The ATP-bound state of P-glycoprotein is closed towards the cell exterior

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The human genome contains 44 ATP-Binding Cassette (ABC) transporters. We focus on the multidrug resistance transporter P-glycoprotein, which is expressed at the blood-brain-barrier, in the intestine, kidney, liver and macrophages. Sav1866 from *Staphylococcus aureus* was the first ABC exporter that was crystallized. The global architecture showed an unexpected twisted architecture. The same fold was observed in MsbA, mouse P-glycoprotein and the human mitochondrial ABCB10 transporter, suggesting that this fold is a common architecture of ABC exporters. The twisted conformation has been confirmed by site-directed mutagenesis and cysteine cross-linking experiments. Although ABC exporters have now been crystallized in several conformations, uncertainty remained with respect to the physiological conformation, because these structures do not seem to be fully compatible with all biochemical evidence. The observed conformation of the ATP-bound state might be a consequence of the crystallization procedure or conditions.

We used homology modeling and MD simulations to determine the equilibrium conformation of the membrane inserted transporter. The aim was to test the hypothesis, if membrane inserted P-glycoprotein would be devoid of the wing-shaped conformation. The transporter was inserted into a pre-equilibrated membrane and long equilibrium simulations were carried out. We could observe the wings to come closer in repeated simulations, indicating that the closed conformation is indeed energetically favored. Water becomes mostly expelled from the hydrophobic region in which the wings come into contact. Thereby, the open passage between the water filled pore and the cell exterior collapses. Our optimized structure shows improved compliance with experimental observations. We then experimentally tested this model by introducing one cysteine on each wing. We found that P-glycoprotein remains active and exports substrates even after cross-linking helix 6 and 12.

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