Supplementary Information

Electronic Polarizability Tunes the Function of the Human Bestrophin 1 Chloride Channel

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Supplementary Methodological Details

Full protein embedded simulations

The full protein systems were prepared using a multiscale procedure. The protein is first coarse-grained (CG) and then embedded into a POPC bilayer and solvating with water and ~ 0.5 M NaCl using Martini version 2.2 (1) and GROMACS 2021 package (2). This system is subject to 100 ns of equilibration in CG before being converted to atomistic representation with the CG2AT protocol (3). An equilibration period of 20 ns followed by a production run of 100 ns using the c36m forcefield (4) and mTIP3P water model was performed. The temperature was maintained at 310 K with coupling constant 1.0 ps by the Nosé-Hoover thermostat (5). Pressure was maintained at 1 bar with coupling constant 5.0 ps by the Parrinello-Rahman barostat (6). Short-range electrostatics were treated with the Verlet cutoff scheme at 1.2 nm cutoff and long-range electrostatics were treated with PME(7). C-alpha atoms were placed under harmonic restraints with a force constant of 1000 kJ/mol/nm^2 to prevent the structure from deviating too much from the experimental structure.

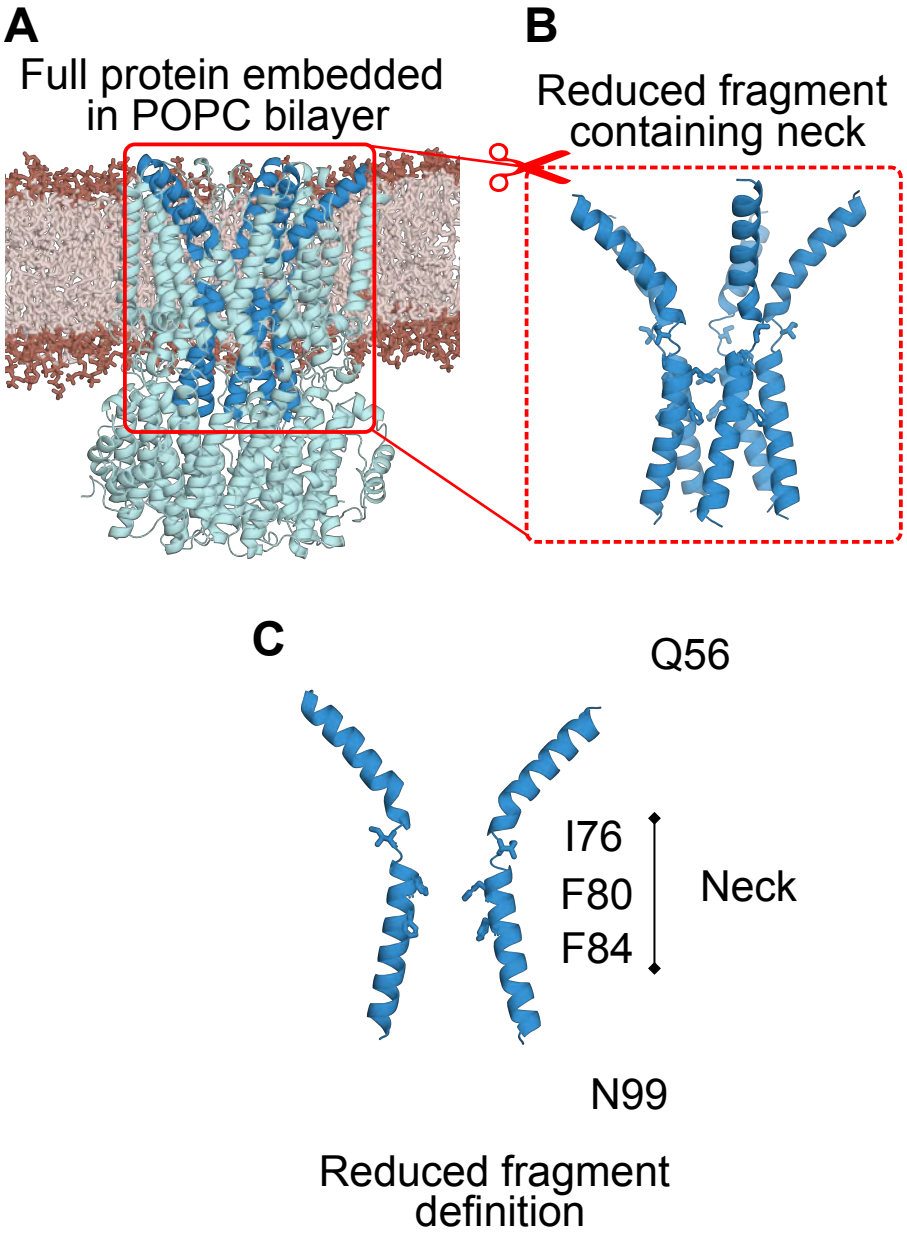


Figure S1: **A** Full protein (cyan) embedded in POPC bilayer (brown). **B** Protein fragment system used in simulations due to the high computational demands of the AMOEBA forcefield. The full protein embedded system in solution contains ~200500 atoms. **C** The protein was truncated at Q56 and N99 such that the remaining fragment system contains the conserved hydrophobic neck region of interest (I76, F80, F84). The reduced protein fragment in solution contains ~57000 atoms. Water and ions are omitted for clarity.

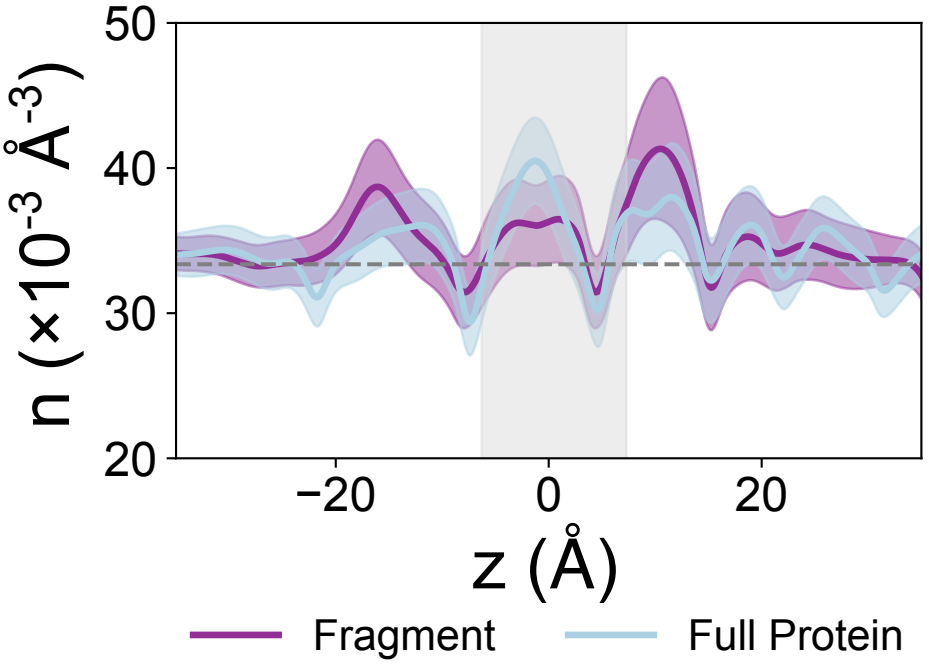
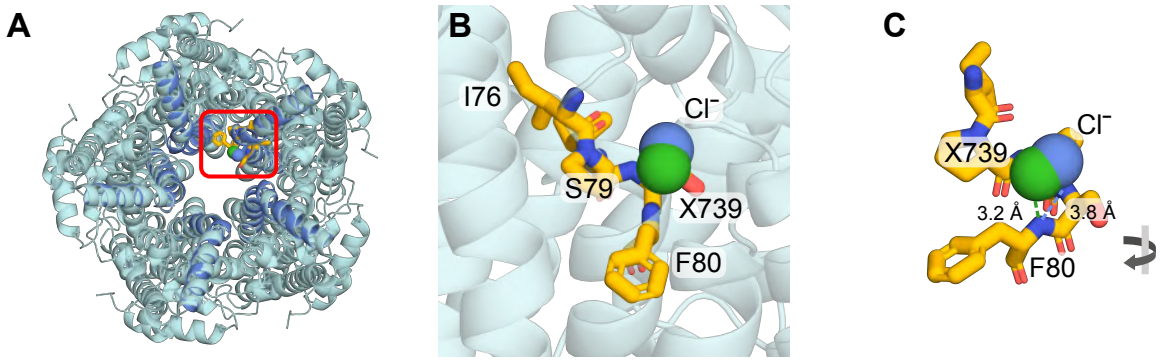


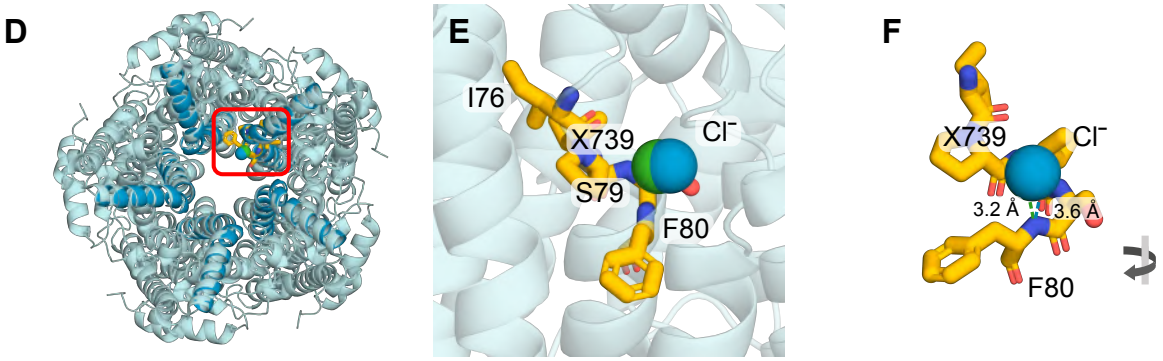
Figure S2: Protein fragment system validation. Time-averaged water density profiles within the pore for the full protein embedded system (light blue) compared with the protein fragment in solution (purple) using c36m. The shaded region represents the neck region, and the dashed grey line corresponds to the density of bulk water (33.37 nm^{-3}). Confidence bands represent the standard deviation over the simulation.

AMOEBA

Binding site 2



Binding site 3



G

Binding Site	Site Residence Time (ns)	k_{off}	Duration (ns)	Occupancy (%)	R-squared	Distance to N of F80 (Å)	Distance to X739 in PDB (Å)
3	0.59	1.7	0.75	14	0.9987	3.8	1.2
12	0.43	2.3	0.63	28	1	3.6	0.7
2	0.34	3.0	0.63	18	0.9999	3.8	0.6

Figure S3: Alternative AMOEBA binding sites of the open state structure (PDB ID 8D1O). **A-C** Binding site 3 and **D-F** show binding site 12. Table **G** gives the binding site statistics where binding site 2 is the site shown in Fig. 2 in the main manuscript.

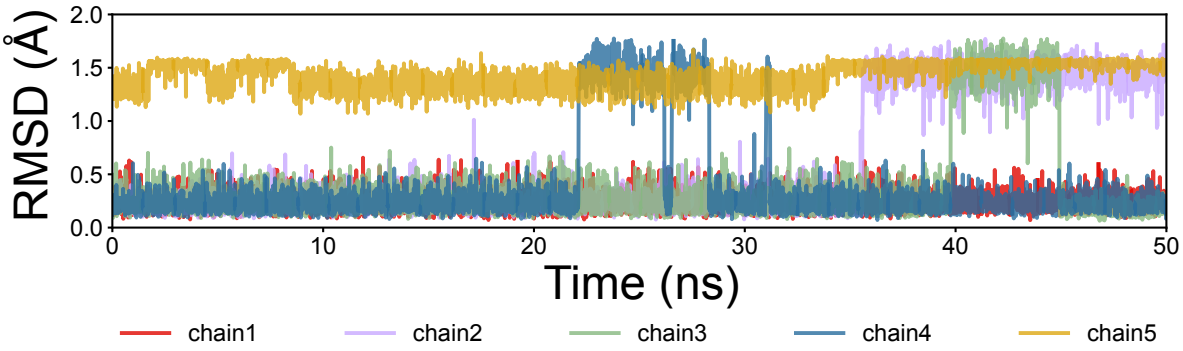


***Figure S4**:* RMSD of F80 sidechain per chain of the open state (PDB ID 8D1O) protein fragment over the AMOEBA simulation.

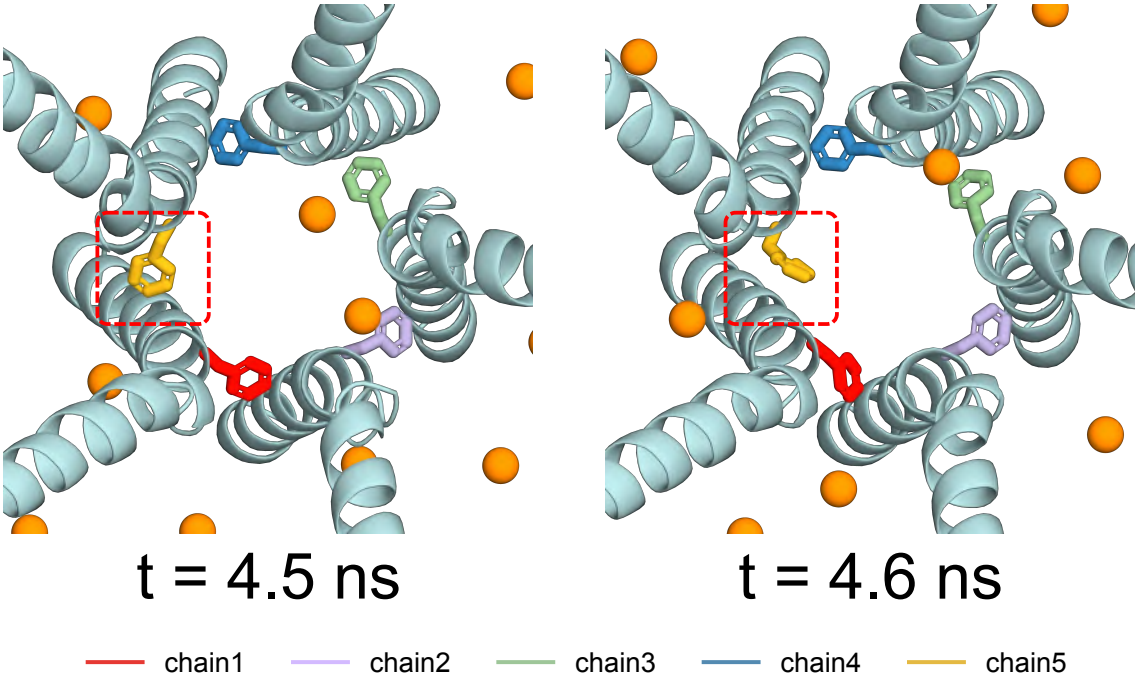


Figure 5: Snapshots demonstrating the spontaneous inwards flipping of an F80 sidechain in the AMOEBA simulation. This motion is not necessarily induced by a passing chloride ion (orange spheres).

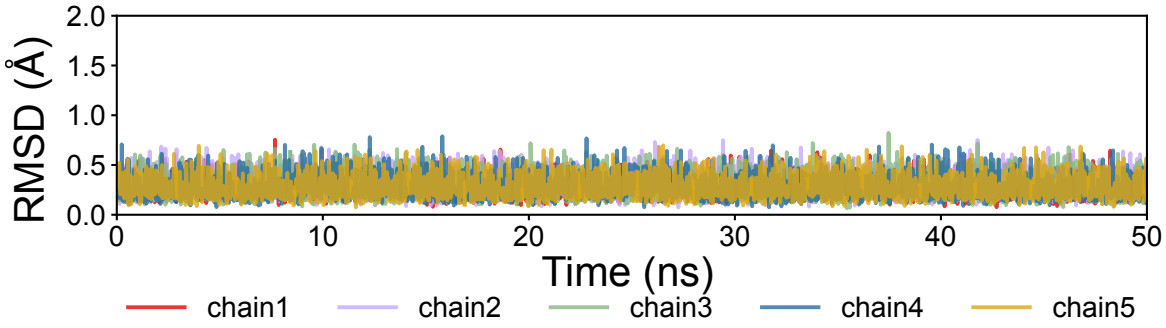


Figure S6: RMSD of F80 sidechains in the partially open state (PDB ID 8D1K). The sidechains remain stable throughout the AMOEBA simulation.

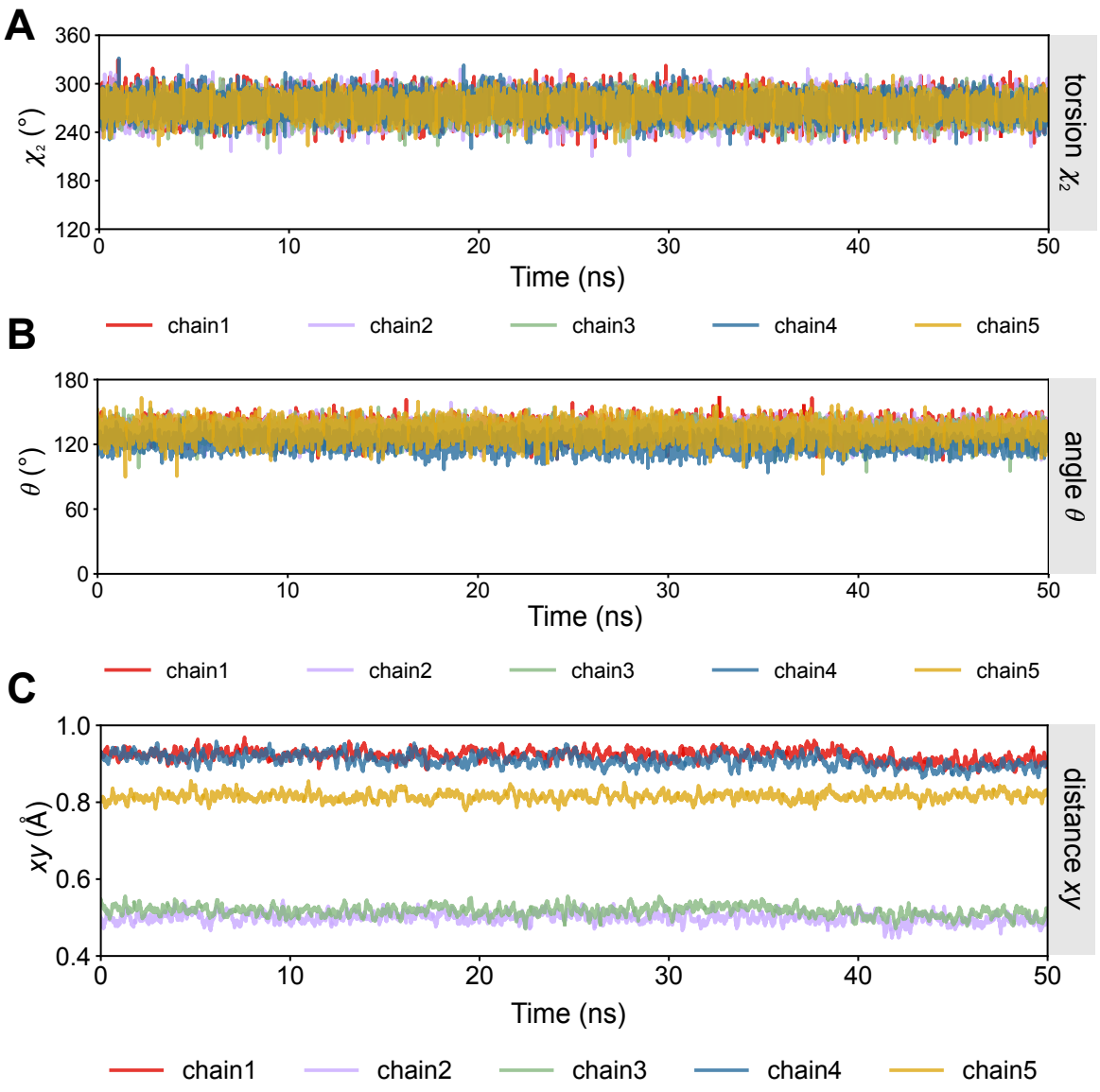


Figure S7: Per chain **A** torsion angle χ_2, **B** angle θ and **C** distance *xy* of F80 in AMOEBA simulation of the partially open state (PDB ID 8D1K). The sidechains remain largely stable in this conformation.

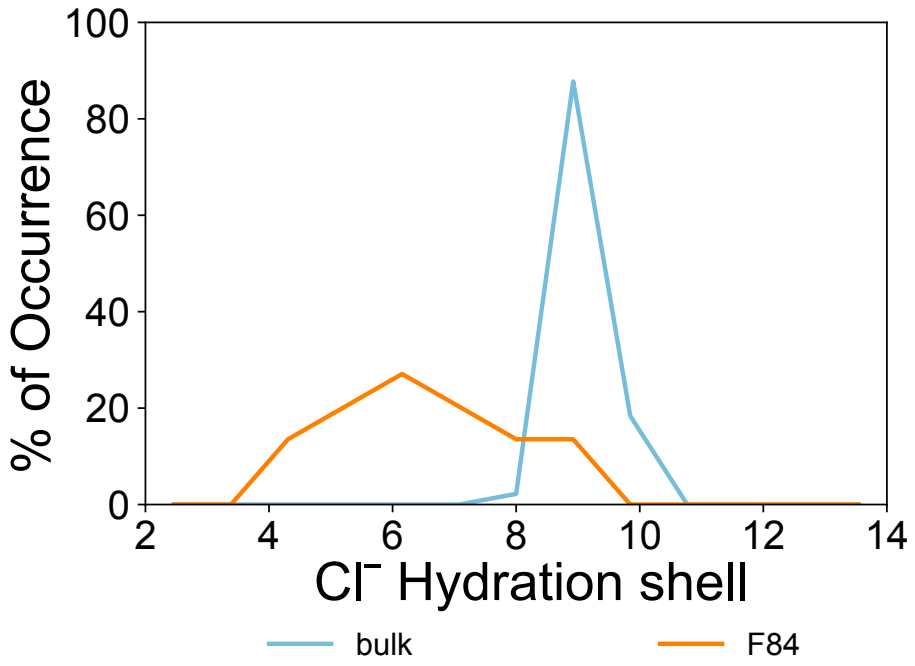


Figure S8: Coordination number of chloride ions in the partially open state structure (PDB ID 8D1K) in bulk (blue) compared with at the z-position corresponding to F84 (orange) within the pore in the AMOEBA simulation. Chloride loses 1-3 water molecules in its first hydration shell at F84 relative to bulk. This is comparable to the dehydration that occurs at F80 in the fully open state (PDB ID 8D1O).

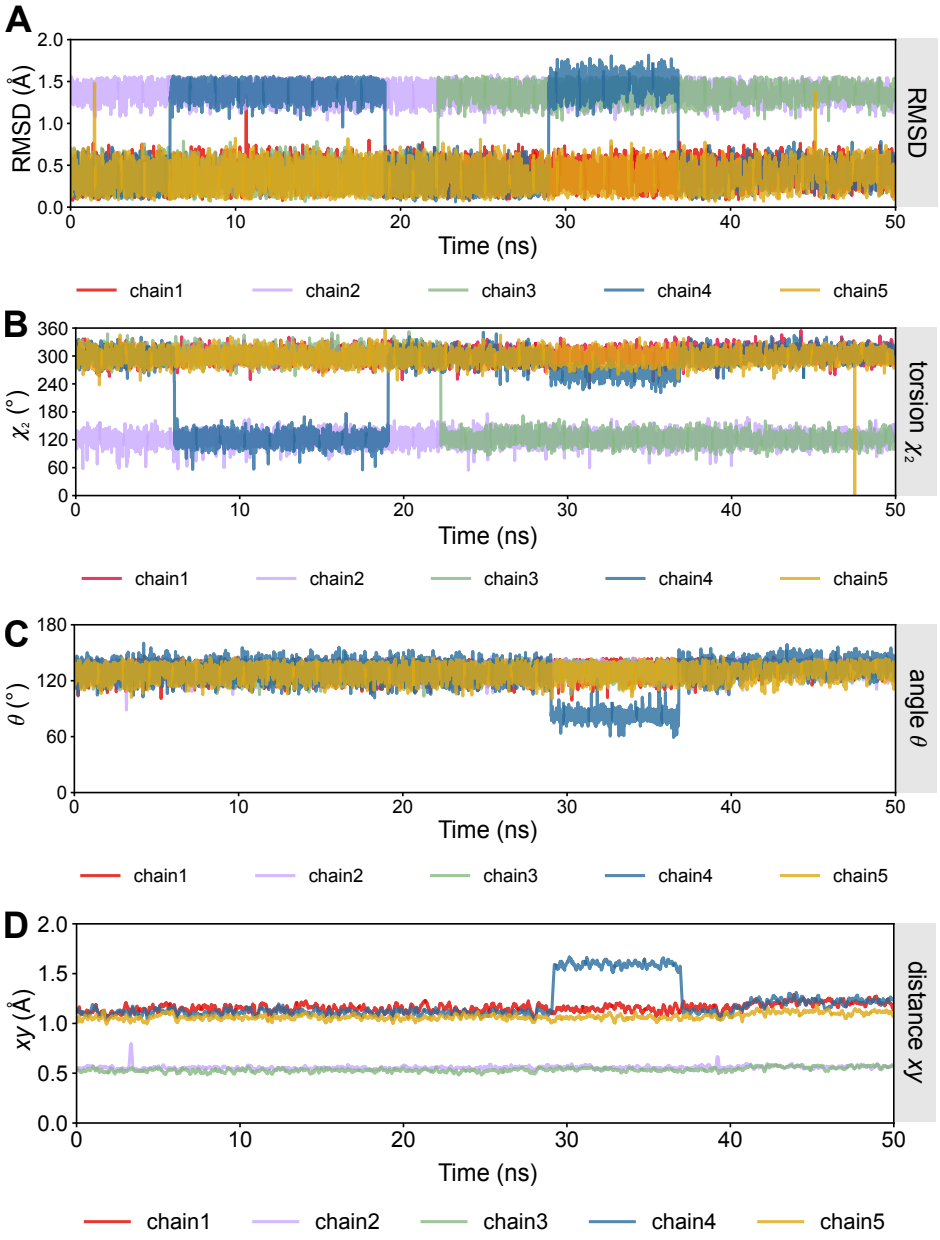


Figure S9: Per chain **A** RMSD, **B** torsion angle χ_2, **C** angle θ and **D** distance *xy* of F84 in the partially open state (PDB ID 8D1K) AMOEBA simulation. The sidechains are largely stable. The two-state behaviour seen in the RMSD and torsion angle correspond to a 180° rotation of the aromatic ring; however, due to the symmetric properties of the aromatic ring, this does not represent an alternative ring conformation. Chain 2 experiences some motion corresponding to an outwards flipping of the sidechain for ~ 7 ns and chain 4 experiences a stochastic downwards flipping motion at ~ 30 – 37 ns.

Supporting information references

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7. U. Essmann, *et al.*, A smooth particle mesh Ewald method. *J Chem Phys* **103**, 8577–8593 (1995).