

Report on experiment MX1090: Online microspectrophotometry: structural basis for lightinduced switching in photochromic fluorescent proteins.

This experiment (in addition to 3 shifts of extra online microspec beamtime kindly granted to us) aimed at investigating the photoswitching mechanisms of two photoswitchable fluorescent proteins named IrisFP and Padron. Concerning Padron, a so-called negatively photoswitchable FP, we solved the x-ray structure of Padron on its way to off-on photoswitching, by illuminating the crystal with green laser light at cryo-temperature. An intermediate state (“A3”) with the chromophore in *cis* conformation was obtained, which spectroscopically relaxes in ~15 min at 100 K. The data were complemented by data sets collected on the A3-equivalent in the protein Dronpa, from which Padron was engineered, for comparative purposes. New data sets were also collected on EYQ1 a mutant of EYFP that exhibits negative photoswitching, and the structure of the protein in its fluorescent on-state was solved.

No data could be collected on IrisFP, due to the lack of time and the poor quality of the crystals.