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Noncovalent Functionalization of Carbon Nanotubes by Fluorescein−Polyethylene Glycol: Supramolecular Conjugates with pH-Dependent Absorbance and Fluorescence

Nozomi Nakayama-Ratchford, Sarunya Bangsaruntip, Xiaoming Sun, Kevin Welsher, and Hongjie Dai*

Department of Chemistry, Stanford University, Stanford, California 94305

Received December 4, 2006; E-mail: hdai@stanford.edu

Carbon nanotubes are interesting 1D nanomaterials,[1] and to explore nanotubes as macromolecules, various functionalization schemes, both covalent and noncovalent, have been developed to impart water solubility and chemical functionalities.[2] Noncovalent modifications of nanotubes include the use of surfactants and aromatic molecules (e.g., pyrene).[3] Here, we report noncovalent functionalization of single-walled carbon nanotubes (SWNTs) by fluorescein−polyethylene glycol (Fluor-PEG) (**1**) based on a serendipitous observation of strong binding of the molecule on SWNTs. The simple functionalization approach imparts aqueous solubility and simultaneously affords fluorescent labels to nanotubes. Interestingly, the optical absorbance and fluorescence of fluorescein bound to SWNTs are distinctly different from those of free fluorescein, displaying pH-dependent noncovalent binding interactions between molecules and nanotubes. The results are important to the supramolecular chemistry of nanomaterials and potential applications such as pH sensing.

By simple sonication of as-grown SWNTs in an aqueous solution of **1** followed by centrifugation to remove large impurities and dialysis of the supernatant to remove free molecules (see Supporting Information), we obtained SWNTs (average length ∼150 nm, Figure 1b) stably suspended in water by physisorbed Fluor-PEG (Figure 1c). The hydrophobic aromatic fluorescein group binds to the sidewall of SWNTs (likely via π-stacking) while the PEG group extends into water. The nanotube suspension was stable in water without aggregation even after heating to 70 °C for 2 days (Figure 1c). High stability was also observed in cell culture medium containing 10% fetal bovine serum and ∼150 mM salt (Figure 1c), suggesting strong binding of Fluor-PEG on SWNTs.

We investigated the optical absorbance and fluorescence characteristics of fluorescein bound to SWNTs in phosphate buffered saline (PBS) at pH 7.4 (note: free unbound fluorescein in all of our SWNT suspensions were removed by dialysis). UV−vis−NIR spectra clearly revealed an absorbance peak (at ∼497 nm) of fluorescein bound to SWNTs (Figure 2a in which the background spectrum with small peaks was due to SWNTs), and the peak was red-shifted (by ∼3 nm) relative to free fluorescein. The number of Fluor-PEG per tube (average length ∼150 nm) was estimated to be ∼90 with a ∼12% coverage of the SWNT sidewall area (see Supporting Information Figure S1). We observed ∼67% quenching of the fluorescence of SWNT-bound fluorescein relative to free fluorescein at the same concentration (495-nm excitation) (Figure 2b) due to interactions between fluorescein and SWNT. Similar fluorescence quenching was reported for SWNT-bound pyrene due to energy transfer.[4,5] The ∼67% quenching effect was observed for various SWNT concentrations up to 10 nM with fluorescein concentrations up to ∼900 nM (Figure 2c).

The absorbance (at 490 nm) and fluorescence of fluorescein are known to increase at higher pHs under higher degrees of deprotonation and saturate around pH ≈ 8.[6−8] Although a similar trend

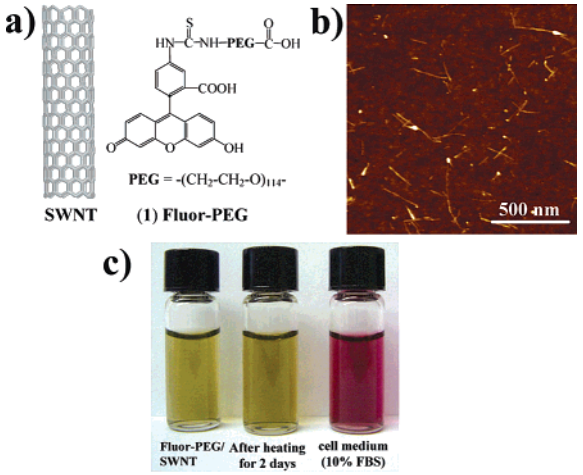

Figure 1. Fluor-PEG-functionalized SWNTs. (a) Schematic showing SWNT and Fluor-PEG (**1**). (b) Atomic force microscopy image of Fluor-PEG/SWNTs deposited on substrate. (c) Photo of Fluor-PEG/SWNT in water (left, yellow-green color due to SWNT-bound Fluor), after heating at 70 °C for 2 days (center), and in cell culture medium supplemented with 10% serum (right, red color due to cell medium). Unbound Fluor-PEG was dialyzed in the starting solution.

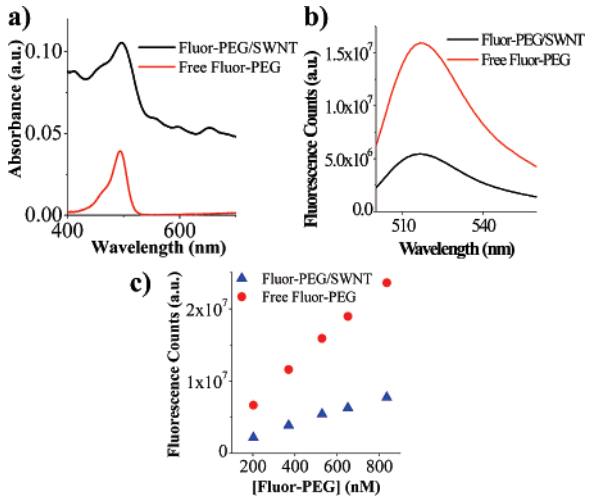

Figure 2. Optical properties of SWNT-bound Fluor-PEG. (a) Absorbance of Fluor-PEG/SWNT and free Fluor-PEG. (b) Corresponding fluorescence emission spectra. (c) Emission peak intensity of SWNT bound Fluor-PEG and free Fluor-PEG in a fluorescein concentration range of 200−900 nM.

was observed for fluorescein bound to SWNTs under increasing pH, no saturation was seen even up to pH ≈ 12 (Figure 3a,c,d). No significant pH dependence was observed in the absorbance (Figure 3b) and photoluminescence (Figure S3) of our SWNTs, in contrast with SWNTs solubilized by surfactants with charged groups and without PEGylation.[9,10] This difference is currently not

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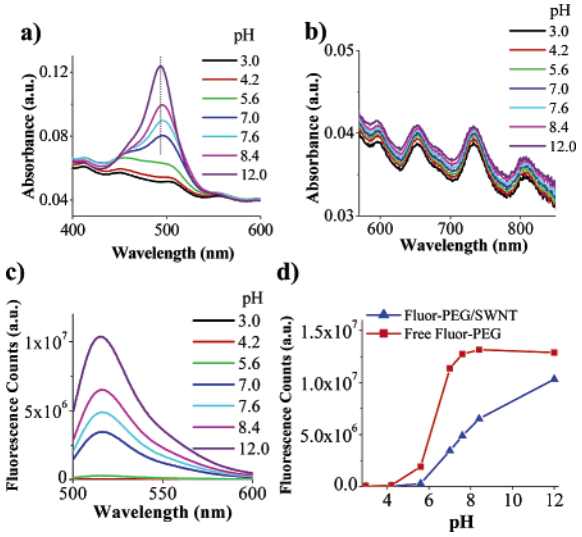

Figure 3. pH-dependent fluorescence and absorbance of Fluor-PEG/SWNT. Absorption curves of Fluor-PEG/SWNT at various pHs in the (a) fluorescein region (dashed line marks the peak at pH ≈ 12) and (b) SWNT region (curves are displaced for clarity). (c) Corresponding fluorescence emission spectra ($\lambda_{excitation}$ = 495 nm). (d) Comparison of emission peak intensity dependence on pH of free Fluor-PEG and Fluor-PEG/SWNT with the same Fluor-PEG concentration.

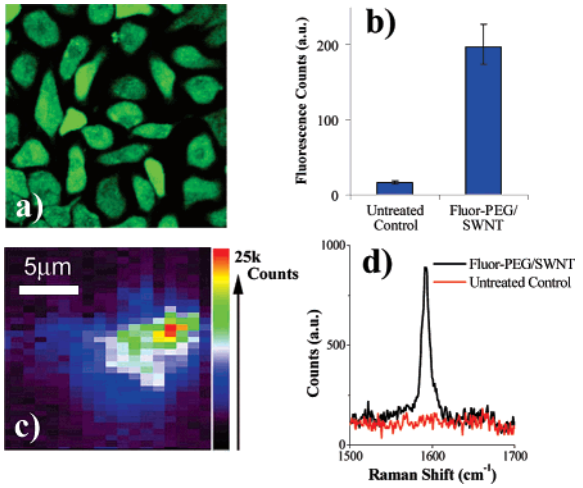

Figure 4. Cellular uptake of Fluor-PEG/SWNT. (a) Confocal fluorescence image of BT474 cells incubated with Fluor-PEG/SWNT. (b) Fluorescence intensities of large population of cells treated with Fluor-PEG/SWNT vs untreated control cells by flow cytometry. (c) Micro-Raman image of the cells incubated with Fluor-PEG/SWNT. (d) Representative Raman spectra showing the presence and absence of the SWNT G-band from Fluor-PEG/SWNT-incubated cells and untreated control cells, respectively.

understood but could be due to the different functionalization schemes used. The absorbance peaks of SWNT-bound fluorescein exhibited monotonic blue-shift (Figure 3a) at higher pHs, approaching the characteristics of free fluorescein (i.e., reversing the effects of fluorescein−SWNT binding). This suggested that the increase in pH led to weakening of the interaction between fluorescein and SWNT, reducing fluorescence quenching, and shifting the spectral peaks back to near that of free fluorescein. At pH 12, we observed slow but noticeable precipitation of Fluor-PEG/SWNTs from the solution after standing for ∼48 h while no precipitates were seen in lower pH solutions (see Supporting Information. Fig. S 2). This confirmed weakened interaction between fluorescein and SWNT at higher pHs, causing Fluor-PEG desorption over time. The mechanism of reduced interaction at higher pHs is not yet fully understood, but we speculate that higher pH and deprotonation impart higher hydrophilicity to fluorescein and thus higher affinity for water solvation. The monotonic increase of Fluor-PEG/SWNT fluorescence without saturation at pH∼8 expected for free fluorescein could also be due to delayed deprotonation of fluorescein when bound to SWNT, or competition of hydroxyl ion with (**1**) for SWNT binding, though further work is needed to fully understand the various possible mechanisms.

Our simple functionalization imparts solubility to nanotubes in physiological buffers and simultaneously affords fluorescent labels. The fluorescence intensity of fluorescein on SWNT is ∼33% of free fluorescein, which can still be utilized in chemical and biological settings. To this end, we investigated the cellular uptake of Fluor-PEG/SWNT and used fluorescence detections for characterization. After incubation of BT474 breast cancer cells in a solution of Fluor-PEG/SWNT, we observed fluorescein signals within cells by confocal fluorescence microscopy (Figure 4a) and flow cytometry (or fluorescence activated cell sorting (FACS), Figure 4b). Further, owing to the strong resonance Raman signatures of SWNTs, we used the G-band Raman peak at ∼1600 cm^{-1}, characteristic of graphitic stretching mode, to directly probe SWNTs in live cells. A micro-Raman image formed by spatially mapping out the G-band-integrated intensity showed the existence of SWNTs at the cells (Figure 4c,d), and the co-localization with fluorescein fluorescence suggested the cellular uptake of Fluor/SWNT conjugates, similar to internalization of other molecular complexes with nanotubes.[11,12] Control cells without exposure to Fluor-PEG/SWNT showed minimal fluorescence and SWNT G-band Raman signal (Figure 4b,d).

We have used PEGylated fluorescein to noncovalently functionalize SWNTs to obtain aqueous stable conjugates with pH-dependent optical properties. We showed that the finite fluorescence intensity of fluorescein-PEG/SWNT can be utilized for detection, imaging and cell sorting in biological applications. Last, since the terminal group of Fluor-PEG on the SWNT conjugate is a carboxylic acid (Figure 1a), one can envision conjugation of various molecules to the conjugates to impart further chemical or biological functionalities.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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