Structural Studies of Gpcrs with Microed

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G-protein coupled receptors (GPCRs) represent a pharmacologically significant superfamily of membrane proteins that are regulating signal transduction across the cell membrane.
GPCRs are typically crystallized in the lipid cubic phase (LCP), which mimics the lipid bilayer environment and stabilizes the membrane proteins.
Approaching the LCP-embedded crystals with MicroED is challenging since LCP has a high viscosity and cannot be blotted away by the regular sample preparation methods.
Previously, we have developed an approach to convert LCP to a less viscous phase by adding a phase-converting reagent such as PEG-400. This approach made it possible to obtain the first GPCR structure by MicroED. However, this technique had high sample consumption and could not be applied to many targets, so our next goal was to extend the sample preparation approach to targeting crystals embedded in the thick media.
Recently, our lab has developed a technique that allows targeting crystals embedded in the lipid cubic phase (LCP) without the need to convert the phase. The target protein was labeled with a fluorescent NHS-ester dye before crystallization. Crystallization is LCP was carried out in 40nL drops overlaid with 400nL of precipitant. LCP with crystals was transferred onto a grid in humidified conditions and flash-frozen in liquid nitrogen. The crystals were located by using fluorescence microscopy and correlated to images taken by the SEM to precisely target the crystals. The lamellae were milled by using xenon and argon plasma beams.
Our approach allowed us to determine the structure of adenosine receptor $A_{2A}AR$ at 2.0 Å resolution. Currently, we are applying this approach to novel GPCR targets that were not accessible by the other crystallographic techniques.