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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.*
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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*
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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.*
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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