ESRF	**Interim report for Long Term Proposal CH–1985 "*Development of Synchrotron Powder Diffraction Methods for Biomolecules*"**	**Experiment number**: CH–1985 (LT)
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Report:

A total of 42 shifts of beamtime have been used between November 14th 2005 and December 5th 2006. Most milestones were achieved. An account of the experiments and the results obtained is given below. However, some changes occurred with respect to the initial timetable. In particular, no beamtime was used on ID11 during this period, and on ID31 a somewhat larger amount of beamtime than initially foreseen was used. This is mainly due to the upgrading and extension of beamline ID11 which was going on for most of 2006. The experiments of this LTP are likely to benefit greatly from the upgrade of the ID11 beamline, which will provide microfocusing capabilities. It was therefore decided, in agreement with the beamline staff, to postpone all experiments on ID11 related to this LTP until 2007, when the extension on ID11 becomes available for users. The part of the LTP which deals with the "Evaluation of area detectors for protein powder measurements" has therefore also been postponed.

Main Scientific Results

Development of methods for *de novo* structure solution of proteins from powder diffraction data.

The preparation of single crystals suitable for x-ray analysis is frequently the most difficult step in structural studies of proteins. If a microcrystalline material can be obtained, it is still possible to perform powder diffraction experiments, but the collapse of the three-dimensional reciprocal space into a one-dimensional powder diffraction pattern gives rise to a severe loss of information. On two examples, we have been able to show that *de novo* solution of the crystallographic phase problem can be achieved at low resolution using

microcrystalline powder samples *via* the single isomorphous replacement (SIR) method. For the protein hen egg white lysozyme HEWL, Gadolinium-containing heavy atom derivatives were prepared by co-crystallising the protein with the complex Gd-Hp-Do3A. For enzyme porcine pancreatic elastase (PPE), a Uranium-containing heavy atom derivative was obtained by soaking a native sample in a solution containing uranyl nitrate. Different methods have been exploited to reduce the problem of overlapping reflections. For HEWL, a series of 6 samples was used, varying both the pH of the crystallisation buffer and the concentration of the heavy atom compound. In the tetragonal polymorph of HEWL there is a systematic dependence of the unit cell parameters on the pH of the crystallisation buffe and we exploited this variation to improve the resolution of accidentally overlapping peaks. Diffraction data were collected at room temperature on the undulator beamline ID31. The parallel beam geometry instrument is equipped with nine Si(111) analyser crystals and provides data with very high angular resolution, with a minimum instrumental contribution to the FWHM of around 0.003°. The analyzer crystal removes the contribution to angular resolution that is due to the sample size and allows irradiation of larger sample volumes (of the order of 3 mm^3), which is important for obtaining good counting statistics from weakly scattering protein samples. The powder patterns obtained from proteins have very narrow diffraction lines, as low as 0.01° FWHM, which is essentially due to crystallite size and microstrain effects. Peak overlap, especially in the low angle region, can therefore be significantly reduced. For PPE, we exploited X-ray induced lattice changes that occur when the samples are irradiated by the high photon flux. The various effects caused by exposing samples to an intense X-ray beam are widely discussed topics in single-crystal protein crystallography since they can seriously hamper structure determination. One common phenomenon is an irreversible radiation-induced lattice expansion, although the mechanism by which this occurs is not fully understood. In many cases the lattice changes are anisotropic and can thus be exploited to improve the resolution of overlapping reflections in a powder pattern. For PPE, four native and five uranyl-derivative powder patterns were collected consecutively at room temperature using a wavelength of 1.25085(3) Å on ID31. Each pattern therefore corresponds to a different exposure to X-rays. Intensities were extracted by a multi-pattern Pawley refinement, in which each diffraction pattern is calculated as a sum of overlapping reflections, the intensities of which are variables in a least-squares procedure. These intensity data were then treated as if they had been collected from a single crystal and no further modifications were made to account for the peak overlap problem. For HEWL, the Gd atoms could be located by direct methods as implemented in the software *SHELXD* For PPE, isomorphous difference Patterson maps revealed clear interatomic peaks in the Harker sections. The parameters of the heavy atom substructures were refined by maximum likelihood techniques as implemented in the program *SHARP*. The resulting data were of sufficient quality to compute molecular envelopes of the protein molecule and to map out the solvent channels in the crystals, which are essential structural data for the characterization of microcrystalline proteins as novel mesoporous materials. The development of *de novo* phasing methods for microcrystalline protein samples offers exciting new opportunities to obtain useful structural information in systems where the growing of large single crystals is problematic.

Future developments. We have now prepared additional heavy atom derivatives for both proteins and collected powder diffraction data from these new samples. The processing of these data is currently in progress. We expect that this additional data will allow us to implement the Multiple Isomorphous Replacement (MIR) method and thus to achieve a substantial quality improvement in the experimental phases. Further, we are also exploring possibilities to extend density modification methods (such as the solvent flattening technique) so that they can be used both to improve the phases and the extraction of overlapping intensities. There is thus scope for further improvements and we anticipate that the implementation of these ideas will increase the resolution of the maps which can be derived by *de novo* phasing of protein powder data.

Radiation damage and cryocooling of protein samples for powder diffraction

A substantial part of our work has been devoted at characterizing radiation damage in protein powder samples. Radiation-induced anisotropic lattice strain can effectively be exploited to help in the deconvolution

of overlapping peaks (see above). However, the concomitant reduction in intensities and the broadening of peaks are major problems and it is thus of paramount importance to explore ways to reduce radiation damage. We have developed protocols for cryo-cooling protein powder samples without loss of diffraction quality. The diffraction line shapes can be monitored in order to optimize the freezing conditions and other post-growth treatments (annealing, desiccation, cross-linking). We have found that samples of microcrystalline tetragonal chicken egg white lysozyme can be effectively cryoprotected for high-resolution synchrotron X-ray powder diffraction studies at 100 K. The survival of the powder in the beam is increased by a factor of around 30. Thus, a high-quality powder diffraction pattern could be collected at 100 K, which attains a resolution of d_{min} asymptotically equal to 2.6 Å, significantly better than the previous limit of ~3.27 Å at room temperature, despite a smaller volume of sample. Systematic variations of the concentration and type of cryoprotectant agent show that the lattice microstrains that accompany cooling, and degrade the quality of the powder diffraction data by broadening the diffraction peaks, are caused by a collapse in the volume of the crystalline unit cell.

Future developments. We will need to assess to what extend the conclusions from this optimization procedure can be transferred to other protein samples. Another major goal will be to investigate the use of area detectors for recording powder spectra and to assess the advantages and drawbacks of this method in comparison with analyzer crystal-point detector based measurement strategies.

Molecular replacement with powder diffraction data

As more and more protein folds become known, the molecular replacement (MR) method becomes a more attractive method for structure solution. Synchrotron powder diffraction data from crystalline proteins were analysed to determine their suitability for MR procedures. A series of examples of small proteins (including lysozyme, trypsin, apoferritin, myoglobin, elastase, insulin and thaumatin) that cover symmetries from cubic down to monoclinic have been studied. The information contained in a powder diagram was shown to be sufficient to position and orient a single protein molecule in an unit cell, when a good model for the protein molecule is available. The challenges encountered in more complex MR problems depend on both the quality of the search model and experimental data. Improvements in powder data quality (reducing peak overlap; increasing counting statistics) lead to corresponding improvements in the performance of MR procedures.

Other experiments (mostly studies in progress)

Solvent contrast variation experiments. Thee intensity of low angle reflections can modulated by varying the mean electron density of the solvent surrounding the protein molecules in the crystal lattice *via* variations in the solvent composition. We have collected a series of measurements on protein powder samples that were soaked in solutions of varying electron density. Clear variations of the intensity of low-angle reflections could be observed. We expect to exploit these intensity modulations to explore the structure of the hydration around a macromolecule and compute a low-resolution envelope of the molecule.

In situ crystallization experiments. We are investigating the crystallization process in protein solutions. As microcrystals start to grow from a supersaturated protein solution, the X-ray spectrum changes from diffuse scattering to powder diffraction, with diffraction lines that become sharper as the growth of the crystal nuclei progresses. We also monitor the the evolution of the small-angle scattering part of the spectrum. Work partly done on the SAXS beamline BM16.

Phase identifications of insulin. Microcrystalline insulin formulations are investigated by X-ray powder diffraction. Characteristic powder patterns can be used as `fingerprints' for different insulin polymorphs. The combination of X-ray powder diffraction and multivariate analysis, such as principal-component analysis, provides a rapid and effective tool for studying the influence of derivatives, additives, ions, pH etc., in the crystallization media. Work done in collaboration with *Novo Nordisk A/S*.

Contribution of the User Group in support of the LTP

In 2005, the Laboratory of Crystallography at EPFL has submitted a grant request to the Swiss National Science Foundation (*SNF*) as a commitment from our laboratory towards the implementation of the LTP (Main applicant: Prof. M. Schiltz, Co-applicant: Dr. P. Pattison). In 2006, the SNF has decided to fund the proposal and has allocated a total sum of CHF 140'172.- over a three-year period (project n° 200021-113339/1). The *SNF* requests this grant to be primarily spend for the funding of a PhD student who is to pursue research work on protein powder diffraction in Lausanne and Grenoble an who is expected to submit a thesis at the *EPFL*. A search process was started in September 2006 in order to find a suitable candidate. The position was eventually offered to Mr Sebastian Basso, who holds an MSc in Chemistry from the University of Bath. He has started working in Lausanne on March 1st 2007. The Swiss-Norwegian Beamlines at ESRF have agreed to provide the necessary infrastructure (office space, lab and computer access *etc*.) in Grenoble.

Scientific event related to the LTP

An international workshop on "Development and Directions of Powder Diffraction on proteins" is going to be organized at the ESRF on 22–23 June 2007, with support from the International Union of Crystallography, the British Crystallographic Association, PANalytical and the EPFL. The workshop will be linked to an EMBO course on anomalous scattering in macromolecular structure determination, which is organised at the EMBL outstation in Grenoble between the 18th and the 22d of June. Therefore we hope to have a few common participants in both workshops. We are aiming for a constructive meeting with powder diffractionists, structural and molecular biologists, people from the pharmaceutical industry and methodological experts and software developers all participating. More than twenty invited speakers have already accepted to attend the workshop.

Publications and Communications arising from the LTP

Papers

J. P. WRIGHT, C. BESNARD, S. BASSO, F. CAMUS, A. N. FITCH, G. FOX, I. MARGIOLAKI, P. PATTISON & M. SCHILTZ (2007). Molecular envelopes from protein powder diffraction data. Submitted to *Journal of the American Chemical Society.*

C. BESNARD, F. CAMUS, M. FLEURANT, A. DAHLSTRÖM, J. P. WRIGHT, I. MARGIOLAKI, P. PATTISON, M. SCHILTZ (2007). Exploiting X-ray induced anisotropic lattice changes to improve intensity extraction in protein powder diffraction: application to heavy atom detection. Accepted for publication in *Z. Kristallographie*.

I. MARGIOLAKI, J. P. WRIGHT, A. N. FITCH, G. C. FOX, A. LABRADOR, R. B. VON DREELE, K. MIURA, F. GOZZO, M. SCHILTZ, C. BESNARD, F. CAMUS, P. PATTISON, D. BECKERS, T. DEGEN (2007). Synchrotron X-ray powder diffraction studies of proteins. Accepted for publication in *Z. Kristallographie*.

J. P. WRIGHT, A. J. MARKVARDSEN & I. MARGIOLAKI (2007). Likelihood Methods with Protein Powder Data. Accepted for publication in *Z. Kristallographie*.

M. J. JENNER, J. P. WRIGHT, I. MARGIOLAKI & A. N. FITCH (2007). Successful protein cryocooling for powder diffraction. *J. Appl. Cryst*. **40**, 121–124

S. BASSO, A. N. FITCH, G. C. FOX, I. MARGIOLAKI & J. P. WRIGHT (2005). High-throughput phase-diagram mapping via powder diffraction: a case study of HEWL versus pH. *Acta Cryst*. D**61**, 1612–1625.

Highlights

J. P. WRIGHT, C. BESNARD, S. BASSO, F. CAMUS, A. N. FITCH, G. FOX, I. MARGIOLAKI, P. PATTISON & M. SCHILTZ. Molecular envelopes from protein powder diffraction data. *ESRF Hihghlights 2006*, pp 61–62.

Communications at international conferences

I. MARGIOLAKI, J. P. WRIGHT, A. N. FITCH, M. J. JENNER, G. C. FOX, M. SCHILTZ, C. BESNARD, F. CAMUS, M. FLEURANT, P. PATTISON, R. KAHN & R. B. VON DREELE (2006). *Synchrotron X-ray powder diffraction as it starts to make an impact in structural biology*. Invited oral communication at the **10th European Powder Diffraction Conference**, 1–4 September 2006, Genève, Switzerland.

C. BESNARD, F. CAMUS, M. FLEURANT, P. PATTISON, M. SCHILTZ, J. P. WRIGHT, I. MARGIOLAKI, A. N. FITCH, M. J. JENNER, & R. KAHN (2006). *Molecular envelopes from protein powder diffraction data*. Invited oral communication at the **10th European Powder Diffraction Conference**, 1–4 September 2006, Genève, Switzerland.

J. P. WRIGHT & A. J. MARKVARDSEN (2006). *Likelihood methods with protein powder diffraction data*. Invited oral communication at the **10th European Powder Diffraction Conference**, 1–4 September 2006, Genève, Switzerland.

F. CAMUS, C. BESNARD, M. FLEURANT, P. PATTISON, M. SCHILTZ, J. P. WRIGHT, I. MARGIOLAKI, A. N. FITCH, M. J. JENNER, & R. KAHN (2006). *Extracting structural information from protein powder diffraction data*. Poster communication at the **23rd European Crystallographic Meeting**, 6–11 August 2006, Leuven, Belgium. Abstract published in *Acta Crystallogr*. (2006), A62, p. s232.

M. J. JENNER, I. MARGIOLAKI, A. N. FITCH & J. P. WRIGHT (2006). *Protein powder diffraction at cryocooled conditions*. Poster communication at the **23rd European Crystallographic Meeting**, 6–11 August 2006, Leuven, Belgium. Abstract published in *Acta Crystallogr*. (2006), A62, p. s234.

I. MARGIOLAKI, J. P. WRIGHT, A. N. FITCH, M. JENNER, S. BASSO, G. C. FOX, M. SCHILTZ, C. BESNARD, F. CAMUS, P. PATTISON, R. KAHN & R. B. VON DREELE (2006). *Synchrotron X-ray Powder Diffraction: Applications in Macromolecular Crystallography*. Invited oral communication at the **5th Pharmaceutical Powder X-ray Diffraction Symposium**, 14–16 February 2006, Somerset, New jersey, USA.

J. P. WRIGHT, I. MARGIOLAKI, G. C. FOX, A. J. MARKVARDSEN & A. N. FITCH (2005). *Molecular Replacement with Powder Diffraction Data*. Invited oral communication at the **XX Congress of the International Union of Crystallography**, 23–31 August 2005, Firenze, Italy. Abstract published in *Acta Crystallogr*. (2005), A61, p. C53.

I. MARGIOLAKI, J. P. WRIGHT, S. BASSO, A. N. FITCH, G. C. FOX, M. SCHILTZ, P. PATTISON & R. B. VON DREELE (2005). *Development of powder diffraction methods for macromolecular crystallography*. Invited oral communication at the **XX Congress of the International Union of Crystallography**, 23–31 August 2005, Firenze, Italy. Abstract published in *Acta Crystallogr*. (2005), A61, p. C54.

I. MARGIOLAKI, J. P. WRIGHT, S. BASSO, A. N. FITCH, G. C. FOX, M. SCHILTZ, P. PATTISON & R. B. VON DREELE (2005). *Powder Diffraction Methods in Macromolecular Crystallography*. Oral communication at the **4th Pharmaceutical Powder X-ray Diffraction Symposium**, 21–24 February 2005, Barcelona, Spain.

Forthcoming

C. BESNARD, J. P. WRIGHT, S. BASSO, F. CAMUS, A. N. FITCH, G. FOX, I. MARGIOLAKI, P. PATTISON & M. SCHILTZ. *Molecular envelopes from protein powder diffraction data*. Invited oral communication at the **24th European Crystallographic Meeting**, 22–27 August 2007, Marrakech, Morocco.

Invited seminars

J. P. WRIGHT (Jan. 2007). School of Chemistry at The University of Edinburgh, UK. Departmental Seminar: *From materials science to structure solution with powder diffraction from proteins*.

I. MARGIOLAKI (Dec 2006). Novo Nordisk, Denmark. *Synchrotron X-ray Powder Diffraction as it begins to make an impact in structural biology.*

J. P. WRIGHT (Dec 2006). Panalytical, The Netherlands. *Likelihood methods with protein powder diffraction data.*

I. MARGIOLAKI (Mar 2006). ESRF seminar: *Synchrotron X-ray powder diffraction: Applications in macromolecular crystallography.*

I. MARGIOLAKI (2006). Annual Meeting of the crystallographic society of Japan- Tokyo. Invited talk: *Complementary techniques in structural biology*

I. MARGIOLAKI (2005). European Molecular Biology Laboratory (EMBL- Hamburg). *High Resolution Powder Diffraction: A useful tool for structural characterisation of materials.*

Forthcoming

I. MARGIOLAKI (Sep 2007). 1st Meeting of the Italian and Spanish Crystallographic Associations (MISCA), Italy. Invited talk: *Biocrystallography via powder diffraction; an emerging method in structural biology.*

I. MARGIOLAKI (Mar 2007). Crystallographic Association of the Netherlands (Nederlandse Vereniging voor Kristallografie), Lunteren, The Netherlands. Invited talk: *Developemnt and future directions of powder diffraction on proteins.*