What Keeps TolC Closed?
Insights from Molecular Dynamics Simulations

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Efflux pumps represent one of the main lines of the defense employed against drugs by Gram-Negative bacteria. A detailed understanding of the opening mechanism of TolC, the outer membrane component of Escherichia coli efflux pump AcrAB-TolC, would be greatly beneficial to the design of new drugs to counter antibiotic resistance.

In this work, the roles of ions, H-bonds and salt bridges in the conformational stability of TolC were investigated by means of molecular dynamics simulations. Lipid embedded TolC was equilibrated for 100 ns in a KCl solution. To identify the most energetically significant hydrogen bonds and salt bridges, the interaction energies of each residue with other residues, as well as with ions and water, were computed. Based on these results and on data found in the literature, several mutations were introduced in the structure with the goal of breaking inter-helix and inter-monomer links, and further simulations were conducted on the mutants, as well as on TolC homologues of other Gram-Negative bacteria for comparative purposes.

Simulations of mutants confirm the importance of the H-bond link near the periplasmic tip, while analysis of the interaction energies suggest salt bridges in the equatorial region could contribute to keeping the protein closed. Results further indicate that ions play a significant role in the conformation of TolC, as removal of K^+ resulted in opening of some mutants, while analysis of ion trajectories revealed three previously unreported ion pockets which could provide a target for blocking compounds. More simulations are being performed to conduct a full comparative study of TolC homologues in other Gram-Negative bacteria.