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# Combination of Graphene Oxide and Thiol-Activated DNA Metallization for Sensitive Fluorescence Turn-On Detection of Cysteine and Their Use for Logic Gate Operations

*Youhui Lin, Yu Tao, Fang Pu, Jinsong Ren,* and Xiaogang Qu**

**In this work, a unique, highly sensitive and selective fluorescence turn-on approach for cysteine detection using an ensemble of graphene oxide (GO) and metallized DNA is reported. The method is based on the extraordinarily high quenching efficiency of GO and the specific interaction between cysteine and metallized DNA via robust Ag–S bonds. In the presence of GO, the dye-labeled single-stranded DNA shows weak fluorescence, while it exhibits a dramatic fluorescence increase upon the formation of the double helix through the "activated" metallized DNA by cysteine. In addition, the protocol shows excellent selectivity for cysteine over various other amino acids found in proteins. Importantly, by exploring GO–DNA interactions and the thiol-mediated DNA hybridization, our sensing system can also be utilized to design the "OR" and "INHIBIT" logic gates using cysteine and DNA as inputs. To the author's knowledge, this method is the first example of combining GO and DNA metallization to fabricate a turn-on fluorescent sensor for cysteine and logic gates.**

## 1. Introduction

Over the past two decades, there has been an explosion of interest in the use of nanomaterials for the development of novel molecular diagnostic tools.[1] These nanomaterial-based biosensors open up a new avenue for simple, sensitive, on-site analysis of specific targets. Due to the excellent optical and electronic and catalytic properties, graphene and its water-soluble derivative, graphene oxide (GO), have received much attention recently in materials science and biotechnology.[2] For instance, GO is a nanoquencher that can quench the fluorescence of various dyes through the long-range energy transfer.[3] Therefore, GO-based nanoprobe has been successfully employed for the fluorescent detection of nucleic acids,[4] proteins,[5] metal ions,[4b,6] small molecules.[4b] Recently, we have found that carboxyl-modified graphene oxide possess intrinsic peroxidase-like activity, which could be utilized in glucose assay[7] and cancer cell detection.[8] Although these GO-based materials hold great promise in nanotechnology and nanomedicine, the exploring of further functions of these novel 2D nanomaterials still remains a big challenge in this field.

As a thiol-containing amino acid, cysteine has attracted much attention in recent years due to its vital biological functions.[9] It plays a valuable role in the human body by providing a modality for the intramolecular crosslinking of proteins.[10] Meanwhile, excess cysteine has been associated with neurotoxicity.[11] It has also been proven to act as the physiological regulator in various diseases, and hence, it is used in medicine and in special nutrition preparations.[12] Given the wide range of physiological and pathophysiological effects, the precise cysteine determination is of great clinical importance.a variety of methods, including liquid chromatography, flow injection, voltammetry, and capillary zone electrophoresis have been developed for the determination. However, most of the approaches involve cumbersome laboratory procedures, require expensive and sophisticated instrumentation, and have relatively high limit of detection. With the development in nanotechnology, new methods have been used for the detection of cysteine.[10,13] For instance, Sudeep et al. reported a strategy for the selective detection of micromolar concentrations of cysteine by exploiting the interplasmon coupling in gold nanorods. However, low sensitivity limited the effectiveness of such strategy.[13b] Carbon nanotube-based fluorescent sensor for cysteine detection was also developed on the basis of the interaction between cysteine and Ag(I).[13a] However, the turn-off assay might significantly compromise their specificity since other quenchers or environmental stimulus might also lead to fluorescence quenching and give ''false positive'' results.

Recently, deoxyribonucleic acid (DNA) has become an extremely favorable tool in nanotechnology and materials science owing to its remarkable molecular recognition properties and structural features.[14] The use of DNA as template appears to be one of the most promising avenues available for fabricating metal nanomaterials and shown wide range of potential applications[14a,15a,16] DNA is rich of phosphate groups, amino groups and heterocyclic nitrogen atoms, it offers multiple binding sites for several metal

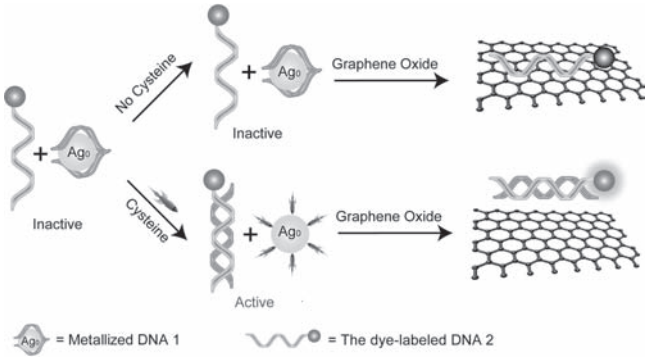
Y. Lin, Y. Tao, F. Pu, Prof. J. Ren, Prof. X. Qu
Laboratory of Chemical Biology and State Key Laboratory of Rare Earth Resources Utilization
Changchun Institute of Applied Chemistry
Chinese Academy of Sciences
Changchun 130022, P.R. China
E-mail: jren@ciac.jl.cn; xqu@ciac.jl.cn
Y. Lin, Y. Tao, F. Pu, Prof. J. Ren, Prof. X. Qu
Graduate School of the Chinese Academy of Sciences
Beijing 100039, P.R. China

DOI: 10.1002/adfm.201101584

ions, and these localized cations could be reduced to form metallic cluster that follow the contour of the DNA template.[15] Braun and co-workers recently reported that DNA metallization could convert the insulating DNA molecules into highly conductive wires, which allowed the application of DNA to the construction of functional nanoelectronic circuits.[15a] Meanwhile, methodology to control the hybridization process has become increasingly important to modulate the functional DNA nanostructures.[17,18] Some strategies have been realized by using strand displacement,[19] artificially synthesized DNA[20] and molecular glue[21] etc. Recently, it has been demonstrated that that thiols have strong affinity with the noble metal surfaces, such as gold nanoparticles[22] and silver nanoparticles[13c,23]. The chemisorbed thiols onto the noble-metal surface have been widely used to fabricate self-assembled monolayers, stably anchor DNA or modify surface properties by ligand exchange.[23] Inspires by these phenomena, we expected that robust interactions between thiols and metallized DNA could provide a novel strategy for controlling DNA hybridization. Modulating metallized DNA hybridization by thiols could provide an additional simple approach for controlling hybridization without a concomitant production of "waste DNA"[19] or the sophisticated synthesis of artificially DNA and small molecules[20,21]. Herein, we demonstrated a new concept for developing a simple, reliable, highly sensitive and selective fluorescence turn-on strategy to detect cysteine by taking advantage the extraordinarily high quenching efficiency of GO and thiol-mediated DNA hybridization. Furthermore, our sensing system can also be utilized to design the "OR" and "INHIBIT" logic gates using cysteine and DNA as inputs.

## 2. Results and Discussion

The working principle of our new assay for cysteine is schematically represented in **Scheme 1**. The detection system consists of metallized DNA, GO and fluorescent DNA probe. The synthesis of metallized DNA 1 can be achieved through the use of single-stranded DNA 1 (ssDNA) as template to control the metal deposition. The dye-labeled DNA 2 strand is designed as the complementary sequence of DNA 1. In the absence of cysteine, the attachment of silver deposition on the DNA 1 scaffolds would disrupt the hybridization of nucleic acids with the dye-labeled DNA 2. When the above solution is incubated with a GO nanosheet, the fluorescence of the dye-labeled DNA 2 can be completely quenched due to the non-covalent π-stacking interaction between GO and ssDNA. In the presence of cysteine, it exhibits intriguing reactivity with metallized DNA 1 by forming Ag-S bonds, which is conjugated much more strongly than DNA, and subsequently liberates DNA 1 from silver surface by ligand exchange.as a result, the "inactive" metallized DNA 1 originally stabilized or protected by silver deposition will become active, and then the binding between the "activated"




**Scheme 1.** Schematic illustration of the strategy for sensitive turn-on fluorescent detection of cysteine by using the ensemble of graphene oxide (GO), thiol-activatable metallized DNA 1 and the dye-labeled DNA 2.

DNA 1 and the dye-labeled DNA 2 will alter the conformation of the fluorescent DNA probe. Upon addition of GO, the fluorescence of such a mixture largely remained because GO possesses different adsorption affinity for ssDNA and dsDNA. Since the fluorescence intensity of the dye-labeled DNA is significantly enhanced after the metallized DNA 1 is "activated" by cysteine, we then sought to take advantage of the observed fluorescence change to develop novel fluorescence turn-on assay for cysteine.

GO was prepared according to a modified Hummer's method.[24] After sonication for 1 h in water, GO was mostly single-layered with a topographic height of ~1.0 nm according to atomic force microscopy (AFM) characterization (**Figure 1**a, b).[2e] The chemically synthesized GO was readily water-dispersible due to the presence of suspended hydroxyl, epoxy and carboxylic groups at the surface, which was confirmed by Fourier transform

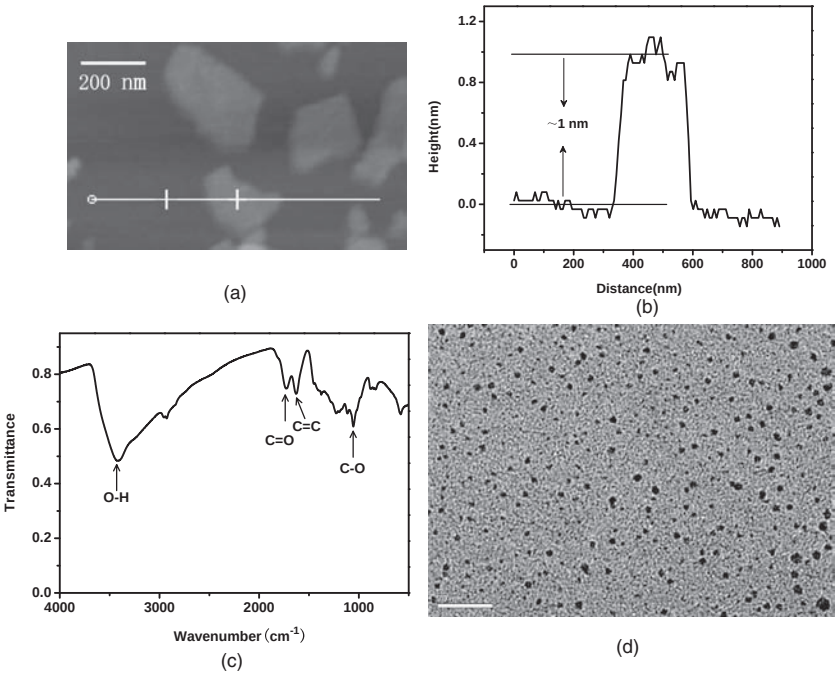



**Figure 1.** a) AFM image and (b) height profile of GO deposited on mica substrates. c) Fourier transform infrared (FTIR) spectrum of GO. d) TEM image of DNA-templated silver deposition. Scale bars, 20 nm.

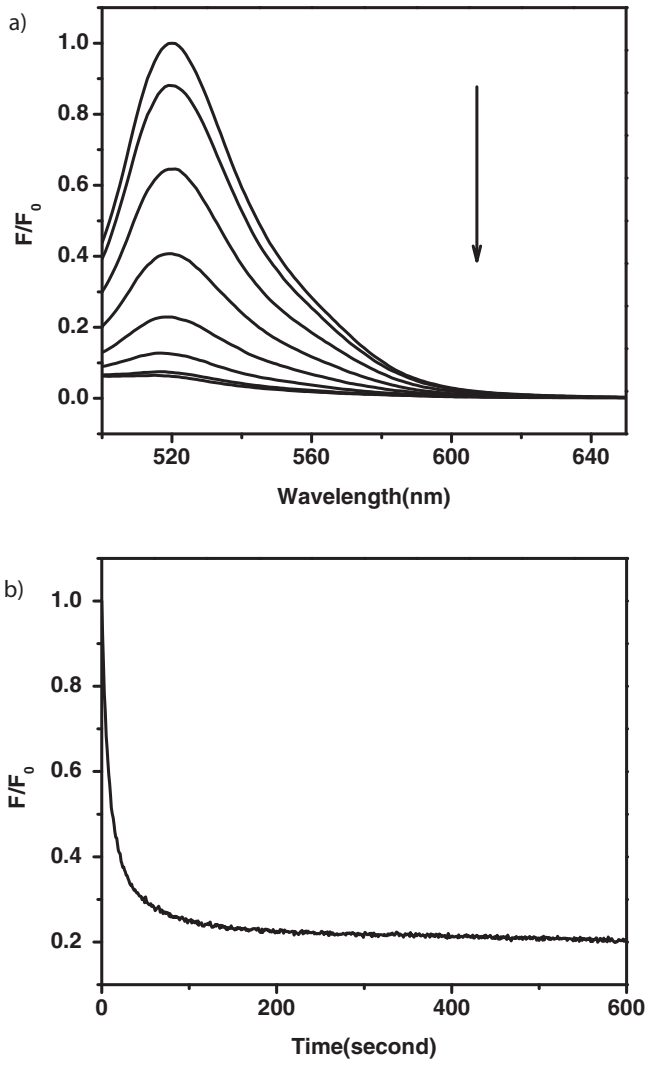



**Figure 2.** a) Fluorescence quenching of the dye-labeled DNA 2 of 25 nM in the absence (black) and presence of GO with a series of concentrations (top to bottom: 1, 3, 5, 8, 10, 13, 15 μg/mL). b) Kinetic study for the fluorescence change at 525 nm of the dye-labeled DNA 2 (25 nM) in the presence of GO (15 μg/mL).

infrared (FTIR) spectroscopy. Several characteristic peaks of functional groups containing oxygen were observed in the FTIR spectrum of GO, including peaks at 1725 and 1056 $cm^{-1}$ that resulted from C=O and C-O stretching, respectively (Figure 1c). The metallized DNA was then synthesized by reduction of Ag(I) bound to the ssDNA with $NaBH_4$. The resulting solutions were studied by transmission electron microscopy (TEM) imaging.as shown in Figure 1d, DNA-templated silver deposition is monodispersed well due to the stronger electrostatic repulsion than van der Waals attraction between DNA-stabilized nanoparticles.

Next, the fluorescence quenching ability of GO was evaluated through fluorescent measurements of the dye-labeled DNA 2 in the presence of GO. In consistent with the previous reports,[4a,4b] the fluorescence of the FAM-tagged ssDNA probe can be effectively quenched by GO, which arose from the strong adsorption of the ssDNA strand on GO and highly efficient long-range energy transfer from the dye to GO (**Figure 2**a). Meanwhile, the quenching reaction reached equilibrium only after several minutes indicating the quenching kinetics were fairly fast (Figure 2b). In contrast, when the dye-labeled DNA 2 was hybridized with its complementary DNA 1, the fluorescence of the FAM-labeled DNA 2 largely remained in the presence of GO (**Figure 3**a, curve 1), which implied that dsDNA bound to GO with much lower affinity than ssDNA under the experimental conditions employed.

In order to prove that DNA metallization can disrupt its hybridization, the fluorescence response of the dye-labeled DNA 2 to metallized DNA 1 was then carried out in the presence of GO. Silver deposition in different molar ratios of Ag(I) to DNA 1 were produced by reduction of Ag(I) bound to the ssDNA with $NaBH_4$. The fluorescence intensity of the FAM-tagged DNA

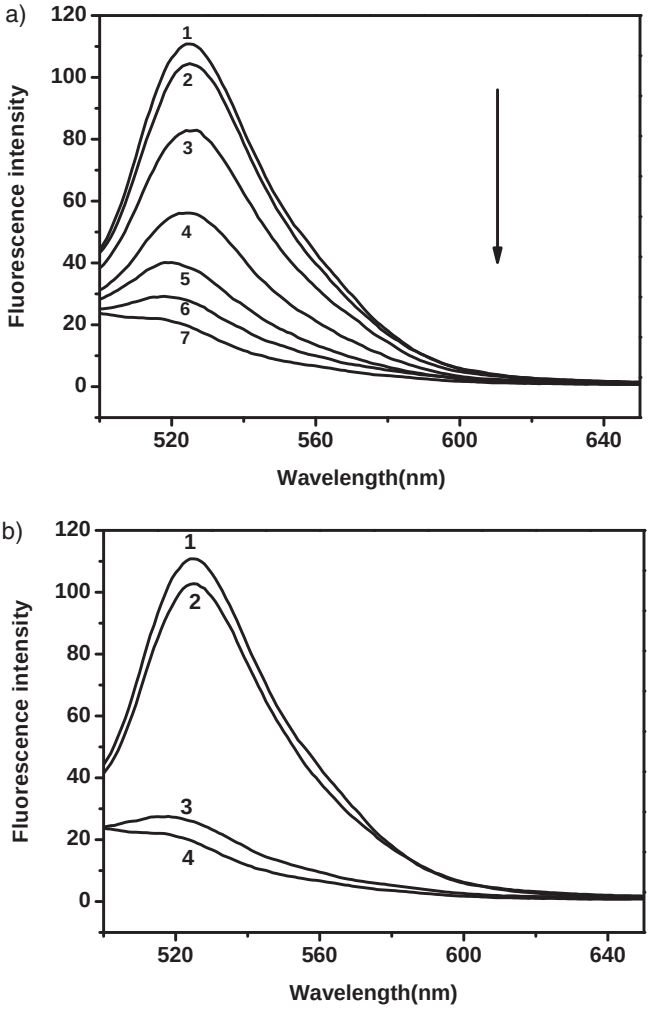



**Figure 3.** a) Fluorescence emission response profiles of the dye-labeled DNA 2 (25 nM) to the metallized DNA 1 (50 nM) in different molar ratios of Ag(I) to DNA 1 in the presence of GO (top to bottom: 0, 5:1, 10:1, 20:1, 40:1, 55:1). Curve 7 at the bottom is fluorescence spectra of the dye-labeled DNA 2 in the presence of GO. b) Fluorescence spectra of solutions containing 1) The dye-labeled DNA 2/DNA 1/GO, 2) The dye-labeled DNA 2/metallized DNA 1/cysteine/GO, 3) The dye-labeled DNA 2/metallized DNA 1/GO and 4) The dye-labeled DNA 2/GO ([The dye-labeled DNA 2] = 25 nM, [Metallized DNA] = 50 nM, [Cysteine] = 1 μM, [GO] = 15 μg/mL).

probe decreased gradually with increasing Ag(I)/DNA molar ratio in the presence of GO (Figure 3a). The fluorescence signal of the mixture is close to the emission intensity of the FAM-tagged ssDNA probe alone at a molar ratio of Ag(I) to DNA equal to 55:1, which supports the notion that DNA hybridization can be disrupted by silver deposition on DNA scaffold. In a controlled experiment, by simple mixing DNA 1, the preformed silver nanoparticles and the dye-labeled DNA 2, the final fluorescence value is very close to the emission intensity of the dye-labeled DNA 2/DNA 1/GO complex (Figure S1), which indicates that the silver nanoparticles existing only in solution can not inhibit the DNA hybridization and affect the fluorescence of the mixture.

We then used the circular dichroism (CD) to study DNA release and the kinetic of ligand exchange. Previous studies have shown that silver deposition on DNA 1 scaffolds can change CD signal of DNA.[25] As shown in Figure S2a, the CD spectra of DNA alone consist of a positive band at 270 nm, whereas the intensity at 270 nm became negative after the formation of silver deposition. Importantly, upon addition of cysteine, the CD spectra of the mixture at the positive band were very similar to that obtained in the bare DNA 1, which indicated that DNA 1 can be effectively released. Meanwhile, the process has almost reached equilibrium within 10 minutes indicating the kinetic of ligand exchange was fast (Figure S2b). More importantly, our experiments verified that addition of cysteine to the mixture of metallized DNA 1/the dye-labeled DNA 2 did turn on the fluorescence of the dye-labeled DNA effectively in the presence of GO.as shown in Figure 3b, a significant increase in fluorescence intensity was observed upon addition of cysteine. This phenomenon suggests that the hybrid nanomaterials composed of metallized DNA, GO and the FAM-tagged ssDNA can be used as fluorometric indicators to detect cysteine. Moreover, the final fluorescence value is very close to the emission intensity expected for the dye-labeled DNA 2 bound to the same amount of DNA 1, which further confirmed that DNA 1 can be effectively released by cysteine from the silver surface.

In addition to fluorescence spectra, the stabilization of the metallized DNA 1/DNA 2 complex was further confirmed by UV melting analysis.as can be seen in **Figure 4**, duplex DNA between metallized DNA 1 and DNA 2 was not formed because no transition was observed in its melting curve (Figure 4, curve 1). For comparison, addition of cysteine to the mixture of metallized DNA 1/DNA 2 (Figure 4, curve 2) led to an obvious transition which has a similar effect of that obtained in the DNA 1/DNA 2 complex without metallization (Figure 4, curve 3). DNA melting results clearly indicate that the DNA hybridization can be effectively inhibited by DNA metallization and cysteine can induce stable duplex formation between the "inactive" metallized DNA 1 and DNA 2. Therefore, the DNA melting results are fully consistent with the CD and fluorescence results, and strongly support our findings and the protocol modulating metallized DNA hybridization by thiols may be an additional simple approach for controlling hybridization without a concomitant production of "waste DNA"[19] or the sophisticated synthesis of artificially DNA and small molecules[20,21].

To further assess the sensitivity of this sensing system, the fluorescence changes were monitored upon addition of

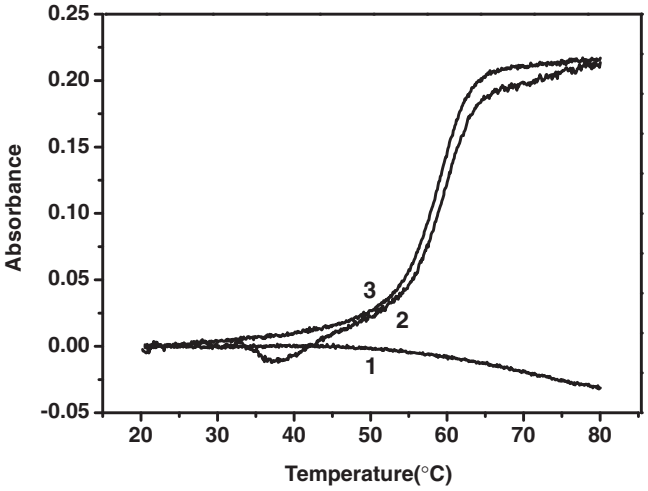



**Figure 4.** Thermal melting profiles of solutions containing 1) The metallized DNA 1/DNA 2 complex, 2) The metallized DNA 1/DNA 2/cysteine complex, 3) The DNA 1/DNA 2 complex ([DNA 1] = 2.5 μM, [Metallized DNA 1] = 2.5 μM, [DNA 2] = 2.5 μM, [Cysteine] = 100 μM). The absorbance at 260 nm was measured in 10 mM Tris–$HNO_3$ buffer (pH 7.4) containing 100 mM $NaNO_3$ and 5 mM $KNO_3$.

increasing concentrations of cysteine.as shown in **Figure 5**a, the fluorescent intensity increased gradually when the concentration of cysteine was raised from 0 to $1 \times 10^{-6}$ M, thus indicating that the release of DNA 1 is highly dependent on the concentration of cysteine. The fluorescence intensity at 525 nm (I525) was plotted as a function of cysteine concentration and a good linear relationship between I525 and cysteine concentration over the range of 0–$1 \times 10^{-6}$ M was obtained (Figure 5a, inset). The detection limit for analyzing cysteine corresponded to 2 nM, which is better than or at least comparable to the values obtained by those previous turn-off assays.[13a,26] More importantly, the turn-on sensing mode offered an additional advantage to efficiently reduce the likelihood of a false positive signal. In addition, this method could also be expended to assay other thiol-containing biomolecules, such as the non-proteinogenic amino acid of homocysteine, the small peptide of glutathione (Figure S3).[13c,22]

For an excellent assay, high specificity is a matter of necessity. To study the selectivity for cysteine determination with current approach, the fluorescent signal of various amino acids found in proteins were measured and compared with that of cysteine at the same concentration ($1 \times 10^{-6}$ M). The results are shown in Figure 5b, it can be seen that only cysteine exhibited a drastic increase in the fluorescence intensity, whereas no obvious enhancement was induced by other amino acids. The excellent selectivity is mainly attributed to the high affinity binding between cysteine and metallized DNA via Ag-S bonds. In addition, DNA appears to be a strong enough binder of the silver surface to effectively compete against other 19 amino acids that lack free thiol units, so the selectivity is further enhanced. To further confirm the mechanism of this turn-on response, we then performed controlled experiments to ensure that the excellent selectivity was attributed to the specific interaction between the Ag and the thiol group of cysteine..as

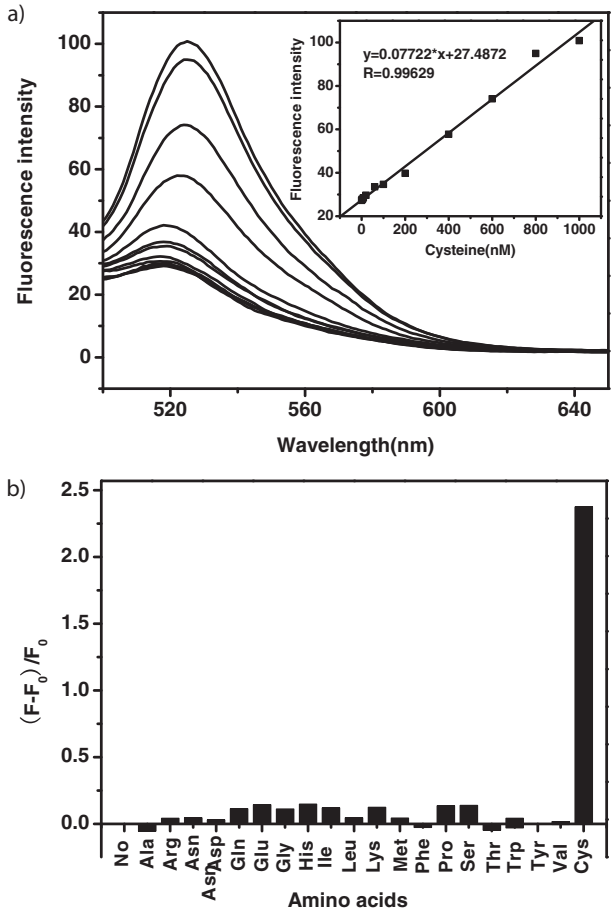
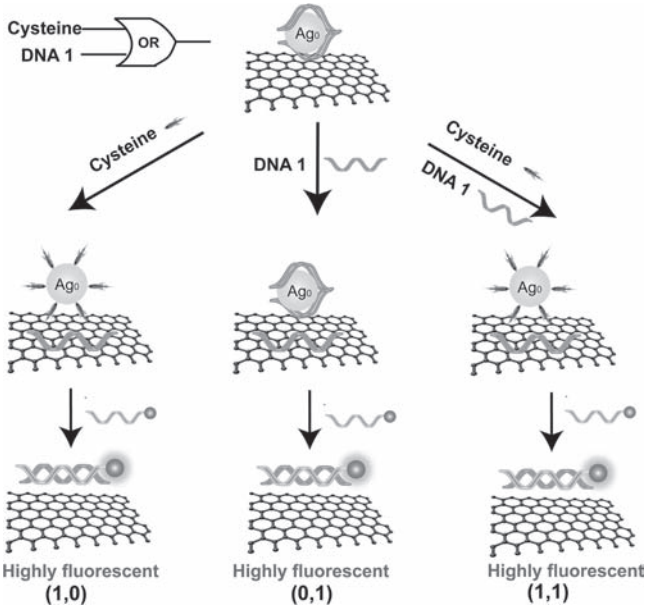



**Figure 5.** a) Fluorescence emission spectra of the dye-labeled DNA 2/metallized DNA 1 complex with the cysteine concentration in the range from 0 to $1.0 \times 10^{-6}$ M in the presence of GO. The inset displays linear plots of fluorescence intensity measured at 525 nm as a function of the cysteine concentration. b) Fluorescence emission response profiles of the dye-labeled DNA 2/metallized DNA 1 complex toward amino acids (1 μM). ([The dye-labeled DNA 2] = 25 nM, [Metallized DNA 1] = 50 nM, [GO] = 15 μg/mL).

can be seen in Figure S4, dramatic fluorescence enhancement was observed in the presence of cysteine, while no significant change occurred in the sample pretreated with thiol-blocking reagent N-ethyl-maleimide (NEM)[27] or NEM alone. The results demonstrated that the observed fluorescence enhancement was actually caused by the robust Ag-thiol interaction.

Besides using the ensemble of GO and DNA metallization for sensitive fluorescent detection of cysteine, the sensing system can also be utilized to design the logic gates using cysteine and DNA as inputs. Logic gates provide the functional units of computers, which are capable of performing Boolean logic.[28] Nucleic acids have recently been recognized as an attractive building material for the construction of logic gates by taking advantage of the programmable sequence-specific recognition property.[28c,28d,29] As far as we know, the ensemble of GO and DNA metallization, and the specific interactions of cysteine with metallized DNA for logic gate operations, have not been reported.




**Scheme 2.** an "OR" logic gate system consisting of metallized DNA 1, GO and fluorescent DNA 2 probe, with DNA 1 and cysteine as inputs and the fluorescent signal as output.

Based on the fact that the dye-labeled DNA 2 can hybridize with either its complementary oligonucleotide strand DNA 1, or the "activated" metallized DNA 1 by cysteine, we designed an OR logic gate using DNA 1 and cysteine as inputs that operate through the ensemble of metallized DNA 1, GO and fluorescent DNA 2 probe **(Scheme 2)**. With respect to input, we defined the presence of DNA 1 or cysteine as 1 and their absence as 0. For output, we define the high fluorescent signal and weak fluorescent signal as 1 and 0, respectively. With no input, the dye-labeled DNA 2 is adsorbed on the GO surface, and its fluorescence is quenched **(Figure 6**a, curve 1**)**. In the presence of either or both inputs (1/0, 0/1, 1/1), the binding between the dye-labeled DNA 2 and DNA 1 (or the "activated" metallized DNA 1 by cysteine) will alter the conformation of the fluorescent DNA probe, and disturb the interaction between the dye-labeled DNA 2 and GO.as a result, the dye-labeled DNA 2 exhibits a dramatic fluorescence increase when it forms a double helix (Figure 6a, curve 2, 3, 4). By observing the fluorescence intensity at 525 nm, the value of I525 for the inputs (1/0, 0/1, 1/1) is much higher than that in the absence of inputs (0/0) (Figure 6b).

When the "activated" DNA 1 was firstly hybridized with the label-free DNA 2 (without the modification of dyes), the interaction of the "activated" DNA 1 with the dye-labeled DNA 2 will be inhibited or blocked. Based on these observations, an INHIBIT logic gate can be created by employing DNA 2 as one input, the addition of cysteine as another input, and the fluorescent signal was defined as output **(Scheme 3)**. The addition of DNA 2 (input = 0/1) cannot lead to the significant fluorescence enhancement of the dye-labeled DNA 2 because duplex DNA was not formed (**Figure 7**a, curve 2). In the presence of both DNA 2 and cysteine, the "activated" DNA 1 formed a double helix with the label-free DNA 2, thus preventing DNA hybridization between the "activated" DNA 1 and the

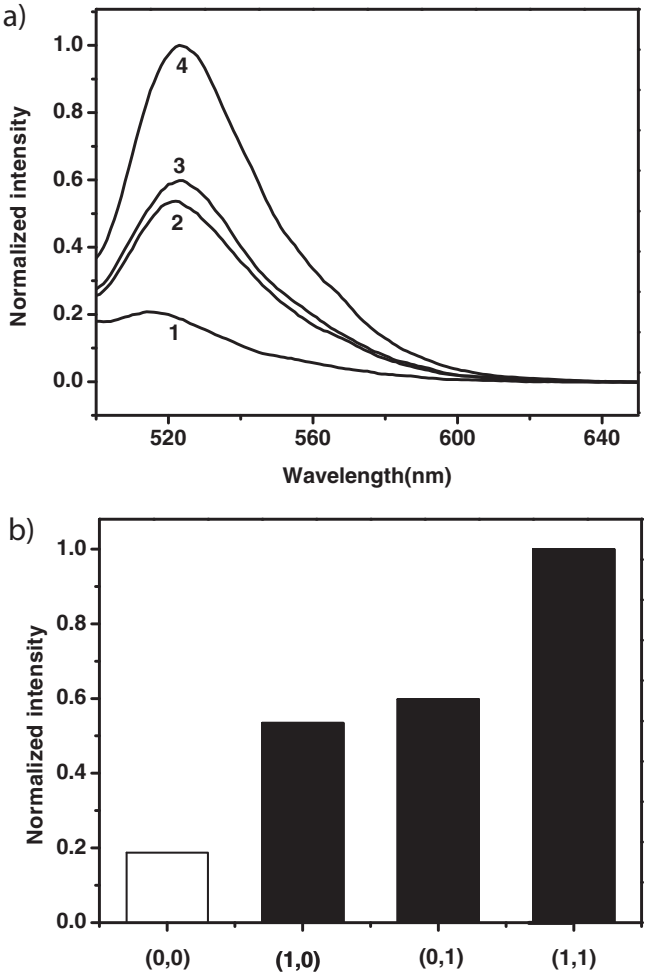
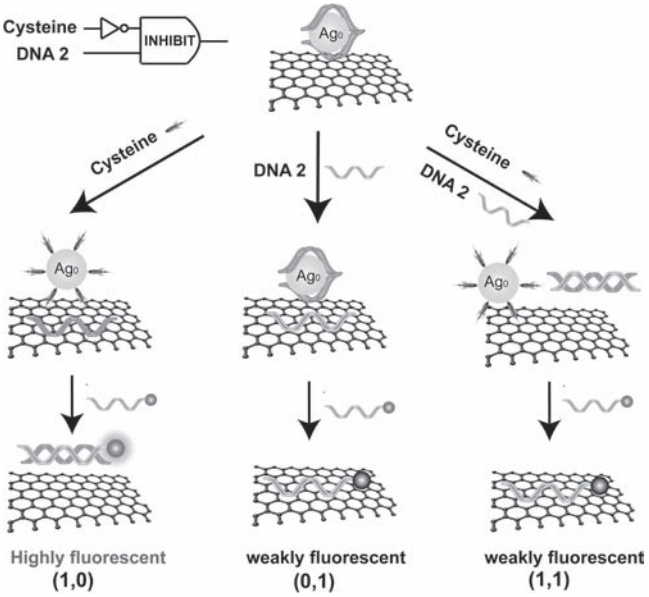



**Figure 6.** a) Fluorescence spectra of the "OR" logic gate system depicted in Scheme 2 and activated by the following inputs: 1) No cysteine, no DNA 1 (0, 0); 2) Cysteine, no DNA 1 (1, 0); 3) No cysteine, DNA 1 (0, 1); 4) Cysteine, DNA 1 (1, 1). ([DNA 1] = 50 nM, [Cysteine] = 2 μM) All systems consisted of the dye-labeled DNA 2 (25 nM), metallized DNA 1 (50 nM), GO (15 μg/mL). b) The corresponding fluorescence response of the "OR" logic gate systems at 525 nm in the presence of different input signals. White and black represent logic value 0 and 1, respectively.




**Scheme 3.** an "INHIBIT" logic gate system consisting of metallized DNA 1, GO and fluorescent DNA 2 probe, with DNA 2 and cysteine as inputs and the fluorescent signal as output.

dye-labeled DNA 2. Only the addition of cysteine (input = 1/0) can cause the high fluorescent signal (output = 1) (Figure 7a, curve 1, 3, 4). By observing the fluorescence intensity at 525 nm, much higher value of I525 for the presence of cysteine (input = 1/0) than those for inputs (0/0, 1/0, 1/1) (Figure 7b). In addition, an INHIBIT gate using thiol-blocking reagent NEM and cysteine as inputs was also designed (Figure S5). For output, we define the high fluorescent signal and weak fluorescent signal as 1 and 0, respectively. When only cysteine were added, the output was 1, otherwise it was 0 (Figure S5).

## 3. Conclusions

In summary, we have demonstrated a novel fluorescence turn-on assay for cysteine by exploring the combination of graphene oxide and thiol-activatable metallized DNA. The protocol relies on the large fluorescence enhancement resulting from the cysteine-mediated DNA hybridization through robust Ag–S bonds and the different binding affinities of GO to single- and double-stranded DNA. The detection limit is better than or at least comparable to the values obtained by those previous turn-off assays. In addition, the turn-on sensing mode offered an additional advantage to efficiently reduce the likelihood of a false positive signal. In addition, the protocol shows excellent selectivity for cysteine over various other amino acids. More importantly, our system can also be utilized to develop "OR" and "INHIBIT" logic gates using cysteine and DNA as inputs. This proof of concept is an important step forward in obtaining DNA-based hybrid nanomaterials with versatile functionalities and reactivities, and will be highly beneficial for a wide range of applications including clinical diagnostics, biosensors, process control, nanomechanical and electronic applications.

## 4. Experimental Section

*Materials and Instrumentation*: All oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). The sequences are as follows: the ssDNA sequence DNA 1 is 5′-AAC AAA CAA GGG AAG AAA GAA-3′, and the complementary ssDNA sequence DNA 2 or the dye-labeled DNA 2 is 5′-TTC TTT CTT CCC TTG TTT GTT-3′, or 5′-FAM-TTC TTT CTT CCC TTG TTT GTT-3′ (FAM = fluorescein-based dye). Graphite was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Alfa Aesar. L-cysteine was obtained from Sigma-Aldrich.all other reagents were of analytical reagent grade, and used as received. Nanopure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiment.

The fluorescence spectra were recorded using a JASCO FP6500 spectrophotometer (JASCO International Co., LTD., Tokyo, Japan). The concentration of DNA was determined using a JASCO V-550 UV/Visible spectrophotometer, equipped with a temperature-controlled

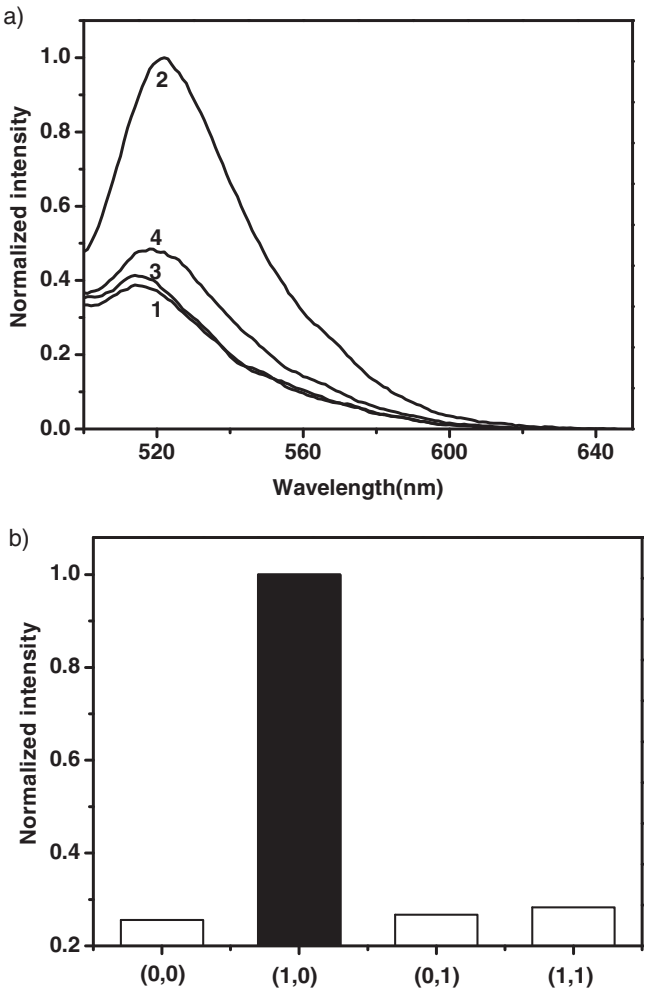



**Figure 7.** a) Fluorescence spectra of the "INHIBIT" logic gate system depicted in Scheme 2 and activated by the following inputs: 1) No cysteine, no DNA 2 (0, 0); 2) Cysteine, no DNA 2 (1, 0); 3) No cysteine, DNA 2 (0, 1); 4) Cysteine, DNA 2 (1, 1). ([DNA 2] = 50 nM, [Cysteine] = 2 μM) All systems consisted of the dye-labeled DNA 2 (25 nM), metallized DNA 1 (50 nM), GO (15 μg/mL). b) The corresponding fluorescence response of the "INHIBIT" logic gate systems at 525 nm in the presence of different input signals. White and black represent logic value 0 and 1, respectively.

cuvette holder. The DNA melting studies was carried out on a Cary 300 UV/Vis spectrophotometer equipped with a Peltier temperature control accessory. The circular dichroism (CD) spectra were carried out on a JASCO J-810 spectropolarimeter equipped with a temperature controlled water bath. The optical chamber of CD spectrometer was deoxygenated with dry purified nitrogen (99.99%) for 45 min before use and kept the nitrogen atmosphere during experiments. TEM images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV.aFM measurements were performed by using a Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). Samples for AFM images were prepared by depositing a dispersed GO/$H_2O$ solution onto a freshly cleaved mica surface and allowing them to dry in air.

*Preparation of GO and DNA metallization*: GO was synthesized from graphite powder based on the Hummer's method [22]. The ssDNA-templated silver deposition was synthesized by reduction of $AgNO_3$ with $NaBH_4$ in the presence of DNA. Briefly, DNA 1 (2 μM) was mixed with excess of $AgNO_3$ (55:1 $AgNO_3$/DNA molar ratio).after 15 min incubation, a freshly prepared $NaBH_4$ ($AgNO_3$ and $NaBH_4$ were mixed in a 1:5molar ratio) was dropped into the above aqueous solution under vigorous stirring.after mixture, the resulting colloidal silver solution was stirred for another 30 min.

*The Fluorescence Assay of Cysteine Detection*: In a typical procedure, metallized DNA 1 ($5 \times 10^{-8}$ M, 400 μL) was incubated with different concentration of cysteine or other amino acids (5 μL) in 10 mM Tris–$HNO_3$ buffer (100 mM $NaNO_3$, 5 mM $KNO_3$, pH = 7.4). The mixture equilibrated for 0.5 h at room temperature, and then the above solution was mixed with the fluorescent DNA 2 probe ($1 \times 10^{-6}$ M, 5 μL). Finally, GO (0.6 mg/ml, 10 μL) was added and equilibrated for 10 min before spectral measurements. The solutions were excited at 490 nm, and emission spectra were collected from 500 to 650 nm.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Acknowledgements

Financial support was provided by the National Basic Research Program of China (Grant 2011CB936004) and the National Natural Science Foundation of China (Grants 20831003, 90813001, 20833006, 90913007, 21072182).

Received: July 12, 2011
Revised: August 23, 2011
Published online: September 22, 2011

[1] a) N. L. Rosi, C.a. Mirkin, *Chem. Rev.* **2005**, *105*, 1547; b) W. Yang, K. R. Ratinac, S. P. Ringer, P. Thordarson, J. J. Gooding, F. Braet, *Angew. Chem., Int. Ed.* **2010**, *49*, 2114; c) S. Song, Y. Qin, Y. He, Q. Huang, C. Fan, H.-Y. Chen, *Chem. Soc. Rev.* **2010**, *39*, 4234.

[2] a) Y. Zhu, S. Murali, W. Cai, X. Li, J. W. Suk, J. R. Potts, R. S. Ruoff, *Adv. Mater* **2010**, *22*, 3906; b) S. Kim, S. H. Ku, S. Y. Lim, J. H. Kim, C. B. Park, *Adv. Mater* **2011**, *23*, 2009; c) Y. Xu, Q. Wu, Y. Sun, H. Bai, G. Shi, *ACS Nano* **2010**, *4*, 7358; d) D. R. Dreyer, S. Park, C. W. Bielawski, R. S. Ruoff, *Chem. Soc. Rev.* **2010**, *39*, 228; e) S. Stankovich, D. A. Dikin, G. H. B. Dommett, K. M. Kohlhaas, E. J. Zimney, E.A. Stach, R. D. Piner, S. T. Nguyen, R. S. Ruoff, *Nature* **2006**, *442*, 282.

[3] a) R. S. Swathi, K. L. Sebastian, *J. Chem. Phys.* **2008**, *129*, 054703; b) R. S. Swathi, K. L. Sebastian, *J. Chem. Phys.* **2009**, *130*, 086101.

[4] a) C.-H. Lu, H.-H. Yang, C.-L. Zhu, X. Chen, G.-N. Chen, *Angew. Chem., Int. Ed.* **2009**, *48*, 4785; b) S. He, B. Song, D. Li, C. Zhu, W. Qi, Y. Wen, L. Wang, S. Song, H. Fang, C. Fan, *Adv. Funct. Mater.* **2010**, *20*, 453; c) K. P. Loh, J. Balapanuru, J. X. Yang, S. Xiao, Q. L. Bao, M. Jahan, L. Polavarapu, J. Wei, Q. H. Xu, *Angew. Chem., Int. Ed.* **2010**, *49*, 6549.

[5] a) D. H. Min, H. Jang, Y. K. Kim, H. M. Kwon, W. S. Yeo, D. E. Kim, *Angew. Chem., Int. Ed.* **2010**, *49*, 5703; b) J. Li, C.-H. Lu, Q.-H. Yao, X.-L. Zhang, J.-J. Liu, H.-H. Yang, G.-N. Chen, *Biosens. Bioelectron.* **2011**, *26*, 3894; c) X. H. Wang, C. Y. Wang, K. G. Qu, Y. J. Song, J. S. Ren, D. Miyoshi, N. Sugimoto, X. G. Qu, *Adv. Funct. Mater.* **2010**, *20*, 3967.

[6] Y. Q. Wen, F. F. Xing, S. J. He, S. P. Song, L. H. Wang, Y. T. Long, D. Li, C. H. Fan, *Chem. Commun.* **2010**, *46*, 2596.

[7] Y. Song, K. Qu, C. Zhao, J. Ren, X. Qu, *Adv. Mater* **2010**, *22*, 2206.

[8] Y. J. Song, Y. Chen, L. Y. Feng, J. S. Ren, X. G. Qu, *Chem. Commun* **2011**, *47*, 4436.

[9] F. Pu, Z. Huang, J. Ren, X. Qu, *Anal. Chem.* **2010**, *82*, 8211.
[10] J. S. Lee, P. a. Ulmann, M. S. Han, C.a. Mirkin, *Nano Lett.* **2008**, *8*, 529.
[11] R. Janaky, V. Varga, A. Hermann, P. Saransaari, S. S. Oja, *Neurochem. Res.* **2000**, *25*, 1397.
[12] H. Xu, M. Hepel, *Anal. Chem.* **2011**, *83*, 813.
[13] a) C. Zhao, K. G. Qu, Y. J. Song, C. Xu, J. S. Ren, X. G. Qu, *Chem. Eur. J* **2010**, *16*, 8147; b) P. K. Sudeep, S. T. S. Joseph, K. G. Thomas, *J. Am. Chem. Soc.* **2005**, *127*, 6516; c) B. Hu, Y. Zhao, H.-Z. Zhu, S.-H. Yu, *ACS Nano* **2011**, *5*, 3166.
[14] a) H. a. Becerril, A. T. Woolley, *Chem. Soc. Rev.* **2009**, *38*, 329; b) D. Li, S. Song, C. Fan, *Acc. Chem. Res.* **2010**, *43*, 631.
[15] a) E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **1998**, *391*, 775; b) C. F. Monson, A. T. Woolley, *Nano Lett.* **2003**, *3*, 359; c) L. Berti, A. Alessandrini, P. Facci, *J. Am. Chem. Soc.* **2005**, *127*, 11216.
[16] a) J. Richter, R. Seidel, R. Kirsch, M. Mertig, W. Pompe, J. Plaschke, H. K. Schackert, *Adv. Mater* **2000**, *12*, 507; b) C. T. Wirges, J. Timper, M. Fischler, A. S. Sologubenko, J. Mayer, U. Simon, T. Carell, *Angew. Chem., Int. Ed.* **2009**, *48*, 219; c) G.a. Burley, J. Gierlich, M. R. Mofid, H. Nir, S. Tal, Y. Eichen, T. Carell, *J. Am. Chem. Soc.* **2006**, *128*, 1398; d) K. Keren, R. S. Berman, E. Braun, *Nano Lett.* **2004**, *4*, 323; e) M. Mertig, L. C. Ciacchi, R. Seidel, W. Pompe, A. De Vita, *Nano Lett.* **2002**, *2*, 841; f) M. Fischler, U. Simon, H. Nir, Y. Eichen, G. a. Burley, J. Gierlich, P. M. E. Gramlich, T. Carell, *Small* **2007**, *3*, 1049; g) J. T. Petty, J. Zheng, N. V. Hud, R. M. Dickson, *J. Am. Chem. Soc.* **2004**, *126*, 5207.
[17] C. Dohno, K. Nakatani, *Chem. Soc. Rev.* **2011** DOI: 10.1039/C1CS15062F.
[18] a) N. C. Seeman, *Nature* **2003**, *421*, 427; b) F. C. Simmel, W. U. Dittmer, *Small* **2005**, *1*, 284; c) H. Yan, X. Zhang, Z. Shen, N. C. Seeman, *Nature* **2002**, *415*, 62; d) J. Sharma, R. Chhabra, A. Cheng, J. Brownell, Y. Liu, H. Yan, *Science* **2009**, *323*, 112.
[19] U. Feldkamp, C. M. Niemeyer, *Angew. Chem., Int. Ed.* **2008**, *47*, 3871.
[20] a) H. asanuma, T. Ito, T. Yoshida, X. Liang, M. Komiyama, *Angew. Chem., Int. Ed.* **1999**, *38*, 2393; b) G. Mayer, A. Heckel, *Angew. Chem., Int. Ed.* **2006**, *45*, 4900.
[21] C. Dohno, S.-n. Uno, K. Nakatani, *J. Am. Chem. Soc.* **2007**, *129*, 11898.
[22] S.-J. Chen, H.-T. Chang, *Anal. Chem.* **2004**, *76*, 3727.
[23] a) C. a. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607; b) W. Gan, B. Xu, H.-L. Dai, *Angew. Chem., Int. Ed.* **2011**, *50*, 6622; c) J. C. Love, L.A. Estroff, J. K. Kriebel, R. G. Nuzzo, G. M. Whitesides, *Chem. Rev.* **2005**, *105*, 1103; d) R. Sardar, J.-W. Park, J. S. Shumaker-Parry, *Langmuir* **2007**, *23*, 11883.
[24] W. S. Hummers, R. E. Offeman, *J. Am. Chem. Soc.* **1958**, *80*, 1339.
[25] a) J. T. Petty, J. Zheng, N. V. Hud, R. M. Dickson, *J. Am. Chem. Soc.* **2004**, *126*, 5207; b) G. Shemer, O. Krichevski, G. Markovich, T. Molotsky, I. Lubitz, A. B. Kotlyar, *J. Am. Chem. Soc.* **2006**, *128*, 11006.
[26] L. Shang, S. J. Dong, *Biosens. Bioelectron.* **2009**, *24*, 1569.
[27] N. M. Alexander, *Anal. Chem.* **1958**, *30*, 1292.
[28] a) D. Miyoshi, M. Inoue, N. Sugimoto, *Angew. Chem., Int. Ed.* **2006**, *45*, 7716; b) B. Shlyahovsky, Y. Li, O. Lioubashevski, J. Elbaz, I. Willner, *ACS Nano* **2009**, *3*, 1831; c) R. Freeman, T. Finder, I. Willner, *Angew. Chem., Int. Ed.* **2009**, *48*, 7818; d) A. Okamoto, K. Tanaka, I. Saito, *J. Am. Chem. Soc.* **2004**, *126*, 9458.
[29] a) A. Saghatelian, N. H. Volcker, K. M. Guckian, V. S. Y. Lin, M. R. Ghadiri, *J.am. Chem. Soc.* **2003**, *125*, 346; b) F. Pu, Z. Liu, X. Yang, J. Ren, X. Qu, *Chem. Commun.* **2011**, *47*, 6024.