Dynamics in the TolC gating region and cavity detection based on protein and water dynamics

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Over-production of multi-drug efflux pumps is a prominent example of how bacteria achieve resistance against antibiotics. In *Escherichia coli* the AcrA/B-TolC efflux pump is capable to expel a broad range of drugs, using the energy of proton-motive force. The detailed functional mechanism of this efflux system is not fully understood yet. While AcrB is the engine in this system, the outer membrane protein TolC acts as an efflux duct that also interacts with a numerous other inner membrane translocases. TolC occurs in at least two states, one that is impermeable for drugs and one where drug passage is possible.

To gain insight into TolC ground state dynamics, we performed a series of 6x 150 ns and 1x 300 ns independent & unbiased molecular dynamics simulations of closed state wild type TolC (PDB ID 1EK9) in a phospholipid / water environment at 0.15 M NaCl concentration. Simulations were performed using GROMACS 4.0.3 and the GROMOS96-53a6 force field.

While TolC remains closed between a "bottleneck region", outlined by Asp-371 and Asp-374, we observe opening and closing motions in a second bottleneck at the AcrB interface region near Gly-365. This local flexibility could be of functional relevance in the formation of the AcrAB-TolC complex.

While we observe frequent and unhindered passage of sodium ions through TolC, in one simulation the successive binding of two sodium ions between Gly-365 and Asp-374 stabilizes a closed conformation of the second bottleneck for more than 150ns.

With dxTuber we introduce a novel tool to analyze protein-internal cavities, clefts and channels based on time-averaged protein & water residence probabilities derived from MD simulations. Resulting cavities are automatically grouped and can be exported as PDB files. Additionally pore-profiles can be computed along the XYZ principal axes.