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Activation and deactivation of vibronic channels in intact phycocyanin rods

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We investigated the excitation modes of the light-harvesting protein phycocyanin (PC) from Thermosynechococcus vulcanus in the crystalline state using UV and near-infrared Raman spectroscopy. The spectra revealed the absence of a hydrogen out-of-plane wagging (HOOP) mode in the PC trimer, which suggests that the HOOP mode is activated in the intact PC rod, while it is not active in the PC trimer. Furthermore, in the PC trimer an intense mode at 984 cm$^{-1}$ is assigned to the C–C stretching vibration while the mode at 454 cm$^{-1}$ is likely due to ethyl group torsion. In contrast, in the similar chromophore phycouromobilin the C$_{5,10,15}$-D wag mode at 622 cm$^{-1}$ does not come from a down-shift of the HOOP. Additionally, the absence of modes between 1200 and 1300 cm$^{-1}$ rules out functional monomerization. A correlation between phycocyanobilin (PCB) and phycopyrrotilbilin (PEB) suggests that the PCB cofactors of the PC trimer appear in a conformation similar to that of PEB. The conformation of the PC rod is consistent with that of the allophycocyanin (APC) trimer, and thus excitonic flow is facilitated between these two independent light-harvesting compounds. This exciton flow from the PC rod to APC appears to be modulated by the vibration channels during HOOP wagging, C = C stretching, and the N–H rocking in-plan vibration. © 2014 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4866293]

I. INTRODUCTION

Phycocyanin is a major component of phycobilisome (PBS), the principal light-harvesting complex in the photosynthetic apparatus of cyanobacteria and red-algae. In the thermophilic cyanobacterium, Thermosynechococcus vulcanus, phycocyanin (PC) is the sole component of the rods and typically has an absorption maximum at 620 nm in its isolated form. PC contains three phycocyanobilin cofactors, which are tuned by the protein environment to absorb different light frequencies. The PBS function is dependent on the correct functionality of each component and the PC absorbs at higher energy than allophycocyanin (APC). PC is typically isolated in low ionic strength buffers in its (αβ)$_3$ trimeric form. As shown by the crystal structure of the rod form of PC from T. vulcanus at 1.5 Å (PDB code:3O2C) the PC subunit has 3 phycocyanobilin (PCB) cofactors and these are well known as (β155, α84, β84) from previous studies. The PCB chromophores are open tetrapyrroles covalently linked via thio-ether bonds to conserve the cysteine residues. In each (αβ) monomer, the α84 and β84 PCBs are located at the inner positions close to the symmetry axis (and almost completely embedded in the protein), whereas β155 is located on the outer circumference of the protein. At RT, the absorption maxima for the β155, α84, and β84 PCBs have been suggested to be 600, 624, and 630 nm, respectively. In contrast to APC, the absorption maxima of the aforementioned cofactors do not change upon an association of the monomers with the trimers. However, the close proximity and the respective orientation of the α84 and β84 PCB cofactors in the adjacent monomers have been suggested to be a major component in the excitonic coupling process in PC.

Recently, Womick and Moran reported different dynamic mechanisms between PC and APC. Using two-dimensional photon echo combined with a transient grating they measured the oscillating excitation dynamics in PC. They did not find interference indicating that excitonic coupling occurs in PC. This result contradicts the sub-100 fs dynamics detected in APC. From an anisotropy experiment these authors obtained an excited mode component at 795 cm$^{-1}$ from a fit. They attributed this excited mode to hydrogen out-of-plane vibration (HOOP) wagging and suggested that the sub-100 fs dynamics found in PC is likely induced by HOOP wagging. More recent femtosecond stimulated Raman spectroscopy of phytochrome detected HOOP relaxation dynamics. The HOOP dynamics was caused by a steric interaction between the C and D rings (see Figure 1) and this was suggested to contribute to photo production with a kinetic component of 500 ± 50 fs. This kinetic component is considerably larger than the sub-100 fs dynamics obtained in PC. Furthermore, the termination of ring D differs in phytochromobilin (PCH)
FIG. 1. Molecular structure. Molecular structure of phycocyanobilin (PCB), phytochromobilin (PCH), and phycoerythrobilin (PEB). The methine bridges are shown using different numbers while further differences with respect to the PCB are shown by hybridization.

compared to PCB in the presence of a vinyl group (see Figure 1). Perhaps the latter difference may be a non-negligible factor in PCH and PCB dynamics.

Previous Raman measurements on isolated PC were not able to determine the HOOP band gap, which is expected to be between 750 cm$^{-1}$ and 850 cm$^{-1}$. To determine the HOOP band gap, we conducted a new Raman investigation into PC in its pure crystalline form. Furthermore, we investigated the conformation of cofactors found in a crystal of PC$_{620}$ and a PC rod, and thereby suggested a route for the excitonic mechanism.

We excited the PC trimer with UV light to trigger vibrational modes related to the S2$^\ast$ excited state of the PC trimer conformation. Using the idea of a recent report showing that near-infrared (NIR) excitation is able to trigger the S1$^\ast$ excited state, which may initiate an intermediate state in the Franck-Condon region (FC) (Figure 6 of Ref. 10) of the open tetrapyrrrole cofactors, we further excited the PC trimer and the PC rod with NIR light. In the framework of this latter approach, in a system containing different PC sub-compounds that absorb the excitation at different band gaps, we intended to monitor the activity of the intermediate state via ground state relaxation in the FC region of the low energy level sub-compound. The ground state relaxation of the PC rod is monitored via pump-probe transient absorption spectroscopy, with excitation at 620 nm (absorption of PC$_{620}$ in the PC rod) and probing at 640 nm (absorption of PC$_{612}$ in the PC rod) to estimate the kinetic component of the intermediate transient state from PC$_{620}$ to PC$_{612}$ in PC rod.

II. MATERIALS AND METHODS

(1) T. vulcanus cells were grown in a 10-L temperature controlled growth chamber on BG11 medium supplemented with 5% CO$_2$ in air at 55°C, and with fluorescent lamp illumination. Cells were grown for 5 to 7 days before collection by centrifugation. Trimeric PC was obtained by resuspending the cells in an isolation buffer (20 mM Tris, 10 mM MgCl$_2$, and 10 mM CaCl$_2$, pH 8) and treating with lysozyme (1 mg/ml) for 1 h at 50°C before passing through a Yeda press cell disruptor under 24.7 bars (25 atm) N$_2$. Following
10 min of centrifugation at 10,000 rpm, the blue supernatant was filtered through an anion-exchange low-pressure liquid chromatography column before further separation by high-pressure anion-exchange chromatography. Fractions were characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and absorption spectroscopy for the selection of samples containing pure PC from APC or linker proteins. The resulting protein was dialyzed against 20 mM Tris at pH 8, and then concentrated by ultrafiltration using a 100-kDa Centricon ultrafiltration device (Amicon). Size-exclusion high-pressure liquid chromatography analysis revealed that the aggregation state of the PC was trimeric and of rod form. PC was crystallized at 20°C by hanging drop vapor diffusion.3

(2) UV Raman measurements were performed using a Horiba Jobin Yvon T64000 triple monochromator system with a tunable solid-state Ti:Sa laser system pumped by a pulsed frequency doubled Nd:YAG laser at 532 nm. With second harmonic generation, wavelengths of 377 nm and 450 nm were obtained. The pulse length was 30 ns with a repetition rate of 10 kHz. For the NIR Raman measurements, a Horiba Jobin Yvon Labram HR with an 830 nm cw diode laser was used. For UV/VIS detection, a liquid-nitrogen cooled back-illuminated UV sensitive CCD detector (2048 × 512) was used and for NIR, an air-cooled CCD detector (1024 × 256) was used (Figure 2(b)). All measurements were done in backscattering geometry at room temperature. The spot size on the sample was approximately 1 μm. The spectral resolution was 5 cm⁻¹ for UV/VIS and 1 cm⁻¹ for NIR. Because PCB cofactors are known to be stable at high laser power density upon excitation either with Raman15 or with synchrotron (PC rod and trimer),3 the UV Raman excitation mean power used was 10 mW higher than the noise signal. Despite the known stability of the PCB cofactor, we noted that the noise was dependent on the crystal size. Therefore, the PC trimer crystal with a larger diameter than the PC rod3 was excited at a power density of 2 mW at 830 nm, while the PC rod was excited at a power density of 5 mW. The illumination time by UV excitation was 20 min and the NIR spectrum was recorded within 10 min for the PC trimer and 15 min for the PC rod. There was no apparent illumination-induced damage to the crystals upon inspection in a light microscope.
To estimate the transient state kinetic component that may be present in the PC rod, we used time resolved pump-probe spectroscopy.

Femtosecond absorption measurements were performed with a commercial titanium sapphire system (Spectra Physics Tsunami, Spitfire and Optical Parametric Amplification (OPA)). The pulses were generated by a Tsunami resonator (Spectra Physics) and were stretched before entering the Ti:sapphire regenerative amplifier (Spitfire, Spectra Physics), which provided an 800 nm beam at 1 kHz with a pulse energy of 1 J and 100 fs FWHM after recompression. The output beam from the Spitfire enters an optical parametric amplifier capable of generating beams in the 475–800 nm region with a pulse energy of 1–35 nJ, at a repetition rate of 1 kHz and 120 fs FWHM. A fraction of the beam entering the OPA was focused onto a sapphire plate to generate a white light continuum (the probe beam). The pulse generated from the OPA was then sent to a variable delay line and made to overlap with the probe beam at the sample spot (150 μm). The fluence was 1.7 × 10¹³ photons/cm². Using the procedure described in Ref. 9, we estimated an average of 0.62 excitations per hexamer. We increased the pulse energy without any observed influence. There was no apparent illumination-induced aggregation formation of PBS in the buffer. At the sample spot, a sample of the PBS antenna complex was located in a 2 mm path silica cuvette and a magnetic stir bar was used to homogenize the sample for illumination. Detection of the probe light was achieved using two photodiodes connected in a differential circuit. The measured FWHM of the beam was ~160–200 fs at the sample spot. Absorption changes were monitored separately, with the polarization of the pump beam set to 54.7° with respect to the polarized probe beam. The latter were done using a π retarder at the pump laser beam.

Following this, the collected ground state relaxation decay trace was then fitted with a multi-exponential decay model:

$$A(\tau) = \sum_{i=1}^{n} a_i(\tau_i)e^{-\frac{\tau}{\tau_i}} + a_0. \quad (1)$$

The values of the decay component $\tau_i$ and the amplitude factor, $a_i(\tau_i)$, were taken to be free parameters.

Exhaustive information about the pump-probe setup can be obtained in the literature. 7

III. RESULTS

A. Absence of HOOP wagging excited mode activity in the PC trimer

Our approach to studying the mechanism of the sub-100 fs relaxation found in PC and the origin of the exciton in PC was to analyze the Raman UV spectra (Figures 3 and 4) and the NIR spectra (Figure 5).

In the Raman spectra (Figure 3) the absence of an excited mode for the HOOP between 795 and 816 cm⁻¹ is apparent. This absence of a HOOP excited mode contradicts the suggestion in the literature.9 These authors suggested that the 795 cm⁻¹ mode obtained from the fit of their anisotropy
Fig. 4. Room temperature UV Raman obtained after background subtraction. Room temperature UV Raman spectra of a PC trimer crystal after 377 nm and 450 nm excitation. The excited modes are labeled (200–700 cm$^{-1}$).

measurement originates from a fast sub-100 fs decay of the excited state in PC. This decay of the 60 fs kinetic component was attributed to solvation.

In addition to the 60 fs component, a 970 fs kinetic component was found and attributed to energy transfer between the cofactors. On the basis of the protein ionization shell that may occur if the solvated charge density is consistent enough to change the electrostatic potential of the shell, perhaps excited state relaxation dynamics may be present within the different kinetic components. However, no quantification of the solvation rate is reported in the above-mentioned reference.

Investigations into open tetrapyrrole cofactors with respect to how they are tied to the protein scaffold may provide additional insights into the influence of assembly in the excited state mechanism of a PC.

Indeed, differences in ring D are apparent between the PCBs in PC and in phytochromes (see Figure 1). The radical ethyl in PCB is substituted by a vinyl group in PCH. The latter may cause the difference in D ring dynamics, as discussed in the literature. Furthermore, after excitation with a NIR laser, the HOOP excited mode shows red-shifted activity with a kinetic component of 500 fs in the phytochrome. Probing the PC rod with FSRS (femtosecond stimulated Raman spectroscopy) will probably help to identify the propagation kinetics of the HOOP along the PC rod in addition to the sub-100 fs component attributed to the PC trimer during solvation. Thus, NIR excitation is sufficient to monitor Raman activity during HOOP wagging between 795 and 816 cm$^{-1}$. With UV excitation, the S2 state of the PC is populated while NIR excitation is sufficient to populate the S1 state (see the Introduction).

Despite the fact that similarities exist between PCH and PCB, the structural difference in ring D in addition to the difference in the surrounding neighboring protein and solvent medium may influence the behavior of propionic acid. This influence may induce different protonation behavior and the band pattern below 1200 cm$^{-1}$ will be subjected to different excitation modes.

Apart from the absence of a HOOP mode, the spectra in Figure 3 show a number of different excited modes, as labeled. The excitations at 377 and 450 nm reveal an unusual prominent excited mode at 984 cm$^{-1}$. The intensity of this excited mode also persists upon 830 nm excitation (see Figure 5) in the PC trimer, which indicates that it is an important excitation band marker, which may be related to the excited-state geometry of the PCB cofactors. According to our knowledge, such a marker has not been detected before. This suggests that crystals of PC have the advantage of retaining the PCB interaction with the surrounding cofactors. Better phonon
coupling in the system assists in identifying additional excited modes that have not been detected before. The absorbance spectrum of the PC trimer (PC\textsubscript{620}) does not advocate the hypothesis of “pre-resonance excitation” (see the supplementary material\textsuperscript{25}). The PC trimer as an intact PC rod of PBS of \textit{T. vulcanus} (see Figure 7) and other PBS species without PE do not absorb below 500 nm and above 700 nm.

The observed modes in the C–C stretching region are at 989, 957, 924, and 865 cm\textsuperscript{-1} in PEB.\textsuperscript{17} PEB differs from PCB in terms of hybridization at C\textsubscript{15} (sp\textsuperscript{3} vs. sp\textsuperscript{2}), which is located between rings C and D. An additional difference is the vinyl group terminating at ring D, as for phytochrome (see Figure 1). These differences may explain the obtained 984 cm\textsuperscript{-1} mode in the case of PCB. We further detected excited modes at 903, 919, and 939 cm\textsuperscript{-1} (Figure 3). In contrast to the 984 cm\textsuperscript{-1} mode of the C–C stretching vibration, the latter excited modes strongly depend on the excitation energy.

This dependence on the excitation energy has been observed in previous work\textsuperscript{13} and it is driven by different coupled excited modes. As observed in the literature\textsuperscript{16} the C–D rocking mode coupled with the C and D rings in-plane rotation is consistent with the bands at 773, 796, and 853 cm\textsuperscript{-1} of PCH. An earlier report found 974, 976, 977, 992, and 994 cm\textsuperscript{-1} in UV-excited spectra at 363.8 nm in native and denatured PCB in 20 mM phosphate buffer, and for free PCB at 20 K.\textsuperscript{11} Only the 992 cm\textsuperscript{-1} marker was found in native and denatured PC. This latter band may be similar to that at 984 cm\textsuperscript{-1}, which was up-shifted at 20 K.

C–N stretching in PC was found to have a sensitive marker between 1070 and 1200 cm\textsuperscript{-1} while C–H and N–H rocking were consistent with the signature of the excited modes between 1200 cm\textsuperscript{-1} and 1500 cm\textsuperscript{-1}.\textsuperscript{17} Because the terminal group of ring D is different in PCB compared to PCH, the coupling mode for C–D rocking between the C and D rings (in-plane rotation) may be up-shifted to 903, 919, and 939 cm\textsuperscript{-1}.\textsuperscript{17} A direct comparison with the PEB excited modes reveal modes at 924, 957, and 989 cm\textsuperscript{-1} (Figure 3), and these are different to the aforementioned excited modes in PC620. The latter difference may also be due to coupling between the C and D rings (in-plane rotation). This hypothesis is strengthened by the detection of a 979 cm\textsuperscript{-1} mode in native phytochrome.\textsuperscript{17} However, the 979 cm\textsuperscript{-1} mode in native phytochrome is of low intensity when compared to the 984 cm\textsuperscript{-1} mode of PC. We, therefore, suggest that the excited mode at 984 cm\textsuperscript{-1} is characteristic of the PC trimer.

We turn our attention now to the C–N stretching modes at 1070–1200 cm\textsuperscript{-1}. The detected excited modes are 1093, 1106, 1130, and 1144 cm\textsuperscript{-1} (Figure 3). The excited mode at 1093 cm\textsuperscript{-1} is present for different excitations, while the excited modes at 1106 cm\textsuperscript{-1} and 1144 cm\textsuperscript{-1} appear upon UV excitation and disappear upon NIR excitation. Instead, a mode at 1130 cm\textsuperscript{-1} appears. This suggests that the 1093 cm\textsuperscript{-1} mode may be a pure C–N vibration while the other excited modes may be coupling between vinyl and a C–H rocking motion, which is delocalized over the D ring as suggested in the literature\textsuperscript{17}.

In earlier work, Szalontai \textit{et al.}\textsuperscript{14} found an amplitude variation of the excited mode at 1095 cm\textsuperscript{-1} upon decreasing the pH from 7.5 to 3. At pH 7.5 the PC is a trimer while between pH 3 and 4 it is a monomer. Denaturation takes place below pH 3. Each pH variation will affect the acid moiety of the PCB cofactor and, therefore, the vibration mode of the ring on to these moieties will also be affected. Those rings are B and C. The obtained 1093 cm\textsuperscript{-1} mode is consistent with C–N stretching in rings B and C (see Figure 1).

One additional characteristic feature in these spectra is the marked trace of the excited mode of the C–H rocking vibration at 1418 cm\textsuperscript{-1} and the C = C stretch at 1666 cm\textsuperscript{-1} (Figure 5). Between 1350 and 1450 cm\textsuperscript{-1} only a slight variation in excited mode amplitudes was observed and the excited modes close to 1400 cm\textsuperscript{-1} were unchanged upon a pH variation from 7.5 to 3.\textsuperscript{14} As already discussed the independence of the excited mode pH at 1418 cm\textsuperscript{-1} suggests that the propionic side chain does not contribute to the vibration. This excited mode is closed to the rocking region of PEB, which is not the case for PCH.\textsuperscript{17} It is likely that ring B does not contribute to C–H rocking in PC.

The 1666 cm\textsuperscript{-1} excited mode is close to the C = C stretching mode at 1664 cm\textsuperscript{-1} that was found in PE by Nostoc sp. at 30 K,\textsuperscript{11} and this was not found in earlier PC and APC Raman investigations. Furthermore, because of the weak interaction in ring D compared to rings A–C and the PCB cofactor interaction with the protein, Szalontai \textit{et al.}\textsuperscript{11} postulated that such a mode arises when the D ends of the chromophore are in the more stable \textit{syn} configuration. They suggest that the C\textsubscript{15} bridge connecting rings C and D may be the origin of a methine stretch found in earlier work. However, because of the absence of a mode around 1666 cm\textsuperscript{-1} in their PC and APC Raman spectra, they concluded that the PE mode at 1664 cm\textsuperscript{-1} did not come from the marker in PC and APC.

The detection reported here in a PC crystal contrasts this earlier suggestion and further supports the assertion that non-bonded interactions play a crucial role in PC as in rhodopsin and phytochrome.\textsuperscript{17} This is highlighted by the absence of an excited state mode in the methine bridge between rings B and C in PC620. This excited mode was suggested to occur around 1594 cm\textsuperscript{-1},\textsuperscript{12} while the mode between rings C and D was shown to be consistent with the 1642 cm\textsuperscript{-1} mode in phytochrome.

A frequency difference was obtained as a component of the computer-assisted decomposition of the Raman spectra around 1649 cm\textsuperscript{-1} by Szalontai \textit{et al.}\textsuperscript{11} and was assigned to the \textit{anti} conformation while the 1621 cm\textsuperscript{-1} mode was assigned to the \textit{syn} conformation. These conformation changes were also discussed by Schirmer \textit{et al.}\textsuperscript{18} who postulated that the \textit{anti, syn, anti} conformation of CPC rules out the presence of a mode at 1666 cm\textsuperscript{-1}. We, therefore, suggest that the PCB cofactor in the PC620 crystal (PC trimer) has a similar conformation to PEB.

Additional excited modes were detected at low wavenumbers (see Figure 4). The modes at 618, 622, and 628 cm\textsuperscript{-1} (Figure 4) appear to depend on the excitation energy. At NIR excitation, the modes at 618 and 628 cm\textsuperscript{-1} obtained upon UV excitation disappear and are replaced by a dominant marker at 622 cm\textsuperscript{-1} (Figure 4).

In the homologue phytochrome species, the C\textsubscript{5,10,15}-D wagging vibrations shift from 633 cm\textsuperscript{-1} to 610 cm\textsuperscript{-1} where they are coupled to the C-ring in-plane rotation.\textsuperscript{17} The
A difference in excited modes between PCH and PCB mostly arise because of the interaction with the terminal group of ring D. However, we do not exclude the possibility that the intervening medium may affect the excited states of the dipole moment through propionic acid, which is very sensitive to any change in the local environment. This may drive further excited modes. The excited modes 618, 622, and 628 cm$^{-1}$ are therefore consistent with C$_{5,10,15}$-D wags.

Another interesting marker of the excited mode of PC620 is the band at 454 cm$^{-1}$ (Figure 6), which is independent of the excitation energy. They suggest that it is vinyl torsion at 461 cm$^{-1}$ in PCH. This mode was suggested to occur between C$_{19}$ and C$_{20}$, which means that it is on the radical R = C$_2$H$_3$ linked to ring D. PCB is functionalized with a radical R = C$_2$H$_5$ connected to ring D, and the observed downshift may be due to this difference. The excited mode may therefore be an ethyl torsion instead of a vinyl torsion. The other detected modes at 372 and 535 cm$^{-1}$ (see Figure 5) may be induced by additional torsions at the propionic side chain.

When comparing the experimental results with calculations, details of the preparation protocol of the phycocyanobilin must be considered. Regarding the reported density functional theory (DFT) study$^{16}$ the authors used a PCB in their calculations. Replacing the PCB linkage to the cysteine moiety at ring A with a hydrogen atom gives ring A a higher degree of freedom than that of ring D. This may induce conformational modes that are absent in the natural PCB environment and may be a reason for the detection of the HOOP excited mode wagging.

Information gathered from the protein data bank (PDB) program ligand explorer$^{19}$ (302C, Figure 7) for PCB from PC620 reveals that different protonations may take place, and these are not only induced by Asp87. Furthermore, the number of identified water molecules is greater around ring A than around the other rings (Figure 6). This is in fair agreement with the different interactions of ring A with the protein compared to that of ring D. The protein and the water molecules that stabilize the protein conformation may influence the excited mode of ring A because this ring is covalently linked to the apoprotein through cysteine. Therefore, the excited mode below 1000 cm$^{-1}$ may be strongly affected by the neighboring medium of the PCB. However, the excited mode at $\sim$622 cm$^{-1}$ that was detected in our investigation shows that in the latter calculation that it is independent of the neighboring medium and can coexist with the HOOP. As a result of this observation, we suggest that the observed 622 cm$^{-1}$ mode is not a downshift of the HOOP but an intrinsic C$_{5,10,15}$-D wag excited mode.

The aim of this section was to examine whether the HOOP vibration mode is activated by UV excitation or by NIR excitation in the PC trimer. The investigations carried out show that the HOOP vibration mode is not active in the PC trimer.

Having discussed the vibronic channel that can be activated by UV and NIR Raman in the PC trimer, we present below the activation of the HOOP excited mode when the PC is constructed as a PC rod.
IV. DISCUSSION

A. Activation of the HOOP in a PC rod

The PC rod consists of PC hexamers where one PC hexamer is created by an association of the double discs with PC trimers. This structure can be monitored by the different linker proteins as suggested in the literature. In the same work, it was shown that the PC trimer has an absorption maximum peak at 620 nm. Our result in Figure 7 qualitatively and quantitatively confirms the data presented in the literature. The absorption maximum of PBS is identical to that of the PC rod at 635 nm. Furthermore, excitation of the PC rod absorption band gap triggers an energy transfer to the acceptor at 650 nm, which is the absorption band gap of APC (Figure 7).

Following our clarification of the PC’s exciton mechanism, we present in Figure 8 the NIR spectrum of the PC rod crystal. This spectrum has a dominant vibrational feature at 816 cm$^{-1}$, which is a signal marker for the HOOP wagging vibration mode. Another remarkable vibration feature is the C = C stretching vibration mode at 1642 cm$^{-1}$, which appears to be similar to that detected in the excitonic band of APC at 650 nm. The C = C stretching vibration mode shift suggests different possible conformations of the PCB cofactors. Consequently, the PC rod appears in the same conformation as the APC trimer.

Furthermore, the dominant activities of HOOP wagging and C = C stretching in-plane vibration suggests a deactivation of the S1 state in the excited state of the PC rod. This deactivation of the S1 state in the PC rod, as a result of the HOOP wagging vibration mode at 816 cm$^{-1}$, shows a correlation with the activation and deactivation of the intermediate state (I$^*$) in PCH and the PC hexamer. The intermediate state in PCH is modulated through HOOP wagging, the redshifted C = C stretch and the N–H in plane rocking vibration modes within 500 fs, as previously reported. However, a kinetic component of 970 fs is present in the PC hexamer. This kinetic component was very slow compared to that assigned to the coupling between the $\alpha$84 and $\beta$84 cofactors.

As with the detection of HOOP wagging and the C = C stretching vibration modes, the vibration mode of N–H in-plane rocking at 1373 cm$^{-1}$ was also detected. Therefore, HOOP wagging, C = C stretching and the in-plane N–H rocking vibration modes are consistent with the deactivation of the S1 state in the PC rod toward the APC activity mode.

A simple comparison between the NIR spectrum of the PC trimers (Figure 5) and the PC rod (Figure 8) shows a C = C stretching vibration mode that is red-shifted from 1666 cm$^{-1}$ (PC trimer) to 1642 cm$^{-1}$ (PC rod). Furthermore, a pump-probe femtosecond transient absorption investigation into an intact PC rod of PBSs, especially the absorption band of the PC rod near the APC core, showed decay kinetic components of 500 fs, 21 ps, and $\tau > 1$ ns, at 640 nm upon excitation at 620 nm (see Figure 9).

The 500 fs decay kinetic component further shows a strong correlation with the corresponding 500 fs$^{21}$ (PC trimers of Mastigocladus laminosus) and 690 fs$^{25}$ (PC $\alpha$ subunits of the AN112 mutant of the cyanobacterium Synechococcus PCC 6301) components, which were found in PC parts without the presence of all the compounds. Previous investigations$^{21,25}$ have been carried out on PC trimers and PC $\alpha$-subunits while PC hexamers have also been investigated$^9,24$ and the presence of a linker was not reported. Furthermore, the kinetic component of 970 fs that was assigned to the energy transfer between the $\alpha$ and $\beta$ cofactors in the PC trimer$^9$ is not consistent with the coupling that exists between the adjacent trimers in the PC hexamer.$^6$ The latter coupling was found to be 400 fs in the PC hexamer of Acaryochloris marina where we suggested the possible formation of an excitonic state at 635 nm.$^6$

Regarding PCH, the 500 fs kinetic component appears to be an activation of the intermediate state (I$^*$), which has...
been suggested to be deactivated in 3 ps in PCH.\textsuperscript{10} In addition, the detection of an excited state decay kinetic component within 500 fs at 640 nm, near the APC band (650 nm), may suggest the deactivation of an intermediate state (I*) in the PC rod. It is known that the PC rod contains PC 612 in addition to PC 620.\textsuperscript{26} When both the PC trimers are isolated, PC 612 shows a blue-shifted absorption compared to that of PC620. However, in the PC rod, PC 620 absorbs at a higher energy state (with a maximum peak at 620 nm\textsuperscript{3}) compared to the absorption energy of PC 612. Furthermore, PC 612 has been suggested to be functional near the APC core,\textsuperscript{26} which means it has an absorption close to 650 nm.\textsuperscript{7} Therefore, when an excited state is formed as a result of light excitation in a PC rod the excited state system may build an intermediate state to mediate the exciton or excitation energy\textsuperscript{6} between PC 620 and PC 612. Consequently, the 500 fs decay kinetics component obtained in the PC rod at 640 nm may be responsible for the deactivation of this intermediate state. This deactivation is faster than the 970 fs found in the Spirulina PC hexamer.\textsuperscript{9} The main difference compared with all the previous investigations is the absence of information regarding the linker protein. This absence of different subcompounds as linker rods, capping linkers, and rod-core linkers in the rod could lead to differences in PC excitation activity. This work provides an apparent insight into the latter statement.

Regarding the mechanism of excitation energy transfer in the isolated PC rod, the kinetics longer than 1 ns may be due to the disruption of excitation energy transfer that is not transferred to APC. The 21 ps that appears when the excitation energy is already transferred from PC620 to the low absorption state of the PC rod (PC612) is likely from the release of heat from the PC rod (protein) to the solvent via hydrogen bonds. This mechanism needs further investigation.

The solvent environment strongly influences coupling between adjacent PC trimers in different species.\textsuperscript{6} The same solvent environment can be used to facilitate the isolation of PC in monomers, trimers, and hexamers.\textsuperscript{3} Excitation of the PC trimer by UV and NIR shows the absence of a HOOP active mode and the presence of a 984 cm\textsuperscript{-1} active mode. Conversely, a feature of the HOOP active mode is apparent upon NIR excitation of the PC rod. We further found that the latter excitation mode (HOOP) is activated by C = C stretching and N–H rocking. These active modes that reflect the APC conformation are likely to be the route of excitation energy transfer and perhaps the exciton from PC rod to APC core.

The deactivation of the S1 state in the PC rod and the APC core mode activity may perhaps be consistent with a strong coupling between adjacent $\beta_{84}$ cofactors in the adjacent trimers, vis-a-vis along the C$_3$ axis of the PC rod. This coupling may be facilitated by the presence of all the components that make a PC rod. Additionally, the absence of HOOP wagging in the PC trimer correlates to the absence of a second PC trimer, which rules out the disruption interaction of their adjacent $\beta_{84}$ cofactors along the C$_3$ axis of the PC rod. This last assertion hinges on the absence of energy transfer via a specific conformation that allows the HOOP wagging active mode.

A further comparison between the NIR spectra of the PC trimers and the PC rods reveals that the C–H rocking vibration at 1418 cm\textsuperscript{-1} (in the PC trimer) is blue-shifted at 1467 cm\textsuperscript{-1}. Furthermore, new vibrational modes are apparent at 1520 and 1584 cm\textsuperscript{-1}. The methine bridge between rings C and D suggests either a syn or anti conformation, and these have been suggested to appear in PCH between 845 and 856 cm\textsuperscript{-1},\textsuperscript{27} while this is likely to be blue-shifted to 876 cm\textsuperscript{-1} in the PC rod.

Despite critical assessments of PC rods and PCH, and of PC rods and PC trimers, and despite the need for further research, analyses undertaken show the presence of HOOP wagging vibration mode activity in the PC rod. HOOP wagging, C = C stretching and N–H rocking vibration modes in-plane appear as active vibronic channels, which contribute to excitation energy flow from the PC rod to the APC core (see Scheme 1 showing the PC rod excitation energy flow).

\begin{center}
\textbf{SCHEME 1.} Showing the rod exciton or excitation energy flow.
\end{center}
V. CONCLUSION AND OUTLOOK

We have shown the absence of an excited state HOOP wagging mode in the excited state dynamics of a PC trimer and its presence in a PC rod. This absence suggests that the HOOP mode does not contribute to the excited state mechanism of the PC trimer. Because the acid moiety is functionally coupled with changes in the surrounding medium and it is bound to rings B and C the excited state factor, which is driven by changes in this medium is suggested to be C–N stretching in rings B and C. In addition, the observed C–H rocking is independent of the medium’s interaction with PCB. The C = C stretching at 1666 cm\(^{-1}\), which was absent in a previous study of PC, was observed in the PC crystal and we suggest that it originates in ring D, where the connected ethyl group may display a torsion excited mode at 454 cm\(^{-1}\). In contrast to PCH, we further show that the C\(_{5,10,15}\)-D wag excited mode at 622 cm\(^{-1}\) is not due to a downshift of HOOP wagging. We further show for the first time that a strong excited mode marker is present at 984 cm\(^{-1}\) instead of a closed vibronic channel, which may indicate strong coupling between \(\alpha_{94}\) and the \(\beta_{94}\) PCB cofactor in the PC trimer. We suggest that this strong excited mode marker at 984 cm\(^{-1}\) comes from C–C stretching. Additionally, we did not observe \(\text{syn}\) and \(\text{anti}\) conformation excited modes driven by a twist of the methine bridge that connects rings C and D in the PCB cofactor of the PC trimer. It is likely that ring D’s methine stretching, and perhaps torsion of the ethyl moiety of ring D, may not be negligible factors in PC trimer dynamics.

Furthermore, the PC rod has the same conformation as the APC trimer. This conformation is suggested by the appearance of a red-shifted C = C stretch at 1642 cm\(^{-1}\) together with HOOP wagging at 816 cm\(^{-1}\) in addition to a N–H rocking in-plane vibration mode. This conformation of the APC trimer in the PC rod facilitates exciton flow between the PC rods and APC. To reach the APC trimer, the exciton route may occur via the deactivation of an intermediate state, which decays in a 500 fs kinetic component.

However, to fully address the excited mode of the PC, the vibration mode of a variant of PC, which displays a blue shift in the absorption spectrum compared to bulk PC (PC\(_{612}\)) due to the lack of methylation in the conserved asparagine residue,\(^\text{28}\) has to be analyzed. Insights gained from the PC\(_{612}\) and PC rod excited modes coupled with the mode of the APC core and the intact PBS of \(T.\) vulcanus will help us understand the vibronic channel of PBS, and to compile a more consistent mechanism for excitation energy transfer (EET) in intact PBS.

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28See supplementary material at http://dx.doi.org/10.1063/1.4866293 for the main information consistent with the absence of absorbance peak below 500 nm and above 700 nm. This PC 620 has its maximum absorption at 620 nm.