Characterization and identification of Ampicillin and Amoxicillin binding sites within the multidrug transporter MexB of *P. Aeruginosa*

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Efflux systems of the Resistance Nodulation-cell Division (RND) superfamily are transmembrane transport proteins playing a key role in Multi-Drug Resistance in Gram-negative bacteria. MexAB-OprM, the major RND efflux pump in *Pseudomonas Aeruginosa*, can extrude a wide range of chemically unrelated compounds, including antibiotics[1]. Up to date, no X-ray MexB structures, in complex with antibiotics, have been solved and the dynamics of the transport process remain largely unknown. Henceforth, the understanding of the molecular determinants regulating recognition, binding, transport and extrusion of the efflux substrates is crucial to design more effective antibiotics and/or inhibitors. In this work, as part of an extensive research activity on RND transporters of Gram-negative bacteria[2], we present a comparative investigation on the interaction between MexB and two penicillins, namely amoxicillin and ampicillin (Fig. 1).

Amoxicillin differs from ampicillin by a hydroxyl group on the 2-amino-2-phenylacetamide substituent bound to the penicillin core. Despite this subtle chemical difference, different experimental studies revealed that only ampicillin is a substrate of MexB[3]. In order to rationalize this different behaviour at an atomistic resolution, we applied a combination of different computational techniques. Several molecular docking poses found inside of the so-called deep binding pocket (DP) of MexB have been selected as starting points to run classical molecular dynamics simulations, followed by binding free-energy calculations (Fig. 2, 3). For each of the two antibiotics, our study revealed a preference for the different DP sub-regions, along with a favoured orientation and representative key interactions with the DP residues. The goal of this work is to include more antibiotics families in order to have a global overview of the transport process in the different sub-regions of MexB distal pocket. Our main findings, in agreement with microbiology and mutagenesis studies, could provide useful information for the design of new compounds able to evade or inhibit the efflux process.

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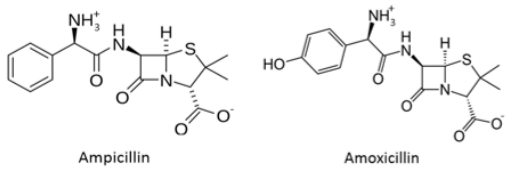

Figure 1. 2D structure of the antibiotics investigated in this study.

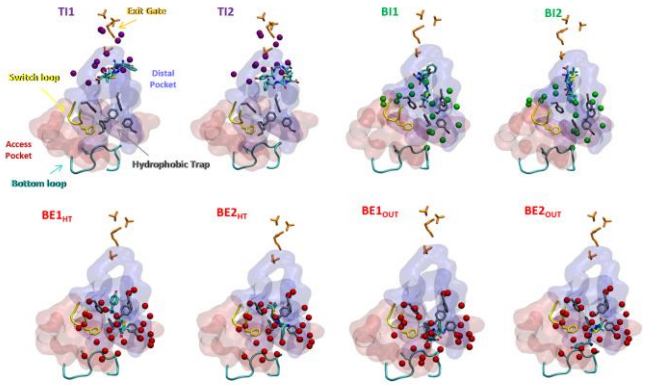

Figure 2. Structural representation of the eight selected docking poses for amoxicillin and ampicillin antibiotics inside the DPT of MexB. The colored spots represent the Cα atoms of the different sub-regions of the pocket (violet for TI, green for BI, and red for BE).

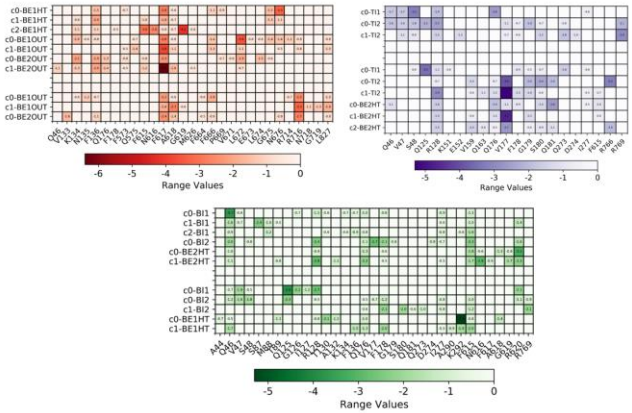

Figure 3. Per-residues energy contribution heat-map of the all clusters found in the MexB BE sub-region (red), TI sub-region (violet) and BI sub-region (green).