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Luminescent Rare-Earth Complex Covalently Modified Single-Walled Carbon Nanotubes: Design, Synthesis, and DNA Sequence-Dependent Red Luminescence Enhancement

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A novel luminescent Eu^{3+}-complex functionalized single-walled carbon nanotube (SWNT) was constructed by covalent linkage through a diaminotriethylene glycol linker. TGA, FT-IR, and SEM demonstrated successful attachment of the Eu^{3+}-complex onto the SWNT surface. Spectroscopic methods showed that the SWNT-Eu^{3+} complex is highly luminescent and DNA can further enhance the red luminescence, and the enhancement depends on DNA sequence and form. The order of the enhancement follows: AT alternative dsDNA > nonalternative AT dsDNA > GC dsDNA > ssDNA dA > ssDNA dT > ssDNA $(GT)_{20}$.

Introduction

Since carbon nanotube (CNT) was discovered[1] by Iijima in 1991, design, synthesis, and application of functionalized CNT have attracted much attention and significant progress has been made.[2] CNTs have shown great potential applications ranging from gene therapy to novel drug delivery, biosensing and clinical diagnosis.[3] To this end, water-soluble, visible fluorescent CNTs are suitable candidates because they can be easily tracked in situ diagnosis or monitored their gene or drug delivery path way in vivo. However, since CNT itself has only a weak infrared fluorescence,[4] much effort has been devoted for developing fluorescent-labeled CNTs either by noncovalent or covalent modifications.[5] Fluorescent organic dye molecules, such as pyrene and porphyrin, are often used for labeling. However, previous studies have demonstrated that most organic compounds have short fluorescence lifetime, and more importantly, their fluorescence are strongly quenched due to photoinduced electron transfer or energy transfer to the nanotube when organic fluorophores are covalently or noncovalently attached to the nanotube surface.[5] It is well-known that rare-earth compounds have been widely used as laser materials, optoelectronic devices and as fluorescence probes in immunoassays.[6] This is because the 4f orbitals of rare-earth elements are shielded by 5s5p6s orbitals, rare-earth compounds exhibit unique spectroscopic characteristics, such as long luminescence lifetime, large Stoke's shift and sharp line-like atomic emission.[7] These features can overcome autofluorescence and light scattering that are often associated with commonly used

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(1) Iijima, S. *Nature* **1991**, *354*, 56–58.
(2) (a) Dresselhaus, M. S.; Dresselhaus, G.; Eklund, P. C. *Science of Fullerenes and Carbon Nanotubes*; Academic Press: San Diego, 1996. (b) Ebbesen, T. W. *Carbon Nanotubes: Preparation and Properties*; CRC Press: Boca Raton, FL, 1997. (c) Yakobson, B. I.; Smalley, R. E. *Am. Sci.* **1997**, *85*, 324–337. (d) Ajayan, P. M. *Chem. Rev.* **1999**, *99*, 1787–1800. (e) Liu, J.; Rinzler, A. G.; Dai, H.; Hafner, J. H.; Bradley, R. K.; Boul, P. J.; Lu, A.; Iverson, T.; Shelimov, K.; Huffman, C. B.; Rodriguez-Macias, F.; Shon, Y. S.; Lee, T. R.; Colbert, D. T.; Smalley, R. E. *Science* **1998**, *280*, 1253–1256.
(3) (a) Wong, S. S.; Joselevich, E.; Woolley, A. T.; Cheung, C. L.; Lieber, C. M. *Nature* **1998**, *394*, 52–55. (b) Zheng, M.; Jagota, A.; Strano, M. S.; Santos, A. P.; Barone, P.; Chou, S. G.; Diner, B. A.; Dresselhaus, M. S.; McLean, R. S.; Onoa, G. B.; Samsonidze, G. G.; Semke, E. D.; Usrey, M.; Walls, D. J. *Science* **2003**, *302*, 1545–1548. (c) Kam, N. W.; O'Connell, M.; Wisdom, J. A.; Dai, H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11600–11605. (d) Singh, R.; Pantarotto, D.; McCarthy, D.; Chaloin, O.; Hoebeke, J.; Partidos, C. D.; Briand, J. P.; Prato, M.; Bianco, A.; Kostarelos, K. *J. Am. Chem. Soc.* **2005**, *127*, 4388–4396. (e) Lin, Y.; Taylor, S.; Li, H. P.; Fernando, K. A. S.; Qu, L. W.; Wang, W.; Gu, L. R.; Zhou, B.; Sun, Y. P. *J. Mater. Chem.* **2004**, *14*, 527–541.
(4) (a) O'Connell, M. J.; Bachilo, S. M.; Huffman, C. B.; Moore, V. C.; Strano, M. S.; Haroz, E. H.; Rialon, K. L.; Boul, P. J.; Noon, W. H.; Kittrell, C.; Ma, J.; Hauge, R. H.; Weisman, R. B.; Smalley, R. E. *Science* **2002**, *297*, 593–596. (b) Lefebvre, J.; Austing, D. G.; Bond, J.; Finnie, P. *Nano Lett.* **2006**, *6*, 1603–1608.
(5) (a) Guldi, D. M.; Rahman, G. M.; Jux, N.; Tagmatarchis, N.; Prato, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 5526–5530 and cited references. (b) Baskaran, D.; Mays, J. W.; Zhang, X. P.; Bratcher, M. S. *J. Am. Chem. Soc.* **2005**, *127*, 6916–6917 and cited references.
(6) (a) Maas, H.; Currao, A.; Calzaferri, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 2495–2497. (b) Enomoto, K.; Nagasaki, T.; Yamauchi, A.; Onoda, J.; Sakai, K.; Yoshida, T.; Maekawa, K.; Kinoshita, Y.; Nishino, I.; Kikuoka, S.; Fukunaga, T.; Kawamoto, K.; Numata, Y.; Takemoto, H.; Nagata, K. *Anal. Biochem.* **2006**, *351*, 229–240. (c) Yu, J.; Parker, D.; Pal, R.; Poole, R. A.; Cann, M. J. *J. Am. Chem. Soc.* **2006**, *128*, 2294–2299. (d) Comby, S.; Imbert, D.; Vandevyver, C.; Bunzli, J. C. *Chem.—Eur. J.* **2007**, *13*, 936–944.
(7) (a) Hanaoka, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 2996–2999. (b) Viguier, R. F.; Hulme, A. N. *J. Am. Chem. Soc.* **2006**, *128*, 11370–11371. (c) Massue, J.; Quinn, S. J.; Gunnlaugsson, T. *J. Am. Chem. Soc.* **2008**, *130*, 6900–6901. (d) Tsukube, H.; Shinoda, S. *Chem. Rev.* **2002**, *102*, 2389–2403. (e) Kupcho, K. R.; Stafslien, D. K.; DeRosier, T.; Hallis, T. M.; Ozers, M. S.; Vogel, K. W. *J. Am. Chem. Soc.* **2007**, *129*, 13372–13373. (f) Hanaoka, K.; Kikuchi, K.; Kobayashi, S.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 13502–13509.

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fluorophores employed in chemical biology. Recently, Shi, Wei, and Prato and co-workers[8] successfully prepared luminescent rare-earth compound modified CNTs by noncovalent adsorption. For in situ diagnosis or monitoring of drug delivery path way in vivo, the noncovalent rare-earth compound modified CNTs will be challenged by adsorption stability and desquamation of rare-earth compounds from CNT surface. This would decrease luminescence and cause the uncertainty when used in vivo. Besides, noncovalent adsorption still suffers from quenching luminescence. Therefore, covalent modification by linker molecule can improve luminescent stability and avoid direct quenching of CNTs.

Herein, we design and synthesize europium (Eu^{3+}) complex covalently modified single-walled carbon nanotube (SWNT), which can emit strong red luminescence upon excitation of the antenna molecule. Since SWNT has been used as gene carries, we studied how luminescence change when this novel luminescent SWNT binding to DNA. DNA can further enhance the red luminescence, and the enhancement depends on DNA sequence and form. Double-stranded DNA (dsDNA) has stronger enhancement than single-stranded DNA (ssDNA). AT alternative dsDNA shows the strongest enhancement while ssDNA $(GT)_{20}$ has the least effect. The order of the enhancement follows: AT alternative dsDNA > nonalternative AT dsDNA > GC dsDNA > ssDNA dA > ssDNA dT > ssDNA $(GT)_{20}$. Since rare-earth elements have similar characteristics, the design presented in this report can be general for preparation of luminescent rare-earth compound covalently modified SWNT. Luminescent SWNT as a potential gene or drug carrier, its visible strong luminescence may provide a probe for monitoring the delivery path way in vivo and for biosensing.

Experimental Section

SWNTs were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). 2,2′-Bipyridine-4,4′-dicarboxylic acid (Bpc), 1-(2-naphthoyl)-3,3,3-trifluoroacetone (TFAcAcN), di-tert-butyl-bicarbonate ($(Boc)_2O$), and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) were purchased from Alfa Aesar. Sulfo-*N*-hydroxy succinimide (Sulfo-NHS) was purchased from Pierce. 1,2-Bis(2-aminoethoxy)ethane was obtained from TCI-EP (Tokyo, Japan). 2-[N-morpholino] ethanesulfonic acid (MES) was purchased from Sigma. CH_2Cl_2, $CHCl_3$, and THF were freshly distilled from CaH_2. The others are of analytical or biochemical grade reagents and used as received.

DNA oligomers (Table S1, Supporting Information) were purchased from Sangon (Shanghai, China) and used without further purification. Concentrations of these oligomers were determined by measuring the absorbance at 260 nm. Extinction coefficients were estimated by the nearest-neighbor method by using mononucleotide and dinucleotide values.[9] Before the sensing experiment, $(A\cdot T)_{17}$, $(G\cdot C)_{17}$, and $A_{22}\cdot T_{22}$ were annealed at 90 °C for 20 min and then slowly cooled to room temperature.

2.1. Preparation of Oxidized SWNT (f-SWNT (1)) (See Scheme S1, Supporting Information). One hundred milligrams of pristine SWNT was suspended in 200 mL of a 3:1 (v/v) concentrated H_2SO_4 (98%) and HNO_3 (70%) solution by sonicating at 35−40 °C for 5 h.[9] The reaction mixture was diluted to 1 L with deionized water, and a black precipitate was obtained by centrifugation. The product was then washed extensively by water until the pH reached neutral conditions. The resulting SWNT was resuspended in 150 mL of 2.6 M HNO_3 and sonicated for 30 min. The mixture was refluxed for about 24 h at 140 °C. The resultant suspension was then diluted by deionized water, filtered through a 0.22 μm polycarbonate membrane, and rinsed thoroughly with deionized water several times until the pH was ∼7.0. The black product obtained was further oven-dried at 50 °C under vacuum and characterized by SEM, FT-IR, and TGA.

2.2. Preparation of Boc-NH$(CH_2CH_2O)_2CH_2CH_2NH_2$. Di-tert-butyl bicarbonate (0.01 mol) dissolved in 50 mL of $CHCl_3$ was added dropwise to a solution of 0.1 mol of 1,2-Bis(2-aminoethoxy)ethane in 100 mL of $CHCl_3$ during 3 h with stirring and cooling in an ice bath. The reaction mixture was stirred for additional 16 h at room temperature and was then washed with 8 × 50 mL of water. The organic phase was dried over Na_2SO_4, evaporated to dryness in vacuo, and obtained as colorless oils.

2.3. Preparation of Amine Modified-SWNT (f-SWNT (4)) (See Scheme S1, Supporting Information). Fifty milligrams of f-SWNT (**1**) were stirred in 20 mL of $SOCl_2$ (containing 0.5 mL of DMF) at 70 °C for 24 h. After centrifugation, the brown-colored supernatant was decanted and the remaining solid was washed with anhydrous THF. After centrifugation, the pale yellow-colored supernatant was decanted. The remaining solid was dried at room temperature under vacuum, giving acyl chloride-modified SWNT (f-SWNT (**2**)).

A mixture of 25 mg of f-SWNT (**2**) and 2 mL of Boc-NH$(CH_2CH_2O)_2CH_2CH_2NH_2$ in 10 mL of dry THF was heated at ∼100 °C for 96 h. After the mixture was cooled to room temperature, solvent was evaporated under vacuum. After it was washed with ethanol four times (5 to 10 min sonication), the remaining solid was dissolved in diethyl ether to remove excess protected amine. After some time, the suspension was centrifuged and the black precipitate was separated out. The resulting Boc-protected amine-modified SWNT (f-SWNT (**3**)) was dried at room temperature under vacuum.

A 20 mg portion of f-SWNT (**3**) was treated with 6 mL of TFA/CH_2Cl_2 (1:1) and then stirred at room temperature for 2 h to cleave the Boc group at the chain-end. The solvent was evaporated in vacuo. The crude product was purified by centrifugation-precipitation, using methanol/ether 5 times (12 mL × 4 and 3 mL × 1) to thoroughly remove organic reactants,[10] and subsequently dried under vacuum. The resulting f-SWNT (**4**) was characterized by SEM, FT-IR, and TGA.

(8) (a) Shi, D. L.; Lian, J.; Wang, W.; Liu, G. K.; He, P.; Dong, Z. Y.; Wang, L. M.; Ewing, R. C. *Adv. Mater.* **2006**, *18*, 189–193. (b) Wei, X. W.; Xu, J.; Song, X. H.; Ni, Y. H.; Zhang, P.; Xia, C. J.; Zhao, G. C.; Yang, Z. S. *Mater. Res. Bull.* **2006**, *41*, 92–98. (c) Accorsi, G.; Armaroli, N.; Parisini, A.; Meneghetti, M.; Marega, R.; Prato, M.; Bonifazi, D. *Adv. Funct. Mater.* **2007**, *17*, 2975–2982.

(9) (a) Li, X.; Peng, Y. H.; Ren, J. S.; Qu, X. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19658–19663. (b) Li, X.; Peng, Y. H.; Qu, X. G. *Nucleic Acids Res.* **2006**, *34*, 3670–3676. (c) Zhao, C.; Ren, J. S.; Qu, X. G. *Chem.—Eur. J.* **2008**, *14*, 5435–5439. (d) Zhao, C.; Song, Y. J.; Ren, J. S.; Qu, X. G. *Biomaterials* **2009**, *30*, 1739–1745. (e) Zhao, C.; Peng, Y. H.; Song, Y. J.; Ren, J. S.; Qu, X. G. *Small* **2008**, *4*, 656–661.

(10) (a) Wu, W.; Wieckowski, S.; Pastorin, G.; Benincasa, M.; Klumpp, C.; Briand, J. P.; Gennaro, R.; Prato, M.; Bianco, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 6358–6362. (b) Singh, R.; Pantarotto, D.; Lacerda, L.; Pastorin, G.; Klumpp, C.; Prato, M.; Bianco, A.; Kostarelos, K. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 3357–3362. (c) Chen, J. Y.; Chen, S. Y.; Zhao, X. R.; Kuznetsova, L. V.; Wong, S. S.; Ojima, I. *J. Am. Chem. Soc.* **2008**, *130*, 16778–16785.

2.4. Preparation of Bipyridine-Modified SWNT (f-SWNT (5)) (See Scheme S1, Supporting Information). Ten milligrams of f-SWNT (**4**) was dissolved in 2 mL of water containing 5% DMF. A solution of 2,2′-bipyridine-4,4′-dicarboxylic acid (Bpc, 20 mg) in 2 mL of 100 mM MES (pH 6.0) was activated with EDC (20 mM) and sulfo-NHS (20 mM) for 1 h. Following the activation step, the pH was raised to 8.0, and subsequently added to the f-SWNT (**4**) solution. The resulting mixture was stirred for 24 h at room temperature, and washed with water by centrifugation. The black precipitated was redissolved in DMSO and filtered through a 0.22 μm polycarbonate membrane. This procedure was repeated two times each with DMSO and CH_2Cl_2/MeOH (10:1) mixture to remove the excess of Bpc. After the final washings with diethyl ether, the black solid was dried under a vacuum,[11] giving f-SWNT (**5**).

2.5. Synthesis of Reference Eu Complex ($Eu(TFAcAcN)_3Bpc$) (See Scheme S2a, Supporting Information). $Eu(TFAcAcN)_3Bpc$ was synthesized according to the conventional route[12] (Scheme S2, Supporting Information) as follows: 1-(2-Naphthoyl)-3,3,3-trifluoroacetone (TFAcAcN, 160 mg, 0.6 mM) and 2,2′-bipyridine-4,4′-dicarboxylic acid (Bpc, 49 mg, 0.2 mM) were dissolved in ethanol (10 mL) under stirring at room temperature. Then five drops of 2 M NaOH were added to adjust the pH level to 8.0. Afterward a $EuCl_3$ (51 mg, 0.2 mM) solution in ethanol (3.5 mL) was added dropwise. After complete addition, the solution was stirred for 1 h to ensure a complete precipitation at room temperature. The precipitate was filtered out, washed repeatedly with ethanol and water and then dried overnight in vacuum.

2.6. Synthesis of Eu Complex-Modified SWNT (f-SWNT (6)) (See Scheme S1, Supporting Information). First, a precursor complex $Eu(TFAcAcN)_3(H_2O)_2$ was prepared according to the standard procedure. In general, the synthesis was very similar to the method described above for $Eu(TFAcAcN)_3Bpc$ complex. The only difference was that no Bpc was added to the reaction mixture (Scheme S2b, Supporting Information).

The SWNT-containing complexes were obtained via ligand exchange reactions. $Eu(TFAcAcN)_3(H_2O)_2$ (5 mg) was codissolved with f-SWNT (**5**) (10 mg) in $CHCl_3$. To ensure completion of the reaction, the reagents were stirred for 24 h at room temperature. Then the solutions were concentrated in vacuum to dryness (on a rotary evaporator), the residues were washed with ethanol and dried in vacuum desiccator to yield f-SWNT (**6**). The resulting Eu^{3+}-complex functionalized nanotubes were characterized by SEM, FT-IR, and TGA.[9,13]

2.7. Physical Measurements. Thermogravimetric analyses (TGA) were performed using a Perkin-Elmer Pyris Diamond TG/DTA Instruments[13d] with a ramp of 10 °C/min under N_2 from 40 to 900 °C. FT-IR characterization was carried out on a BRUKE Vertex 70 FT-IR spectrometer using the potassium bromide (KBr) pellet method.[9] SEM images of f-SWNTs were obtained on a HITACHI S-4800 scanning electron microscope. Samples were prepared by pipetting 5 μL of colloid solution onto a silicon substrate pretreated with piranha etch solution (4:1 concentrated H_2SO_4/30% H_2O_2) for 1 h at room temperature. After evaporation of the solvent, the substrate was dried overnight under vacuum. UV−vis absorbance experiments[9,13] were carried out on a Cary 300 UV/vis spectrophotometer. All the spectra were measured in 1.0-cm-path-length cell at room temperature. Fluorescence measurements[13] were carried out on a JASCO FP-6500 spectrofluorometer. The fluorescence emission was monitored from 550 to 700 nm using an excitation wavelength of 372 nm; the fluorescence excitation was monitored from 220 to 500 nm using an emission wavelength of 615 nm. The slit width for the excitation and emission are of 5 nm.

2.8. Dna Luminescence Enhancement. The f-SWNT (**6**) was dissolved in DMSO at a concentration of 200 mg/L by sonicating for 30 min at room temperature in a water bath and briefly sonicated once again prior to use. Five microliters of the solutions were drawn out and diluted with 200 μL of Tris-HCl buffer (5 mM Tris-HCl 100 mM NaCl, pH 7.0) to a final concentration of 5 μg mL^{-1}. The resulting solutions were mixed with the target DNA ($(A\cdot T)_{17}$, $(G\cdot C)_{17}$, $T_{22}\cdot A_{22}$, $(GT)_{20}$, T_{22} and A_{22}) at varying concentrations from 0 to 2 μM. The mixture was allowed to react for 12 h at 4 °C and then fluorescence spectra were recorded. The control experiments using PAA and TMPyP4 instead of DNA at varying concentrations from 0 to 1.0 wt % and from 0 to 3.0 μM were also carried out under the same conditions.

2.9. Determination of DNA Binding Constants (K_b). The binding constants (K_b) of different DNA sequences with SWNT-Eu^{3+} complex were estimated from the luminescence titration curves.[13a,b,14] Luminescence titrations were carried out in Tris-HCl buffer (5 mM Tris-HCl, 100 mM NaCl, pH 7.0) and were titrated by different DNA. The excitation wavelength was 372 nm;, the emission spectrum was recorded from 550 to 700 nm at 20 °C. Emission intensity at 615 nm was used for data analysis.[13a,b,14]

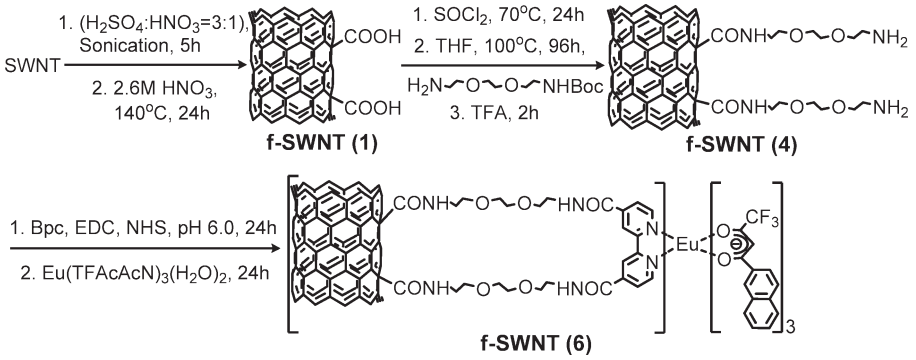
Results and Discussion

The basal unit of nanohybrid SWNT is composed of a carboxyl-modified SWNT, a luminescent Eu^{3+}-complex tag, and a diamine linker, that are linked together by the acylamide bonds (Scheme 1, f-SWNT (**6**)). In addition, 1,2-bis(2-aminoethoxy)ethane linker can also efficiently prevent SWNT luminescence quenching effect on Eu^{3+}-complex. The alignment of the units makes the SWNT emit red luminescence (Scheme 2).

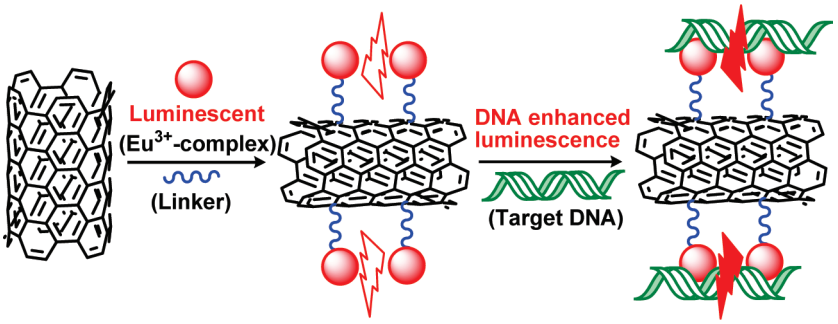
Luminescent Eu^{3+}-complex covalently modified SWNTs (f-SWNT **6**) were synthesized through the sequence of reactions (Scheme 1). First, purified and oxidized f-SWNT **1** was obtained after modification of previously reported procedures involving reaction of pristine SWNTs.[9] The strong acid treatment generates defects on the side walls and forms open ends that are both terminated by carboxylic acid groups.[9c−e] The loading (amount of functional groups) was calculated after derivatization of the carboxylic groups (activated as acid chlorides) with Boc-monoprotected diaminotriethylene glycol. The Boc group was removed with TFA solution to afford f-SWNT **4**. Then, 2,2′-bipyridine-4,4′-dicarboxylic acid (Bpc) was activated using standard coupling reagents and then condensed with f-SWNT **4** to afford f-SWNT **5** (Scheme S1, Supporting Information). Finally, Eu^{3+}-complex $Eu(TFAcAcN)_3(H_2O)_2$ (Scheme S2,

(11) Singh, P.; Kumar, J.; Toma, F. M.; Raya, J.; Prato, M.; Fabre, B.; Verma, S.; Bianco, A. *J. Am. Chem. Soc.* **2009**, *131*, 13555–13562.
(12) Fuchsbatter, A.; Troshina, O. A.; Troshin, P. A.; Koeppe, R.; Lyubovskaya, R. N.; Sariciftci, N. S. *Adv. Funct. Mater.* **2008**, *18*, 2808–2814.
(13) (a) Zhang, H. Y.; Yu, H. J.; Ren, J. S.; Qu, X. G. *Biophys. J.* **2006**, *90*, 3203–3207. (b) Zhang, H. Y.; Yu, H. J.; Xu, H. X.; Ren, J. S.; Qu, X. G. *Polyhedron* **2007**, *26*, 5250–5256. (c) Xu, H. X.; Zhang, H. Y.; Qu, X. G. *J. Inorg. Biochem.* **2006**, *100*, 1646–1652. (d) Zhang, H. Y.; Yu, H. J.; Ren, J. S.; Qu, X. G. *FEBS Lett.* **2006**, *580*, 3726–3730. (e) Peng, Y. H.; Song, Y. J.; Feng, L. Y.; Ren, J. S.; Qu, X. G. *J. Inorg. Biochem.* **2009**, *103*, 1675–1679.
(14) (a) Yu, H. J.; Ren, J. S.; Qu, X. G. *ChemBioChem* **2008**, *9*, 879–882. (b) Qu, X. G.; Chaires, J. B. *Methods Enzymol.* **2000**, *321*, 353–369.

Scheme 1. Synthetic Scheme for Luminescent, Eu^{3+}-Complex Covalently Modified SWNTs



Scheme 2. Schematic Representation of the Luminescent Eu^{3+}-Complex Covalently-Modified SWNT and Its Luminescence Enhanced by DNA



Supporting Information) was used as a precursor in the ligand exchange reactions with f-SWNT **5** to form the f-SWNT **6**.

The degree of functionalization for the f-SWNT **1**, **4**, **6**, and the reference Eu^{3+}-complex have been evaluated by widely used thermogravimetric analysis (TGA) under N_2 atmosphere (Figure 1). The TGA curves of the Eu^{3+}-complex and f-SWNT **6** show a gradual weight loss of about 41.2% and 58.7% at 900 °C, respectively, as compared to 92.6% of f-SWNTs **4** and 95.3% of the oxidized f-SWNT **1**, respectively. On the basis of weight loss, we can estimate that the degree of functionalization for f-SWNT **6** is of one Eu^{3+}-complex each, 48 carbon atoms.[11]

The infrared spectra[9e] (as KBr pellets) of f-SWNTs **1**, **4**, and **6** and Eu^{3+}-complex also confirmed covalent modifications that occurred. FT-IR spectrum of f-SWNT **4** shows two strong and broad absorption bands at 1577 and 1675 cm^{-1}, corresponding to the N−H bending and the C=O stretching of CONH, respectively (Figure 2). In all spectra, two weak signals around 2860 and 2920 cm^{-1} were detected and assigned to symmetric and asymmetric $-CH_2-$ stretching because of the diaminotriethylene glycol linker. Furthermore, the characteristic absorption bands at 686, 794, 1137, 1299, and 1533 cm^{-1} of Eu^{3+}-complex were also observed in the FT-IR spectrum of f-SWNT **6**, indicating the attachment of the Eu^{3+}-complex to SWNT surface.

f-SWNT **6** was also characterized by scanning electron microscopy (SEM); Figure 3 showed nanotubes with pendent complexes, and almost no independent Eu^{3+}-complex agglomerates were observed (Figure 3). The nanotube diameter of 15−20 nm suggested that the covalently modified Eu-complex caused the aggregation of the modified nanotubes. The attached complex not only increases the nanotube diameter, but also makes the nanotubes more likely aggregate into nanotube bundles for the existence of the aromatic groups. Furthermore, it was in sharp contrast with that of just a mixture of f-SWNT (**1**) and Eu^{3+}-complex (Figure S1a−h, Supporting Information), further indicating that f-SWNT (**6**) is covalently modified.

Spectroscopic properties of f-SWNT **1**, **4**, and **6** and Eu^{3+}-complex have also been investigated. Both f-SWNT **6** and Eu^{3+}-complex have strong absorbance at 350 nm (Figure 4). Their photoluminescence (PL) spectra in DMSO show the characteristic sharp emission lines in the red light emitting region that are typical for Eu^{3+} ions (Figure 5, left). There are four apparent bands in the PL spectra centered[7,13] at 579, 592, 615, and 652 nm. These lines are usually attributed to the $5D_0-7F_0$, $5D_0-7F_1$, $5D_0-7F_2$, and $5D_0-7F_3$ transitions in the Eu^{3+} ion. Meanwhile, there is no peak exist in f-SWNT **1** and **4**. Red PL is observable by naked eyes upon UV excitation (Figure 5, right). Therefore, a red PL rare-earth complex covalently modified SWNT has been designed and synthesized according to Scheme 1.

Since SWNTs have been used as gene carriers, here we studied how luminescence change when this novel luminescent SWNT binding to DNA. Intriguingly, DNA can enhance the luminescence[13] of the newly synthesized SWNT, and the enhancement is related to DNA sequence and double-stranded or single-stranded DNA. Luminescence titrations[13a,b,e] were conducted in the presence of

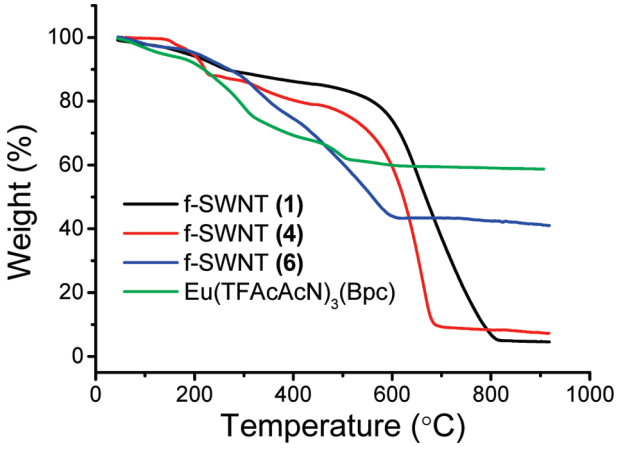


Figure 1. Thermogravimetric analysis for f-SWNT **1** (black), f-SWNT **4** (red), f-SWNT **6** (blue), and Eu(TFAcAcN)$_3$(Bpc) itself (green) in N_2 atmosphere with a ramp of 10 °C/min.

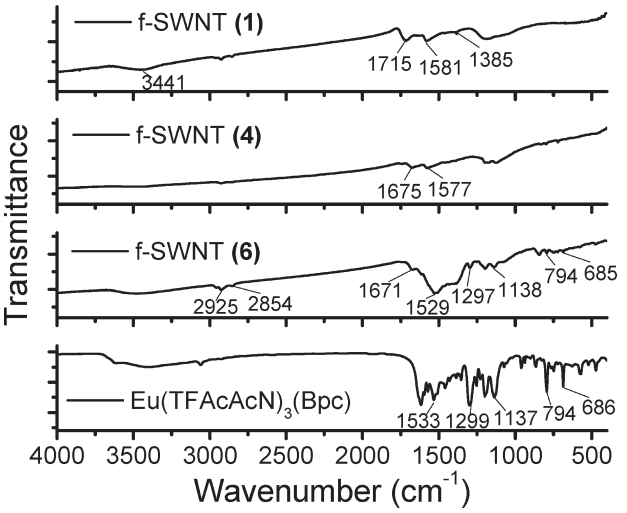


Figure 2. FT-IR spectra of (from top to bottom) f-SWNT **1**, f-SWNT **4**, f-SWNT **6**, and Eu(TFAcAcN)$_3$(Bpc) itself. The principle absorption features are highlighted with the correspondent wavenumbers.

SWNT-Eu^{3+} complexes at pH 7.0 titrated by different DNA, respectively. As shown in Figure 6a, when titrated by alternative AT double-stranded DNA $(A\cdot T)_{17}$, the luminescence intensity of rare-earth complex covalently modified SWNT was highly sensitive to $(A\cdot T)_{17}$ DNA and increased with increasing $(A\cdot T)_{17}$ DNA concentration.[13] Figure 6b shows the correlation between the emission intensity at 615 nm and the concentration of $(A\cdot T)_{17}$ DNA in the range from 0 to 2 μM. Under our experimental conditions, the luminescence intensity becomes maximum when $(A\cdot T)_{17}$ DNA concentration reached 2 μM, the intensity increases over 4-fold.

For clarification of DNA enhancement,[13a−c,e] a polyanionic polymer, polyacrylic acid (PAA) and a porphyrin compound (TMPyP4) were selected as control samples. As seen in Figures S2 and S3, Supporting Information, the intensity of the fluorescence emission of SWNT-Eu^{3+} complexes was sensitive to PAA or 5,10,15,20-tetrakis-(1-methyl-4-pyridino)porphyrin (TMPyP4) and decreased upon increasing concentration of PAA or TMPyP4. This may be caused by the noncovalent interactions between

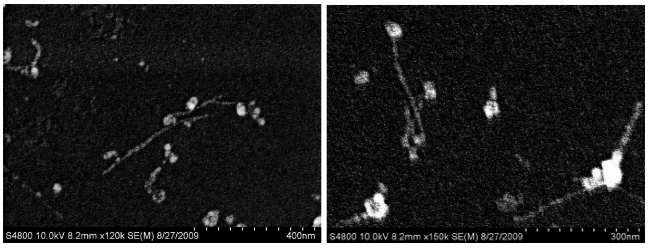


Figure 3. SEM images of f-SWNT **6**.

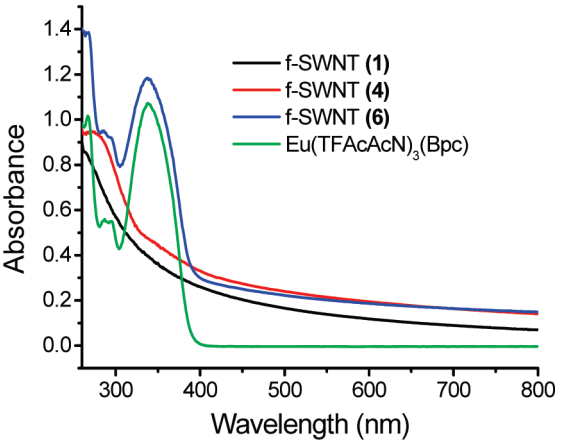


Figure 4. UV−visible absorption spectra of f-SWNT **1** (black), f-SWNT **4** (red), f-SWNT **6** (blue), and Eu(TFAcAcN)$_3$(Bpc) itself (green) in DMSO solution at room temperature.

PAA or TMPyP4 and SWNTs. The noncovalent wrapping or $\pi-\pi$ stacking effects make the Eu^{3+}-complexes more compact onto the SWNTs surface and lead to fluorescence quenching. Figures S2b and S3b, Supporting Information, show the correlation between the maximum emission intensity at 615 nm and the concentration of PAA and TMPyP4 over the range from 0 to 1.0 wt % and from 0 to 3.0 μM, respectively. Fluorescence quenching upon addition of PAA or TMPyP4 indicates that in addition to noncovalent interactions between SWNTs and the target DNA, DNA hydrophobic microenvironment, specific conformation and the interactions between Eu^{3+}-complex and DNA base pairs and phosphate and sugar backbone[13a−c,e] can be responsible for the fluorescence enhancement upon Eu^{3+}-complex binding to DNA.

We also used other DNA,[13] such as nonalternative AT dsDNA, $A_{22}\cdot T_{22}$; double-stranded GC DNA, $(G\cdot C)_{17}$; single-stranded DNA including dA_{22}, dT_{22}, and $(GT)_{20}$. DNA sequences used were summarized in Table S1, Supporting Information. Each luminescence titration was carried out following the same procedure as alternative AT dsDNA $(A\cdot T)_{17}$ (Figure 6a and b), and the luminescence spectra and the concentration dependence were shown in Figures S4−S8, Supporting Information, respectively. It can be seen that all the DNA we used can enhance the luminescence of rare-earth complex[13] covalently modified SWNT; however, the enhancement is different and dependent on DNA sequence and its form, double-stranded or singled-stranded DNA[13] (Figure 7), consistent with our previous results that rare-earth complex has DNA sequence and form preference.[13] The increase can be varied

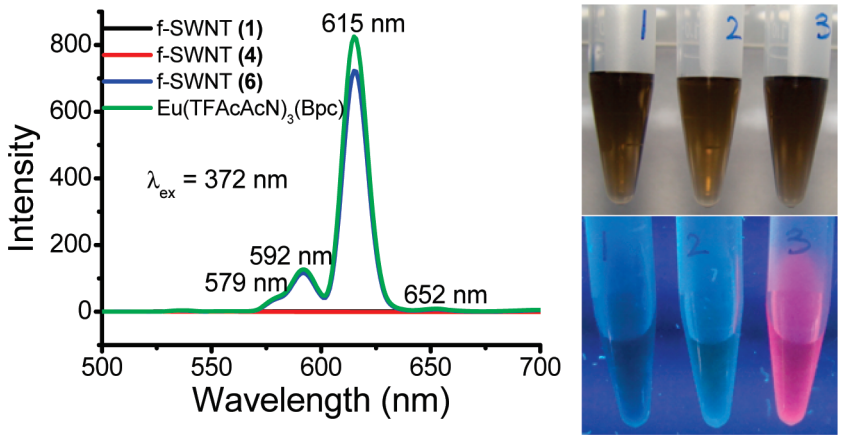


Figure 5. (Left) Emission spectra of f-SWNT **1** (black), f-SWNT **4** (red), f-SWNT **6** (blue), and Eu(TFAcAcN)$_3$(Bpc) itself (green) in DMSO solution at room temperature (λ_{ex} = 372 nm). (Right) Photographs of (1) f-SWNT **1**, (2) f-SWNT **4**, and (3) f-SWNT **6** in DMSO solution taken under daylight lamp (top) and 365 nm UV light (bottom).

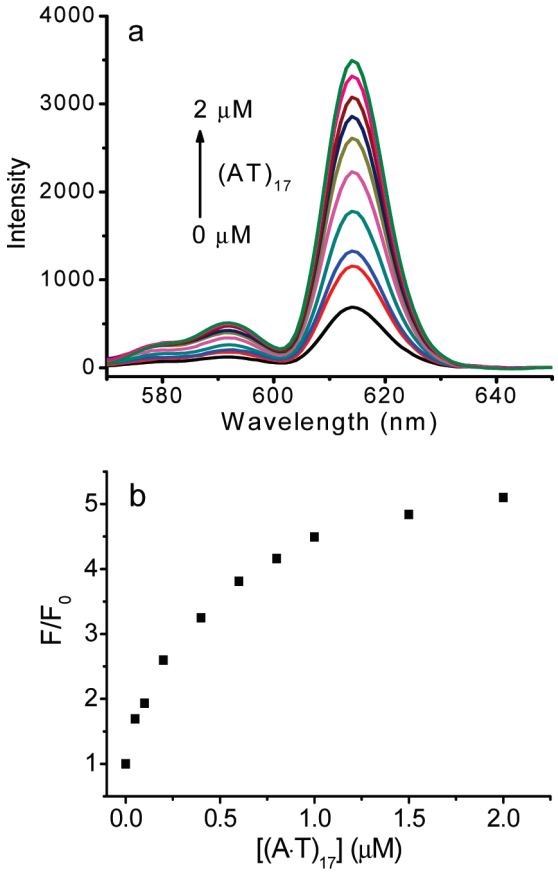


Figure 6. (a) Emission spectra of Eu^{3+}-complex covalently modified SWNTs in the absence or presence of different concentration of $(A\cdot T)_{17}$ dsDNA in 5 mM Tris-HCl 100 mM NaCl, pH 7.0 buffer. (b) Plot of the relative luminescence intensity change against $(A\cdot T)_{17}$ dsDNA concentration.

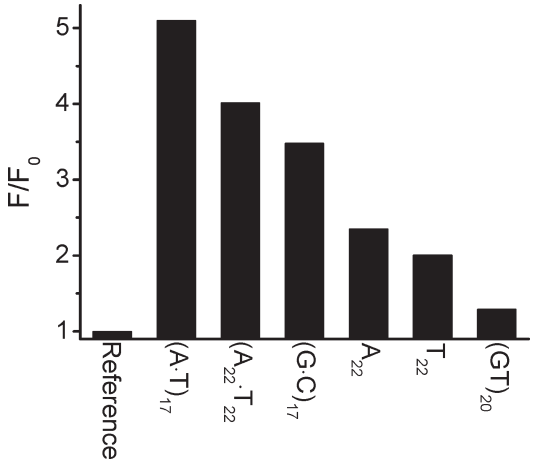


Figure 7. Luminescence enhancement depends on different DNA used. All DNA was kept at 2 μM, and the luminescence measurements were performed in Tris-HCl buffer (5 mM Tris-HCl, 100 mM NaCl, pH 7.0). λ_{ex} = 372 nm, λ_{em} = 615 nm.

Table 1. Summary of the Relative Luminescence Increase (F/F_0) and Binding Constants (K_b) of Eu^{3+}-Complex Covalently-Modified SWNTs Bound to Different DNA in 5 mM Tris-HCl, 100 mM NaCl, pH 7.0 Buffer

DNA sequences	F/F_0	K_b (M^{-1})[a]
$(AT)_{17}$	5.10	6.14×10^5
$A_{22}\cdot T_{22}$	4.01	7.24×10^5
$(GC)_{17}$	3.48	7.50×10^5
A_{22}	2.35	1.14×10^6
T_{22}	2.01	2.23×10^6
$(GT)_{20}$	1.29	5.91×10^7

[a] Binding constants were calculated according to refs 13a, 13b, 14. Details as described in Experimental Section. Errors in the binding constants were within 15%.

from 0.2-fold for ssDNA $(GT)_{20}$ to 4.1-fold for AT alternative dsDNA when the maximum enhancement was achieved. The enhancement follows: AT alternative dsDNA > nonalternative AT dsDNA > GC dsDNA > ssDNA dA_{22} > ssDNA dT_{22} > ssDNA $(GT)_{20}$. Generally, dsDNA has stronger enhancement than ssDNA.

This may suggest that the luminescence enhancement is in contrast to their DNA binding affinity to SWNTs because previous studies have shown that dsDNA binds weaker to SWNTs than ssDNA.[3b] Our quantitative binding data[13a,b,14] further support this assumption (Table 1). Under our experimental conditions, AT alternative dsDNA, $(A\cdot T)_{17}$, has the strongest luminescence enhancement and the least binding constant to SWNTs while ssDNA $(GT)_{20}$ has the weakest enhancement and the largest binding constant, consistent with that ssDNA $(GT)_{20}$ binds tightly to SWNTs.[3b] In addition to consideration of binding affinity,

the difference in DNA sequence, conformation, single strand or double strand provides different DNA binding sites and hydrophobic microenvironments. Therefore, the interactions among different DNA and SWNTs and Eu^{3+} complex can result in different luminescence enhancement. These results are in agreement with previous studies that both SWNTs and europium complex have DNA sequence preference,[13] and different DNA has different luminescence enhancement upon europium complex binding.

Conclusion

In summary, we have designed and synthesized red luminescent europium (Eu^{3+}) complex covalently modified SWNTs. This method can be applied to synthesize other luminescent rare-earth compound covalently modified SWNTs because of rare-earth elements with similar characteristics. DNA can enhance SWNT luminescence, the enhancement is related to DNA sequence and dsDNA or ssDNA. dsDNA has stronger enhancement even if dsDNA binds weaker to SWNTs than ssDNA. By taking advantage of unique rare-earth spectroscopic properties, this type of covalently modified luminescent SWNT can be a gene or drug carrier, its visible strong luminescence may provide a probe for monitoring the delivery path way in vivo and for biosensing.

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Supporting Information Available: Table of oligonucleotide sequences, reaction schemes illustrating preparation of Eu^{3+} complexes and the targetable Eu^{3+}-complex functionalized SWNTs, SEM images and UV−vis spectra of functionalized SWNTs, and fluorescence spectra changes upon addition of PAA, TMPyP4, and different DNA are available. This material is available free of charge via the Internet at http://pubs.acs.org.