s1.m7.p17 **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. E-mail: muzzolini.laura@hsr.it*

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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.

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s1.m7.p18 **Structure of the *Bacillus brevis* acetolactate decarboxylase: a bifunctional enzyme with a novel fold.** Shabir Najmudin[a/c], David H.G. Crout[b] and Vilmos Fülöp[a], [a]*Department of Biological Sciences and* [b]*Department of Chemistry, University of Warwick, Coventry CV4 7AL, England,* [c]*Departamento Química / Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal*. *E-mail: shabir@dq.fct.unl.pt*

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Acetolactate decarboxylase (ALDC) has the unique ability to decarboxylate both enantiomers of acetolactate to give a single enantiomer of the decarboxylation product, (*R*)-acetoin. The enzyme decarboxylates the normal substrate (*S*)-α-acetolactate. It then catalyses a tertiary ketol rearrangement of the (*R*)-enantiomer with the migration of the carboxylate group. Because this degenerate rearrangement proceeds via a transition state with a *syn* arrangement of the oxygen functions, the product is (*S*)-α-acetolactate which is then decarboxylated in the normal way [1,2]. The enzyme also catalyses the decarboxylation of (*S*)-α-acetohydroxybutyrate. (*S*)-α-Acetolactate and (*S*)-α-acetohydroxybutyrate, the products of decarboxylation of β-ketocarboxylates are the biosynthetic precursors of the essential branch-chain amino acids valine and isoleucine respectively.

Details of the overexpression, purification and crystallisation of α-acetolactate decarboxylase are given in Najmudin *et al.* [3]. Phases were obtained from a SAD experiment to give a partial model to 2.3Å. The structure was completed using higher resolution data (2.0Å) in both orthorhombic (I222) and trigonal ($P3_221$) forms. α-Acetolactate decarboxylase is a 2 domain α/β protein with no significant structural similarity to any other protein. The N-terminus domain comprises a 7 β-strand mixed β-sheet, which is extended into second molecule of the dimer related by 2-fold symmetry to give a 14 β-strand β-sheet. The C-terminus domain is a β-cylinder comprising 5 anti-parallel β-strands and a long α-helix. It provides the three highly conserved histidines, which coordinate the metal ion (Zn/Cd). The coordination of the metal is completed by a conserved glutamate from the C-terminus and two water molecules. The likely catalytic site is completed by the highly conserved arginine, threonine and a further glutamate in the vicinity of the metal.

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