Supporting Information

Gu *et al.* 10.1073/pnas.0812299106

SI Text

How does the direction of force affect the selection of pathways? In the steered MD simulations, the force direction was perpendicular to the membrane (along the *z* axis) as an initial plausible direction to drive substrate translocation. The underlying assumption in steered MD simulations is that upon adopting a soft deformation (via AFM pulling with a soft spring; see *Methods*), the substrate can adjust its conformation as well as orientation, and eventually find its energetically favorable pathway. Both pathways P1 and P2 indeed followed a convoluted pattern showing that the aspartate sampled radial displacements, as well, while moving along the z direction. However, the overall direction of P1 was observed to deviate by about 20° degrees from the *z* axis; whereas P2 was almost parallel to the *z* axis. We repeated the second phase of our simulations by tilting the direction of the pulling force toward P1. This time, the substrates in all three subunits selected P1 and the corresponding pulling forces and work done were significantly reduced (Fig. S2). *D–F* therein compare the force, position and work profiles obtained for the second phase by adopting this intrinsically preferred pulling direction, as opposed to that exerted along the vertical *z* axis (displayed in *A–C*). These additional simulations indicate that the pathway P1 is preferred by substrate in the context of the local transporter structure and energetics (Movie S1).

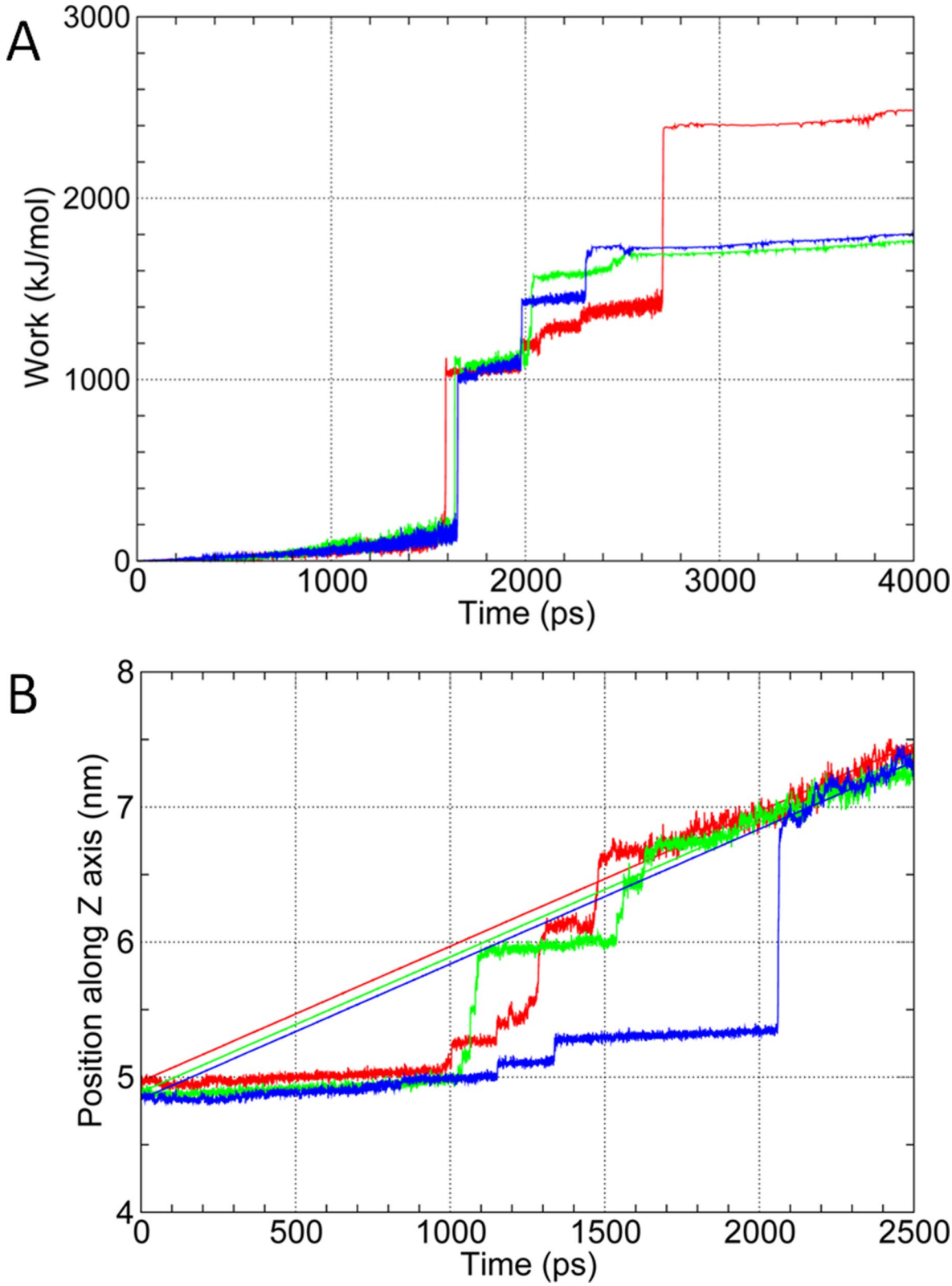


Fig. S1. Substrate profiles of the 3 subunits. Work (*A*) and displacement (*B*) profiles of substrates in the 3 subunits during the steered MD simulations (corresponding to Fig. 2*A* and *B*). Results for the subunits A, B, and C are colored in red, green, and blue, respectively. (*B*) The result from the steered MD run of the second phase. The $t = 1.6$ ns snapshot of the preceding run is used as the initial structure, after removal of the exerted force, and energy minimization.

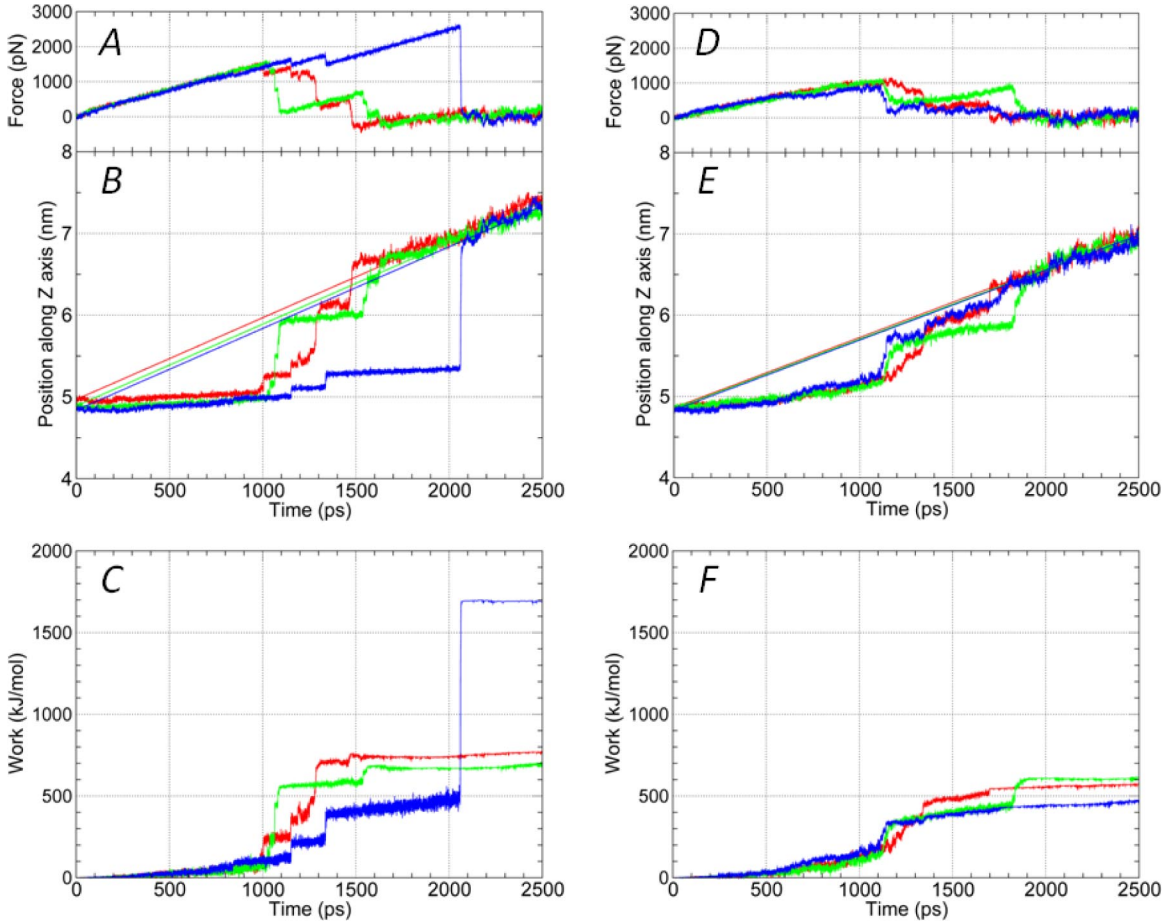


Fig. S2. Force, displacement, and work profiles of substrates in the three subunits during the second phase of steered MD simulations. Results for the subunits A, B, and C are colored in red, green, and blue, respectively. In panels *A–C* the pulling forces were along the *z* axis. Substrates in subunits A (red) and B (green) permeated through P1, while the substrate in subunit C (blue) went through P2. In panels *D–F*, the pulling forces were tilted toward P1 and substrates in all three subunits translocated through P1. The required pulling forces and work done were reduced.

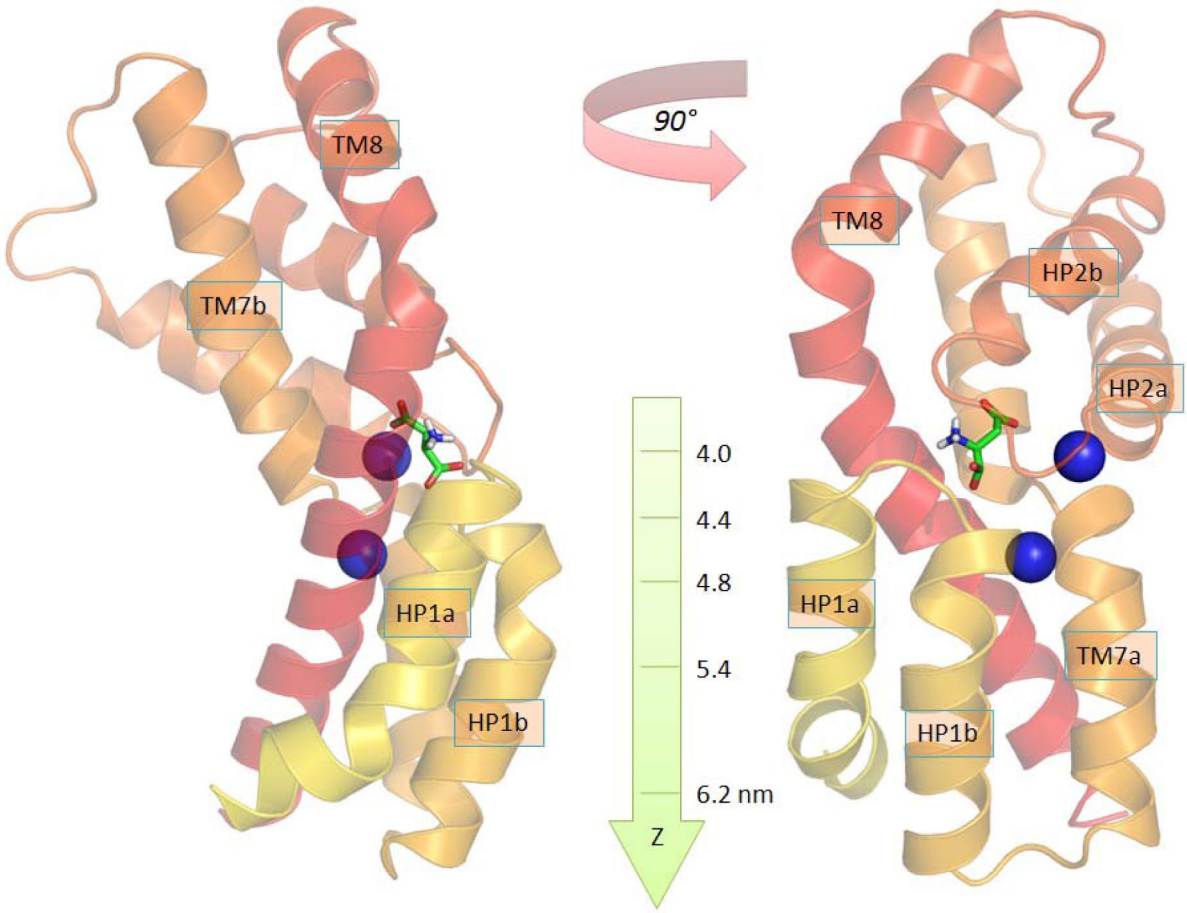
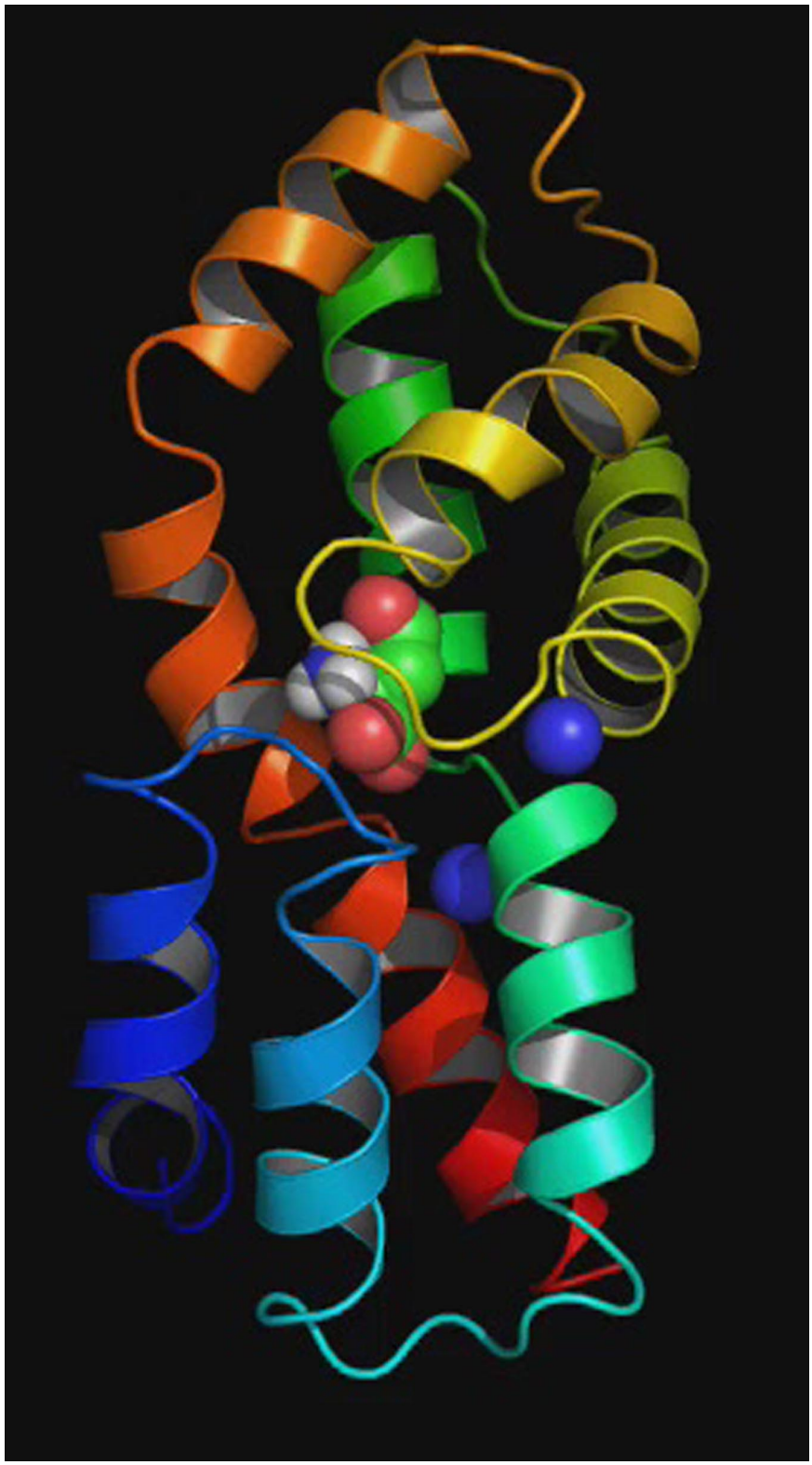


Fig. S3. Direction of the constant-velocity pulling force exerted via an elastic spring on the substrate in steered MD simulations. The same representation as in Fig. 1*D* is used. The figure on the right is rotated by 90° with respect to that on the left. Direction of the pulling force, parallel to the *z* axis, is shown as a gradient arrow. HP2a, HP2b, and TM7a are not shown in the left figure for clarity.



Movie S1. Translocation of aspartate along a putative permeation pathway observed in the steered MD simulations. Only the core domain of a subunit is shown for clarity. Aspartate and sodium ions are displayed as spheres.

Movie S1 (MOV)

Table S1. Sequences of glutamate transporters from different organisms and their sequence identities with respect to the archaeal transporter Glt_{Ph}

Accession no.	Protein Name	Organism	Identity, %
O59010	Glt_{Ph}	*Pyrococcus horikoshii* (Archaea)	100
Q9V0E7	Sodium dicarboxylate symporter family protein	*Pyrococcus abyssi* (Archaea)	91
Q8U0W2	Glutamate/aspartate transport protein	*Pyrococcus furiosus* (Archaea)	83
Q5JID0	Proton/glutamate symporter, SDF family	*Pyrococcus kodakaraensis* (Archaea)	75
A8TH68	Sodium:dicarboxylate symporter	*Methanococcus voltae* (Archaea)	59
Q9Y8Q0	Proton glutamate symport protein	*Aeropyrum pernix* (Archaea)	59
A3DPQ3	Sodium:dicarboxylate symporter	*Staphylothermus marinus* (Archaea)	58
P43003	EAAT1	*Homo Sapiens* (Human)	28
P24942	EAAT1	*Rattus norvegicus* (Rat)	27
P43005	EAAT3	*Homo Sapiens* (Human)	28
P51907	EAAT3	*Rattus norvegicus* (Rat)	29
P43004	EAAT2	*Homo Sapiens* (Human)	27
P31596	EAAT2	*Rattus norvegicus* (Rat)	26

All data are retrieved from UniProt (www.uniprot.org).