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

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The Structure of an Ester Synthesising Peptidase
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X-prolyl dipeptidyl peptidase (PepX) is a dipeptidase that appears to be ubiquitous in dairy lactic acid bacteria. PepX is best characterised for its highly specific peptidase activity, namely the ability to remove dipeptides from the N-terminus of larger peptides, where proline is residue 2 in the peptide sequence. PepX however is also able to synthesise esters via a transferase mechanism.

The structure of PepX from *Streptococcus thermophilus* has been solved by molecular replacement methods to 1.9Å resolution using PepX from *Lactococcus lactis* [1] as a model. The refined structure has an R factor of 18.2% and R_{free} of 23%.

Characterisation of the ester synthetic activity showed that PepX was capable of producing ethyl butanoate, only if the synthetic triacylglyceride tributyrin was the donor molecule. The basis for this specificity is discussed in terms of the structure of the enzyme, and the topology of the active site. A model for the catalytic activity that is in agreement with the observed kinetic data is presented.

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Keywords: peptidase, transferase, enzyme kinetics

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Protein Crystallography with Spallation Neutrons
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The Protein Crystallography Station (PCS) at the Los Alamos Neutron Scattering Center, is a high performance neutron protein crystallography beam line.[1] Beam time is free and is allocated by a peer review process.

The beam line exploits the pulsed nature of spallation neutrons with a large position sensitive electronic neutron detector that allows time resolved collection of Laue patterns. The data collected uses neutrons with wavelength of 0.7 to 6 Angstroms. The neutron optics employs a partially decoupled moderator with a conical beam line, collection all useful neutrons from the whole moderator surface with a beam divergence matched to the average mosaic of typical protein crystals. [2]

Some typical results from the user program will be presented illustrating data collected for protein crystals with molecular weights from a few kD to over 500kD.

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Keywords: protein crystallography, neutron diffraction, time resolved

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Crystal Structure of *Moronis saxatilis* F-lectin
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F-lectins are a novel carbohydrate recognition domain first identified in *Anguilla anguilla* agglutininin (AAA), a 17 kDa serum fucolectin from European eel [1]. Because AAA specifically recognizes fucosylated oligosaccharides has been used extensively as a reagent in blood typing and histochemistry. F-lectins from invertebrate and vertebrate species function as innate immunity recognition molecules.

The serum of *M. saxatilis*, striped bass, contains a fucolectin (MsFBP32) that displays two distinct F-lectin sequences in tandem. The crystal structure of MSFB32 complexes with fucose and Lewis-a trisaccharide were determined. The MsFPB32 crystal structure shows a 83 Å long trimer with each distinct monomer CRD segregated to opposite sides. Despite trimers are not observed in solution, each half of the crystal asymmetric unit present striking similarities with the AAA physiological trimer. This arrangement of CRDs suggests a specific function for the recognition of carbohydrates structures on the cellular wall of fish pathogens. Although the two carbohydrate recognition sites of MsFBP32 are F-type carbohydrate binding sites, differences between them suggest that this is a divalent lectin that may recognize and link self to non-self carbohydrate structures.

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Keywords: lectin, innate immunity, crystal structure

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Producing Diffraction Quality Powders from Soluble Lysozyme and Thaumatin
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The pioneer effort on insulin and lysozyme has revealed the possibility of acquiring powder diffraction profile from proteins. These powder profiles were shown to be of sufficient quality to extract structural information. These results imply the idea of using protein powder diffraction for the identification of ligand complexes.

Our effort is to develop a general method to obtain polycrystalline powder from protein in solution. Our approach takes advantage of the crystallization conditions known to produce single crystal. Lysozyme and thaumatin were used as test case in this study. The crystallizaton conditions explored for lysozyme were from NaCl in Acetate buffer pH 4.5 and the Na/K tartrate in MES buffer pH 6.5 for thaumatin.

In order to generate protein powder, we increased the number of nucleation sites by increasing the concentration of protein and/or precipitant. In both cases, the proteins were first dissolved in the appropriate buffer and then the precipitant was added. Powder diffraction profiles were collected on the high-resolution powder beam line X3B1 at the National Synchrotron Light Source and could be interpreted from the known single crystal lattice. Our results suggest that polycrystalline powder can be produce from soluble lysozyme and thaumatin and further analysis is in progress to apply this approach to other proteins.
Keywords: protein crystallography, powder diffraction, protein crystallization

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A Novel Chlorophyll-binding Mode of Water-soluble Chlorophyll Protein (WSCP)
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Chlorophyll (Chl), the most important pigment in photosynthesis, is known as a generator of oxygen radical under excess light. Since the oxygen radical is harmful on plant cellular component, plants need to quench it. In the photosynthetic apparatus, carotenoid quenches the overexcited Chl by xanthophyll cycle. However, it still remains to be seen that how plants prevent the Chl-mediated oxygen radical formation at the stage of Chl biosynthesis and Chl transport pathway.

The putative Chl carrier, water-soluble chlorophyll protein (WSCP), prevents Chl-mediated oxygen radical formation without carotenoid in an as yet unknown manner [1]. To elucidate this mechanism, we crystallized the WSCP from *Brassica oleracea* var.