Report on ps time-resolved WAXS of the Green Fluorescent Protein SC-2082 beamline ID09B 17/10/2006

In collaboration with Michael Wulff, Philip Anfinrud, Friedrich Schotte and Marco Cammarata we have performed pump-probe experiments of the Green Fluorescent protein. Because of irreversible photodegradation of the sample, a flow system was used that allowed replacing the sample on a slow time-scale. This flow system was a syringe pump driven by a stepper motor used at a forward speed of 12 μm per displacement at 50 Hz and used a 1 ml volume of 2.0 mM GFP per 4 hours. In addition to flowing, the goniometer head was continuously and reversibly translated in the y-direction. The X-ray beam from U17 and U20 was focussed on the edge of a 300 μm radius capillary. The ground state optical absorption at the wavelength used was 60 cm-1 and optical excitation was with 390 nm pulses with 70 μJ energy in a 300 μm spot, temporally stretched in fused silica. This excitation regime assumed a complete and instantaneous ground-state bleach on the basis of published data (Kennis et al. 2004. PNAS 2004. 101: 17988) A 150 ps delay was chosen targeting the maximal temporal population of the deprotonated radiative intermediate I* in the fluorescence photocycle. In addition, 3 ns and 10 ns delays were included to follow the decay of the I* state which has a 3 ns lifetime.The detector distance was 175 mm, corresponding to a 2Φ=24˚ angular range and a 2Å resolution at the edge at 15KeV. With several re-alignments, datasets were collected that averaged about 50 images with 500 shots per image. The data were processed and subtracted from a dark image that had a negative excitation time-point at -3 ns. Comparison with similar experiments conducted with myoglobin, we conclude that the difference signal at small angle is assigned to swelling of the macromolecule after optical excitation and the feature at high angle is the solvent response whereas at intermediate values the superimposed high resolution contributions from structural response of the protein is not resolved relative to the other features with these number of pump-probe cycles.

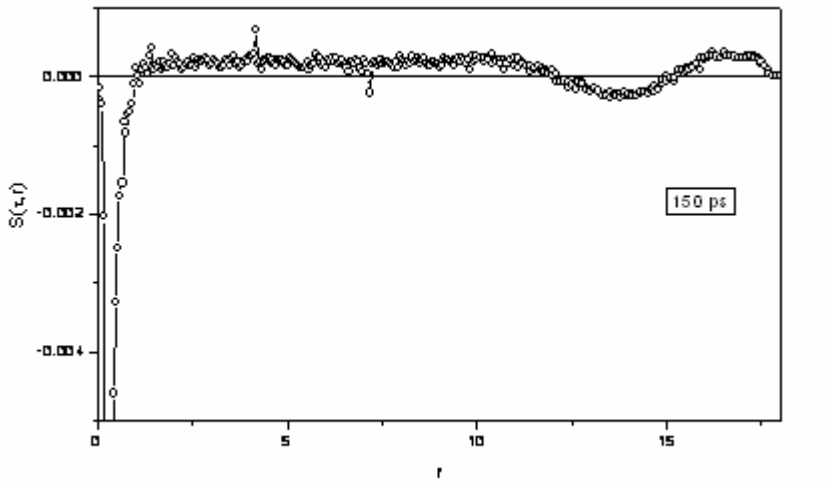

Figure 1. Pump-probe WAXS response of GFP at 150 ps delay with 390 nm excitation of the neutral A state.