resolution of 1.4 Å using a synchrotron radiation source. Crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 25.76, b = 45.38 and c = 56.77 Å. Initial phases were calculated by molecular replacement using an edited rhodniin molecule as the search model. Currently the structure is under the final stages of refinement with an R = 0.203 and R_{free} = 0.214. We shall discuss the implications of this structure in the light of its biological inhibitory function against factor XIIa.

[1] Campos I.T.N., et al., *Insect Biochem. Mol. Biol.*, 2002, **32**, 991.
Keywords: infestin, *Triatoma infestans*, serine-protease inhibitor

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Structural Studies on 3-hydroxyanthranilate-3,4-dioxygenase
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3-Hydroxyanthranilate-3,4-dioxygenase (HAD) catalyzes the oxidative ring opening of 3-hydroxyanthranilate in the tryptophan-based quinolinate biosynthetic pathway. The enzyme requires Fe^{2+} as a cofactor and is inactivated by 4-chloro-3-hydroxyanthranilate (ClHAA). The structure of HAD from *Ralstonia metallidurans* was determined at 1.9 Å resolution. The structures of HAD complexed with the inhibitor ClHAA and either molecular oxygen or nitrous oxide were determined at 2.0 Å resolution, and the structure of HAD complexed with the substrate 3-hydroxyanthranilate was determined at 3.2 Å resolution. HAD is a homodimer with a subunit topology that is characteristic of the cupin barrel fold. Each monomer contains two iron binding sites. The catalytic iron is buried deep inside the cupin barrel. The other iron site forms an FeS_4 center close to the solvent surface. The two iron sites are separated by about 24 Å. Based on the crystal structures of HAD, mutagenesis studies were carried out and a new mechanism for the enzyme inactivation by 4-chloro-3-hydroxyanthranilate is proposed.
Keywords: dioxygenase, cupin, enzyme mechanism

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Stucture of *S. typhimurium* Formylglycinamide Ribonucleotide amidotransferase
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Formylglycinamide ribonucleotide amidotransferase (FGAR-AT) catalyzes the ATP-dependent conversion of formylglycinamide ribonucleotide, and glutamine to formylglycinamidine ribonucleotide (FGAM) and glutamate in the purine biosynthetic pathway. In eukaryotes and Gram negative bacteria, FGAR-AT is encoded by the purL gene as a multidomain protein. In Gram positive bacteria and archaebacteria FGAR-AT is a complex of three proteins: PurS, PurL and PurQ. We have determined the structure of FGAR-AT from *Salmonella. typhimurium* at 1.9 Å resolution. The structure reveals four domains: an N-terminal domain structurally homologous to a PurS dimer, a linker region, an FGAM synthetase domain homologous to an aminoimidazole ribonucleotide synthetase dimer, and a triad glutaminase domain. A structural ADP molecule was found bound. A glutamylthioester intermediate was found in the glutaminase domain at C1135. The N-terminal domain is hypothesized to form the channel through which ammonia passes from the glutaminase domain to the FGAM synthetase domain.
Keywords: formylglycinamide ribonucleotide amidotransferase, ATP-grasp motif, protein evolution

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Towards the Structure of the BchI Hexameric Complex of Magnesium Chelatase
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The insertion of Mg^{2+} into protoporphyrin IX is catalyzed by the enzyme magnesium chelatase and represents the first committed step in the chlorophyll and bacteriochlorophyll biosynthetic pathways. Magnesium chelatase, in organisms that synthesize bacterio-chlorophyll, consists of three subunits, known as BchI, BchD and BchH. The structure of the monomeric BchI has been determined by X-Ray crystallography and it belongs to the chaperone-like, ATPase associated with a variety of cellular activities, (AAA) family of ATPases. Examination by electron microscopy of BchI solutions in the presence of ATP demonstrated that BchI, like other AAA proteins, forms oligomeric ring structures, [1]. The ATP hydrolysis is proposed to trigger major conformational changes in the hexamer, as has been shown for other AAA modules, [2]. Thus, it is crucial to fixate the complex in one single conformational state using non-hydrolysable substrates to create a homogenous environment. Needle shaped protein crystals have been obtained in different conditions. Ongoing optimization of crystallization conditions will hopefully give single crystals suitable for X-ray diffraction.

[1] Fodje M. N., Hansson A., Hansson M., Olsen J. G., Gough S., Willows R. D., Al-Karadaghi S., *J. Mol. Biol.*, 2002, **311**, 111-122. [2] Rouiller I., DeLaBarre B., May A.P., Weis W.I., Brunger A.T., Milligan R.A., Wilson-Kubalek E.M., *Nat. Struct. Bio.l*, 2002, **9(12)**, 950-7.
Keywords: AAA-domain, ATPase, biosynthesis

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Crystal Structure of Glutaredoxin of CxxC1 Type from Poplar
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Glutaredoxins are small ubiquitous oxidoreductases of the thioredoxin family. The major function of glutaredoxin and thioredoxin is to selectively reduce disulfide bridges of target proteins. One of the major differences between glutaredoxin and thioredoxin is that the latter is reduced by various thioredoxin reductases whereas the former requires glutathione. The functions of plant glutaredoxins are not well understood compared to those of *E. coli* and animals. Nineteen poplar glutaredoxins have been annotated based on its genome information and they are classified into three classes [1]. We started crystal structure analysis of poplar glutaredoxin of CxxC1 type belonging to one of the three classes for deepening our understanding of plant glutaredoxins.

The glutaredoxin was overexpressed in *E.coli* cells and crystals were obtained by the hanging drop vapor diffusion method. X-ray diffraction data with three wavelengths were collected at 100K on beam line BL5A of the Photon factory, Tsukuba up to 2.3 Å resolution. The crystals belonged to space group $P6_1$ with unit cell dimensions a=b=97.5 Å and c=91.7 Å. The structure has been determined by the multiple-wavelength anomalous dispersion method. The polypeptide chain was traced in the electron density map and is now being refined. The molecule has a structure typical of the glutaredoxin fold but contains several new features.

[1] Rouhier N., Gelhaye E., Jacquot J.P., *Cell Mol Life Sci*, 2004, **61**, 1.
Keywords: redox enzyme, protein structure determination, MAD

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The Structure of *Yersinia pestis* Dehydroquinase
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Type II dehydroquinase (EC 4.2.1.10) catalyzes the third step in the shikimate pathway by dehydrating 3-dehydroquinate to dehydroshikimate. *Y. pestis* dehydroquinase is formed by monomers of 150 amino acid residues that assemble as dodecamers. The enzyme was purified by size-exclusion and ion-exchange chromatography and crystallized using 16% PEG 3350. The crystals diffracted to 2.9Å resolution and belong to space group $I4_1$ (a=b= 132.4 Å, c = 187.2 Å) with six monomers in the crystallographic asymmetric unit. The dodecamer is built up by a crystallographic two-fold axis that operates on two- and three-fold non-crystallographic operators. The structure was determined by molecular replacement techniques and refined. The monomers display a typical flavodoxin-like fold with a central parallel β-sheet connected by helices. The active site is on the C-terminal end of the parallel β-sheet, at the trimer interface, and near the external surface of the dodecamer.

The structure of the active site is similar to that reported for the enzyme in other species. A peculiar feature of the enzyme is a loop, residues 18 to 29, that closes down on the active site when substrates and inhibitors are bound, and is often ordered only in such cases. Loop residue Tyr 28 (24 in *M. tuberculosis* numbering) is critical in the dehydration step according to the proposed mechanism. In the *Y. pestis* enzyme the loop is ordered in only one out of six subunits, and is in a completely open conformation with a displacement of main chain atoms of up to 11 Å, relative to the closed state.

Keywords: shikimate pathway, Type II dehydroquinase, *Yersinia pestis*

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Crystal Structure of HAV 3C Protease Complexed with a β-lactone Inhibitor: a New Crystal Form of HAV 3C and Its Application for Studying Enzyme-inhibitor Interaction

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We obtained a new crystal form of Hepatitis A virus (HAV) 3C protease in complex with a serine-derived β-lactone inhibitor (N-benzyloxycarbonyl-L-serine-β-lactone). The crystals, which diffract to the highest resolution known to date (1.4 Å) for HAV and related viral proteases, are of space group $P2_12_12_1$ and contain one molecule per asymmetric unit. Serendipitously, the inhibitor covalently binds to a histidine residue (His102) on the surface of the enzyme opposite to the active site and leaves the catalytic residue Cys172 unmodified. Moreover, this is the first time that a functional arrangement of the catalytic triad (Cys172, His44, Asp84) was observed in HAV 3C crystals, making them ideal for the study of enzyme-inhibitor interactions in atomic detail.

The new crystal form seems to result at least partly from the binding of the benzene moiety of the lactone inhibitor to a hydrophobic pocket of a neighboring molecule. Structural alignment with previously solved HAV 3C crystal structures indicates significant conformational changes occur beyond the site of chemical modification. The two anti-parallel β-strands (aa 139-158) between the N- and C-terminal domains of HAV 3C exhibit less flexibility than previously observed in other crystal forms, which may have an important role in the organization of a catalytically competent triad. In particular, the hydroxyl of Tyr143 forms a hydrogen bond with $O^{\delta 2}$ of Asp84, facilitating the latter to interact with $N^{\varepsilon 2}$ of His44, the general base in catalysis. We subsequently soaked HAV 3C-lactone crystals in solution containing another irreversible inhibitor N-iodoacetyl-Val-Phe-amide to explore the feasibility of using these new HAV 3C crystals for the study of interactions between the enzyme and active site-reacting inhibitors.

Keywords: hepatitis, protease, inhibitor

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Crystal Structure of a Protein Disulfide Oxidoreductase from *Aquifex aeolicus*

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Protein disulfide oxidoreductases (PDO*s*) are ubiquitous redox enzymes that catalyse dithiol-disulfide exchange reactions. These enzymes include the families of thioredoxin (TRX), glutaredoxin (GRX), protein disulfide isomerase (PDI), disulfide bond forming (Dsb) and their homologues. In 1995 a hyperthermostable PDO has been purified from the archaeon *Pyrococcus furiosus* (*Pf*PDO). This protein has an unusual molecular mass of 26 kDa, compared to the small sizes of most GRX*s* and TRX*s*, and its amino acid sequence shows no overall similarity to previously studied PDO*s*. Interestingly, it presents two active sites with the conserved CXXC sequence motif. The resolution of its three-dimensional structure revealed important conformational details suggesting that *Pf*PDO may be related to the PDI, known only in eukaryotes.

Comparison of the genomes from archaea and bacteria showed the existence of a group of redox proteins similar to *Pf*PDO. The unusual features of these enzymes suggest that they could constitute a new family of PDO*s*. We have recently focused our attention on a protein isolated from the thermophilic bacterium *Aquifex aeolicus* (*Aa*PDO) and belonging to this putative new enzyme family. In order to provide insights into the function, structural diversity and evolution of PDO*s*, a structural and functional study on this protein has been carried out.

Keywords: crystal structure, PDO, thermostable enzymes

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***Pseudomonas aeruginosa* PA3859: from Structure to Function**

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We have recently purified a carboxylesterase from *Pseudomonas aeruginosa* that corresponds to the open reading frame PA3859 [1]. The biochemical characterization of this enzyme showed that it hydrolyzes short chain fatty acid esters with a broad substrate specificity. However, its biochemical characterization did not provide insights into a clearly defined *in vivo* function.

Neither bioinformatics tools did provide hints on its physiological function: useful information could not be gained by data mining for homologs or orthologs.

The successful crystallization and 3D structure determination of PA3859 at 2.1Å resolution provided new insights into the structural basis for its *in vivo* substrate(s) specificity. The presence on the protein surface, next to the active site, of an hydrophobic cleft exposed to the solvent has been hypothesized as the possible protein *hot spot* binding site that may accommodate an alkylic chain. This hypothesis was also supported by the localization of genes involved in lipid metabolism in the vicinity of the PA3859 locus. Docking of a variety of phospholipids suggested lysophopsphatidylcholine as potential substrate. We have established, by an enzymatic bioassay, that PA3859 is indeed able to release free fatty acid from lysophosphatidylcholine.

[1] Pesaresi A., Devescovi G., Lamba D., Venturi V., Degrassi G., *Curr. Microbiol.*, 2005, **50**, 102.

Keywords: *Pseudomonas aeruginosa*, carboxylesterase, lysophospholipase