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 translocation in TonB dependent transporters. PfeA, a TonB dependent transporter, translocates an enterobactin molecule and is composed of three domains: plug, β-barrel and external loops. Crystallographic and mutagenesis data provide us valuable information regarding the enterobactin binding site, but further steps in the translocation remain unknown. With the enterobactin being the most efficient iron chelator, its binding and translocation trough the PfeA could serve as a role model for the possible antibiotic-iron complexes. In order to get detailed description of the processes underlying the binding and translocation of the enterobactin we use a combination of computational techniques, including cavity detection algorithms, molecular docking, molecular dynamics and methadynamics simulations, to investigate the PfeA-enterobactin complex. In particular, we impose perturbations on our system by removing the enterobactin molecule or mutating R481 and Q483, amino-acids which abolish binding as shown in experiments. This perturbations cause changes in the protein dynamics which we quantify by employing the linear mutual information as a measure of amino-acid correlation. Protein conformations extracted from the dynamics are used in extensive rigid docking of enterobactin aimed at detecting most favorable binding sites. We additionally detect cavities within PfeA capable of hosting the enterobactin molecule and measure a correlation between cavity fluctuations. To encourage the enterobactin to explore more intensively the free-energy surface we perform metadynamics simulations to quantify the free-energy of unbinding for both wild-type and double-mutant PfeA.