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On the Unusual Temperature-Dependent Emission of the CP47 Antenna Protein Complex of Photosystem II

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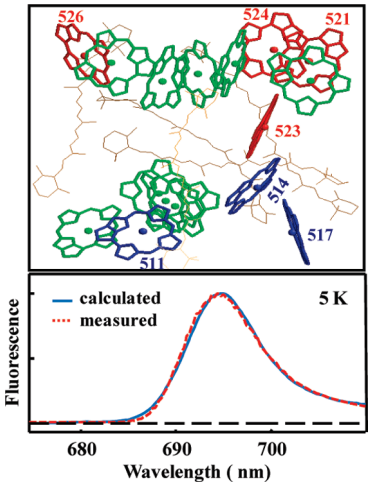
ABSTRACT It is shown that the fluorescence origin band maximum (~695 nm) of the intact CP47 antenna protein complex of PSII from spinach does not shift in the temperature range of 5−77 K. However, emission shifts continuously to shorter wavelengths (~692 nm) if high fluence is used, and hole burning takes place. If permanent damage does not occur, this process is reversible by cycling the temperature. In contrast, the emission peaks previously observed near 685 and 691 nm are characteristic of destabilized complexes and cannot be eliminated by temperature cycling. We argue that the CP47 complex is extremely light sensitive at low temperatures and that its 695 nm emission band in the PSII core, in contrast to several literature reports, does not arise from excitations that are trapped on red-absorbing chlorophyll of the ~690 nm band, as 5 K emission of intact (nonaggregated) CP47 also peaks near 695 nm.

SECTION Biophysical Chemistry



Although Photosystem II (PSII) has been studied for decades, some aspects of the electronic structure and excitation energy transfer (EET) of PSII remain controversial. For example, even though many emission spectra at different temperatures from both the intact (oxygen evolving) and isolated reaction center (RC) and CP43 and CP47 inner antenna protein complexes of PSII from plants[1−15] and cyanobacteria[16−18] have been published, no coherent picture emerges when the published emission spectra of the above complexes are compared. This is in part due to the fact that complex biological systems are prone to photodamage and/or destabilization even at extremely low doses of photon density during the isolation and/or interrogation procedures. In this regard, we showed recently[19] that the isolated (intact) CP47 antenna complex from PSII has a 5 K fluorescence emission maximum near 695 nm[19] and not, as previously reported, near 690−692 nm.[3,6,16] We have suggested that the previously measured 690−691 nm CP47 emission peak is a combination of emissions from the 695 nm emission of intact CP47 complexes with the lowest-energy state possessing very weak oscillator strength[19,20] and from the two trap emissions near 685 and 690 nm originating from photodamaged/destabilized complexes.[19,20] This interpretation is consistent with reports by Huyer et al.[8] (isolated CP47) and Komura et al.[12] (PSII core complexes) of multiple CP47 low-T emission bands in the 685−695 nm range resolved by time-domain spectroscopy. Note that only the 695 nm emission of CP47 is consistent with data obtained for more native (i.e., PSII core) complexes at 77 K.[9,11,13,18]

To shed more light onto the emission of isolated CP47 complexes (the possibility of aggregation has been excluded), we measured fluorescence spectra at different temperatures and excitation wavelengths, as well as different levels of photobleaching of the lowest-energy state. We demonstrate that intact CP47 complexes have very different temperature-dependent emission in comparison with the previously published fluorescence spectra.[3] In particular, we show that the 695 nm emission peak of intact isolated CP47 does not shift significantly in the 5−77 K temperature range. This finding calls into question the previous interpretation[9,18] (used to explain spectroscopic data of CP47[21] and the PSII core.[9,11,13,18]) that the 77 K emission peak near 695 nm in the CP47-RC complex originates from a subset of CP47 pigment(s) (assigned to the 690 nm band), which are unable to transfer energy uphill to the RC, whereas the low-temperature (5 K) CP47 emission peak near 690−691 nm originates from the entire distribution of lowest-energy pigment states. In addition, we demonstrate that nonphotochemical HB can produce a blue-shifted (reversible upon temperature annealing) contribution to the low temperature emission spectrum, which may help to explain some literature data. We argue that considerable reinterpretation of the low energy states and their emissive properties in this important antenna complex is warranted, as only a proper understanding of CP47 emission will explain the long-standing puzzle of PSII core emission and its temperature--dependent profile,[9,11,13,18] where an unusual "red" shift of the PSII core fluorescence origin band with increasing temperature was observed. Figure 1 shows the arrangement of CP47 Chls and carotenes (in brown) on the

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stromal and lumenal side of the membrane.[22] Very recently, we have suggested that most likely Chl 523 (not Chl 526)[4] contributes strongly to the lowest-energy state, providing the low oscillator strength and specific excitonic interactions needed to fit the absorption and red-shifted (695 nm) emission spectra as well as the persistent hole-burning (HB) spectrum.[20] Figure 2 (frame A) shows typical temperature-dependent emission spectra of the CP47 complex obtained from ref 3. For comparison, frames B and C show temperature-dependent emission spectra for the destabilized and intact CP47 complexes, respectively, obtained recently in our laboratory. Note the 680.3 nm emission band (see frame B) was observed only in some preparations containing a small fraction of destabilized CP47 complexes in which energy transfer to the lowest- energy traps is disrupted. In all frames, at higher temperatures (i.e., above 100 K), the increase in temperature results not only in temperature broadening but also in a blue shift of the spectra due to the equilibrium of the excitation over the antenna pigments.

Interestingly, both the fluorescence maxima and bandwidths of the spectra shown in frame C (intact sample) are drastically different from those shown in frames A and B. The fluorescence maximum at 4 K obtained from ref 3 (Figure 2A; top spectrum) lies near 690 ± 1 nm with a full width at half-maximum (fwhm) of ~12.5 nm. Similar spectra were reported in ref 6, while an even larger fwhm (~16.5 nm) was observed in ref 7. Likewise, our 5 K emission spectrum for destabilized CP47 (frame B) peaks at 691.3 nm and has an fwhm of 13.3 nm. The above-mentioned fwhm are by a factor of ~1.3−1.7 larger than that shown in frame C for intact CP47, which peaks at 694.8 nm and has a width of ~9.5 nm. In addition, as illustrated in Figure 3, the integrated (normalized) intensity of the fluorescence spectra from frames A, B, and C of Figure 2, plotted as black squares, red

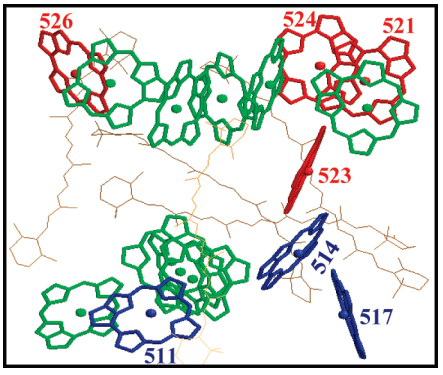

Figure 1. Arrangement of CP47 Chls and carotenes (in brown) on the stromal and lumenal side of the membrane. Chls 521, 523, 524, and 526 are shown in red. Chls 511, 514, and 517 are in blue. Remaining Chls are in green; the pigments are numbered as in ref 22.

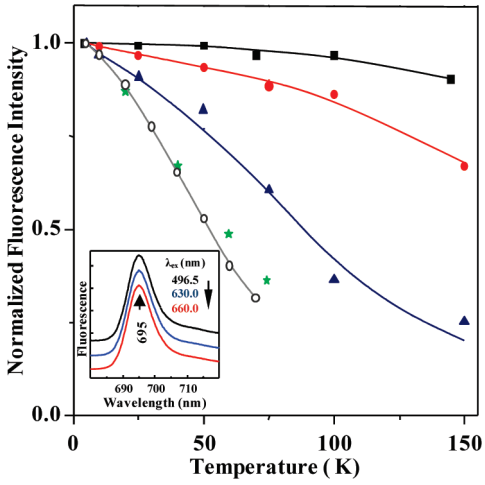

Figure 3. Normalized (integrated) fluorescence intensity of CP47 complexes shown in Figure 2 plotted as a function of temperature: black squares, red circles, and blue triangles represent data shown in Figure 2, frames A, B, and C, respectively. The inset shows fluorescence of intact CP47 at excitation wavelengths of 496.5 nm (top), 630.0 nm, and 660.0 nm (bottom curve). The green stars and open circles, shown for comparison, correspond to the normalized relative quantum yields of PS II core reported in refs 18 and 11, respectively.

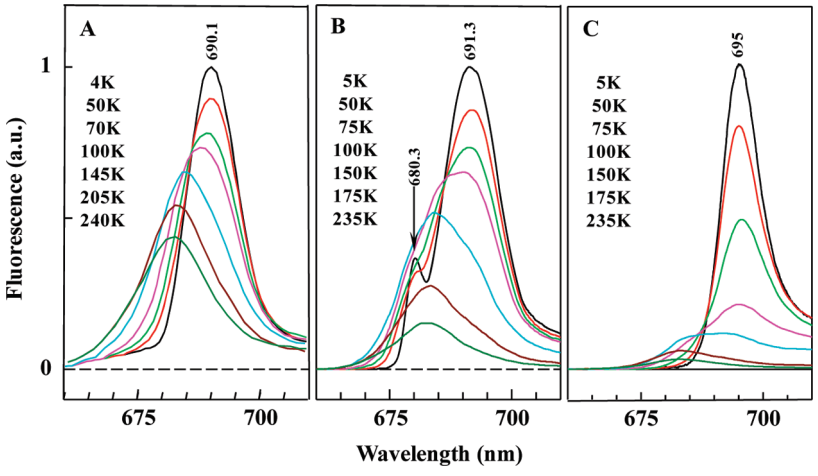

Figure 2. Temperature-dependent emission spectra of various CP47 complexes. Frame A: digitized spectra as presented in ref 3. Spectra shown in frames B and C represent spectra of destabilized and intact CP47 complexes (this work), respectively.

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dots, and blue triangles, respectively, show very different behavior when plotted as a function of temperature.

The strong decrease of CP47 fluorescence as a function of temperature (particularly for the intact sample of Figure 2C and PSII core that must contain intact CP47) is most likely due to strong temperature-dependent internal conversion. For comparison, the data points labeled by open circles show the integrated (normalized) fluorescence intensity for a PSII core in the temperature range of 5−70 K taken from ref 11, while the green stars correspond to the normalized relative fluorescence quantum yields of the PSII core as reported in ref 18. One must take some care in comparing the PSII and CP47 data: since all data sets are normalized to a value of unity close to 0 K, the values reported represent relative, not absolute, fluorescence yields; thus these data do not, for example, allow the determination of the absolute EET efficiency to the RC. Nonetheless, it is interesting to note that the relative yields of PSII core emission from refs 11 and 18 decrease even faster than that of CP47. Although the presence of additional quenching processes in the PSII core cannot be excluded, this behavior suggests that in the intact PSII core complex, the fluorescence efficiency is determined by the rate of EET to the RC, rather than by competition with nonradiative processes. This in turn implies that the nonradiative decay processes, which quench fluorescence in the isolated CP47 protein, are not so fast as to quench EET to the RC in the intact PSII core complex (which would impair CP47 function as a light-harvester).

As seen in Figure 2C, the fluorescence origin band of the intact CP47 lies at ∼695 nm, and its maximum does not shift significantly in the temperature range of 4−77 K. (A small shift in some samples from ∼694.8 nm at 5 K to ∼695.6 nm at 50 K is due to reversible HB as discussed below.) The observation of the 695 nm emission band at 5 K in isolated CP47 complex is in contrast to 5 K spectra previously reported in the literature[3,6,7] (similar to those of frames A and B of Figure 2) and calls into question several previous interpretations of such experimental data.[9,11,13,18] For example, the puzzling observation in ref 18 of an unusual red-shift of CP47-RC fluorescence between 4 and 77 K (from 691 to 694 nm) led to the interpretation that, while emission at 5 K (691 nm) originated from all lowest-energy pigments in the CP47 subunit, the 694 nm 77 K emission peak was due only to pigments that are unable to transfer energy to the RC (leading to selective emission from red-absorbing Chls). The presence of 695 nm emission at 5 K from *isolated* CP47 complexes (for which EET to the RC is obviously impossible) indicates that this explanation (while feasible for the previously obtained data) is most likely incorrect since one should otherwise expect 691 nm emission at 5 K from the isolated complex as well. Similarly, since 685 nm emission at 77 K observed in previously studied samples, as shown in ref 19, originates from destabilized CP47 complexes, we also reject the suggestion put forward in refs 11 and 18 that the intact CP47 complex possesses a state (i.e., the 683/684 nm state) that slowly transfers energy to the RC. This is consistent with our previous findings that intact CP47 complexes do not show a transient hole near 684 nm,[19] which is, however, clearly observed in destabilized complexes.[21,23] Note that the emission maximum is independent of the excitation wavelength as shown in the inset of Figure 3. Thus we conclude that, in the intact CP47 antenna, excitation energy is very efficiently transferred to the lowest-energy state at ∼693 nm resulting in a narrow (fwhm ∼9.5 nm) 695 nm emission band, and we see no reason to assume that, in intact CP47 (residing in the intact PSII core complex) at ∼5 K, a considerable part of the absorbed energy cannot be used for charge separation as suggested in refs 11, 13, and 18. These findings emphasize that, in order to elucidate the true EET dynamics in CP47 (and other photosynthetic complexes) the samples studied have to be free from contributions of partly destabilized/photodamaged complexes, especially when data are obtained in fluorescence and/or fluorescence excitation mode. This is because small subpopulations of disordered/photodamaged complexes with relatively large fluorescence quantum yield may strongly affect the composite emission signal.

Some of these effects become clearer when one considers emission originating from complexes with a modified (by HB) low-energy state, referred to in ref 19 as $A1_{mod}$. In ref 19 we showed that, in the first approximation, one can say that this is the lowest-energy state in CP47 in which the A1 state at 693 nm has been saturated, i.e., A1 ceases to be the lowest-energy state. In reality, of course, due to the continuous HB process (CP47 is quite sensitive to light and heat),[24] the lowest-energy state at 693 nm and the resulting fluorescence continually shift "blue" as a function of fluence (f). This is one reason why in some samples the emission origin band was slightly blue-shifted. We hasten to add that the $A1_{mod}$ band can be eliminated by temperature cycling (recreating the original A1 state) proving that no irreversible changes are introduced ($f < 4$ kJ/cm^2). It should be emphasized that these HB-induced shifts are distinct from the emission peaks near 685 and 691 nm observed, for example, in Figure 2A, B, due to destabilized complexes; these contributions (referred to as FT2 and FT1 in ref 19) cannot be annealed by raising the temperature. The latter is consistent with ref 25, where the heterogeneity of CP47 was also revealed via observation of different fluorescence decay components. These authors suggested, in agreement with our recent HB and fluorescence studies,[19,20] that in destabilized CP47 samples the interchromophoric interactions must be disrupted and/or perturbed in different way(s) depending on the subpopulation of the CP47 complexes.

The temperature dependence of CP47 emission from samples with saturated lowest-energy state ($A1_{mod}$) is shown in the main frame of Figure 4; the 5 K emission peaks near 692 nm, having been shifted from ∼695 nm by HB.

The inset in Figure 4 shows that the modified lowest-energy $A1_{mod}$ state shifts blue as revealed by the HB spectra; although the HB spectra are not shown here, the blue squares in the inset (that correspond to the spectral position of the hole maximum burned under nonresonant conditions using λ_{ex} of 496.5 nm) shift to shorter wavelengths as a function of fluence. A similar "blue" shift is observed in emission maxima (see the red circles in the same inset). The emission spectra shown in the mainframe of Figure 4 clearly indicate that, upon temperature annealing, the emission band shifts back to 695 nm as observed in intact (unburned) samples (see the

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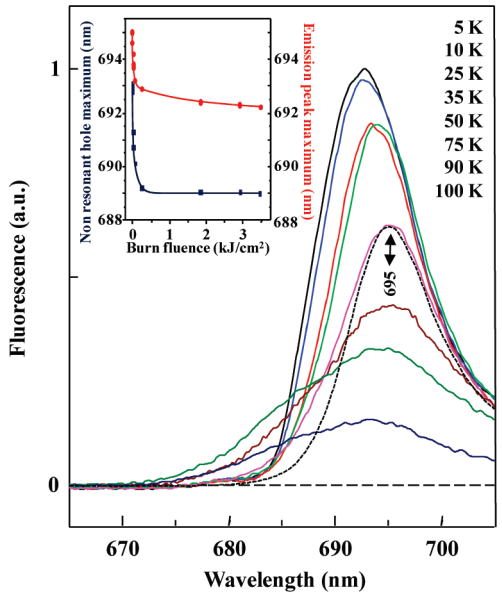

Figure 4. Temperature dependence of the fluorescence spectrum (top curve) obtained for a sample with saturated nonresonant hole at 5 K; its maximum is near 692 nm. The remaining spectra were obtained at higher temperatures. The dotted curve is the preburn 50 K spectrum from Figure 2C scaled for comparison with the postburn curve. The inset show the shifts of the broad nonresonant holes (blue squares) and positions of the corresponding maxima of the origin bands (red circles), obtained at different burn fluences.

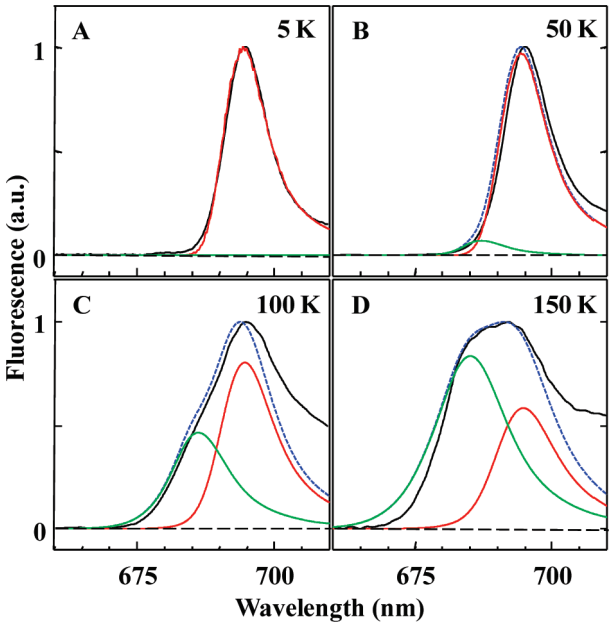

Figure 5. Comparison of experimental CP47 fluorescence spectra (black curves) with calculated emission spectra (blue dashed curves) at 5, 50, 100, and 150 K. The red and green curves correspond to the calculated lowest-state emission and higher-states emissions, respectively.

double sided arrow) as the hole is thermally refilled. For comparison with the 50 K post-HB spectrum, the dotted curve in Figure 4 shows the 50 K emission spectrum from a fresh (unburned) sample. The two spectra peak at 695 nm, indicating that thermal processes have already mostly refilled the burned hole. The slight broadening of the post-HB spectrum can be attributed to incomplete refilling due to complexes in which 50 K is not a high enough temperature to completely refill the spectral hole on the experimental time scale.

The observed shift of these HB spectra from ~692 nm at 5 K to ~695 nm at 50 K may help to explain the unusual temperature dependence observed in refs 11, 13, and 18. In fact the behavior reported in ref 18, where the emission peak of the CP47-RC complex was observed to shift from ~691 nm at 4 K to 694 nm at 77 K, is almost identical to the behavior seen in Figure 4. The similarity of these data sets suggests that the CP47-RC data in ref 18 may be affected by HB contributions in the 4 K spectrum (which are erased as the temperature is raised), although new experiments with fresh CP47-RC preparations are necessary to confirm this suggestion. In general, depending on sample exposure to light and experimental conditions during emission measurements, the blue-shifted emission observed in various CP47 samples reported over the years,[3,6,7] is most likely caused by two effects: (1) a contribution from reversible photobleaching due to high photon densities used for excitation, and/or (2) a contribution from the differently destabilized (or photodamaged) complexes (irreversible damage) characterized by the FT1 and FT2 trap emissions.[19]

Finally, we note that the *T*-dependence of our intact (and unburned) CP47 sample is in excellent agreement with the theoretically predicted behavior for temperatures up to ~150 K. Figure 5 shows calculated (blue curves) and experimental (black) fluorescence spectra obtained at four temperatures: 5, 50, 100, and 150 K (see figure caption for details). Note that, especially at low temperatures (5 and 50 K), the calculated spectra agree very well with the experimental curves (the discrepancy on the long-wavelength side at $T = 100$ and $T = 150$ K can be attributed to vibrational modes, which are not included in the calculations). The red curve in each frame shows emission from the lowest excitonic state, while the green curve shows the Boltzmann population-weighted emission from higher excitonic states, which is negligibly small, even up to 50 K. As a result, up to about 80 K, the maximum remains near 695 nm, in agreement with our experimental data. All spectra were calculated using the excitonic states composition from ref 20 and the standard expression for the emission line shape function, which (after applying a series expansion to the exponent in the expression shown in ref 26) reads

$$D(\omega;T) \propto e^{-S(T)} \sum_{R=0}^{\infty} \frac{S(T)^R}{R!}\, l_R(-\omega;T)$$

where $S(T) = \int_{-\infty}^{\infty} p(\omega;T)\mathrm{d}\omega$, $P(\omega;T) = (1 + n(\omega;T))J(\omega) + n(-\omega;T)J(-\omega)$, $J(\omega)$ is the spectral density function, $l_0(\omega;T)$ is a delta function representing the zero-phonon line (ZPL), $l_1(\omega;T) = S(T)^{-1} \cdot p(\omega;T)$, and, for $R > 1$, $l_R(\omega;T)$ is the convolution $l_1(\omega;T)$ with itself $R - 1$ times. For numerical calculations, the sum was truncated after a number of terms sufficient to account for 99.99% of the total intensity of the phonon-sideband. The best results were obtained with the Huang–Rhys factor $S = 1$ for all states (as obtained for the lowest state

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in ref 20; for details, see the Supporting Information). Note that, at higher temperatures (70−150 K), as expected, the emission band shifts to higher energies (blue shift) due to emission from higher states caused by the equilibrium of the excitation energy over the antenna Chls; this behavior is in contrast to the unusual red-shift observed in Figure 4 due to HB. Above 100−150 K, the deviation of the simulated spectra from the experimental ones increases significantly, most likely due to thermal activation of vibrational modes, *T*-induced changes in pigment/protein/solvent interactions, and/or quadratic/anharmonic electron−phonon coupling effects not accounted for in our simulations. These effects are beyond the scope of this communication and will be addressed elsewhere.

We anticipate that these data will provide more insight into the unusual temperature dependence of PSII core emission[11,13,18] and ultimately shed more light into the nature of EET from CP47 (and/or CP43) to the RC in PSII core complexes. Here, in light of the data discussed above, we only suggest that one of the conclusions in refs 11, 13, and 18 that low-temperature PSII emission originates from CP43 and CP47 "slow-transfer" states may have to be revisited. That is, given the observed emission near 695 nm from *isolated* CP47 at all temperatures between 5 and 77 K, we do not think that the strong temperature dependence seen in PSII core, including the red shift of the emission observed between 4 and 77 K, can be attributed solely to the lowest-energy CP47 pigments maintaining some "slow transfer" character or being thermally excited in a PSII assembly that becomes increasingly equilibrated.[13] Instead, we suggest that the multiple emission bands observed from the isolated PSII core complex[12] may originate from destabilized or photodamaged subunits, leading to the unusual *T*-dependent profile.

In summary, we have shown that the fluorescence origin band of the intact CP47 antenna protein complex of PSII from spinach does not shift in the temperature range of 4−77 K, and has very different temperature dependence than previously reported in ref 3. Temperature-dependent emission spectra of CP47 obtained at different saturation levels of the lowest-energy state(s) near 693 nm offer more insight into the origin of the previously published CP47 fluorescence spectra. It has conclusively been shown that the emission maximum of CP47 and its bandwidth strongly depend on the degree of bleaching of the low-energy state(s) and/or destabilization of the protein complex. That is, the 695 nm emission shifts continuously to shorter wavelengths reaching, due to photobleaching, a position of ∼692 nm at saturated HB conditions. This process is reversible by cycling the temperature. In contrast, the emission peaks previously observed near 685 nm and ∼691 nm characteristic of destabilized complexes[19] cannot be eliminated by temperature cycling. Thus we conclude that the well-known 695 nm emission of CP47 previously observed only at 77 K[11,13] does not arise from excitations that are trapped on red-absorbing CP47 Chls of the 690 nm band, as 5 K emission of intact CP47 also peaks near 695 nm.[19,20] Likewise, we argue that the 685 nm emission in PSII cores does not arise from excitations that are transferred slowly from 683 nm states in CP47 to the RC, as suggested in refs 11 and 18, since the 683/684 nm state and its emission near 685 nm are absent in intact CP47. Instead we suggest that both the ∼685 nm and ∼690 emissions in the PSII core could originate from destabilized or photodamaged complexes. Thus the slow EET dynamics observed in ref 13 may not be characteristic of the intact CP47 antenna, showing again that the EET dynamics in PSII deserves further study. These results highlight that it is essential that general criteria of intactness are established; otherwise it will be difficult to compare data generated in different laboratories.

SUPPORTING INFORMATION AVAILABLE Spectroscopic measurements and details of the calculated temperature-dependent emission spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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