QSAR, Molecular Graphics and Modeling Study on β-Lactam Antibiotics as Substrates of the Multidrug Resistance Efflux AcrB Pump

Rudolf Kiralj (PQ) and Márcia M. C. Ferreira (PQ)*, Laboratório de Quimiometria Teórica e Aplicada, Instituto de Química, Universidade Estadual de Campinas, Campinas, SP, 13083-970, Brazil.

marcia@iqm.unicamp.br, rudolf@iqm.unicamp.br, http://lqta.iqm.unicamp.br

INTRODUCTION

AcrAB-TolC is the most important multidrug resistance efflux pump system of gram-negative bacteria.[1] This resistance includes β-lactam antibiotics, known as the most widely used antibacterial agents which primarily inhibit penicillin-binding proteins responsible for the construction and maintenance of the bacterial cell wall. AcrAB-TolC pump consists of transport protein AcrB, linker lipoprotein AcrA, and the channell-tunell TolC. It is supposed that drugs from cytoplasm or periplasm enter into AcrB, and then by proton motive force are transferred to TolC, from where they are excreted directly to the bacteria exterior. Bacterial resistance is initiated by high concentration of drugs and their metabolites which induce overexpression of efflux pumps. Besides pronounced lipophilic/amphiphilic character and the presence of multiple charges, there is no obvious structural similarity among exctreted drugs, including β-lactam antibiotics. The primary purpose of this work[2] is to establish relationships between activity expressed as log of minimal inhibitor concentration (pMIC) elevated by three strains of *Salmonella typhimurium* (HN891, SH7616, SH5014)[1], and lipophilicity, electronic and hydrogen bond descriptors for 16 PM3 geometry optimized penicillins and cephalosporins at neutral pH. The next aim is to visualize pump – drug molecular recognition mechanism, using crystal structure of AcrB transporter from *Escherichia coli*.[3] These results can aid in explaining bacterial drug efflux mechanism, and design of novel β-lactams which would not be excreted from bacterial cells.

[1]Nikaido, H., Basina, M., Nguyen, V., Rosenberg, E. Y. *J. Bacteriol*. **1998**, 180, 4686. [2]Kiralj, R.; Ferreira, M. M. C., J. Bacteriol., submitted. [3]Koronakis, V., Sharff, A., Koronakis, E., Luisi, B., Hughes, C. *Nature*, **2000**, 405**,** 914.

METHODS

MICs for bacterial strains: negative logarithm of minimum molar inhibitor concentration (pMIC) for 16 penicillines and cephalosporins, increased by bacterial strains *Salmonella typhimurium* SH5014 and its mutants SH7616 and HN891, as well as the ratio of MICs for SH5014 and SH7616, denoted as A/B, was from from Nikaido *et al*.[1]

Modeling of drugs: molecular structures of 16 β-lactam antibiotics, *n*-hexane, erythromycin and rifampicin were refined/modeled by PC Spartan Pro using atomic coordinates from the 3D Pharmaceutical Plug-in Structure Database or the Cambridge Structural Database (NCSA ChemViz).

Lipophilicity parameters: logarithm of the octanol-water partition coefficient logK_{OW} was from Nikaido *et al.*[1] and also calculated by various programs and methods. These different scales of lipophilicity were analyzed by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

Other molecular descriptors: geometrical, electronic and hydrogen bond molecular properties were calculated using 2D or 3D geometry of the antibiotics.

QSAR studies: Partial Least Squares Regression (PLS) models were built to predict pMICs from lipophilicity and all descriptors, using some of them in non-linear form.

Molecular graphics and docking studies: 2D and 3D docking of some drugs into an AcrB vestibule from AcrB cystal structure was performed. 3D docking study of some drugs to the pore was also carried out.

SOME RESULTS & COMMENTS

To see related Figures and Tables below the text.

Chemometric analysis of pMICs: HCA and PCA revealed that lipophilicity and charges are important in excretion of β-lactams by bacterial strains. The presence of three charges in a molecule cause all strains to act the same way. β-Lactams were classified as good, moderately good to poor, and bad AcrB substrates.

Chemometric analysis of lipophilicity: 9 lipophilicity parameters, mostly logP values calculated by various methods, analyzed by HCA & PCA, show heterogenicity described by the first three principal components and a few clusters of lipophilicity parameters.

QSAR studies. PLS models for calculation of pMICs included only 2 lipophilicity descriptors in parabolic form, and 2 electronic and HB descriptors. Molecular dipole moment and its Y-component are essential for the drug action with respect to AcrB.

2D and 3D docking to vestibules: through 3 AcrB vestibules drugs can come inside the transporter. Vestibules' structural and electronic properties, especially of their 2D projection BRAMLA (Brazil Map-Like Area), agree with chemometric - QSAR study. 2D docking of molecular images of some β-lactams, rifampicin, erythromycin to BRAMLA, as well as docking of nafcillin to 3D vestibule structure, confirm the above findings.

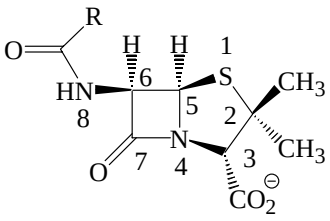
3D docking to the pore: the pore is the AcrB channel connected to TolC. Docking of selected β-lactams, *n*-hexane and rifampicin to the pore recognition site is in accord to the above analyses. The pore – drug complexes optimized by molecular mechanics MMFF94 field, reveal that poor AcrB substrates bind to the pore recognition site *via* hydrogen bonds and do not enter the pore channel.

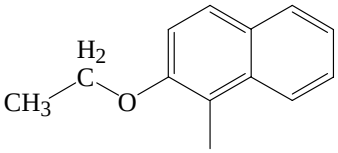
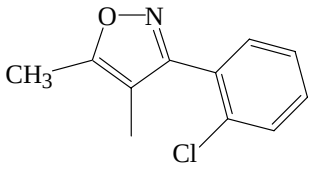
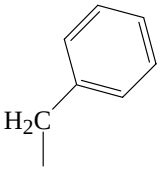
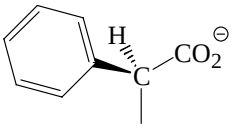
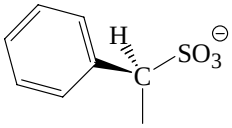
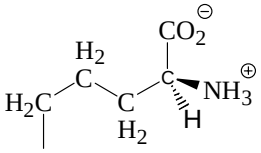
CONCLUSIONS

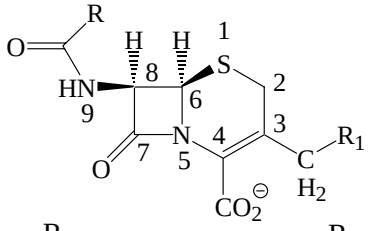
Lipophilicity parameters in parabolic form, electronic and hydrogen bonding properties of β-lactams determine their efflux elevated by AcrB pumps. Steric properties play an important role in pore-drug recognition mechanism. Based on all studies performed in this work, a rationale for drug efflux mechanism can be proposed in terms of drug and vestibule/pore molecular properties. However, the study demonstrated that various lipophilicity scales do not contain necessarily the same information, neither are sufficient for prediction of antibiotic pMICs.

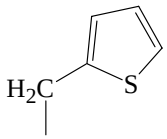
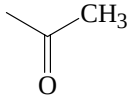
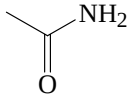
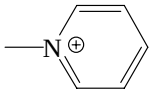
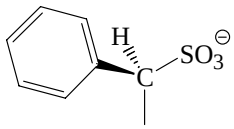
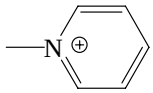
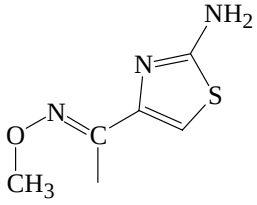
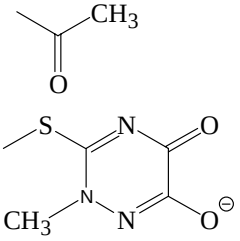
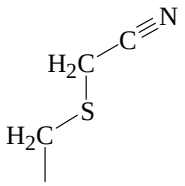
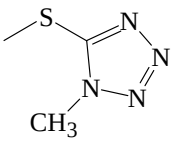
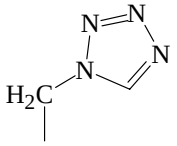
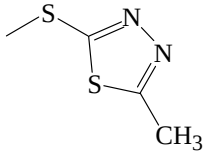
ACKNOWLEDGEMENT

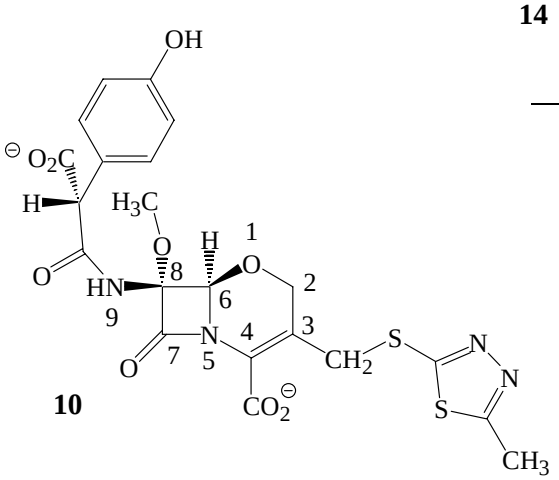
The authors thank to FAPESP.



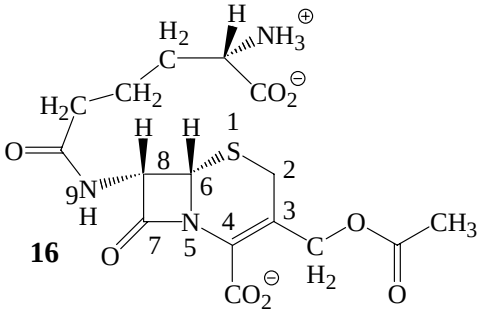
No.	R
1	
2	
3	
7	
8	
15	



No.	R	R_1
4		
5		
6		
9		
11		
12		
13		
14		

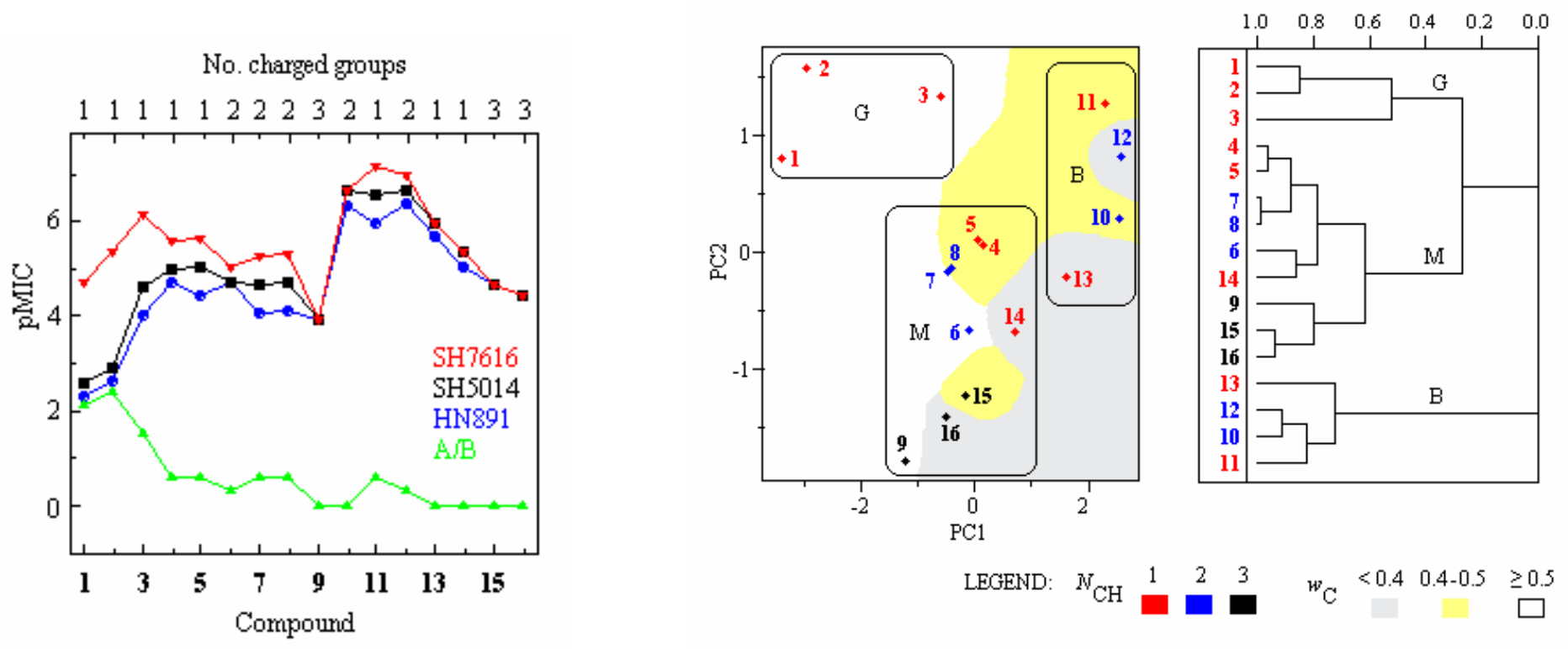


10

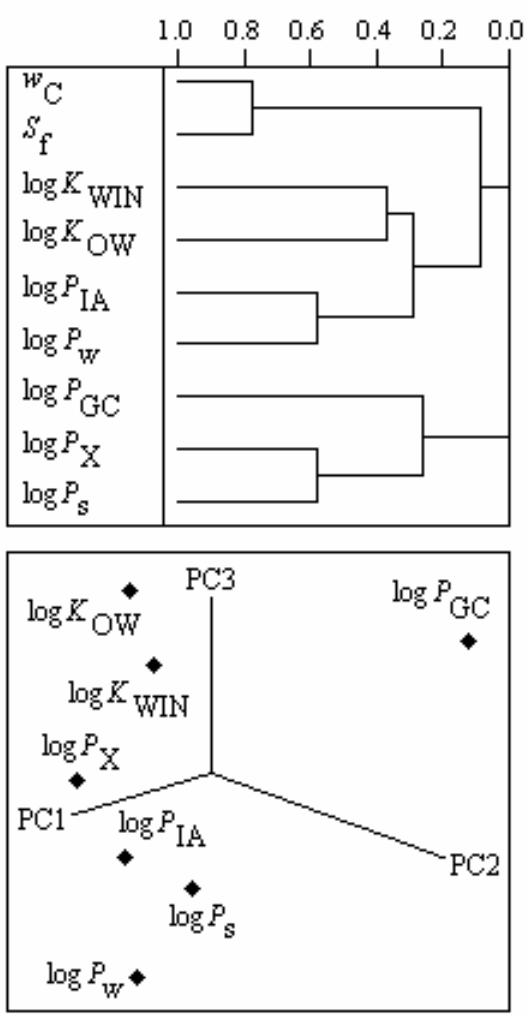


16

Antibiotics under study

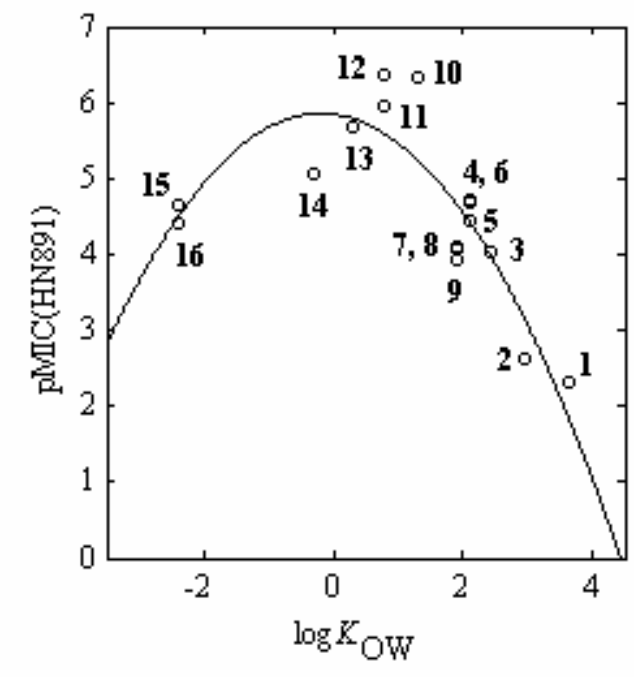


Left: Comparison of pMICs for β-lactams. pMIC for *S. typhimurium* SH5014 strain is placed in between the pMICs for its mutants SH7616 and HN891. pMIC(A/B) has significantly different characteristics. The number of charged groups in antibiotic molecules at neutral pH shows that different bacterial strains are not distinguished well when exctreting highly charged antibiotics Right: PCA scores plot shows the position of β-lactams in the space defined by principal components PC1 and PC2. HCA dendogram of samples (β-lactams). Both plots exhibit clustering of good (G), moderately good to poor (M), and bad (B) AcrB substrates. Also, other clustering of the antibiotics with respect to their molecular properties (N_{CH}, w_C), is visible. PCA and HCA was performed using only pMIcs data.

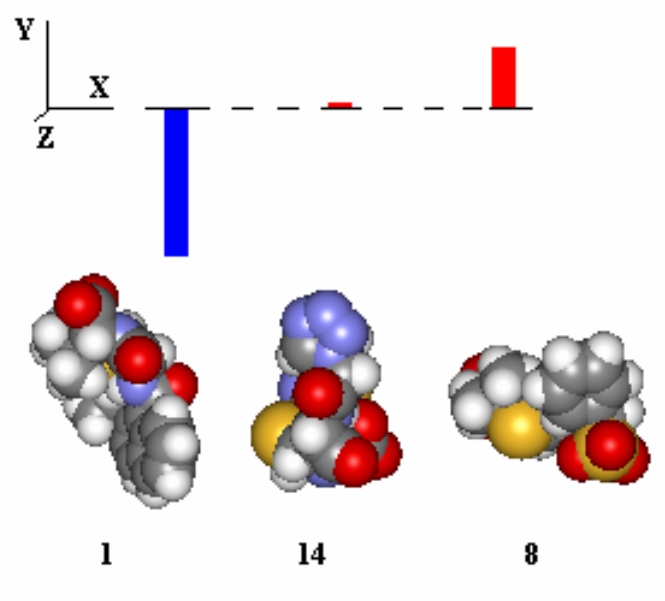


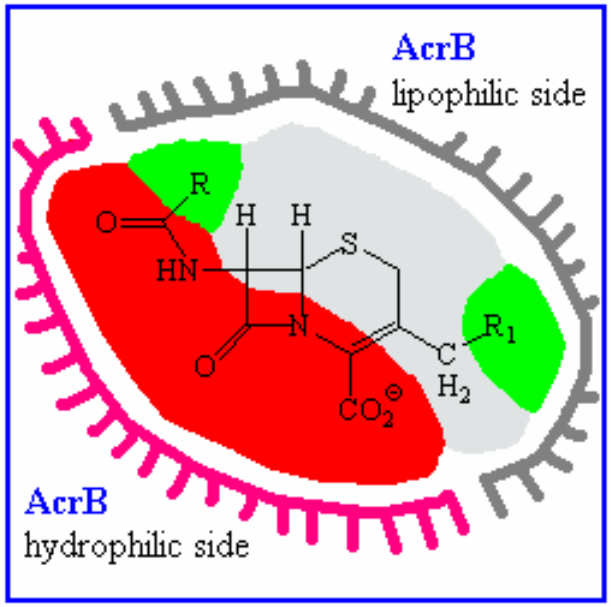
HCA dendogram on (top) and PCA loadings plot (bottom)

for lipophilicity parameters

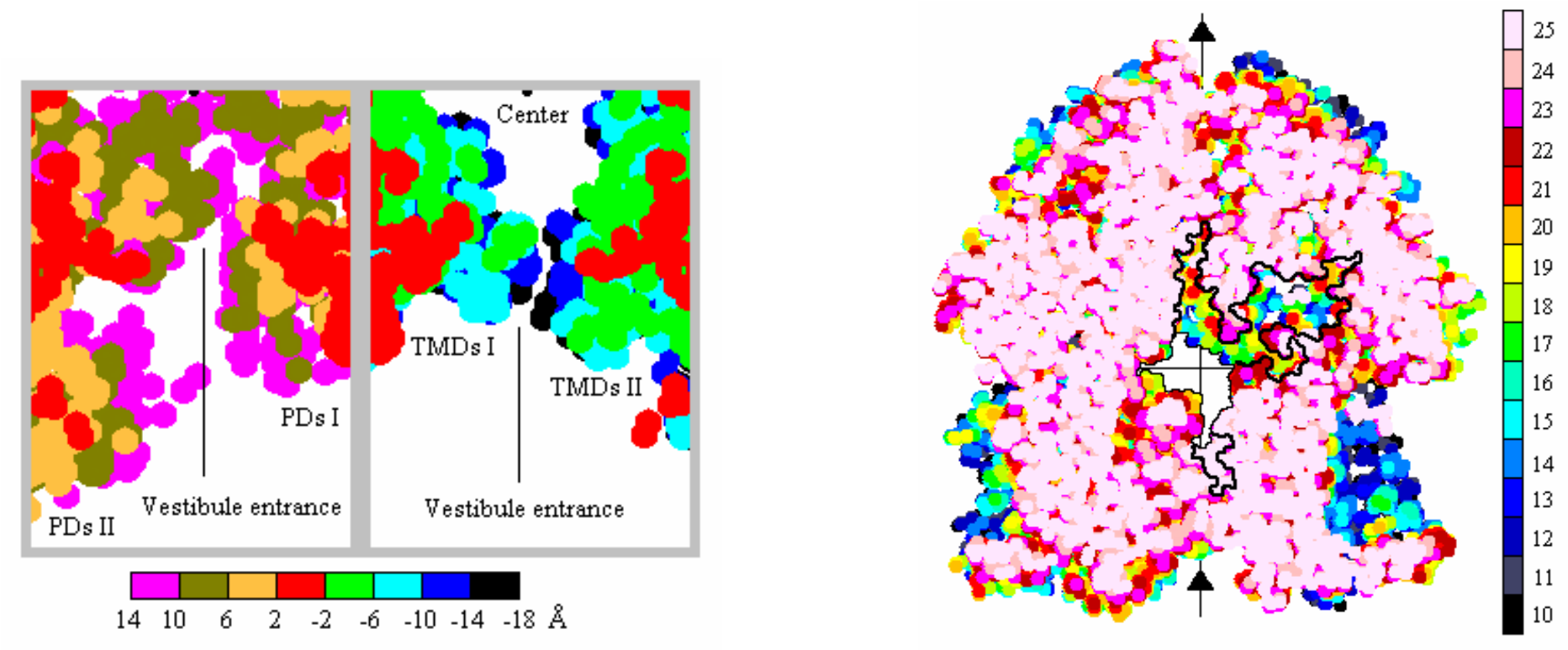


An example of non-linear pMIC – lipophilicity relationship.

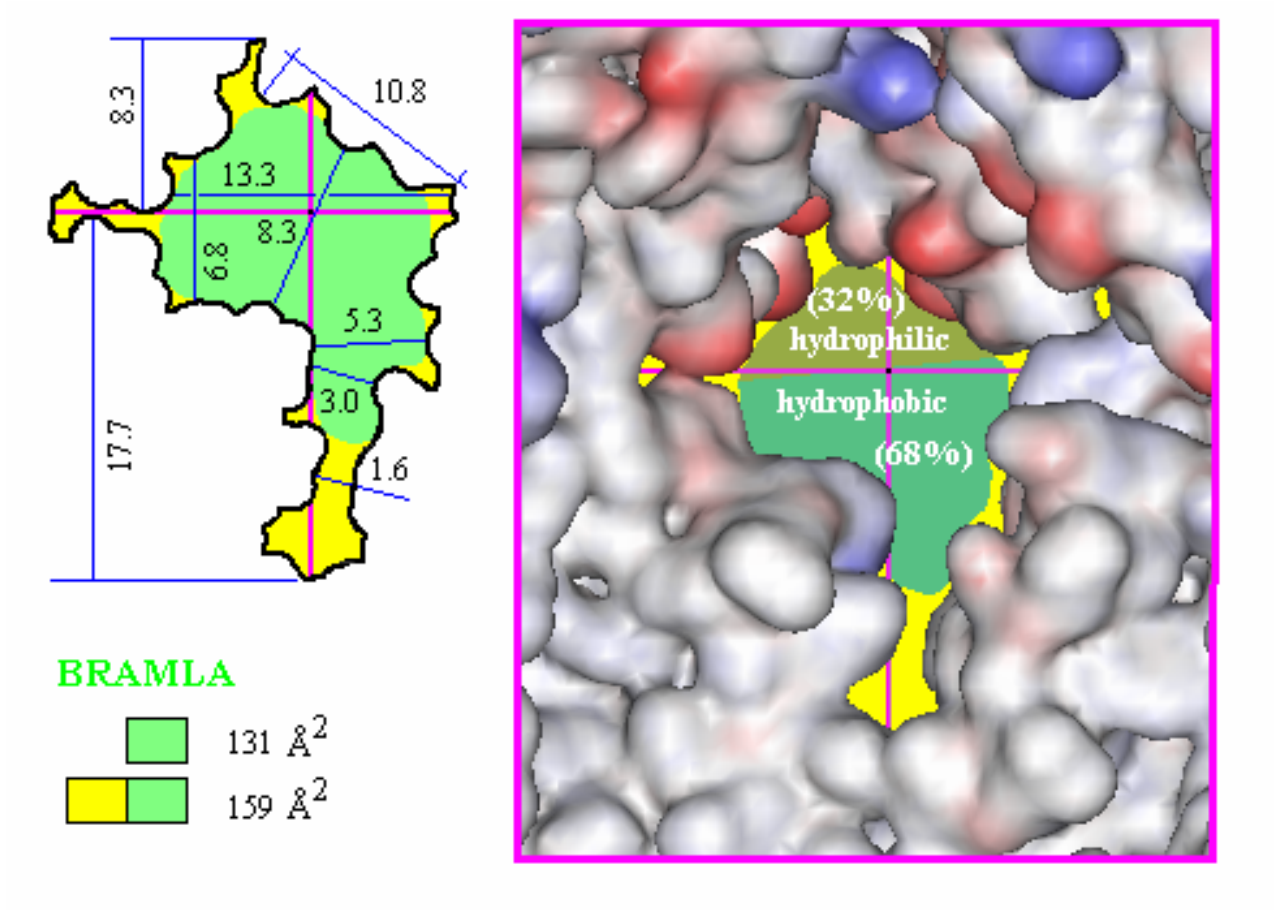




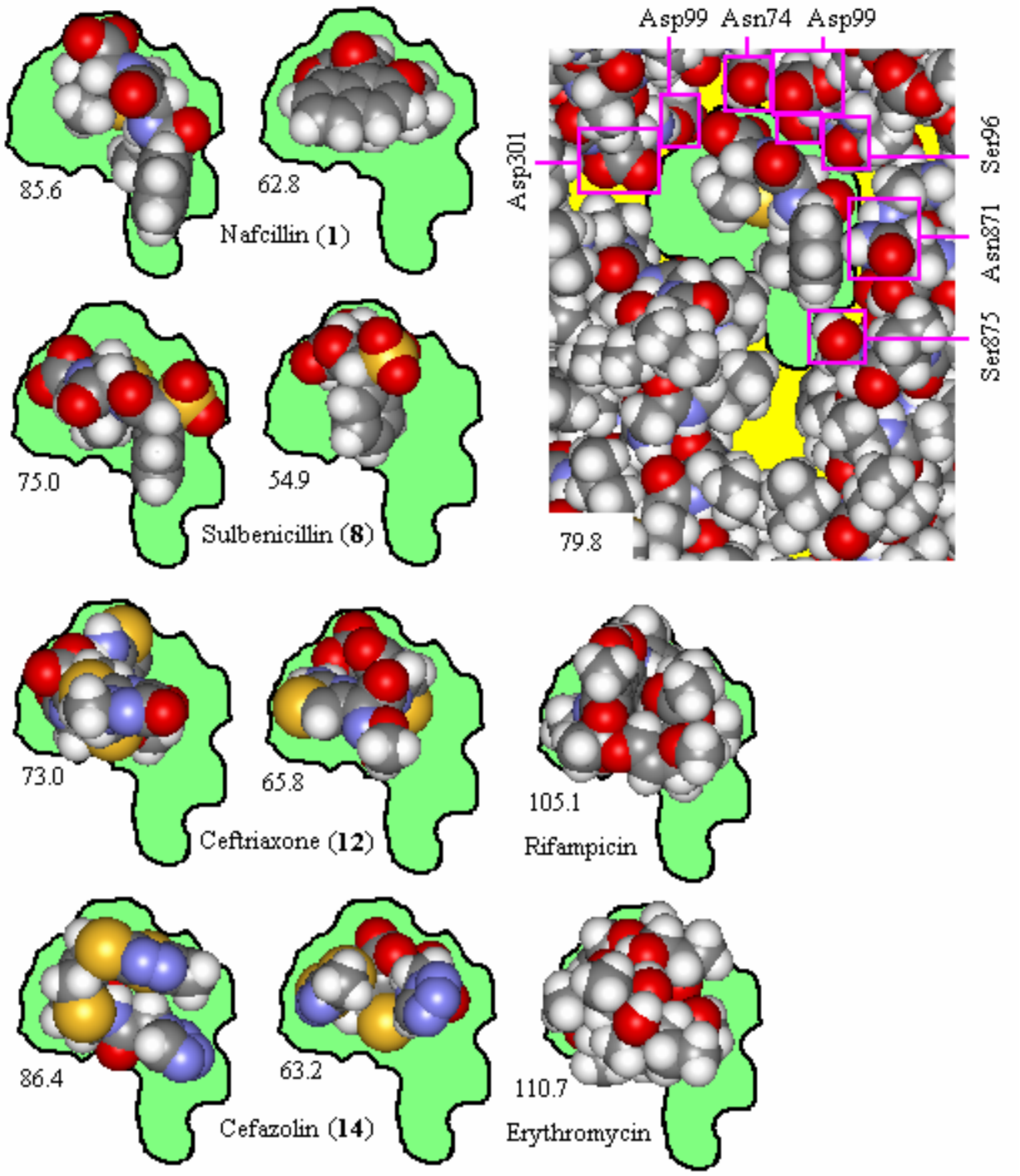
Left: β-lactam molecules with the most negative (**1**), close to zero (**14**) and the most positive (**8**) dipole moment component along the Y – axis (Dipy). The molecules lie in the XY – plane. Right: A simplified 2D representation of stereoelectronic AcrB pump – chephalosporin fit. The predominantly polar (red) and hydrophobic (gray) drug regions align along the compatible domains of the pump. The R and R_1 substituents (green) can be polar, amphiphilic or hydrophobic, and consequently, influence the drug – receptor fit.



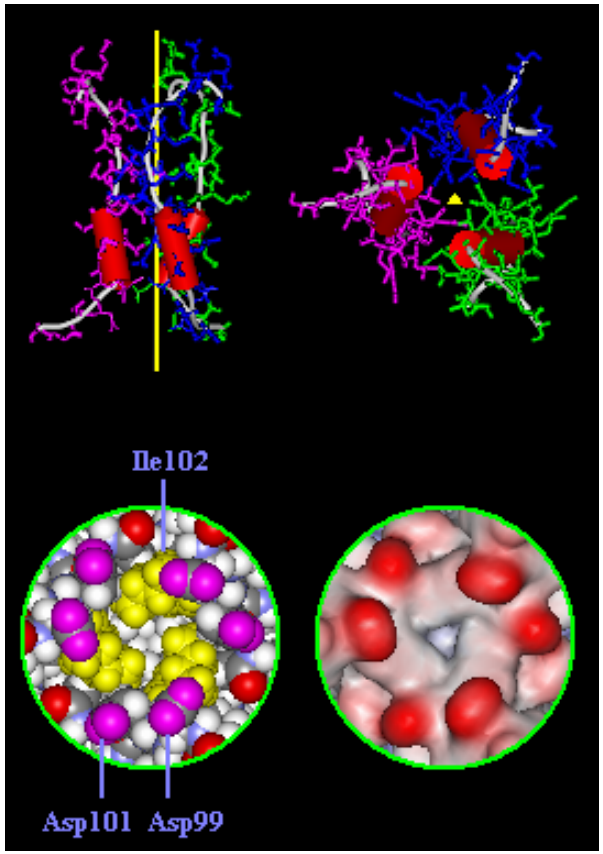
Left: Topographic map of a vestibule and its surroundings upwards (left) and downwards (right) the three-fold rotation axis of the functional trimer. The heights of the layers are given with respect the vestibule plane. Right: Topographic map of AcrB transport protein presented by 15 slices. The heights are the slice distances from the trimer axis (in Å). Two pockets in the PDs region (slice at 22 Å), and the inner cleft at the end of the vestibule (slice at 19 Å), are drawn as solid curve lines.

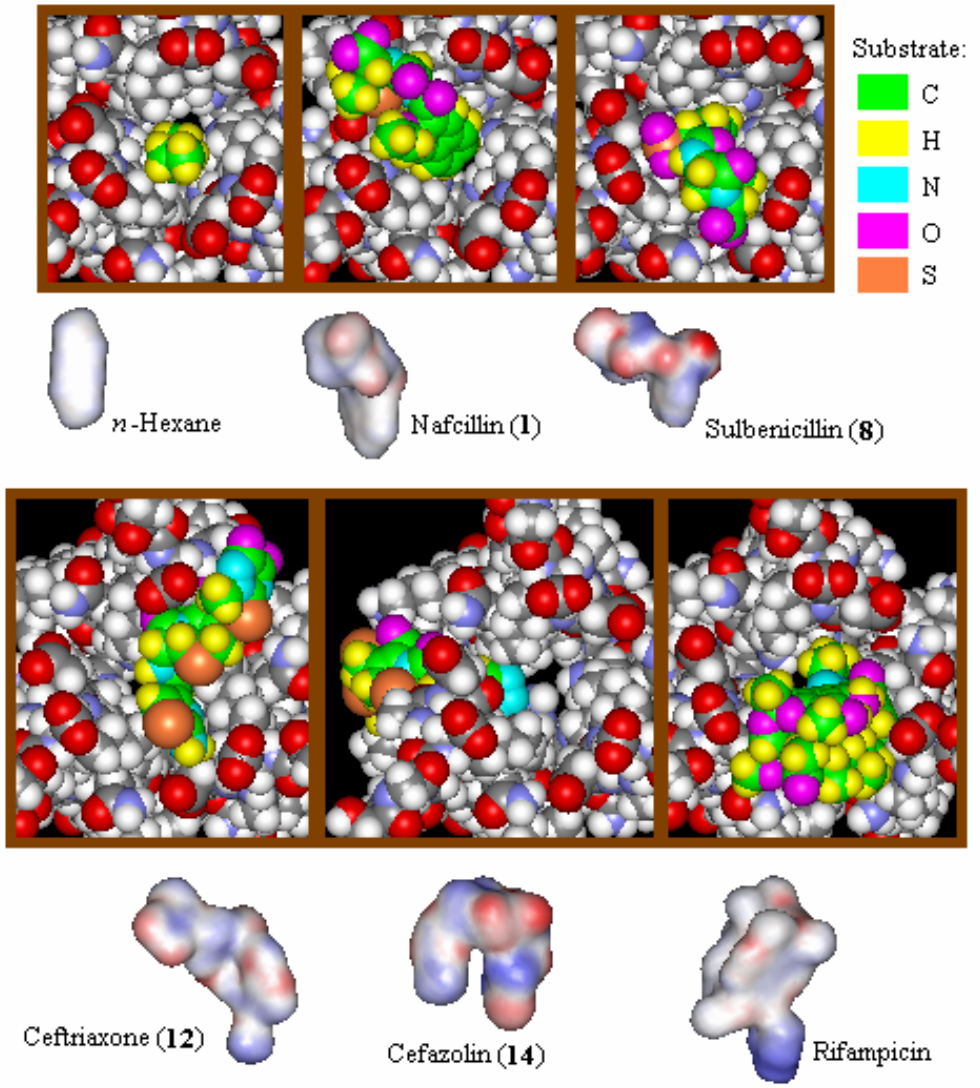


Left: Definition of the BRAMLA region (Brazil Map-Like Area, green) and the vestibule's dimensions. Right: 3D molecular electrostatic representation of a vestibule with the BRAMLA hydrophilic and hydrophobic areas.



Small figures: 2D docking of selected AcrB substrates to BRAMLA area, using maximum (left) and minimum (right) stereoelectronic fit approach. Big figure: 3D docking of nafcillin to a vestibule at 25 Å from the trimer axis, with labelled lipophilic residues (marked but not named is Glu95). Molecular image surface areas are in $Å^2$.





Left: The structure of the pore channell viewed perpendicularly to and along the trimer axis. The protomer segments are colored differently. The structure of the pore recognition site represented by CPK model and electrostatic potential surface. **Right:** Docking of selected substrates to the pore recognition site and their electrostatic potential surfaces. The substrates are colored differently to be distinguished from the pore residues.