Experimental report

15/02/2021

Proposal: TEST-3114 **Council:** 4/2020

Title: PHOTOSYSTEM Il CONFORMATIONIN THE PRESENCE OF DETERGENTS

Research area:

This proposal is a new proposal

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Samples: D2O
Detergent
PS II protein

Instrument	Requested days	Allocated days	From	To
D22	1	1	23/08/2020	24/08/2020

Abstract:

Exp. Report: TEST-3114

Photosystem II conformation in the presence of detergents

Photosynthesis has shaped the atmosphere by massive O_2-formation from water and the biosphere by facilitating the large-scale production of primary biomass and energy-rich carbohydrates [1, 2]. This process is initiated by photosystem II (PSII), a multisubunit pigment-protein complex located in the thylakoid membrane of cyanobacteria, algae, and plants. Photosystem I (PSI) is a large membrane-bound pigment-protein supercomplex found in higher plants, algae, and cyanobacteria. It is a photoactive enzyme that carries out the conversion of solar energy into storable chemical energy. To this end, a frequent approach is to isolate PSII and PSI from *T. elongatus* using the detergent n-dodecyl-ß-D-maltoside (DDM), whereby PSII and PSI retain their full oxygen-generating activity [3, 4]. It is assumed that above the critical micelle concentration of DDM, all PSII and PSI molecules are surrounded by a DDM belt and almost no free micelles are present in the solution.

Another object of our interest was the orange carotenoid protein (OCP), which plays a key role in cyanobacterial photoprotection [5]. Photoconversion entails structural rearrangements in OCP that are required for its binding to the phycobilisome light-harvesting complex, thereby inducing excitation energy dissipation in case of light stress. Detachment of OCP from the phycobilisome requires binding of the fluorescence recovery protein (FRP) [6-10]. It is assumed that OCP interacts with FRP only in the photoactivate state, whose structure is still unknown.

During beamtime TEST-3114, we performed a SEC-SANS study of the solution structure of the PSII-DDM and PSI-DDM complexes from the cyanobacterium *T. elongatus*, where regular DDM detergent was exchanged for its deuterated analog (dDDM). By using 100% D_2O-based buffer, we were able to match the detergent belt of the PSII-DDM complex as well as free DDM micelles (Fig. 1).

Our results suggest that contrast matching using deuterated DDM works extremely well for this system. Using this approach in combination with SEC-SANS, we obtained SANS data without DDM micelle contributions. This allows strong simplification of the data analysis, which can be done in the approximation of a monodisperse system. The analysis of the PSII-dDDM SANS data was performed using ATSAS [11]. From the analysis of the low q data, we could obtain an ab-initio structural model, which fits well to the known crystal structure of PSII. Our further analysis will include the fit of the PSII-dDDM SANS curve over the entire q range measured. A manuscript based on this data is in preparation [12].

During the TEST-3114 measurement, we also investigated the interactions of FRP with OCP mutants (OCPdeltaNTE orange and pink), which are lacking the N-terminal domain. To study each component of OCPdeltaNTE_orange_FRP and OCPdetlaNTE_pink_FRP separately, a series of samples with deuterated parts were prepared. This allowed for selective contrast matching of the components of the complexes. From the analysis of the SANS curves obtained, we claculted the structure of the orange complex by the Pepsi Flex program [13]. (see Fig. 2). A manuscript based on this data is in preparation [14].

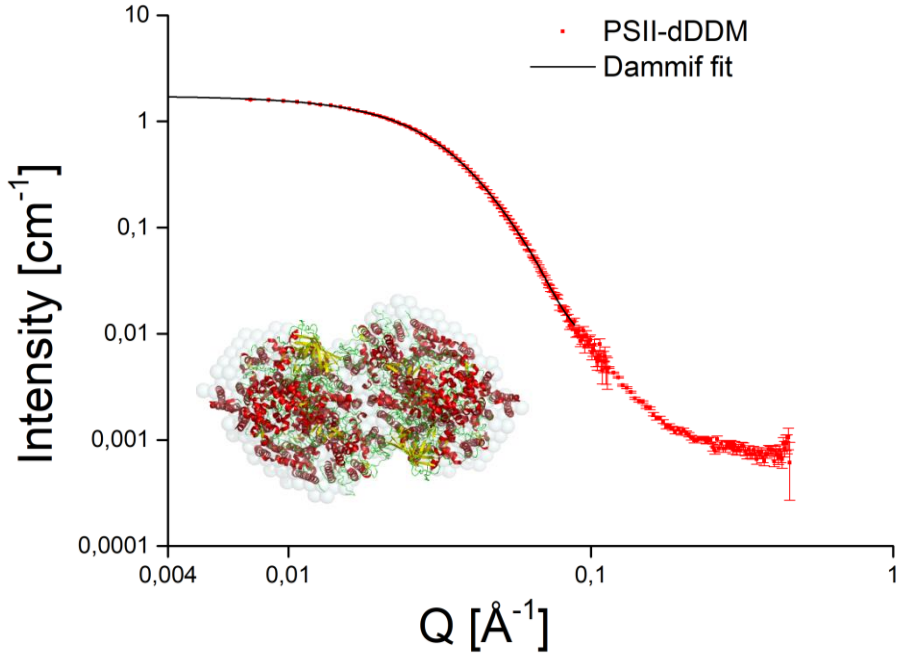

Figure 1. Dammif (ATSAS) fit (black line) of the low-q region of the PSII-dDDM SANS curve (red dots) measured in 100% D_2O. The fit corresponds to a chi^2 of 1,22. The inset shows the superposition of the known crystal structure of PSII (pdb code 3WU2 [15]) and the sphere structure obtained by Dammif.

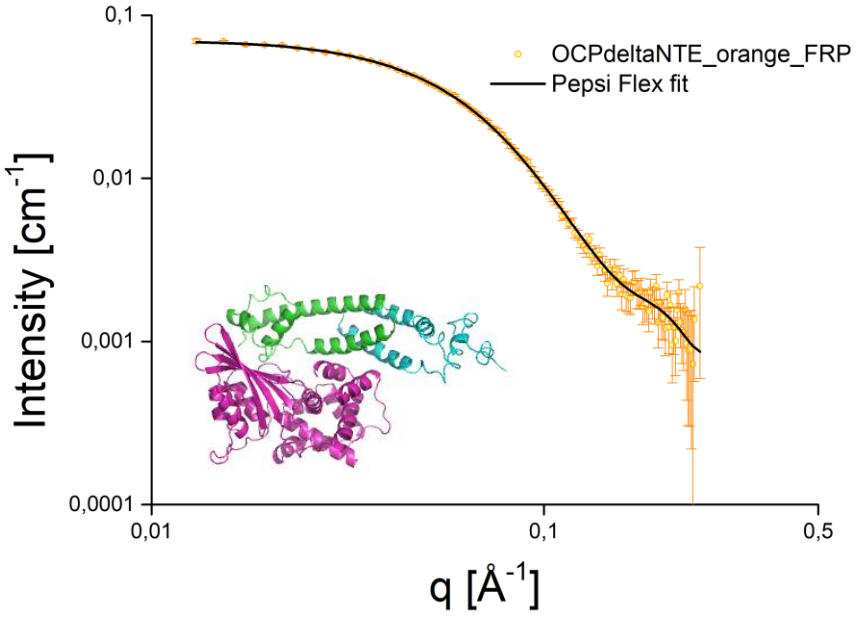

Figure 2. Pepsi Flex fit (black line) of the OCPdeltaNTE_orange_FRP SANS curve (orange dots) measured in 100% D_2O buffer. The fit corresponds to a chi^2 of 0,66. The inset shows the best structural model obtained by Pepsi Flex: the purple cartoon represents OCPdeltaNTE orange protein and the green and cyan cartoons show the two monomers of FRP.

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