MBG FOCUS TALK

hosted by Thomas Boesen

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Direct detection, phasing, and membrane protein crystallography using MicroED

Membrane proteins serve diverse and important physiological functions and are a common target for developing novel therapeutics. Structure determination is however severely hampered as these are notoriously difficult to crystallize and often only grow a few micron in size, typically requiring the use of lipidic cubic phase (LCP) to facilitate crystallization by mimicking the native lipid bilayer. Microcrystal electron diffraction (MicroED) is an electron cryo-microscopy (cryo-EM) method suitable for such crystals that are too small for conventional X-ray crystallography. However, the high viscosity of the gel-like LCP means any excess material can not be removed using standard blotting methods, and an additional complication is locating crystals embedded in thick layers of LCP.

Here, we demonstrate targeting and accessing these crystals directly within the LCP using plasma focused ion beam milling and scanning electron microscopy (pFIB/SEM) with an integrated fluorescence light microscopy (iFLM) module. Fluorescently labeled crystals of a G protein-coupled receptor (GPCR) grown in LCP were identified by correlating the fluorescent signal with SEM images. The localized crystals were then machined into thin lamellae of optimal thickness using a pFIB. High-quality MicroED data were collected using electron counting on a direct electron detector, resolving the structure of the human adenosine receptor and revealing the bound ligand. Optimizing the sample preparation was key in deriving the structural model, as well as improvements made in both data quality and resolution by using direct electron detection. This was feasible after significantly lowering the exposure to ensure a linear response of these cameras when operated in electron counting mode.

Furthermore, electron counting enabled *ab initio* phasing of two macromolecular structures, triclinic lysozyme at 0.87 Å and proteinase K at 1.5 Å resolution, and allowed visualization of individual hydrogen atom positions at subatomic resolution. Taken together, these advances in MicroED methods open up a new path for routine high-quality structure determination of difficult to crystallize membrane proteins and create novel opportunities for drug discovery.