# MicroED of membrane proteins

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Membrane proteins typically have large hydrophobic surfaces and require detergents and lipids for stabilization during purification and crystallization. A large number of membrane protein structures was obtained using lipidic cubic phase (LCP). Some classes of membrane proteins require LCP to form a tight crystal packing that results in high-resolution diffraction.

Sample preparation for MicroED from LCP sample has a number of hurdles. LCP has high viscosity and has to be removed in order to make a thin sample suitable for MicroED. Isolating a crystal from LCP can result in the dehydration of crystal and loss of diffraction quality. A number of lipidic phase converting reagents was explored for the sample preparation. Sample preparation is carried out in hydrated environment in presence of PEG400 as a cryoprotectant and a component to dissolve the lipidic phase.

After the vitrification, grids are loaded into a dual-beam focused ion-beam, scanning electron microscope (FIB/SEM). Crystals are located and milled into thin lamellae. During the milling process, the voltage is decreased while getting closer to the final sample thickness in order to minimize the damage. Final step of thinning is carried out separately after all lamellae are made. The final sample thickness is 200nm. Grids are loaded into electron microscope and checked for diffraction quality.

Current sample preparation protocol allowed to determine the structure of adenosine A2A receptor in complex with an antagonist ZM2485 at 2.8 Å resolution.