

Harnessing low background data collection at the nano-focus beamline, VMXm, for structure determination of membrane proteins from microcrystals.

Structure solution of membrane bound proteins remains a significant challenge. These targets, such as potentially druggable GPCRs, represent ~3% of the X-ray diffraction structures in the PDB. Crystallisation often requires the use of lipid cubic phase (LCP) to maintain the hydrophobic environment, protein stability and promote crystallisation. Membrane protein LCP crystals are generally smaller and more fragile than other protein crystals. As such, a microfocus beamline is often needed for data collection.

The Versatile Macromolecular Crystallography beamline (VMXm) provides a stable X-ray beam of 0.4-10 μm x 1.5-5 μm (V x H), an in-vacuum sample environment and integrated scanning electron microscope to enable high quality, low background, single crystal data collection from crystals measuring 0.5-10 μm . Significant effort is made to ensure that the sample is mounted in such a way as to minimise additional X-ray scatter. Together the beamline apparatus and standard sample mount contribute minimal background noise.

However, LCP generates a significant amount of X-ray scatter, so it is essential to remove as much of the LCP as possible. This is challenging for LCP derived microcrystals, where providing a suitable, low noise sample mount is difficult while also minimising the amount of LCP material all without damaging the fragile microcrystals. Some of these challenges have already been addressed for LCP sample preparation for electron diffraction [1].

We will outline the sample preparation process for LCP derived membrane protein crystals for VMXm and examples of its deployment at the beamline. We will also demonstrate how the use of VMXm and these sample preparation methods enable data collection from previously very difficult to access membrane protein samples.

1. Zhu, L. et al. Structure Determination from Lipidic Cubic Phase Embedded Microcrystals by MicroED. *Structure/Folding and Design* 1–16 (2020) doi:10.1016/j.str.2020.07.006.

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