Experimental report

24/08/2022

Proposal: 8-04-924 **Council:** 4/2021

Title: Global diffusion and solution structure of photo-switchable proteins from cyanobacteria

Research area: Biology

This proposal is a resubmission of 8-04-902

Main proposer: Marcus MOLDENHAUER

Experimental team: Maksym GOLUB
Joerg PIEPER
Mina HAJIZADEH

Local contacts: Olga MATSARSKAIA
Tilo SEYDEL
Markus APPEL
Lionel PORCAR

Samples: orange carotenoid protein

Instrument	Requested days	Allocated days	From	To
D22	1	0		
IN16B	3	3	27/09/2021	30/09/2021
D11	1	1	29/09/2021	30/09/2021

Abstract:

In cyanobacteria, photosynthesis is initiated by light-absorption in protein complexes referred to as phycobilisomes. In the presence of excess light, photodamage is prevented by so called non-photochemical quenching (NPQ). This regulative process is realized by the interplay between the light harvesting phycobilisomes and a light-sensitive effector of NPQ referred to as the Orange Carotenoid Protein (OCP). OCP has to undergo a light-induced structural change from its inactive orange state OCPO to an active red state OCPR, which can be initiated by illumination using a laser wavelength of 450 nm at 300 K. It is important to emphasize that the rate of NPQ is limited by the diffusion of the water-soluble OCPs towards the membrane-extrinsic phycobilisomes so that an accurate determination of the diffusion constants is pivotal for a quantitative understanding of NPQ. Here, we propose to study the global diffusion in the inactive orange state OCPO, in the active red state OCPR induced by in-situ illumination, and using a mutant assumed to mimic the active state in the dark. Complementary SEC-SANS experiments using D22 are meant to establish the respective solution structures.

Exp. Report: 8-04-924

Global diffusion and solution structure of photo-switchable proteins from cyanobacteria

In cyanobacteria, photosynthesis is initiated by light absorption in protein complexes called phycobilisomes (PBs). In the case of excess light, photodamage to the photosynthetic apparatus is prevented by so-called non-photochemical quenching (NPQ). This adaptive process responsible for high light tolerance is realized by the interplay between the light-harvesting PBs, a light-sensitive effector of NPQ called Orange Carotenoid Protein (OCP), and a regulatory Fluorescence Recovery Protein (FRP). The underlying structural processes are currently a field of intensive research (see, e.g., [1, 2]).

During the experiment 8-04-924 at IN16B, two water-soluble proteins were probed at dark conditions and under blue-light illumination. The first protein was photo-sensitive OCP, which undergoes a structural change from the ground state (OCP^O) to the active form (OCP^R) under blue light and remains in the active state over the timescale of a backscattering experiment at a temperature lower than 15 °C. The second protein probed in this experiment is the mutant of OCP (OCP^{W288A}), which is not photo-active and is assumed to resemble the structure of the active state OCP^R in the dark.

The dynamics of both OCP preparations was characterized by QENS measurements using the Si 111 monochromator and analyzers of IN16B, corresponding to a neutron wavelength of 6.271 Å. Therefore, we can reach the momentum transfer range from 0.19 $Å^{-1}$ to 1.83 $Å^{-1}$. QENS spectra were recorded by mechanically Doppler-shifting the incident energy through a movement of the monochromator. The experimental elastic resolution ΔE of 0.88 μeV was defined by a vanadium standard run. The measurement time per one QENS measurement was about 6 hours. The scattering function S(Q,w) was obtained from raw data using Mantid.

The studied proteins were diluted in a D_2O buffer with a volume of 1ml, the protein concentration was 65 mg/mL and 45 mg/mL for OCP^O and OCP^{W288A}, respectively. Samples were filled in between two sapphire windows, which were sealed together with the help of indium wire and aluminum frames. We used a defocused blue laser to fully illuminate the samples through the sapphire windows. The specially designed aluminum sample holder was cooled to the temperature of 285K by a Julabo device. The temperature control was performed by a thermocouple connected to the aluminum frames of the sample cell. Standard measurements of buffer, vanadium, and empty cell were performed to permit a correct data treatment.

The results of the experiments are summarized in Figure 1A. The data reveal an enhancement of the OCP dynamics under illumination, which is seen as broadening the QENS spectrum. Interestingly, the QENS spectrum of OCP^{W288A} is drastically different from that of OCP^R. Most interestingly, there is no effect of illumination on the dynamics of the mutant OCP^{W288A}. Further analysis of the QENS data after buffer subtraction (Figure 1B) will be performed to

define the type of observed dynamics and evaluate the effect of illumination in the case of OCP.

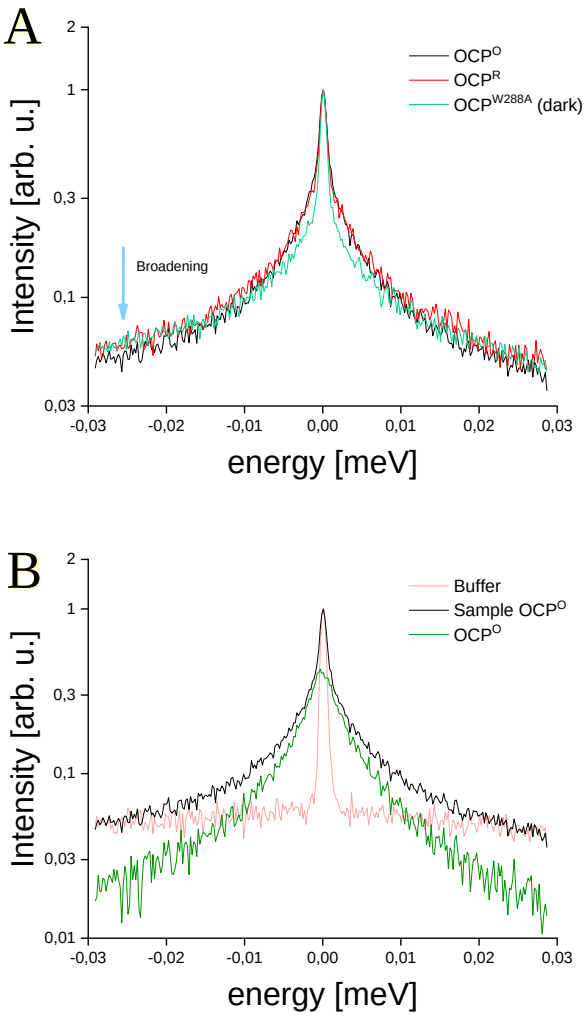

Figure 1. Panel A: Summed intensities of the OCP^{W288A} (dark), $OCP^{O,}$ and OCP^{R} samples (green, black and red curves, respectively). Panel B: Summed intensities of the OCP^{O} sample (black curve) and the buffer (light-red curve). The green curve represents the buffer subtraction with the prefactor of 0.894.

References:

1. Kirilovsky, D.; Kerfeld, C. A. Cyanobacterial Photoprotection by the Orange Carotenoid Protein. *Nat. Plants* **2016,** *2*, 1-7.
2. Gupta, S.; Guttman, M.; Leverenz, R. L.; Zhumadilova, K.; Pawlowski, E. G.; Petzold, C. J.; Lee, K. K.; Ralston, C. Y.; Kerfeld, C. A. Local and Global Structural Drivers for the Photoactivation of the Orange Carotenoid Protein. *Proc. Natl. Acad. Sci. U. S. A.* **2015,** *112*, 5567-5574.