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A Reusable DNA Single-Walled Carbon-Nanotube-Based Fluorescent Sensor for Highly Sensitive and Selective Detection of Ag^+ and Cysteine in Aqueous Solutions

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Abstract: Here we report a reusable DNA single-walled carbon nanotube (SWNT)-based fluorescent sensor for highly sensitive and selective detection of Ag^+ and cysteine (Cys) in aqueous solution. SWNTs can effectively quench the fluorescence of dye-labeled single-stranded DNA due to their strong π–π stacking interactions. However, upon incubation with Ag^+, Ag^+ can induce stable duplex formation mediated by C–Ag^+–C (C = cytosine) coordination chemistry, which has been further confirmed by DNA melting studies. This weakens the interactions between DNA and SWNTs, and thus activates the sensor fluorescence. On the other hand, because Cys is a strong Ag^+ binder, it can remove Ag^+ from C–Ag^+–C base pairs and deactivates the sensor fluorescence by rewrapping the dye-labeled oligonucleotides around the SWNT. In this way, the fluorescence signal-on and signal-off of a DNA/SWNT sensor can be used to detect aqueous Ag^+ and Cys, respectively. This sensing platform exhibits high sensitivity and selectivity toward Ag^+ and Cys versus other metal ions and the other 19 natural amino acids, with a limit of detection of 1 nM for Ag^+ and 9.5 nM for Cys. Based on these results, we have constructed a reusable fluorescent sensor by using the covalent-linked SWNT–DNA conjugates according to the same sensing mechanism. There is no report on the use of SWNT–DNA assays for the detection of Ag^+ and Cys. This assay is simple, effective, and reusable, and can in principle be used to detect other metal ions by substituting C–C base pairs with other native or artificial bases that selectively bind to other metal ions.

Keywords: DNA · fluorescence · nanotubes · sensors · silver

Introduction

Silver is widely distributed in nature and has been used for many years. The most important applications of silver are in the electrical industry, the photographic and imaging industry, and the pharmaceutical industry.[1] However, silver ions inactivate sulphydryl enzymes and combine with amine, imidazole, and carboxyl groups of various metabolites. The increasing amount of information on the interaction of silver with essential nutrients, especially selenium, copper, and vitamins E and B12, has focused attention on its potential toxicity.[2,3] Thus, the development of sensitive and selective methods for the determination of trace amounts of silver ions (Ag^+) in aqueous media is of considerable importance for the environment and human health. A number of methodologies, such as electrothermal atomic absorption spectrometry (ETAAS),[4–6] voltammetry,[7–9] inductively coupled plasma atomic emission spectrometry (ICP-AES),[10] inductively coupled plasma mass spectroscopy (ICP-MS),[11–13] potentiometry,[14–16] and the fluorescence method[17–21] have been reported in the past few years. Though these methods have high selectivity and sensitivity, they are somewhat complex, costly, and time consuming.

DNA–metal base pairs currently attract considerable attention because of their potential in sensing applications. Some metal ions can selectively bind to a few native or artificial bases in DNA duplexes to form metal-mediated base pairs.[22] This generally gives rise to an increase in the thermal stability of DNA duplexes.[23–30] In particular, DNA–

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metal base pairs are often utilized to promote a structural change in single- or double-stranded oligonucleotides, which enables the detection of metal ions (e.g., Hg^{2+} and Ag^{+}) by various means.[30–36] Hg^{2+} is found to specifically interact with the thymine–thymine (T–T) mismatch in DNA duplexes,[23] which provides a rationale for highly selective Hg^{2+} detection by using T-containing oligonucleotides.[37–40] Similarly, cytosine–cytosine (C–C) pairs in DNA duplexes are able to exclusively capture Ag^{+} to form C–Ag^{+}–C base pairs.[30] Based on this feature, a direct DNA-based fluorescent Ag^{+} sensor has been recently reported.[30] However, this sensor exhibits a poor selectivity toward other heavy metal ions.[30] Therefore, it is highly desirable to develop a detection system that is not only sensitive and reliable but also selective, simple, practical, and economical in operation.

Herein, we design a reusable fluorescent sensor for the first time for highly sensitive and selective detection of Ag^{+} in aqueous solution using DNA/single-walled carbon nanotube (SWNT) conjugates and C–Ag^{+}–C coordination chemistry. This sensing platform can also be used to probe cysteine (Cys), which can bind Ag^{+} through thiol–Ag^{+} interaction,[41] based on the previously described competition mechanism.[42] Cys plays a crucial biological role in the human body by providing a modality for the intramolecular cross-linking of proteins through disulfide bonds to support their secondary structures and functions.[43] It is also a potential neurotoxin,[44–46] a biomarker for various medical conditions,[47,48] and a disease-associated physiological regulator.[49–51] Compared with reported methods such as fluorometry based upon fluorescent dyes,[52–55] electrochemical voltammetry,[56–60] fluorescence-coupled HPLC techniques,[61,62] and chromophoric colorimetric sensors,[52,53,63,64] this sensing platform is simple and economical, and has high sensitivity and selectivity.

As the leading nanodevice candidate, SWNTs have shown great potential applications ranging from molecular electronics to ultrasensitive biosensors.[65] Single-stranded DNA (ssDNA) has recently been demonstrated to interact noncovalently with SWNTs, and forms stable complexes with individual SWNTs by wrapping around them by means of π–π stacking between nucleotide bases and SWNT sidewalls.[66] Double-stranded DNA (dsDNA) has also been proposed to interact with SWNTs, but its affinity is significantly weaker than that of ssDNA.[67] Also, scatter examples of noncovalent interactions of SWNTs with organic dyes or dye-labeled biomolecules have now been reported,[68–70] and SWNTs can act collectively as fluorescence quenchers for dyes.[70] In the present study, a new fluorescent sensing platform for Ag^{+} and Cys was constructed by FAM (fluorescein derivative)-labeled C-rich oligonucleotide (DNA-1, Table 1) and SWNTs. A reusable sensing platform was also demonstrated that utilizes the covalently linked SWNT–DNA conjugates. The sensing mechanism is based on the difference between the interactions of carbon nanotubes with ssDNA and dsDNA, as shown in Figure 1. In the absence of Ag^{+}, the single-stranded FAM-labeled DNA-1 wrapped around the SWNTs, FAM fluorescence was completely quenched, and no apparent fluorescence enhancement was observed when the semicomplementary C-rich DNA-2 was added, thereby indicating that FAM-labeled DNA-1 and DNA-2 cannot form a stable duplex without Ag^{+}. However, in the presence of Ag^{+}, double-stranded DNA was formed by C–Ag^{+}–C coordination chemistry that suppresses the strong fluorescence quenching effect of SWNTs. Therefore the fluorescence intensity was significantly increased relative to that in the absence of Ag^{+}. Because Cys is a strong Ag^{+} binder and can remove Ag^{+} from C–Ag^{+}–C base pairs, this will break up double-stranded DNA to single-stranded DNA. Finally, the detached FAM-labeled DNA-1 rewraps around the SWNTs, and consequently the fluorescence is quenched again.

Table 1. Oligonucleotide sequences of the probes and the control DNAs used in this study.

Probe and control DNA	Sequence
DNA-1	5′-CTC TCT CTC TCT CTC TCT CTC-FAM-3′
DNA-2	5′-CAC ACA CAC ACA CAC ACA CAC-3′
DNA-3	5′-CCA ACC CCC CAG AAA GAA-3′
DNA-4	5′-CTC TCT CTC TCT CTC TCT CTC- 3′
DNA-5	5′-NH_2-TTT TTT CAC ACA CAC ACA CAC ACA CAC-FAM-3′

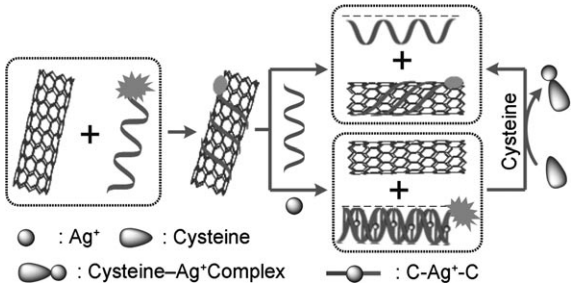

Figure 1. Schematic representation of the Ag^{+} and Cys detection mechanism using the SWNT–DNA system based on a fluorescence spectroscopy method.

Results and Discussion

SWNT–DNA-based sensing strategy: We employed two separate 21-base C-rich oligonucleotides as Ag^{+} binding sequences. First, the FAM-labeled DNA-1 and SWNTs readily form stable SWNT–DNA complexes in aqueous solution by means of π–π stacking interactions between nucleotide bases and SWNT sidewalls; this results in complete fluorescence quenching. In the absence of Ag^{+}, when the semicomplementary C-rich DNA-2 was added, duplex DNA was not formed because no transition was observed in its melting curve (Figure S5 in the Supporting Information), and no fluorescence enhancement was observed. However, in the presence of Ag^{+}, DNA-1 and DNA-2 form stable double-stranded DNA through the specific C–Ag^{+}–C interactions

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(Figure 1) because an apparent melting transition with a melting temperature, T_m (T_m is about 60 °C when 1 μM Ag^+ was added, and 70 °C when 5 μM Ag^+ was added) is observed (Figure S5 in the Supporting Information). As a result, the duplex DNA formed in this way cannot interact with SWNTs effectively. It suppresses the fluorescence quenching effect of SWNT and FAM fluorescence is significantly increased compared to that without Ag^+. At this moment, the addition of Cys can remarkably decrease fluorescence since Cys can remove Ag^+ from C–Ag^+–C base pairs and destroys the duplex structure. The detached random coiled DNA-1 wraps around the SWNTs again, and consequently turns off FAM fluorescence. Figure 2 shows

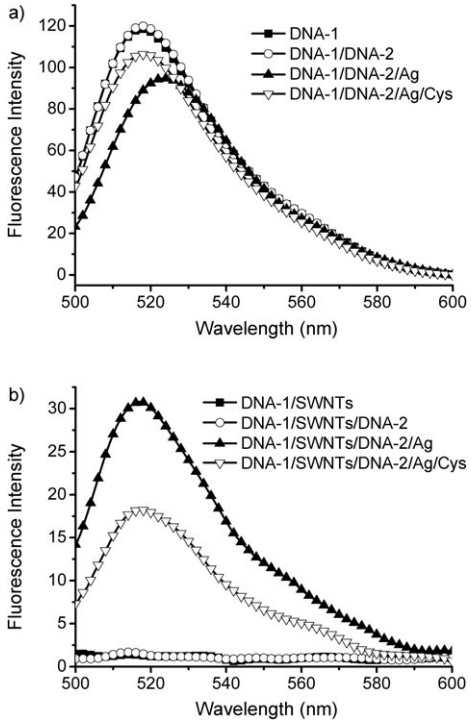

Figure 2. Changes in the fluorescence emission spectra of DNA-1 with DNA-2 in the a) absence and b) presence of 1.5 μg mL^{-1} SWNTs in HEPES buffer (10 mM HEPES 200 mM $NaNO_3$, pH 7.4) caused by Ag^+ and/or Cys ([Ag^+] = [Cys] = 100 nM). The concentration of DNA-1 and DNA-2 were 10 nM, and the excitation wavelength was 480 nm.

fluorescence spectra in the absence and presence of 1.5 μg mL^{-1} SWNTs before and after the addition of 100 nM Ag^+. Without Ag^+, SWNTs almost completely quench FAM fluorescence, even in the presence of its semicomplementary DNA-2. However, in the presence of Ag^+, FAM fluorescence is greatly increased, although little quenching of FAM fluorescence is observed upon addition of 100 nM Ag^+ without SWNTs. About twentyfold fluorescence enhancement is observed compared with that without Ag^+ (Figure 3). Addition of Cys to the DNA/SWNT/Ag^+ system leads to remarkable decrease in FAM fluorescence, thereby indicating that the C–Ag^+–C mediated duplex is destroyed, and the detached DNA-1 wraps around the SWNTs again.

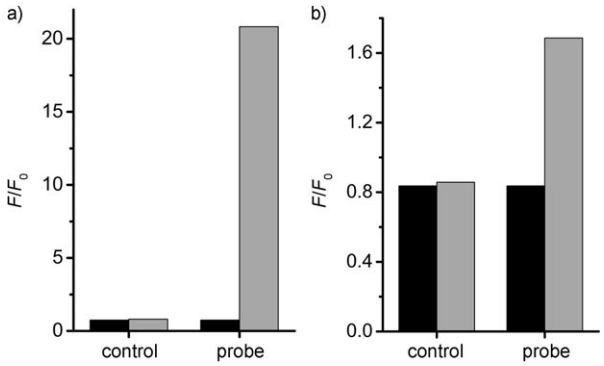

Figure 3. Comparisons of the signal-to-background ratio (F/F_0) of DNA-1 with DNA-2 or the control DNA-3 generated by a tenfold excess amount of a) Ag^+ and b) Cys in the absence (black bars) and presence (gray bars) of 1.5 μg mL^{-1} SWNTs. The concentration of DNA-1, DNA-2, and the control DNA-3 were 10 nM, and the excitation and emission wavelengths were 480 and 518 nm, respectively.

But in the absence of SWNTs, we only observe a little increase in the fluorescence intensity. About twofold fluorescence enhancement is observed compared with that without Cys (Figure 3). These observations clearly suggest that our designed fluorescence-labeled SWNT–DNA system can detect both Ag^+ and Cys. To exclude the possibility that Ag^+ may directly prevent DNA-1 from adsorbing onto the SWNT surface, we performed control experiments using a control oligonucleotide DNA-3 that cannot form the duplex structure with DNA-1 through specific C–Ag^+–C binding (Table 1). Almost no fluorescence change is observed regardless of the absence or presence of Ag^+ or Cys (Figure S6 in the Supporting Information). Taken together, these observations suggest that the fluorescence signal enhancement compared to that without Ag^+ is indeed attributed to the formation of stable C–Ag^+–C base pairs in the DNA duplex.

Sensitivity and selectivity toward Ag^+: As indicated in Figure 4, the fluorescence emission intensity of the sensor is sensitive to Ag^+ and increases as the concentration of Ag^+ increases. A linear correlation exists between the emission intensity at 518 nm and the concentration of Ag^+ over the range of 0–150 nM ($R^2 = 0.992$) (Figure 4b). This sensor has a detection limit of 1 nM based on 3σ/slope, which is ten times higher than that of a recently reported directly DNA-based fluorescent Ag^+ sensor[30] and is even comparable to that of graphite furnace atomic absorption spectrometry.[71] When using a higher concentration of DNA-1/DNA-2 (50 nM), the linear correlation range can expand to 0–400 nM (Figure S7 in the Supporting Information). These data show ultrahigh sensitivity and good linearity for the quantitative analysis of Ag^+ by using the SWNT–DNA platform.

We then evaluated the selectivity of this system for Ag^+ by testing the response of the assay toward other environmentally relevant metal ions, including Hg^{2+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Eu^{3+}, and Fe^{3+} (Figure 5) at the concentration

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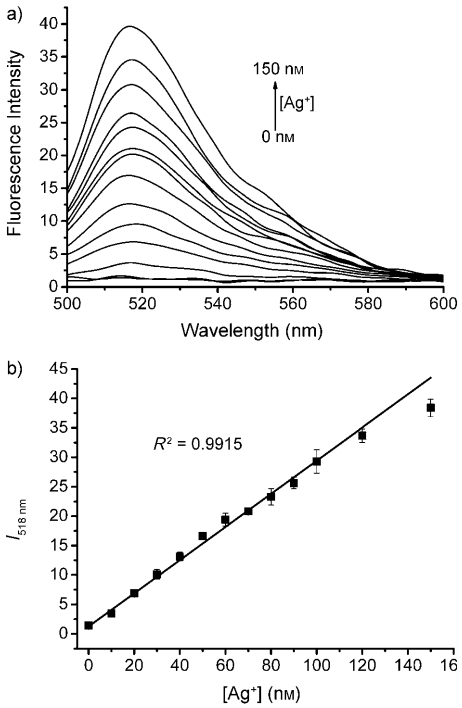

Figure 4. a) Fluorescence emission spectra of DNA-1/SWNTs/DNA-2 (10 nM/1.5 μg mL^{-1}/10 nM, λ_{ex} = 480 nm) in the absence and presence of different concentrations of Ag^+. b) The fluorescence intensity at 518 nm plotted against the concentration of Ag^+.

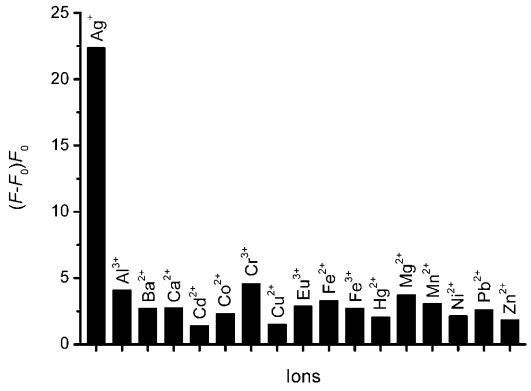

Figure 5. Selectivity of the Ag^+-ion sensor. All competing metal ions were tested at 1 μM. For comparison, the sensor response to 100 nM Ag^+ is also presented. The DNA-1, DNA-2, and SWNT concentrations were 10 nM, 10 nM, and 1.5 μg mL^{-1}, respectively.

of 1 μM under the same conditions as those used with Ag^+. Remarkably, no apparent fluorescence signal changes of the solutions are observed with these metal ions, even at high concentrations; only Ag^+ results in a significant increase in the fluorescence signal. These results clearly demonstrate that the SWNT–DNA-based Ag^+ sensor is highly selective toward Ag^+ over the other metal ions and better than a direct DNA-based fluorescent Ag^+ sensor.[30]

Detection of Cys with high sensitivity and selectivity: Another sensing application of the SWNT–DNA platform is to detect Cys, based on a competition mechanism previously described.[42] As shown in Figure 6, the SWNT–DNA/Ag^+ system shows strong fluorescence in the absence of Cys.

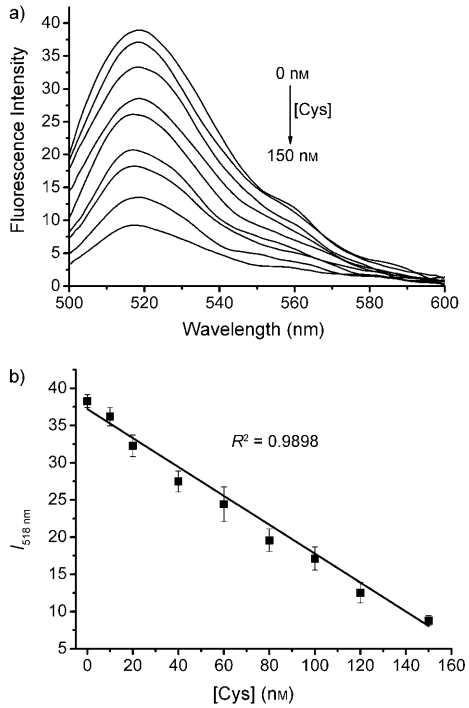

Figure 6. a) Fluorescence emission spectra of DNA-1/SWNTs/DNA-2/Ag^+ (10 nM/1.5 μg mL^{-1}/10 nM/150 nM, λ_{ex} = 480 nm) in the absence and presence of different concentrations of Cys. b) The fluorescence intensity at 518 nm plotted against the concentration of Cys.

Upon addition of Cys to the solution of SWNT–DNA/Ag^+, a dramatic deactivation of the fluorescence response is observed. This suggests that Ag^+ is removed from C–Ag^+–C base pairs through competitive binding with Cys. As indicated in Figure 6a, the fluorescence intensity of SWNT–DNA/Ag^+ system is sensitive to Cys and decreases as the concentration of Cys increases. The fluorescence intensity at 518 nm exhibits a linear correlation to Cys concentration over the range of 0–150 nM (R^2 = 0.990) (Figure 6b). The present limit of detection for this sensor is 9.5 nM (based on 3σ/slope), which reveals a high sensitivity for the analysis of Cys by using the SWNT–DNA/Ag^+ sensing platform.

To determine the selectivity of this assay, we studied the fluorescence response to the other 19 essential amino acids at a concentration of 1 μM (Figure 7). It is clear that only Cys can significantly decrease fluorescence. The other α-amino acids do not interfere in detection of Cys, that is, only Cys is able to remove Ag^+ from C–Ag^+–C base pairs. Other amino acids, including lysine, arginine, and methionine, have been found to combine with Ag^+ as well,[41] but such interactions are not significant enough to interfere with the Cys detection here. This suggests that the C–C mismatch site binds Ag^+ strongly enough to effectively compete against all tested amino acids other than Cys, which contrib-

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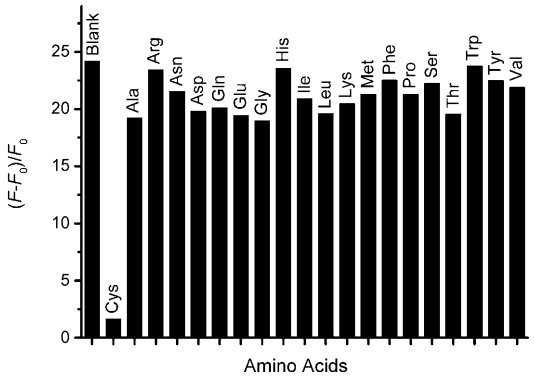

Figure 7. Selectivity of the Cys sensor. All competing amino acids were tested at 1 μM. For comparison, the sensor response to 100 nM Cys is also presented. DNA-1, DNA-2, SWNTs, and Ag^+ concentrations were 10 nM, 10 nM, 1.5 $\mu g\,mL^{-1}$, and 100 nM, respectively.

utes to high selectivity of this SWNT–DNA/Ag^+-based sensing platform for Cys.

Sensing applications of the covalently linked SWNT–DNA probe: To demonstrate the practicality of the SWNT–DNA-based sensors, we constructed a reusable sensing platform using the covalent linked SWNT–DNA conjugates. As shown in Figure 8, a 27-mer C-rich oligonucleotide with 5′-NH_2 and 3′-FAM-labeled DNA (DNA-5) was covalently attached to the carboxyl-modified SWNTs by means of carbodiimide-assisted amidation. Because of the strong π–π stacking between DNA bases and SWNTs, the flexible DNA chains adsorb on the SWNT surface and the fluorophore fluorescence is quenched completely. Basically, no apparent fluorescence enhancement is observed, even in the presence of its semicomplementary DNA-4 on account of the lack of stable duplex formation. However, in the presence of Ag^+, the duplex DNA was formed by C–Ag^+–C coordination chemistry and suppresses the fluorescence quenching effect of the SWNTs. Therefore the fluorescence intensity increases relative to that in the absence of Ag^+. The same is found with the noncovalent SWNT–DNA-based Ag^+ sensor, in which Cys can also remove Ag^+ from C–Ag^+–C base pairs,

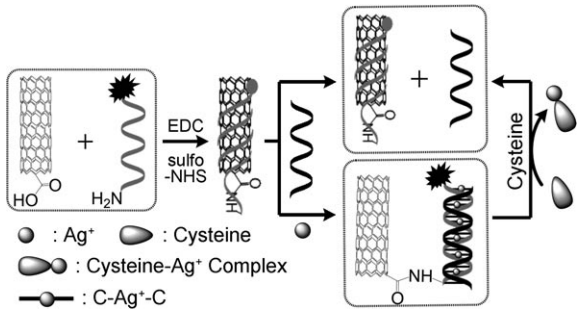

Figure 8. Schematic representation of the reusable fluorescent sensor for Ag^+ and Cys using the covalently linked SWNT–DNA sensing platform (EDC = 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride; sulfo-NHS = sulfo-*N*-hydroxy succinimide).

which weakens the Ag^+-mediated duplex, and finally destroys the duplex. Therefore the covalently attached DNA-5 is in its random coiled state again; it will readsorb onto the surface of the SWNTs and consequently deactivate the fluorescence. Figure 9 shows the fluorescence emission spectra

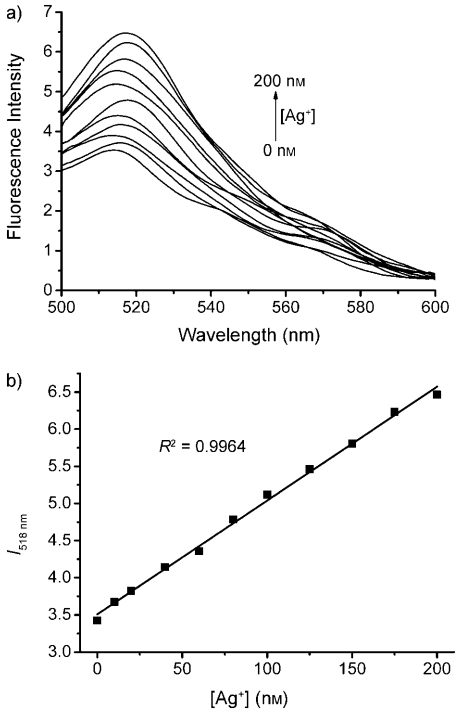

Figure 9. a) Fluorescence emission spectra of SWNT–DNA-5 conjugates with DNA-4 in the absence and presence of different concentrations of Ag^+. The concentration of DNA-4 was 50 nM, and the excitation wavelength was 480 nm. b) The fluorescence intensity at 518 nm plotted against the concentration of Ag^+.

of the SWNT–DNA-5 conjugates with DNA-4 in the absence and presence of different concentrations of Ag^+. In the absence of Ag^+, the mixture of SWNT–DNA-5 and DNA-4 has weak fluorescence, which can be attributed to the incomplete fluorescence quenching. Upon increasing additions of Ag^+, the fluorescence is gradually enhanced because of the formation of an Ag^+-mediated duplex. Figure 9b shows a fluorescence intensity change at $\lambda = 518$ nm versus Ag^+ concentration. A linear relationship ($R^2 = 0.996$) is observed in the concentration range from 0 to 200 nM. The detection limit of Ag^+ is as low as 6.2 nM based on a 3σ/slope, thereby showing ultrahigh sensitivity and good linearity for the quantitative analysis of Ag^+ by using the covalent SWNT–DNA conjugates.

This covalent SWNT–DNA-conjugate-based sensing platform was also used to detect Cys. Figure 10 depicts the quantitative analysis of Cys by detecting fluorescence change. As the concentration of Cys increases, fluorescence is gradually decreased. This suggests that Ag^+ is removed from C–Ag^+–C base pairs through competitive binding with Cys. Figure 10b shows a dependence of $I_{518\,nm}$ on the Cys

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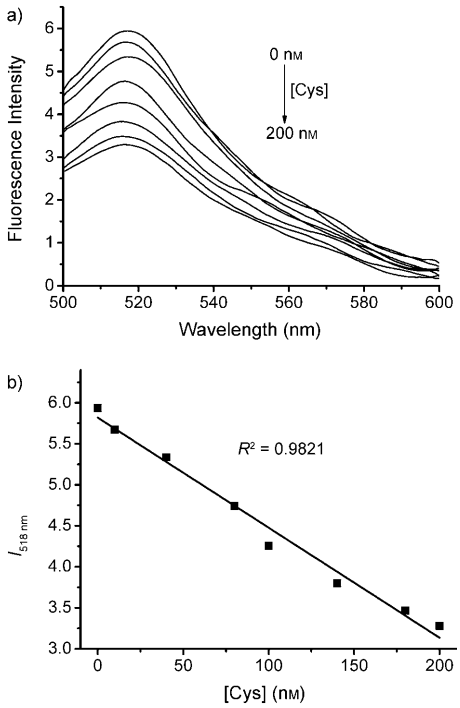

Figure 10. a) Fluorescence emission spectra of SWNT–DNA-5/DNA-4/Ag^+ (10 μL/50 nM/200 nM, λ_{ex} = 480 nm) in the absence and presence of different concentrations of Cys. b) The fluorescence intensity at 518 nm plotted against the concentration of Cys.

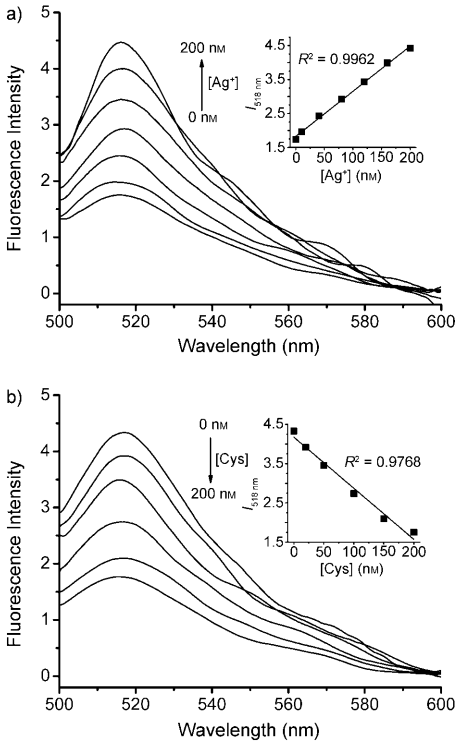

Figure 11. a) Fluorescence emission spectra of SWNT–DNA-5 conjugates with DNA-4 in the absence and presence of different concentration of Ag^+ in the second sensing cycle. The inset shows a Ag^+-concentration-dependent increase in the fluorescence intensity at λ = 518 nm. b) Fluorescence emission spectra of SWNT–DNA-5/DNA-4/Ag^+ (10 μL/50 nM/200 nM, λ_{ex} = 480 nm) in the absence and presence of different concentrations of Cys in the second sensing cycle. The inset shows a decrease in $I_{518\,nm}$ that is dependent on the Cys concentration.

concentration, which has a linear relationship (R^2 = 0.982) in the range from 0 to 200 nM and a detection limit of 17.9 nM based on a 3σ/slope, thus indicating high sensitivity for the analysis of Cys by using covalent SWNT–DNA conjugates.

Testing the reusability of the covalently linked SWNT–DNA sensor: To test the reusability of this sensing platform, we precipitated SWNT–DNA conjugates by ultracentrifugation after the first sensing of Ag^+ and Cys. After washing with deionized water and 0.5 M $NaNO_3$ by several centrifugation cycles at 13000 rpm, the as-prepared SWNT–DNA conjugates were redispersed in deionized water and were used for the second sensing cycle. Figure 11 shows the fluorescence emission spectra change of the reusable SWNT–DNA-conjugate-based sensor against Ag^+ or Cys concentration. Both of them show a linear response upon an increase of Ag^+ or Cys concentration; this demonstrates the reusability of our covalent SWNT–DNA-conjugate-based sensing platform and its superiority in practical and economical applications relative to other ion sensors.

Conclusion

In summary, we report for the first time a reusable DNA–SWNT fluorescent sensor for highly sensitive and selective detection of Ag^+ and Cys. Ag^+ can induce a duplex formation by means of C–Ag^+–C coordination chemistry; this was further confirmed by DNA melting studies. This activates the sensor fluorescence and shows high sensitivity and selectivity for Ag^+. On the other hand, Cys is a strong Ag^+ binder and can remove Ag^+ from C–Ag^+–C base pairs; this turns off the sensor fluorescence again and exhibits high sensitivity and selectivity for Cys compared to the other 19 natural amino acids. Based on these results, we have constructed a reusable fluorescent sensor by using the covalently linked SWNT–DNA conjugates according to the same sensing mechanism, and demonstrate that the covalent SWNT–DNA conjugates are reusable, simple, and economical. This assay has several important features. First, use of water-soluble carboxyl-modified SWNTs offers a convenient "mix-and-detect" approach for the rapid detection of Ag^+ in aqueous solutions. Second, use of an Ag^+-responsive oligonucleotide probe imparts extraordinarily high sensitivity and selectivity to the sensor. Third, strong binding between Cys and Ag^+ enables a "turn-off step" to be applied to the sensing of Cys with high selectivity and sensitivity. This method can in principle be used to detect other metal ions by substituting C–C base pairs with other native or artificial bases that selectively bind to other metal ions.[25,27,71–73]

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Experimental Section

Materials: SWNTs ($\phi = 1.1$ nm, purity $> 90\%$) were purchased from Aldrich (St. Louis, MO), purified as described previously by sonicating SWNTs in a 3:1 v/v solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) for 10 h at 35–40 °C, and washed with water, thereby leaving an open hole in the tube side and functionalizing the open end of SWNTs with a carboxyl group to increase their solubility in aqueous solution.[74–77] The stock solution of SWNTs (0.2 mg mL^{-1}) was obtained by sonicating the SWNTs for 8 h in pH 7.0 aqueous solution.

1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) was purchased from Alfa Aesar, sulfo-*N*-hydroxy succinimide (sulfo-NHS) was purchased from Pierce. 2-(*N*-Morpholino) ethanesulfonic acid (MES), *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), sodium dodecyl sulfate (SDS), and Triton X-100 were purchased from Sigma and used as received. Other chemicals used in this study were analytical or biochemical grade reagents.

DNA oligomers (Table 1) 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.[74,76] All experiments were carried out in aqueous HEPES buffer (10 mM HEPES 200 mM $NaNO_3$, pH 7.4) unless stated otherwise.

Ag^+ was prepared by dissolving some $AgNO_3$ in water and was diluted into a specific concentration when used. The stock solution of Cys was prepared in water, and tris-(2-carboxyethyl)phosphine (TCEP) was added to activate the thiol. All solutions were prepared with MilliQ water (18.2 MΩ cm) from a Millipore system.

Other metal salts used in this work include $BaCl_2$, $CaCl_2$, $CdCl_2$, $CoCl_2$, $CuCl_2$, $FeCl_2{\cdot}4H_2O$, $Hg(NO_3)_2$, $MgCl_2$, $MnSO_4$, $NiCl_2$, $Pb(NO_3)_2$, $ZnSO_4$, $AlCl_3$, $CrCl_3$, $EuCl_3$, and $FeCl_3{\cdot}6H_2O$.

Other amino acids used in this work include Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val.

Preparation and characterization of SWNT–DNA-5 conjugates are described in detail in the Supporting Information.

Assay for Ag^+: DNA-1 (10 nM) was mixed with SWNTs (1.5 μg mL^{-1}) for 1 h, then DNA-2 (10 nM) was added. The resulting solution was mixed with different concentrations of Ag^+ ion. The mixture was allowed to react for 1 h and then fluorescence spectra were recorded. Control experiments with the control DNA-3 were carried out under identical conditions. The selectivity for Ag^+ was confirmed by adding other metal-ion stock solutions instead of Ag^+ in a similar way. All experiments were performed at room temperature.

Assay for Cys: DNA-1 (10 nM) was mixed with SWNTs (1.5 μg mL^{-1}) for 1 h, then DNA-2 (10 nM) was added. The resulting solution was mixed with Ag^+ ion (150 nM). After 1 h, the mixture was allowed to react with different concentrations of Cys for over 2 h and then fluorescence spectra were recorded. Control experiments with the control DNA-3 were carried out under identical conditions. The selectivity for Cys was confirmed by adding other amino acid stock solutions instead of Cys in a similar way. All experiments were performed at room temperature.

Assays for Ag^+ and Cys using the reusable covalently linked SWNT–DNA-5 conjugates: To detect Ag^+, the as prepared SWNT–DNA-5 conjugates (10 μL) were diluted into 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (250 μL; 10 mM HEPES, 200 mM $NaNO_3$, pH 7.4), then DNA-4 (50 nM) was added. The resulting solution was mixed with different concentrations of Ag^+ ions. The mixture was allowed to react for 1 h and then fluorescence spectra were recorded.

To detect Cys, the as-prepared SWNT–DNA-5 conjugates (10 μL) were mixed with 50 nM DNA-4 and Ag^+ ion (200 nM). After 1 h, the mixture was allowed to react with different concentrations of Cys for over 2 h and then fluorescence spectra were recorded. All experiments were performed at room temperature.

Renewal of the SWNT–DNA-5 conjugates: After the first sensing cycle of Ag^+ and Cys, the SWNT–DNA-5 conjugates were renewed with the following processes. The sensing solution was first centrifuged for 30 min at 13000 rpm. Then, the sediments were washed with deionized water and 0.5 M $NaNO_3$ by several centrifugation cycles at 13000 rpm. After several cycles of washing, the sample was redispersed in deionized water and left to stand at room temperature for few hours, and the supernatant fraction were collected and used for the second sensing cycle.

Physical measurements: Fluorescence measurements were carried out using a JASCO FP-6500 spectrofluorometer. An excitation wavelength of 480 nm was used, and the fluorescence emission was monitored from 500 to 600 nm with the slit width for the excitation and emission of 10 nm.

UV melting experiments were carried out using a Cary 300 UV/Vis spectrophotometer equipped with a Peltier temperature control accessory. All UV melting curves were measured in a 1.0 cm-path-length cell with the same concentration of Ag^+ aqueous solution as the reference solution. Absorbance changes at 260 nm versus temperature were collected at a heating rate of 1 °C min^{-1}. Primary data were transferred to the graphics program Origin for plotting and analysis.[78]

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