COUPLING OF LASER EXCITATION AND INELASTIC NEUTRON SCATTERING MEASUREMENT

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Light-harvesting antennae are pigment-protein complexes involved in light-absorption and excitation energy transfer (EET) to the so-called "reaction center" complexes, where the photochemical processes of photosynthesis take place. C-phycocyanin (C-PC) is one component of the phycobilisome, the light-harvesting system of cyanobacteria [1]. In phycobilisomes, EET is a highly efficient key event [2-3], where light-induced dynamics of the antenna pigment/protein complexes may play a role [4-7].

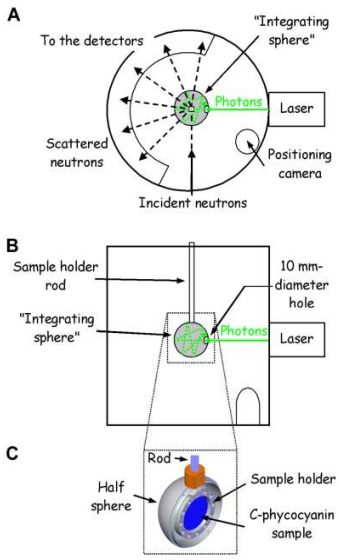

Fig. 1 *Schematic experimental setup of the "laser/neutron" coupling on the time-of-flight MIBEMOL neutron spectrometer : top (A) and side (B) views. The neutron and photon beams are perpendicularly oriented. Homogeneous illumination of the protein is provided by an "integrating sphere" (a spherical and hollow Al chamber) containing the sample holder (C). Photons aim on the sample through a 10 mm-diameter hole in the sphere. Height and orientation positioning of the sphere inside the sample well is checked using a camera.*

To detect fast and localized protein motions at room temperature, related to light absorption and EET in the isolated pigment/C-PC protein system, as well as to investigate the timescale of such dynamical changes, we developed a new experimental setup on the time-of-flight spectrometer MIBEMOL (LLB, France). This new "time-resolved" method was technically challenging, since we had to synchronize pulsed inelastic neutron scattering measurements with repetitive light excitations. The principle of the experimental setup is shown in Fig. 1. The laser was fixed outside the sample well of MIBEMOL spectrometer, perpendicularly to the neutron beam and in front of the detectors (Fig. 1A). The photon beam was directly aimed on the sample through the 10 mm-diameter hole of an "integrating sphere" (Fig. 1, B and C), without any additional optical device. We used a spherical and hollow aluminium chamber, containing a highly reflective and diffusing interior coating, to illuminate the sample inside uniformly (Fig. 1C).

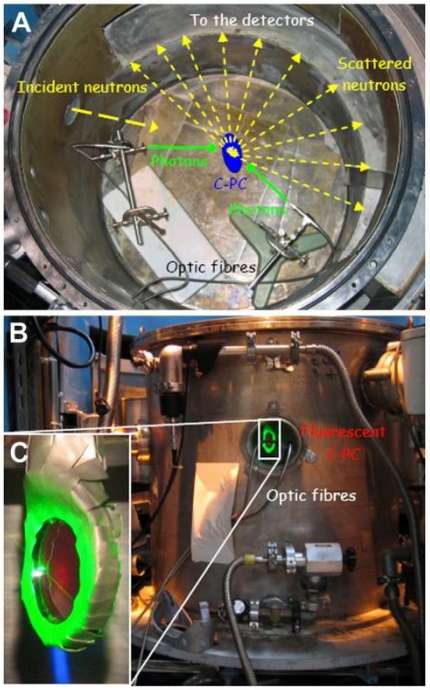

Fig. 2 *Top (A) and side (B) views of the sample environment on MIBEMOL neutron spectrometer during illumination experiments on C-PC. To check that the protein solution was entirely illuminated, we made direct observation of the sample, without the sphere, by using optic fibres. Inset (C): fluorescence of the C-PC (red colour) in the sample holder with the green laser beam reflected on the cadmium mask.*

The "integrating sphere" was positioned in front of the laser head using a camera located inside the sample well. First, we positioned and aligned the experimental setup without the "integrating sphere" but using optic fibres illuminating both sides of the sample container (Fig. 2). In that configuration, we were able to check that the sample was entirely illuminated, since fluorescence was homogenously emitted from the surface of the sample (Fig. 2C).

MIBEMOL data acquisition system has been modified to synchronize the laser excitation flashes (6 ns duration) with the neutron pulses (~87 µs duration) at the sample position, in order to perform "time-resolved" measurements. Successive neutron scattering measurements (few hours duration), with or without the laser, gave access to the "light" and "dark" spectra respectively. "Light" and "dark" spectra were separated by 50 ms in the case of a 10 Hz laser frequency (Fig. 3). The quasi-simultaneous process of such "light" and "dark" relative measurements and their evolution as a function of time could be compared to measurements performed in a "double beam" spectrometer (Fig. 3A). The flash lamp pumping and the laser triggering have been controlled by a special device and checked all along the experiment by an oscilloscope (Fig. 3B).

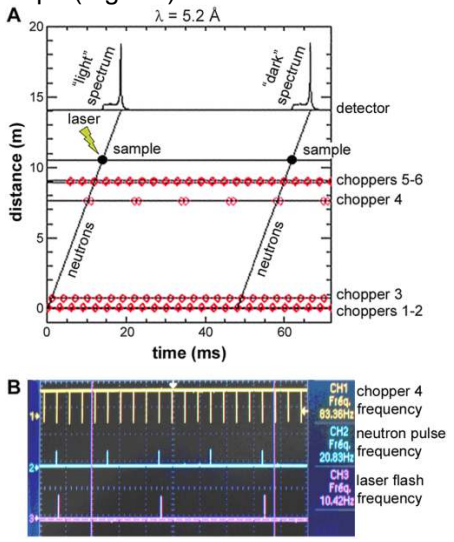

Fig. 3 *A): Diagram of the chopper velocities on MIBEMOL spectrometer for the "laser/neutron" setup, at λ = 5.2 Å configuration (~140 µeV resolution). The incident and scattered neutrons are represented by a solid line and typical TOF spectra have been drawn at the detector. The "light" spectrum was obtained when the beginning of the neutron test pulse (~87 µs duration) was synchronized with the laser flash, symbolized by a lightning symbol (~25 mJ, 10 Hz, λ = 532 nm, 6 ns duration). The "dark" spectrum, resulting from each neutron test pulse between two laser flashes, was considered as the reference without laser. B):Oscilloscope screen showing the neutron pulse frequency at chopper #4 (80 Hz, channel #1), the recorded neutron pulse frequency (20 Hz, channel #2), and the laser flash frequency (10 Hz, channel #3).*

This procedure of alternating "light" and "dark" measurements was novel and eliminated many spurious effects that could occur in the sample during the experiment (e.g. positioning of the sample holder or undesired laser-induced heating of the sample). For the present study, no time delay has been introduced between the beginning of the laser flash and the beginning of the neutron pulse. However, for future measurements, a time delay between the laser flash and the neutron pulse can be introduced.

Measurements have been performed at constant temperature (298 K) and pressure. The light source was a 6 ns pulsed Nd:YAG laser, at λ = 532 nm, with maximum frequency and energy of 14 Hz and ~25 mJ, respectively. We chose a circular Al sample holder surrounding 50 mm diameter sapphire glasses with a 0.5 mm sample thickness. Such dimensions optimized the intersection with the neutron beam. Besides, the elastic intensity level from the Al/sapphire sample holder was very weak and, in the Q-window of our measurements, no Bragg peak was observed for sapphire glass, nor Al. The Al "integrating sphere" surrounding the sample holder was also almost "transparent" for neutrons.

We obtained a set of energy-transfer spectra measured at different scattering angles ($0.5 < Q < 2.1$ $Å^{-1}$). We used an incident wavelength of 5.2 Å to have the maximum neutron flux with a reasonable resolution (~140 µeV). Such a resolution gives access to motions on 0.2-20 ps timescale. Data collection times were about 10-20 h to obtain sufficient statistics. In the case of C-PC, the incoherent dynamical structure factor was not significantly altered by light; 2 modes within the vibrational density of states were slightly increased upon illumination. New applications using other stimuli on sample like electric or magnetic excitations are now under development. We have in particular already undertaken to probe the dynamics of a polymer electrolyte (lithium salt embedded in a polymer matrix used as electrolytes in batteries) submitted to an electric field, so far in the 1 Hz-30 MHz range.

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