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i-Motif Quadruplex DNA-Based Biosensor for Distinguishing Single- and Multiwalled Carbon Nanotubes

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Abstract: The increasing worldwide demand for carbon nanotubes (CNTs) and increasing concern regarding how to safely develop and use CNTs are requiring a low-cost, simple, and highly sensitive CNT detection assay for toxicological evaluation and environmental monitoring. However, this goal is still far from being achieved. All the current CNT detection techniques are not applicable for automation and field analysis because they are dependent on highly expensive special instruments and complicated sample preparation. On the basis of the capability of single-walled carbon nanotubes (SWNTs) to specifically induce human telomeric i-motif formation, we design an electrochemical DNA (E-DNA) sensor that can distinguish single- and multiwalled carbon nanotubes both in buffer and in cell extracts. The E-DNA sensor can selectively detect SWNTs with a direct detection limit of 0.2 ppm and has been demonstrated in cancer cell extracts. To the best of our knowledge, this is the first demonstration of a biosensing technique that can distinguish different types of nanotubes. Our work will provide new insights into how to design a biosensor for detection of carbon nanotubes.

Introduction

The worldwide demand for carbon nanotubes (CNTs) has increased dramatically for electrical, mechanical, energy storage, and health and medical applications. The global CNT market is expected to grow to between $1 billion and $2 billion by 2014.[1] So, not surprisingly, people are becoming increasingly concerned regarding how to safely develop and use CNTs. Issues of human health and the environment, even occupational health for factory workers who directly interact with CNTs,[2] have been raised because of poor understanding of the toxicity and biological effects of CNTs. Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes have been reported.[2b] Two recent comments[3,4] on the research progress[5,6] of the toxicological and pharmacological effects of CNTs appeal for more attention on the safety because needlelike CNTs may produce asbestos-like pathogenic behavior. Therefore, a low-cost, simple, and highly sensitive CNT detection assay is highly desirable for toxicological evaluation and environmental monitoring. However, this goal is still far from being achieved, and all the current CNT detection techniques are not applicable and dependent on highly expensive special instruments as recently reviewed by Tantra and Cumpson.[2] These instruments include transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), NMR, Raman spectroscopy, and near-infrared fluorescence spectroscopy, which need special training to operate and prepare the sample. Therefore, they are not suitable for automation and field analysis.[2] Here, we report an electrochemical DNA (E-DNA) sensor that can distinguish single- and multiwalled carbon nanotubes. This biosensor can selectively detect carboxyl-modified single-walled carbon nanotubes (SWNTs) with a direct detection limit of 0.2 ppm and has been demonstrated in cancer cell extracts.

Results and Discussion

Human telomeres[7] are composed of tandem repeats of the double-stranded DNA sequence (5-TTAGGG):(5-CCCTAA). The G-rich strand can form a four-stranded G-quadruplex consisting of G-quartets, whereas its complementary C-rich strand may adopt i-motif structures with intercalated $C{\bullet}C^+$ base pairs.[7] The sensor we present here is based on the fact that SWNTs can specifically induce human telomeric i-motif DNA formation.[7−9] SWNTs are the only reported ligand that can selectively stabilize and facilitate i-motif DNA formation.[7−9]

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Table 1. Sequences of the Oligomers Used in This Study

oligomer	sequence
oligo 1	5′-HS-C6-TTTTTCCCTAACCCTAACCCTAACCC-MB-3′
oligo 2	5′-AGGGTTAGGGTTAGGGTTAGGG-3
oligo 3	5′-HS-C6-AAAAAGCTTTTT-3
oligo 4	5′-HS-C6-TTTTTC*T*CTCAC*T*CTCAC*T*CTCCA*C*C-MB-3′

The E-DNA sensor[10−20] used in our studies consists of a short single-stranded DNA (oligo 1, Table 1) containing a human telomeric i-motif DNA sequence as well as a linker sequence to ensure solvent exposure and an unfolded state at pH 7.0. This DNA, modified with a redox-active methylene blue (MB) at its 3′-terminus, can be covalently attached at its 5′-terminus to a gold electrode[20] via a thiol−gold bond (Scheme 1, middle). To determine optimal redox tag and electrode attachment geometries, we performed the hybridization of the immobilized DNA with a cDNA target, human telomeric G-quadruplex DNA. The E-DNA sensor preparation, coverage, and characterization were performed as described previously.[15,20] In the absence of target, the immobilized 26-mer DNA is thought to remain unfolded in the buffer (pH 7.0). The observed Faradaic current is due to the attached MB tag (Figure 1A), which comes within close proximity of the electrode surface and collides with (or weakly binds to) the electrode and transfers electrons.[20] In the presence of the i-motif cDNA (G-quadruplex DNA, oligo 2, Table 1), the signal gain of the Faradaic current decreased about 67%. The signal decrease indicates the formation of telomeric DNA duplex (Scheme 1, left), which prevents the MB tag from approaching the electrode surface and suppresses Faradaic current. Additionally, the sensor is reversible (Figure 1A). A brief, low ionic strength wash to dissociate the duplex (30 s in room temperature ultrapure water) is sufficient to recover ∼100% of the original signal. In order to confirm that the signal decrease is caused by the hybridization between i-motif DNA and its complementary G-quadruplex DNA, we use dA_{22} DNA as a noncomplementary control. All the experiments were carried out under the identical conditions. However, no signal decrease was observed (Figure S1 of the Supporting Information) indicating that the observed decrease is due to the duplex formation between i-motif DNA and its complementary G-quadruplex DNA.

SWNTs can selectively induce i-motif formation[7−9] of the immobilized human telomeric DNA on the surface. When the i-motif DNA modified electrode is immersed in buffer (pH 7.0) containing 5 ppm SWNTs for 1 h at room temperature, the Faradaic current decreases dramatically (Figure 1B) and falls to ∼16.8% of the original current, suggesting that the MB tag is held far away from the electrode surface[20] upon SWNT binding. We assume that the immobilized DNA is in a conformational equilibrium between its unfolded state and a folded i-motif conformation; the presence of SWNTs can drive the equilibrium toward the folded state through its binding to the i-motif DNA 5′-end major groove by facilitating base pairs $C•C^+$ formation.[7−9] Therefore, the formed i-motif structure holds the MB tag away from the electrode surface (Scheme 1, right), thereby producing the suppressed signal. This assumption is supported by our confocal fluorescence microscopy results. At pH 7.0, the immobilized DNA is in its unfolded random state,[21] which brings the 3′-end attached MB into close proximity of the gold surface and quenches MB fluorescence[22−25] (Figure 2A). However, we cannot preclude the possibility that the densely packed MB-modified DNAs prevent the 3′-end attached MB from approaching the surface. When hybridized with the complementary G-quadruplex DNA under the same conditions, G-quadruplex/i-motif DNA forms a rigid duplex. This hybridization will lift up the MB away from the gold surface,[25] leading to strong fluorescent emission (Figure 2C). When parallel substrate (Figure 2B) was treated with SWNTs at pH 7.0, MB fluorescence is also increased (Figure 2D), indicating that the i-motif DNA induced by SWNTs is formed[25] and the labeled MB is pushed away from the gold surface. Quantitive analysis of the average fluorescence intensity calculated from these images (Figure 2) indicates that formation of the rigid duplex DNA leads to a stronger MB fluorescence enhancement than the i-motif formation (Figure 2E).

The i-motif based sensor exhibits a rapid response and a low detection limit in comparison to using highly expensive spectroscopic and microscopic instruments and complicated sample preparation.[2] The influence of the incubation time on the signal gain of this biosensor is shown in Figure S2 of the Supporting Information. For example, the electrochemical signal attenuates to 89.8% of its initial value after a 60 min incubation in 0.2 ppm SWNTs (Figure S2A of the Supporting Information). During the next 30 min incubation, the decrease is only 0.6% (Figure S2A of the Supporting Information). Therefore, a 60 min equilibration time is enough to nearly reach the maximal signal change induced by SWNTs (Figure S2B of the Supporting Information). Further electrochemical studies indicate that the detection limit of SWNTs can be as low as 0.2 ppm at room temperature after 60 min incubation (Figure S2C of the Supporting Information).

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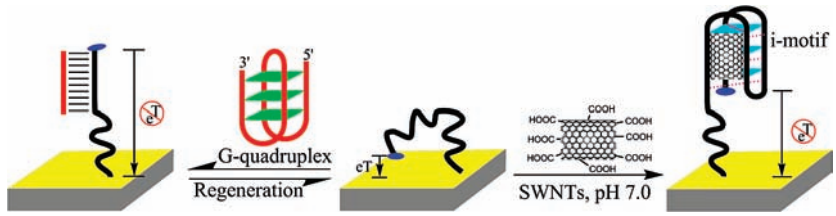
Scheme 1. Schematic of the i-Motif Telomeric DNA-Based Electrochemical DNA (E-DNA) Sensor[a]



[a] E-DNA sensor is based on a conformation change of an electrode-bound, methylene blue-modified human telomeric C-rich sequence. The plain and dashed lines show the hydrogen bonding formed by the C•C$^+$ hemiprotonated base pair of the "building blocks" for i-motif formation on right panel.

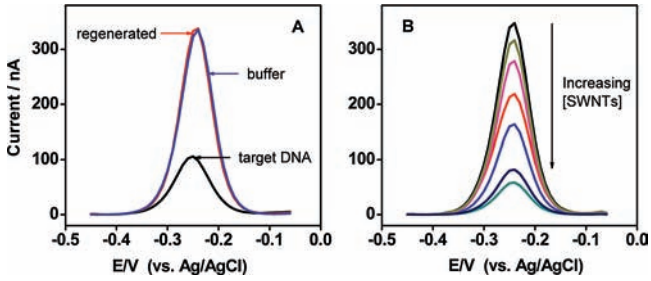


Figure 1. Alternating current (AC) voltammograms of the E-DNA biosensor. (Left) In the presence of the complementary G-quadruplex DNA (1 μM), the Faradaic current decreases dramatically, reaching 33% of the original peak current. After regeneration, the current recovers completely. (Right) A similar response is obtained with addition of single-walled carbon nanotubes at concentrations of 0, 0.2, 0.5, 1, 2, 3, and 5 ppm (from top to bottom). Incubation was performed in 0.1 mM cacodylic acid/sodium cacodylate containing 100 mM NaCl (pH 7.0). The incubation time was fixed at 1 h. Then, measurements were carried out in 100 mM phosphate salt, 100 mM NaCl, pH 7.0.

The electrochemical signals in the current system were generated via a specific target binding-induced conformational change. Control experiments were used in order to exclude the possibility that SWNTs may quench the electroactivity of MB. The thiolated oligo 3 is self-cDNA that is attached on the gold surface through the thiol−gold linkage. The immobilized oligo 3 is immersed in 20 μM MB solution for 30 min with stirring to fabricate a MB-labeled sensor. MB molecules can be oxidized via charge transfer[26,27] through the DNA (Figure S3 of the Supporting Information). Then, the sensor was immersed in SWNT-containing samples (1 ppm) for another 30 min. A negligible change is observed (Figure S3 of the Supporting Information), indicating that SWNTs did not quench the electroactivity of MB. These results also demonstrated that the signal drop of the i-motif based sensor in the presence of SWNTs comes from the specific target binding-induced DNA conformational change that has been verified in our previous studies.[7] Using another control sequence, MB-conjugated oligo 4, produced similar results (Figure S4 of the Supporting Information). A slight change can be due to nonspecific SWNT binding.

The specific target binding-induced conformational change can be used to distinguish between single-walled and multi-walled CNTs, further demonstrating that this structural transition is target-specific. Figure 3A shows the contrasting results obtained from the two different types of carbon nanotubes. Obviously, the signal decrease is strongly dependent on the type of carbon nanotube. At the same concentration of 1 ppm, the signal decrease is about 39% for SWNTs and only 7% for multiwalled carbon nanotubes (MWNTs). The difference becomes even more clear at 2 ppm. The signal decreased by 69.2% for SWNTs but only by 11.6% for MWNTs. A 5-fold difference

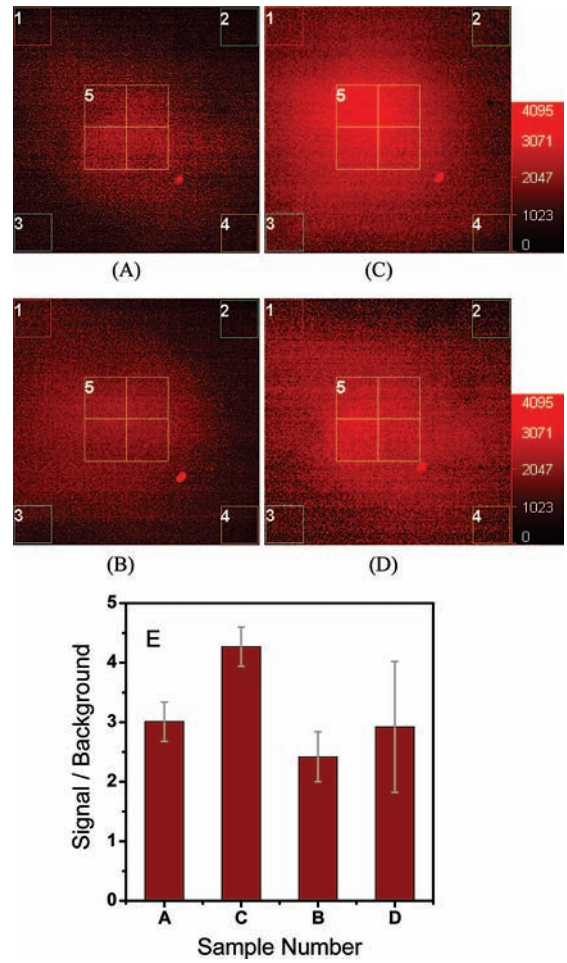


Figure 2. Confocal fluorescence images of the MB-labeled i-motif telomeric DNA modified gold surface. (A, B) Images of MB-labeled i-motif telomeric DNA alone. (C) Sample (A) after hybridization with 1 μM complementary G-quadruplex DNA for 0.5 h. (D) Sample (B) after treatment with 50 ppm SWNTs for 0.5 h. All images were recorded at the same sample surface region at room temperature, and before each sample collection, the surface was thoroughly rinsed with PBS (pH 7.0). (E) Illustrated on the histogram (from left to right) are the signal (fluorescence intensity calculated from the selected cDNA or SWNTs treated region, square 5, Figure 2) to background (the average of the fluorescence intensity calculated from the four selected squares 1, 2, 3, and 4, Figure 2A, B) ratio (*S*/*B*) changes as a function of the sample number depicted in Figure 2. For samples A and C, the average of the fluorescence intensity calculated from squares 1, 2, 3, and 4 of Figure 2A was set as the background value, while for samples B and D, the average of the fluorescence intensity calculated from squares 1, 2, 3, and 4 of Figure 2B was set as the background value. The error bar is determined from the average of three independent experiments under the same conditions.

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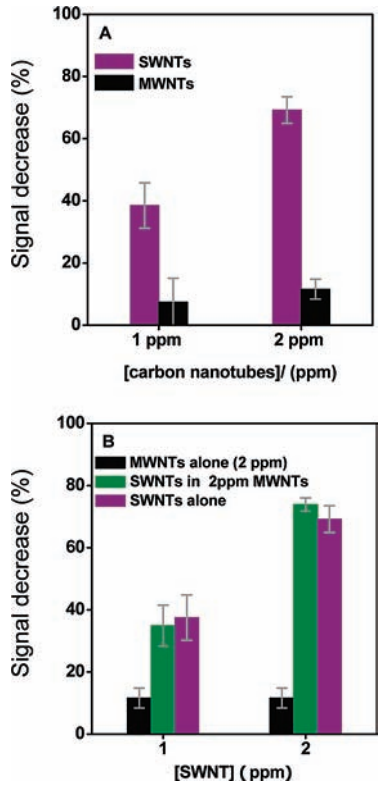

Figure 3. (A) E-DNA sensor can readily discriminate between SWNTs (purple columns) and MWNTs (black columns) at the same concentration of either 1 ppm (left) or 2 ppm (right). (B) SWNTs can be detected in the mixture of SWNTs and MWNTs. Signal decrease for SWNTs alone (1 ppm or 2 ppm, purple column) and 2 ppm MWNTs alone (black column) is shown for comparison with a mixture of 2 ppm MWNTs with addition of 1 or 2 ppm SWNTs (green column). The signal decrease of mixtures is similar to that of SWNTs alone.

in signal can make it easy to distinguish the SWNTs and MWNTs at the same concentration. Since MWNTs are a mixture and contain a small amount of SWNTs that are difficult to separate, this small amount of SWNTs can cause partial DNA conformational transition evidenced by circular dichroism (CD) studies (Figure S5 of the Supporting Information) and can lead to a slight signal decrease. We also used this sensor to detect gold nanoparticle (about 1 nm sized) whose size and surface modification are both similar to the SWNT we used and found no signal change (Figure S6 of the Supporting Information), further supporting the change is from SWNT specific binding induced conformational transition and our previous studies.[7] As indicated in our previous studies,[7−9] in addition to the favorable electrostatic interactions between the positively charged C•C$^+$ base pairs and the carboxyl groups on SWNTs, various interactions of i-motif DNA bases and backbone with SWNTs, such as $\pi-\pi$ stacking on the wall of the nanotube, hydrophobic, and van der Waals interactions, can increase i-motif stability. According to the i-motif structure and our nuclease cleavage and fluorescence studies,[7−9] the minor groove of i-motif DNA is too narrow to fit a rigid nanotube (1.1 nm sized). The SWNT rather binds to the DNA major groove.[7−9] Since a MWNT (10−20 nm sized) is too large to bind to the major groove, it cannot induce i-motif DNA formation (Figure S7 of the Supporting Information).

The E-DNA sensor can detect SWNTs in a mixture of SWNTs and MWNTs (Figure 3B). MWNTs were fixed at 2 ppm, SWNTs were varied from 1 ppm to 2 ppm. The signal decrease is close to the value with SWNTs alone. Figure S8 of the Supporting Information shows similar results obtained from a mixture of SWNTs and MWNTs at 10 ppm, which is a more biologically relevant concentration,[3,4] indicating that presence of MWNTs cannot influence detection of SWNTs and this E-DNA sensor can be used to detect SWNTs in a CNT sample.

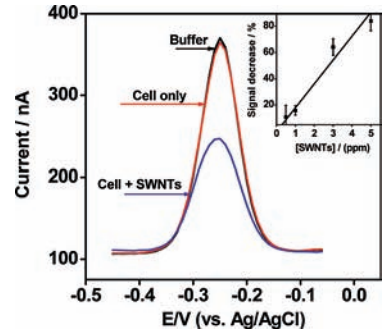

Figure 4. E-DNA sensor can detect intracellular SWNTs through analysis of cell extracts. The electrodes were incubated in either phosphate buffer (black line) or the supernatant of K562 cells cultured with (blue line) or without (red line) SWNTs. The control solution (red line) was cell supernatant without adding SWNTs during cell culture. The insert shows the calibration curve for the analysis of different concentrations of SWNTs, which were prepared by adding 0.5, 1, 3, and 5 ppm SWNTs into cell supernatant (cell cultured without SWNTs; for details see the Supporting Information). A linear equation is obtained that $y = 0.99 + 18.3x$. On the basis of this equation, we can estimate the amount of intracellular SWNTs to be 2.4 ppm. The incubation solutions have the same cell density of about 1×10^4 cell/mL.

Since SWNTs have been widely used as gene vector or drug delivery carriers, we extend our work using this E-DNA sensor to detect SWNTs in cancer cell extracts. Cell culture and cell extracts were prepared as previously described.[28] The E-DNA signal is significantly decreased after incubation in cell extracts for 1 h, indicating the detection of intracellular SWNTs in the extracts of the human chronic myelogenous leukemia K562 cells (Figure 4). The calibration curve is shown in Figure 4 as insert; data was measured in 1×10^4 cell/mL cell supernatant (cells were cultured in the absence of SWNTs) with the addition of different amounts of SWNTs. According to the calibration curve, the concentration of SWNTs in the supernatant of the cells is 2.4 ppm. We also used this biosensor to measure the mixture of SWNTs and MWNTs in cell extracts. The results (Figure S9 of the Supporting Information) clearly show that this biosensor can distinguish SWNTs and MWNTs even at a 10 ppm mixture of SWNTs and MWNTs in cell extracts, demonstrating that this sensor can cover the concentration range of SWNTs used in biotechnology.[3,4] In comparison with SWNTs alone in buffer, at the same SWNT concentration, the signal decrease in cell extracts is smaller than in buffer. This difference can be caused by proteins, DNA, and RNA in cell extracts bound to the SWNTs. Our results obtained in fetal calf serum (Figure S10 of the Supporting Information) show that loss in sensitivity can be mainly due to protein adsorption on nanotubes. Even so, this E-DNA sensor is sensitive enough to detect SWNTs in cell extracts and provides the potential application in clinical diagnosis and environmental monitoring.

In summary, we report here that a novel, low-cost E-DNA sensor can be used to detect SWNTs in both buffer and cancer cell extracts. In contrast to microscopy and spectroscopy instruments, this E-DNA sensor is easy to operate, economic, sensitive, and suitable for automation, miniaturization, and field

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analysis. Since the DNA structural transition is induced by specific SWNTs binding, this biosensor is capable of distinguishing carboxyl-modified single- and multiwalled carbon nanotubes in buffer and in cell extracts. To the best of our knowledge, this is the first demonstration of a biosensing technique that can distinguish different types of nanotubes. Our results will shed light on future research on this issue.

Materials and Methods

Materials. The methylene blue (MB)-labeled DNA oligonucleotide[15] was synthesized by Biosearch Technologies, Inc. (Novato, CA). Other sequences were synthesized by Sangon (Shanghai, China). The sequences of the oligomers are summarized in Table 1. All oligomers were used as received. Tris-(2-carbozyethyl) phosphine hydrochloride (TCEP) was purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. 6-Mercaptohexanol (MCH) was purchased from Fluka and used as received. SWNTs (φ = 1.1 nm, purity >90%) purchased from Aldrich (St. Louis, MO) were treated as described previously by sonicating SWNTs in a 3:1 v/v solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) for 24 h at 35−40 °C and washing with water. The stock solution of SWNTs (0.15 mg mL^{-1}) was obtained by sonicating the SWNTs for 8 h in pH 7.0 aqueous solution.[7−9] Multiwalled carbon nanotubes (MWNTs, φ = 10−20 nm) were purchased from Nanotech Port Co. Ltd. (Shenzhen, China), and the treatment process was the same as above. In order to prevent aggregation, we sonicated the stock solution of SWNTs and MWNTs about 10 min each time before use. Fetal bovine serum was purchased from TBD (Tianjin, China).

Electrode Cleaning and E-DNA Sensor Preparation. The oligo 1 modified sensor[15,20] was fabricated by using gold disk electrodes (Φ = 2 mm, CH Instruments, Austin, TX). The electrodes were prepared by polishing with 0.3 and 0.05 μm deagglomerated γ alumina (BUEHLER, UAS) suspensions followed by sonication in water and multiple steps of electrochemical cleaning described in the literature[15] before modification with the thiolated MB-tagged probe DNA. The clean gold surface was incubated with a 0.1 μM solution of thiolated MB-labeled DNA oligomer pretreated with TCEP in buffer (100 mM phosphate, 1.5 M NaCl, 1 mM $MgCl_2$, pH = 7.0) for 12 h at room temperature. The surface was then rinsed with buffer and subsequently passivated with 6-mercaptohexanol in the phosphate buffer for 1 h. Then, the electrodes were rinsed again with buffer (100 mM phosphate, 100 mM NaCl, pH 7.0) and stored in the same phosphate buffer prior to measurements.

Electrochemical Measurements. All measurements were performed by alternating current voltammetry (ACV) with a CHI 660B electrostation in a standard cell with a platinum counter electrode and an Ag/AgCl reference electrode. The E-DNA sensor measurements were conducted by monitoring the modified working electrode in the phosphate buffer (100 mM phosphate salt, 100 mM NaCl, pH 7.0) using ACV with a step potential of 10 mV, amplitude of 25 mV, and a frequency of 10 Hz. For all SWNT detection measurements, SWNTs were diluted with the aqueous cacodylic buffer (0.1 mM cacodylic acid/sodium cacodylate/100 mM NaCl, pH 7.0), and then, the modified electrodes were incubated in each sample containing different concentrations of SWNTs for 1 h at room temperature except the time-course study. Prior to measurement, washing with the measured buffer is necessary. Measurements were carried out either in phosphate buffer or in fetal calf serum diluted to 5 and 50% by phosphate buffer of varying salt concentrations to control the pH and ionic strength so that the final NaCl concentration is 100 mM. For the target hybridization measurements, the electrodes were incubated in 1 μM complementary G-quadruplex DNA or 1 μM dA_{22} oligomer solution for 1 h,[25] and the regeneration was done with a simple 30 s ultrapure water rinse at room temperature.

Confocal Fluorescence Measurements. The gold substrates[25,29,30] (flat transparent glass chips were coated with a layer of 50 nm thick gold film) were carefully cleaned with piranha etch solution (4:1 concentrated H_2SO_4/30% H_2O_2) for 1 h at room temperature and then thoroughly rinsed with ultrapure water and blown dry under a stream of N_2 before use. The freshly prepared gold substrates were incubated in a solution of the thiolated MB-labeled i-motif DNA (2 μL diluted in 100 μL of phosphate buffer (PBS) (pH 7.0) pretreated with TCEP) for 1 h at room temperature. After being rinsed with PBS (pH 7.0), the surface was passivated with MCH (1 mM in PBS, pH 7.0) for 1 h.[27] After a thorough rinse with PBS (pH 7.0), the i-motif DNA modified substrate was hybridized with its cDNA (AG3) (1 μM in PBS, pH 7.0) for 0.5 h, and another one was incubated with 50 ppm (50 μg/mL) SWNTs in PBS (pH 7.0) for 0.5 h. All experiments were performed at room temperature.

Fluorescence images were recorded on an Olympus Fluoview FV1000 confocal microscope,[25] using the excitation wavelength at 515 nm. The DNA chip was inverted on a clean coverslip on top of a 10× air objective. In order to track the fluorescence of a certain area, a pattern "○" was scratched on the opposite side of the gold surface so that the same area could be examined before and after hybridization or treating with SWNTs for comparison.[25] The images were collected at 512 × 512 pixels per image with an integration time of 10 μs per pixel and were processed using the FV10-ASW 1.6 Viewer program (Olympus, Japan). The average fluorescence intensity of the selected region from the fluorescence image was calculated using the same program.

Cell Culture and Treatment. In order to examine the usefulness of the method presented here in biological samples, we have applied it to the extracts of cells for intracellular detection of SWNTs.

The human chronic myelogenous leukemia K562 cells[28] were grown in Iscove's modified Dulbecco's medium (Gibco BRL) supplemented with 10% fetal calf serum in a humidified 37 °C incubator with 5% CO_2. Cells were passaged three times per week. Exponentially growing cells were used for the experiment. Briefly, 50 ppm SWNTs were administered to K562 cells at a density of 10^5 cells/mL in Iscove's modified Dulbecco's medium without serum. The cells were incubated at 37 °C for 6 h, harvested by centrifugation, and washed three times with phosphate buffered saline (PBS). Cell numbers were determined by Trypan blue exclusion in a hemocytometer chamber. Cell pellets were resuspended in buffer containing 100 mM phosphate salt, 100 mM NaCl, pH 7.0, and then sonicated. Cell lysates were centrifuged at 10 000*g* for 5 min, and the supernatants were collected for analysis.

The detection of intracellular SWNTs was carried out by diluting the cell supernatant to 1 × 10^4 cells/mL. The control experiment was also performed, whereby SWNTs were not added into the cell culture.

The addition of different amounts of SWNTs into the cell supernatant was also measured in order to give a calibration curve. The modified electrodes were incubated in these solutions for 1 h and measured in phosphate buffer. All the solutions have the same cell density of 1 × 10^4 cells/mL.

Circular Dichroism Measurements. CD spectra were measured[7−9] on a JASCO J-810 spectropolarimeter. The optical chamber of the CD spectrometer was deoxygenated with dry purified nitrogen (99.99%) for 45 min before use and kept the nitrogen atmosphere during experiments. Three scans were accumulated and automatically averaged.

Preparation of Carboxylate-Modified Gold Nanoparticles. Hydrogen tetrachloroaurate(III) trihydrate (99.99%) and sodium borohydride (98%) were purchased from Alfa Aesar (Ward Hill, MA). Mercaptosuccinic acid (97%) was obtained from Sigma-Adrich (St. Louis, MO). Methanol (HPLC-grade) was purchased from Aladdin Reagent Database Inc. (Shanghai, China). These

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reagents were used without further purification. High purity water (18.2 MΩ cm) from Milli-Q Synthesis was used in all of the experiments.

Gold nanoparticles (about 1 nm sized) were prepared and characterized (Figure S11 of the Supporting Information). Briefly, 0.25 mmol of $HAuCl_4$ aqueous solution was first mixed with 0.625 mmol of mercaptosuccinic acid (MSA) in 50 mL of methanol. Freshly prepared 0.2 M $NaBH_4$ aqueous solution (12.5 mL) was then added at a rate of 5 mL/min under vigorous stirring. After further stirring for 1 h, the resulting solution was centrifuged, and the supernatant was decanted. The precipitate was then sequentially washed with 20% (v/v) water/methanol solution (3 times) and methanol (2 times). Finally, the precipitate was dried under vacuum at room temperature. The as-prepared carboxylate-modified gold nanoparticles were characterized and confirmed by UV−visible absorption spectroscopy and X-ray diffraction (XRD). The powder of carboxylate-modified gold nanoparticles was dissolved in ultrapure water and then diluted with the aqueous cacodylic buffer. The modified electrodes were incubated in Au nanoparticle solutions for 1 h and measured in phosphate buffer.

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Supporting Information Available: AC voltammograms, CD spectra, UV-visible absorption spectrum, X-ray diffraction pattern, DNA UV melting curves, figure showing incubation time dependence, and charts showing biosensor interaction with SWNTs and MWNTs and that SWNTs can be detected in a mixture of SWNTs and MWNTs are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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