Quantitative Drug Structure – Complex Geometry Relationships in β-Lactam Efflux by Bacterial Multidrug Resistance Pump AcrAB-TolC

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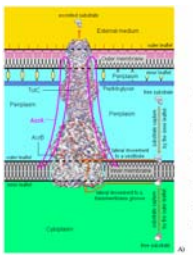
THE PRIMARY OBJECTIVES OF THIS WORK

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 were performed;

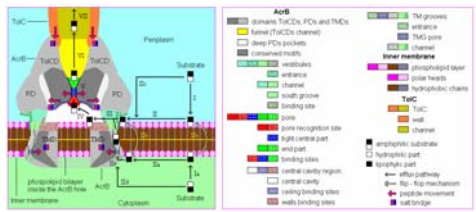
2) To show that the β-lactams and the organic dyes, although structurally diverse, have properties in common that are responsible for their active efflux;

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

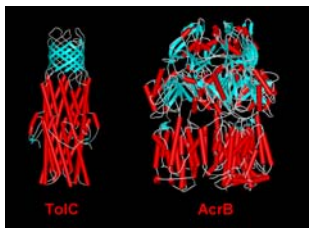
BACTERIAL MULTIDRUG RESISTANCE *VIA* ACTIVE DRUG EFFLUX BY MEANS OF A PROTON-DEPENDENT AcrAB-TolC PUMP



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.

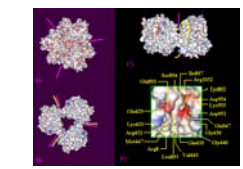


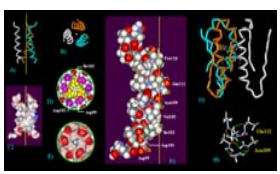
The AcrB mediated drug efflux from a Gram-negative bacteria cell (AcrA is excluded) in steps I - VII. Hydrophilic/lipophilic orientation of an amphiphilic drug is also visible. Conserved motifs, salt bridges and principal peptide movements are marked with special symbols. The efflux mechanism consists 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 *R3*. V. Koronakis *et al*., *Nature* 305, 2000, 914) and AcrB (PDB: 1IWG, space group *R32*. S. Murakami *et al*., *Nature* 419, 2002, 587) showing α-helices, β-sheets and coils.

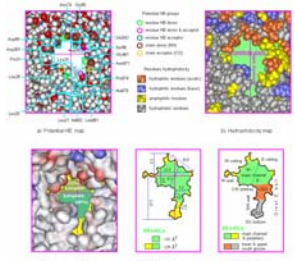
ESSENTIALS OF THE RECEPTOR, SUBSTRATE AND COMPLEX STRUCTURE



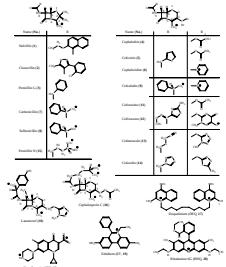


Electrostatic potential of the A) pore domains (PDs) and B) transmembrane domains (TMDs) viewed upwards and downwards the vestibules plane, respectively. C) The transmembrane groove (TMG) circular shape and position at the TMDs surface. The position of the vestibules (pink line) and the TMGs (yellow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

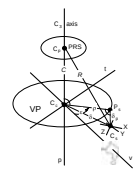
The pore channel viewed perpendicularly to (A) and along with (B) the symmetry axis. C) The pore channel interior excluding a pore helix. The pore recognition site represented by CPK model (D) and electrostatic potential (E). F) A pore helix from a protomer represented by CPK model and hydrogen bond donor (D's) and acceptor (A's) groups from residues (yellow) and the polypeptide chain (green). G) Three RND conserved motifs 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 residues and polypeptide chains. b) The hydrophobic character of the residues. c) The electrostatic potential map. d, e) BRAMLA area with its dimensions (d) and regions of its interior and exterior (e).

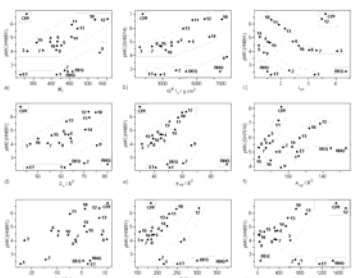


Chemical structures of studied AcrAB-TolC substrates: β-lactam antibiotics (1-16) with atomic numbering, dequalinium (17), ciprofloxacin (18), ethidium (19) and rhodamine 6G (20).



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 1-20.

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



Representative efflux activity-drug molecular descriptor plots important for elucidation of the efflux mechanism: a) pMIC(HN891) - M_1; b) pMIC(SH5014) - I_x; c) pMIC(HN891) - I_{yz}; d) pMIC(HN891) - S_x; e) pMIC(SH5014) - A_{yz}; f) pMIC(SH7616) - A_{xy}; g) pMIC(HN891) - D_y; h) pMIC(HN891) - α; i) pMIC(HN891) - $|\beta_y|$. See the next Table for definition of molecular descriptors that were calculated in this and previous work (M. M. C. Ferreira, R. Kiralj, J. Chemometr., 18, 2004, 242-252).

List of molecular descriptors for drugs 1 - 20

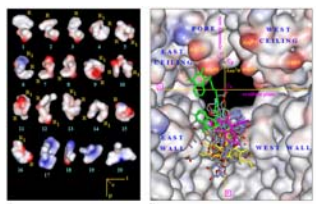
Symbol	Name and description	Nature[a]	Origin[b]	Ref.[c]
M_1	relative molecular mass	steric	comput	pw, tw
I_x	1st principal moment of inertia (around axis X)	steric	comput	pw, tw
I_y	2nd principal moment of inertia (around axis Y)	steric	comput	pw, tw
I_z	3rd principal moment of inertia (around axis Z)	steric	comput	pw, tw
I_{xy}	ratio between the principal moments I_x / I_y	steric	comput	tw
I_{xz}	ratio between the principal moments I_x / I_z	steric	comput	tw
I_{yz}	ratio between the principal moments I_y / I_z	steric	comput	tw
S_x	molecular area projected on the YZ plane (along X axis, in the CPK molecular model)	steric	molgrp	tw
S_{Cx}	ratio between the area S_x and the area of the YZ face of the molecular box defined by the farthest CPK spheres (in the CPK molecular model), expressed as %	steric	molgrp	tw
α	molecular polarizability	electron	comput	pw, tw
$\|\beta\|$	absolute 2nd-order molecular polarizability β	electron	comput	pw, tw
$\|\beta_y\|$	absolute X component of β	electron	comput	pw, tw
γ	3rd-order molecular polarizability	electron	comput	pw, tw
D_x	X component of molecular dipole moment	electron	comput	pw, tw
D_y	Y component of molecular dipole moment	electron	comput	pw, tw
D_z	Z component of molecular dipole moment	electron	comput	pw, tw
n_e	surface molecular density projected on the YZ plane, defined as N / S_x, where N is the number of atoms	steric	molgrp	tw
Δ_1	the length of the molecular box along the axis X (in the CPK molecular model)	steric	molgrp	tw
Δ_{12}	the width (Y)/height(Z) ratio (of the YZ face of the molecular box (in the CPK molecular model)	steric	molgrp	tw
W_{Dx}	maximum of (X coordinate ± vdW radius)	steric	comput	tw
W_{VD}	difference between the maximum and minimum of (Y coordinate ± vdW radius)	steric	comput	tw
W_{DV}	ratio between the W_{Dy} and W_{Dy} differences	steric	comput	tw
A_{yz}	area of the XY face of the molecular box defined by the minimum and maximum atomic coordinates	steric	comput	tw
A_{Dx}	area of the YZ face of the molecular box defined by the minimum and maximum atomic coordinates	steric	comput	tw
A_{Dy}	area of the molecular box defined by the minimum and maximum atomic coordinates	steric	comput	tw
Δ_{xy}	ratio between the X and Y edges of the molecular box defined by the minimum and maximum atomic coordinates	steric	comput	tw
Δ_{xz}	ratio between the X and Z edges of the molecular box defined by the maximum and maximum atomic	steric	comput	tw
Δ_{yz}	ratio between the Y and Z edges of the molecular box defined by the maximum and maximum atomic	steric	comput	tw

[a]Nature of molecular descriptors: steric or electron. [b]Origin or calculation method: compos = compositional, comput = computed and molgrp = molecular graphics description. [c]References: tw - this work, pw - previous work.

Experimental and predicted AcrB - substrate geometry

Parameter	Experimental (for 17 - 20) DEQ	CPF	ET	RHQ	Predicted (for β-lactams 1 - 16) Molecular descriptors	range	mean
$D'/$ Å	7.24	6.29	6.34	7.30	$+I_{xz}+A_{Dx}+A_{Dy}$	6.0 - 6.7	6.3
δ_p / Å	2.64	7.20	8.28	15.02	$-\Delta_{xz}-\|\beta_y\|-n_e$	6.5 - 14.0	11.5
D / Å	4.48	13.09	5.09	8.55	$+\delta_p-S_x-\beta+\alpha^2+\delta_p^2$	7.6 - 9.7	9.1
δ / Å	5.20	14.94	9.72	17.29	$-\Delta_{xz}+\|\beta_y\|-n_e$	10.0 - 17.1	14.7
R / Å	10.85	18.80	15.48	23.91	$-\Delta_{xz}+\|\beta_y\|-n_e$	15.4 - 23.1	20.4
A_{xy}	88.3°	73.0°	90.8°	87.2°	$-D_x$	81° - 106°	94°
A_{yz}	70.3°	149.7°	73.1°	99.3°	$-D_x$	70° - 150°	83°
A_{xz}	160.2°	65.7°	19.3°	9.8°	$+I_{xz}+\Delta_{xz}$	0° - 55°, 130° - 210°	13°
A_{xy}	151.0°	158.6°	82.5°	97.9°	$-I_{xy}$	82° - 180°, −171° - 180°	151°
A_{yz}	116.5°	110.8°	19.7°	168.2°	$-\Delta_{12}-\Delta_{yz}+W_{yz}$	77° - 110°, −145° - 180°	146°
A_{xy}	100.9°	94.9°	71.5°	98.9°	$-I_{yz}+(W_{Dx})^2-\gamma$	83° - 99°	92°
α	78.3°	4.0°	−11.4°	−1.4°	$-(W_{yz})^2+(\Delta_{xz})^2$	0° - 65°, −28° - 0°	−1°
Π_{xyz}	15.1°	77.4°	168.4°	171.7°	$-I_{yz}-I_{xz}-S_{Cx}$	44° - 162°	111°
Π_{xyz}	116.5°	111.0°	80.2°	82.8°	$+I_{xz}-I_{xy}$	77° - 116°	100°
Π_{xyz}	100.9°	155.2°	95.3°	85.9°	$-(I_{xz})^2-(W_{yz})^2$	78° - 149°	115°
Π_{xyz}	100.9°	21.3°	96.1°	86.8°	$+D_x$	54° - 115°	92°

Experimental and predicted values of the dependent variables for the four drugs (DEQ, CPF, ET, RHQ) and β-lactams (1-16), respectively.



a) Electrostatic potential of 1-20. 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.