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# Detection of phospholipid-carbon nanotube translocation using fluorescence energy transfer

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Single-walled carbon nanotubes (SWNTs) and lysophospholipids readily assemble into supramolecular complexes in aqueous solutions. Upon light excitation the fluorescence of rhodamine-labeled lysophospholipids was redshifted and quenched due to the optical absorption of the SWNTs. Utilizing fluorescence energy transfer, the authors detected the translocation and disassembly of SWNT complexes in MCF breast cancer cells. These lipid-coated SWNT complexes enable drugs to be delivered at an effective dose and their subsequent release to be monitored in real time. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2360228]

Single-walled carbon nanotubes (SWNTs) are mosaics of carbon atoms synthesized into single tubules.[1] The simple and plain structures of SWNTs constitute one of the most important classes of nanomaterials owing to their unsurpassed stiffness, easy accommodation of chemical and biological versatilities, and rich optical and quantum electronic properties.[2–7] Toward nanomedicine, an emerging field of utilizing nanomaterials for medicinal benefits, SWNTs have been transformed into substrates for detecting antibodies associated with human autoimmune diseases with high specificity[8] and carriers of contrast agent aquated $Gd^{3+}$-ion clusters for greatly enhanced magnetic resonance imaging.[9] When covalently or noncovalently attached by nucleic acids (DNA or RNA including short-stranded RNA for gene silencing),[10–13] vaccines,[14] and proteins,[15,16] SWNTs have been shown as effective gene and drug transporters.[12,17,18]

Isolated SWNTs have been recently reported as long-term fluorescent tags for cellular imaging and sensing.[19,20] These applications are inspired based on the understanding that semiconducting SWNTs have very large spectral shifts between excitation and emission wavelengths when they are excited at second van Hove absorption transitions (typically between 500 and 900 nm) and detected through first van Hove emission (typically between 800 and 1600 nm).[21] Recent photophysical studies have found that SWNTs can act collectively as quenchers for their covalently tethered and/or $\pi$-stacked pyrenes and chromophores.[22–25] These phenomena were attributed to electron transfer or energy transfer from the photoactive tethers to the SWNTs, resulting from their tight binding as well as the broad absorption spectrum of SWNTs. In this letter, we show that lysophospholipid 1,2-dipalmitoyl-*sn*-glycero-3-lysophosphoethanolamine-N-(Lissamine rhodamine B sulfonyl), or Rd-LPE, solubilizes SWNTs in aqueous solution via pure hydrophobic interactions and that these self-assembled supramolecular complexes, once excited, readily undergo fluorescence energy transfer from the Rd-LPE to the SWNTs. Utilizing this energy transfer we show sensitive detection of the translocation of lipid-SWNT assembly and the dissociation of Rd-LPE from SWNTs in cells. This study advances our understanding of cellular responses to nanomaterials and facilitates the development of supramolecular nanodevices.

Lipid Rd-PE were purchased from Avanti. Our previous study[26] found that lysophospholipids or single-tailed phospholipids could self-assemble into highly organized striations on SWNTs [illustration see Fig. 1], rendering SWNTs biocompatible and a SWNT solubility significantly higher than that offered by nucleic acids, proteins, and surfactants such as sodium dodecyl sulfate. We envisioned that Rd-LPE, a lysophospholipid functionalized by a rhodamine dye, would yield satisfactory SWNT solubility due to its conical shape[26] and its amphiphilic structure. To obtain Rd-LPE, a cleavage procedure[27] was performed to remove one fatty acyl tail of the Rd-PE using enzyme phospholipase A2 (Sigma), which hydrolyzes the acyl group attached to the SN2 carbon of Rd-PE. At the conclusion of the digestion, four products remained: Rd-LPE, fatty acyl tails, undigested Rd-PE, and free PE head groups. These groups were separated by thin layer chromatography (TLC).

The pink band containing the Rd-LPE scrapped off the TLC plate, placed into an Eppendorf tube, dissolved with

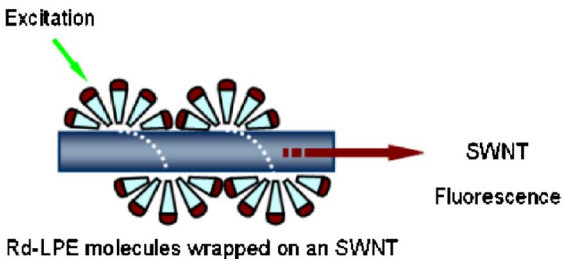


FIG. 1. (Color online) Scheme of Rd-LPE ("cones" with red heads and blue tails) bound to a SWNT ("gray cylinder"). Rd-LPE molecules self-assemble around the SWNT forming striations as described in Ref. 26.

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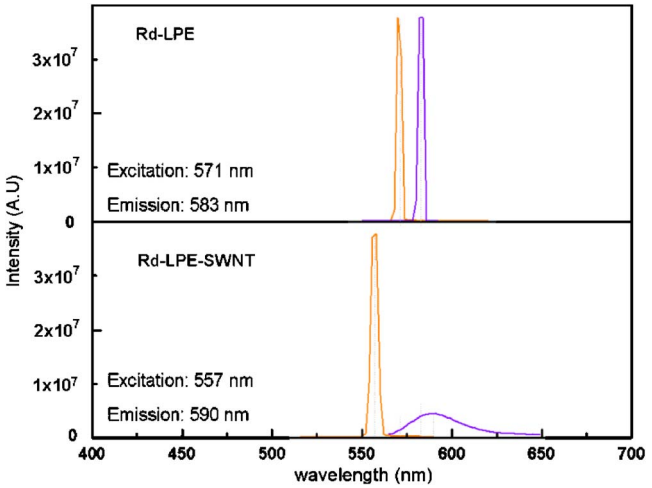


FIG. 2. (Color online) Energy transfer from Rd-LPE to SWNTs. A sharp decrease and broadening of the emission peak (purple) is observed in the bottom panel when compared to the data depicted in the top panel (absorption in orange).

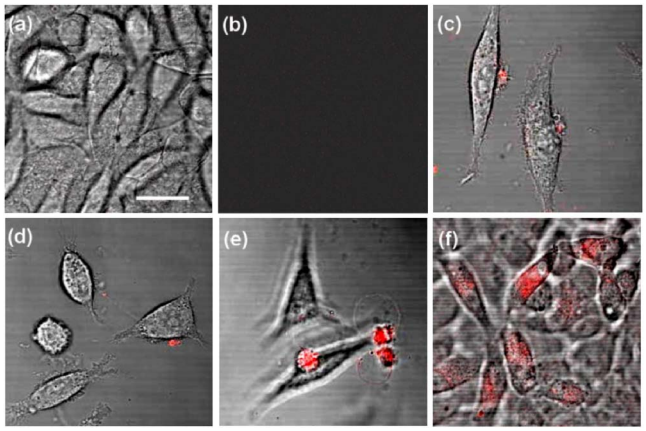


FIG. 3. (Color online) (a) Image of control 1 without the use of SWNTs. (b) Image of control 2 without the presence of MCF7 cells. Images (c)–(f) show increased translocation of Rd-LPE across MCF7 cells with incubation times of 0.5, 1, 2, and 3 h. The red spots in the images suggest Rd-LPE were dissociated from SWNTs. Scale bar: 10 $\mu$m.

chloroform/methanol solvent (50:50), vortexed, and then centrifuged for 5 min at 10 000 rpm. The supernatant was extracted and placed in an Eppendorf tube to dry under inert gas. This process was repeated until the silica was no longer pink, indicating the successful removal of most Rd-LPE lipids from silica scrapings. Note that a small sample from each of the four bands observed from TLC was removed and run through a mass spectrometer (Q-Tof micro™) to confirm substances present. After being completely dried, SWNTs (arc deposition, diameter ~1.4 nm) were added to the Rd-LPE at approximately a weight ratio of 1:5. Then 300 $\mu$l of water was added and the solution was probe sonicated (VC 130 PB, Sonics & Materials) at 8 W for 15 min. The solution was then centrifuged for 5 min at 13 000 rpm and the supernatant then disposed off to remove the free lipids and SWNT catalysts.

As expected, the mixing of SWNTs and Rd-LPE produced a stable and homogeneous solution. We noticed a concomitant color change of the solution from pink to gray once SWNTs were added to Rd-LPE. This could be due to the occurrence of fluorescence resonance energy transfer (FRET), a physical process resulting from induced dipole-induced dipole interactions at a close proximity (<10 nm).[28,29] To confirm this, a spectrofluorometer (QM-8/2005, PTI, resolution <1 nm) was used to measure the fluorescence emission of Rd-LPE and Rd-LPE-SWNT, as shown in the top and bottom panels of Fig. 2, respectively. The absorption and emission peaks for Rd-LPE were at 571 and 583 nm. In contrast, the absorption peak for Rd-LPE-SWNT was blueshifted to 557 nm and the emission peak was redshifted to 590 nm. The quenching of rhodamine fluorescence was also observed for dried Rd-LPE-SWNT sample indicating the stable binding of Rd-LPE and SWNTs without water. By adding chloroform or other amphiphiles to Rd-LPE-SWNT solution, we could remove Rd-LPE from SWNT surfaces and consequently observe the recovery of rhodamine fluorescence (data not shown).

The fluorescence quenching and recovery of Rd-LPE upon binding to and dissociation from SWNTs can be utilized for sensing the translocation of SWNTs and their interactions with cell membranes and organelles, an area of great interest to researchers in integrating nanomaterials and biological systems. To explore this prospect we imaged the translocation of Rd-LPE-SWNT complexes across MCF7 breast cancer cells. The MCF7 cell line (ATCC) was cultured in DMEM with 1% penicillin streptomycin, 1% sodium pyruvate, and 10% fetal bovine serum. Approximately 10 000 MCF7 cells were seeded in each (200 $\mu$l) of an eight-chambered glass slide and allowed to attach overnight at 37 °C with 5% $CO_2$. Approximately 10 $\mu$l of 2 $\mu$g/$\mu$l of Rd-LPE-SWNT was then added into each chamber and incubated for 0.5, 1, 2, and 3 h, respectively. After incubation, the chambers were washed three times with 1×PBS and kept in PBS for imaging using a confocal fluorescence microscope (LSM510, Zeiss).

Two controls including Rd-LPE incubated with MCF7 cells for 3 h and Rd-LPE-SWNT without MCF7 cells were imaged. Our first control found no uptake of Rd-LPE without SWNTs [Fig. 3(a)]. Our second control without the addition of cells to chamber glass showed no fluorescence from Rd-LPE-SWNT complexes [Fig. 3(b)]. This observation reinforced the idea that fluorescence was quenched when Rd-LPE molecules were assembled onto a SWNT. The time course study in Figs. 3(c)–3(f) demonstrates increased rhodamine fluorescence with incubation time. In particular, the red spots appearing on the peripheries of the cell membranes in Figs. 3(c)–3(e) suggest that some Rd-LPE molecules could be stripped off SWNTs when crossing cell membranes. The cells in Fig. 3(f) are largely fluorescent despite being imaged at various focal depths, indicating high translocation efficiency for 3 h incubation and the physical separation of SWNTs and Rd-LPE.

Micro-Raman spectroscopy was conducted to probe the presence of SWNTs in cells. In this experiment Rd-LPE was replaced by lysophosphotidylcholine or LPC to avoid fluorescence overwhelming much weaker Raman signals. The selection of LPC over LPE was because the former offers a SWNT solubility similar to that by Rd-LPE. SWNTs coated by LPC were incubated with MCF7 cells for 2 h, thoroughly washed with 1×PBS before being dried on silicon substrates prior to the measurement. Raman spectra (data not shown) were recorded for pristine SWNTs in isolated form (top), SWNT coated by LPC (middle), and SWNT-LPC incubated with MCF7 cells (bottom). *G* bands around 1590 $cm^{-1}$ were easily identified in all three cases, indicating the presence of

isolated SWNTs in all samples and confirming that SWNTs were indeed translocated into MCF7 cells.

The peak fluorescence intensity of Rd-LPE in Fig. 2 was more than eight times higher than that of Rd-LPE-SWNT due to strong fluorescence quenching by SWNTs. This phenomenon can be understood from the hypothesized mechanism of FRET where a Rd-LPE acted as a fluorescence donor and a SWNT as a fluorescence acceptor. Upon green light excitation, fluorescence emission of Rd-LPE (Fig. 2, top panel, in purple) is quenched or drained by the absorption of SWNTs due to their spectral overlap and their nanometric spatial proximity (a Rd-LPE molecule is ~2 nm in length). The blueshift of the absorbance peak (in orange) and the redshift of the emission (in purple) in Fig. 2 further suggest that, due to SWNT absorption, more photon energy is required for fluorescence excitation, while rhodamine emits photons of lower energy. The broadening of the Rd-LPE-SWNT emission spectrum was possibly due to the nonhomogeneity of isolated or small bundled SWNTs, as well as the varying physical distances between Rd-LPE molecules in striations and SWNT surfaces. The weak fluorescence emission of SWNTs was unobservable, overwhelmed by the fluorescence of free Rd-LPE in solution.

The controls in Figs. 3(a) and 3(b) indicate that SWNTs are necessary for the translocation of Rd-LPE. Without SWNTs, conical-shaped Rd-LPE might readily form micelles. Alternatively, while endocytosis[17,18] and passive ratchet diffusion[12] could play significant roles in the translocation of Rd-LPE-SWNT complexes, the hydrophobic moieties of exposed/uncoated SWNTs could prompt interactions with the amphiphilic cell membranes leading to translocation. SWNTs may also affect the electrochemical properties of lipid membranes and the organization of the membrane lipid matrix by varying the lipid/membrane microviscosity and rigidity, and therefore facilitating the translocation of Rd-LPE or other drugs into cells. The exchange of Rd-LPE with lipid bilayers of cell membranes needs to be explored for potential applications in molecular signaling.

We speculate that the strong fluorescence seen in Fig. 3(f) is emitted by Rd-LPE molecules dissociated from SWNTs after their translocation across MCF cell membranes. This scheme simulates the ready release of drug loads of their noncovalently bound SWNT transporters and shows promise for developing alternative gene and drug delivery using SWNTs. We noticed that the fluorescence in Fig. 3(f) was nonuniformly distributed inside cells while being mostly kept outside cell nuclei.

In summary, we have measured fluorescence energy transfer within supramolecular assembly of Rd-LPE-SWNT. The formation of Rd-LPE-SWNT complexes is realized by hydrophobic interactions in nature, and thus the mechanism of its energy transfer differs from that in the covalent or $\pi$-stacking schemes as previously reported.[22–25] This robust energy transfer has been utilized for the detection of SWNT biomolecular complexes in MCF cells. The lack of rhodamine fluorescence in extracellular space suggests the high binding efficiency and stability of Rd-LPE-SWNT complexes prior to their translocation. In contrast, the presence of strong rhodamine fluorescence in intracellular space simulates the simple/efficient release of drug loads (Rd-LPE in this case) from SWNT transporters, a feature unavailable from covalent methods and is desirable for gene and drug delivery. Based on our hypothesized FRET mechanism, the nonuniform distribution of rhodamine fluorescence inside cells is understood as SWNTs located beyond proximity (>10 nm) from the Rd-LPE molecules. Since lysophospholipids, lysophosphatidic acid, and sphingosine 1-phosphate can be used as receptors for signal transduction[30] and the head groups of lysophospholipids offer great versatility in biofunctionalization of genes and drugs, our method provides an optical indicator for detecting the real-time interaction of SWNTs and biomolecules and shows the promise of using SWNTs as energy quenchers for biosensing and nanomedicine.

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