Structure of YbeK pyrimidine hydrolase from *E. coli*: understanding a possible new helper in cancer gene therapy. Laura Muzzolini[a], Wim Versees[b], Jan Steyaert[b], Massimo Degano[a]. [a]*Biocrystallography Unit, Dibit San Raffaele Scientific Institute, Milan, Italy.* [b]*Laboratorium voor Ultrastructuur, Vrije Universiteit Brussel and Vlaams Interuniversitair Instituut voor Biotechnologie, Brussels, Belgium.*

Keywords: Nucleoside hydrolase; Fluorouracil; Cancer gene therapy

Nucleoside hydrolases (NH) are a family of enzymes that catalyse the cleavage of the N-glycosidic bond between the base and the ribose of nucleosides. NHs were first characterized in protozoan parasitic organisms like Leishmanias and Trypanosomes, where they are central enzymes of the purine-salvage pathway. In fact, these parasites rely on nucleobases salvaged from the host for DNA, RNA and cofactor biosynthesis. Structural studies on NHs from kinetoplastids have revealed a common feature of all these enzymes that is a cluster of aspartate residues at the N-terminal region of the protein that chelate a calcium ion necessary for catalysis [1]. NH activity was revealed in other organisms like bacteria [2], yeast [3] and *Caenorhabditis elegans* [4] in which ribonucleosides are predominantly metabolised by nucleoside phosphorylases. In these organisms the role of NHs is yet to be determined. Comparative analysis of known genome sequences revealed the presence of genes bearing the aspartate cluster also in other organisms like plants, insects and helminth parasites. Neither the encoding genes nor nucleoside hydrolase activity have ever been observed in mammalian cells. Here are reported the structural studies on YbeK protein, one of the three NHs identified in *E. coli*. The crystal structure of YbeK to 1.8Å is the second bacterial NH characterized after the YeiK enzyme also from *E. coli* (PDB code: 1Q8F), which was previously solved by our group [5]. YbeK has an high enzymatic specificity towards pyrimidine nucleosides and in particular its activity on the chemotherapeutic agent 5-fluorouridine could be utilized for a suicide gene therapy approach in cancer treatment. YbeK crystals belonging to the orthorhombic I222 space group were grown in presence of the catalytic product ribose. Structure was determined with the molecular replacement technique using the YeiK monomer as search model. One protein molecule was found in the asymmetric unit, and the physiological tetramer obeys the space group symmetry. The electron density map to 1.8Å resolution clearly showed the presence of an ion and a ribose molecule bound to the active site, and an overall structure that resembles the NH fold [1]. Refinement of the model is currently underway. The high resolution structure of this pyrimidine-specific NH will allow a closer look into the active site and catalytic mechanism of this enzyme class, leading to a more specific usage of compounds in anticancer therapy.

[1] Degano, M. et al. (1996). *Biochemistry*
[2] Petersen, C. and Moller, L.B. (2001). *J Biol Chem*
[3] Magni, G. et al. (1975). *J Biol Chem*
[4] Versees, W. et al.. (2003). *Acta Crys D*
[5] Giabbai, B. and Degano, M. (2004). *Structure*. In press.