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Chemometric and Molecular Graphics and Modeling Study on Bacterial β-Lactam Efflux Mechanism by Multidrug Resistance AcrB Pump

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ABSTRACT

The primary purposes of this work

- To establish relationships between activity expressed as log of minimal inhibitor concentration (pMIC) elevated by three strains of *Salmonella typhimurium* (HN891, SH7616, SH5014), and lipophilicity, electronic and hydrogen bond descriptors for 16 PM3 geometry optimized penicillins and cephalosporins at neutral pH.

- To visualize pump – drug molecular recognition mechanism, using crystal structure of AcrB transporter from *Escherichia coli*.

- These results can aid in explaining bacterial drug efflux mechanism, and design of novel β-lactams which would not be excreted from bacterial cells.

INTRODUCTION

- Antibiotics are characterized by their chemical composition and mode of action.
- Penicillins and cephalosporins have the cell wall as target for their action.
- β-lactam antibiotics are the most used antibacterial inhibitors of the Penicillin-Binding-Proteins (PBPs), which are responsible for the construction and maintenance of bacterial cell wall.
- There are different mechanisms by which bacteria exhibit resistance to antibiotics:

  1- Bacteria produce β-lactamases which hydrolyze the β -lactam antibiotic ring before their binding to PBPs.

  2- Bacteria change their permeability to the drug (passive membrane transport).

  3- Bacteria develop a structurally altered PBP that is still able to perform its metabolic function, but less affected by the drug.

  4- Bacteria change their express transport system that actively pump the drug to the outer cellular environment (Multi Drug Resistance MDR efflux pump).

- Most drug-resistant microorganisms emerge as a result of genetic change.

The major mechanism of MDR in bacteria is the pump drug efflux. In general this is accomplished by the presence of AcrAB-TolC efflux systems, which are responsible for the unidirectional pumping of a wide variety of lipophilic and amphiphilic compounds out of the cell.

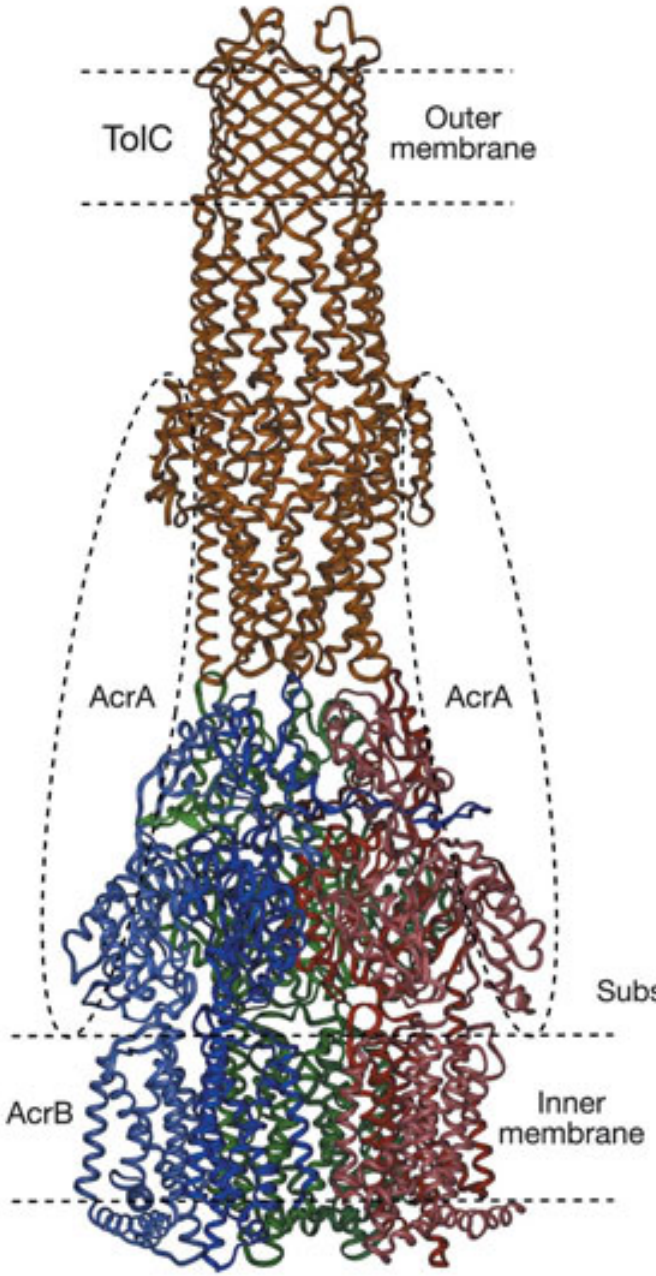
MDR PUMPS consist of 3 components:

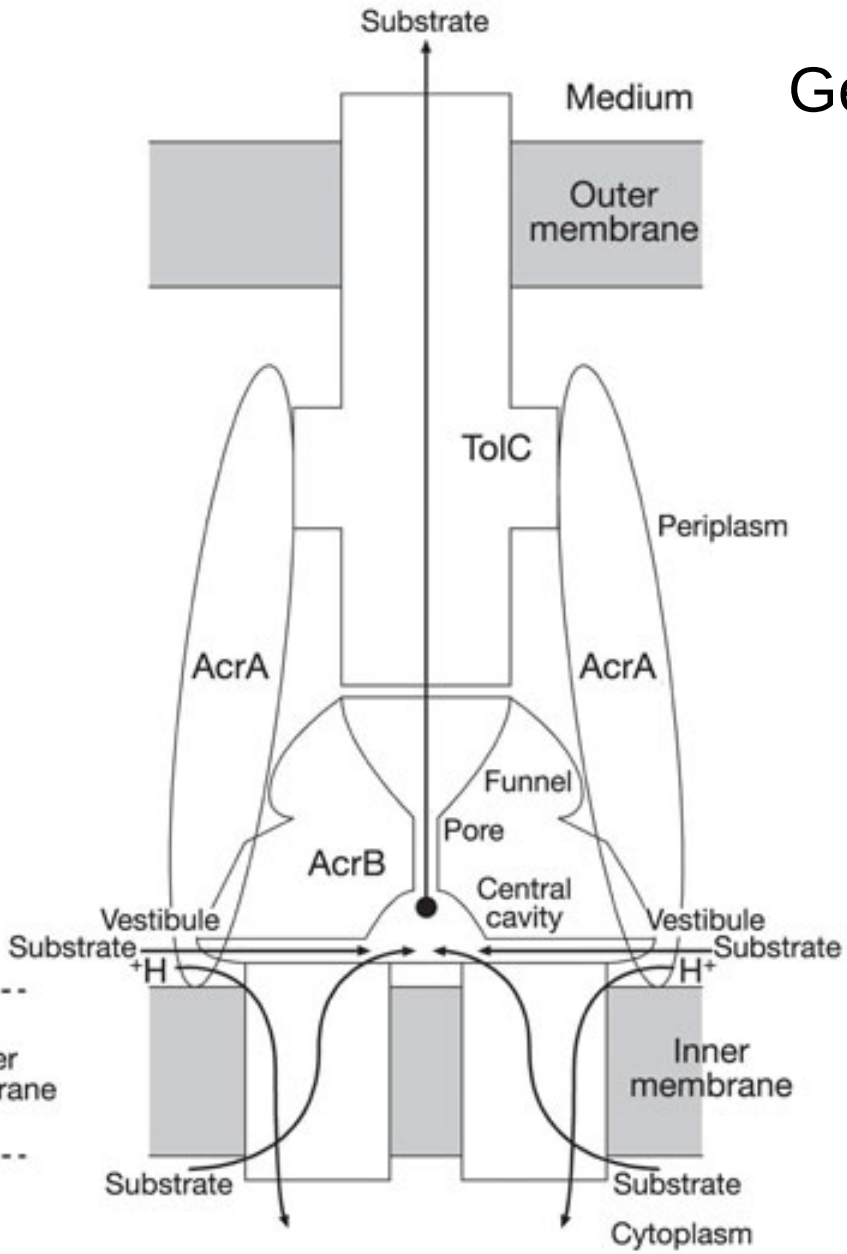
1- a resistance-nodulation-cell division transporter **AcrB** (trimeric)
2- an outer membrane channel protein of the family **TolC** (trimeric)
3- a membrane fusion lipoprotein **AcrA** (probably trimeric also)

FACTORS THAT INFLUENCES THE MULTI DRUG EFFLUX RATE

Pumps number
Substrate concentration
pH
Highly charged residues
Substrate charged groups

General





AcrAB-TolC bacterial pump.

S. Murakami *et al*., *Nature*, **419** (2002) 587.

METHODOLOGY

MICs for bacterial strains → Mass concentration MICs (from literature) for 16 β-lactams effluxed by bacterial strains *S. typhimurium* SH5014 (parent strain), SH7616 (an *acr* mutant) and HN891 (an overproducer of the Acr pump).

Drugs Modeling → Molecular structures were refined or modeled by Spartan Pro using atomic coordinates from PPSD, CSD or 2D formula. Conformational search was done by Montecarlo method and the most stable conformers were optimized by PM3.

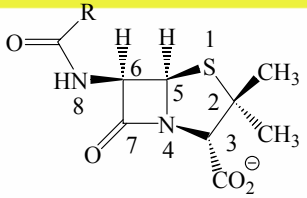
Lipophilicity Parameters → $\log K_{OW}$ was from Nikaido *et al.*; gas-phase lipophilicity ($\log P_{GC}$) was calculated by Spartan; several octanol-water partitition coefficients were calculated by free web programs using different approaches: $\log P_{w}$; $\log P_{s}$, $\log P_{IA}$, $\log K_{WIN}$ and $\log P_{X}$.

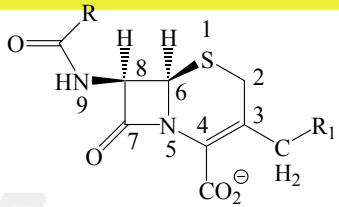
w_C, S_f → are the number fraction and surface fraction of hydrophobic carbon atoms, respectively.

Electronic properties → dipole moment D and its components; gap between frontier molecular orbitals Δ, third-order molecular polarizability γ; number fraction of heteroatoms H_f; the number of charged groups N_{CH}; the number of nitrogen and sulfur atoms N_{NS}; the number of all π- and lone pair electrons divided by molecular surface σ_π; the sum of overall atomic numbers for substituents R and R_1 Z.

Hydrogen bond (HB) parameters → the number of hydrogen bond acceptors A_{HB},; the number of hydrogen bonds divided by the number non-H atoms <HB>.

Molecules





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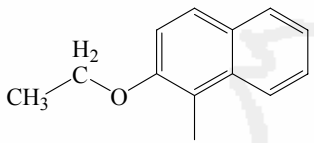
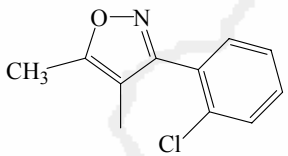
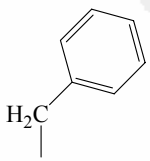
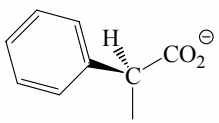
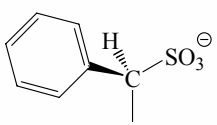
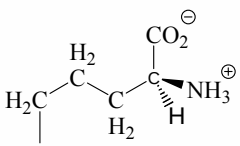
1	
2	
3	
7	
8	
15	

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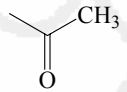
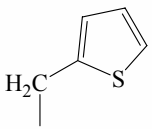
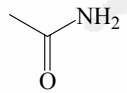
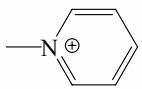
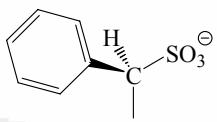
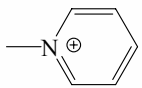
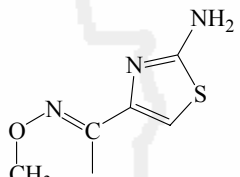
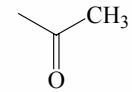
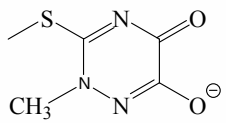
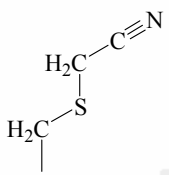
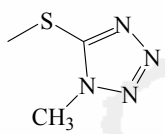
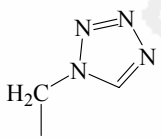
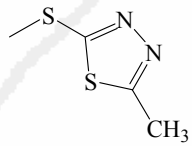
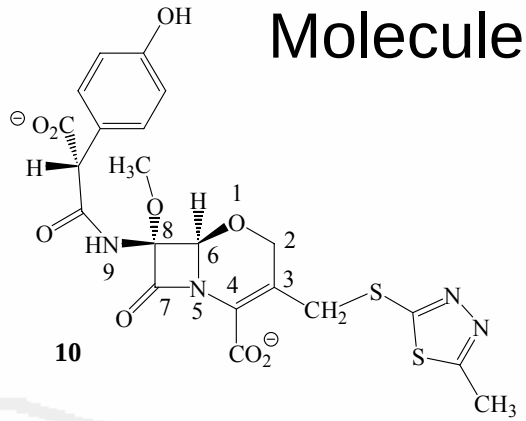
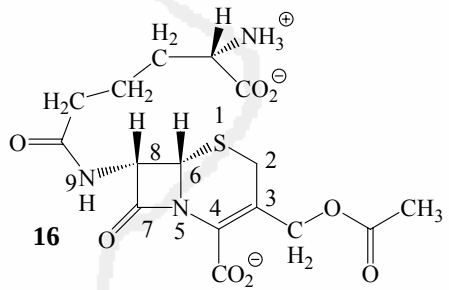
4		
5		
6		
9		
11		
12		
13		
14		

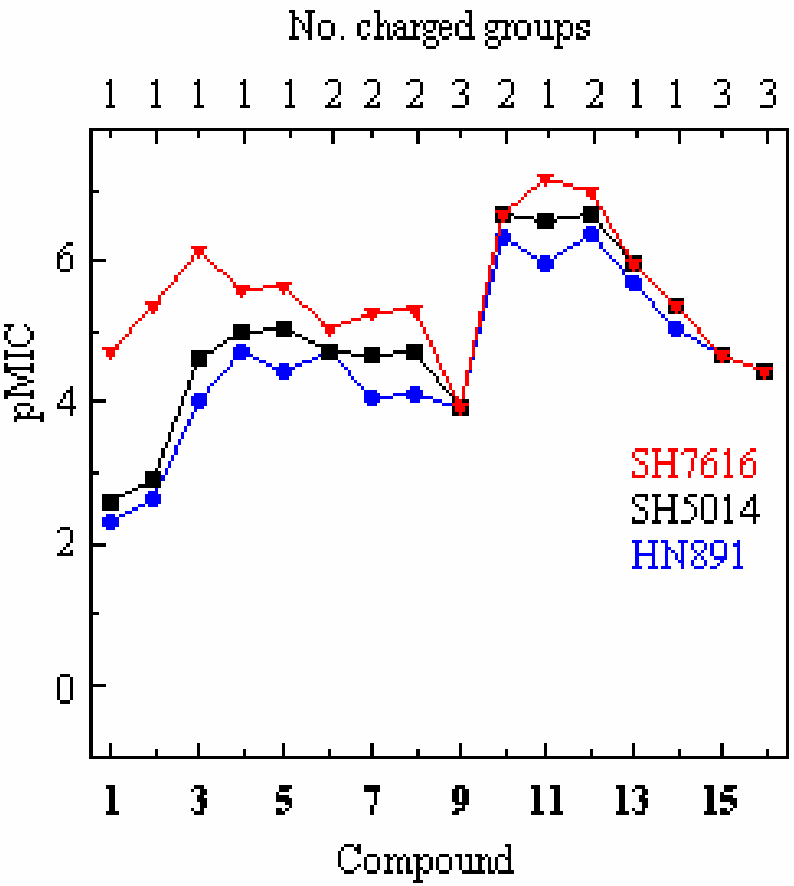


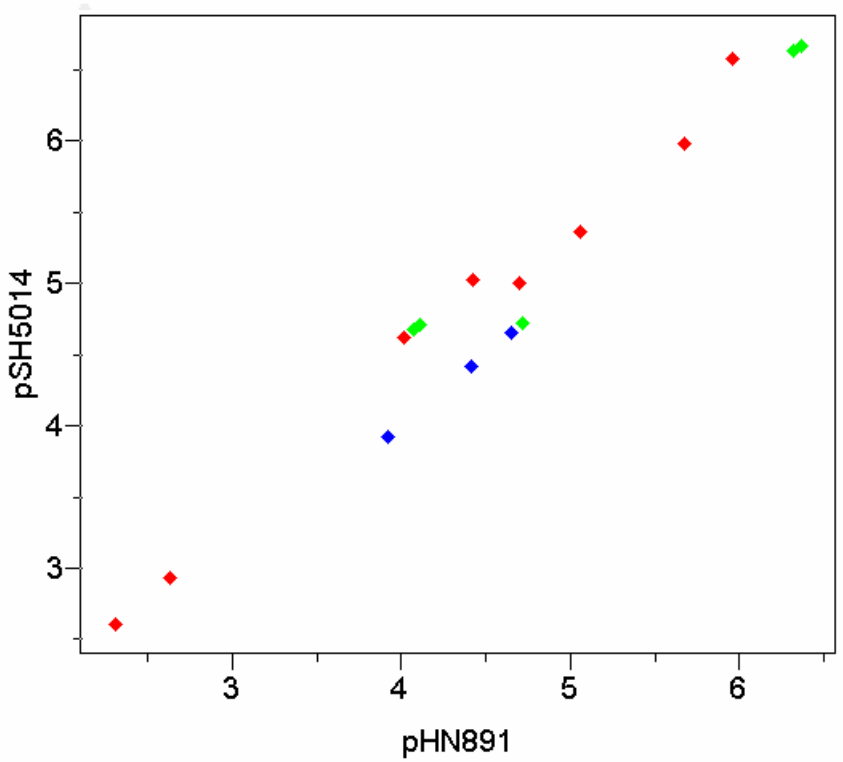


1: Nafcillin
2: Cloxacillin
3: Penicillin G
4: Cephalothin
5: Cefoxitin
6: Cephaloridine
7: Carbenicillin
8: Sulbenicillin
9: Cefsulodin
10: Latamoxef
11: Cefotaxime
12: Ceftriaxone
13: Cefmetazole
14: Cefazolin
15: Penicillin N
16: Cephalosporin C

16 antibiotics (penicillins and cephalosporins) as AcrB substrates

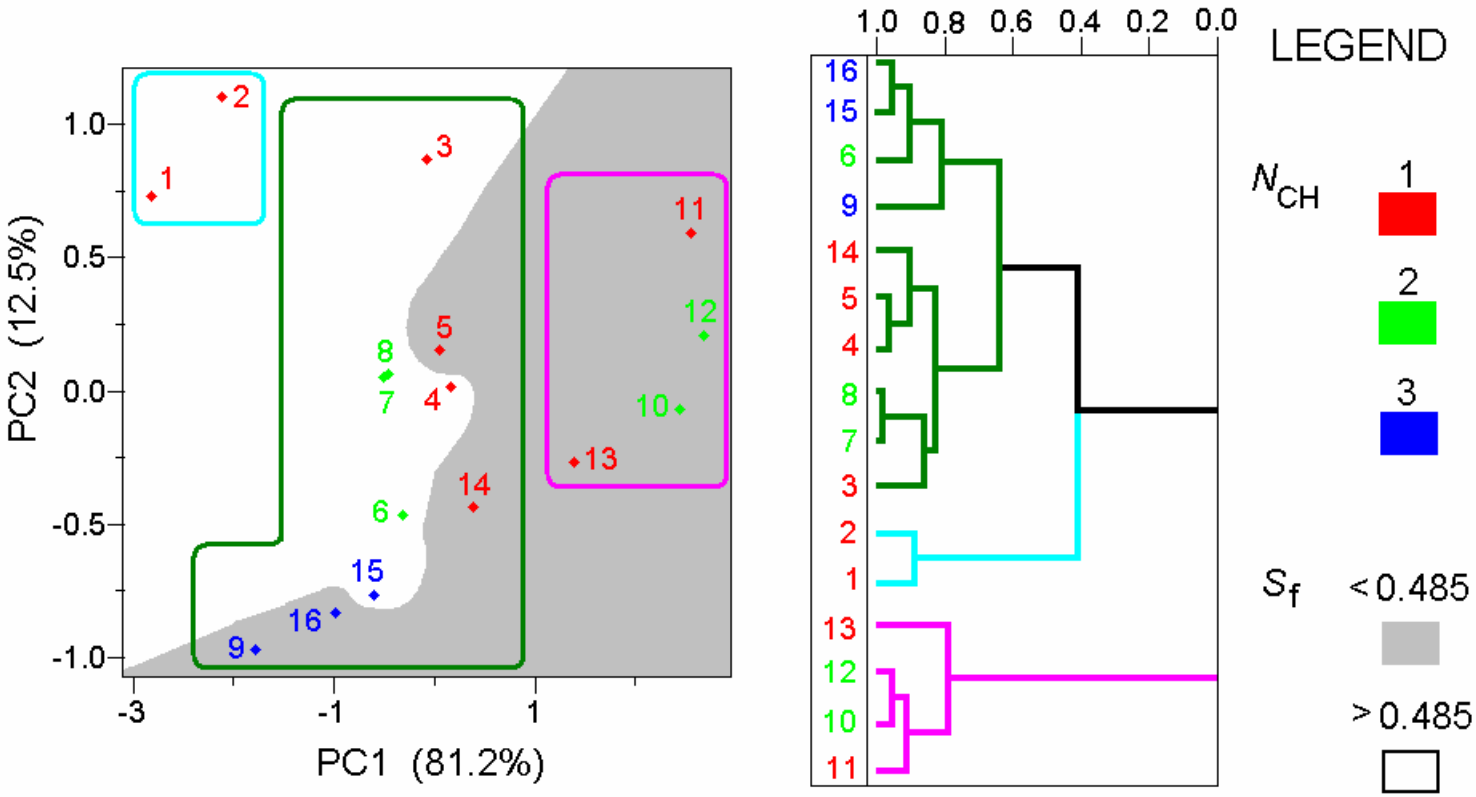
Correlation of pMICS





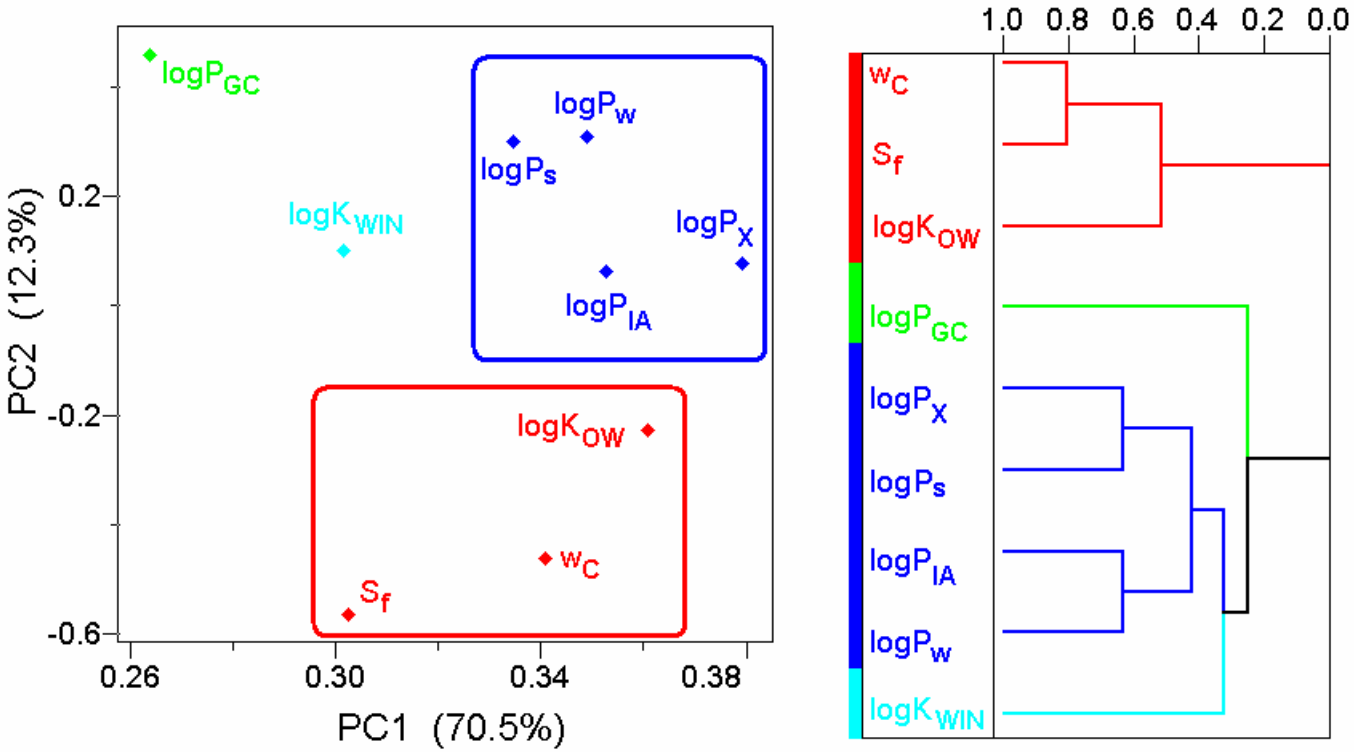
Correlation between the three pMICs. pHN891 and pSH5014 are highly correlated (right). pSH7616 shows different trend (left). Antibiotics which bear 3 charges are effluxed by the three strains in the very same way.

Chemometrics of pMICs



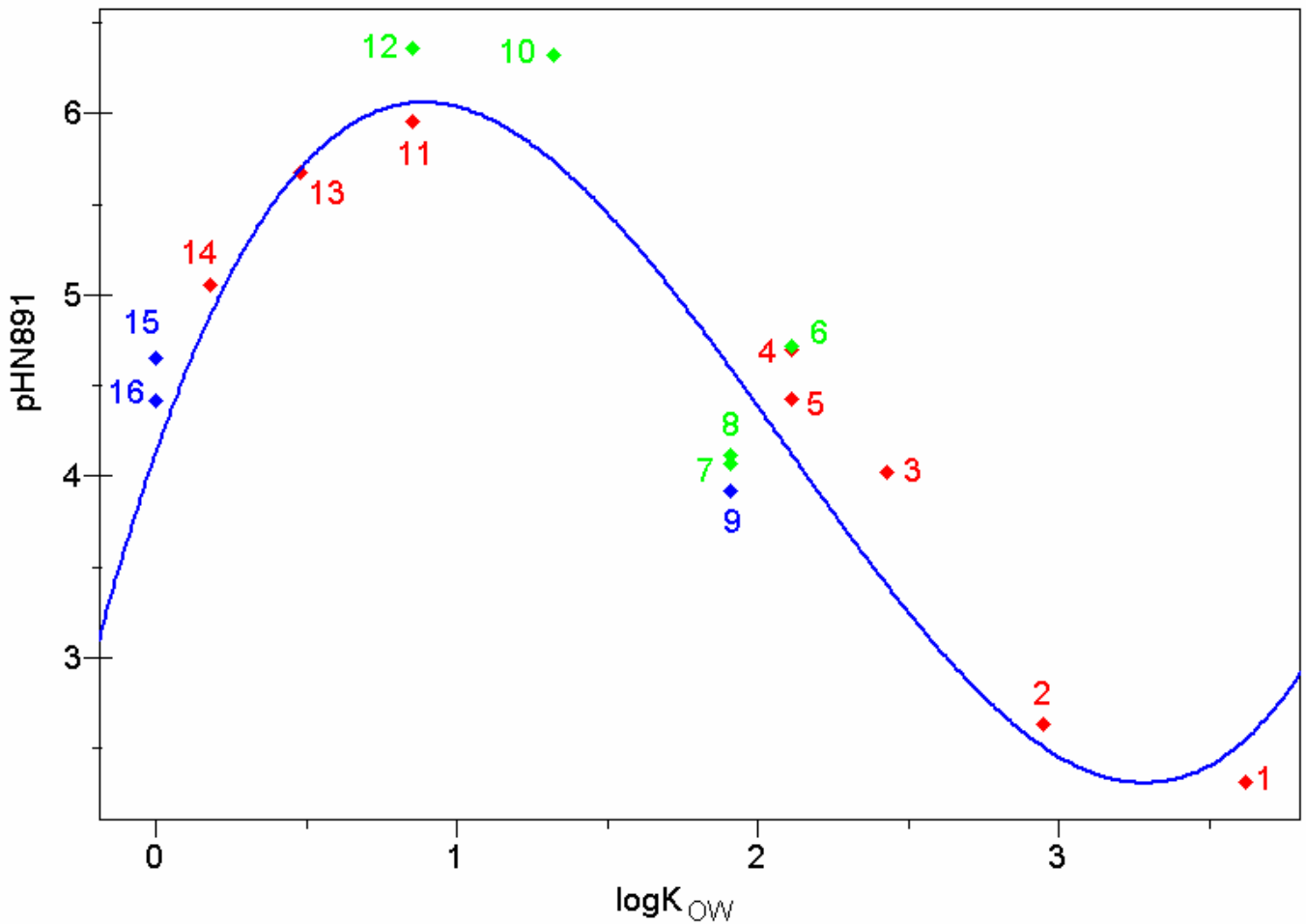
β-Lactams were classified as **good**, **moderately good to poor**, and **bad** AcrB substrates. Clustering of β-lactams with respect to the number of charged groups N_{CH} and hydrophobic surface fraction S_f is visible. PCA and HCA were performed using only pMIcs data.

Chemometrics of lipophilicity descriptors



PCA (left) and HCA (right) analysis of 9 lipophilicity descriptors: logP for gas-phase ($logP_{GC}$) and liquid chromatography ($logK_{WIN}$, $logP_s$, $logP_w$, $logP_{IA}$, $logP_x$), $logK_{OW}$, surface fraction (S_f) and number fraction (w_C) of hydrophobic carbons. Two clusters and two isolated logPs are visible. The lipophilicity descriptors do not contain the same information (82.8% of the variance contained in PC1 + PC2).

Lipophilicity – pMIC relationships



An example of non-linear lipophilicity-activity relationship. 3rd order polynomial fits pMIC = a + b logP + c $(\log P)^2$ + d $(\log P)^3$ were used to generate new lipophilicity descriptors $L(\log P) = \log P + (c / b)(\log P)^2 + (d / b)(\log P)^3$ for QSAR study.

PLS regression models for pMICs

pMIC	Parameters	SEP	*Q*	*R*	PCs
HN891	$\log P_{IA}$, $L(\log K_{OW})$, $L(\log K_{WIN})$, $L(\log P_X)$ $L(\log P_s)$, w_C, S_f	0.369	0.946	0.984	4 (84%)
	Z, H_f, <HB>, γ, N_{NS}	0.699	0.784	0.850	1 (65%)
	$\log P_{IA}$, $L(\log K_{OW})$, S_f, H_f, Z, <HB>, γ	**0.276**	**0.969**	**0.989**	**4** (91%)
SH5014	$\log P_{IA}$, $L(\log K_{OW})$, $L(\log K_{WIN})$, $L(\log P_X)$ $L(\log P_s)$, w_C, S_f	0.529	0.893	0.977	3 (82%)
	Z, H_f, <HB>, γ, N_{NS}	0.773	0.745	0.821	1 (65%)
	$\log P_{IA}$, $L(\log K_{OW})$, S_f, H_f, Z, <HB>, γ	**0.405**	**0.943**	**0.980**	**4** (87%)
SH7616	$\log P_{IA}$, $L(\log K_{OW})$, $L(\log K_{WIN})$, $L(\log P_X)$ $L(\log P_s)$, w_C, S_f	0.640	0.694	0.788	1 (52%)
	Z, N_{CH}, <HB>, γ, N_{NS}	0.627	0.714	0.883	3 (81%)
	$L(\log K_{WIN})$, $L(\log P_X)$, S_f, γ, Z,	**0.508**	**0.821**	**0.893**	**3** (82%)

It is visible that the best PLS models are obtained when all types of parameters are used: lipophilic, electronic and hydrogen bonding.

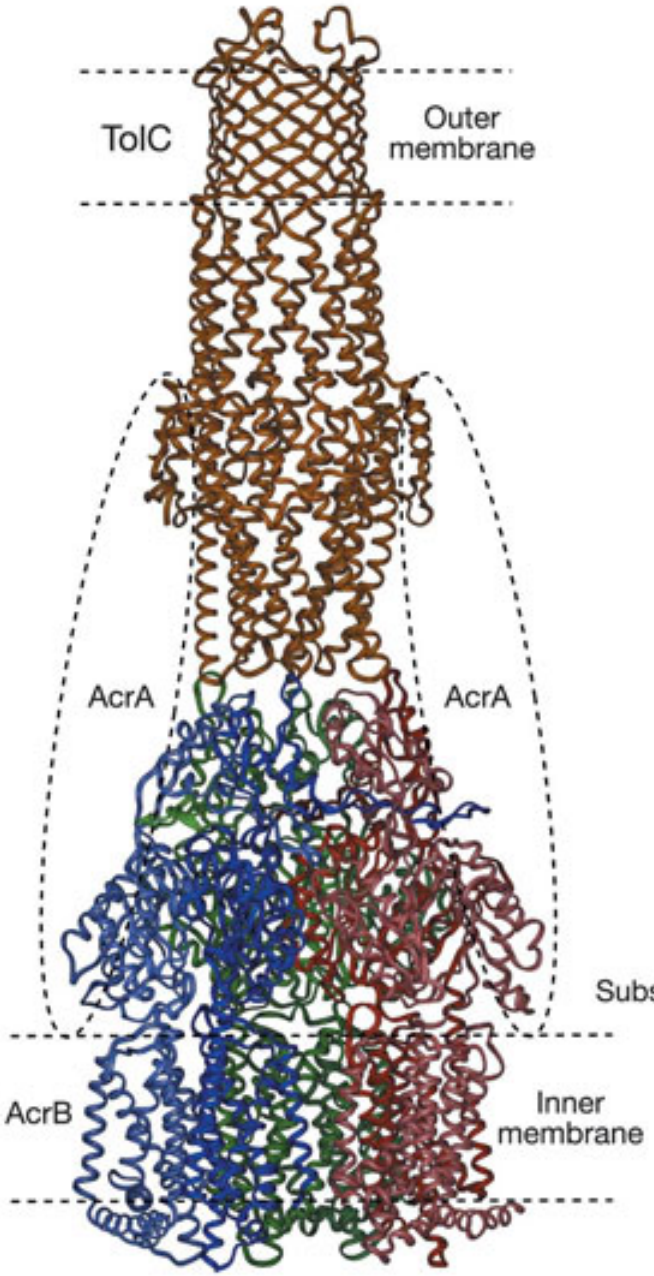
Experimental[a] and Predicted pMICSH5014[b]

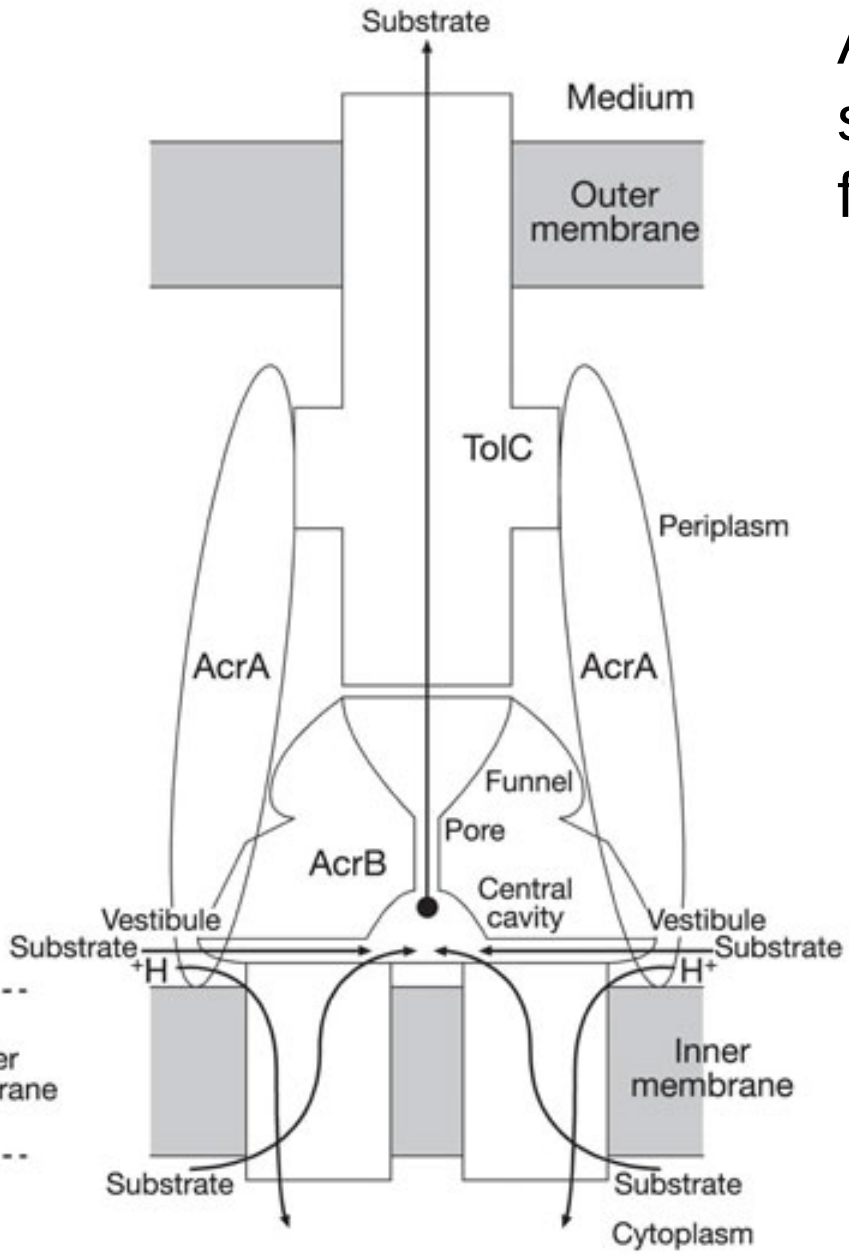
Nafcillin (**1**)	2.607	2.894
Cloxacillin (**2**)	2.930	2.786
Penicillin G (**3**)	4.621	**3.844**
Cephalothin (**4**)	4.996	4.930
Cefoxitin (**5**)	5.029	4.984
Cephaloridin (**6**)	4.715	5.153
Carbenicillin (**7**)	4.675	5.088
Sulbenicillin (**8**)	4.714	4.969
Cefsulodin (**9**)	3.919	**3.104**
Latamoxef (**10**)	6.637	6.254
Cefotaxime (**11**)	6.579	6.440
Ceftriaxone (**12**)	6.665	7.071
Cefmetazole (**13**)	5.975	5.981
Cefazolin (**14**)	5.357	5.334
Penicillin N (**15**)	4.652	4.319
Cephalosporin C (**16**)	4.414	**5.033**

Except for 3 samples, exp-cal differences are smaller than 10%.

[a]H. Nikaido *et al*., *J. Bacteriol*., **180 (**1998**)** 4686. [b]MIC are in mols per liter.

AcrAB-TolC structure and function

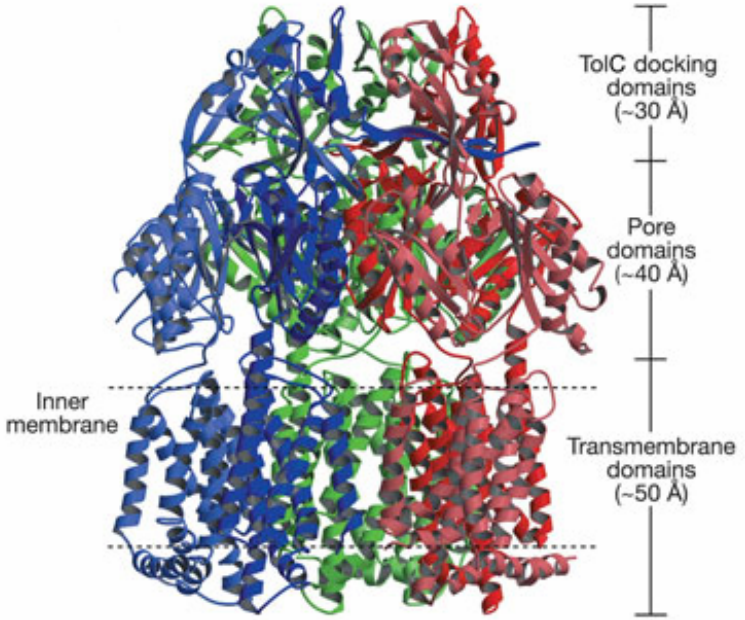




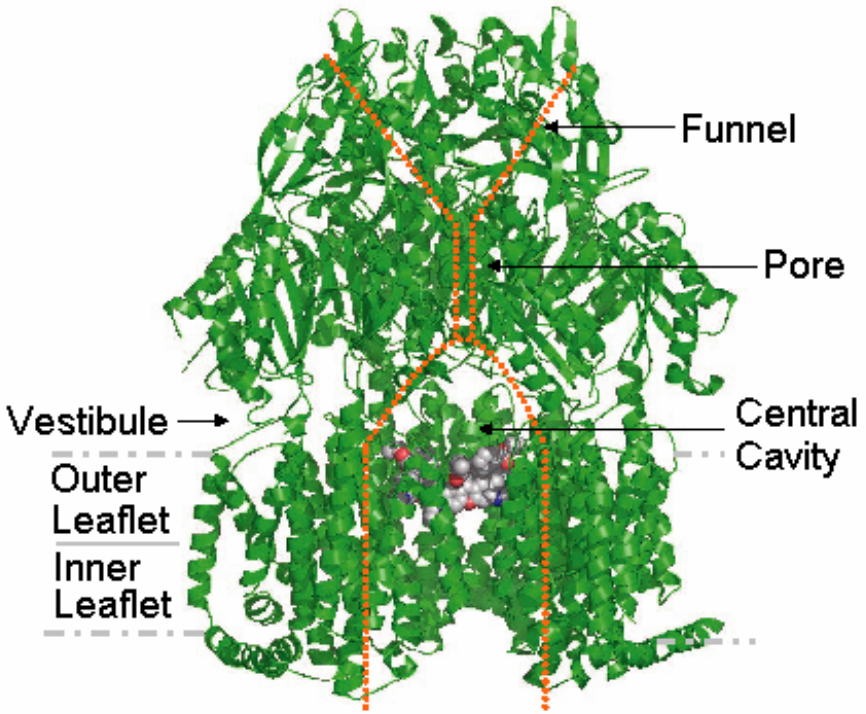
AcrAB-TolC bacterial pump.

S. Murakami *et al*., *Nature*, **419** (2002) 587.

AcrB crystal structure



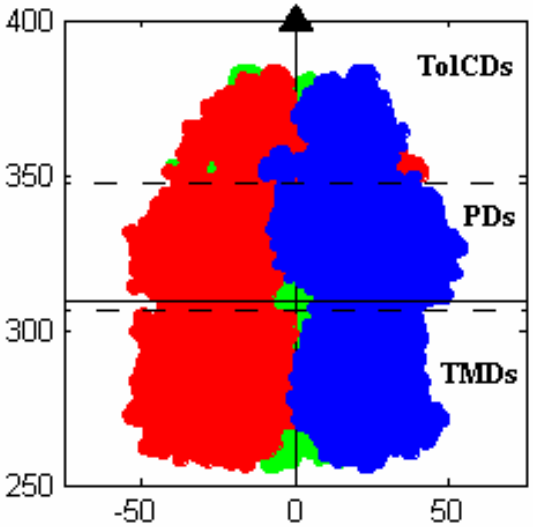
Nature **419** (2002) 587.

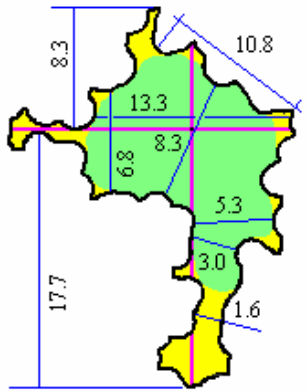


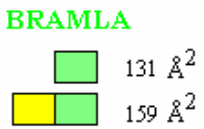
Science **300** (2003) 976.

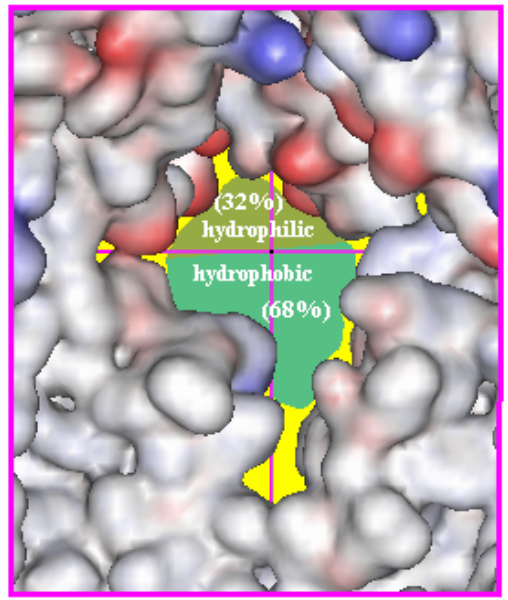
Crystal structure of the AcrB trimer determined by X-ray diffraction: protein without (left) and with a ligand (right). Three distinctive units are visible: **TolC docking domains**, **Pore domains** and **Transmembrane domains**. The system of cavities and channels for drug efflux can be also noted: **the three vestibules**, **the large central cavity**, **the narrow pore**, and **the cone-like funnel**.

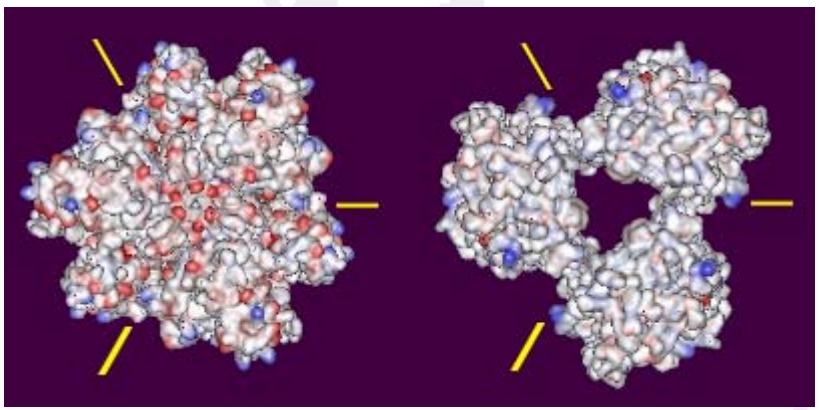
The vestibule structure







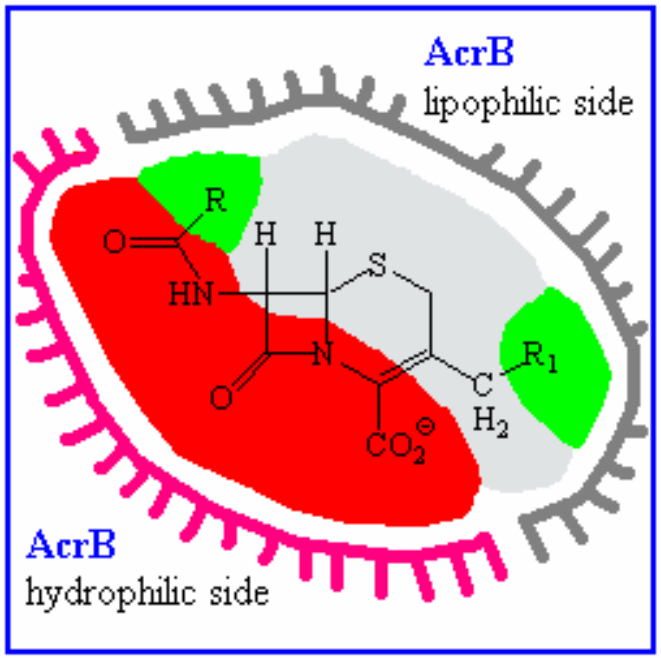


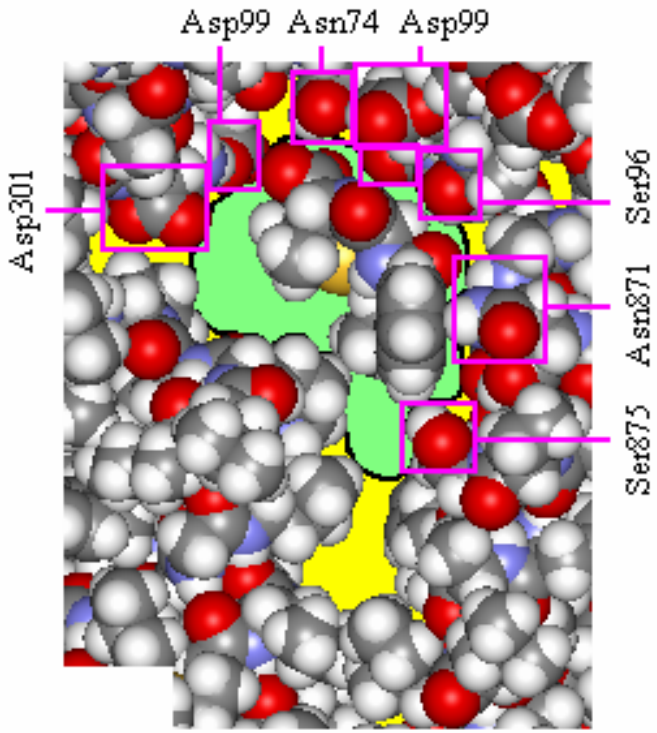


Left: Electrostatic potential of the pore anf the transmembrane domains.

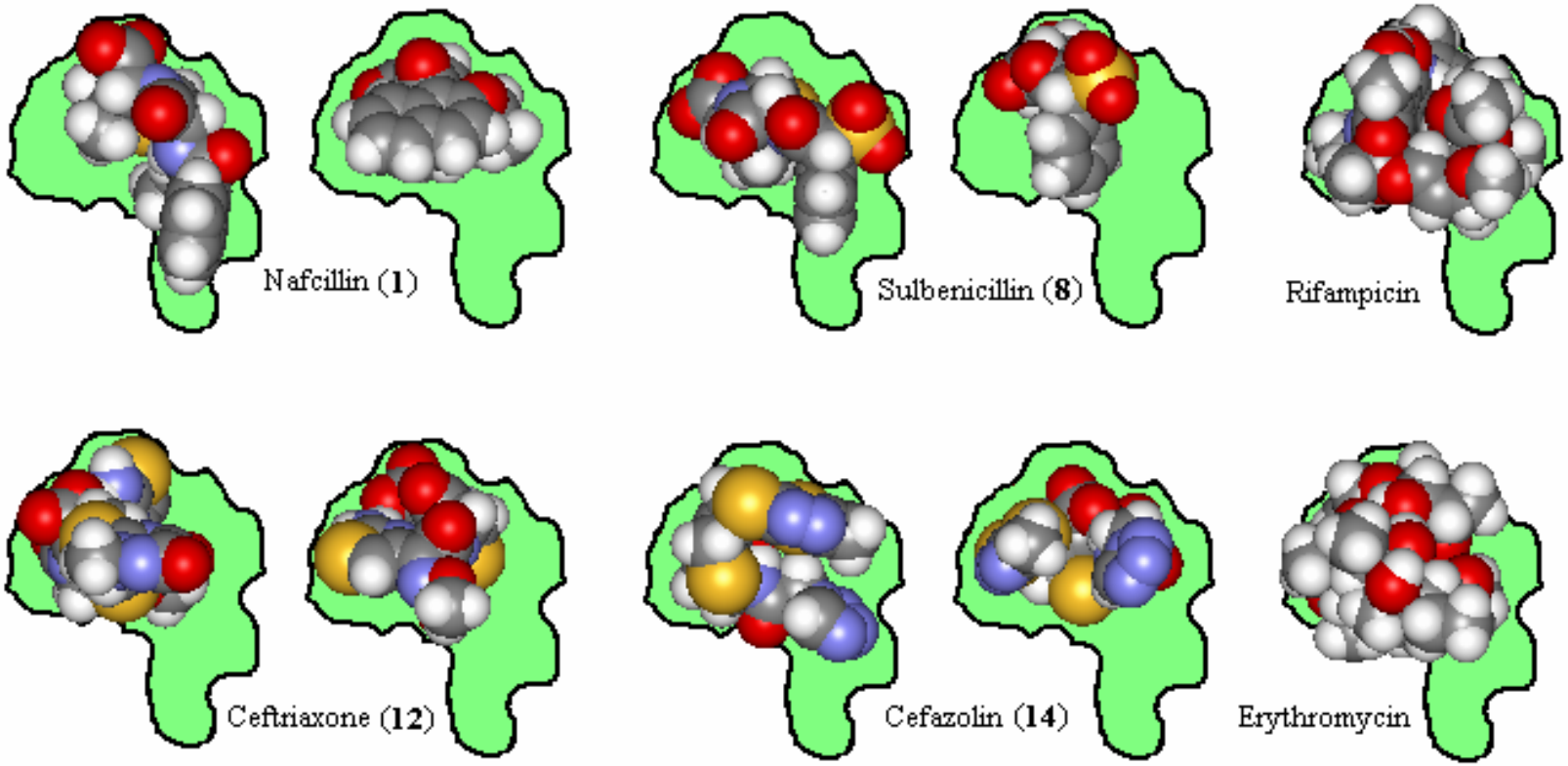
The vestibule's projection has functional surface through which the drug can pass without difficulty. This area is called BRAMLA, due to its resemblance with the map of Brazil (**BRA**zil **M**ap-**L**ike **A**rea). The upper third of BRAMLA is surrounded by hydrophilic and the other two thirds by hydrophobic residues of the AcrB.

The vestibule-drug interactions



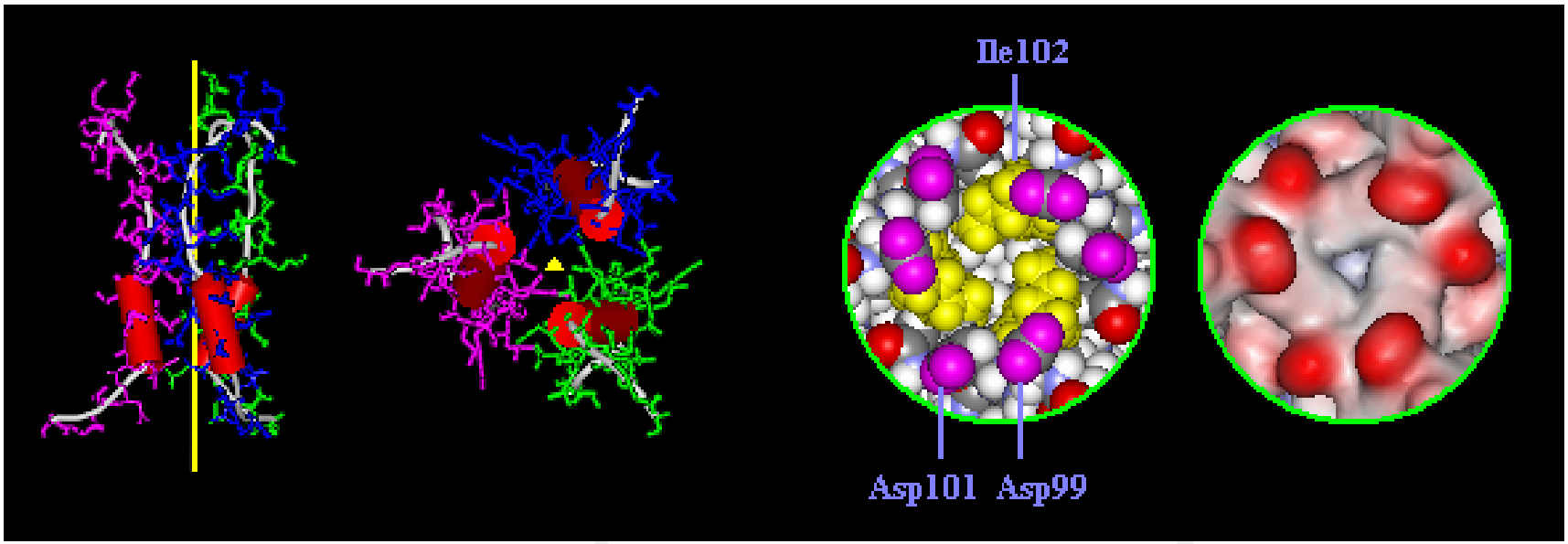


Left: Schematic representation of drug-vestibule stereolectronic complementarity that was deduced from similarity of the 16 antibiotic structures and importance of lipophilic, electronic and hydrogen bonding molecular parameters. Molecular recognition is obvious, and it can be weaken or enhanced by the nature of R and R_1 side chains. Right: 3D docking of nafcillin (**1**) to the vestibule. Interactions between hidrophilic AcrB residues (in rectangles) and nafcillin polar groups are visible.



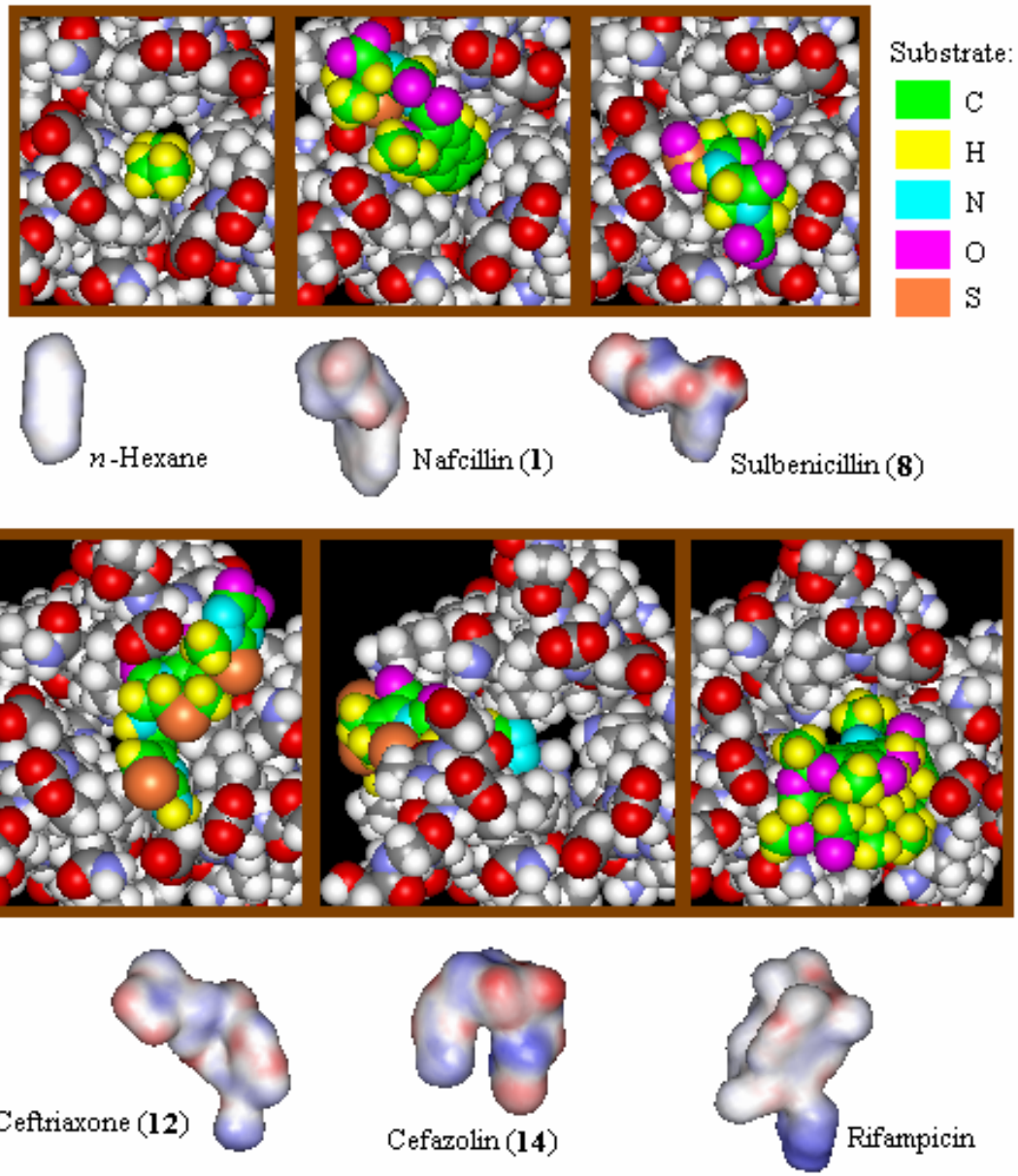
2D docking of selected AcrB substrates to the BRAMLA area, using maximum and minimum (right) stereoelectronic fit approach for some antibiotics. It can be noticed that the antibiotic molecules differ in how well then can fit sterically and electronically to the vestibule. These fittings correspond to biological activities for the presented antibiotics.

The pore structure



The structure of the pore channell (left figures) and the pore recognition site (right figures) viewed perpendicularly to or along the three-fold axis of the AcrB protein. The pore channel consists of three short α-helices and three random coils. The pore recognition site contains highly hydrophobic (yellow) and hydrophilic (red or pink) residues: these residues are selective with respect to drugs due to hydrophobic, polar and hydrogen bond interactions.

The pore-drug interactions



Some drugs docked to the pore recognition site.

Lipophilic drugs enter the pore channel easier than hydrophilic ones due to:

1) weaker intermolecular interactions;

2) more favourable drug-pore recognition.

These conclusions, based on 3D docking of the presented drugs, are in agreement to chemometric results.

CONCLUSIONS

PLS models of good quality were obtained using lipophilic, electronic and hydrogen bond descriptors for 16 β-lactams.

Proposed efflux mechanism based on chemometrics and molecular graphics and modeling methods:

1) a drug molecule comes from periplasmic space and interacts with a vestibule through a mechanism of molecular recognition → large and highly hydrophilic molecules hardly enter the vestibule and come to the central cavity of AcrB protein.

2) a drug molecule from the central cavity comes to the pore recognition site and through a mechanism of molecular recognition enters the pore channel → again large and highly hydrophilic molecules hardly enter the pore channel to be excreted from the cell.

ACKNOWLEDGEMENT

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