Selective Enhancement of Carbon Nanotube Photoluminescence by Resonant Energy Transfer

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We report on a simple method for enhancing the efficiency of photoluminescence (PL) emission from selected chiral forms of semiconducting single-wall carbon nanotubes (SWCNTs). The method is based on the use of a fluorescent dye (Nile blue A) that shows the capability of resonant energy transfer on to nanotubes. The excitation of Nile blue A in the presence of SWCNTs results in the quenching of its fluorescence and the energy is resonantly transferred to certain chiral forms. The PL emission from these chiral forms shows a marked increase in efficiency signifying the occurrence of Förster type resonant energy transfer (FRET). Due to its simplicity, this procedure has widespread implications for the detection of carbon nanotubes as well as for their use as fluorophores in FRET-based in vivo and in vitro biological applications.

1. Introduction

Single-wall carbon nanotubes (SWCNTs) are emerging as prospective candidates for a variety of applications.[1] They find widespread interest in a number of experimental and theoretical studies due to their interesting electrical[1] and optical[2] properties. The optical properties are now much widely pursued mainly after the observation of band-gap photoluminescence[3] and the ensuing use of photoluminescence excitation (PLE) spectroscopy[4, 5] as a suitable characterization tool. In this technique, the band-gap fluorescence of semiconducting CNTs in dispersion is mapped as a function of the incident laser wavelength that is either continuously tuned or discretely varied over the entire visible range, delivering valuable information regarding the distribution of various chiral forms in dispersion[6] and the dispersion efficiency.[7] Chiral form-specific data extracted using this technique at a dispersion level is very vital for the successful realization of a number of applications, such as the use of SWCNTs as fluorogenic probes for biological purposes.[8] In spite of the huge promise of this method, one aspect that has been hampering widespread deployment is the low emission efficiency of individual SWCNTs in dispersion due to their low quantum yields.[9, 10] In addition, bundling of the tubes in suspension leads to broadening and a reduction in emission intensity thereby lowering the efficiency further.[11, 12] Here we show that with the help of an appropriate "enhancer" or "sensitizer" utilizing resonant energy transfer, the emission efficiency of a desired subset of chiral forms can be selectively enhanced. In addition, the improvement in PL emission efficiency paves way for the detection of certain chiral forms that otherwise go undetected.
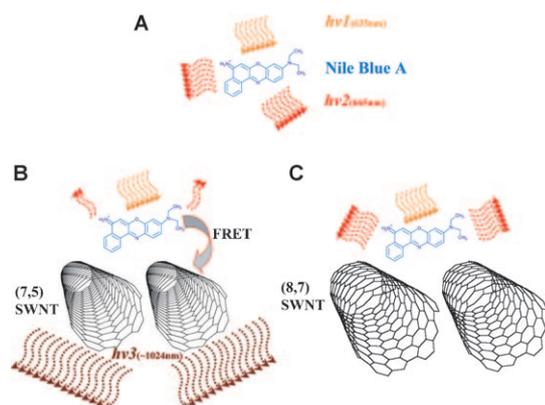
Resonant energy transfer involves the transfer of energy from a donor molecule to an acceptor molecule via a number of interactions.[13] The most common form is Förster resonant energy transfer (FRET), where dipole–dipole interactions play a major role.[14–16] The main requirements for FRET include the overlap of emission spectra of the donor and the absorption spectra of the acceptor, in addition to a close spatial proximity (< 10 nm) between the two. Resonant energy transfer on to SWCNTs has been observed using conjugated systems, where the donor was anchored to the acceptor tubes. For example, quantum dots have been complexed to CNTs[17] or dyes acting as photosensitizers have been bound to nanotubes.[18] These systems however require extensive chemical functionalization, which does not preserve the native character of the tube. Charge transfer effects may also play an important role during the photosensitization process. In some cases dyes have been used as surfactants and the occurrence of energy transfer has been proposed as a possible mechanism.[19] With nanotube–porphyrin complexes, enhancement in nanotube PL has been reported at high donor concentrations (1 to 2 M) without any selectivity.[20] For applications such as in vivo imaging, it is important to have a very low concentration of the donor to avoid any kind of (bio)chemical interactions. FRET is a well-established technique for labelling biomolecules and used widely in molecular diagnostic studies.[21] For a successful FRET experiment, it is important to be highly selective in addition to being sensitive to just one or a few of the chiral forms.[22] In this paper, we show that with a smart choice of the donor, energy transfer can be maximized to certain chiral forms and thereby the efficiency of PL emission from these species can be improved even at very low donor concentrations (μM).

The principle behind our technique is shown in the schematic in Figure 1. The excitation of the free dye ($\lambda_{exc}^{NB} =$

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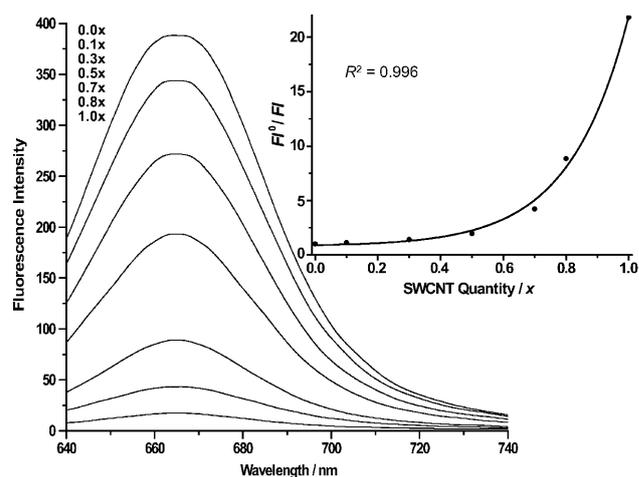1015 Lausanne (Switzerland)

Figure 1. Schematic representation showing the selective enhancement of carbon nanotube photoluminescence by Förster resonant energy transfer (FRET). A) Nile blue A fluoresces at an emission maximum of 665 nm upon excitation with light at 635 nm. B) The (7,5) SWCNT is in resonance with the excited state of Nile blue A, and hence, upon excitation of Nile blue A, the PL from the (7,5) tube is enhanced by FRET. C. In contrast, the (8,7) tube is not in resonance, and hence, its PL remains unaffected.

Figure 2. The emission spectrum of 2.5 μM (final concentration) Nile blue A in the presence of different CoMoCAT nanotube concentrations (excitation wavelength: 635 nm). 1x refers to the starting concentration after centrifugation. The inset is a Stern–Volmer plot showing the ratio of the emission intensity (at 665 nm) in the absence of nanotubes (Fl^0) to the intensity in the presence of nanotubes (Fl).

635 nm) results in PL emission with a maximum at 665 nm (Figure 1 A). A (7,5) SWCNT has an absorption maximum at 660 nm corresponding to its second van-Hove energy ($E_{22}^{(7,5)} =$ 1.87 eV) and is hence in resonance with the excited state of Nile blue A. As a result of this, the excitation of Nile blue A in the presence of (7,5) tubes results in resonant energy transfer as shown in Figure 1 B. This can be corroborated with the fluorescence quenching of Nile blue A and an enhancement in the PL emission of the (7,5) tube. On the other hand a (8,7) tube absorbs at much longer wavelengths (maximum 740 nm) and hence there is no occurence of FRET as depicted in Figure 1 C. Accordingly, the PL emission from these tubes is hardly affected. In general, by choosing an appropriate donor fluorophore that has an emission maximum overlapping with the absorption maximum of any (*n*, *m*) semiconducting tube, the PL emission from that specific tube can be significantly enhanced.

2. Results and Discussion

We first present the quenching behaviour of Nile blue A in the presence of CoMoCAT SWCNTs. For this purpose, 10 μL of diluted dye dispersion was mixed with 1 mL of surfactant solution and with 1 mL of each of the SWCNTs dispersions of different concentrations. Figure 2 shows a collection of the emission spectra for each of the concentrations of CoMoCAT tubes. It can be inferred that with increasing nanotube concentration, the emission intensity reduces continuously, signifying that the nanotube raw-material quenches the fluorescence from Nile blue A. This is further apparent from the Stern–Volmer plot[23] in the inset of Figure 2, showing the ratio of the fluorescence intensity (at the emission maximum of Nile blue A 665 nm) without nanotubes (Fl^0) to the intensity with nanotubes (Fl) as a function of nanotube concentration.

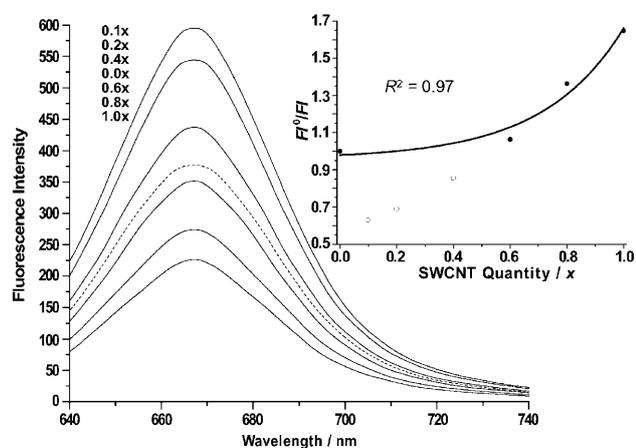The non-linearity and the shape of Stern–Volmer (SV) plot suggest that there is more than one species involved simultaneously in the quenching process. This can be understood by considering that the nanotube raw material comprises amorphous carbon and catalyst particles in addition to metallic and semiconducting tubes, all of which might contribute to the fluorescence quenching of Nile blue A. In addition, the probability of having bundles in the dispersion is more at higher concentrations.[24] This, in connection with the curvature of the SV plot suggests that bundles might contribute more to quenching than individual SWCNTs. However, the proportion of semiconducting tubes is much higher in the nanotube raw material than all the other components.[25] Further, the SV plot presents a very high value of Fl^0/Fl, at the highest concentration of nanotubes, corresponding to a reduction in fluorescence intensity of more than 95 %. This signifies a very high quenching efficiency characteristic of resonant energy transfer[26] by at least one kind of species present in the nanotube raw-material. By comparing the absorption spectrum of CoMoCAT tubes A (as shown in Figure 3) with the emission spectrum of Nile blue A, it follows immediately that this species which is responsible for the high quenching efficiency must be the semiconducting tubes, whose second van-Hove energies (E_{22}^s) overlap largely with the emission energy range for Nile blue A.

On the other hand, the E_{22}^s of HiPCO tubes occur at wavelengths slightly shorter than that for CoMoCAT tubes[27] and hence the overlap of the E_{22}^s nanotube absorption band with the emission band of Nile blue A is much lower. Furthermore, the relative proportion of tubes that can absorb in this range is much lower in the HiPCO raw material as is apparent from the absorption spectrum (see Figure 3). The quenching pattern and the SV plot for the interaction of Nile blue A with HiPCO tubes are shown in Figure 4. It is apparent that the SV plot for HiPCO tubes is close to linear suggesting that the quenching effect of semiconducting SWCNTs is relatively the same as that of the other components in the raw material. In addition, the quenching coefficient (Fl^0/Fl) at the highest concentration is much weaker (around 45 %) than that for CoMoCAT (95 %), al-
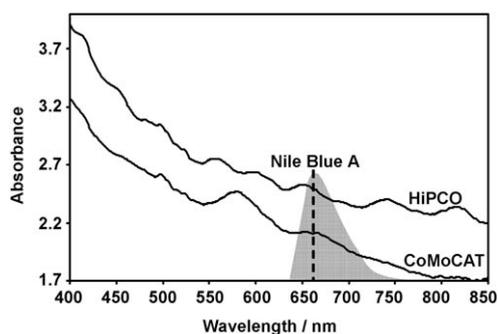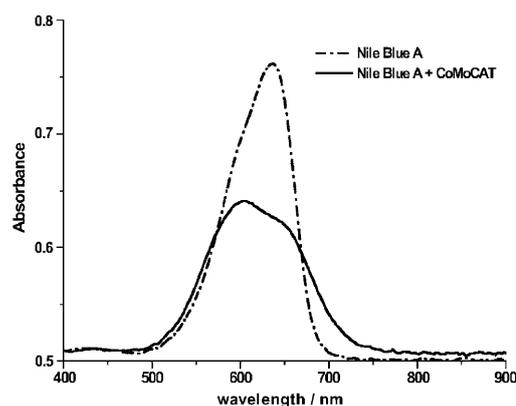
Figure 3. Optical absorption spectra of CoMoCAT and HiPCO SWCNTs (1x in both cases). The shaded areas show the emission spectrum of Nile blue A, indicating the spectral overlap with the second van Hove energy (E_{22}^{s}) of the SWCNTs in the respective samples. The dashed line represents the wavelength of the emission maximum for Nile blue A (665 nm).

Figure 5. Absorption spectra of 3.0 μM (final concentration) Nile blue A in the absence and presence of CoMoCAT nanotubes. The absence of a red-shift signifies that the quenching of Nile blue A is dynamic in nature.

Figure 4. Emission spectrum of 2.5 μM (final concentration) Nile blue A in the presence of different HiPCO nanotube concentrations (excitation wavelength: 635 nm). 1x refers to the starting concentration after centrifugation. The inset shows the Stern–Volmer plot containing the ratio of emission intensities at 665 nm as a function of the nanotube concentration (Fl^0, Fl: fluorescence intensities in the absence and presence of nanotubes respectively). Black dots signify that the emission from Nile blue A is quenched, whereas white dots correspond to the case when the emission is enhanced, most likely due to increased scattering effects at low concentrations.

though the total concentration of HiPCO raw material is higher than that of CoMoCAT (as inferred from the absorption spectra in Figure 3). These aspects indicate a low efficiency of energy transfer, which can be corroborated with a low concentration of tubes that have an absorption band overlapping with the emission spectrum of Nile blue A. It is noteworthy that at low HiPCO concentrations (less than 0.5x), the fluorescence of Nile blue A is not quenched but rather enhanced. One possibility is that the dilution of HiPCO dispersions might lead to more effective scattering[28] of the emitted light, thereby enhancing the dye fluorescence.

Further support for the occurrence of FRET is obtained by observing the absorption spectrum of the dye in the presence and absence of nanotubes as shown in Figure 5. The absence of a strong red shift in the overall spectrum suggests that

there is minimal complexation of the dye with the nanotubes.[18] The slight broadening could be due to the interaction of the dye with the other components of the raw material. This is consistent with previous observations that molecules with an anthracene type backbone (such as Nile blue A) are quite rigid and have a low probability of π-stacking and complex formation with the nanotube.[19] The reduction in the absorbance can be corroborated with increased scattering due to the low-aspect-ratio components present in the dispersion. This along with the quenching pattern suggests that the quenching is dynamic or collisional and that the mechanism is based on the quenching sphere of action model.[29,30] This type of quenching involves the collision of an excited dye molecule with quenchers (nanotubes) that are in its proximity (within the quenching sphere[29]). As a result, the excitation energy is transferred resonantly to the nanotubes and the dye molecule returns to its ground state without emitting light. In this process, neither a chemical alteration nor complex formation takes place. The energy transfer is most likely through long-range dipole–dipole interactions between the fluorophore and the quenchers.[16]

A third important aspect for FRET is the spacing between the dye molecule and CNTs. Unlike complexed systems, in our case there is always a statistical distribution of dye molecules around the nanotube in the illumination area. By choosing a high enough concentration of the dye solution and simultaneously low enough to avoid self-quenching and other kinds of interactions,[31] the possibility of energy transfer can be optimized. It is noteworthy mentioning that the concentration of the dye used here [0.7 to 3.5 μM] is six orders of magnitude lower than the porphyrin concentration [> 1 M] used in the study of SWCNT–porphyrin complexes[20] and this is key to maximizing a Förster kind of energy transfer. This is the main advantage of this technique, not requiring extensive chemistry, and hence the interaction of the dye with the nanotubes can be followed in their native state. Functionalizing the tubes could lead to a change in their electronic and emission properties[32] and hence may not be ideally suited for certain applications.

Now, we turn towards the effect of energy transfer on the photoluminescence from semiconducting SWCNTs. Figure 6 shows the PL emission spectra ($\lambda_{exc} = 638$ nm) from semiconducting tubes before and after addition of Nile blue A. Unlike
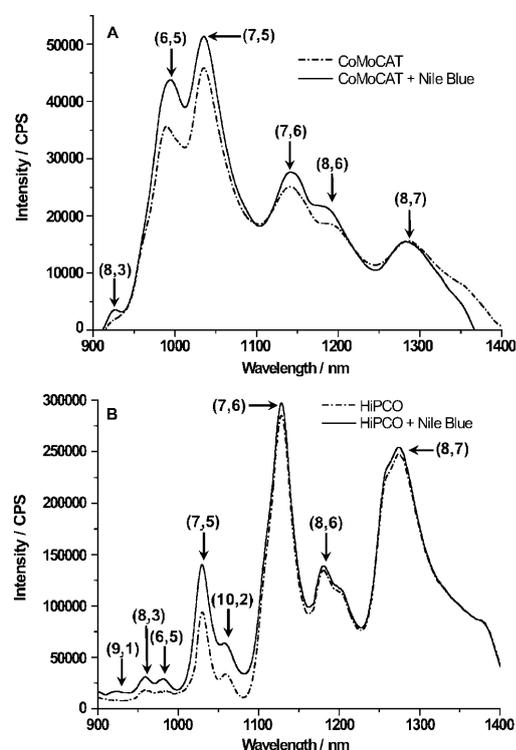
Figure 6. Selective enhancement of nanotube photoluminescence PL spectrum of HiPCO (A) and CoMoCAT (B) tubes with (dash-dot line) and without (solid line) Nile blue A in the near-infrared range. Nile blue A final concentration was 3.5 μM and the excitation source was a 638 nm diode laser. In both cases, the PL emission from selected chiral forms [e.g. (7,5), (7,6) and (8,6) tubes] are enhanced upon addition of Nile blue A. Most notably, the PL of (8,7) that is not in resonance with the excited dye remains almost unaffected. Furthermore, (8,3) [absorption maximum at 665 nm] and (9,1) [absorption maximum at 691 nm] tubes, could not be or poorly detected without Nile blue A, because they are not in resonance with excitation source (638 nm). But upon Nile blue addition both chiral forms can be detected, that is, enhancement in PL signifying an efficient energy transfer from Nile blue A on to these chiral forms.

previously reported experiments,[20] the measurements were obtained immediately after addition of the dye signifying the simplicity of this technique. It is apparent that the PL emission from nanotubes is enhanced in the presence of Nile blue A signifying the transfer of energy from the excited state of the dye resonantly on to the nanotubes. Specifically, 1) in the presence of Nile blue A, there is a clear enhancement in the efficiency of PL emission from only those tubes whose absorption maximum overlaps with the emission spectrum of Nile blue A [such as for the (7,5), (7,6) and (8,6) tubes]; 2) the PL emission from tubes that are not in resonance with the excited state of Nile blue A [such as the (8,7) tube] remains almost unaffected in the presence of the dye; 3) PL emission is observed from tubes that were not detectable before the addition of Nile blue A [such as the (8,3) tube], signifying a remarkable improvement

in sensitivity. While on one side these observations underline the occurrence of FRET, on the other side they demonstrate clearly that the PL emission from certain chiral forms can be selectively enhanced. The emission efficiency (in the whole spectral range 900–1400 nm) upon addition of Nile blue A is found to increase by up to 10 times for HiPCO tubes and 2 times for CoMoCAT. The difference in the increase in emission efficiency between HiPCO and CoMoCAT could arise from the different proportion of non-fluorescent components (amorphous carbon, catalyst particles and metallic tubes) present in the respective raw material. This increase in efficiency has been attained with a low concentration of the dye (1.5 μM) suggesting a strong improvement in nanotube detection sensitivity using this technique.

3. Conclusions

We have introduced a simple scheme based on FRET representing a very efficient method for enhancing the band-gap fluorescence of selected SWCNTs without disturbing their native state. It neither requires elaborate chemistry nor any control over conditions such as incubation time, pH or temperature. In addition, inexpensive dyes that are commercially available can be utilized for this purpose. The improved sensitivity opens up the possibility to work with very low concentrations of nanotubes. It is important to keep in mind that the choice of the dye might be critical, so as not to undergo any chemical interaction with the nanotubes and that the major proportion of energy transfer goes towards populating the E_{22}^s of the desired chiral forms. By choosing fluorophores with very narrow emission spectra such as fluorescent nanoparticles it is possible to enhance the emission efficiency of very specific tubes. This could then be used as a tool to estimate the concentration of a specific chiral form of nanotubes in dispersions. The detection of the distribution of chiral forms can thus be improved by using a mixture of non-interacting dyes and the concentration of CNTs established. This might represent a major step in overcoming a fundamental bottleneck in a range of CNT-based activities, mainly separation science. Finally, our demonstration of the ability to deploy SWCNTs in a FRET-based scenario is expected to provide an impetus for molecular diagnostics and biological applications involving in vitro and in vivo imaging.

Experimental Section

0.04 mg mL^{-1} HiPCO (High pressure Pyrolysis of CO from Unidym, Inc.) and CoMoCAT (Co and Mo Catalyst process from SouthWest NanoTechnologies Inc.) SWCNTs in aqueous 0.1% Triton-X-100 (Acros) were separately sonicated (SONOPULS Ultrasonic Homogenizer HD-3100) for 40 sec with 1 sec impulse and 2 sec rest intervals. SWCNT dispersions were centrifuged (ROTANTA 460 RS, Hettich) at 4637 g (4600 rpm) for 30 min, and the supernatant was used as the stock dispersion assuming that the final SWCNTs concentration is 1x. Diluted SWCNT dispersions (0.8x, 0.7x, 0.5x …) were prepared just before the experiments by mixing the stock dispersion with the 0.1% surfactant solution in appropriate ratios.

No further sonication was used in the case of freshly prepared stock dispersion.

A stock solution of the fluorescent dye [0.5 mg mL^{-1} Nile blue A (Sigma)] was prepared in absolute ethanol. A 10–50μl aliquot of 10x diluted stock was added to 1.0 mL of blank and sample (0.7–3.5 μM final concentration). The mixing of the solutions and dispersions were performed just before the measurements. The fluorescence emission from solutions (in the visible range) in standard cuvettes (10 mm path length) containing only the dye solution and with added SWCNTs were measured using a standard luminescence spectrometer (PerkinElmer, LS50B). PLE spectra (in the near infra-red range) were collected using the NS1 Nanospectralyzer (Applied Nanofluorescence) using a 638 nm laser diode as the excitation source. Optical absorption spectra were obtained using an Implen Nanophotometer (standard cuvettes, path length 10 mm).

Acknowledgements

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