Keywords: membrane proteins, high-throughput, green fluorescent protein

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A systematic approach to membrane protein crystallization in bilayers. Xiang-Yu Liu[a], Pontus Gourdon[a], Jacob Lauwring Andersen[a], Maike Bublitz[a], Bjørn P. Pedersen, Laure Yatime, Maria Nyblom,Claus Olesen, Jesper V. Møller, Poul Nissen, J. Preben Morth. [a]*Centre for Membrane Pumps in Cells and Disease - PUMPKIN. Danish National Research Foundation, Department of Molecular Biology, University of Aarhus, Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark.* E-mail: xyliu@bioxray.au.dk

Methods to get high quality membrane protein crystals are in high demand and in a constant development. A preferred method of choice is to maintain low levels of detergent, near the Critical Micelle Concentration (CMC), to shield the hydrophobic regions throughout the purification and crystallization process. This approach typically results in 'Type II' crystals that are built up through interactions between the hydrophilic surfaces of the molecules [1]. In contrast, 'Type I' crystals (often described as stacked 2D crystals) [2, 3] are characterized by continuous bilayers, formed by a lipid/detergent saturated environment, in which the proteins are packed. We present an improved method to induce growth of bilayer membrane protein crystals in high concentrations of lipids and detergent. The straightforward procedure includes a systematic screening approach for lipidation and crystallization, and subsequent improvement of diffraction properties of membrane protein crystals by optimization of added amounts of detergents and lipids.

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Keywords: Membrane proteins, crystallization, bilayers, type I crystals

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Access Membrane Transport by the Sodium-Hydantoin Transporter Mhp1. Simone Weyand[a,b,d*], Tatsuro Shimamura[a,b,c*], Oliver Beckstein[e*], Nicholas G. Rutherford[f], Jonathan M. Hadden[f], David Sharples[f], Mark S. P. Sansom[d], So Iwata,[a,b,c,d,g], Peter J. F. Henderson[f], Alexander D. Cameron[a,b,d]. [a]*Division of Molecular Biosciences, Membrane Protein Crystallography Group, Imperial College, London SW7 2AZ, UK.* [b]*Japan Science and Technology Agency, Exploratory Research for Advanced Technology, Human Receptor Crystallography Project, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan.* [c]*Department of Cell Biology, Graduate School of Medicine, Kyoto University, Yoshida-Konoe-cho, Sakyo-Ku, Kyoto 606-8501, Japan.* [d]*Membrane Protein Laboratory, Diamond Light Source, Harwell Science and Innovation Campus, Chilton, Didcot, Oxfordshire OX11 0DE, UK.* [e]*Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK.* [f]*Astbury Centre for Structural Molecular Biology, Institute for Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK.* [g]*Systems and Structural Biology Center, RIKEN, 1-7-22 Suehiro-cho Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan.*
*These authors contributed equally to this work.
E-mail: s.weyand@imperial.ac.uk

Membrane transport proteins are classified into different groups. However, the common molecular mechanism of all of them is based on the alternating access model [1]. Mhp1 belongs to the nucleobase–cation–symport-1 family of secondary active transporters with indolyl methyl- and benzyl-hydantoins as substrates in *M. liquefaciens*. Two crystal structures of this protein were already solved and present the outward facing and occluded forms [2]. Recently, a crystal structure in a third conformation, inward facing, was solved and revealed detailed insights into the alternate access model. The strucure was first solved by molecular replacement and later on by SAD and refined at 3.8 Å resolution to R=27.3% and R_{free}=31.3.1% [3].
Mhp1 comprises a five-helix inverted repeat, a common motif among secondary transporters. This new crystal structure is complementing its previously described structures in outward-facing and occluded states. From analyses of the three structures and molecular dynamics simulations, a mechanism for the transport cycle in Mhp1 could be proposed. The switch from the outward- to the inward-facing state, to effect the inward release of sodium and benzylhydantoin, is primarily achieved by a rigid body movement of transmembrane helices 3, 4, 8, and 9 relative to the rest of the protein. This forms the basis of an alternating access mechanism applicable to many transporters of this emerging superfamily.

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