SUPPLEMENTAL INFORMATION

Conformational dynamics on the extracellular side of LeuT controlled by Na^+ and K^+ ions and the protonation state of E290

George Khelashvili[1], Solveig Gaarde Schmidt[2], Lei Shi[1,3], Jonathan A. Javitch[4], Ulrik Gelather[2], Claus J Loland[2], Harel Weinstein[1,5]

[1]Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University, New York, NY, 10065, USA
[2]Department of Neuroscience and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen N, Denmark
[3]Computational Chemistry and Molecular Biophysics Unit, National Institute on Drug Abuse - Intramural Research Program, National Institutes of Health, Baltimore, MD 21224, USA
[4]Departments of Psychiatry and Pharmacology, Columbia University College of Physicians and Surgeons, New York, New York 10032; Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, New York 10032.
[5]Institute for Computational Biomedicine, Weill Medical College of Cornell University, New York, NY 10065, USA

SUPPLEMENTARY FIGURES

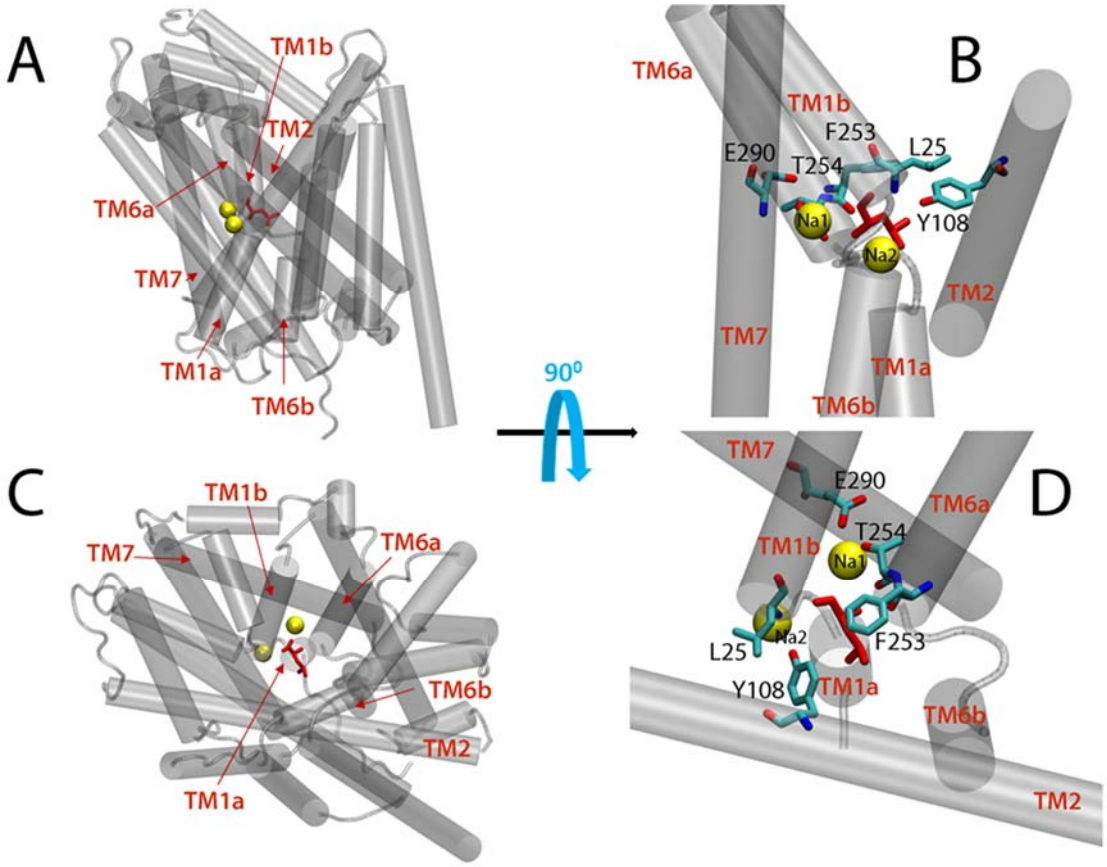

Figure S1: (A-C) Side and top (extracellular) views of LeuT (cartoon) in the occluded state (PDBID: 3GJD) is bound to two Na^+ ions in Na1 and Na2 sites (yellow) and substrate Leucine (red licorice). (B-D) Side and top (extracellular) views of functionally important regions and residues in LeuT. Functional Na^+ ions in Na1 and Na2 sites and substrate Leucine are shown as in panel A. L25, Y108, F253, T254, and E290 residues are depicted in licorice. Relevant helices are labeled.

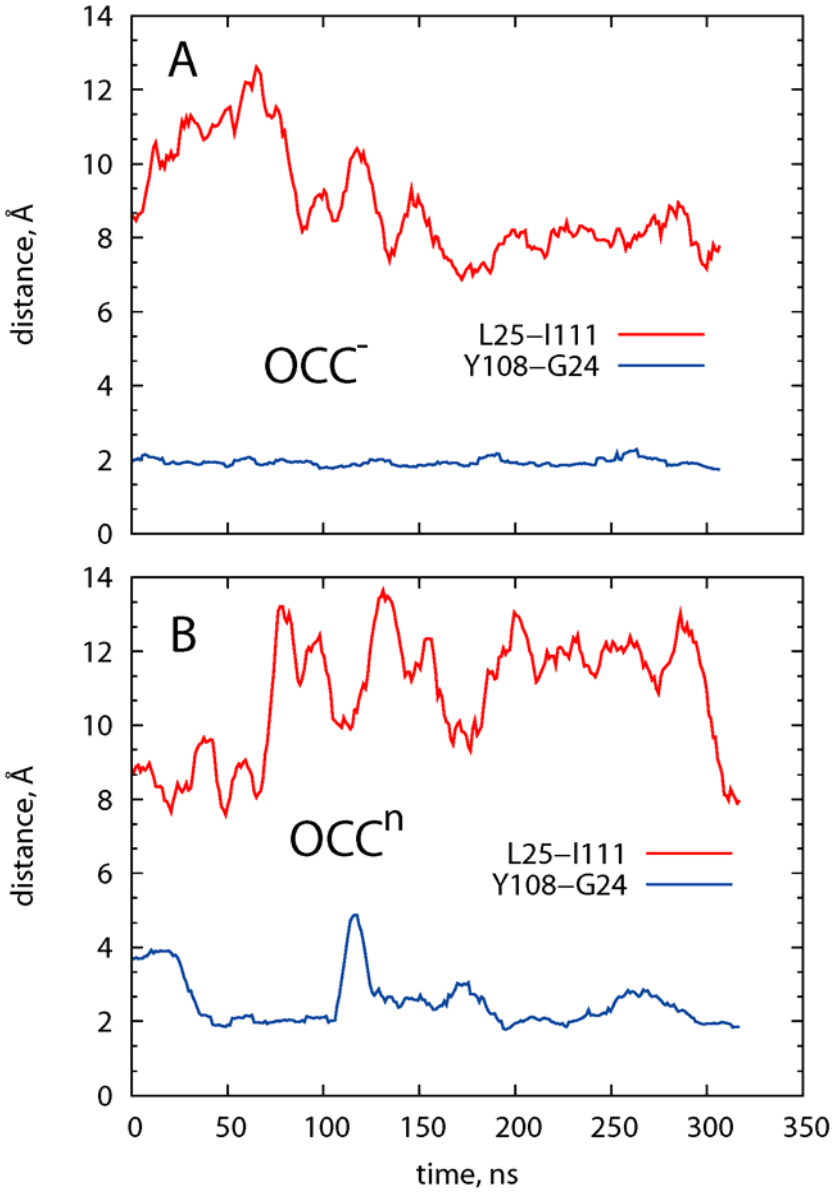

Figure S2: L25-I111 (red) and Y108-G24 (blue) distances as a function of time in the OCC^- (A) and OCC^n (B) trajectories.

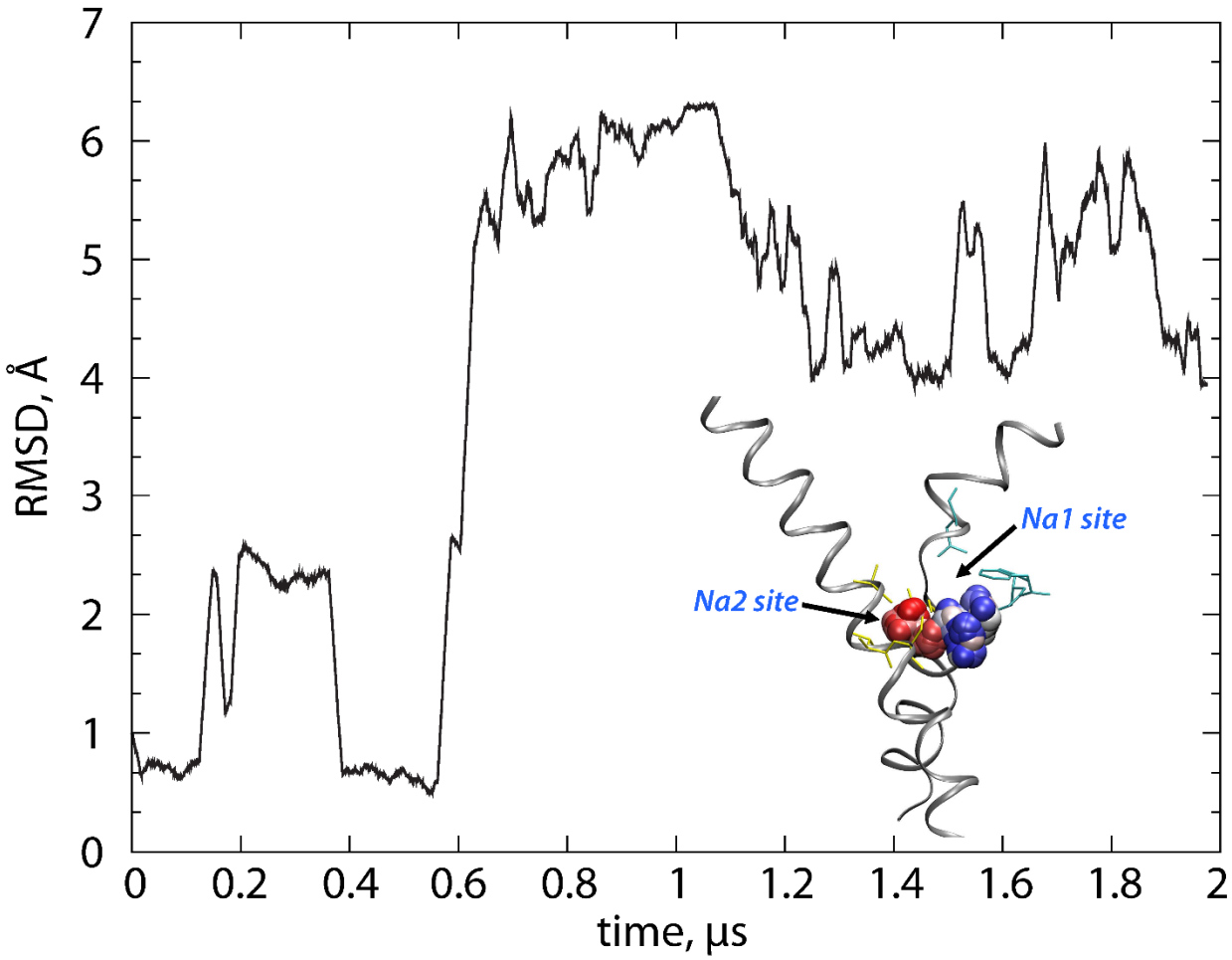

Figure S3: RMSD vs. time for K^+ ion initially bound at the Na2 site of LeuT in the OCC^-K simulation. The inset illustrates volume sampled by the K^+ ion during the trajectory. Different colored sphere represent positioning of the K+ at various stages of the simulation (with the red-white-blue gradient indicating locations during initial, middle, and final time intervals of the trajectory). TM1 and TM6 segments of LeuT are shown are cartoons. For completeness, the residues comprising the Na1 and Na2 sites in LeuT are depicted in sticks. The RMSD was calculated with respect to K^+ positioning in the initial frame of the simulation after the trajectory was aligned using the backbone atoms of the TM helical segments.

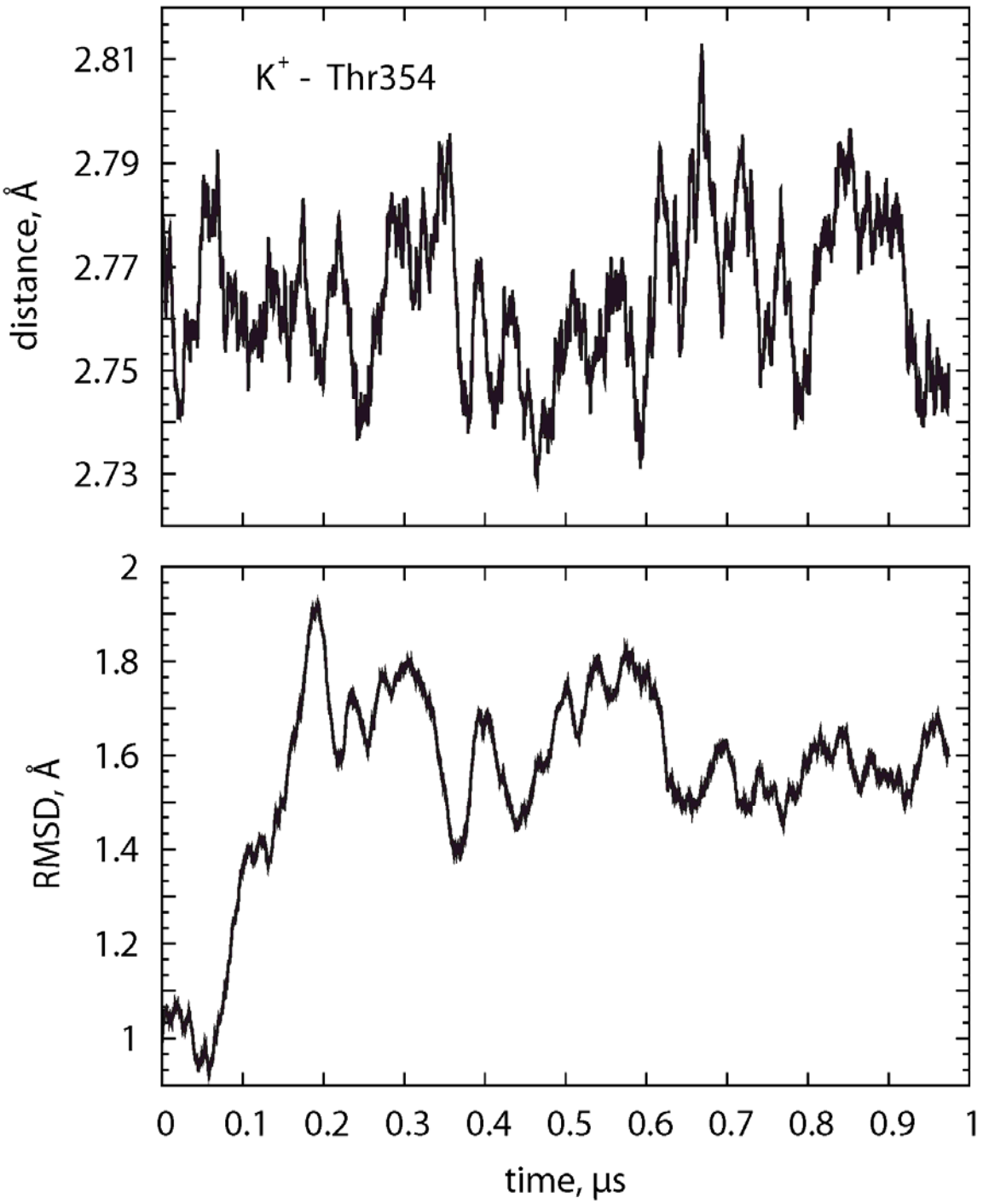

Figure S4: (A) Time evolution of the minimal distance between K^+ ion in the Na2 site and Thr354 in the SMD^-K simulation. (B) RMSD vs. time for the K^+ ion initially bound at the Na2 site of LeuT in the SMD^-K simulation. The RMSD was calculated with respect to K^+ positioning in the initial frame of the simulation after the trajectory was aligned using the backbone atoms of the TM helical segments.

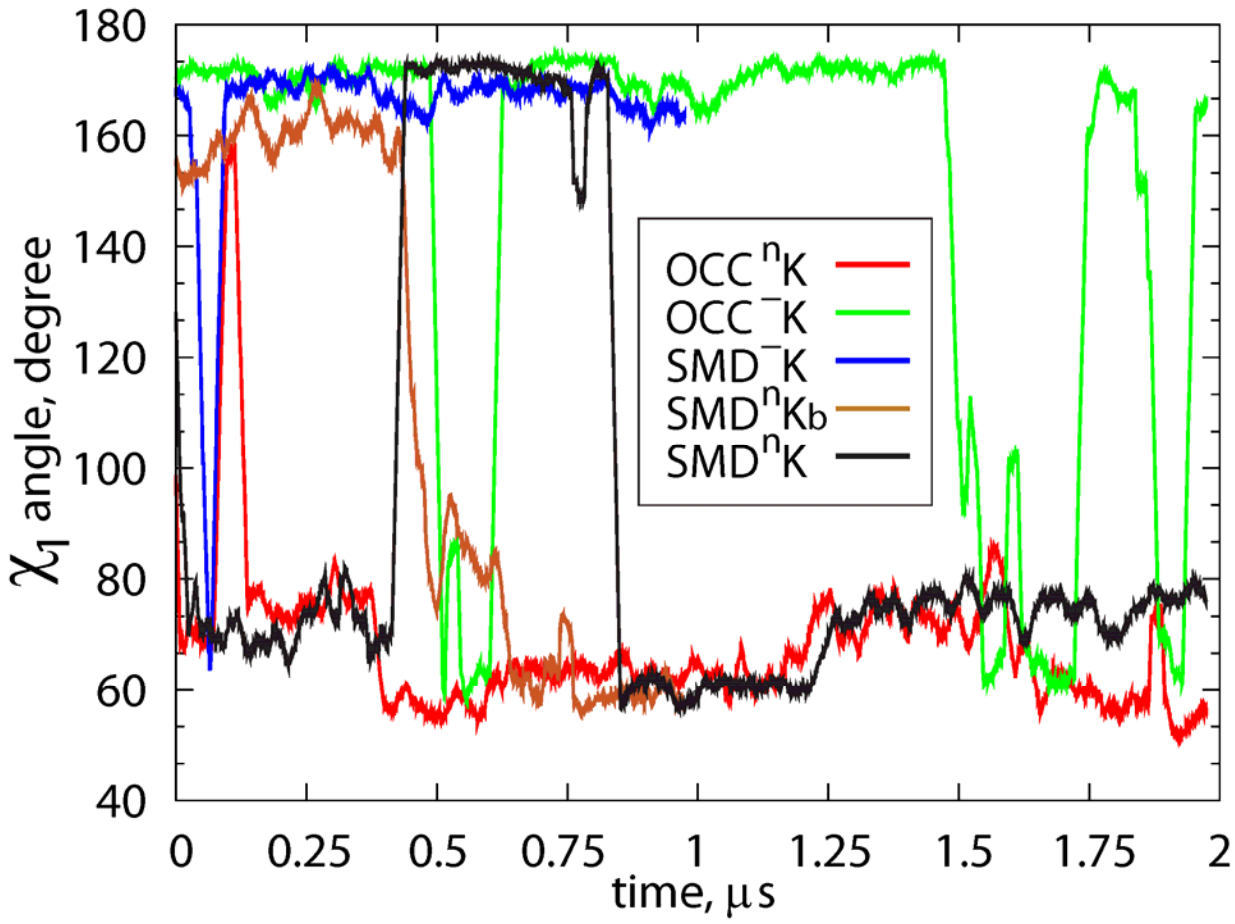

Figure S5: Time evolution of the χ_1 dihedral angle of F253 residue in the different MD simulations.

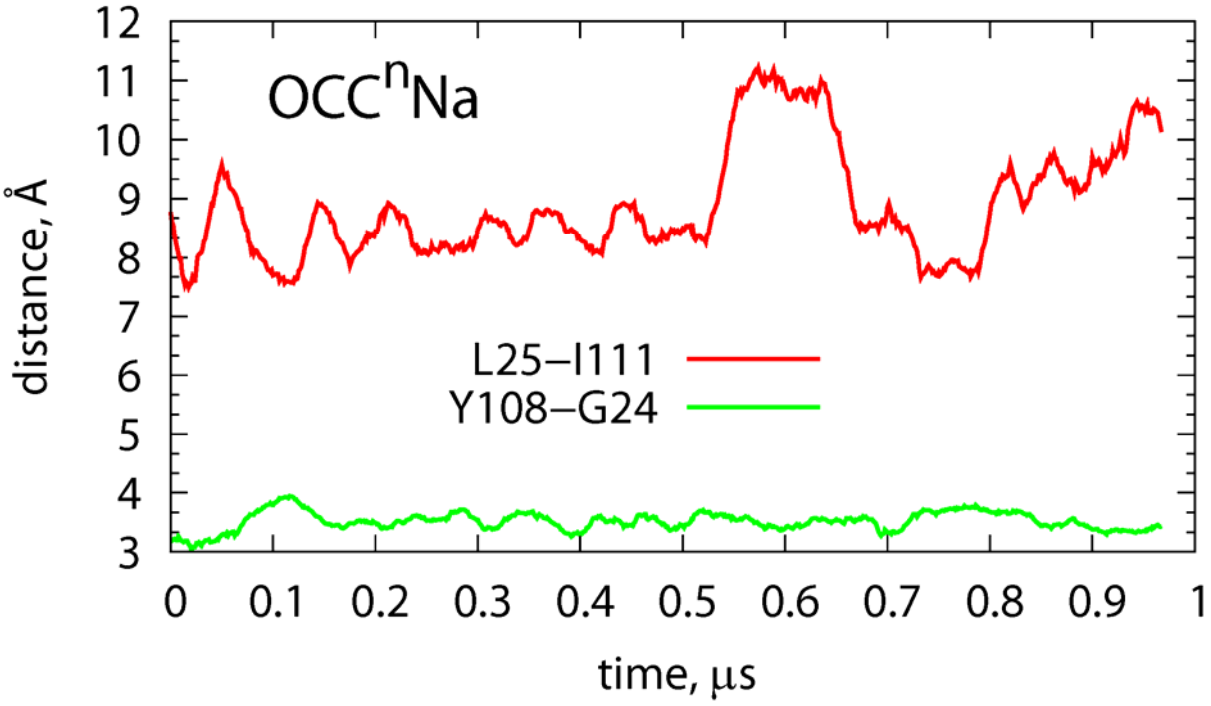

Figure S6: L25-I111 (red) and Y108-G24 (green) distances as a function of time in the OCC^{n}Na trajectory.

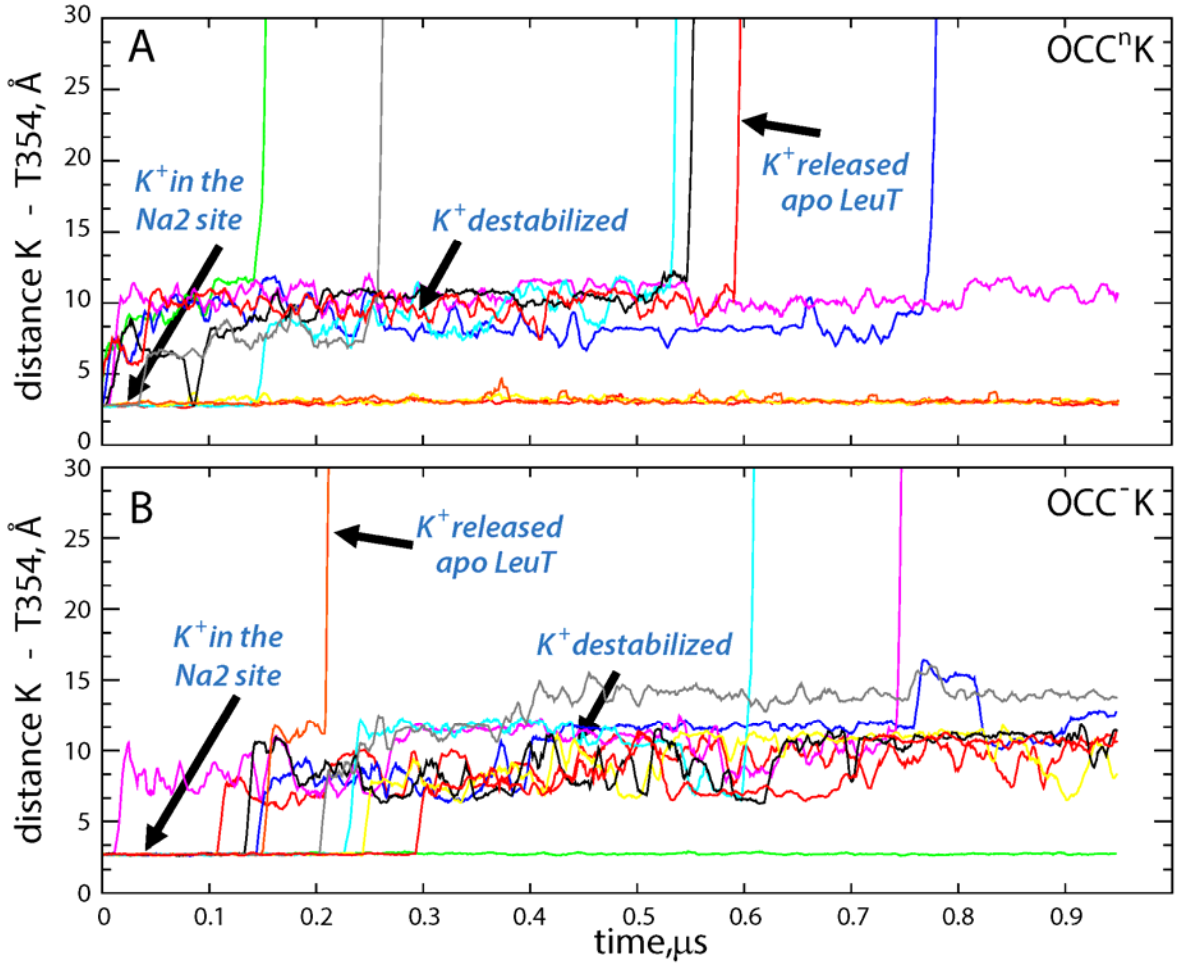

Figure S7: Time evolution of the minimal distance between K^+ ion in the Na2 site and Thr354 in the OCC^nK (A) and OCC^-K (B) ensemble simulations. Different trajectory replicas are shown in different colors. Destabilization and release of the K^+ ion can be assessed by monitoring gradual increasing in the distance. The range of *y* (vertical) axis on the two panels was truncated at 30 Å for better resolution of distance changes.

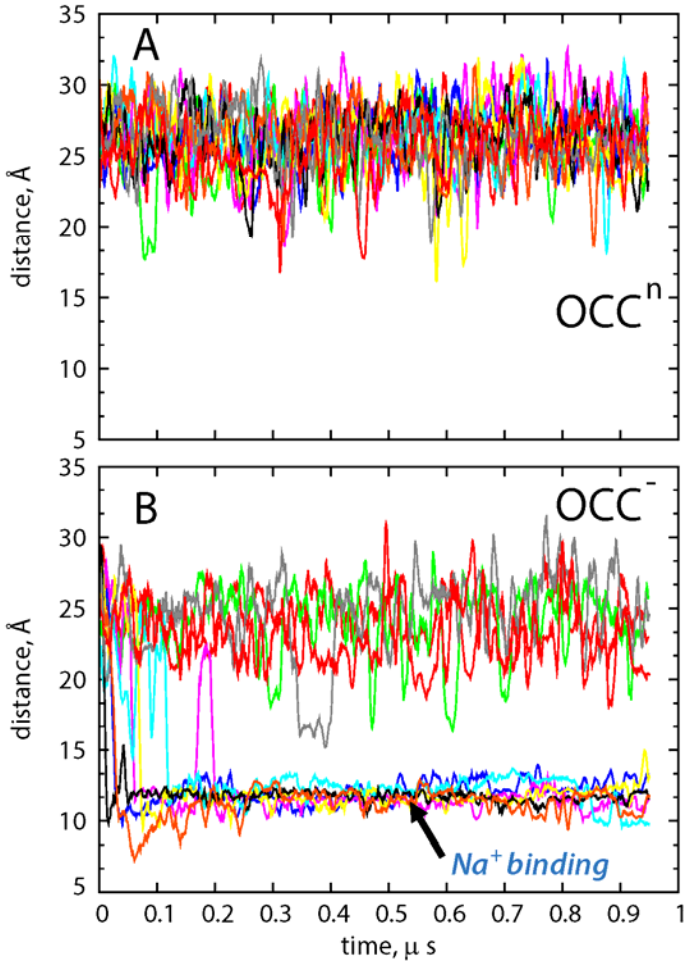

Figure S8: Time evolution of the minimal distance between Thr354 and the nearest Na^+ ion in the OCC^n (A) and OCC^- (B) ensemble simulations. Different trajectory replicas are shown in different colors. Conformations of LeuT with Na^+ are characterized by relatively short (≤12Å) distance with respect to Thr354.

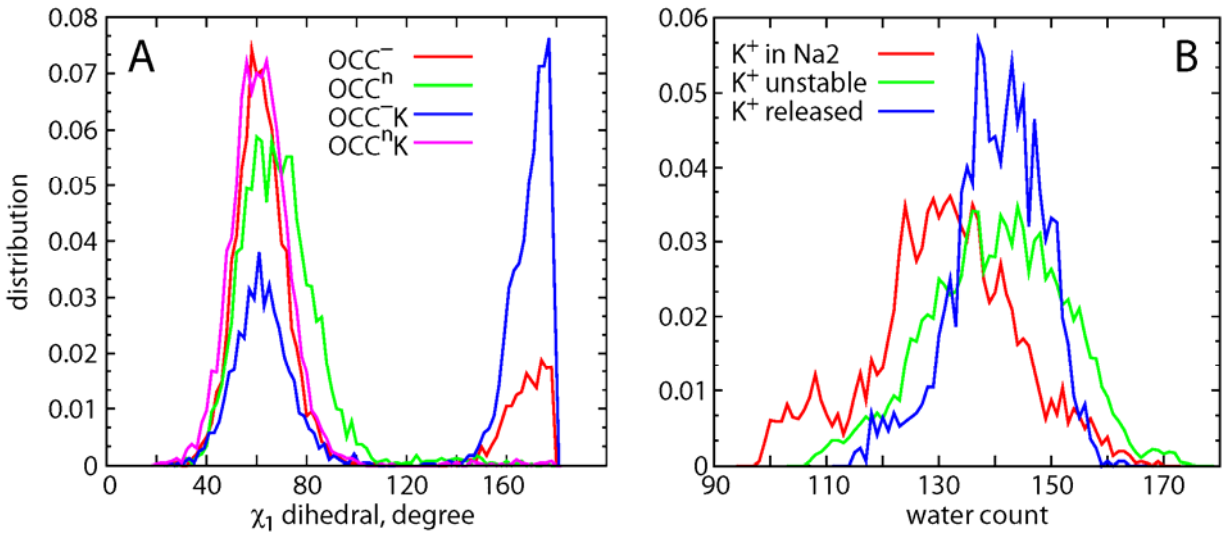

Figure S9: (A) Probability distribution of χ_1 dihedral angle of F253 residue in different ensemble MD simulations. For a particular construct, the profile was obtained by analyzing combined trajectory that included last halves of all the replica simulations (10 per construct). (B) Distribution of water molecule count in the EC vestibule of LeuT in the OCC^nK ensemble simulations. The combined trajectory used in panel A was analyzed to identify time-frames in which K^+ was stably bound in Na2 site (K^+ in Na), K^+ was destabilized in Na2 but still within confines of Na1 and Na2 sites (K^+ destabilized, see Fig. S7), and in which K^+ was released (K^+ released). Water count for these three different sets of trajectory frames were performed separately and the resulting data was binned to obtain the distribution profiles.

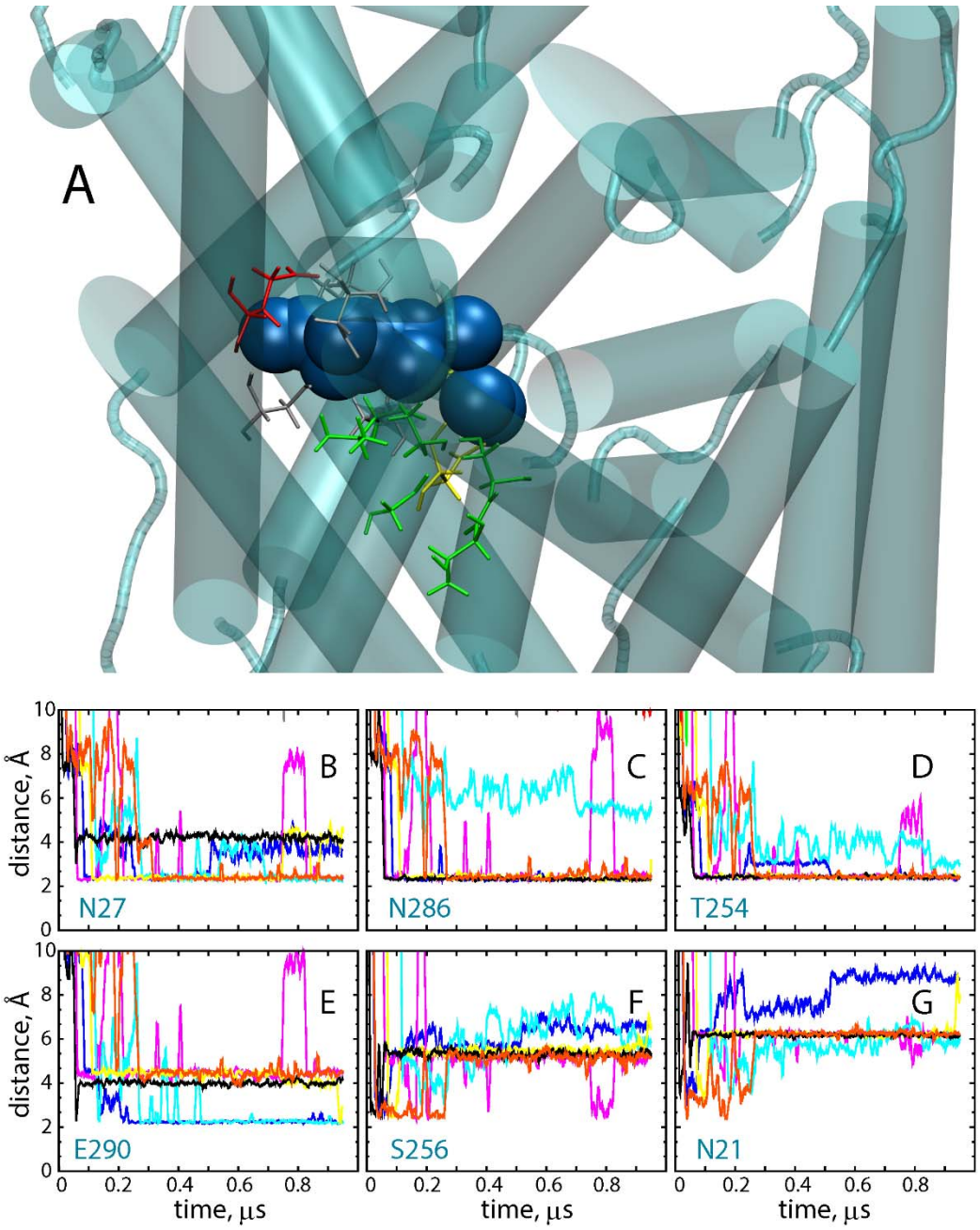

Figure S10: (A) Na^+ ions binding LeuT from the EC solution sample a region overlapping with ion binding sites. Snapshots combine positioning of the partitioned Na^+ ions (blue spheres) from all 10 replicates of the OCC^- construct (only trajectory frames are shown in which Na^+ ions were within confines of the Na1 and Na2 sites). The residues comprising Na1 site (A22, N27, T254, N286) and Na2 site (G20, V23, A351, T354, S355) are depicted in grey and green respectively. In addition, E290 residue is shown in red, and residues N21 and S256 – in yellow. (B-G) Time evolution of the minimum distance between selected residues in LeuT and the nearest Na^+ ion in the OCC^- ensemble simulations. Different trajectory replicas are shown in different colors. The range of *y* (vertical) axis was truncated at 10 Å for better resolution of distance changes.