Supplementary information S2 (box) | An idealized kinetic model for ABC transporters

An idealized, and highly simplified, two-state kinetic model for ABC transporters may be used to illustrate general mechanistic features of the transport cycle (scheme S1). For the purposes of this analysis, the transporter is assumed to adopt two distinct conformations, designated outward (E_o) and inward (E_i) facing, that are stabilized by ATP (T) and ADP (D), respectively. To evaluate the rate of translocation of substrate from the outside pool (S_o) to the inside pool (S_i), the following simplifying assumptions are imposed on the kinetic model:

- ATP binds exclusively to the outward-facing conformation in states E_oT and E_oTS_o.
- ADP binds exclusively to the inward-facing conformation in states E_iD and E_iDS_i.
- ATP hydrolysis drives the conversion from outward- to inward-facing states, with rate constants k_T,S and k_T in the liganded and unliganded states, respectively. For this exercise, no ‘slippage’ is assumed; i.e. ATP hydrolysis is completely coupled to the conformational change. No assumption is made concerning the ATP stoichiometry.
- The exchange of ADP is associated with conversion from the inward- to outward-facing states, with pseudo first-order rate constants k_x,S and k_x in the liganded and unliganded states, respectively.
- Substrate binding steps are at equilibrium, with dissociation constants K_i and K_o for binding to the inward- and outward-facing states, respectively, with the slow kinetic steps corresponding to interconversion of inward- and outward-facing conformations.
While these assumptions are too restrictive to describe the observed complexities of actual ABC transporters, this model does serve as a useful starting point to address two important mechanistic features of ABC transporters: (i) the relationship between importers and exporters and (ii) how efficient coupling between substrate translocation and ATP hydrolysis can be achieved.

Using the rapid equilibrium, steady-state approximation\(^1\), the rate of substrate translocation across the membrane from the outside to the inside of the cell may be evaluated from the differences between the rates of import and export:

\[
\frac{d(S_i)}{dt} = k_{T,S}(E_o TS_o) - k_{i,S}(E_i DS_i)
\]

\[
= k_{T,S}k_x \left( \frac{S_o}{K_o} - k_{i,S} \frac{S_i}{K_i} \right)
\]

\[
(k_T + k_x) + (k_{T,S} + k_x) \frac{S_o}{K_o} + (k_{i,S} + k_T) \frac{S_i}{K_i} + (k_{T,S} + k_{i,S}) \frac{S_o}{K_o} \frac{S_i}{K_i} E_T
\]

The overall rate of ATP hydrolysis is given by:

\[
- \frac{d(ATP)}{dt} = k_{T,S}(E_o TS_o) + k_T(E_o T)
\]

\[
= k_{T,S}k_x \left( \frac{S_o}{K_o} + k_T k_x + k_{i,S}k_T \frac{S_i}{K_i} + k_{T,S}k_{i,S} \frac{S_o}{K_o} \frac{S_i}{K_i} \right)
\]

\[
(k_T + k_x) + (k_{T,S} + k_x) \frac{S_o}{K_o} + (k_{i,S} + k_T) \frac{S_i}{K_i} + (k_{T,S} + k_{i,S}) \frac{S_o}{K_o} \frac{S_i}{K_i} E_T
\]

Optimization of the rate of substrate transport while minimizing the rate of ATP hydrolysis is equivalent in this model to maximization of the following ratio:

\[
\frac{\text{transport rate}}{\text{ATP hydrolysis rate}} = \frac{k_{T,S}k_x \left( \frac{S_o}{K_o} - \left\{ k_{i,S}k_T \frac{S_i}{K_i} \right\} \right)}{k_{T,S}k_x \left( \frac{S_o}{K_o} + k_T k_x + k_{i,S}k_T \frac{S_i}{K_i} + k_{T,S}k_{i,S} \frac{S_o}{K_o} \frac{S_i}{K_i} \right)}
\]

which corresponds to minimization of the bracketed quantities (which are always positive).
For an importer engaged in active transport (with \((S_o) < (S_i)\)), this optimization may be achieved by having a higher affinity for substrate in the outward-facing conformation than in the inward-facing conformation \((K_o < K_i)\), a stimulation of ATPase activity in the substrate-bound conformation \((k_{T,S} > k_T)\) and a higher rate of nucleotide exchange in the unliganded state \((k_x > k_{x,S})\), where the optimization is subject to the equilibrium constraints imposed on these constants. For exporters, the opposite set of relationships would hold. The key to minimizing the futile cycling of nucleotide then is to keep the rate of ATP hydrolysis minimal until the proper state of the transporter is achieved.
Scheme S1  An idealized two-state kinetic model for the mechanism of ABC transporters. In this highly simplified model, the transporter is assumed to exist in two states, outward and inward facing (E₀ and Eᵢ, respectively), where the outward-facing conformation is stabilized by ATP (T) and the inward-facing conformation by ADP (D). In the absence of nucleotide and substrate (and out of the membrane), E₀ and Eᵢ may be approximately in equilibrium, based on the observed conformations of detergent-solubilized transporters⁴. K₀ and Kᵢ represent the dissociation constants for substrate (S) binding to the outward- and inward-facing conformations, while the rates of ATP hydrolysis in the appropriate states are denoted by kₜₕₛ and kₜ, and the rates of nucleotide exchange by the pseudo first-order rate constants kₓₛ and kₓ.

References