Prediction of β-lactam position and orientation in the central cavity of the component of bacterial AcrAB-TolC multidrug efflux pump AcrB

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THE OBJECTIVES OF THIS WORK

1) To predict position and orientation of 16 β-lactam antibiotics in the central cavity of the multidrug efflux pump AcrB (a component of AcrAB-TolC membrane transporter) that exists in several Gram-negative bacteria → using only known molecular structures and calculated properties of drugs (β-lactams and four organic dyes: dequalinium, ethidium, ciprofloxacin, rhodamine 6G) and protein-drug (AcrB-organic dyes) complexes → no protein-drug complex geometry optimizations nor molecular dynamics simulations;

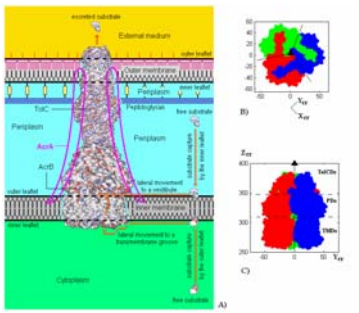
2) To explain the efflux mechanism of β-lactams and other drugs by AcrAB-TolC;

3) To explain structure-function relationships for AcrB and TolC that are relevant for the efflux mechanism;

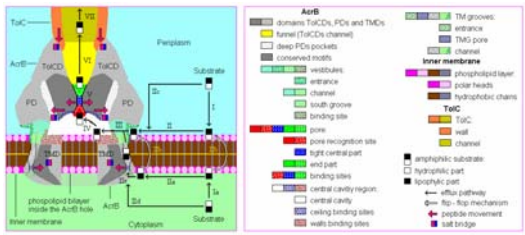
4) To show that the β-lactams and the organic dyes, although structurally diverse, have properties in common that are responsible for their active efflux;

5) To use this similarity and the AcrB-organic dye complexes to predict AcrB-β-lactam geometry → Quantitative Drug Structure – Complex Geometry Relationships.

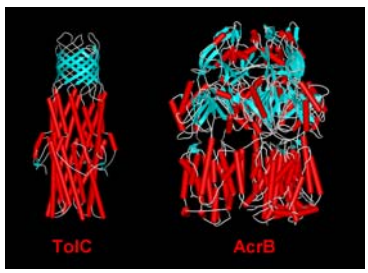
BACTERIAL MULTIDRUG RESISTANCE *VIA* ACTIVE DRUG EFFLUX BY MEANS OF A PROTON-DEPENDENT AcrAB-TolC PUMP



A): Combined 3D - schematic representation of AcrAB-TolC pump in *E. coli*. Two main drug efflux mechanism are shown, one starting in the periplasm, and other in the cytoplasm. B) and C): Blue, red and green AcrB protomers around the symmetry axis.

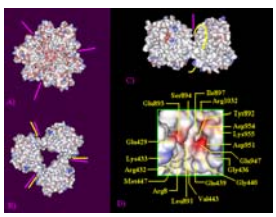


The AcrB mediated drug efflux from a Gram-negative bacteria cell (AcrA is excluded) in steps I - VII. Hydrophilic/lipophilic orientation of an amphiphilic drug is also visible. Conserved motifs, salt bridges and principal peptide movements are marked with special symbols. The efflux mechanism consists of a series of allosteric effects initiated by proton influx, braking of salt bridges and hydrogen bonds, changing of pump conformation and opening its efflux channel components, binding drugs in the central cavity and extruding them through the pore and TolC.

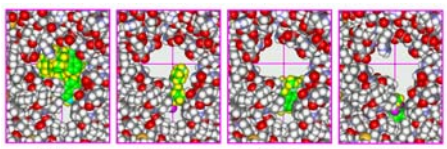


Schematic representation of the 3D structure of trimmer TolC (PDB: 1EK9, space group *R*3. V. Koronakis *et al*., *Nature* 305, 2000, 914) and AcrB (PDB: 1IWG, space group *R*32. S. Murakami *et al*., *Nature* 419, 2002, 587) showing α-helices, β-sheets and coils.

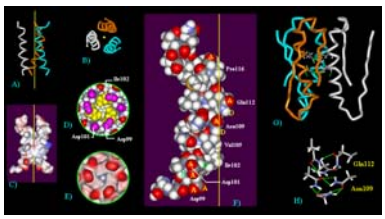
ESSENTIAL AcrB AND AcrB-DRUG COMPLEX STRUCTURE



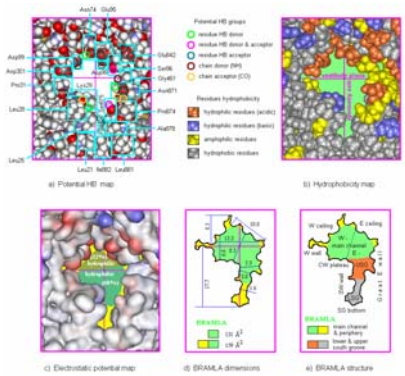
Electrostatic potential representation of A) the pore domains (PDs) and B) the transmembrane domains (TMDs) viewed upwards and downwards the vestibules plane, respectively. C) The transmembrane groove (TMG) circular shape and position at the TMDs surface. The position of the vestibules (pink line) and the TMGs (yellow circle). D) The TMG pore at its entrance, with essential residues.



Structure of AcrB complexed with dequalinium (DEQ), ciprofloxacin (CPF), ethidium (ET) and rhodamine 6G (RHQ). The drugs are viewed along the vestibule axis. (PDB: 1OYD, 1OYE, 1OY9 and 1OY8. E. W. Yu *et al*., *Science*, 300, 2003, 976.

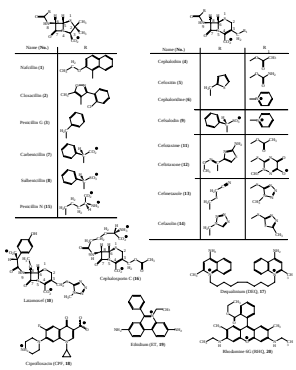


The structure of the pore channel viewed perpendicularly to (A) and along with (B) the symmetry axis. C) The pore channel interior excluding a pore helix. The structure of the pore recognition site represented by CPK model (D) and electrostatic potential (E). F) A pore helix from a protomer represented by CPK model and hydrogen bond donor (D's) and acceptor (A's) groups from residues (yellow) and the polypeptide chain (green). G) Three RND conserved motifs called A including the pore with hydrogen bonding residues that keep the pore to be tight. H) These residues with their hydrogen bond network.

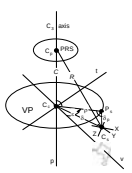


Vestibule and its 2D representation BRAMLA (BRAzil Map-Like Area). a) Potential hydrogen bonding groups from amino-acid residues and polypeptide chains. b) The hydrophobic character of the essential residues. c) The electrostatic potential map. d, e) BRAMLA area characterized by its dimensions (d) and distinct regions of both its interior and exterior (e).

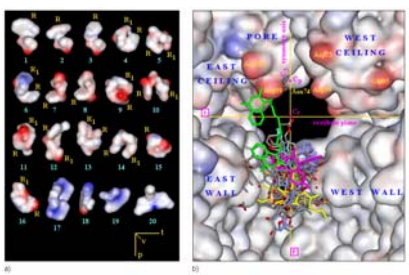
PREDICTION OF β-LACTAM POSITION AND ORIENTATION IN THE CENTRAL CAVITY OF AcrB



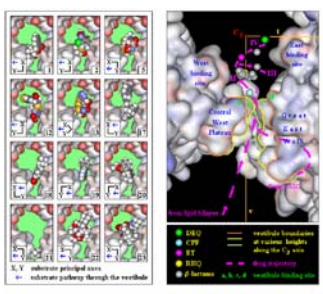
Chemical structures of studied AcrAB-TolC substrates: β-lactam antibiotics (1-16) with atomic numbering, dequalinium (17), ciprofloxacin (18), ethidium (19) and rhodamine 6G (20).



Parameters of the AcrB-drug complex geometry, defined by the intersection of the vestibule axis (v) and protein symmetry axis (p). Distance and angle parameters of the drug are dependent variables that are quantitatively related to several steric and electronic molecular descriptors of drugs 1-20.



a) Electrostatic potential of 1-20 in the central cavity. Most drugs expose positive, amphiphilic or hydrophobic heads (R or R_1) toward predominantly negatively charged ceiling and the pore. b) 16 β-Lactams in predicted position/orientation in the central cavity, with experimental location of DEQ (green), CPF (light blue), ET (pink) and RHQ (yellow). Important charged residues are labeled at the pore region. Linear relationships between the dependent variables (protein-drug complex geometry parameters) and various electronic (dipole moment components, polarizabilities) and steric (principal moments of intertia and their ratios, molecular box size parameters) molecular descriptors of drugs 17-20 were established. These relationships were then used to predict the orientational/positional parameters for β-lactams.



a) Representative drug molecules at the vestibule entrance. Their orientation on the way towards the central cavity is qualitative. b) Probable drug pathway through the vestibule and binding to the central cavity/vestibule binding sites. Both experimental and predicted positions of center of mass reveal four possible pathways in the binding area: I - binding to the vestibule binding sites a-d (E and SW walls at the vestibule end); II - drug binding to the W binding site in the central hole; III - drug positioning in the middle of the binding area including interactions with two or three binding sites; IV - binding to the E binding site. It is supposed that a drug molecule moves along these trajectories until reaching its best binding position.