

Supplementary Information for: Locking Two Rigid-body Bundles in an Outward-Facing Conformation: The Ion-coupling Mechanism in a LeuT-fold Transporter

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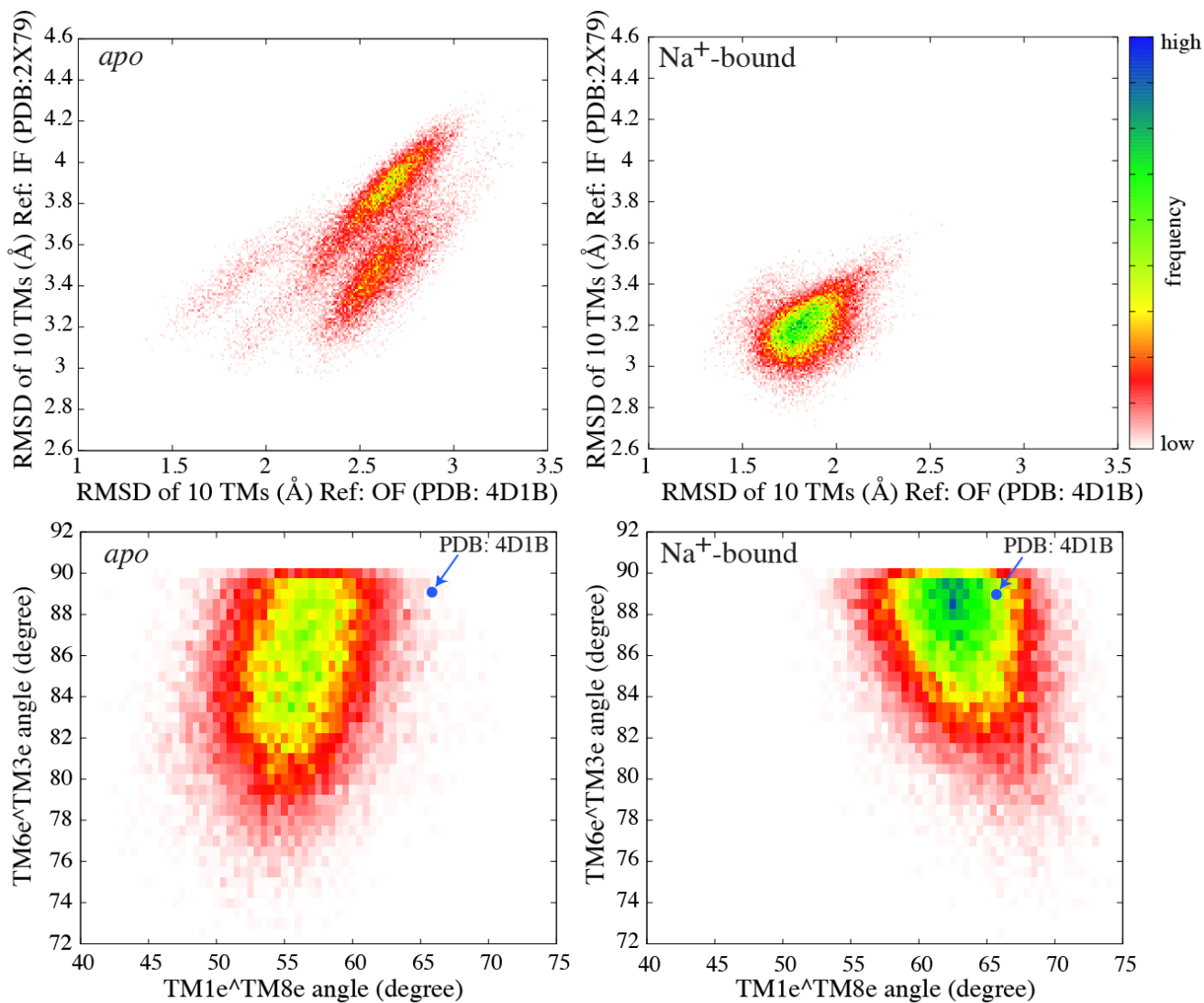


Figure S1. Na⁺-binding effect on the global protein conformation and dynamics in the OF state. (Top) Distribution of backbone RMSDs of 10 TMs in the *apo* (Traj.3, left) and Na⁺-bound (Traj.2, right) simulations with reference to OF Na⁺/substrate-bound (PDB ID code 4D1B) and IF *apo* (PDB ID code 2X79) crystal structures. (Bottom) Distribution of angles between TM1e and TM8e (x axis) and TM3e and TM6e (y axis) in the *apo* (Traj.3, left) and Na⁺-bound (Traj.2, right) trajectories.

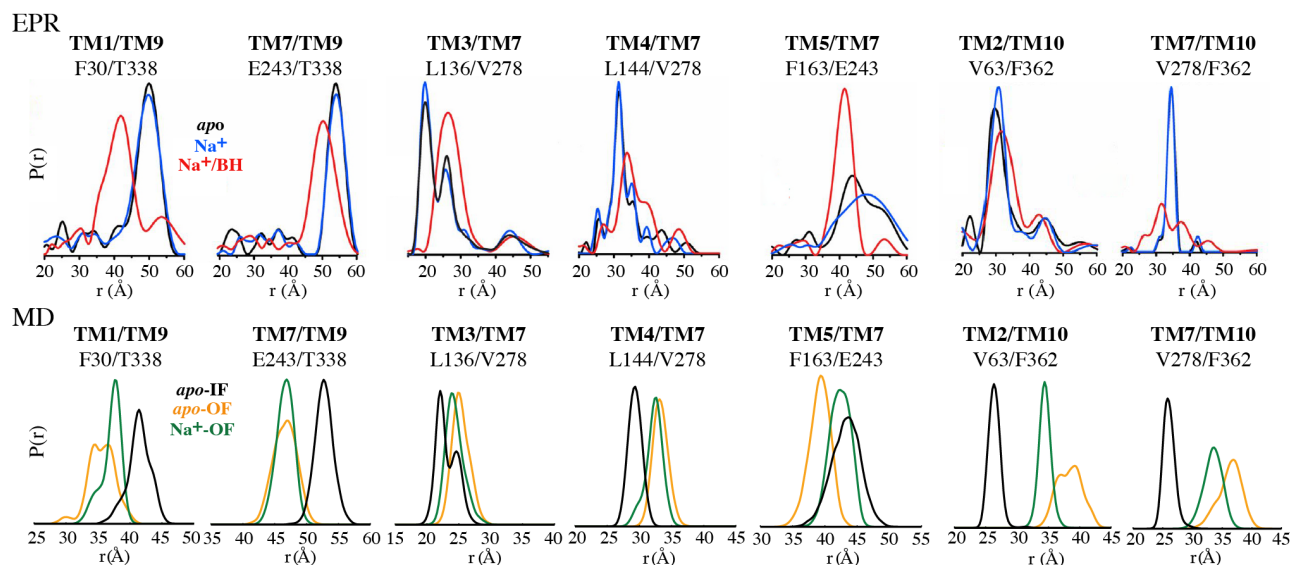


Figure S2. Comparison of EPR distance distributions adapted from Kazmier et al.¹ with permission (copyright 2014 National Academy of Sciences) (top), and the distance distributions calculated from MD simulations of IF apo, OF apo, and OF Na⁺-bound states (Traj.4, Traj.3, and Traj.2 respectively) (bottom). EPR distance distributions for each pair were obtained in the *apo*, Na⁺-bound (Na⁺), and Na⁺- and benzyl-hydantoin-bound (Na⁺/BH) intermediate states. The distances from MD simulations are those between the last heavy atoms on the side chain of the two residues (CZ for Phe, CG2 for Thr and Val, OE2 for Glu, CD2 for Leu), which are expected to be slightly deviated from those between the EPR spin labels in experiments. Both distributions from EPR experiments and MD simulations are shown as a probability, P(r), relative to distance, r.

TM3		220	230	240
Transporter				
LeuT	89	F A K I L G V F G L W I P L V . V A I Y Y V Y I E S W T L G F A I K F		
DAT	106	. F K G I G Y A V V L I A F Y . V D F Y Y N V I I A W S L R F F F A S		
MhsT	89	P W K V A G L M G V A A G F L . I L S F Y G V I A G W I L F Y L F N Y		
Mhp1	102	G S L I P I T L K A L L S L F . W F G F Q T W L G A L A L D E I T R L		
vSGLT	126	L K T I L A V F W I S L Y I F V N L T S V L Y L G G L A L E T I L G .		
BetP	234	L G K L I D I L A I I A T V F . G T A C S L G L G A L Q I G A G L S A		
ApeT	85	I T G A L S I L L W M S Y V I . S I A L F A K G F A G Y F L P L I N .		

TM8		570	580	590	600
Transporters					
LeuT	336	G G T F L G F L W F F L L F F A G L T S S I A . I M . Q P M I A F L E			
DAT	402	A S T F W A L I F F M M L A T L G L D S S F G . G S . E A I I T A L S			
MhsT	305	L G P I V G I A F F I L L G A A A L S S A V S . L L . E V P V A Y F M			
Mhp1	295	S . I P M A I L F Q V F V L L A T W S T N P A A N L . L S P A Y T L C			
vSGLT	348	V . . G V K G V V F A A L A A A I V S S L A S . M L . N S T A T I F T			
BetP	449	G G Q I X G I I A X I L L G T F F I T S A D S . A S . T V X G T X S Q			
ApeT	268	L G N L G F L L I S I G A L F S I S S A M N A . T I Y G G A N V A Y S			

Figure S3. Structural based sequence alignment for the symporters in LeuT-fold transporters, for the TM3 (top panel) and the TM8 (bottom panel). The conserved motif in TM8 for the Na₂ site is highlighted in the red box. The sequence alignment is performed with the MultiSeq module of VMD and using Stamp Structural Alignment.

References

1. Kazmier, K., Sharma, S., Islam, S. M., Roux, B. & Mchaourab, H. S. Conformational cycle and ion-coupling mechanism of the Na⁺/hydantoin transporter Mhp1. *Proc. Natl. Acad. Sci. USA* **111**, 14752–14757 (2014).