Structural studies of purine nucleoside phosphorylase inhibitors

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Purine nucleic acid metabolism is an essential process which occurs in almost all the cells. Synthesis can occur in two ways: from precursors in time and energy-consuming *de novo* pathway or through much preferred salvage pathway which recycles intermediates of purine degradation pathway [1].

Purine nucleoside phosphorylase (PNP) is an enzyme that hydrolyzes ribose from inosine and guanosine in presence of inorganic phosphate producing hypoxanthine and guanine which are further degraded to xanthine which can be recycled via salvage pathway. Activity of human PNP (hPNP) is increased in various pathologies, such as different types of cancer and autoimmune diseases and this makes PNP a target in drug discovery. Additionally, salvage pathway is essential for parasites, such as *Mycobacterium tuberculosis* (tuberculosis causing pathogen), where PNP (MtPNP) activity is important for transition from latent to active infection [2].

Both hPNP and MtPNP form homotrimers from monomers with molecular weight of 32 kDa and 28 kDa respectively. These enzymes share low sequence homology (35%), but overall fold and active site are conserved. Each subunit forms an active site in proximity of subunit-subunit interaction region, where all but one residue belong to the parent subunit. Active site can be divided into three regions based on the substrate moieties and inorganic phosphate binding positions.

Currently, there are several inhibitors that entered clinical trials in the last decade. Overall, they are composed from purine-moiety linked connected to a sugar-region-binding moiety. All of these inhibitors are characterized by low selectivity and specificity which leads to serious side-effects [2].

In this project we utilize X-ray crystallography to gain structural information for design of PNP inhibitors with high specificity and selectivity. Our compounds contain three moieties that occupy all three regions of the active site and their affinity and selectivity to hPNP and MtPNP is optimized through structure-assisted inhibitor design approach.

Both enzymes were prepared by heterologous expression in *E. coli* in high yields and purity required for crystallographic studies. Crystallization conditions were identified through wide screening and optimization and diffraction data were collected at synchrotron BESSY II, Helmholtz-Zentrum, Berlin. We solved and refined eight crystal structures to resolutions 1.6-2.6 Å, five structures of hPNP and three structures of MtbPNP.

Structure analysis showed that all the inhibitors bind to the active sites in the expected way: mimicking binding of substrate. Overall, modifications at different positions of central sugar-mimicking moiety increase inhibitor affinity towards MtbPNP while decreasing the affinity towards hPNP. These changes can be explained by differences in flexibility of the active sites that allows MtbPNP to accommodate these modifications.

This structural information is crucial for in understanding structure-activity relationship and can further be used to modify this class of compounds

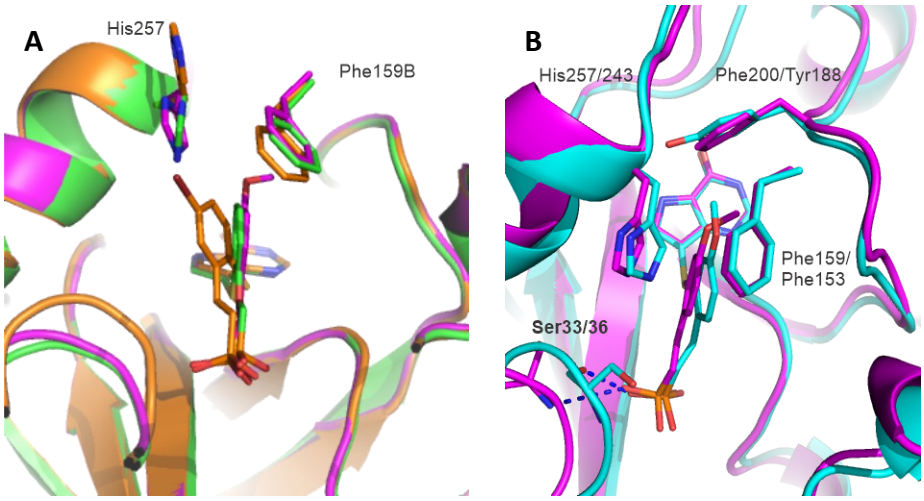

Figure 1: A. Overlay of three hPNP active sites with different inhibitors bound; B. Overlay of hPNP (magenta) and MtbPNP (cyan) with JS-375 bound to the active site.

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