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Energy transfer from natural photosynthetic complexes to single-wall carbon nanotubes

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ABSTRACT

Combination of fluorescence imaging and spectroscopy results indicates that single-walled carbon nanotubes are extremely efficient quenchers of fluorescence emission associated with chlorophylls embedded in a natural photosynthetic complex, peridinin-chlorophyll-protein. When deposited on a network of the carbon nanotubes forming a thin film, the emission of the photosynthetic complexes diminishes almost completely. This strong reduction of fluorescence intensity is accompanied with dramatic shortening of the fluorescence lifetime. Concluding, such thin films of carbon nanotubes can be extremely efficient energy acceptors in structures involving biologically functional complexes.

1. Introduction

Carbon nanomaterials have been since decades considered to be promising structures in constructing devices for sensing, photovoltaics, optoelectronics. In most cases the underlying physicochemical properties are related to large surface to volume ratio, ability to functionalize the surface with various active groups, strong potential of being an acceptor of both, charge and energy. Among the most illustrative examples are electron transfer in assemblies of fullerens and organic dyes [1], energy transfer between semiconductor quantum dots and carbon nanotubes [2], acceptor properties of graphene and reduced graphene oxide visualized using fluorescence microscopy [3,4], sensing of biologically relevant molecules with graphene oxide and its derivatives [5,6], and many others.

In the context of artificial photosynthesis, several attempts were made to assembly a structure, where naturally evolved photosynthetic complexes were attached to carbon nanotubes [7,8]. It required proper functionalization of the nanotubes in order to facilitate efficient coupling between the two nanostructures. Moreover, restrains are imposed on the conditions for sample preparation as photosynthetic complexes are proteins that require proper buffer conditions for preserving their function, whether it concerns energy harvesting, energy transfer, or electron transfer. On the other hand, while demonstrated on a single nanotube level, extension of the concept of constructing functional hybrid nanostructures composed of carbon nanotubes and photosynthetic complexes is limited by technology of surface deposition of carbon nanotubes with controlled chirality and their resulting electrical properties.

In this work we explore the possibilities to construct a hybrid assembly of semiconducting single-wall carbon nanotubes (SWCNTs) coupled with light-harvesting photosynthetic proteins responsible – in natural environment – for sunlight absorption and energy transfer. Fluorescence microscopy imaging and spectroscopy indicate that the energy transfer from the photosynthetic complexes to carbon nanotubes is extremely efficient, pointing towards high potential of such nanostructures for designing functional hybrid devices.

Carbon nanotube thin films were produced using the water solution of separated semiconducting SWCNTs from NanoIntegris (Iso-Nanotubes-S, 99% purity) with the tube diameters from 1.2 nm to 1.7 nm and the length in the range of 0.1–4 μm. Initially, nanotubes were stabilized by a combination of ionic surfactants, while the concentration of the SWCNTs in the solution was 0.01 mg/ml. However, to prepare small CNT thin films we removed

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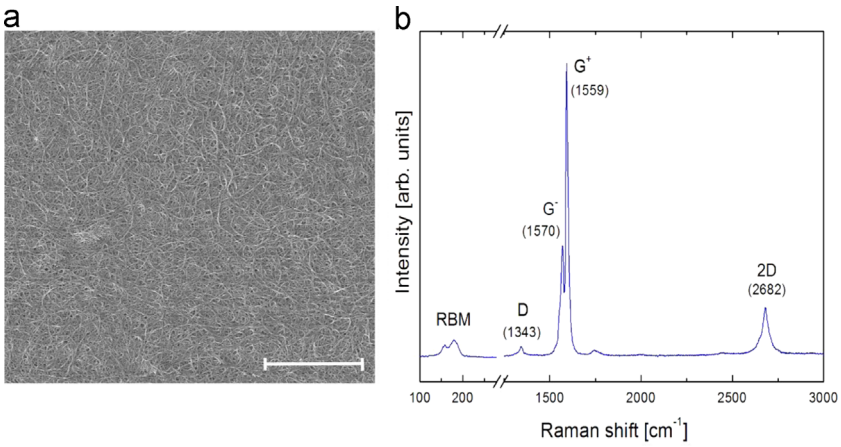

Fig. 1. (a) SEM image of a SWCNT thin film on glass; the scale bar represents 2 μm, and (b) Raman spectra of semiconducting SWCNT thin film showing typical Raman modes.

surfactants from the solution of the nanotubes. One part of the CNT solution was added to 2 parts of acetone. After 2 h nanotubes were precipitated from the solution. In the next step, tubes were collected and centrifuged for 10 min at 10,000 rpm to separate them from the acetone. During centrifugation, the nanotubes were re-bundled and collected to the bottom of the centrifuge flask. Acetone was removed and replaced by the water, and solution was mixed vigorously. After this process water was replaced by the methanol and centrifuged, again. The entire procedure was repeated several times to remove residual surfactants. At the end small and clean CNT bundles were kept in the methanol and next the mixture was dropped on a glass substrate (at temperature 50 °C). After methanol evaporation small SWCNT films were placed on the substrate. To increase the adhesion between the thin films and the substrate the samples were immersed in water by 30 min and then gently rinsed by methanol and dried by the nitrogen stream. Fig. 1a shows typical SEM image of the produced thin film and Raman spectroscopy confirms the presence of clean single-walled carbon nanotubes (see. Fig. 1b). From the SEM image we can confirm that a thin film of SWCNTs is formed, although the network is not self-organized, and the nanotubes are randomly oriented onto the surface. The thickness of the film depends on the exact location and is in the range of 10–100 nm.

To probe interactions between photosynthetic complexes and SWCNTs we chose peridinin-chlorophyll-protein (PCP) light-harvesting complex from *Amphidinium carterae*. This complex has simple structure, is soluble in water, absorbs light in a broad spectral range and is characterized with relatively high quantum yield of fluorescence emission. Native PCP has a trimeric form with each monomeric protein subunit consisting of eight peridinins and two chlorophyll *a* molecules [9]. In this work, for simplicity, we used reconstituted PCP complexes (monomers) obtained as described previously [10]. Absorption spectrum of PCP shows three main bands (Fig. 2, black line): from 350 nm to 550 nm, due to carotenoids–peridinins, as well as at 440 nm and 670 nm, ascribed to Soret band and Q_y band of chlorophyll *a* molecules, respectively. The emission of PCP complexes (Fig. 2, red line), associated with fluorescence of chlorophylls, occurs at 673 nm [11].

For characterization of the SWCNT thin films we used scanning electron microscopy (SEM, Raith eLINE Plus) and Raman spectroscopy. Raman spectra were collected by the Renishaw spectrometer using a 514 nm Ar laser excitation line and a laser power of 41 μW.

Absorption spectrum of PCP was measured using Varian Cary 50 UV–vis spectrophotometer. Fluorescence intensity maps (90 μm × 90 μm in size) were acquired with Nikon Eclipse Ti inverted wide-field microscope equipped with Andor iXon Du-888 EMCCD camera. The samples

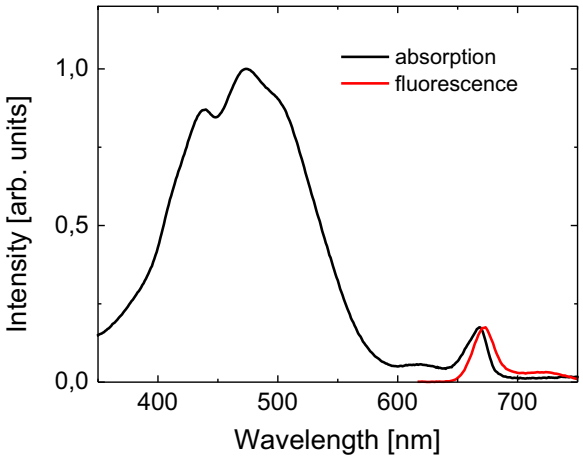

Fig. 2. Absorption (black) and fluorescence (red) spectrum of the PCP solution. Excitation wavelength of 485 nm was used. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were excited at 480 nm using a light-emitting diode illuminator through a microscope objective (Plan Apo, 100×, NA 1.4). Fluorescence was extracted with a combination of a dichroic mirror T505LP (Chroma) and a narrow bandpass filter (Thorlabs FB670-10). Acquisition time and electron multiplying gain of 0.5 s and 300, respectively were used. Prior to fluorescence measurements, we collected light transmission images to locate areas where single-walled carbon nanotubes were present. Time- and spectrally-resolved fluorescence measurements were performed using a home-built confocal fluorescence microscope described in detail elsewhere [12]. The sample was excited at 485 nm with a pulsed laser characterized with a repetition rate 20 MHz and excitation power of 20 μW. The excitation beam was focused on the surface of the sample by LMPlan 50x microscope objective with numerical aperture of 0.5 (Olympus). Fluorescence was filtered by longpass filter (HQ665LP Chroma) and then detected by Amici prism coupled with Andor iDus DV 420A-BV CCD camera. Fluorescence transients were collected by time-correlated single photon counting module SPC-150 (Becker & Hickl) with fast avalanche photodiode (idQuantique id100-20) as a detector.

Fluorescence intensity maps were obtained for two different concentrations of PCP complexes diluted in 0.02% polyvinyl alcohol (PVA from Sigma-Aldrich): 2 μg/ml and 0.2 μg/ml. 30 μl of each solution was spin-coated at 20 rps on a glass slide (reference) and SWCNT thin films. For fluorescence spectra and fluorescence transients measurements we used the solution of PCP complexes diluted in water with a concentration of 2 μg/ml. 1 μl of the

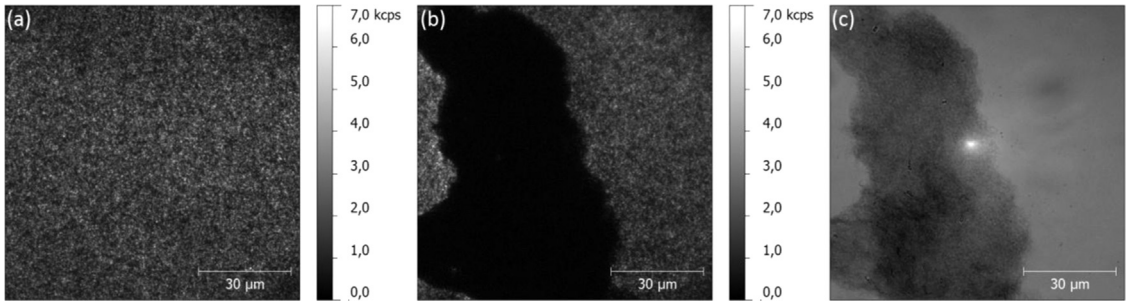

Fig. 3. Fluorescence maps of PCP (with concentration of 2 μg/ml) spin-coated on a reference sample (a), and on a SWCNT thin film (b) with transmission image of the same area (c). The excitation wavelength was 480 nm.

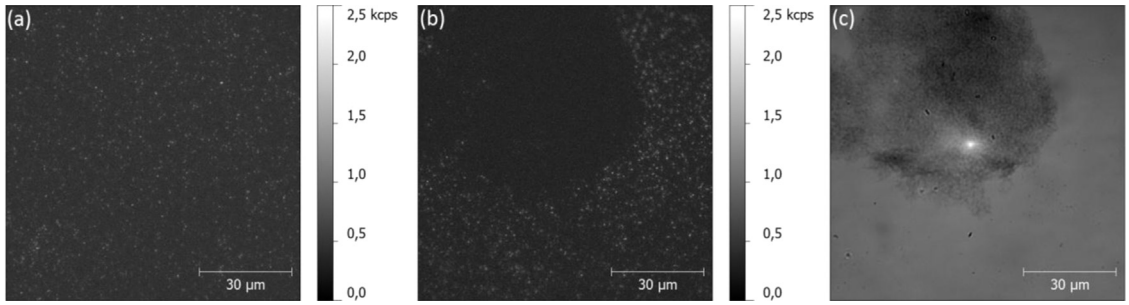

Fig. 4. Fluorescence maps of PCP (with concentration of 0.2 μg/ml) spin-coated on a reference sample (a), and on a SWCNT thin film (b) with transmission image of the same area (c). The excitation wavelength was 480 nm.

solution was drop-casted on the substrate covered with SWCNT and reference sample.

To obtain fluorescence intensity maps first we had to find areas with SWCNT. For this purpose we recorded transmission images and then corresponding fluorescence maps. In Fig. 3 we show representative sequence of images obtained for the sample where the PCP concentration of 2 μg/ml was used. For this concentration the fluorescence image obtained for the PCP complexes deposited on glass substrate (Fig. 3a) is highly uniform with intensities reaching up to 7000 cps. This indicates that our method of sample preparation results in highly homogeneous coverage. This is important for comparing the results obtained for PCP/SWCNT hybrids, displayed in Fig. 3b and c. Transmission image (Fig. 3c) exhibits well-defined dark areas, which we attribute to bundles of SWCNTs. The remaining part of the sample corresponds to pure glass substrate. The fluorescence image collected for the same area (Fig. 3b) is qualitatively similar, with the bright regions attributable to fluorescence of PCP complexes deposited on glass, as for the reference, and dark regions where no emission is present.

The intensity of fluorescence emission in the bright areas is similar to the reference sample, while regions containing SWCNT thin film are dark. This observation is similar to the one reported for the PPC complexes deposited on reduced graphene oxide [13]. Based on fluorescence intensity images we are not able to discriminate between plausible explanation which would assume fluorescence quenching of PCP fluorescence due to energy transfer to carbon nanotubes, and absence of PCP complexes onto a layer of carbon nanotubes due to some unspecific interactions that take part during sample preparation. We note however that the boundary between bright and dark areas visible in the fluorescence image is strongly correlated with the transmission of this area of the sample. Consequently, taking into account irregular shape of the SWCNT layer it would be highly challenging to define processes that would lead to such a strict interface formation between a PVA layer containing PCP complexes and areas containing carbon nanotubes.

In addition, transmission image of the sample shows that the thickness of the SWCNT thin film is not uniform, as it transmits light differently across the area covered by the nanotubes. Surprisingly it has no effect on the fluorescence intensity of the PCP complexes deposited on the SWCNT layer.

We have carried out analogous experiments for a layer where PCP complexes were diluted by an order of magnitude, to a concentration of 0.2 μg/ml. This concentration allows for obtaining a layer, where isolated spots are visible in the fluorescence image, as shown in Fig. 4a. These spots correspond to the emission of individual PCP complexes embedded in the PVA layer. For highly diluted sample the result is qualitatively the same, namely, for areas where in transmission a layer of carbon nanotubes is visible, the emission is completely none. Again the boundary between emitting and dark areas is very well defined and correlates perfectly with the location of the SWCNT thin film.

The results shown in Fig. 4 were obtained for highly diluted solution of the PCP complexes, the concentration was similar to the one used previously for detecting fluorescence if individual PCP complexes [11]. The emission intensity of individual complexes can be used as a local probe of the environment. We analyzed over a 100 isolated spots visible in Fig. 4 in order to compare intensities for both samples: PCP complexes embedded in PVA matrix and PCP complexes deposited on SWCNT thin film, but away from the film itself. It was prompted by the impression that the spots visible for the sample, where PCP complexes were deposited on the SWCNT thin film, are brighter than for the reference. Importantly, in the case of ensemble sample, shown in Fig. 3, the intensities for both samples are identical. In contrast, when comparing the data obtained for isolated spots, tentatively attributed to individual PCP complexes, as displayed in Fig. 5, there is substantial difference between bot samples. While in the case of the reference sample, i.e. PCP complexes on glass, the distribution is roughly normal, with an average value of approximately 2000 cps, for the sample, in which the PCP complexes in the sample with the SWCNT thin film, the distribution is much broader, with significant number of isolated spots having

intensities higher than for the reference. As a result, the average value of the intensity measured for isolated spots in this sample amounts to 2400 cps, inspite of the fact, that in both cases the matrix (PVA) is the same. Furthermore, the number of emitting spots in the areas away from the SWCNT thin film and in the reference sample are also comparable. Therefore, we can neglect any clustering effects that would result in fluorescent spots in the case of the hybrid sample being due to two or more single PCP complexes. The origin of this difference is not clear at present, and it requires further studies.

There is also another issue with the results obtained using wide-filed fluorescence imaging. In this experiment the excitation is passing through the SWCNT layer before exciting the molecules placed onto it, and furthermore, the emission also passes the SWCNT layer on its way to the detection system. Consequently, both the excitation efficiency and detection efficiency are reduced for the PCP complexes deposited on the carbon nanotube layer as compared to the complexes placed on the bare glass substrate. This effect could contribute to some degree to the observed differences in fluorescence intensity of the PCP complexes on and off the SWCNT thin film.

Important insight into the mechanism responsible for disappearing of the fluorescence emission of the PCP complexes deposited on SWCNT thin film was obtained using confocal fluorescence spectroscopy. In this experiment, in contrast to the wide-field microscopy results, the PCP complexes are excited first with no excitation beam passing through the nanotubes thin film, and in addition the emission is also collected directly from the PCP layer. In particular, the influence of SWCNT on the optical properties of PCP complexes was determined by the spectrally- and time-resolved measurements. The data were obtained by collecting 50 spectra and 25 transients, all from different spots across the actual sample. In this way we have obtained information not only about the properties of PCP/SWCNT hybrid, but also about the homogeneity of the samples. In Fig. 6 we present all acquired emission spectra of PCP complexes drop-casted on glass (a) and SWCNT thin film (b). As both the excitation power and the acquisition time was kept the same for both series, this result indicates that – in accord with the wide-field fluorescence imaging – the emission of the PCP complexes is efficiently quenched (by approximately factor of 50) when deposited on SWCNT layer. At the same time, the shape of the fluorescence spectrum is not affected by the presence of SWCNTs, which implies that the protein maintains its functionality when coupled to the carbon nanostructure. From a broader perspective, we can conclude that the PCP protein is very robust, as the proximity of carbon nanostructures, while changing the intensities of emission, leaves no effect on the functionality of light-harvesting and energy transfer between the pigments comprising the complex.

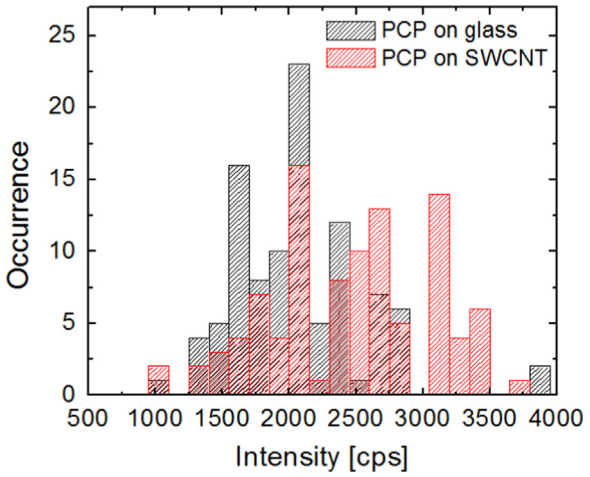

Fig. 5. Histograms of fluorescence intensity obtained for isolated emission spots for PCP complexes deposited on a glass substrate (black) and the SWCNT thin film (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fluorescence intensities obtained by integrating emission spectra are compared for both substrates in Fig. 7. Typically, coupling emitters with other nanostructures, such as metallic films or nanoparticles [14,15] results in significant broadening of the intensity distribution of the emission. In contrast, in the case of PCP/SWCNT hybrid nanostructure, the distribution of fluorescence intensity is very narrow, indicating exceptionally good homogeneity of the sample, both in terms of morphology of carbon nanotubes, and interaction between them and PCP complexes. In order to elucidate this effect in greater detail, it is required to fabricate SWCNT substrates with much lower density, thus with more spacing between the nanotubes. In this way it should be possible to observe both coupled and isolated PCP complexes (or other fluorophores) within the areas where the nanotubes are present. In the case of the structures studied in this work, we find no emission out of the regions with nanotubes, although, judging from the transmission of the sample, the thickness of the SWCNT layer is not uniform, and even very thin areas are visible.

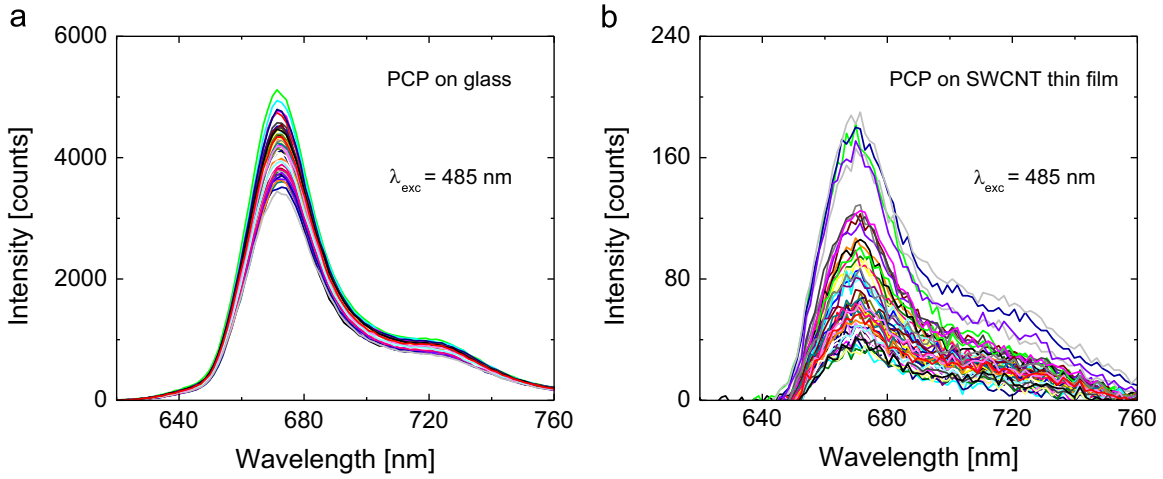

Fig. 6. Emission spectra of PCP complexes drop casted on (a) reference sample and (b) SWCNT. The excitation wavelength was 485 nm.

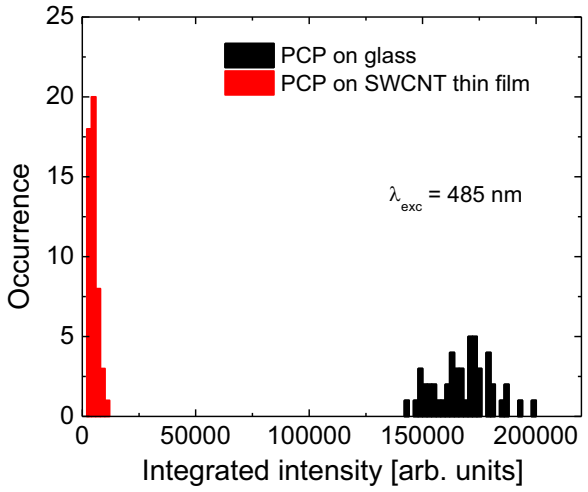

Fig. 7. Histograms of fluorescence intensity obtained from emission spectra of PCP complexes deposited on a glass substrate (black) and the SWCNT thin film (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

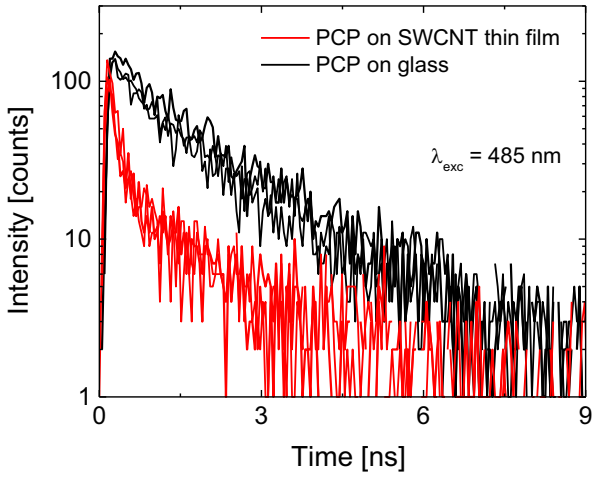

Fig. 8. Representative fluorescence decay curves obtained for PCP complexes deposited on a glass substrate (black) and the SWCNT thin film (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The emergence of energy transfer from PCP complexes to SWCNT is probed most directly using time-resolved fluorescence experiment. In Fig. 8 we compare decay curves obtained for PCP complexes deposited on glass (black) and SWCNT (red). The data is not normalized, and three curves shown as representative ones for a given substrate, are very similar, both in terms of intensity and decay character. In the case of PCP complexes deposited on glass we find long decay, approximately monoexponential, similar to previous experiments [10]. In contrast, for the PCP complexes deposited on the SWCNT thin film the decay changes qualitatively its character and is becoming more of two-exponential. While the longer decay is comparable with the behavior found for the reference sample, it also features extremely short decay, close to our temporal resolution limit. We attribute this short decay to the PCP complexes that are coupled to the SWCNT film and efficiently transfer their energy. Indeed, the fast decay is approximately 10 times shorter than the one of the reference, suggesting that the efficiency of the energy transfer reaches 90% in this hybrid assembly. As the PCP complex is a light-harvesting antenna and not a charge-separating photosynthetic complex, we expect the process responsible for the decrease of fluorescence intensity and shortening of the decay time to be associated with excitation energy transfer, rather than an electron transfer.

In conclusion, the results of fluorescence imaging and spectroscopy indicate that the energy can be efficiently transferred from natural light-harvesting complexes to carbon nanotubes deposited on a surface. The process is very uniform and its efficiency reaches 90%, as determined from fluorescence transients. Our observations can be potentially important for constructing artificial photosynthetic systems based on carbon nanotubes.

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