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**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<sub>6</sub> 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<sup>3</sup> 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 (R3, 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.

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**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 homogeneity 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<sup>3</sup> 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 its 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 results 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