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.

[1] Marshall M., Cohen P. P., *Methods Enzymol.*, 1970, **17**, 229.
Keywords: carbamate kinase, mechanism, structure

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The First Structure from Ultralente Insulin Microcrystals
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Ultralente human insulin is one of the commercially available preparations for the treatment of diabetes type 1. Its long-acting activity arises from rhombohedral microcrystals, which are directly injected as an insulin-zinc suspension into the blood stream [1]. The slowly dissolving crystals provide a gradual release of insulin and hence a constant blood level over several hours.

The hexameric arrangement of the insulin heterodimers in Ultralente microcrystals has been elucidated by atomic-force microscopy [2]. Powder diffraction studies proposed the T_6 conformer by comparison of the unit cell constants with those obtained from bigger insulin crystals grown from different crystallization conditions [3]. However due to the small crystal size of approximately 25 x 25 x 5 μm^3 the three-dimensional structure has not been determined yet.

A suspension of Ultralente human insulin was provided by Novo Nordisk. A complete data set from one crystal (*R*3, 81.03 Å, 33.57 Å) could be collected up to a resolution of 1.9 Å at the micro diffractometer MD2 of beamline X06SA, Swiss Light Source, PSI. The structure was solved by molecular replacement. Results from the structure analysis will be discussed on the poster.

[1] Hallas-Møller K., Petersen K., Schlichtkrull J., *Science*, 1952, **116**, 394. [2] Yip C. M. et al., *Biophys. J.*, 1998, **75**, 1172. [3] Richards J. P. et al., *J. Pharm. Sci*., 1999, **88**, 867.
Keywords: macromolecular crystallography, microcrystals, ultralente insulin

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Characterization of Insulin NPH Microcrystals in Pharmaceutical Suspensions
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Insulin preparations with an extended action profile consist of microcrystalline suspensions that slowly dissolve and release the insulin into the blood stream. The action profiles are partly dependent on the crystal form and on the composition of crystals and soluble insulin. The intermediate-acting preparation, NPH (Neutral Protamine Hagedorn) is an insulin-zinc solution co-crystallised with the basic peptide protamine, consisting mainly of arginine residues. The crystals form hexameric insulins with two zinc atoms and one protamine peptide per hexamer. Structural characterisation of the crystals in the pharmaceutical preparations have until now been hampered, mainly due to their microcrystalline nature. Single crystal x-ray analysis has been used after modifications of the crystal growing media to promote larger crystals. However, such modifications may influence the crystal contacts, packing and structural arrangement and may therefore not reflect the true structure in the microcrystals.

In this study, we have used x-ray powder diffraction to analyse several insulin products, and in house preparations of microcrystals. We are able to distinguish between different crystal systems and to check for homogeneousness between different batches/preparations. Furthermore, the micro diffractometer at SLS (Swiss Light Source, Switzerland) has been used to collect single crystal data of the 25 x 5 x 5 μm^3 large NPH microcrystals to a resolution of ~3Å.
Keywords: microcrystals, crystal characterization, macromolecular crystallography

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Femtosecond Laser Processing of Protein Crystals
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It is difficult to process protein crystals by mechanical tools due to their softness and fragility. In this work, the non-contact processing is successfully demonstrated by using multi-photon absorption of focused femtosecond laser beam. We call this technique the femtosecond laser induced cut and cleave operation (fs-CACO). By precisely controlling the laser fluence and the position of the laser focal point, we were able to perform accurate and reproducible processing of hen egg-white lysozyme (HEWL) crystals with little damage in the sealed growth vessels. Using XRD measurements, we confirmed that the processed HEWL crystal retained adequate quality and that there was no deterioration induced by the femtosecond laser irradiation. This technique enables us to process protein crystals without troublesome treatment such as unsealing of the vessels and removal of solutions surrounding the crystals. Fs-CACO procedure will be a powerful tool for making problematic protein crystals suitable for XRD measurements. In the future, this technique could be applied to various processing techniques (e.g., processing protein crystals that overlap each other in the growth vessels or utilizing processed crystals as seeds for protein crystallization).
Keywords: femtosecond laser, protein crystal, laser processing

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Validation of the Molecular Replacement Solutions with the OMIT Procedure
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In the method of molecular replacement, the phases for the initial map of the unknown structure are computed by rotating and translating a trial molecular entity to estimate it's location and orientation within the unit cell of the unknown structure.

The usefulness of this initial map is determined by the molecular entity actually being present in the unknown structure, by the fraction of the unknown that this entity represents, and by accuracy with which it is positioned within the cell. With the omit validation procedure a small fraction of the trial model is deleted before the translation-rotation search. The validity and usefulness of the search resalts are judged by quantitatively evaluating the region of the molecular-replacement map corresponding to the omitted entity. This procedure was found to be useful when evaluating the structure of the maltose binding protein that was thought to have a Ru(II) group bound to it. The validation procedure indicated that the Ru(II) group was not bound to the maltose binding protein molecule in an ordered fashion.
Keywords: molecular replacement, model building, ligand

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Estimate the Time of Soak Simulating Small Molecule Diffusion in Protein Crystal
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For a biocrystallographer, incubating a protein crystal in a solution containing a diffusive compound is a recurring operation. The soaking technique is widely used for preparation of heavy-atom derivatives, cryoprotection of crystals and introduction of inhibitors or substrates in crystal structure of enzymes. However, very few experimental values of the diffusion times in protein crystals are reported in literature. The methods used to detect diffusion of substrates are mainly: X-ray diffraction (monitoring changes in X-ray intensities for some selected reflections as substrates are diffused into the crystal); birefringence of the crystal; and spectroscopy (monitoring specific