efficient drop inspection and analysis, these technological advancements have initiated the development of microplates with low birefringent background (LBR plates) to allow more effective use of polarized light in protein crystal detection. LBR plates are especially beneficial for identification of crystals out of focus, very small microcrystals, microcrystals hidden in or resembling precipitate or phase seperation and crystals located at the edge of droplets or crystallization wells.

As an alternative to classical microplates, plastic microstructured devices for liquid-liquid diffusion crystallography offer the benefits of low protein and reagent consumption, ease of handling and time conservation. Further advantages of plastic microstructures devices are a broad selection of available raw materials and surface treatments as well as reasonable costs of manufacture.

Keywords: high throughput crystallography, polarized light microscopy, microfluidics

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Lactate Dehydrogenases from Extremophile Organisms: Clues for Radio-resistance?

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Lactate dehydrogenases (LDH) catalyse the last step of glycolysis in which pyruvate is reduced by NADH to lactate. Our structural study of LDH from *D. radiodurans* finds its place in a broader project aimed at investigating the effect of ionising radiation on many enzymes of the malate/lactate dehydrogenase family from extremophile organisms (thermophiles, psychrophiles, halophiles and radio-resistants).

Ionising radiation, including synchrotron radiation, has specific effects on structure, activity and stability of biological macromolecules and represents at the same time the cause of the damage and the tool to study it [1].

In order to better understand how molecular adaptation to extreme environments is achieved, and if it confers radio-resistance, the structure of LDH from *D. radiodurans* has been solved, both in its native form and in complex with the allosteric cofactor fructose 1,6-biphosphate (FBP). A structural comparison with LDHs from other extremophile organisms is under way. Furthermore, the sensitivity to ionising radiation of the two allosteric forms and the possible protective effect of FBP and NADH against radiation damage is currently being investigated.

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Keywords: LDH, radiation damage, allostery

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Interactions of Phospholipids with Integral Membrane Proteins; Use of Brominated Lipids

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The lipid environment of integral membrane proteins is important to their structure and function, but little is known about specific protein-lipid interactions. Knowledge of these will help in devising better protocols for solubilisation and crystallisation of membrane proteins. Although some lipids may co-purify with the protein and show as residual electron density, the detergent used in solubilisation can confuse the interpretation. By co-crystallising the protein with brominated lipids their binding sites can be distinguished.

We have located two lipid binding sites in a complex of the reaction centre (RC) from *Rb. sphaeroides* with the 1-Palmitoyl-2-Stearoyl(6,7)-dibromo-sn-glycero-3-Phosphocholine (Br-PC) based on single wavelength data. We are now producing complexes of RC with variety of brominated lipids changing the lipid headgroup, the number and positions of bromine atoms, and concentration of the lipid in co-crystallisation with RC. We would also like to perform MAD experiments for some of these complexes at the Br K-edge (around 13.47 keV) to improve the anomalous signal and also to assess the possibility of using this method to phase structures of integral membrane proteins. Results of these experiments will be described.

The authors are members of the membrane protein structure initiative (MPSI), supported by the BBSRC.

Keywords: membrane protein crystallisation, brominated phospholipids, MAD and SAD experiments

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Structural Studies of Water-soluble Chlorophyll Protein from *Chenopodium album*

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Generally, chlorophyll (Chl) molecules functioning in photosynthesis are associated with hydrophobic integral membrane proteins. Water-soluble Chl protein (WSCP) was first found in *Chenopodium album* in 1963. WSCPs have then been detected in several species classified in the Polygonaceae, Chenopodiaceae, Amaranthaceae and Brassicaceae families. Although the physiological function of WSCPs has not yet been cleared, these WSCPs can be categorized into two classes according to their photoconvertibility: *Chenopodium*-type (Class I) and *Brassica*-type (Class II). The absorption spectrum of a Class I WSCP changes drastically on exposure to visible light, while a Class II WSCP does not. And there is no significant sequence homology between Classes I and II WSCPs. The X-ray structure analysis of Class II WSCPs containing *Lepidium*-, *Raphanus*- and Kale-WSCPs reveals that these WSCPs consist of 4 subunits and a Chl is contained in each subunit. In order to determine the crystal structure of a Class II WSCP and elucidate the photoconversion mechanism, *Chenopodium*-WSCP was extracted from leaves, purified, and crystallized in a dark room. Green rod crystals appeared in a week. A native data set was collected to 3.0 Å resolution at 100 K with synchrotron radiation at PF. The space group of the crystal was determined to be orthorhombic *I*222 with unit-cell parameters a = 47.08, b= 61.42, and c = 107.0 Å. Heavy atom derivative screening for structure determination is in progress. The photoconversion mechanism and the interaction between Chl and the protein are being studied.

Keywords: water-soluble chlorophyll protein, photoconvertibility, pigment protein

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Structure of the C-terminal Domain of DipZ from *Mycobacterium tuberculosis*

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DipZ (Rv2874) from *M. tuberculosis* is a member of the CcdA protein family. This family of proteins shares a conserved transmembrane electron transport domain with similarity to CcdA from *Rhodobacter capsulatus* and varies in size from 190 aa to over 750 aa. It has been proposed that the function of the larger proteins has been modified by the acquisition of extra-cytoplasmic protein domains. The transmembrane region functions by passing electrons from the cytoplasm of the cell across the membrane for use by these extra-cytoplasmic domains [1].

The C-terminal soluble domain from DipZ has been crystallised and the structure determined by SAD methods from crystals soaked in K_2PtCl_4. The model reveals a dimeric structure. Each subunit is comprised of two domains, an N-terminal thioredoxin-like fold predicted from earlier sequence alignments, and a C-terminal jelly-roll fold with similarity to the family 6 carbohydrate binding modules. A

large cavity is formed at the dimer interface and extends between the two domains of each subunit. Binding studies have demonstrated the ability of this protein construct to bind to cellulose.

These observations suggest a broadening in the functional repertoire of CcdA-like proteins and possible role in carbohydrate processing.

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Keywords: protein structures, structural biology, carbohydrate binding proteins

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Structure of the N-terminal domain of PEX1 AAA-ATPase

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Peroxisomes are responsible for several pathways in primary metabolism, including beta-oxidation and lipid biosynthesis. PEX1 and PEX6 are hexameric AAA-type ATPases, both of which are indispensable in targeting over 50 peroxisomal resident proteins from the cytosol to the peroxisomes. Although the tandem AAA-ATPase domains in the central region of PEX1 and PEX6 are highly similar, the N-terminal sequences are unique. To better understand the distinct molecular function of these two proteins, we analyzed the unique N-terminal domain (NTD) of PEX1. Extensive computational analysis revealed weak similarity of PEX1 NTD to the N-terminal domains of other membrane related type II AAA-ATPases, such as VCP / p97 and NSF. We have determined the crystal structure of mouse PEX1 NTD at 2.05 Å resolution, which clearly demonstrated that the domain belongs to the double-psi-barrel fold family found in the other AAA-ATPases. The N-domains of both VCP and NSF are structural neighbors of PEX1 NTD with a 2.7 Å and 2.1 Å r.m.s.d. of backbone atoms, respectively. Our finding suggest that the supra-domain architecture, which is composed of a single N-terminal domain followed by tandem AAA domains, is a common feature of organellar membrane-associating AAA-ATPases. We propose that PEX1 functions as a protein unfoldase in peroxisomal biogenesis, using its N-terminal putative adaptor-binding domain.

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Keywords: PEX1, N-terminal domain, AAA-ATPase

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Hyperthermostable Ferredoxin from *Pyrococcus furiosus*

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Pyrococcus furiosus is a hyperthemophilic archaeon with a growth optimum of 373 K. Its ferredoxin is a 66 amino acid electron transfer protein, which contains one $[Fe_4S_4]$-cluster. An aspartate residue is coordinating one of the irons, which is easily lost so that the cluster converts to an $[Fe_3S_4]$-cluster under oxidizing conditions. The protein also contains a disulfide bond, which is redox active at approximately the same potential as the $[Fe_4S_4]$-cluster and exists in equilibrium between two conformations [1].

The structure of *pyrococcus furiosus* ferredoxin was determined to 1.5 Å by molecular replacement with ferredoxin from *Termotoga maritima* [2], [3]. It reveals an extensive hydrogen-bonding network, which provides an explanation for the thermostability. It has been suggested that *pyrococcus furiosus* ferredoxin is a dimer under physiological conditions [4]. The packing of the two molecules in the asymmetric unit indicates the intermolecular contacts in such a dimer. The disulfide bond is seen in two conformations.

Recently, we have managed to crystallize the $[Fe_4S_4]$-form of the protein. We are currently working on optimizing these crystals.

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Keywords: metalloprotein, iron sulfur cluster, thermostable proteins

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Crystal Structure of the NgcE Protein of the *Streptomyces* ABC transporter

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The NgcE protein binds *N*-acetylglucosamine (GlcNAc) as well as *N,N'*-diacetylchitobiose and is a component of the ABC transporter Ngc for GlcNAc-uptake in *Streptomyces olivaceoviridis*. The NgcE protein was overproduced in a soluble and purified to homogeneity. Crystals of NgcE, which grew in the presence of 1 m*M* GlcNAc, 20 %(w/v) PEG MME 2000, and 100 mM Tris-HCl (pH 8.5), showed plate-like form, and belonged to either space group $P2_12_12$ (a=59.9, b=153.0, c=41.7 Å) or $P2_12_12_1$ (a=58.1, b=96.3, c=151.7 Å). The former crystals diffracted to 2.2 Å resolution and the latter to 1.8Å. The MAD phasing and the initial model building were performed using 2.0Å data sets of a selenomethionine-derivative $P2_12_12_1$ crystal. The structure of the NgcE protein containing GlcNAc was solved as well as the structure containing *N,N'*-diacetylchitobiose. The overall structure shows a two-domain joined by a hinge-bending sugar binding region, which is similar to the maltose binding protein MalE of *Escherichia coli* and other solved sugar-binding protein of ABC transporter.

Keywords: ABC transporter, solute-binding protein, *N*-acetylglucosamine

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Crystal Structure and Structural Stability of Acylphosphatase from Hyperthermophilic Archaea *Pyrococcus horikoshii* OT3

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Pyrococcus horikoshii OT3 is a hyperthermophilic archaea that grows at temperatures between 88 ºC and 104 ºC. Proteins produced by this archaea possess high thermostability. To elucidate the structural basis for the high stability of acylphosphatase (AcP) from *P. horikoshii* OT3, we determined its crystal structure at 1.72 Å resolution. *P. horikoshii* AcP possesses high stability despite its approximately 30% sequence identity with eukaryotic enzymes that have moderate thermostability. Comparison with the crystal structure of eukaryotic AcP revealed some significant characteristics in *P. horikoshii* AcP that likely play important roles in structural stability: (i) shortening of the flexible N-terminal region and long loop; (ii) an increased number of ion pairs on the protein surface; (iii) stabilization of the loop structure by hydrogen bonds. In *P. horikoshii* AcP, two ion pair networks were observed, one located in the loop structure positioned near the C-terminus, and other on the β-sheet. The importance of ion pairs for structural stability was confirmed by site-directed mutation and denaturation induced by guanidium chloride.

Keywords: structure and stability of protein, thermostable, X-ray crystallography