**Supporting Information**

**Molecular Mechanism Underlying ABC Exporter Gating:**

**A Computational Study**

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**Figure S1.** The coarse-grained (CG) systems containing the MsbA exporter (grey spheres), POPC lipid bilayer (orange, salmon and light grey spheres), and watermolecules (dark blue spheres), a total of 58379 MARTINI particles are used.

# Several mechanistic models shown in Figure S2-S5, respectively, are compared with our model presented in Figure S6 (see Figure 4 in the main text). In Figure S2, a mechanistic model

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# Figure S2. A mechanistic model for the MsbA ABC exporter [1]

# of the alternating access with a twist was proposed for the gating movements in the MsbA ABC exporter, based on two highly twisted inward facing (IF) conformations, IF1 (PDB: 3B5X ) and IF2 (PDB: 3B5W), and an outward facing (OF) conformation(PDB: 3B60) [1]. This model suggested that the twisting motion of the NBDs drives the closing of the internal gate and the opening of the external gate, resulting in the transition. Also using these three structures as references, Moradi and Tajkhorshid proposed a doorknob mechanism for the conformational state transition in the MsbA ABC exporter from their nonequilibrium-driven molecular dynamics (MD) simulations [2]. In this doorknob mechasim, the NBDs acts as a “doorknob” that needs to be twisted before the internal gate can be opened, in agreement with the above X-ray crystallography experiments. However, this mechanistic view was challenged by recent strucral experiments [3-5]. In particular, the potential of mean force (PMF) obtained in the simulations [2] was unable to capture the transition, as discussed in the main text. Furthermore, careful examination shows that the 3BX5 structure represents a state, in which the internal and external gate are both open. This is contradictory to the unidirectionality (i.e., from intracellular to extracellular) of transporting substrates by an exporter[3].

# Recently, a consensus model was proposed [4], in which the NBD motion drives the closing

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# Figure S3 A consensus model for ABC exporters [4]

# of the internal gate and the opening of the external gate, as displayed in Fig. S3. However, this mechanistic model is not consistent with the structure of an ABC exporter, whose transmember domains (TMDs) are characterized by the domain-swapped arrangement as discussed in the main text.

# Figures S4-S5 show mechanistic models, which were published most recently for a bacterial ABC exporter, Atm1[5], and the peptidas-containing ABC transporter 1(PCAT-1) [6],

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# Figure S4. A mechanistic model for a bacterial ABC exporter, Atm1[5].

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**Figure S5.** A simple mechanistic model for the peptidas-containing

ABC transporter 1(PCAT-1)[6]

# respectively. These two models are basically similar to that shown in Figure S3. As mentioned above, these mechanical models misrepresent the key structural feature of the TMDs, and therefore were unable to elucidate the gating mechanism of the TMDs in response to the NBD motion.

# As comparision, the mechanical model from Figure 3 in the main text is given in Figure S6. The model captures the crucial feature of the domain-swapped arrangement, which allows the internal and external gate to be arranged approximately perpendicular to each other. Thus, the NBD motion drives the two gates to move in a highly cooperative manner, as dicussed in the main text.

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**Figure S6**. A mechanical model proposed for the MsbA exporter based on the coarse-grained

MD (CG-MD) simulations, see detailed discussion in the main text.

# References

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