Experimental report

28/08/2022

Proposal: 8-04-887 **Council:** 4/2020

Title: Following the formation kinetics of a transient intermediate of a photoreceptor during its photocycle with time-resolved QENS

Research area: Biology

This proposal is a new proposal

Main proposer: Andreas STADLER

Experimental team: Andreas STADLER
Igor GRAF VON WESTARP
Luman HARIS

Local contacts: Tilo SEYDEL

Samples: D2O
PpSB1-LOV photoreceptor

Instrument	Requested days	Allocated days	From	To
IN16B	4	3	26/06/2021	29/06/2021

Abstract:

Members of the LOV protein family are blue-light photoreceptors employing LOV domains as photosensory modules. LOV domains are structurally well-conserved that typically bind a flavin chromophore. Upon light absorption, they undergo a covalent bond formation between the C4a atom of the flavin ring and the sulfur of the neighboring cysteine residue. The photocycle is thermally reversible and in the dark, the FMN-cysteinyl adduct decays to the ground state. In this proposal we suggest to investigate changes of molecular dynamics through the dark recovery process of the photoreceptor PpSB1-LOV by QENS experiments on IN16B. In a previous experiment on IN16B we found a transient intermediate during the dark-recovery process that is formed with a kinetic relaxation time of 11 h. That intermediate has different dynamic behaviour than the light-excited state and comparatively similar dynamic behaviour as the fully dark-adapted ground state that is formed with significantly slower relaxation time of 39h. In the present proposal we would like to use elastic & inelastic fixed-windows scans to observe initial dynamical changes and to identify dynamical transitions more rapidly.

Quasi-elastic neutron scattering (QENS) experiments were performed on the high flux backscattering spectrometer IN16B at the ILL in Grenoble, France. The recorded data was analyzed between $q = 0.19\,\text{Å}^{-1}$ and $1.90\,\text{Å}^{-1}$. All protein samples used during the QENS experiment were prepared in a D_2O based buffer and had a protein concentration of $65\,\frac{mg}{ml}$. Flat rectangular aluminum sample containers with an inner thickness of 1 mm were placed at an angle of $135°$ with respect to the incident neutron beam. The PpSB1-LOV samples were filled in the sample holders in a dark environment before the experiment started to ensure the presence of the pure dark state. PpSB1-LOV dark state was measured first as a reference. Afterwards, the protein solution was taken out of the container in a transparent syringe. It was then illuminated with a blue light LED for 2 min, before being returned to the sample holder. A total time of around 15 min was needed to seal the sample holder containing the illuminated protein solution, to put it in the cryostat of the IN16B instrument and to start the recording of QENS data. The dark-recovery process of the PpSB1-LOV light state was consequently observed for 66 hours during a first QENS experiment (proposal: 8-04-828). A second QENS experiment was performed on IN16B to focus on the first 10 hours and to confirm the observations (proposal: 8-04-887). Pure D_2O buffer was measured to subtract the background, while the instrumental resolution was obtained from a vanadium standard. Sample temperature was $T = 289$ K for all measured samples.

Additionally, residue resolved NMR experiments have been performed as function ot time. To obtain time-resolved information on the structural recovery process, we measured a ^{1}H-^{15}N HSQC spectrum of the light state (t=0) and subsequent 2D spectra with experimental times of 7 min 22s. Due to the slow nature of the exchange process, on the NMR chemical shift timescale, we observe two distinct peaks for each amide group of numerous residues, one for the light state and one for the dark state.

The combination of time-resolved QENS and NMR experiments allowed us to follow the light-induced changes in the internal dynamics of the photoreceptor on multiple time scales. A scientific publication that is based on the QENS and NMR experiments is currently in preparation.

Figure 1 shows a compilation of representative QENS spectra, obtained HWHM as well of EISF of the fully dark-adapted and light-light after 1.5 h.

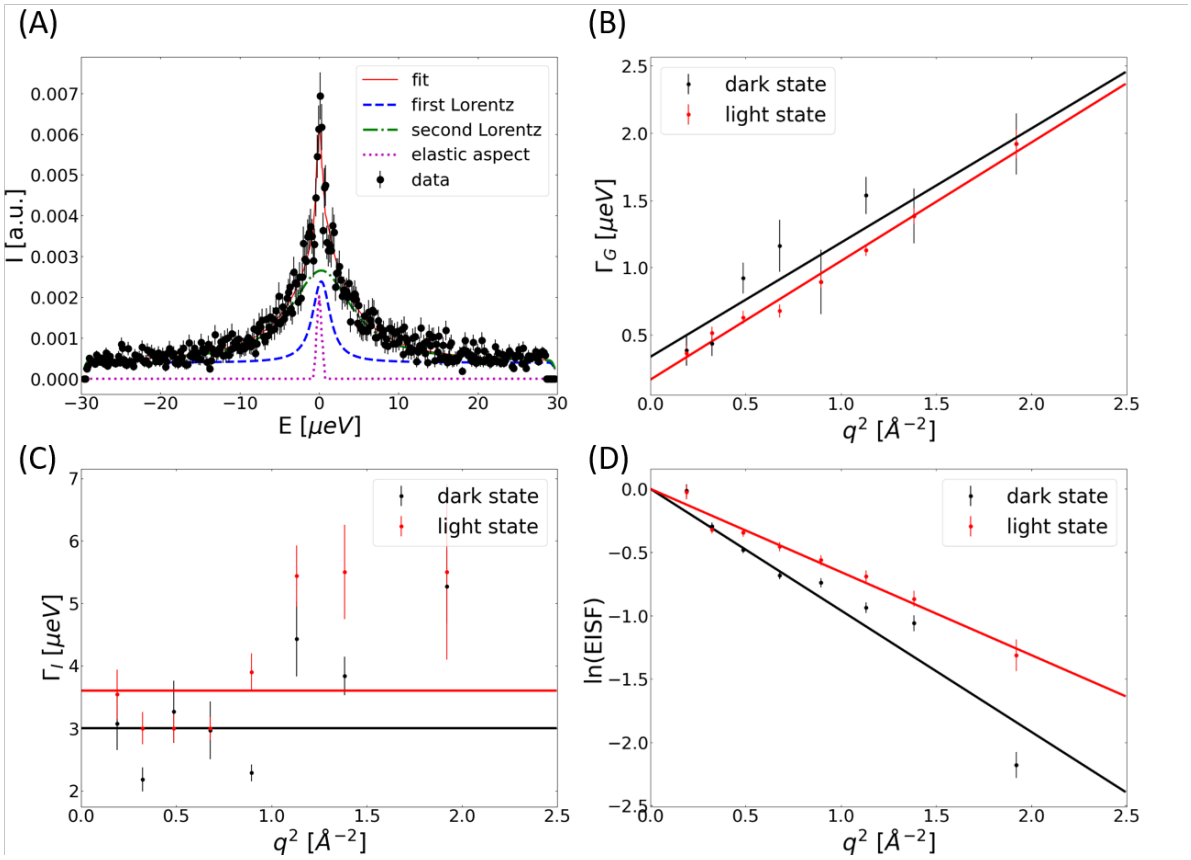

Figure 1. (A) Experimental QENS spectrum of PpSB1-LOV in the dark state including theoretical fit shown as red solid line. The fitted narrow and broad Lorentzians contain information on global protein diffusion and the convolution of global and internal protein dynamics, respectively. A residual elastic aspect caused by elastic scattering of the sample holder is discernible. All theoretical curves are convoluted with the instrumental resolution function. The scattering vector shown is q= 1.17 Å^{-1}. In (B) the line-widths $\Gamma_G(q)$ informing on global protein diffusion are shown for the PpSB1-LOV dark state, as well as for the light state at t = 1.5 h after light-activation. Solid lines are linear fits to the data to extract the effective diffusion coefficients informing on global protein diffusion. No relevant difference in global diffusive behaviour was observed for all samples at all time-points. Panel (C) shows the peak broadening $\Gamma_I(q)$ of the PpSB1-LOV dark-state and the light-state at t = 1.5 h after photo-illumination. The values of $\Gamma_I(q)$ are indicative on internal diffusive motions in the protein. The solid lines correspond to the fitted average values of the $\Gamma_I(q)$. No significant changes have been observed for internal diffusive dynamics between the light- and dark-states. (D) EISF of PpSB1-LOV dark- and light-state at t = 1.5 h after blue-light illumination. Linear fits were used to obtain the MSD as function of time.

Figure 2 shows the MSD(t) of the PpSB1-LOV protein after photo-illumination. The MSDs reveal a simple-exponential dark recovery process with an exponential recovery time of 12.3 +/- 2.3 h. The blue square represents the MSD of the dark-adapted PpSB1-LOV and it is the average value of both experiments (8-04-828 and 8-04-887) performed on IN16B.

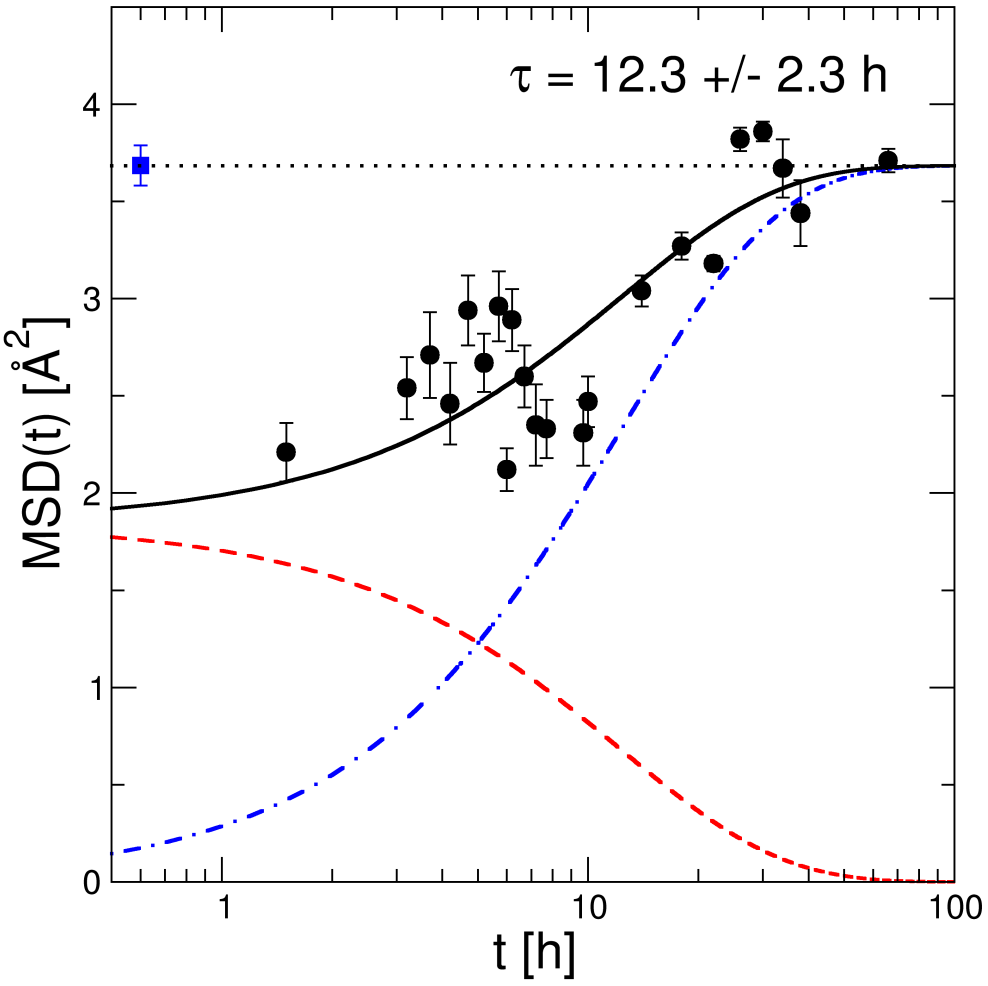

Figure 2. MSD versus time of PpSB1-LOV for up to 70 h after blue-light illumination (black symbols). The blue square indicates the MSD of the PpSB1-LOV dark state. The solid black line is a fit using a kinetic model for a first order transition of the dark recovery process. The obtained dark recovery time is $\tau = 12.3 \pm 2.3\ h$. The red dashed and blue dashed-dotted lines represent the time behaviour of the light- and dark-state components, respectively.