Supplementary Information

ABC transporters: The power to change

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Box S1. A brief history of ABC transporter crystallography

Paralleling the general experiences in structural biology, progress in the crystallographic analysis of ABC transporters has been largely driven by sample preparation considerations. Consequently, the earliest crystal structure determinations targeted the periplasmic binding proteins, because these components were released upon osmotic shock and hence relatively readily purified. In 1981, Quiocho and co-workers reported the structure of the arabinose binding protein[1], and established the characteristic bilobal architecture of these proteins. The ligand binding site was identified in the cleft between the two lobes or domains, and the association/dissociation of ligand was observed to be accompanied by inter-domain hinge bending motions.

The first structure of an ABC subunit, HisP of the histidine uptake system, was published in 1998 from the groups of Kim and Ames[2]. (The structure of RbsA, the ABC subunit of the ribose transporter, was also reported in 1998 meeting abstracts by Hermodson and Stauffacher[3], but the structure was subsequently neither published nor deposited in the PDB). In addition to establishing the polypeptide fold of the defining component of ABC transporters, a point of great

interest related to the relative positions of the conserved sequence motifs was the dimeric arrangement of the ABCs. Unexpectedly, different sets of subunit-subunit interactions were observed for HisP and in the subsequently solved structure of MalK[4]. It was not until the structure determination by Hunt's group[5] of a variant of the archaeal ABC subunit MJ0796 with bound ATP, following the crystal structure of the non-transporter ABC protein Rad50[6], that the functionally relevant dimeric arrangement was observed for the ABC subunits of a transporter. The various intermolecular interactions observed in the initial structures of ABC subunits reflected the influence of crystal contacts and the generally weak association of isolated subunits in the absence of the TMDs.

The structure determination of complete ABC transporters was complicated by the challenges of membrane protein overexpression and purification. The first structure of an intact ABC importer was published in 2002 for the *E. coli* vitamin B_{12} importer BtuCD[7], while the first exporter structure was reported in 2006 of the multidrug efflux pump Sav1866 from *Staphylococcus aureus*[8]. These early targets were not selected on the basis of previous biochemical characterization, but rather were identified through a screen of multiple homologues that were amenable to expression, purification and crystallization. In many ways, this is analogous to Kendrew's survey of myoglobin from various diving mammals to find the one providing the best diffracting crystals[9]. The structure of the MalFGK transporter[10] represents an example of a transporter system specifically targeted because of the extensive prior biochemical and genetic characterization. The structure of an intact eukaryotic ABC transporter, particularly a human transporter, has yet to be achieved, reflecting the challenges of preparing adequate quantities of homogenous and functionally active recombinant eukaryotic membrane proteins.

As a microcosm of structural biology, there are general lessons to be drawn from the crystallography of ABC transporters, including the central roles of sample preparation and characterization, and the recognition that crystallization conditions and crystal contacts can influence the conformational states and associations of components. While common to all crystallographic studies, these considerations are exacerbated with membrane proteins by the common utilization of detergents that are not completely faithful mimics of the membrane bilayer environment. The importance of high resolution and high quality data collection cannot be overemphasized, particularly since membrane protein crystals are typically (although not universally) characterized by modest diffraction quality. A cautionary tale in the ABC transporter field is provided by the MsbA crystal structure determinations[11, 12], where an unfortunate error in the initial data processing led to an incorrect structure determination that went undetected at 4.5 Å resolution. Although this was an extreme example, the important point is that the higher the resolution, the more objective criteria there are for assessing the correctness of the structural analysis. These issues will remain relevant as more and more complex structural assignments are pursued based on low resolution x-ray crystallography, electron microscopy, small angle scattering, spectroscopic studies and computational modeling.

Box S2: 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; ie 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[13-15], 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:

$$\frac{d(S_i)}{dt} = k_{T,S}(E_o TS_o) - k_{x,S}(E_i DS_i)$$

$$= \frac{k_{T,S}k_x\frac{(S_o)}{K_o} - k_{x,S}k_T\frac{(S_i)}{K_i}}{(k_T + k_x) + (k_{T,S} + k_x)\frac{(S_o)}{K_o} + (k_{x,S} + k_T)\frac{(S_i)}{K_i} + (k_{T,S} + k_{x,S})\frac{(S_o)}{K_o}\frac{(S_i)}{K_i}} E_T$$

The overall rate of ATP hydrolysis is given by:

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

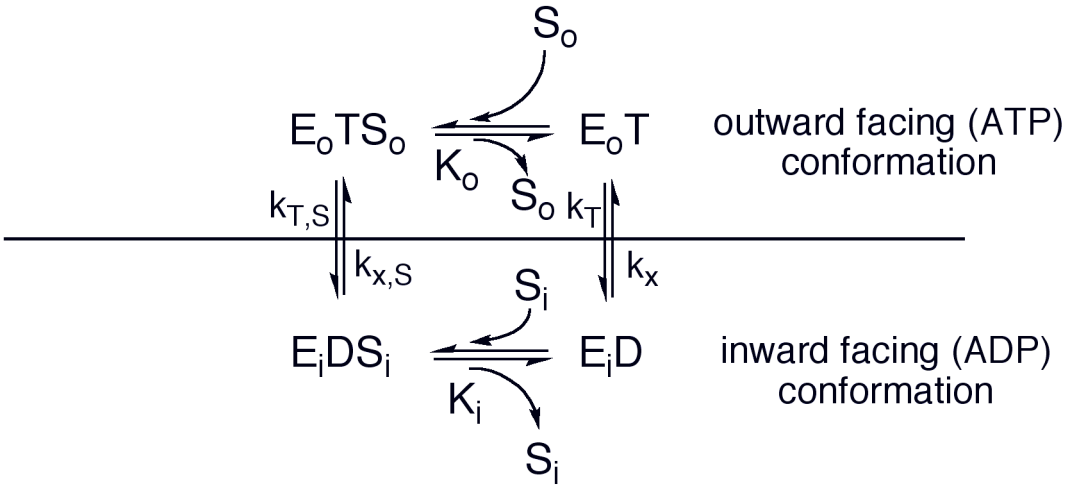
$$= \frac{k_{T,S}k_x\frac{(S_o)}{K_o} + k_T k_x + k_{x,S}k_T\frac{(S_i)}{K_i} + k_{T,S}k_{x,S}\frac{(S_o)}{K_o}\frac{(S_i)}{K_i}}{(k_T + k_x) + (k_{T,S} + k_x)\frac{(S_o)}{K_o} + (k_{x,S} + k_T)\frac{(S_i)}{K_i} + (k_{T,S} + k_{x,S})\frac{(S_o)}{K_o}\frac{(S_i)}{K_i}} E_T$$

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:

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

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 (E_o and E_i, 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), E_o and E_i may be approximately in equilibrium, based on the observed conformations of detergent solubilized transporters[16]. K_o and K_i 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 $k_{T,S}$ and k_T , and the rates of nucleotide exchange by the pseudo-first order rate constants $k_{x,S}$ and k_x.

Box S3: Structure Comparison

When structures are available for more than one homologous protein, an inevitable consideration concerns the conformational relationships between them. Although conceptually this comparison should be a straightforward process, a number of subjective decisions are involved that can influence the final conclusions. At the heart of these comparisons is the rigid body superposition between the coordinate sets for the two conformations, *x* and *x'*, which may be described in terms of a rotation matrix *R* and a translation vector *d* by the equation: $x' = Rx + d$. The calculation of this transformation is incorporated into a number of superposition programs and is unambiguous for a pair of truly rigid body structures. With real coordinate sets, the key operation is to identify structural elements that are essentially unchanged in the two conformational states, ie that behave as rigid bodies. A sensitive way to identify approximately rigid elements is with difference distance plots[17] to find regions with conserved intramolecular distances. In practice, an iterative algorithm is used to find equivalent residues that superimpose within a certain limit. For the ABC subunits of ABC transporters, the secondary structure elements of the catalytic domain represent a commonly maintained rigid element. The TMDs are more variable, but conserved cores have been have been identified[16, 18] for the TMDs of both type I and type II ABC importers that can serve as a basic rigid scaffold.

Another aspect to characterizing conformational transformations is the choice of reference frame to compare the structures. For ABC transporters exhibiting two-fold molecular symmetry (which is approximately the case for all transporters solved to date), two principal reference frames are typically employed: the use of the entire transporter (all four domains of each transporter) in the superposition so that the symmetry axes coincide (the "symmetric frame"), or

the use of only an individual domain (or part of a domain) in the superposition (the "single domain frame"). This problem has counterparts in the analysis of conformational transitions in any system, particularly symmetric, oligomeric assemblages[19]. Since the use of difference reference frames for the superposition will generally lead to different results for the calculated transformation, the details of any coordinate comparison must be clearly specified.

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