Supplementary information S1 (box) | A brief history of ABC transporter crystallography

In parallel to 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 coworkers 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 by 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 Protein Data Bank (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 *Escherichia coli* vitamin B$_{12}$ importer BtuCD$^7$, whereas 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$_2$ 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\textsuperscript{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 modelling.

References
