ESRF	**Experiment title: Elucidation by SAXS of Light-Induced Oligomerization of the Blue-Light Receptor Aureochrome**	**Experiment number**: LS-2643
Beamline: ID02	**Date of experiment**: from: 03.05.2017 to: 05.05.2017	**Date of report**: 28.07.2017
Shifts: 6	**Local contact(s)**: Dr. Thomas Zinn	*Received at ESRF:*

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Report:

The development of new synthetic receptors attracts growing interest among scientists in the field of optogenetics, as the activation of those receptors in living organisms in a spatio-temporally precise manner may be the answer to neuronal diseases such as Parkinson. Due to their unique arrangement of the C-terminal sensor (LOV) and N-terminal effector (bZIP) domain, aureochromes have recently drawn increasing attention. In stramenopile algae they act as blue-light-regulated transcription factors [1]. The knowledge of the mechanism of their regulation may promote the development of new synthetic receptors [2,3]. For this reason we and others have characterized aureochromes and functional segments of the photoreceptor by UV/Vis spectroscopy, light-induced FT-IR difference spectroscopy, size exclusion chromatography, X-ray diffraction and SAXS experiments [4-8].

In May 2017 we conducted a SAXS experiment at the ID02 beamline. We were assigned 6 shifts. The specific objectives of the beamtime were the investigation of the solution structure of

i) aureochrome in the absence of DNA under rigorous dark and light conditions,
ii) aureochrome in the presence of DNA under rigorous dark and light conditions,
iii) the sensory LOV domain at very low concentrations under rigorous darkness.

For the experiment we designed a sample chamber, which allowed a controlled illumination of the sample within the capillary. In each performed experiment, fifteen frames were taken

with exposure times of 0.05 s. The samples revealed some radiation damage (Figure 1a), which is why only the first two frames were used for further evaluation. Four different concentrations were investigated for i). After data reduction, absolute scaling and concentration normalization, the dark state SAXS data revealed no significant concentration-dependent changes (Figure 1b). Similarly promising results were obtained with the data for the light state and in the presence of DNA. Four different concentrations were used for objective iii). As expected, the normalized sample data revealed a strong concentration dependency, from which a dissociation constant for monomerization was estimated. However, the high noise level at very low sample concentrations prevents a final evaluation of the data to reveal the solution structure of the monomeric state of the LOV domain in the dark.

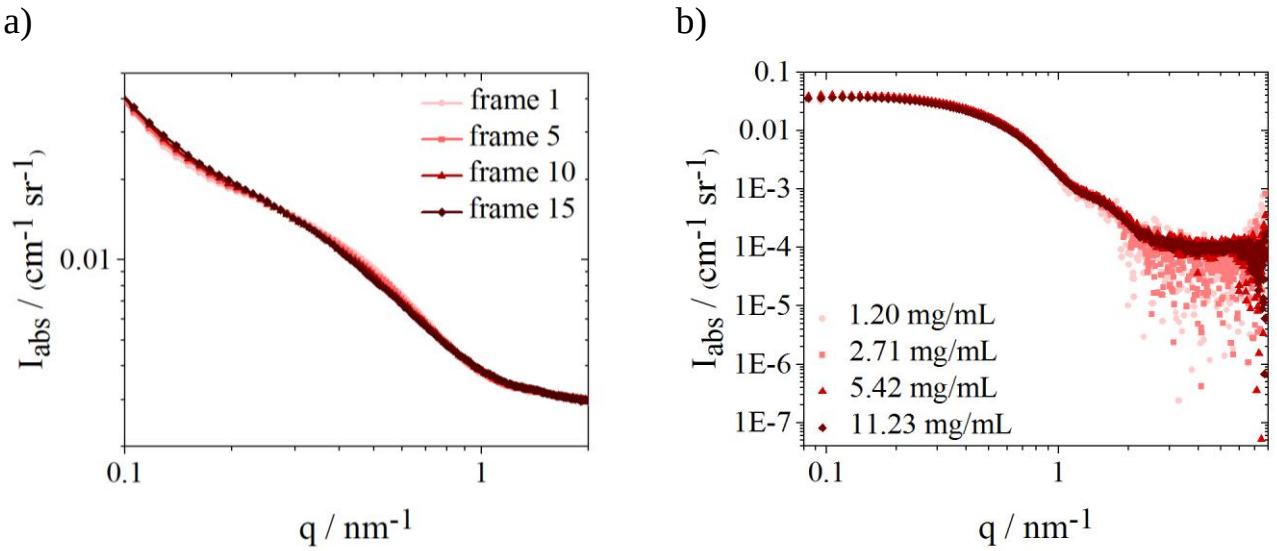

Figure 1: Dark state aureochrome at 1 m detector distance. a) Raw data (at 5.42 mg/mL) revealing frame-dependent radiation damage. b) Concentration dependency.

In conclusion, the SAXS experiments at the ID02 beamline provided us with promising results concerning objectives i) and ii). Further in-depth analysis is currently being performed.

References:

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