Inhibition of S-adenosyl-L-homocysteine hydrolase from *Pseudomonas aeruginosa* by targeting the enzyme dynamics

Bacterial infections are a significant and growing medical problem. Moreover, drug discovery efforts against numerous human pathogenic microorganisms are very often of limited success. On one hand, the explosion of biological and structural information has led to the selection of potential targets for discovery of new antimicrobial lead compounds. However, the emergence and spread of drug resistance make the struggle with pathogens unequal, as many disease-causing microbes have become resistant to a wide range of antibiotics. The presence of multiple antibiotic resistance plasmids among distinct bacterial species is one spectacular example.

For rational antibacterial drug design purposes, the target selection is a crucial step. Ideally, a developed inhibitor should target an essential biosynthetic pathway which occurs only in the bacteria (pathogen) but not the human (host) cells. However, experience with commercially available antibiotics clearly indicates that the emergence of drug resistance is just a matter of time.

The key factors in *Pseudomonas aeruginosa* pathogenesis are numerous virulence factors, a large number of multi-drug efflux systems, as well as low permeability of the outer membrane. Moreover, this pathogen is capable of forming complex bacterial communities, called biofilms. This process makes them more resistant to antibiotics than single-growing cells. A comprehensive analysis of numerous molecular mechanisms related to the pathogenic perfection of *P. aeruginosa* shows that they all depend on the same biochemical pathway, namely SAM-dependent methylation. In *P. aeruginosa* a protein called *S*-adenosyl-L-homocysteine hydrolase (SAHase) is the only enzyme involved in the regulation of SAM-dependent methylation reactions. This fact indicates that SAHase is an essential element of *P. aeruginosa* metabolism and SAHase inhibition will result in the accumulation of SAH in the cell. As a consequence, vital SAM-dependent processes will be stopped. Thus, selective inhibition of SAHases in the targeted cells is an excellent possibility for drug intervention at the molecular level of cell metabolism. Unfortunately, SAHases are also highly-conserved enzymes with almost identical organization of the active site in eukaryotic and prokaryotic organisms. This fact practically precludes the design of highly selective inhibitors against the enzymes of pathogenic origin that would not affect humans. Thus, such inhibitors are not considered for treatment due to cytotoxicity against human cells.

In this project the Principal Investigator proposes to focus on new mechanism of *S*-adenosyl-L-homocysteine hydrolase inhibition based on targeting the enzyme dynamics. Such an approach to SAHase inhibition is innovative and has not been exploited before. We foresee that targeting PaSAHase dynamics will be a promising strategy for the development of new inhibitors of SAHases from pathogenic organisms. Within this proposal two major types of inhibition will be considered: (1) disturbing the domain oscillation frequency by targeting the monovalent cation binding site of the hinge region, and (2) arresting the enzyme in its closed conformation by targeting the surface area between the two main domains. To develop new routes for enzyme inhibition through targeting the PaSAHase dynamics, a broad range of methods from the intersection of microbiology, cell biology, biophysics, biochemistry, NMR spectroscopy, crystallography and structural chemistry must be applied. Therefore, in this proposal we will apply a combination of state-of-the-art experimental techniques to reach our goals.