s1.m7.p29 **Structural Genomics of DNA Damage Response in *Escherichia coli.***
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Keywords: DNA repair; Structural genomics; Phosphatase

The aim of the project is to identify, characterize and determine the three-dimensional structures of new proteins involved in DNA repair in *E. coli*. The candidates are uncharacterized proteins that show significant increase in their expression levels in response to DNA damage caused by mitomycin C [1]. Following the genotoxic stress, nearly 400 genes induced more than twofold as detected by DNA microarrays. Out of them, 48 genes have been selected for structural and functional studies. All targets form functional families represented in bacteria and in some cases also in archaea and eukaryotes. In the first round of the project, four proteins have been crystallized, and their X-ray structures were determined. Analysis of the structure and subsequent biochemical studies have led to the initial functional hypotheses for these proteins that are now being tested. One of the proteins, the YcdX gene product, have a beta/alpha-barrel structure with 7 beta/alpha units [2] as compared to the more common 8-stranded TIM barrel. The trinuclear zinc binding site indicates the putative active center of the protein. The metal site geometry and the overall fold resemble endonuclease IV, a TIM-barrel enzyme, and suggest a similar (phosphatase) activity for YcdX. Enzymatic assays proved that YcdX is a pyrophosphatase. Analysis of the genomic context suggests that it is probably related to yet unidentified DNA polymerase. Their putative complex may have a role in DNA repair.

[1] Khil PP, Camerini-Otero RD. (2002) Over 1000 genes are involved in the DNA damage response of Escherichia coli. *Mol. Microbiol*. **44**:89-105.
[2] Teplyakov A, Obmolova G, Khil PP, Howard AJ, Camerini-Otero RD, Gilliland GL. (2003) Crystal structure of the *Escherichia coli* YcdX protein reveals a trinuclear zinc active site. *Proteins* **51**:315-318.

s1.m7.p30 **Structure/function studies on a S-adenosyl-L- methionine dependent uroporphyrinogen-III C-methyltransferase (SUMT), a key regulatory enzyme of tetrapyrrole biosynthesis.**
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Keywords: Tetrapyrrole biosynthesis; uroporphyrinogen-III methyltransferase; Cobalamin

The biosynthesis of vitamin B12, "the anti-pernicious anaemia factor", requires about 30 enzymes, and is further complicated by the appearance in nature of two separate pathways, representing aerobic and anaerobic routes, where the major difference seem to be concerned with the process of cobalamin ring contraction and cobalt chelatation. *Pseudomonas aeruginosa* is able to synthesise the vitamin in the absence of oxygen. However, the bacterium can also make B_{12} when grown aerobically. Thus, there must exist a pathway that can operate both in the presence and absence of molecular oxygen.

Uroporphyrinogen (uro'gen) III methyltransferase, a key enzyme in the biosynthetic pathways of vitamin B12 and siroheme, catalyzes the S-adenosyl-L-methionine (SAM)-dependent bismethylation of its substrate, uro'gen III, resulting in the formation of dihydrosirohydrochlorin (precorrin-2). The enzyme exists in at least two forms. One form, encoded by the *cob*A gene, is required for vitamin B_{12} synthesis in *Pseudomonas denitrificans*. The second form, encoded by the *cys*G gene, is required for siroheme in *E. coli*. Both forms of the enzyme perform the *in vivo* synthesis of precorrin-2, but in addition, CysG has NAD^+-dependent precorrin-2 oxidase and ferrochelatase activities. The CysG enzyme mass is ~52 kDa, whereas the smaller CobA protein mass is of ~30 kDa and is homologous only to the C-terminal region of CysG.

CobA is a key regulatory enzyme in the branched tetrapyrrole biosynthetic pathway, and is sensitive to both substrate and product inhibition. To gain some molecular insight into how this enzyme exerts its control, we have crystallised the CobA protein and collected data to 2.7Å resolution on the SRS synchrotron in Daresbury (UK). The molecular replacement method (AMoRe) has been used for the phase problem solution with the C-terminal domain of CysG as a search model.

[1] E. Raux, H.L. Schubert, J.M. Roper, K.S. Wilson, M.H. Warren *Bioorganic chemistry* **27** (1999) 100-118.
[2] E. Raux, H.L. Schubert, M.J. Warren Cell. *Mol. Life Sci.* **57** (2000) 1880-1893.
[3] M.E. Stroupe, H.K. Leech, D.S. Daniels, M.J. Warren, E.D. Getzoff *Nature Structure Biology* **10** (2003) 1064-1073.