MS5-P58 Structural investigations of purine nucleoside phosphorylase from *Helicobacter pylori* I

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Helicobacter pylori is bacterial pathogen known for its ability to colonize and persist in human stomach (Makola et al., 2007). It is estimated that today *H. pylori* infects more than half of the world's population. Severe impact that *H. pylori* has on human health, makes the search for effective drugs to fight this pathogen of the utmost importance.

Purine nucleoside phosphorylase (PNP) represents one promising drug target for this pathogen, as it is the key enzyme in the purine salvage pathway. *H. pylori* PNP is a homo-hexameric protein, and can be regarded as a trimer of dimers (Fig. 1a). The active site of each monomer can be in either open or closed conformation. Although the first solved crystal structure of very similar *E. coli* PNP, complexed with its ligands showed 3 open + 3 closed conformations (Koellner et al., 2002) we have recently determined several structures of *E. coli* PNP with 4 open + 2 closed sites conformations (Mikleušević et al., 2011).

Two crystal structures of the PNP from the clinical isolate of *H. pylori* have been determined. Both belong to the orthorhombic crystal system and space group $P2_12_12_1$. The crystal cells are different though: unit cell axes are 74, 129, 155 Å (crystal grown at pH 7) and 104, 120, 139 Å (crystal grown at pH 4.5). Interestingly, it was found in both structures, that the protein has 5 open + 1 closed conformations, which is the first such case, to the best of our knowledge. In the structure from pH 7 crystallization conditions, the closed active site is occupied with one phosphate ion (substrate) and one hypoxanthine molecule (product) in the so-called *dead-end complex*, while all five open sites are empty. The structure at pH 4.5 has the closed active site fully occupied (Fig. 1b), while the open sites are partially occupied with hypoxanthine and phosphate. Differences in active site conformations between *H. pylori* and *E. coli* PNP structures will be discussed as well as possible implications on the PNP mechanism of action.

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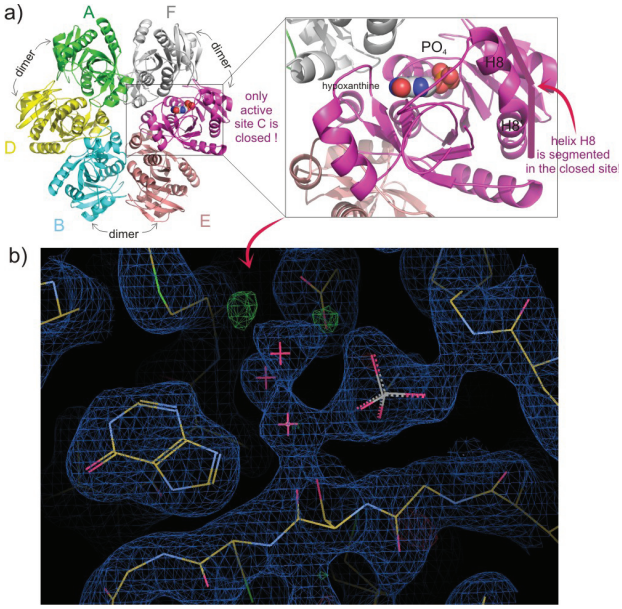

Figure 1. a) Hexameric structure of *H. pylori* PNP b) closed active site of the *H. pylori* PNP containing one hypoxanthine molecule, and one phosphate ion, forming a *dead-end complex*.

Keywords: Purine nuceoside phosphorylase, Helicobacter pylori, dead-end complex