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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.

[1] Rigolet P., Mechin I., Delage M.M., Chich J.F., *Structure*, 2002, **10**, 1383.
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.

[1] Langan P., Greene G., Schoenborn B.P., *J. App. Cryst*., 2004, **37**, 24. [2] Benno P. Schoenborn, John D. Court, Allen C. Larson, Phil Ferguson, *J. of Neutron Research*, 1999, **7**, 89-106.
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.

[1] Bianchet M.A., Odom E.W., Vasta G.R., Amzel L.M., *Nature & Structural Biology*, 2002, **9**, 628-634.
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.

acephala (kale) [2] and solved the crystal structure by molecular replacement method. The model structure, Lepidium WSCP (PDB code: 1WYA), shares 41% identity of primary sequence. Kale WSCP possesses a homo-tetrameric structure consisting of 19 kDa subunits, and each monomer contains one Chl but no carotenoid, as in the case of Lepidium WSCP.

The remarkable structural feature is that all four Chls are packed in a hydrophobic core at the inter-subunit interface. Because the Chls are secluded from solvent, it is unlikely that the excitation energy of Chl transfers to oxygen and generates radical species.

[1] Schmidt K. *et al*., *Biochemistry*, 2003, **42**, 7427. [2] Horigome D., Satoh H., Uchida A., *Acta Cryst*., 2003, **D59**, 2283.

Keywords: WSCP, chlorophyll, oxygen radical

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Group-subgroup Relations, Twinning, and Rigid-body Vibration (TLS) in a Bio-crystal: Analogy to Inorganic Structures

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The crystal structure of native methylenetetrahydromethanopterin dehydrogenase, **Mtd-nat**, from *Methanopyrus kandleri* (a= 120 Å, b= 151 Å, c= 220 Å, β=90.0º, *mmm* Laue symmetry due to twinning) was shown to own monoclinic symmetry (C2) by group theory arguments. The structure is closely related to that of the Se-methionine labelled protein, **Mtd-Se**, (a= 120 Å, b=151 Å, c= 110 Å, $C222_1$, structure solution by MAD) differing only by small reorientations of about 1° of the hexameric structural units. Standard tests for twinning were negative; the twinning was recognized using l=odd reflections only.

The structural units in the Mtd-Se crystals feature a striking anisotropic rigid body libration of the hexameric units as shown by TLS refinement (at 1.55 Å resolution) which is consistent with the static reorientation in the Mtd-nat crystals [1].

The relation between the two crystal structures, the rigid body libration in one, as well as the characteristic twinning of the other suggest an analogy to the structural changes at certain kinds of phase transitions described by group-subgroup relations which imply twinning, 'soft' lattice vibration modes, and which are well studied in inorganic structural chemistry and solid state physics.

[1] Warkentin E., Hagemeier C. H., Shima S., Thauer R. K., Ermler U., *Acta Cryst*., 2005, D**61**, 198-202.

Keywords: group-subgroup relations, twins, TLS refinement

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Phasing with Iodine and an X-ray Home Source

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The goal of the present work is focused on the phasing strategy employed to elucidate the crystal structure of the protein N-acetylglucosamine-6 phosphate (GlcNAc6P) deacetylase from *E. coli* [1]. GlcNAc6P deacetylase is an enzyme of the amino sugar catabolism pathway, catalyzing the conversion of the GlcNAc6P in to GlcN6P. The crystal structure was phased by SIRAS using low resolution (2,9Å) iodine anomalous scattering. Native crystals[1] were soaked in a cryo-solution consisting of 1.2 M NaH_2PO_4 and 0.7 M NaI for 10 min. A high redundancy dataset (694° angular sector) was collected on a rotating anode at 100K, resulting in 1,676,880 observed and 21,619 independent reflections. Seventeen iodine sites of partial occupation (1.0-0.3) were found with SHELXD and the output correlation coefficients between the observed and calculated SFs differences were 34.73% (all) and 18.93% (weak data). Phase calculation was carried out with the program SOLVE. Phase extension to 2Å resolution, based on a native data set collected at a synchrotron source [1], and succeeding density modification steps were performed with program RESOLVE. An initial hybrid model was built by merging residues traced in different runs and sub cycles of ARP/WARP model building. Some insights on the refined structure will be presented.

[1] Ferreira F. M., *et al.*, *Acta Cryst.*, **D56**, 670.

Keywords: phasing, SIRAS, GlcNAc6P deacetylase

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Structural Comparison and Analysis of the Substrate Specificities of Purine Nucleoside Phosphorylases

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The reversible phosphorolysis of purine and pyrimidine nucleosides is an important reaction in the salvage pathway, where cleavage of glycosidic bond yields a free base and ribose-1-phosphate. Structural studies reveal that only two folds exist, which provides the basis to classify the nucleoside phosphorylases into two families: nucleoside phosphorylase-I and nucleoside phosphorylase-II. Nucleoside phosphorylase-I enzymes share a common single-domain subunit, have either a homotrimeric or a homohexameric quaternary structure, and accept both purine and pyrimidine substrates. Nucleoside phosphorylase-II enzymes share a common two-domain subunit fold with a dimeric quaternary structure, and are specific for pyrimidine nucleosides [1]. Purine nucleoside phosphorylases (PNPs) belong to the nucleoside phosphorylase-I family. Typically, homohexameric PNPs cleave inosine, guanosine and adenosine, while homotrimeric PNPs cleave guanosine and inosine but not adenosine; however, exceptions have been observed.

Fifteen known structures of homohexameric and homotrimeric PNPs from bacterial and mammalian species are analyzed based on sequence alignment, phylogenetic analysis and substrate specificity. While conservation of key active site residues is observed in both bacterial and mammalian PNPs, there is significant sequence divergence between the two classes of PNP. Comparison of the active sites from known structures of the trimeric and hexameric PNP family members provides insight to the structural basis of substrate specificity.

[1] Pugmire M., Ealick S.E., *Biochem. J*, 2002, **361**, 1.

Keywords: purine nucleoside phosphorylase, active site, substrate specificity

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Examination of the Mechanism of Carbamate Kinase by Structural Analyses

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Carbamate kinase (EC 2.7.2.3) catalyzes the reversible reaction $NH_2COO^- + ATP \leftrightarrow NH_2COOPO_3^{2-} + ADP$ serving to synthesize ATP from carbamoyl phosphate in microorganisms [1].

Since CK catalysis involve phosphoryl group transfer, the enzyme CK may have the residues which stabilize intermediate during phosphate transfer. To clarify this point we have determined the three-dimensional structure of carbamate kinase of *Pseudomonas aeruginosa* bound to carbamoyl phosphate and ADP by X-ray crystallography. The structural analysis provides the information on substrate binding and catalysis in CK.

Comparing Apo form of CK with ADP&CP bound form, there's a large conformational changes that cover CP binding pocket. Detailed examinations of the part where the conformational changes happened showed some H-bond and ion pair with Phosphate group of Carbamyl phosphate drove these changes.

Through these structural data, we could suggest a procedure of Pa Carbamate kinase reaction and provide some insights of mechanism of reaction.