

Electronic Supplementary Information

Improving Fluorescence Detection Limit with Positively Charged Carbon Nanostructure as a Low Background Signal Platform

Xiulan He,^{a,b} Li Zhang,^b Hetong Qi,^b Ping Yu,^{b,*} Junjie Fei,^{a,*} Lanqun Mao^{b,*}

^a College of Chemistry, Xiangtan University, Xiangtan, Hunan 411105, China

^b Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, The Chinese Academy of Sciences, Beijing 100190, P. R. China.

Table of Contents

Experimental section.....	S1
Schematic illustration of ATRP.....	S2
XPS results.....	S3
FT-IR results.....	S4
Fluorescence spectra for DNA sensor with Pim-graphene.....	S5

S1- Experimental section

Materials. Multiwalled carbon nanotubes (MWNTs, purity, >95%; length, 5-15 μm ; diameter, <10 nm) were provided by Shenzhen Nanoport Co. Ltd. (Shenzhen, China). Thrombin aptamer was synthesized by Sangon Biotech (Shanghai, China) and labeled at 5' end with FAM dye. DNA sequence of thrombin aptamer was 5'-FAM-GGTTGGTGTGGTTGG-3'. The sequence of FAM-labeled single strand DNA(FAM-sDNA) was 5'-FAM-TTTTTTTTTTTTTTTTT-3' and was purchased from Invitrogen Company. The sequence of the targeted complementary strand DNA (tcDNA) was 5'-AAAAAAAAAAAAAAAAA-3'. Thrombin was bought from Sigma. Other reagents were purchased from Beijing Chemical Company (China). All chemicals are of at least analytical grade reagents and used without further purification. All aqueous solutions were prepared with Milli-Q water (18.2 M Ω ·cm). Unless otherwise noted, all experiments were carried out at room temperature.

Apparatus. Fluorescence emission/excitation spectra were measured on an F-4600 Fluorescence Spectrophotometer (Hitachi). FT-IR spectra were obtained on a Tensor-27 FT-IR spectroscopy (Bruker) with KBr pellet. X-ray photoelectron spectroscopy (XPS) was performed on an ESCALab220I-XL electron spectrometer from VG Scientific using 300 W Al K α radiation.

Synthesis of 1-Vinyl-3-Butylimidazolium Chloride: Synthesis of 1-Vinyl-3-Butylimidazolium chloride was performed according to the previous report.¹ Briefly, 1-vinylimidazole (9.6 g, 102 mM) and 1-chlorobutane (29.6 g, 320 mM) were added to a three-necked flask, and the mixture was then stirred vigorously for 75 h in an oil bath of 70 °C under nitrogen atmosphere. After cooling to room temperature, the top phase was decanted and the bottom viscous liquid was first washed by ethyl acetate for three times and then filtered and dried under vacuum at 50 °C overnight to obtain [Vbim][Cl] as a white solid. [Vbim][Cl]: ¹H NMR (400 MHz, D₂O) δ = 0.92 (t, 3H), 1.34 (m, 2H), 1.88 (m, 2H), 4.23 (t, 2H), 5.42 (dd, 1H), 5.80 (dd, 1H), 7.14 (dd, 1H), 7.57 (s, 1H), 7.76 (s, 1H).

Synthesis of 2-Bromo-2-Methyl-propionic Acid 2-Hydroxy-Ethyl Ester: Synthesis of 2-

bromo-2-methyl-propionic acid 2-hydroxy-ethyl ester was performed according to the previous report.² Briefly, anhydrous ethylene glycol (112.5 mL, 2.05mol) was added to a 250 mL three-neck round bottom flask that had been flame-dried under vacuum and purged with 3 times with argon. The flask was equipped with a magnetic stir bar and rubber septum. The flask was then cooled to 0 °C in an ice bath. Slowly, α -bromoisobutyryl bromide (10 mL, 80.9 mmol) was added dropwise to the stirring ethylene glycol. The reaction was stirred at 0 °C for 3 h. The reaction was quenched with 50 mL H₂O and extracted with CHCl₃ (3 \times 50 mL). The combined organic extracts were dried over MgSO₄, filtered, and the CHCl₃ was removed by a rotary evaporator. The subsequent liquid was purified by distillation to yield a viscous, clear, colorless liquid.

Preparation of Acidic Treated MWNTs: The chemical oxidation of the pristine MWNTs (1.0 g) was carried out by cutting them in 60% HNO₃ under the sonication for 1.5 h at 50 °C.³ The black mixture was diluted with water, centrifuged at 14000 rpm for 1 h, and washed with distilled deionized water and acetone several times successively. The oxidized MWNTs were washed with distilled water and vacuum-filtered through a 0.2- μ m Millipore polycarbonate membrane until the pH of the filtrate was 7.0. The filtered solid was dried under vacuum for 24 h at 60 °C.

Preparation of Graphene Oxide: Graphene was prepared from reduction of exfoliated graphite oxide. Graphite oxide was obtained through natural graphite oxidation based on Hummer's method.⁴ Generally, the preoxidized graphite powders were put into concentrated H₂SO₄ at 0 °C with gradual addition of KMnO₄ under stirring. The mixture was kept at 35 °C for several hours and diluted gradually in an ice bath with deionized water. The mixture was re-diluted, followed by addition of H₂O₂. The mixture was filtered when the color changed to brilliant yellow and washed with aqueous solution of HCl and deionized water. The obtained graphite oxide powder was dialyzed in graphite oxide dispersion. The graphite oxide was exfoliated under sonication for about 2 h to ensure most graphite oxide was exfoliated to single layer graphene oxide. The graphite oxide was vacuum-filtered through a 0.2- μ m Millipore polycarbonate membrane. The filtered solid was dried under vacuum for 24 h at 60 °C.

Surface-Initiated Atom Transfer [Vbim][Cl] Radical Polymerization.⁵ A sample of MWNTs or graphene oxide (100 mg) was refluxed with 30 mL of thionyl chloride at 70 °C. After 24 h, the excess thionyl chloride was removed under vacuum. The activated MWNTs or graphene oxide were washed with anhydrous THF and dried under vacuum. Hydroxyethyl-2-bromoisobutyrate (2.3 mL) in toluene (5 mL) was added to the flask that contained MWNTs–COCl or graphene–COCl and the reaction was stirred at 100 °C for about 24 h under a pure N₂ atmosphere. After the reaction finished, the solvent was removed under vacuum, and the products were washed several times with ethanol (250 mL) and filtered. The initiator-attached nanotubes and graphene were dried at 40 °C for 10 h under vacuum.

In a typical polymerization, initiator-attached nanotubes or graphene (20.5 mg) were placed in a clean glass ampoule attached with a septum adaptor connected to both nitrogen and a vacuum system. [Vbim][Cl] monomer solution mixed with CuBr was first introduced into the reaction tube and then degassed by purging with N₂ for 15 min to remove dissolved O₂. N,N,N',N'',N''-Pentamethyldiethylenetriamine (PMEDTA) was then added to the monomer solution at a [Vbim][Cl]/CuBr/PMEDTA molar ratio of 50:5:15, and the mixture was degassed for a further 15 min. The initiator-modified carbon nanostructures were then placed in the reaction tube. Immediately, the tube was sealed under an N₂ atmosphere. The polymerization started when the reaction tube was immersed in an oil bath at 60 °C. It was stopped by removing the reaction vessel and cooling it to room temperature after a predetermined time. The sealed ampoule was placed in an oil bath that was maintained at 100 °C and the reaction stirred for 24 h.

References:

- 1 X. D. Mu, J. Q. Meng, Z. C. Li and Y. Kou, *J. Am. Chem. Soc.*, 2005, **127**, 9694.
- 2 M. A. White, J. A. Johnson, J. T. Koberstein and N. J. Turro, *J. Am. Chem. Soc.*, 2006, **128**, 11356.
- 3 (a) Y. Lin, A. M. Rao, B. Sadanadan, E. A. Kenik and Y. P. Sun, *J. Phys. Chem. B*, 2002, **106**, 1294.
(b) J. Liu, A. G. Rinzler, H. J. Dai, J. H. Hafner, R. K. Bradley, P. J. Boul, A. Lu, T. Iverson, K. Shelimov, C. B. Huffman, F. Rodriguez-Macias, Y. S. Shon, T. R. Lee, D. T. Colbert and R. E. Smalley, *Science*, 1998, **280**, 1253.

- 4 W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339.
5 D. Baskaran, J. W. Mays and M. S. Bratcher, *Angew. Chem., Int. Ed.*, 2004, **43**, 2138.

S2-Schematic Illustration of ATRP

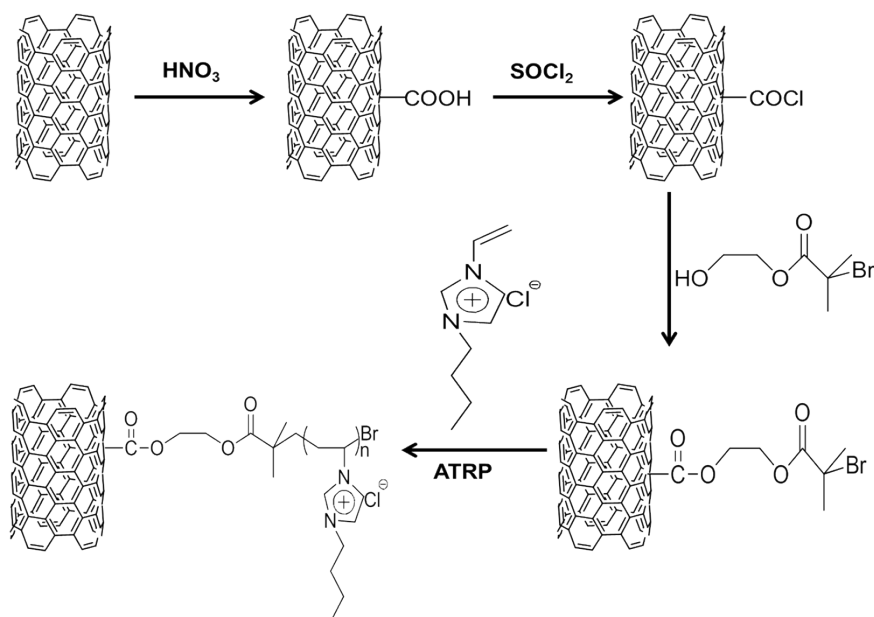


Fig. S1 Schematic illustration of grafting Pim to CNTs by ATRP.

S3-XPS Results

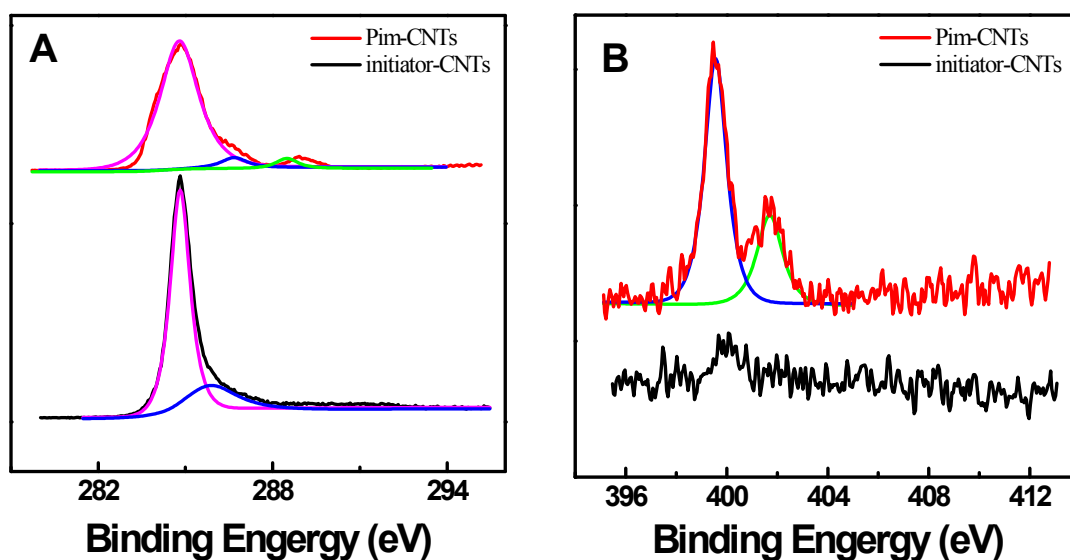


Fig. S2 X-ray photoelectron spectroscopy results: C 1s (A) and N1s (B) core-level spectra of an initiator-CNTs (black curve) and Pim-CNTs (red curve).

As shown, there was a new peak observed for C 1s in 289.0 eV for Pim-CNTs (Fig. S2A, red curve), which was attributable to carbon (i.e., N=C-N) species on imidazolium. Meanwhile, an obvious N1s peak was observed for the Pim-CNTs (Fig. S2B, red curve), which was not observed for the initiator-CNTs.

S4-FT-IR Results

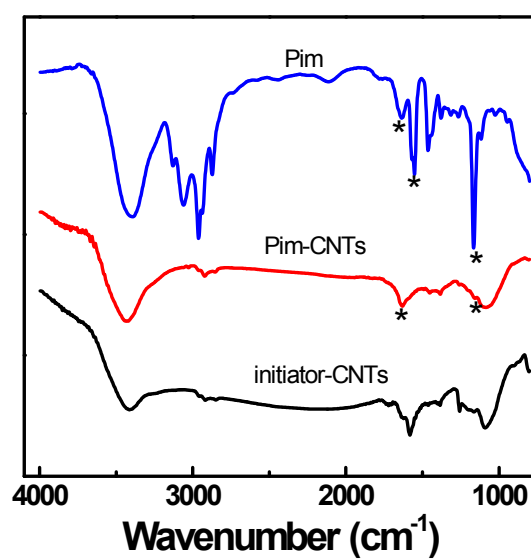


Fig. S3 FT-IR spectra of pure Pim (blue curve), initiator-CNTs (black curve) and Pim-CNTs (red curve).

FTIR characteristic peaks of imidazolium at 1630.4 and 1158.1 cm⁻¹ were also observed in the spectrum of the Pim-CNTs, demonstrating the successful functionalization of CNTs with polyimidazolium.

S5-Fluorescence Spectra for DNA Sensor with Pim-graphene

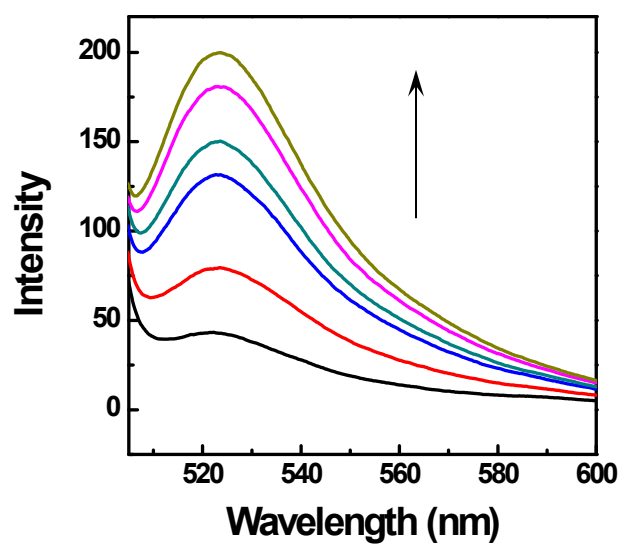


Fig. S4 Fluorescence spectra of the DNA sensor upon addition of different concentrations of tsDNA: 0, 10, 20, 30, 40, 50 nM. Excitation wavelength: 490 nm. Measurements were performed in 10 mM HEPES buffer containing 75 mM NaCl and 4 mM MgCl_2 (pH 7.4).