

**Structural and functional characterization of bacterial YeiK nucleoside hydrolase from *E. coli*: catalytic mechanism and possible role in cancer gene therapy.** Barbara Giabbai<sup>a</sup>, Michela Savoldi Boles<sup>a,b</sup>, Massimo Degano<sup>a</sup>. <sup>a</sup>*Biocrystallography Unit, DIBIT San Raffaele Scientific Institute, Milan, Italy.* <sup>b</sup>*Bicocca University, Milan, Italy*

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Enzymes with nucleoside hydrolase (NH) activity are crucial for the salvage of nucleic acid components in purine auxotrophic protozoan parasites. Several genes encoding for putative NH proteins have been annotated in genomes from both prokaryotes [1] and eukaryotes [2] [3] based on the presence of a consensus sequence formed by a N-terminal aspartate cluster [4]. Here we show the characterization of the YeiK (RihB) gene product from *Escherichia coli* as a calcium-dependent, pyrimidine-specific NH. We obtained single crystals of YeiK belonging to the triclinic crystal system in space group P1, with unit-cell parameters  $a=44.81$ ,  $b=85.71$ ,  $c=90.68$  Å,  $\alpha=112.95^\circ$ ,  $\beta=101.95^\circ$ ,  $\gamma=85.92^\circ$ . The structure of YeiK was solved using the molecular replacement technique for phase determination. The crystal structure of YeiK to 1.7 Å defines the basis for its substrate specificity, and identifies new residues involved in the catalytic mechanism. Large variations in the tetrameric quaternary structure compared to non-specific protozoan NHs are brought forth by minor differences in the interacting surfaces. The overall structure of the YeiK monomer resembles the NH fold [4], with an open ( $\alpha,\beta$ ) structure. To best characterize the catalytic mechanism of this pyrimidine specific enzyme, we performed kinetic studies on both native and site-specific mutants of the protein. The structure-based mutagenesis of YeiK supports a different mechanism for pyrimidine nucleoside hydrolysis compared to both non-specific and purine-specific NHs [5]. The first structural and functional characterization of a non-parasitic NH suggests a possible role for these enzymes in the metabolism of unusual tRNA nucleosides. The high catalytic efficiency of YeiK towards 5'-fluorouridine could be exploited for a suicide gene therapy approach in cancer treatment.

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