

## **Report on experiment MX769: X-ray induced photobleaching mechanism of fluorescent proteins investigated by combined diffraction and online spectroscopy.**

This experiment aimed at investigating the loss of fluorescence observed in fluorescent proteins upon x-ray exposure. We noticed that upon opening the x-ray shutter there was a very rapid decay of Fluorescence in the fluorescent protein IrisFP. We monitored this decay as well as the absorption decay by online microspectrophotometry. During this experiment, we successfully collected composite data sets allowing us to compare the x-ray structure of IrisFP under either a very low x-ray dose, or a more pronounced x-ray dose. The difference electron density map between these two data sets revealed pronounced structural distortion of the IrisFP chromophore. This was an important result, suggesting the accumulation of a radical state in the crystal. This radical state was further substantiated by Raman data. Radical state formation in fluorescent molecules has been already known in the case of organic dyes. The results coming from this experiment are presently under review by JACS.