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Max is a postdoctoral scholar in the lab of Tamir Gonen at the Dept of Biological Chemistry at the University of California, Los Angeles (UCLA). His research is primarily involved with developing novel methods in electron crystallography for structure determination of (in-) organic compounds, natural products, and macromolecules from small crystals. He obtained his PhD in structural biology at the Biozentrum, University of Basel, in the group of Jan Pieter Abrahams, and spent two years working as a postdoc in the lab of Xiaodong Zou at Stockholm University, before moving to UCLA. Currently, his research focuses on developing methods that integrate cryogenic electron microscopy (cryo-EM), focused ion-beam milling (FIB), and microcrystal electron diffraction (MicroED) to determine atomic resolution structures of membrane proteins and identify the ligands that bind and regulate them.

Recent advances in MicroED: membrane protein structure determination, direct electron detection, and *ab initio* phasing

Structure determination in membrane protein crystallography is often hampered by failed crystallization attempts that can be notoriously difficult and typically require the use of highly viscous lipidic mesophases to facilitate crystallization by mimicking the native lipid bilayer. This complicates sample handling, the crystals often are only very small, and they are difficult to locate and access from the crystallization matrix they are embedded in. MicroED is a cryo-EM method suitable for structure determination of small microcrystals that are beyond the reach of conventional X-ray crystallography. Here, we demonstrate that fluorescently labeled crystals of a G protein-coupled receptor (GPCR) grown in lipidic cubic phase (LCP) can successfully be localized and machined into thin lamellae using plasma FIB and scanning electron microscopy (pFIB/SEM) with an integrated fluorescence light microscopy (iFLM) module. 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 a structural model, as well as improvements made in both data quality and resolution employing direct electron detection. Electron counting data collection was feasible after significantly lowering the exposure to ensure a linear response of the camera and minimize coincidence loss. 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 sub-atomic 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.