Determining the physiological state of a membrane protein: investigating the P-gycoprotein crystal structure

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P-glycoprotein is one of the major multi-drug transporters in humans. Expressed primarily in barrier tissues, P-glycoprotein (P-gp) exports a wide range of substrates and is a cellular first defence mechanism against xenotoxins. P-gp is a member of the ABC transporter superfamily, utilising the energy released from Mg^{2+} catalysed ATP binding and hydrolysis between the two nucleotide binding domains (NBDs) to induce a conformational change across the two transmembrane domains (TMDs). This conformational change drives the translocation of substrate across the membrane. Recent studies have crystallized homologues of P-gp in an ATP bound conformation (Dawson & Locher, 2006). Here the two TMDs are in an outwardly splayed conformation and are connected to the tightly coupled NBDs which form a characteristic nucleotide sandwich dimer, with nucleotide analogues sandwiched between them. In 2009 a medium resolution crystal structure of murine P-gp was solved in an alternate conformation, with inwardly facing TMDs and a wide gap separating the two NBDs (Aller *et al.*, 2009). The large conformational differences between the structures gave rise to questions as to whether this nucleotide-free structure represents an alternative conformation in the transport cycle, or whether this structure arose as a crystallization artefact. Here we address these questions *via* two sets of molecular dynamics (MD) simulations: one of P-gp in a cholesterol enriched POPC bilayer, and the second in the crystallographic mother liquor.

Simulations of the crystallographic mother liquor demonstrate that the nucleotide-free structure of P-gp is stabilized primarily by protein-protein contacts in the unit cell crystal lattice. Removal of these contacts is sufficient to destabilize the P-gp crystal structure. The instability arises from an attractive potential between the NBDs, resulting in a salt bridge between D558 and H1228. Experimentally, cholesterol is necessary for the functional activity of P-gp, however MD simulations demonstrate that the presence of cholesterol alone in a POPC membrane is not sufficient to stabilize the P-glycoprotein crystal structure. We are identifying the underlying factors producing the instability of the P-gp crystal structure and examining the effects of different ionic solutions on structural stability of P-gp.

Dawson RJ. Locher KP. (2006) Structure of a bacterial multidrug ABC transporter. *Nature* **443:** 180-5.

Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, Chang G. (2009) Structure of P-Glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* **323:** 1718-1722.