## The effect of ATP binding on the conformation of P-glycoprotein

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The ABC transporter P-glycoprotein exports over 120 drug-like compounds and natural substrates out of the cell. Both ABC importers and exporters share two highly conserved nucleotide binding domains (NBDs) that bind and hydrolyse ATP, and two non-conserved transmembrane domains (TMDs) that facilitate the transport of substrate. Crystal structures of isolated ABC transporter NBDs, in complex with ATP or its analogues, display a characteristic nucleotide sandwich dimer conformation in which two molecules of ATP are tightly bound between the two NBDs. The current transport model for ABC exporters proposes that the two ATP molecules bind simultaneously to form this nucleotide sandwich dimer. The formation of the nucleotide sandwich dimer induces a conformational change in the TMDs, allowing the extracellular release of substrate. However two recent structures of the homologous ABC exporters, ABCB1 and TM287/288, show variability in the ATP-bound conformation of the NBDs, giving rise to conformations where the nucleotide sandwich dimer does not form. This has raised questions regarding the molecular mechanism and sequence of events involved in ATP binding, hydrolysis and substrate transport.

In this study we use non-biased molecular dynamics simulation techniques to examine the conformational changes associated with ATP binding in the human ABC transporter, P-glycoprotein. We find that ATP-bound P-glycoprotein adopts two dominant conformations in simulations, but does not form a nucleotide sandwich dimer during 100 ns of simulation time. The P-glycoprotein conformations from these simulations correspond to the crystal structures of homologous ABC exporters in which a nucleotide sandwich dimer does not form. We examine the processional motion of the NBDs and the effect this motion has on the conformation of the TMDs, in the context of substrate transport. Our results indicate that ATP binding in P-glycoprotein is an asymmetric process in which high-affinity ATP binding occurs more readily at the NBD1 binding site than in the NBD2 site.