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The High-throughtput Membrane Protein Structural Biology Pipeline at the SGC. Liz Carpenter. *Structural Genomics Consortium, Nuffield Department of Clinical Medicine, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Headington, Oxford, OX3 7DQ, England.*
E-mail: liz.carpenter@sgc.ox.ac.uk

The Structural Genomics Consortium is a public-private partnership which produces structures of human proteins with immediate release of the structure and experimental details as soon as the structure is complete. Mammalian membrane proteins remain one of the most difficult areas for structural biology due to the hydrophobicity and instability of these proteins. Since more than 50% of all drugs bind to IMPs, it is imperative that we develop systems for producing and solving the structures of these proteins. The integral membrane protein (IMP) group at the SGC has developed a pipeline for the production of human membrane proteins in the baculovirus/insect cell system, using a multiple construct approach. Our targets include G-protein coupled receptors (GPCRs), channels, GAP junctions, solute carriers, ABC transporters and enzymes. We have screened 150 IMP targets in 10 months, identified proteins that show significant solubility on detergent extraction and screened these with a variety of detergents to find the conditions which give monodisperse protein in one or more detergents. Crystallisation of these proteins is underway and we have initial crystallization hits for proteins in 3 families. This pipeline has therefore delivered the throughput and quality control needed for production of protein for functional, biophysical and structural studies.
In addition to the pipeline for the production of integral membrane proteins, we are also working with the extracellular domains (ECDs) of GPCRs. We have solved the structures of two Class B GPCR ECDs to date, the vasoactive intestinal peptide receptor and the growth hormone releasing hormone receptor, revealing the conformation of the binding site for the peptide to the ECD prior to peptide binding.

Keywords: Membrane Proteins, pipeline, baculovirus

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Automated high-throughput crystallisation suited for membrane proteins. Yvonne Thielmann[a], Jian Xu[b], Matthew Lundy[b], Mandel Mickley[b], Ute Boronowsky[c], Jan Kubicek[c], Jürgen Köpke[a]. [a]*Department for Molecular Membrane Biology, Max-Planck-Institute of Biophysics, Max-von-Laue-Strasse 3, 60438 Frankfurt am Main, Germany.* [b]*Rigaku Automation , Inc., 5999 Avenida Encinas, Suite 150, CA 92008, Carlsbad, USA.* [c]*QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany*
E-mail: yvonne.thielmann@biophys.mpg.de

Membrane protein crystallisation still remains a challenging task. To optimise the usage of time and sample amount in our "Membrane Protein Core Centre" the Rigaku CrystalMation™ system was setup to fully automate the crystallisation process while dealing with sample volumes of 100 nl per experiment. Three different crystallisation strategies will be applied to offer a broad range of crystallisation conditions for membrane proteins: First commercially available membrane protein crystallisation screens and second a series of in-house produced screens will be provided. These cover a pH screen and screens depending on tolerated pH and used detergent, a set of PEGs and salts generates conditions close to phase separation. The third method applied will be the fully automated setup of cubic phase crystallisation which is in preparation by a cooperation with Qiagen and Rigaku. Additionally we are using various temperatures to suit the need of the individual membrane protein. This enables us to provide a quite diverse range of crystallisation conditions together with a fully automated setup which is also capable of optimisation.

Figure 1 Setup of CrystalMation™ system

Keywords: membrane proteins, automation, cubic phase crystallisation

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Crystal structure of oxygen evolving Photosystem II at atomic resolution. Yasufumi Umena[a], Keisuke Kawakami[b], Jian-Ren Shen[b], Nobuo Kamiya[a]. [a]*Osaka City University, Japan.* [b]*Okayama University, Japan.*
E-mail: yas6374@protein.osaka-u.ac.jp

Photosystem II (PSII) is a multi-subunit membrane protein complex functioning in photosynthesis. It is a large homo-dimer complex composed of 38 protein subunits and 114 co-factors, and performs a series of light-induced electron transfer reactions leading to the splitting of water and the generation of molecular oxygen. The catalytic center is composed of four manganese atoms and one calcium atom, which are linked by oxo-bridges, and is generally called the Mn_4Ca cluster. Various studies including X-ray crystallographic and spectroscopic analyses have been reported on the structure and function of the Mn_4Ca cluster, but the precise organization of the metal cluster and the mechanism of water-splitting reaction are not fully understood. So far, the crystal structure of oxygen-evolving PSII has been reported at 2.9 Å resolution [1], which was not high enough to resolve the individual metal atoms as well as the oxo-bridges in the Mn_4Ca-cluster. We have succeeded in improving the resolution and diffraction quality of PSII crystals significantly by optimizing the crystallization conditions as well as by employing a post-crystallization dehydration procedure. As a result, we were able to solve the crystal structure of PSII from *Thermo-synechococcus vulcanus* at 1.9 Å resolution. The diffraction data of PSII crystal was collected at the beamline BL44XU of

SPring-8 in JAPAN. Since previous report has shown that the Mn_4Ca-cluster suffer from serious X-ray radiation damage during data collection of PSII crystals [2], we adopted a slide-oscillation method to collect the low-dose X-ray diffraction data using large PSII crystals (length: ~1mm), which allowed us to collect a full data set from nine irradiation points of one single crystal in a range of 180 degree. The X-ray dose irradiated onto each point was estimated to be half of the value determined by the spectroscopic method where the radiation damage begins to occur [2]. In the crystal structure obtained at 1.9 Å resolution, the electron density distributions for each of the 5 metal ions were clearly separated, which were also confirmed by the anomalous difference Fourier map. Five oxygen atoms forming the oxo-bridges between the metal ions were clearly identified by the omitted Fourier map, and several water molecules were found to be associated with the metal cluster, which may function as substrates for the oxygen-evolving reaction. In addition, thousands of water molecules were found in the whole structure of PSII dimer. Some of the water molecules form a network linking the catalytic site and two chloride ion sites identified previously [3]. Including protein subunits and co-factors, we will discuss the atomic resolution structure of PSII complex.

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Keywords: membrane protein complex, photosynthesis, metal clusters

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A neutron-diffraction study of the low-cycle fatigue behaviour of an austenitic stainless steel 316.

Adelaide Dubreuil[a,b], Shu Yan Zhang[b], Sophie Eve[a] and Alexander Korsunsky[c], [a]*ENSICAEN, 14000 Caen, France,* [b]*ISIS Facility, STFC, Rutherford Appleton Laborator, Chilton Oxfordshire, OX11 0QX, UK,* [c]*University of Oxford, Oxford, OX1 3PJ, UK*
E-mail: shu-yan.zhang@stfc.ac.uk

Type 316 stainless steel considered to be one of the leading candidate materials for the first wall and blanket structure of future fusion reactors because of its well-characterized properties such as high temperature strength, good compatibility with coolant and irradiation properties for fast breeder reactors.
The present work describes the behavior of this steel under low cycle fatigue at different temperatures.
The in situ neutron diffraction fatigue test was conducted on the ENGIN-X diffractometer at the ISIS spallation neutron source in Chilton, UK. The fatigue tests were performed at room temperature and at 550°C, where the minimum strain and the maximum strains were -1.1% and 1.1%, respectively. Diffraction spectra were acquired at a series of load levels within selected fatigue cycles, enabling measurement of lattice strain parallel and perpendicular to the loading axis.The measurements were used to quantify the evolution of internal microstrain within crystallites as a function of applied macrostress through the fatigue cycles. The measurement has shown that during low cycle fatigue, grain families show different responses to applied stress, i.e. anisotropic behavior is observed. Some grain groups undergo elastic cycling whereas others exhibit plastic deformation characterized by a significant hysteresis fatigue loop.
The cyclic hardening and softening behavior during fatigue are discussed and comparison is made between the experimental results and finite element crystal plasticity modelling.

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Keywords: Neutron diffraction, Low cycle fatigue, Strain measurement.