Supplementary Information

Drug export and allosteric coupling in a multidrug transporter revealed by molecular simulations

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Supplementary Figures S1-S6, Supplementary Tables S1-S2, Supplementary Methods, and Supplementary References.
Supplementary Figures

Figure S1: CG contact energy of different types of interfaces. The ensemble-averaged energy based on the samples derived from the “control” (see the main text for details) is shown, and the magnitude is expressed by colors. Energy is in the unit of $4.17k_B T$, where $k_B$ is the Boltzmann constant ($k_B$ was set to 1 in the current unit of temperature) and $T$ is the simulation temperature.
Figure S2: Phase diagrams with different parameters. $r_{\text{diff}}$ is the ratio of coefficient constants between the “com3” and the “spec” contacts. $f_{\text{inter}}$ characterizes the strength of overall inter-protomer interaction: $f_{\text{inter}} = 1.0$ means the average depth of each contact energy on the interface is the same to that of intra-protomer interaction. (a) Phase diagrams with the contour plots representing the probability of AAA and AAE states, respectively. (b) Phase diagrams with the contour plots representing the probability of BEA state. “TS” means the transition state, in which one or more protomers stay in none of the three native states. We saw in the phase diagrams that the region of the BEA state became wider or narrower, and even disappeared in some cases, but very interestingly, it was always true that, if the BEA state was found, the right side of the BEA state had high probability for the AAA state.
Figure S3: Construction of an internal coordinating system for tracking drug export. The mass center of three groups of atoms in the protomer I, colored respectively pink, dark-red (represents the “exit”), and red (“cleft”), are used to construct the X-, Y-, and Z-axis. The yellow numbers show the distance between groups measured along X- and Z-axis. For clarity, the “capping” residues and PN2 sub-domain of protomer I are removed, except for the beads involved in forming the three groups.
Figure S4: Construction of the porter domain assembled with capping residues. (a) TolC docking domain with cutted capping residues. (b) Porter domain. (c) Porter domain assembled with capping residues. (d) Coarse-grained porter domain and capping residues.
Figure S5: Schematic energy functions for the native contacts of the protomer-protomer interface. (a) A Lennard-Jones-type (LJ-type) potential function for the “spec” contact, i.e., the residue pairs that are in contact only in one interface. The reference distance is indicated by $r_1$, and the well-depth $\epsilon_{\text{spec}}$. (b) A two-well energy function for the “com2” contact, i.e., the residue pairs that are in contact only in two interfaces. Each well corresponds to a LJ-type potential with reference distance $r_v$ ($V=1, 2$) and well-depth $\epsilon_{\text{com2}}$. The target function is constructed by merging the two LJ-type potentials through Equation (S11). (c) A three-well energy function for the “com3” contact, i.e., the residue pairs that are in contact in all the three interfaces. Three LJ-type potentials, each with reference distance $r_v$ ($V=1, 2, 3$) and well-depth $\epsilon_{\text{com3}}$, are calculated first and are merged to construct the target function through Equation (S11). (See Supplementary Methods for details).
Figure S6: Phase diagrams of the statistical model. (a) The calculation started with a model that the free energy of the interface, $\Delta U_{ij}$, was approximated by the average energy of interface derived from the coarse-grained simulation, where $i,j=A$ (access), B (binding), and E (extrusion). (b) The parameters were then calibrated to generate a similar phase diagram to that obtained from the simulation. We see that by using a proper set of parameters, the statistical model could capture the essential properties of the phase diagram obtained from the simulation of the structural model (Fig. 2a). All the phases in Fig. 2a were observed at the similar location here; especially the “AAA” phase is on the right-hand of “BEA” phase, which is the major finding of the thermodynamic study in this work. However, there are still some differences between the two phase diagrams, e.g., the “AAB” phase shown here was not observed in Fig. 2a. Such difference is possibly caused by the higher ordered correlation among degrees of freedom, which was missed in the simple statistical model.
**Supplementary Tables**

**Table S1. Comparison of C$_\alpha$-RMSD (Å)**

|          | Access | Binding | Extrusion |
|----------|--------|---------|-----------|
| Access   | 2.199  | 2.769   | 3.246     |
| Binding  | 2.199  |         | 3.246     |
| Extrusion| 2.769  | 3.246   |           |
| 1IWG     | 1.231  | 2.172   | 3.037     |

Results on the last row are based on the comparison between the porter domain of PDB structure 2DRD and that of 1IWG; all other results are solely based on the structure 2DRD. C$_\alpha$-RMSD: The root-mean square-deviation calculated based on C$_\alpha$ atoms.
Table S2. All-atom energy (kcal/mol) and CG constants for inter-protomer interaction

| # Contact | Com3  | Com2  | Spec  |
|-----------|-------|-------|-------|
| All-atom average energy per contact | -1.170 | -0.748 | -0.420 |
| CG constant ($\epsilon$): |       |       |       |
| $r_{\text{diff}}=30$, $f_{\text{inter}}=0.5$ | 0.300  | 0.137  | 0.010  |
| $r_{\text{diff}}=30$, $f_{\text{inter}}=0.7$ | 0.420  | 0.192  | 0.014  |
| $r_{\text{diff}}=30$, $f_{\text{inter}}=1.0$ | 0.600  | 0.274  | 0.020  |
| $r_{\text{diff}}=10$, $f_{\text{inter}}=0.5$ | 0.281  | 0.139  | 0.028  |
| $r_{\text{diff}}=10$, $f_{\text{inter}}=0.7$ | 0.393  | 0.194  | 0.039  |
| $r_{\text{diff}}=10$, $f_{\text{inter}}=1.0$ | 0.561  | 0.277  | 0.056  |

$r_{\text{diff}}$ is the ratio of the largest CG constant to the smallest CG constant and $f_{\text{inter}}$ is the average inter-protomer CG contact energy relative to the average intra-protomer CG contact energy.
Supplementary Methods

Reference structures

The crystal structure with the PDB entry 2DRD\textsuperscript{14} was adopted as the reference for models of both the protein, AcrB, and the drug, minocycline. In the present work, three constructs were used: Construct 1 is the single protomer of the AcrB porter domain that contains L30-A183, G271-F332, P565-Q726, and E810-A873, construct 2 is the trimer complex of the AcrB porter domain that contains the same residues as 1, and construct 3 is the trimer complex of the AcrB porter domain plus some capping residues and the minocycline. The capping residues, M184-W187, A753-M781, and A215'-A236', belonging to the TolC docking domain participate to the exit tunnel, where the prime means the residue belonging to the neighboring protomer (See Supplementary Fig. S4).

For convenience, in the trimer, we named the protomers initially in the binding (B), extrusion (E), and access (A) states as ‘I’, ‘II’ and ‘III’, respectively (See Fig. 1d), and called the trimer state by the three characters, such as the BEA state meaning that the protomers I, II, and III take B, E, and A states, respectively. The symmetric structure with the PDB entry 1IWG\textsuperscript{13} was also considered merely for a comparison with 2DRD.

Coarse-grained model

The protein was simplified by using one-bead, placed on the position of C\textalpha atom, per one amino acid. For the constructs 1 and 2, a group of beads belonging to the same protomer were considered as one continuous chain. For the construct 3, in addition to the chain for the porter domain, the capping residues were grouped into two independent chains: a chain that includes M184-W187 and A757-M781, and another chain that includes A215'-A236'. In the construct 3, the minocycline (drug) in the protomer I was represented by six beads connected by pseudo-bonds between neighbors and placed at the positions of the carbon atoms (in a bonded order) C21, C20, C17, C8, C13, and C71.

The energy function is structure-based and thus was built based on the reference structure. For the three constructs, we have the energy function in the following forms:

\[ V_{\text{constr } 1} = V_{\text{porter},I}, \]  
\[ V_{\text{constr } 2} = \sum_{J=I,II,III} \left( V_{\text{porter},J} + V_{\text{inter},JJ+1} \right). \]
\[ V_{\text{constr} 3} = \sum_{J=I, II, III} \left( V_{\text{porter}+\text{cap}, J} + V_{\text{inter}, JJ+1} \right) + V_{\text{drug}} + V_{\text{AcrB-drug}}. \]  

(S3)

Here, \( V_{\text{porter}, J} \) is the intra-protomer potential for the porter domain in the protomer \( J \), \( V_{\text{inter}, JJ+1} \) is the inter-protomer interaction potential between the protomers \( J \) and \( J+1 \) (The protomer IV is the same as the protomer I), \( V_{\text{porter}+\text{cap}, J} \) is basically similar to \( V_{\text{porter}, J} \), but includes some additional terms for the capping residues, \( V_{\text{drug}} \) is the potential for the minocycline, and \( V_{\text{AcrB-drug}} \) is the interaction potential between the porter domain of the AcrB and the minocycline. In the following, we describe each of these terms in details.

**Intra-protomer interaction potential of the porter domain**

In the constructs 1 and 2, for each protomer \( J \) (\( J=I, II, \) or III) of the porter domain, we defined the potential energy, \( V_{\text{porter}, J} \) by the multiple-basin model\(^2\). Namely, \( V_{\text{porter}, J} \) is the lowest energy solution of the following secular equation,

\[
\begin{vmatrix}
V(R \mid R_A) + \Delta V_A - V_{\text{porter}, J} & \Delta & \Delta \\
\Delta & V(R \mid R_B) + \Delta V_B - V_{\text{porter}, J} & \Delta \\
\Delta & \Delta & V(R \mid R_E) + \Delta V_E - V_{\text{porter}, J}
\end{vmatrix} = 0
\]  

(S4)

Here, \( V(R \mid R_v) \) (\( v=A, B, \) or E) is the off-lattice Go potential energy defined below, where \( R \) and \( R_v \) are the coordinates for the protomer \( J \) and the reference \( v \), respectively. The parameter \( \Delta V_v \) (\( V=A, B, \) or E) characterizes the relative stability of the state \( v \) (here we set \( \Delta V_A = 0 \) and the other two can be changed freely), and \( \Delta \) is the coupling constant between the corresponding two states (for simplicity, we considered an identical \( \Delta \) for all pairs of states). Because of the symmetry of the matrix, Equation (S4) has always three real-value solutions. Among the three roots of Equation (S4), always the smallest one was chosen as the multiple-basin model. Because it always has the three real-roots, the energy is continuous with the coordinates of residues.

The potential energy \( V(R \mid R_v) \) (\( v=A, B, \) or E) is based on the off-lattice Go model developed by Clementi, Nymeyer, and Onuchic\(^2\) and consists of local and non-local interactions:

\[
V(R \mid R_v) = V_{\text{local}}(R \mid R_v) + V_{\text{nonlocal}}(R \mid R_v).
\]  

(S5)

Specifically,
\[ V_{\text{local}}(R \mid R_\nu) = \sum_{\text{bonds}} K_b (b_i - b_{ij})^2 + \sum_{\text{angles}} K_\phi (\theta_i - \theta_{ij})^2 + \sum_{\text{dihedral}} \{ K^{(1)}_\phi [1 - \cos (\phi_i - \phi_{ij})] + K^{(3)}_\phi [1 - \cos 3(\phi_i - \phi_{ij})] \}, \] (S6)

where \( b_i, \theta_i, \) and \( \phi_i \) are the \( i \)-th bond length, bond angle, and dihedral angle, respectively, and the subscript \( \nu \) means the variable of reference. Generally, the coefficient constants in Equation (S6) were set as following: \( K_b=100.0, K_\phi=20.0, \) and \( K^{(1)}_\phi=2K^{(3)}_\phi=1.0. \) For some portions of protein where strong local strain energies were involved, the interactions were weakened and site-specific constants were applied as in the previous work\(^{25} \). In the case of the triple-basin model, the maximum pairwise strain energy was considered. For example, for the bond angle \( \theta_i \), we first calculated \( E_{\theta_i} = \max\{ K_\theta (\theta_{\mu,i} - \theta_{ij})^2 \} \), where \( \mu \) and \( \nu \) are different states. Then the site-specific constant was determined by \( K_{\theta,i} = \min\{ K_\theta, K_\theta \epsilon_\theta / E_{\theta,i} \}, \) where the threshold \( \epsilon_\theta=1.0. \)

Similarly, we calculated site-specific constants for the bond length \( (K_{b,i}) \) and the dihedral angle \( (K^{(1)}_{\phi,i} = 2K^{(3)}_{\phi,i}) \), in which the thresholds were set as \( \epsilon_b=10.0 \) and \( \epsilon_\phi=0.5, \) respectively.

The energy function for the non-local interaction was defined by

\[ V_{\text{nonlocal}}(R \mid R_\nu) = V_{\text{native-attr}}(R \mid R_\nu) + V_{\text{repul}}(R \mid R_\nu) \]

\[ = \epsilon_1 \sum_{i < j < 3} \min \left\{ 1.5 \left( \frac{r_{ij}}{r_{ij}} \right)^{12} - 6 \left( \frac{r_{ij}}{r_{ij}} \right)^{10} + 1 \right\}, \]

\[ + \epsilon_2 \sum_{i < j < 3} \max \left\{ 0.5 \left( \frac{r_{ij}}{r_{ij}} \right)^{12} - 6 \left( \frac{r_{ij}}{r_{ij}} \right)^{10} \right\} + \epsilon_3 \sum_{i < j < 3} \left( \frac{C}{r_{ij}} \right)^{12}, \] (S7)

where \( r_{ij} \) is the distance between residue \( i \) and \( j \), and

\[ r_{ij} = \min \left\{ \epsilon_{i,j} \{ r_{ij} \} \right\} \] (S8)

is the minimal native distance between \( i \) and \( j \) among the reference structures \( R_\nu \). In the fully atomistic reference structure, a pair of residues \( i \) and \( j \) is defined as “native contact” when at least one non-hydrogen atom in residue \( i \) is within 6.5Å from a non-hydrogen atom of residue \( j \). Based on this definition, all residue pairs were classified into three types: Type 1 means residue pairs that make contacts in all references, type 2 means residue pairs that make contacts in some but not all of the references, and type 3 means those that do not make contact in any reference. The coefficient constants were set to: \( \epsilon_1=0.3, \epsilon_2=0.2, \) and \( C=4.0. \) In Equation (S7) the zero
energy is shifted to be at the bottom of the energy curve instead of infinite distance.

Inter-protomer interaction potential of the porter domain

We modeled the inter-protomer interaction energy, \( V_{\text{inter}, j/l+1} \), using the asymmetric AcrB structure, 2DRD, which has the three inter-protomer interfaces: For the pairs that are in contacts in all the three interfaces (the com3 set as introduced in main text), we used a triple-well pairwise potential where each minimum corresponds to the native distance in one interface and all the depths were identical (the precise definition given below. This is somewhat similar to \(^{40-41}\) (See Supplementary Fig. S5). In a similar way, the “com2” pairs (i.e., the pairs that are in contact only in two interfaces) were modeled by a double-well pairwise potential. The “spec” pairs (i.e., the pairs that are in contact only in one interface, \( V \)) were modeled by the Lennard-Jones-type potential,

\[
V(r_{ij} | r_{v,ij}) = 5\left(\frac{r_{v,ij}}{r_{ij}}\right)^{12} - 6\left(\frac{r_{v,ij}}{r_{ij}}\right)^{10}.
\]  

(S9)

where \( r_{ij} \) is the distance between particle \( i \) and \( j \) (\( i, j \) belonging to different protomers) and \( r_{v,ij} \) is the corresponding distance in the reference interface structure \( R_v \). Thus, the energy function becomes

\[
V_{\text{inter}, j/l+1} = \varepsilon_{\text{com3}} \sum_{i,j}^{\text{com3}} v_{ij}^{(3)} + \varepsilon_{\text{com2}} \sum_{i,j}^{\text{com2}} v_{ij}^{(2)} + \\
\varepsilon_{\text{spec}} \sum_{i,j}^{\text{spec}} v(r_{ij} | r_{v,ij}) + \varepsilon_2 \sum_{i,j}^{\text{type3}} \left(\frac{C}{r_{ij}}\right)^{12}.
\]  

(S10)

where, the constants \( \varepsilon_2=0.2 \) and \( C=4.0 \) are the same as those in the intra-protomer potential. The definition of “type 3” pairs is also the same as that for the intra-protomer interaction. The coefficient constants, \( \varepsilon_{\text{com3}}, \varepsilon_{\text{com2}}, \) and \( \varepsilon_{\text{spec}} \), were determined by a multiscale method (see below).

The triple-well and double-well potential energy, \( v_{ij}^{(3)} \) and \( v_{ij}^{(2)} \), were defined in the following procedure. In a similar way to the multiple-basin model, we defined all the structure-based energy surfaces by solving the following secular equation:
where \( n \) is the number of wells, \( n = 2 \) or 3. The coupling constant \( \Delta' \) was introduced to merely smooth the potential surface and hence was set to be small in the absolute value: \( \Delta' = -0.1 \) (see later section for the reason of choosing negative value in \( \Delta \)).

Some additional terms from the capping residues in the construct 3

In the construct 3, the energy function for each protomer is slightly different from that of the constructs 1 and 2 because of the capping residues. We note that the capping residues were introduced merely to make the exit “tunnel” better defined (The capping residues augment the “wall” of the exit tunnel). The details of these treatments are not essential at all. Here, for completeness, we describe it in details.

In the construct 3, the energy function for protomer \( J \) was separated into two parts:

\[
V_{\text{porter-cap}, J} = V_{\text{porter}, J} + V_{\text{cap}, J}.
\] (S12)

First, \( V_{\text{porter}, J} \) is the solution of a secular equation similar to that for constructs 1 and 2 (Equation (S4)) except that the energy terms, \( V(R \mid R_v) \) and \( V_{\text{porter}, J} \) were replaced by \( V'(R \mid R_v) \) and \( V_{\text{porter}, J}' \), \( (V=A, B, \text{ or } E) \), respectively. Here,

\[
V'(R \mid R_v) = V(R \mid R_v) + V_{\text{porter-cap}, J}(R \mid R_v).
\] (S13)

The last term of Equation (S13) considers the non-local interaction between the porter domain and the capping residues, which has the same functional form as that of the intra-porter domain and was multiplied by a factor 0.7. (The capping residues were introduced to merely form a natural “exit” tunnel for the drug export and so the porter-cap interaction was not essential in the current work. The factor used here was set somewhat arbitrarily.)

Second, in the construct 3, we considered the interaction within the capping residues, which were modeled as an independent multiple-basin system. The secular equation used for capping residues was:
\[
\begin{bmatrix}
V_{\text{cap}}(R | R_A) - V_{\text{cap}, j} & \Delta'' & \Delta'' \\
\Delta'' & V_{\text{cap}}(R | R_B) - V_{\text{cap}, j} & \Delta'' \\
\Delta'' & \Delta'' & V_{\text{cap}}(R | R_E) - V_{\text{cap}, j}
\end{bmatrix} = 0
\]

where the Go potential for the capping residues, \( V_{\text{cap}}(R | R_v) \) \((v=A, B, \text{or} E)\), has the same functional form as Equation (S5). Since the conformational change of capping residues is small, the absolute value of the coupling parameter, \( \Delta'' \) is not important. In this work we set \( \Delta'' = -18.0 \).

*The potential for the drug*

The minocycline, drug, was modeled as an essentially rigid linear chain by using quite large spring constants in bond-stretching, bond-angle changes, and the dihedral-angle changes. The energy function is simply

\[
V_{\text{drug}} = \sum_{\text{bonds}} K_{b,i} (b_{i,j} - b_{i,0})^2 + \sum_{\text{angles}} K_{\theta,i} (\theta_{i,j} - \theta_{i,0})^2 + \sum_{\text{dihedral}} K_{\phi,i} (\phi_{i,j} - \phi_{i,0})^2 ,
\]

where \( b_{i,j}, \theta_{i,j}, \text{and} \phi_{i,j} \) are the \( i \)-th bond length, bond angle, and dihedral angle of the drug, respectively, and the variables with subscript “0” mean the values of reference structure.

\( K_{b,i}=120.0 \), \( K_{\theta,i}=24.0 \), and \( K_{\phi,i}=24.0 \). The choice of these parameters does not matter, as far as they are sufficiently large.

*The interaction potential between AcrB and the drug*

Interactions between the drug and AcrB consist of two terms: 1) A generic repulsive term was applied for all the pairs between the drug beads and the AcrB amino acids and 2) the hydrophobic attraction similar to that used was applied between the drug beads and the hydrophobic residues in the binding pocket of AcrB, namely, A47, Y49, P50, L177, F178, Y275, I277, I278, A279, A286, L289, F610, A611, V612, F615, and I626.

Explicitly, the energy function is

\[
V_{\text{AcrB−drug}} = V_{\text{ex}} + V_{\text{HP}} .
\]

The excluded volume term is a simple repulsion defined as,
\[
V_{\text{ex}} = \varepsilon_2 \sum_{i,j \text{ are not in the same molecule}} \frac{C_i}{r_{ij}^{12}},
\]

where \(r_{ij}\) is the distance between the drug bead \(i\) and the protein bead \(j\), \(\varepsilon_2 = 0.2\), and \(C_i = 4.6\). The hydrophobic interaction between the drug and the binding pocket was modeled in the same way as before\(^7\) and was expressed as:

\[
V_{\text{HP}} = - \sum_{i \text{ in drug or hydrophobic residues}} \varepsilon_{\text{HP}} S_{\text{HP}}(\rho_i),
\]

where \(\varepsilon_{\text{HP}} = 0.58\). The “buriedness” of bead \(i\), \(S_{\text{HP}}(\rho_i)\), is defined by

\[
S_{\text{HP}}(\rho_i) = \begin{cases} 
1.0, & \rho_i \geq 1.0 \\
0.5(1 - c_{\text{linear}}) \left[ 1.0 + \cos \left( \frac{1 - \rho_i}{1 - \rho_{\text{min}}} \right) \right], & \rho_{\text{min}} < \rho_i < 1.0 \\
c_{\text{linear}} \rho_i, & \rho_i \leq \rho_{\text{min}} 
\end{cases}
\]

where \(c_{\text{linear}} = 0.2\) and \(\rho_{\text{min}} = 0.3\). \(\rho_i\) represents local atom density around bead \(i\) and is defined by

\[
\rho_i = \frac{\sum_{i,j \text{ not in the same molecule}} u_{\text{HP}}(i,j)}{n}
\]

where the maximum coordination number \(n = 3\). The degree of contact between bead \(i\) and \(j\) (\(i\) and \(j\) are either the hydrophobic residues or drug beads, but are not of the same type), \(u_{\text{HP}}(i,j)\), is defined by

\[
u_{\text{HP}}(i,j) = \begin{cases} 
1.0, & r_{ij} \leq d_{\text{min}} \\
0.5 \left[ 1.0 + \cos \left( \frac{r_{ij} - d_{\text{min}}}{d_{\text{max}} - d_{\text{min}}} \right) \right], & d_{\text{min}} < r_{ij} < d_{\text{max}} \\
0.0, & r_{ij} \geq d_{\text{max}}
\end{cases}
\]

where \(r_{ij}\) is the distance between bead \(i\) and \(j\), \(d_{\text{min}} = 6.0\), and \(d_{\text{max}} = 10.0\).

Setting free parameters in the CG model

The CG model has several parameters that crucially affect the results. Here, we describe how to treat them in the current study. These essential parameters can be classified into two categories, those for the intra-protomer energy and those for the inter-protomer interaction energy.

In the intra-protomer energy, the multiple-basin model contains two types of parameters, \(\Delta\) and \(\Delta V\) in Equation (S4), that crucially affect the dynamics and the thermodynamics of the protomer, respectively. Here, we explain how to treat them. In principle both the \(\Delta\) and \(\Delta V\), \((V = B\ or\ E)\) can be determined by experiments or by all-atom simulations, but, in practice, these
data are not available at the moment and so we need to adjust them empirically.

As in the main text, the coupling constant $\Delta_{ij}$ controls the barrier height between the states $i$ and $j$. Thus, this constant affects the rate between the two states. Without a prior knowledge, we set all $\Delta_{ij}$ equal (denoted as $\Delta$). The change in $\Delta$ basically changes the time-scale for all the transitions in the same way, but does not affect the thermodynamics or relative order of events.

In the case of the double-basin potential tested before, the sign of $\Delta$ did not matter because all the expressions depend only on the square of $\Delta$. However, in the triple-basin model, the solution contains the cubic form of $\Delta$, so that the sign of $\Delta$ matters. A priori, we did not know which is appropriate, but our preliminary study unambiguously indicated that use of a positive $\Delta$ makes it hard to observe the conformational change of a single protomer of the porter domain. On the other hand, a negative $\Delta$ generated frequent conformational changes much more easily. Thus, we decided to use a negative $\Delta$.

The second type of essential parameters for the intra-protomer energy is the energy depths of the three basins, $\Delta V_B$, $\Delta V_E$, and $\Delta V_A$, which controls the relative stabilities. One of the three parameters can be set as the reference (here $\Delta V_A=0$), so that the remaining two parameters ($\Delta V_B$, $\Delta V_E$) are essential.

We decided $\Delta$ and $(\Delta V_B, \Delta V_E)$ in the following way. First, beginning with a very small $|\Delta|$ and $\Delta V_B=\Delta V_E=0.0$, we performed simulation on a single protomer of the porter domain. Because of the high-energy barriers, conformational change was difficulty to observe in this situation. Then, by gradually increasing $|\Delta|$, the conformational change occurred, and the process continued until all possible transitions between states were obtained within a reasonable simulation time. Too large value of $|\Delta|$ makes the coupling too strong, which, in the end, leads to coalescence of three basins into one. This is apparently not appropriate. We thus chose a minimal $|\Delta|$ by which all the transitions could be seen within the feasible time. Next, we tuned the $\Delta V_B$ and $\Delta V_E$ in an iterative way, until the population of the three states became approximately equal. The resultant parameters, $\Delta=-188$, $\Delta V_B=-8.6$, and $\Delta V_E=-2.5$, were denoted as “control” and were used as reference for other studies. Then, in the trimer complex studies, we scanned the $(\Delta V_B, \Delta V_E)$ plane relative to the control set.

Next, we describe the parameter setting for the inter-protomer interaction potential where the essential parameters are the strengths, i.e., the depths of the wells for these inter-protomer interactions, $\varepsilon_{\text{com3}}$, $\varepsilon_{\text{com2}}$, and $\varepsilon_{\text{spec}}$. To infer the nature of these interactions, we first estimated the residue-residue pairwise energies of the corresponding pairs at the asymmetric structure using the all-atom energy function, AMBER (version 10) ff99sb force field$^{42,43}$ with a GB/SA implicit
solvent model. The whole molecule of AcrB (without drug) was adopted as input, in which the missing residues were rebuilt as loop by using MODELLER. The 300-step steepest descent followed by 300-step conjugate gradient energy minimization procedure was performed. Then, the calculated total energy was decomposed into residue pairwise energies and they were averaged in each of the contact sets (i.e., com3, com2, and spec. See Supplementary Table S2). Interestingly, we found significant difference in the average contact energies among the three classes: The contact energy in the com3 set was on average stronger, whereas the contact energy in the spec set was on average weaker. Since this was quite a noticeable feature, we decided to include it in the current CG modeling.

Based on the all-atom contact energy estimate, we took a linear mapping between the all-atom contact energies and the CG contact energies. Thus, there are two parameters that define the linear mapping. As the two free parameters, we used the ratio of the strongest inter-protomer contact to the weakest inter-protomer contact \( r_{\text{diff}} \) and the average inter-protomer CG contact energy \( f_{\text{inter}} \) relative to the average intra-protomer CG energy. Since we did not know what are the best values of them \( a \ priori \), we repeated simulation in some different choices of the parameters \( r_{\text{diff}} \) and \( f_{\text{inter}} \) (see Supplementary Table S2 and Supplementary Fig. S2).

Quantitative results, of course, depended on the parameters, but we focused on the qualitative results that were robust over different parameter sets. In the main text, we used \( r_{\text{diff}} = 30 \) and \( f_{\text{inter}} = 0.7 \) as the representative case. Results for some other parameter sets are presented in Supplementary Fig. S2. Most importantly, as in the main text, we saw in the phase diagrams that the BEA phase appeared in a reasonable place in the diagram, and that the area of the BEA phase became larger or smaller, and even disappeared in some parameter cases. But, very interestingly, it was always true that, if the BEA state was found, the right side of the BEA state had high probability for the AAA state. This was the most robust result and thus is the main result here.

**Unit system**

For length, the angstrom (\(10^{-10} \text{m}\)) unit was used. The energy unit was not calibrated to map it with the real values. In terms of \( k_B T \), where the \( k_B \) is the Boltzmann constant (\( k_B \) was set to 1 in the current unit of temperature) and the \( T \) is the simulation temperature, the unit of the energy corresponds to \( \sim 4.17 k_B T \). 

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Simulation protocols

All the CG simulations were performed by the CafeMol package (http://www.cafemol.org). We conducted three sets of simulations.

1) In the stage of setting the parameters, $\Delta$, $\Delta V_B$, and $\Delta V_E$, we performed single-protomer simulations (using construct 1). The 20 independent trajectories were run for each trial of $\Delta$, $\Delta V_B$, and $\Delta V_E$, each with $1.2 \times 10^6$ steps for the equilibration and $1.9 \times 10^7$ steps for the production, and structures were saved every 2000 steps.

2) Studying the thermodynamics of the trimer, we performed simulations on the trimer complex of the AcrB porter domain (construct 2). Initially, $\Delta$, $\Delta V_B$, and $\Delta V_E$ of each protomer were set to the “control” values and the interaction strengths (see Equation (S10)) between protomers were set to $\varepsilon_{\text{com3}}=0.3$, $\varepsilon_{\text{com2}}=0.03$, and $\varepsilon_{\text{spec}}=0.03$, which are rather weak. This is because these weak interactions between protomers made protomers quite independent each other and thus made the sampling over all possible trimer conformations possible. Using this parameter set, we performed 100 independent trajectories, with each having $2.0 \times 10^6$ steps for the equilibration followed by $1.9 \times 10^7$ steps for the production, and structures were saved every 2000 steps. This set of simulations provided sufficient conformational samples for all the possible trimer states, i.e., $3 \times 3 \times 3 = 27$ states.

Then, for drawing phase diagrams, we used the standard histogram re-weighting method for obtaining thermodynamic results in the 2-dimensional parameter space ($\delta B$, $\delta E$). Here $\delta B=\Delta V_B-\Delta V_{c,B}$ and $\delta E=\Delta V_E-\Delta V_{c,E}$, respectively, where the subscript “c” means the “control”. Namely, the samples generated by the above simulations were re-weighted to obtain the statistics for other cases with more reasonable inter-protomer interaction (i.e., the constants were set to be those derived from the all-atom energy calculation) and with different $\Delta V_B$ and $\Delta V_E$ from the “control” value. For the purpose, the standard histogram re-weighting method was employed:

$$
\langle A \rangle = \frac{\langle Ae^{-\beta E} \rangle}{\langle e^{-\beta E} \rangle},
$$

where $A$ is the quantity of interest, $\beta$ is the inverse product of Boltzmann’s constant $k_B$ and temperature $T$, $\Delta E=E-E_0$ is the energy difference between the energy $E_0$ used for sampling and the energy $E$ of the target parameter case, and $\langle \cdot \rangle_0$ and $\langle \cdot \rangle$ mean the ensemble averages over the energy functions $E_0$ and $E$, respectively.

3) In the simulation of the drug export dynamics (construct 3), 10 trajectories were run for each type of “driving force” (see the main text). Each trajectory has $2.0 \times 10^5$ steps before the
switching of energy landscape and $6.0 \times 10^6$ steps afterwards, with structures saved every 1000 steps.

As was written in the main text, we set the initial state as the protomer I, II, and III being in the B, E, and A states, respectively. For the protomer I, the drug was explicitly included and thus the unbound parameter $\delta_B = 1.0$ was used, whereas for other protomers, we did not include the explicit drug and thus assumed the bound (optimized) parameter $\delta_B = -0.5$ that effectively includes drug-bound energy in the B state. In all the three types, i.e., “B $\rightarrow$ E”, “E $\rightarrow$ A”, and “A $\rightarrow$ B” types, simulations before the switching were identical. In the “B $\rightarrow$ E” type simulation, the $\Delta V_B$ of the protomer I was suddenly decreased by 10 at the time of switching. Similarly, in the “E $\rightarrow$ A” type simulation, the $\Delta V_A$ of the protomer II was decreased by 10 at the time of the switching. In the “A $\rightarrow$ B” type simulation, the $\Delta V_B$ of the protomer III was decreased by 10 at the time of the switching.

All the CG simulations were carried out in the constant temperature ensemble with Berendsen’s thermostat at the temperature $T=0.24$. The mass of each CG particle was set to 10. The Verlet algorithm was employed for the numerical integration of Newton’s equation, in which the time step was set to be 0.2.

**Trajectory analysis**

**Q-score**

The state of a protomer was quantitatively measured by the fraction of formation of certain native contacts, the so-called Q-score (see Fig. 1e). Namely, to monitor the closeness to, say, the E state, we used a set of residue pairs that are in contact in the E state, but not in contact in the B or the A state. These “E-specific” contacts monitor the closeness to the E state. The expression of Q is defined as

$$Q_v = \frac{\sum_{i,j} q(r_{ij}, r_{v,ij})}{N_v},$$

(S23)

where $q(r_1, r_2) = 1$ if $r_1 \leq 1.2r_2$, or $=0$ otherwise, $r_{ij}$ is the distance between particle $i$ and $j$ ($r_{v,ij}$ is the corresponding C$_\alpha$-C$_\alpha$ distance in the reference structure $V$), $\Omega_v$ is a subset of the “spec”
contacts of the state \( V \) in which the members satisfying \( q(r_{\mu,ij},r_{v,ij})=1(\mu \neq V) \) are removed,

\( N_v \) is the size of \( Q_v \) and \( \mu, V=“A”, “B”, or “E”. \)

In this work, a protomer is identified to reach the state \( V \) when \( Q_v \) is dominant and becomes larger than 0.5.

**Measurement of the conformational change and drug export**

The trajectories in the drug export dynamic simulations were monitored by the two quantities: the conformational change of protein and the drug export (see Fig. 3 a-c). The first was defined by

\[
x = Q_{E,I} + Q_{A,II} + Q_{B,III}
\]

where \( Q_{v,j} \) is the \( Q \)-score of state \( V \) for the protomer \( J \). The second quantity was calculated by

\[
y = Z_i - X_i
\]

where \( Z_i \) and \( X_i \) are the internal coordinates of the mass center of drug with respect to the protomer I (see Supplementary Fig. S3 for the definition of the internal coordinating system). It shows that at the “exit” and “cleft”, the values of \( y \) are about 28Å and -20Å, respectively, which were adopted as two thresholds for identifying the events of drug export.

**Ternary plot**

The ternary plot is a convenient way to present the conformational change of a system with three states. In this work, the three-dimensional \( Q \)-score (see above) was projected onto a two-dimensional plane by following equations,

\[
x_j = \frac{0.5Q_{B,j} + Q_{A,j}}{Q_{B,j} + Q_{E,j} + Q_{A,j}},
\]

\[
y_j = \frac{\sqrt{3}Q_{B,j} / 2}{Q_{B,j} + Q_{E,j} + Q_{A,j}},
\]

where \( Q_{v,j} \) is the \( Q \)-score of protomer \( J \) with respect to state \( V, V=A, B, \) or \( E \) and \( J=I, II, \) or \( III. \)

After the mapping, the trajectory is restricted within a triangle, whose vertices located at (0.5,\( \sqrt{3}/2 \)), (0,0), and (0,1) represent “binding”, “extrusion”, and “access” states, respectively (see Fig. 3d-f).
Supplementary References

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