Simulated substrate binding to the inner membrane translocase AcrB

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One of the most common ways of cells to protect themselves against cytotoxic substances are active transporters which transport the harmful substances out of the cell as soon as they enter. In *Escherichia coli*, one of the proteins involved in this process is the inner membrane translocase AcrB that is part of the AcrAB-TolC efflux pump, whose over-expression is a major cause of multi-drug resistance. AcrB occurs as trimer, where each monomer has a different conformation: loose (L), tight (T) or open (O) – each representing the different consecutive states in the transport cycle.

Here we report molecular dynamics simulations of the asymmetric 2GIF AcrB x-ray structure in a phospholipid bilayer / 150 mM NaCl environment. To study the interaction with one of AcrB's simplest substrate, 25 hexane molecules were added to the system, with three hexanes directly placed in front of each monomer's porter domain. Using GroMACS 4.0.3 and the GROMOS96 53a6 force field we performed 5 independent MD runs, each 50 ns long.

During one of the simulations we observe one hexane entering the presumed drug transport channel of the (L) monomer. Binding occurs in a stepwise process during which the hexane moves towards the hydrophobic binding pocket inside the protein before reaching a final position after 25 ns, 5 Å away from Phe-628. For the (T) monomer we also observe hexane binding during one of the simulations. However, here diffusion into the binding pocket occurs in a single step reaching a final position after 5 ns, 8 Å away from Phe-628. In none of the runs substrate binding takes place in the (O) monomer. Yet, an accumulation of hexane molecules in front of the closed porter domain could be observed that might allow for a faster binding and therefore faster expulsion of hexane from the periplasmic space of the bacteria when the open state changes into loose conformation during the AcrB transport cycle.