Transport mechanism in the RND transporter AcrB of E. coli

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The efflux pump AcrAB-TolC of the Resistance-Nodulation-cell Division (RND) is responsible for intrinsic and acquired antibiotic resistance in *E. coli* [1]. The core of this machinery is the proton-gradient-driven antiporter AcrB, a homotrimeric protein with an unreached ability to recognize antibiotics belonging to many different families [2-3].

On the basis of available experimental data, a functional rotation mechanism has been hypothesized for this transporter, in which recognition and expulsion of substrates are coupled to concerted conformational changes occurring in each monomer of AcrB [3]. While several computational studies have been performed aiming to confirm and detail this mechanism [3-6], no protocol has been designed to address at a molecular level the energetics associated to the whole translocation process.

In this work, we devise an original protocol that combines several enhanced-sampling techniques to smoothly couple large-scale conformational changes of AcrB to full translocation of the substrate doxorubicin from the distal pocket of monomer B to the TolC docking domain. Our method allows to characterize the mechanism and the free energy profile associated to the full translocation of doxorubicin within AcrB. In particular, our results show that translocation occurs via a full rotation of the drug before it enters the putative Gate to the proximal region of AcrB. Moreover, irrespective of simulation details, the functional rotation mechanism crucially lowers the main free energy barrier associated with extrusion with respect to the same process with the protein in equilibrium. The role of different drug-protein interactions and that of water molecules to assist the process is detailed and reveals interesting features of the process. Finally, perspectives of this method in the coarse-grained framework will be discussed.

References

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