Affinity sites in E. coli transporters: structural and dynamical features

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The *Escherichia coli* AcrAB-TolC efflux pump is the archetype of the resistance nodulation cell division (RND) exporters from Gram-negative bacteria [1,2]. Overexpression of RND-type efflux pumps plays an important role in multidrug resistance (MDR), which makes these pumps important targets for antibacterial drug discovery. The core of this system is the proton-gradient-driven antiporter AcrB, a homotrimeric protein able to recognize and transport antibiotics belonging to many different families [2-3]. The low specificity of AcrB makes the identification and characterization of affinity sites particularly challenging.

Experimental data extracted from different techniques have identified several relevant locations on the transporter, but the lack of crystal structures of AcrB in complex with a wide range of substrates still hampers a full characterization of the affinity sites [1-3]. To gain insights into these aspects we performed extensive molecular docking runs with Autodock Vina [4]. Within the ensemble-docking framework, we used several structures of AcrB extracted from both X-ray and MD simulations, as well as representative structures of the configurations sampled by a set of compounds during long MD trajectories in explicit water [5]. The results of the docking runs were considered in terms of distributions of affinities, contacts, and locations assumed by the compounds interacting with a large set of configurations assumed by AcrB. A subset of the poses was selected as starting conformations for MD simulations.

Although docking runs were performed for the following compounds: imipenem, meropenem, meropenem 2, cefepime, ceftazidime, fleroxacin, ciprofloxacin, fusidic acid, oxacillin, piepracillin, avibactam and tazobactam, we focused our attention on the data for imipenem, meropenem, meropenem 2. The scoring function distribution of meropenem is shifted toward more negative values than that of imipenem. Furthermore, for meropenem poses are found within the Distal Pocket of monomer T, where imipenem is hardly accommodated. For imipenem, MD simulations seem to point out a tendency of the compound to escape the transporter and/or to remain close to cleft, in a region in contact with the solvent. In the Distal Pocket, imipenem is less stable than meropenem and undergoes large displacement with respect to the selected docking pose. The observed behaviour of imipenem and meropenem in these preliminary simulations seemingly agree with that extracted form a previous study of us on the interactions of imipenem and meropenem with MexB, the homologous protein of AcrB in *P. aeruginosa* [6].

References

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