SI Appendix

Supplementary Figures

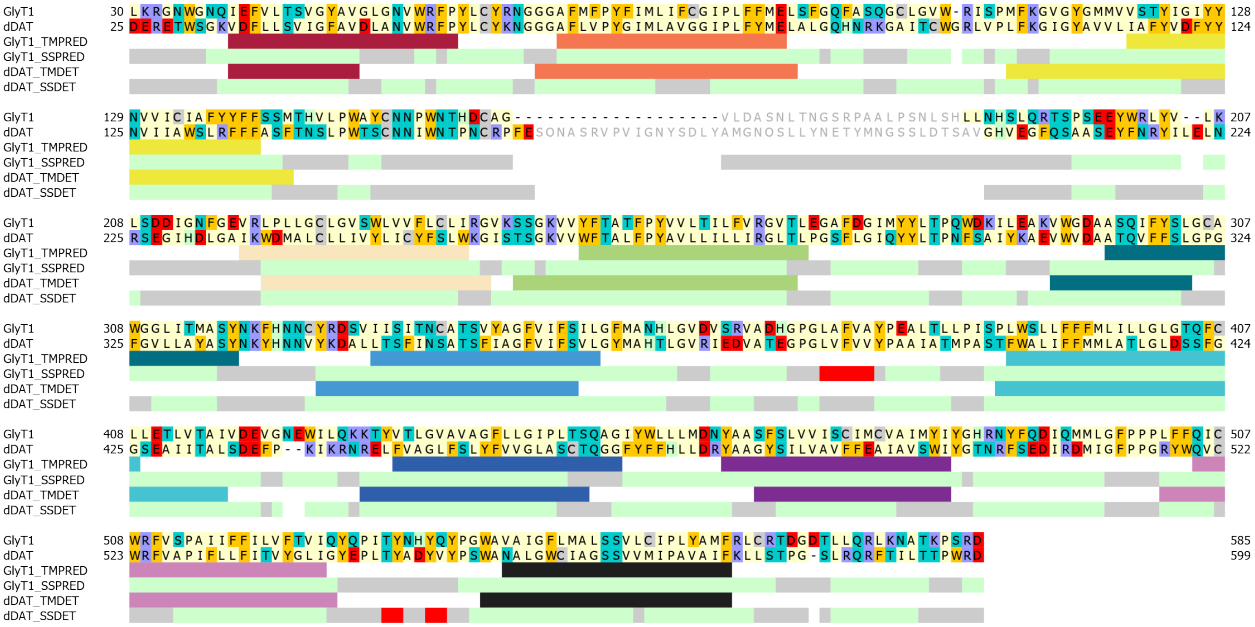

Figure S1. Alignment between dDAT and GlyT1 used to guide the homology modeling. Residues in gray font are not present in the template or are not built into the model. Amino acid background coloring indicates chemical properties, i.e., red for acidic (Asp, Glu), blue for basic (Arg, Lys), teal for polar (Asn, Gln, Ser, Thr), gold for aromatic (Phe, Tyr, Trp), gray for cysteine, green for histidine, and pale yellow for all hydrophobic residues, proline, and glycine. To assess the quality of the alignment we compared the transmembrane spanning regions of dDAT ("TMDET", computed by OPM for PDB ID 4M48) (77) and GlyT1 ("TMPRED", predicted by TOPCONS (78)), shown underneath the sequences. For those segments, the repeat containing TMs 1-5 is indicated with boxes colored in red-to-green while the repeat containing TMs 6-10 is indicated with boxes colored in blue tones. The secondary structure of dDAT ("SSDET", computed with DSSP v3.0.1 (79, 80)) and GlyT1 ("SSPRED", predicted with PSIPRED v4.0 (81)) is also provided; in those lines, the alpha-helical portion of the sequence is shown as light green boxes, the beta-stranded segments with red boxes and the regions without defined secondary structure (coil) with grey boxes.

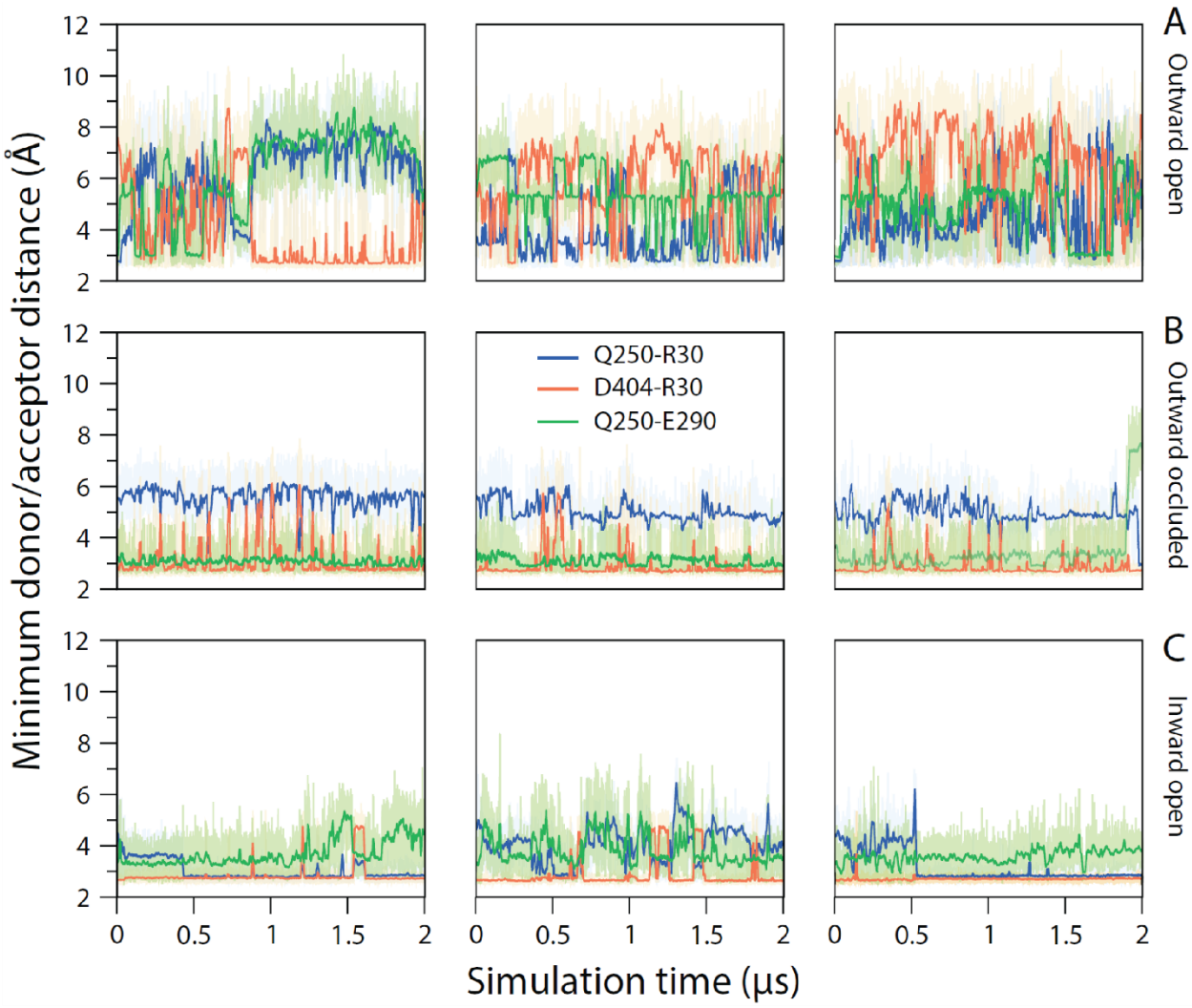

Figure S2. Time-course of the interactions between residues in the extracellular pathway and the chloride binding site during MD simulations of LeuT. Data represent distances between the closest donor and acceptor atom of Arg30 with Gln250 (blue), with Asp404 (orange), and between Gln250 and Glu290 (green). Simulations are for LeuT in (A) outward-open apo (3TT1), (B) outward-occluded holo (3F48), and (C) inward-open apo (3TT3) conformations. Each simulation was repeated three times (columns).