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Structure of a multidrug transporter:

Crystal structures of a mammalian multidrug efflux pump bound to peptide inhibitors may reveal drug binding sites

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Multidrug transporters are the bane of pharmacologists, as they diminish the efficacy of many drugs by pumping them out of bacteria and mammalian cells¹. The multidrug efflux pump P-glycoprotein (P-gp), for example, contributes to multidrug resistance in about half of human cancers. In a recent paper in *Science*, Aller *et al.*² reported x-ray crystal structures of mouse P-gp to 3.8 Å resolution—the highest-resolution structures of a mammalian multidrug transporter to date. The three structures show P-gp alone and P-gp bound to two stereoisomers of a novel cyclic hexapeptide inhibitor, all in the absence of ATP. These results reinforce many of the conclusions derived previously from detailed biochemical studies and illustrate the pitfalls of using static structures to understand conformationally dynamic molecules such as ATP-binding cassette (ABC) transporters.

The mammalian P-gp transporters (encoded by the *MDR1* (*ABCB1*) gene in the human and the *mdr1a* and *mdr1b* genes in the mouse) were first cloned in 1986^{3,4}, and their essential physiological, biochemical and pharmacologic features have been well described⁵. However, controversy remains over how P-gp recognizes hundreds of different hydrophobic drugs and pumps them out of the cell and how similar it is to the 47 other known members of the human ABC transporter family. High-resolution crystal structures of P-gp would be invaluable for investigating these questions and for designing therapeutic P-gp antagonists.

Although the structures solved by Aller *et al.*² lack the high resolution needed to settle most of the remaining controversies, they do provide useful new information. The essential features are a large, 6000 Å³ internal cavity to which hydrophobic drugs can bind in various orientations and at different locations, as well as two portals in the inner leaflet part of the protein that allow entry of hydrophobic drugs from the lipid bilayer. The structure supports the “hydrophobic vacuum cleaner” model first proposed to explain the observation that P-gp can remove hydrophobic drugs directly from the membrane⁶. The drug-binding cavity is composed of parts of the transmembrane segments from both halves of P-gp, especially transmembrane helices 4, 5 and 6 and 10, 11 and 12, as predicted from previous photoaffinity labeling studies^{7,8} and cross-linking analyses⁹. Although not of sufficiently high resolution to discern individual side chains, the structure makes many clear predictions about residues likely to be involved in drug binding that can and will be tested by mutational analysis. Interestingly, the three deposited structures in the absence of any compound (PDB: 3G5U) and in the presence of one (PDB:3G60), or two compounds (PDB:3G61) in the central cavity display identical crystal forms, with few changes to crystal cell parameters and to overall conformation of the protein (0.6 Å root-mean-square deviations for all C alpha

atoms), and to the local environment of the binding sites, except for a few with direct contact with the bound inhibitor.

Several features of the model will undoubtedly stimulate more questions than they answer. To crystallize unglycosylated mouse P-gp purified from the yeast *Pichia pastoris*—an accomplishment that had eluded all others in the field—Aller *et al.*² had to eliminate ATP, ATP analogs and magnesium from the crystallization medium. The structure (Fig. 1a) shows the two halves of the potential ATP-binding sites 30 Å apart—a considerable distance given that these regions must interact to bind ATP during the catalytic cycle.

The authors speculate that the structure in Fig. 1a represents mouse P-gp in a “pre-transport state” and binding of ATP stimulated by drug-substrate during the catalytic cycle likely results in dimerization of the ATP sites. However, since ATP concentrations in the cell (3–5 mM) always far exceed the affinity of the transporter for ATP (K_{mATP} 0.3–1 mM), it seems unlikely that P-gp ever exists in the cell in a ‘nucleotide-free’ state. Thus, the structure may represent a crystallization artifact or a non-functional conformation that has only very transient existence. The lack of significant conformational changes in the transmembrane domains upon inhibitor binding also supports this concern. The current hypothesis that ATP binding induces dimerization of the two nucleotide-binding domains is also not supported by the authors’ model.

The structure of Aller *et al.*² differs in substantial ways from previous models of P-gp (Fig. 1b) based on the so-called ‘closed conformation’ of an ABC transporter from *Staphylococcus aureus* SAV1866 (ref. 10). In addition to the above-mentioned 30 Å distance between the two NBDs and the lack of nucleotide binding, the other major difference is that Aller’s structure has a transmembrane domain (TMD) conformation open to the inside, whereas the model based on SAV1866 has a conformation open to the outside. Furthermore, as noted, although the cyclic hexapeptides used as inhibitors may turn out to be transport substrates, they are apparently incapable of inducing expected conformational changes in P-gp. It also remains to be seen whether these hexapeptides interact with human P-gp to the same extent that they interact with mouse P-gp, as there are significant differences in substrate specificity between mouse and human multidrug transporters¹¹.

A major technical problem in this field has been the difficulty of crystallizing P-gps from humans and other mammals. This has been attributed to the extreme conformational flexibility of these transporters. That Aller *et al.*² discovered conditions for the crystallization of mouse *mdr1a* P-gp but not human P-gp, which is 87% identical in amino acid sequence, reinforces the mystery surrounding the challenge of crystallizing the mammalian ABC transporters. The authors’ results could be related to the unstable nature of these proteins when expressed in *Pichia* yeast or to structural differences among the transporters. Nevertheless, this study gives hope that persistence will be rewarded and encourages the field to continue to seek crystals of other mammalian P-gps that diffract x-rays to higher resolution and that represent more physiologically relevant conformations.

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Figure 1.

Ribbon renditions of the structure of P-glycoprotein embedded in a membrane bilayer. ATP (red) and ADP (blue) molecules are shown present in the cytoplasm and two representative human P-gp substrate/inhibitor molecules (black) are shown in the extracellular space. **(a)** Mouse P-gp at 3.8Å resolution (PDB: 3G60) as described by Aller *et al.*². The N-terminal transmembrane domain and nucleotide-binding domain are shown in red and yellow, respectively, and the C-terminal transmembrane domain and nucleotide-binding domain are in cyan and magenta, respectively. The bound QZ59-SSS inhibitor in the TM region is shown as a stick model in black. The red double-arrow indicates a distance of 30Å between the two 'nucleotide-free' nucleotide-binding domains. The dashed black circle indicates an area proposed to be one of the portals for entry of substrates or modulators directly from the membrane. **(b)** Model of human P-gp based on the structure of *S. aureus* SAV1866 bound to ADP and open to the extramembrane space (PDB: 2HYD) from ref. 10. Two bound ADP molecules are shown in black. The color code follows that in **a**.