| ESRF | **Experiment title:**<br>**Real time investigations of Dronpa structural switching mechanism** | **Experiment number**:<br>SC3061 |
|---|---|---|
| **Beamline**:<br>ID09B | **Date of experiment**:<br>from: 19. Oktober **2010** to: 21. Oktober **2010** | **Date of report**:<br>31/01/2011 |
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## Report:

Dronpa is a green fluorescent protein (GFP)-like protein and from the sea anemone *Anemonia sulcata*, can be reversibly photo-switched between a fluorescent "on" to a non-fluorescent "off" state by the use of green light. At room temperature, Dronpa recovers back to its ground state reversibly, making it ideal for stroboscopic experiments. In **SC-3061** we have studied the x-ray scattering behaviour of Dronpa upon photoexcitation with an optical laser pulse. Photo-crystallography relying on the cryo-trapping techniques has revealed that the photo-switching of Dronpa is based on a cis/trans isomerisation of the chromophoric unit coinciding with a proton transfer process in the centre of the protein. The secondary structure of Dronpa is characterised by a beta barrel in which the chromophore unit is buried inside the barrel (Figure 1A).

During out test beamtime (**SC-3061**) we could reveal **by time-resolved x-ray scattering experiments** that upon photoexcitation in the optical resonance of the chromophore, the beta barrel starts to deform. One typical x-ray scattering difference map is shown in Figure 1A (50 us time point (**on**) **-** (- 10 ns time point) (**off**)) for the scattering area where scattering signals are dominated by correlation between the sheets of the beta barrel of Dronpa. These x-ray scattering differences rise from typical deformations as shown in Figure 1A. They have been derived by **quantitative analysis of the x-ray scattering data with protein refinement programs (DAMMIN) adapted to ID09B parameters and conditions (i.e. including excitation yields)**. The refinement includes scattering features up to q = 20 $A^{-1}$. Figure 1A shows the difference in structural changes between the off structure (corresponding to a negative time point in the time-resolved experiment) and the on structure (50 microsecond time point).

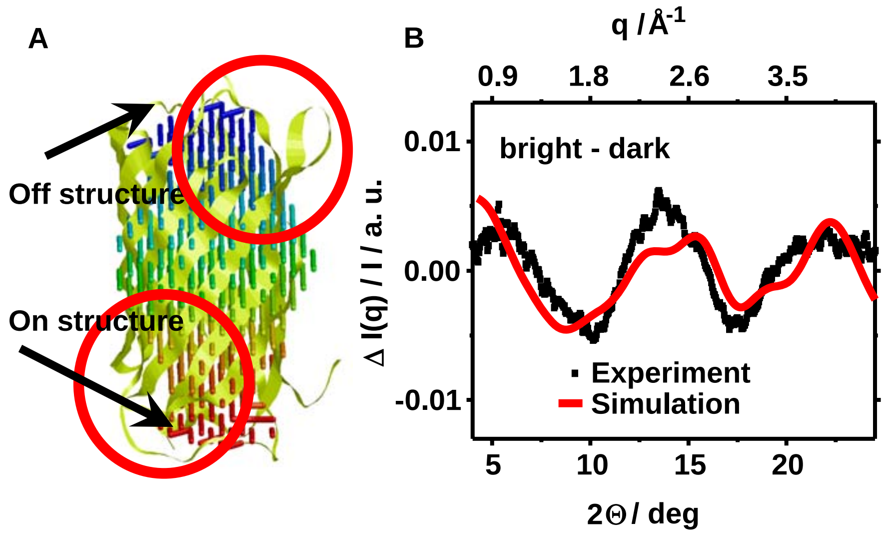


Figure 1: Structural changes on the beta barrel secondary structure as been derived by data evaluation of the test beamtime at ESRF in October 2010 (program package DAMMIN), including the structures derived from the time-resolved small angle x-ray scattering experiments as initial starting structures. B) A typical x-ray scattering difference map for the difference of (50 us (**on**) - (- 10 ns (**off**)). The scattering area is dominated from scattering signals rising from the correlations between the sheets of the beta barrel of Dronpa.

In order to make the chances more visible, the beta barrel and backbone amino acids of the off structure are shown in a crystallographic presentation and the on structure (refinement with slightly lower resolution) in form of barrels from blue to red. As been highlighted by the red circles, the main differences between the ground state and 50 us state structure is given in the edges of the beta barrel structure. The data were of so excellent quality that this kind of refinement was possible. So in the excited state structure, the beta barrel rearranges dominantly in the area of the secondary structure turns. For the refinement we have started with on and off state structures derived from small angle x-ray scattering experiments *as reference.* There structural changes are expressed as time-dependent radius of gyration changes in Figure 2.

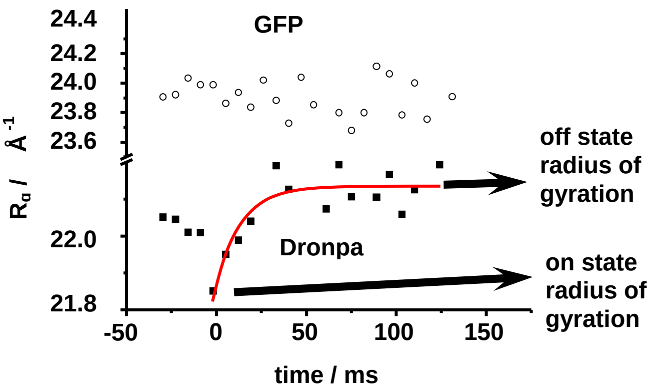


Figure 2: Photo-induced effects on the radius of gyration of Dronpa as been revealed by time-resolved small angle x-ray scattering experiments.

Unfortunatelly we were not able to derive the structural response function for the time scales from picoseconds to microseconds due to the time limitation of the test beamtime. Nonetheless, the scattering experiments were thought to be a preparation step and our on-line data analysis gave such nice structural changes that we decided to stay with scattering experiments in Dronpa during the october run 2010.