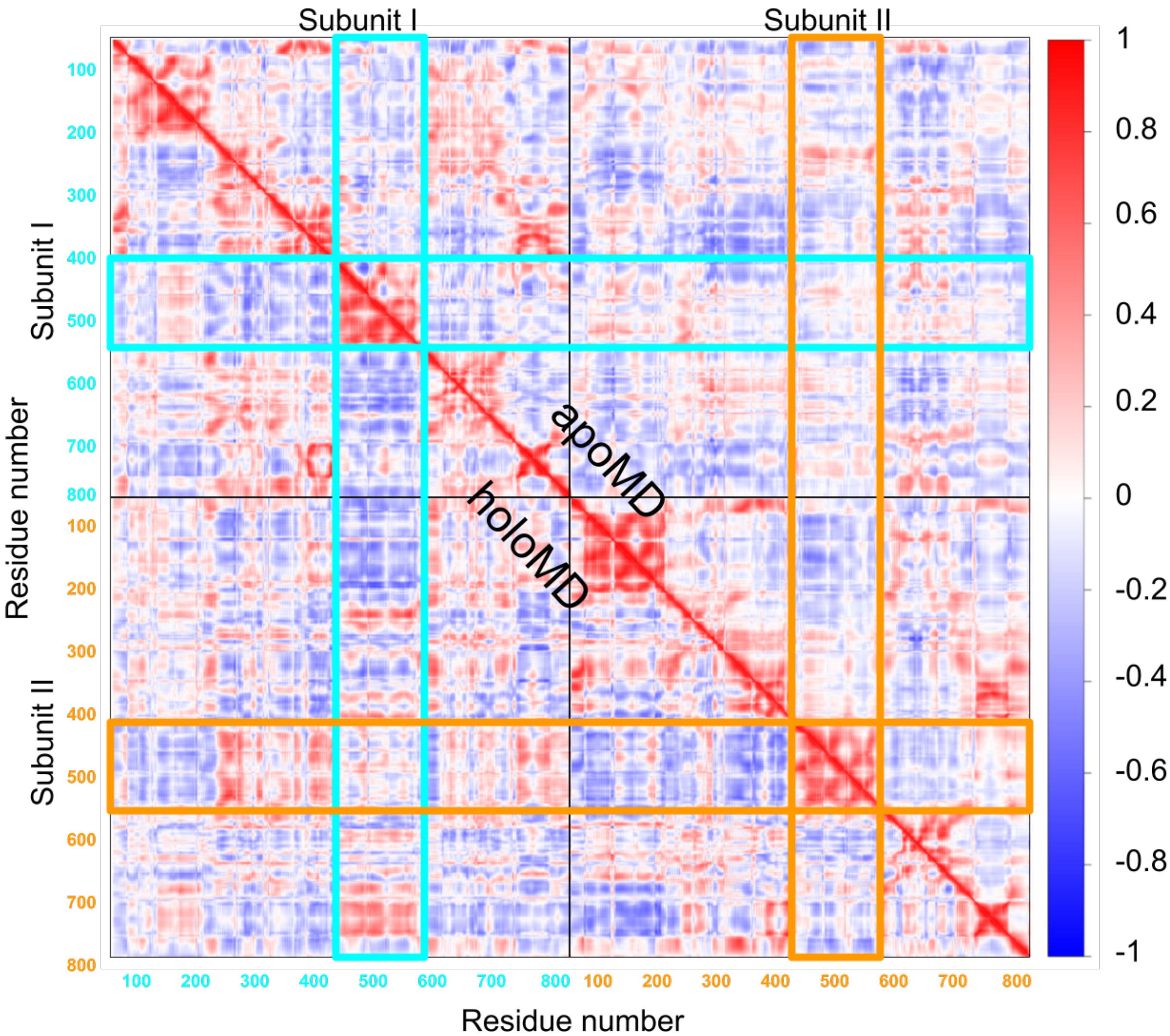


S2. Cumulative plot of instantaneous number of water molecules inside the HG during the three independent holoMD simulations.



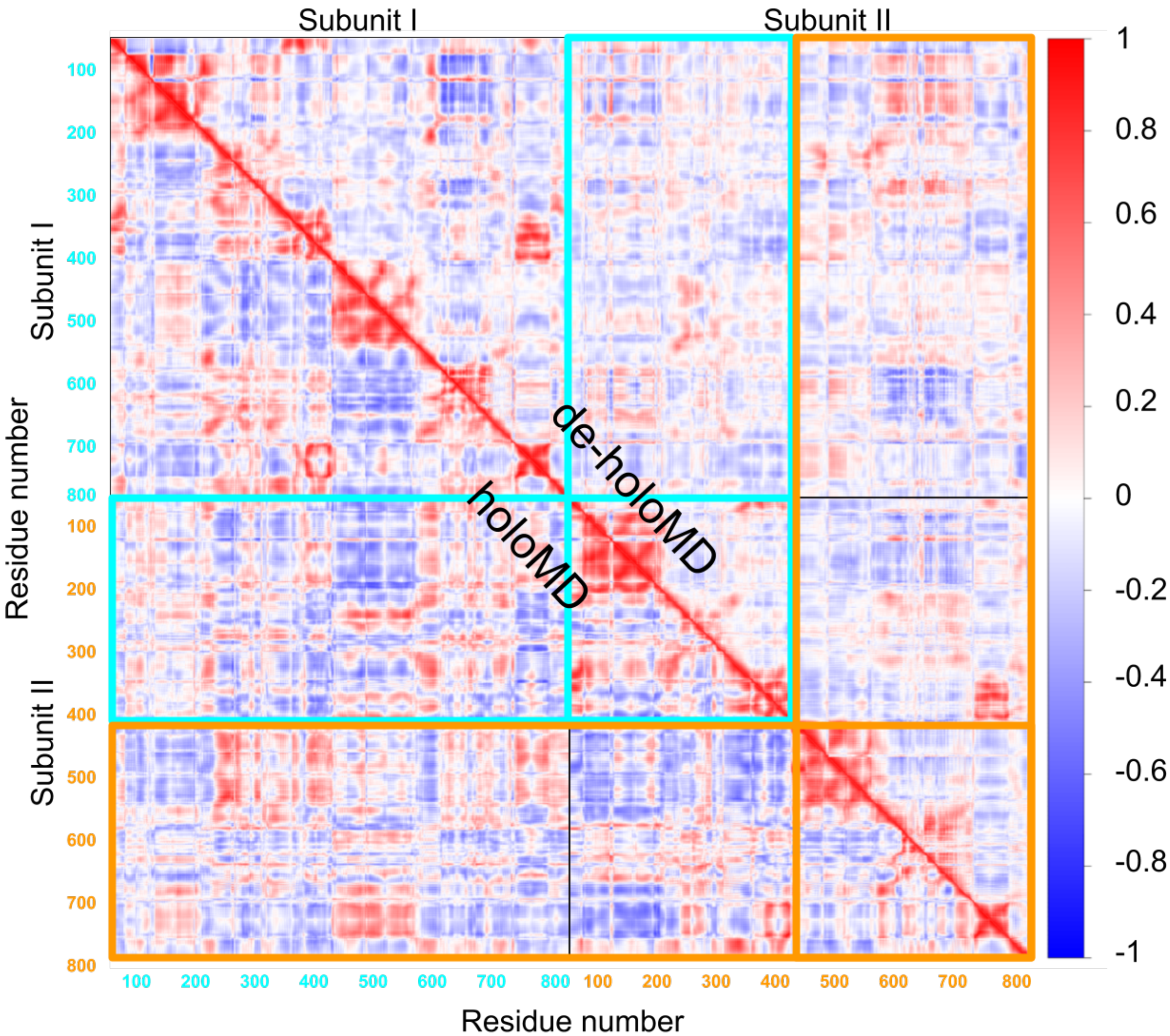
S3. Average number of water molecules along the channel calculated in molecular dynamics simulations performed at different voltages.

A

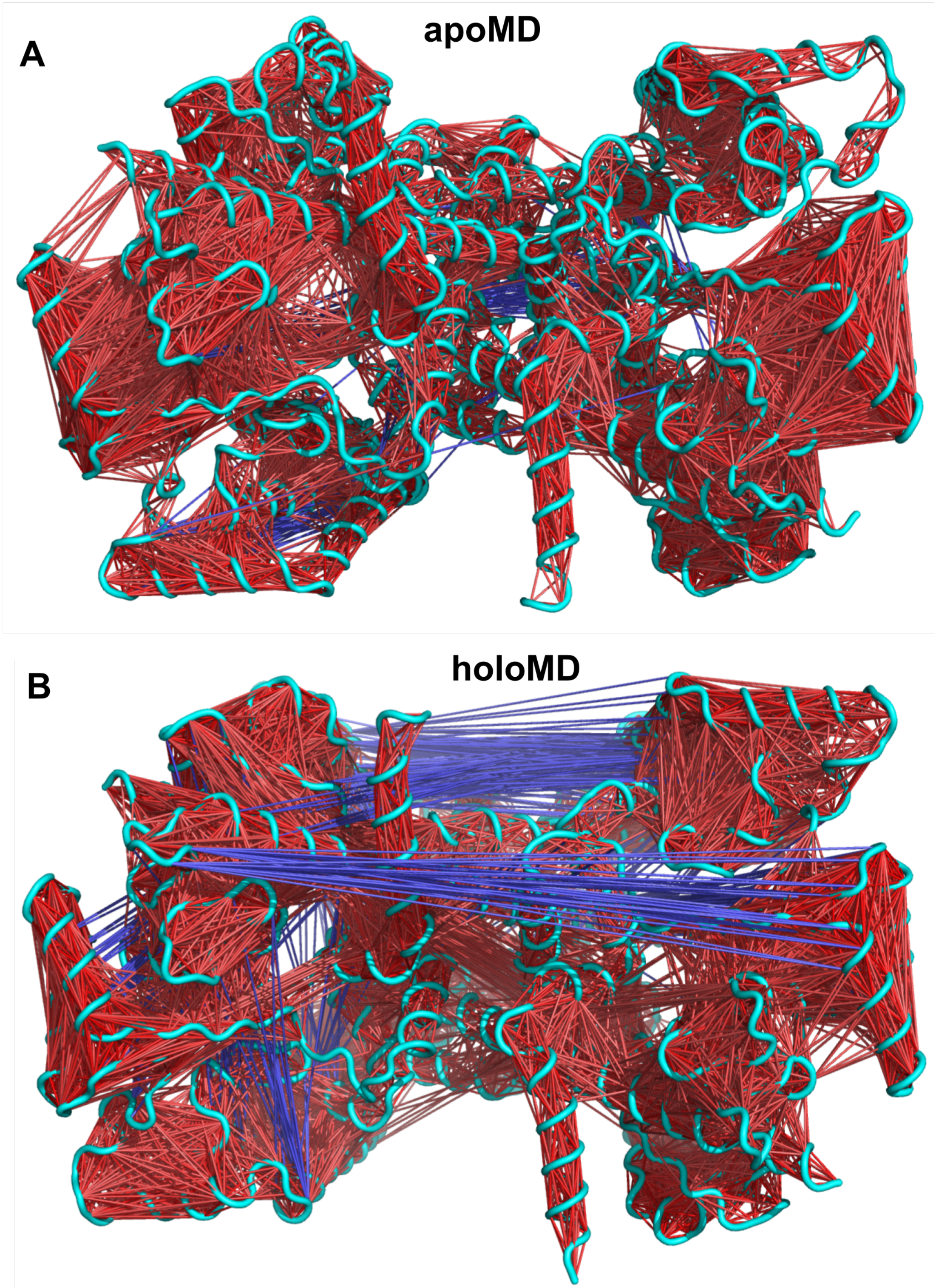


Subunit I
Subunit II
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
100
200
300
400
500
600
700
800
Subunit I
Subunit II
Residue number
apoMD
holoMD
Residue number

B

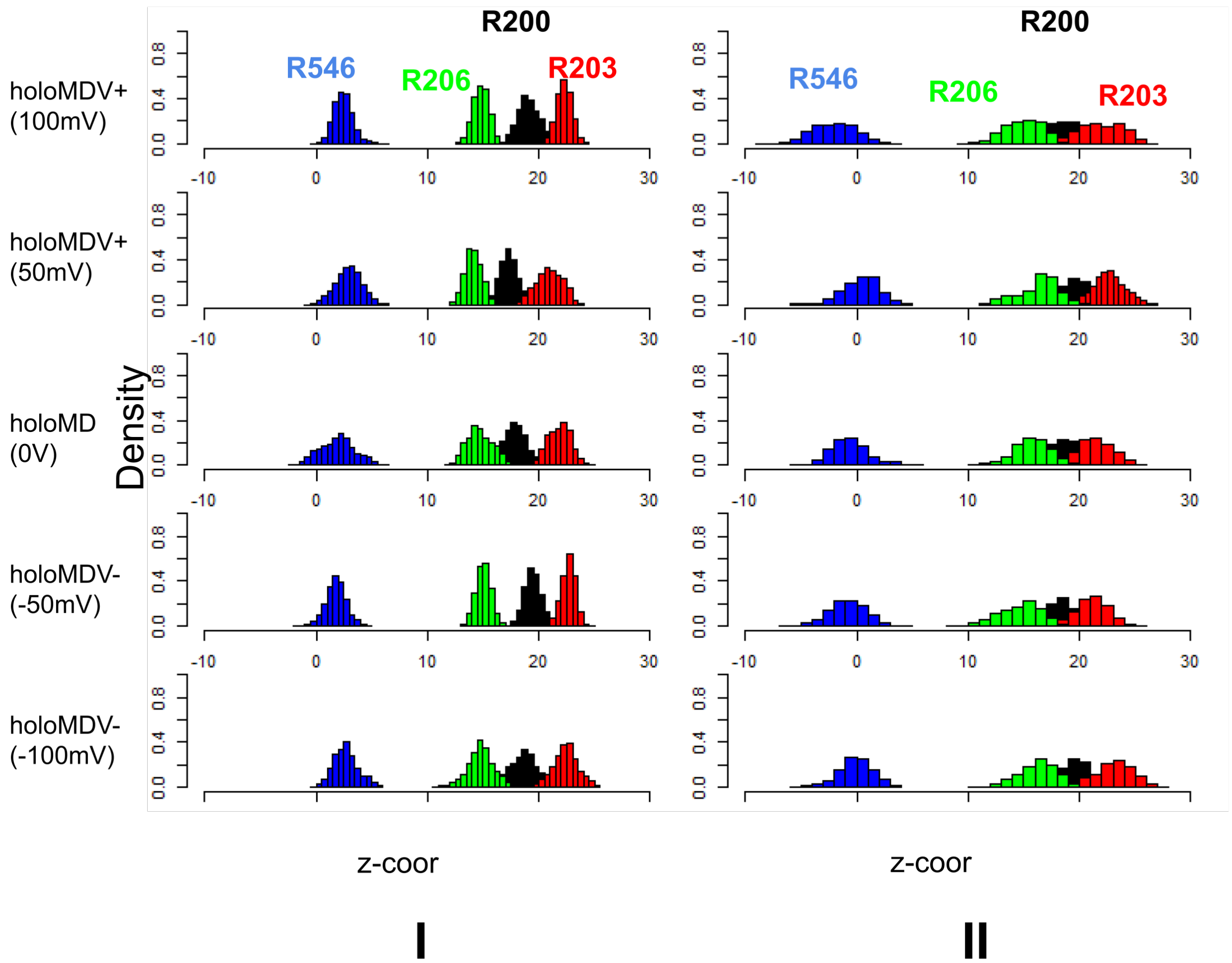


S4. DCCMs of C-α atoms. Panel A shows the holoMD and apoMD, respectively below and above the diagonal. We observe a larger number of highly (anti-)correlated amino-acid residues in holo than in apo simulations. Panel B shows the holoMD and de-heloMD, respectively below and above the diagonal. DCCM of de-holoMD simulation exhibits a behaviour similar to the apoMD simulation suggesting that the effect of ligand is indeed detectable in the collective dynamics of the mTPC1.

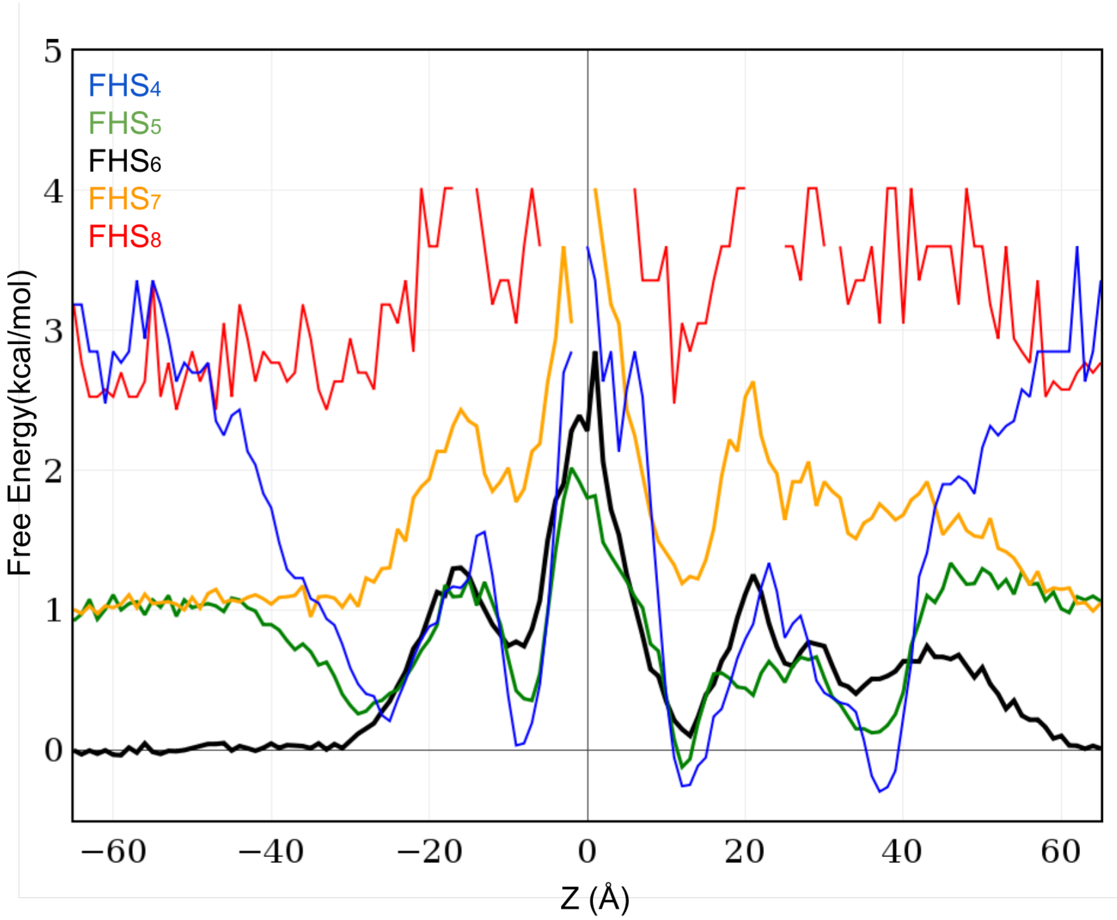


S5. C-α correlation calculated from apoMD (Panel A) and holoMD (Panel B) simulations. Drawn lines connect two C-α atoms having absolute values for correlation (red) and anticorrelation (blue) higher than 0.6.

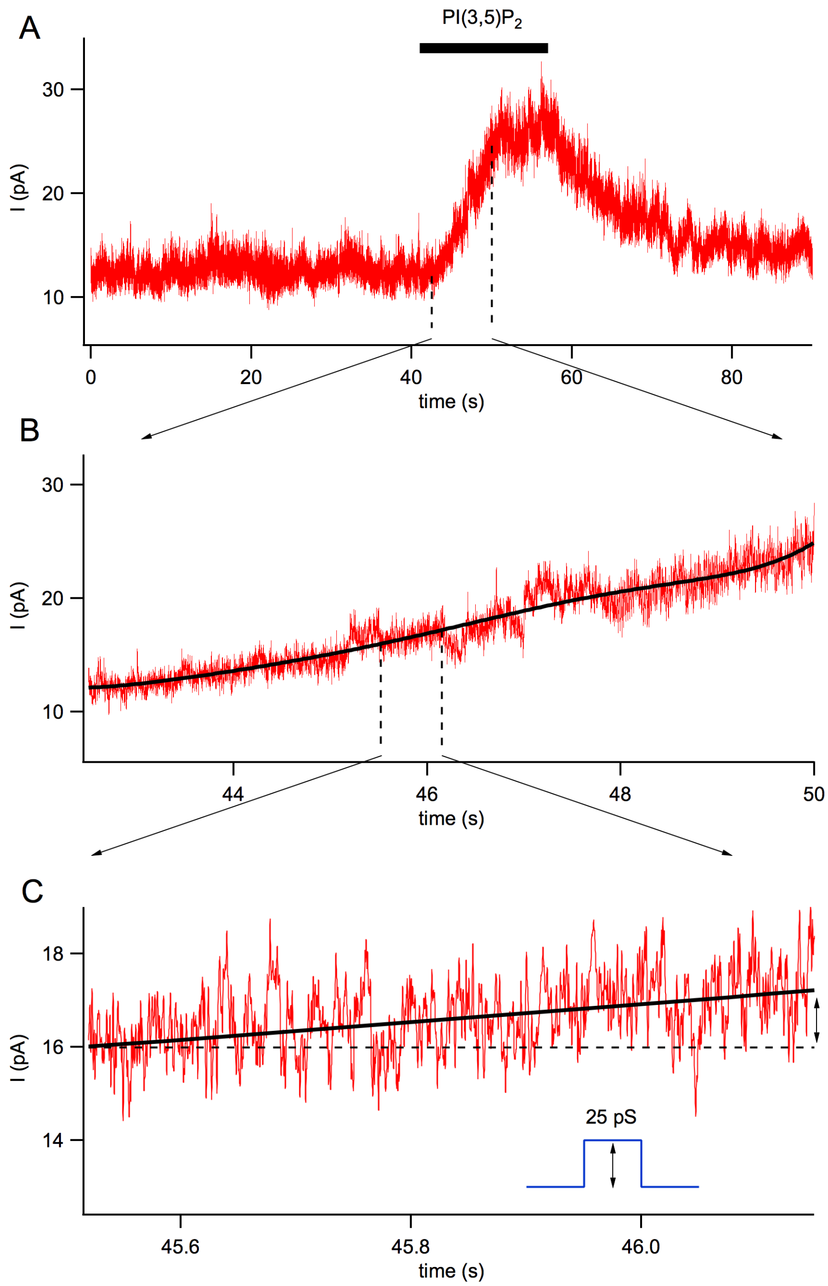
VSD ARGININES



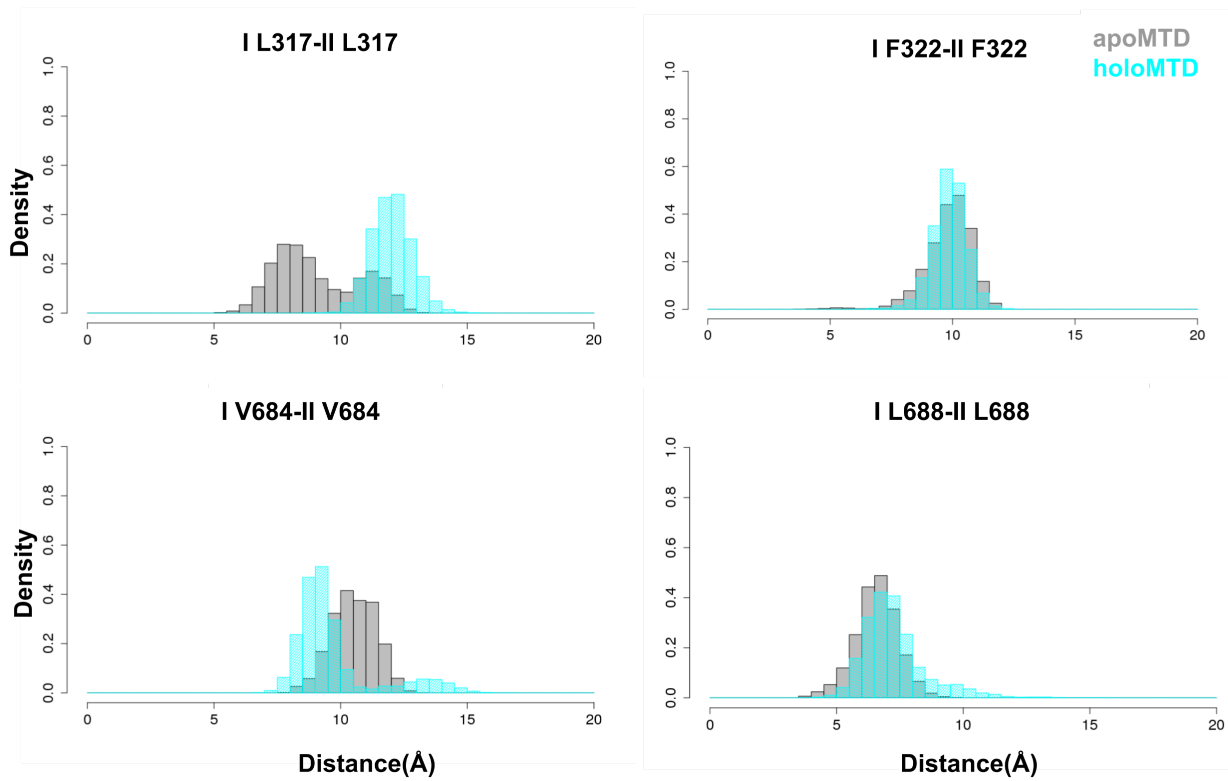
S6. Density distributions of the Z-coordinates recorded for guanidino carbons of VSD arginines of subunit I (left) and subunit II (right) on the molecular dynamics simulations performed under different voltages.



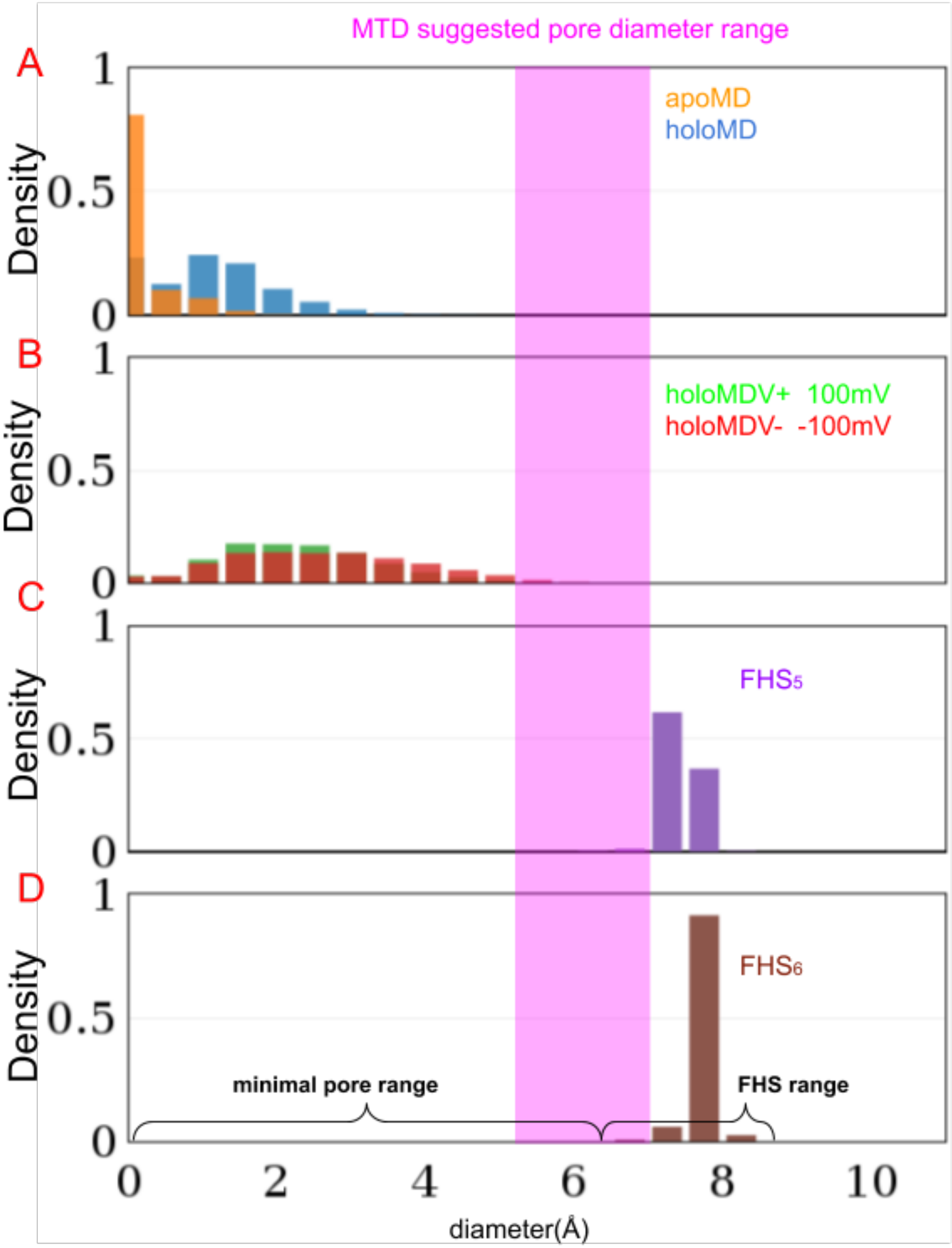
S7. Free energy profiles of sodium ions position along the Z-axis with a different FHS numbers (4 water molecules/blue, 5 water molecules/green, 6 water molecules/black, 7 water molecules/orange, 8 water molecules/red). The FHS_6 value in the bulk is taken as the zero energy. FHS_4, FHS_7, and FHS_8 profiles have gaps due to the lack of the statistics in some region around or at the hydrophobic gate.



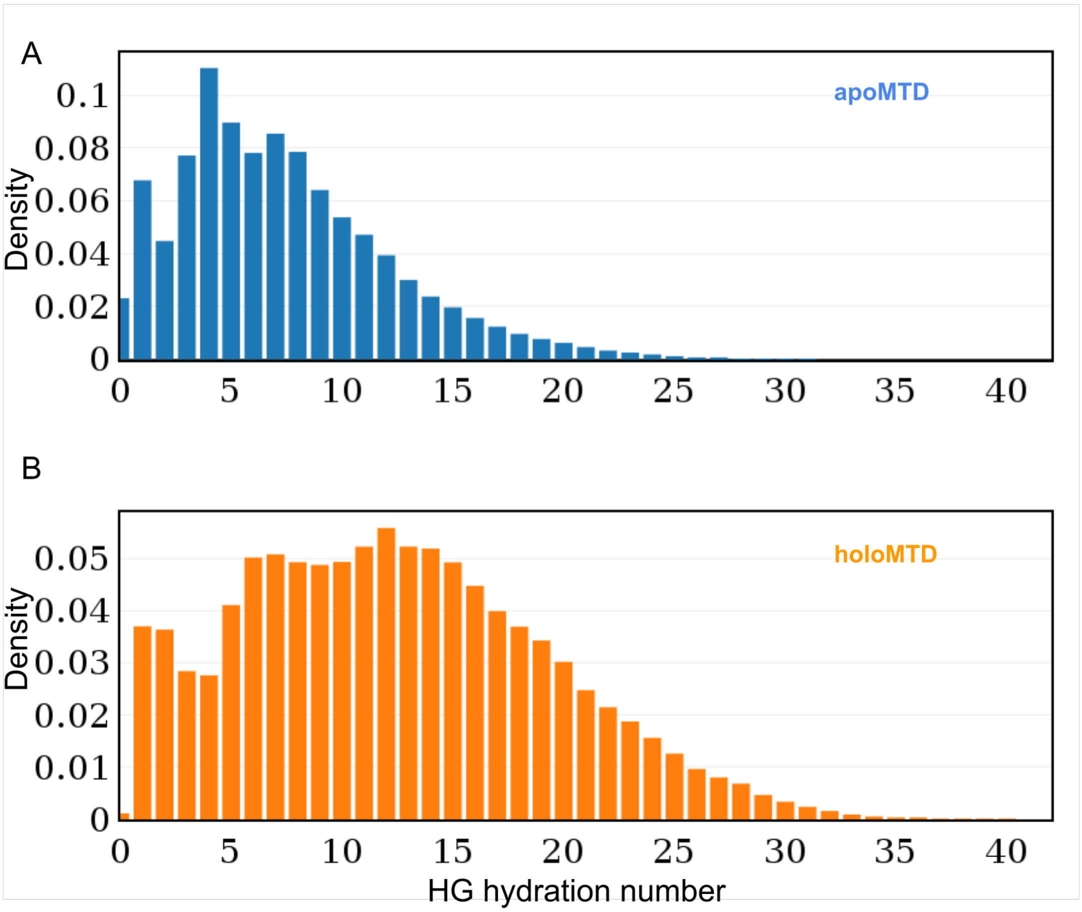
S8. Upper limit estimation of hTPC1 single channel conductance. A) Addition of PtdIns(3,5)P_2 at 100 nM in the cytosolic bath solution reversibly activated hTPC1 channels expressed in vacuoles isolated from Arabidopsis mesophyll cells. Data recorded at a constant voltage of +40 mV from a small tonoplast vesicle in whole-vacuole configuration. B) Current increase due to the progressive activation of hTPC1 channels by PtdIns(3,5)P_2 is shown versus the time interval between the two dashed lines of panel A. Data were fitted with a polynomial function (black line). C) The current between the two dashed lines of panel B displays a linear increase of about 1 pA in the selected time interval (black trace: polynomial fitting); no clear single channel opening can be distinguished (the blue trace is an example of a single channel opening of the same amplitude). Therefore, the value of the hTPC1 single channel conductance should be lower than 25 pS.



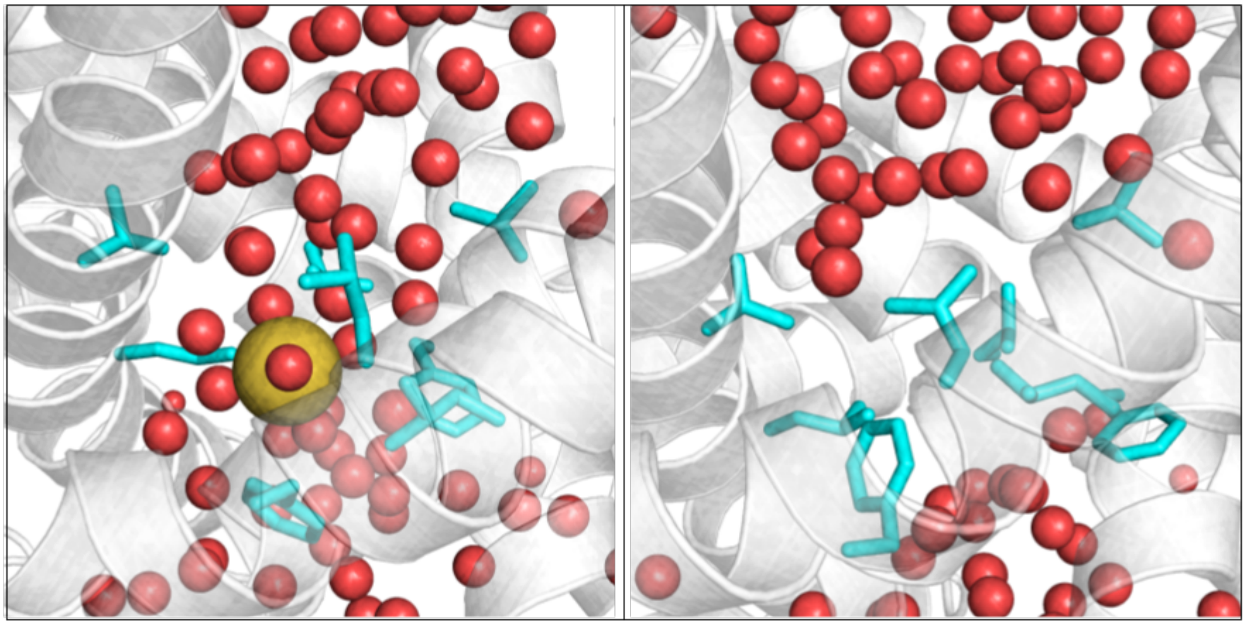
S9. Density distribution of the HG pairs' minimum distances (see Fig. 2B) calculated on apoMD (grey) and holoMD (cyan) simulations.



S10. Distribution of minimal pore diameters for different simulations- apo (orange), holo (blue), holo 100mV (green), holo -100mV (red)- compared with FHS diameter distributions for 5 (violet) and 6 (brown) water molecules. Magenta bar marks the size of the pore at the moment of sodium passage as obtained in MTD simulations.



S11. Density distributions of the number of water molecules in touch with HG amino-acid residues for apo(top) and holo(down) MTD simulations. This number was used as the second collective variable in the MTD simulations.



S12. Showcases of open (left) and closed (right) HG. Closed state is characterized by the absence of water molecules inside the gate.