Exploring PfeA-Enterobactin interactions with molecular simulations

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Aiming to find efficient Trojan-horse candidates we must gather existing and acquire an additional knowledge about binding to and translocation by TonB dependent transporters. Binding and translocation of the enterobactin molecule (ENT) through the cathecolate transporter PfeA of *P. aeruginosa* could serve as a test-case for this biological process. In order to get a detailed molecular description of the PfeA-ENT complex, we used a combination of computational techniques including cavity detection algorithms, molecular docking, molecular dynamics, and enhanced sampling simulations.

A correlation analysis reveals that the distributions and sizes of the detected cavities are controlled by the collective H-bonding interactions formed between the plug and the rest of the protein. Our findings agree with the experimental results and show how the establishment of the above H-bonds network would represent a unique signal of ENT binding, which is indeed completely absent for the PfeA-apo simulation and appears to be reduced for a double-mutant. Further, with metadynamics simulations we quantified the residence time of ENT in the binding site. In agreement with experimental data, we found marked differences between the wild-type and double-mutant variants of PfeA, with the latter showing a weak binding.

Eventually, by combining metadynamics, cavity analysis and a completely blind docking search, we propose a possible translocation pathway for ENT, from extracellular to periplasmic space, along which free-energy barrier is crucially controlled by plug-barrel H-bond interactions.

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