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Quenching the Chemiluminescence of Acridinium Ester by Graphene Oxide for Label-Free and Homogeneous DNA Detection

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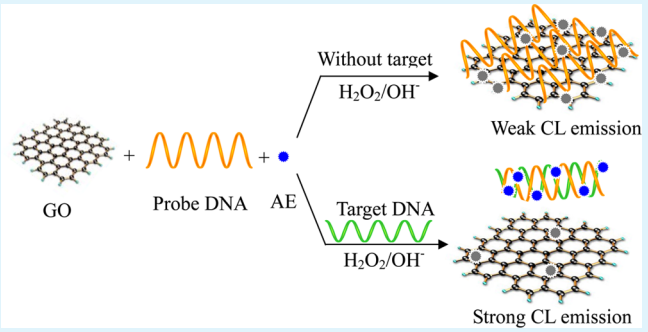
Supporting Information

ABSTRACT: It was found that graphene oxide (GO) could effectively quench the chemiluminescence (CL) emission from a acridinium ester (AE)-hydrogen peroxide system. By taking advantage of this quenching effect, as a proof of concept, a label-free and homogeneous DNA assay was developed for the detection of *Mycobacterium tuberculosis* DNA. In the absence of target DNA, both probe DNA and AE were absorbed on the surface of GO, producing a weak CL emission owing to the CL quenching effect of GO. However, in the presence of target DNA, a double-stranded structure of DNA was generated, leading to the release of the oligonucleotide from the GO surface. AE favors binding with double-stranded DNA, which will be released from the GO surface; thus, the quenching effect of GO will be no longer effective and a strong CL signal can be observed. This assay can detect *M. tuberculosis* DNA with a detection limit of 0.65 nM. This sensitivity is lower than that of previously reported electrochemical detection.



KEYWORDS: *chemiluminescence, DNA, label free, graphene oxide, acridinium ester, detection*

INTRODUCTION

The development of novel methods for rapid, cost-effective, specific, and sensitive DNA detection is extremely important because of their various applications in gene-expression profiling, biomedical diagnosis, forensic, and food analysis technology.[1] To date, specific and sensitive DNA detection mainly depends on the signal transduction of the hybridization of complementary DNA strands by chemiluminescence (CL), fluorescence, atomic force microscopy, surface plasmon resonance spectroscopy, and electrochemical techniques.[2−7] Among others, CL has shown great potential in DNA sensors for point-of-care and ultrasensitive assays because of its low background, inherent sensitivity, and simple instrumentation.[8−10] However, current CL DNA assays suffer from tedious labeling processes. Hence, it is highly desirable to develop novel label-free CL methods for DNA assays.

Graphene oxide (GO) can not only differentiate single- and double-stranded DNA structures but also can quench the fluorescence of nearby organic dyes,[11] quantum dots, and upconverting nanocrystals,[12,13] resulting in extensive studies for the development of fluorescent biosensors.[14] However, few CL DNA assays based on GO have been reported in which GO indirectly quenches CL emission of organic dyes from the luminol-H_2O_2 system by resonance energy transfer[15] or GO indirectly quenches CL emission from a luminol-H_2O_2-horseradish peroxidase mimicking DNAzyme system by inhibiting the DNAzyme activity.[16] Although methyl red and gold nanoparticles were found to directly quench the CL of a classic acridinium ester (AE) CL system by virtue of resonance energy transfer,[17,18] the direct quenching effect of CL emission by GO has not been reported to date. Herein, we report GO as an effective CL emission quencher for an AE-hydrogen peroxide system. The quench effect is possibly attributed to either photoinduced electron ransfer or long-range resonance energy transfer between the GO and chemiluminescent luminophor of the AE-H_2O_2 system. Furthermore, we demonstrate the proof of concept for using GO as a CL-sensing platform for DNA (*Mycobacterium tuberculosis* (*M. tuberculosis*) DNA) detection, which has the following advantages. (i) The DNA probe does not need to be labeled (label-free), which is in favor of DNA hybridization reaction. (ii) The assay occurs in a homogeneous liquid phase, which avoids the separation and washing steps. (iii) The detection limit (0.65 nM) of this DNA assay is lower than that of previously reported methods for the detection of *M. tuberculosis* DNA.[19]

EXPERIMENTAL SECTION

Materials and Reagents. GO was synthesized from natural graphite powder by a modified Hummers method,[20] and its structure is shown in Figure S1. AE was purchased from Cayman Co., Ltd. Sodium citrate, sodium chloride, and disodium hydrogen phosphate

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were obtained from Shanghai Reagent (Shanghai, China). Other chemicals were of analytical-reagent grade or better. Ultrapure water with a resistivity of 18.2 MΩ cm was produced by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA). The oligonucletides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the oligonucleotide used in this work are as follows:

(1) 5′-GGTCTTCGTGGCCGGCGTTCA-3′ (*M. tuberculosis* DNA probe);
(2) 5′-TGAACGCCGGCCACGAAGACC-3′ (complementary *M. tuberculosis* DNA target);
(3) 5′-TGAACGCCGACCACGAAGACC-3′ (one base mismatched *M. tuberculosis* DNA target);
(4) 5′-TCAACGCCGACCACGAAGACC-3′ (three bases mismatched *M. tuberculosis* DNA target);
(5) 5′-TCAACACCGACCGCGAAGACC-3′ (five bases mismatched *M. tuberculosis* DNA target);
(6) 5′-TCAACAGCGACCACGTAAACG-3′ (seven bases mismatched *M. tuberculosis* DNA target);
(7) 5′-ATGTCTCAAGCCAGCTGCTG-3′ (noncomplementary *M. tuberculosis* DNA target).

Apparatus. UV−vis spectra were obtained on a UV8453 spectrophotometer (Agilent). Atomic force microscopy (AFM) measurements were performed using DI Multimode V atomic force microscope (Bruker). The fluorescence spectra were measured on a model F-7000 spectrofluorometer (Hitachi). The CL was detected by a microplate luminometer (Centro LB 960, Berthold) equipped with an injector, which was used to inject the alkaline hydrogen peroxide. A PST-60 HL plus Thermo Shaker (Biosan) was used to control the temperature of the DNA hybridization.

Quenching of the CL of AE by GO. Fifty microliters of AE (0.4 nM) and 50 μL of a GO solution with different concentrations from 0 to 1 mg/mL were successively added to a well. The CL reaction was triggered by injecting 50 μL of H_2O_2 (0.2 mM, 0.05 M NaOH). The light emission was measured by a microplate luminometer.

DNA Detection Based on Quenching AE CL by GO. Thirty microliters of DNA probe (20 nM in phosphate buffer solution (PBS), (disodium hydrogen phosphate (Na_2HPO_4)/citric acid, 20 mM, pH 5.2)) and 20 μL of GO solution (250 μg/mL) were successively added to a well for 10 min followed by the addition of 40 μL of AE (4.5 nM). The final *M. tuberculosis* DNA concentrations in the samples ranged from 1 to 200 nM, and the GO concentration was maintained at 25 μg/mL. After allowing this mixture to hybridize for about 60 min at 37 °C and natural cooling to room temperature, the CL of the mixture was detected when 50 μL of H_2O_2 (0.2 mM, 0.05 M NaOH) was injected into each well. The quantitative determination was based on the peak height of the total CL emission.

RESULTS AND DISCUSSION

GO-Induced CL Quenching of AE. GO was found to be able to quench the CL emission of an AE-H_2O_2 system, as illustrated in Figure 1. The CL emission of the AE-H_2O_2 system in the absence and presence of GO (GO sheets were prepared by a modified Hummer's method[20] and are characterized in the Supporting Information, Figures S2 and S3) in aqueous solution were measured, respectively. As shown in Figure 1b, with GO a remarkable CL decrease is observed for the AE system. The CL of this system is quenched by up to 99.8% of its original signal in the presence of 0.5 mg/mL GO. Under optimized conditions (0.05 M NaOH, and 0.2 mM H_2O_2; Figures S4 and S5), the CL emission of the AE-H_2O_2 system quenched by GO follows a typical Stern−Volmer type equation

$$I_0/I = 1 + K_{sv}[GO]$$

where I_0 and I are the CL intensities of AE in the absence and presence of GO, respectively, K_{sv} is the Stern−Volmer quenching constant, and [GO] is the concentration of GO.

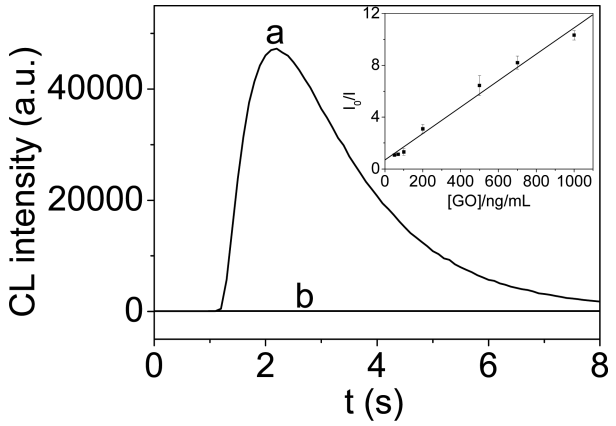

Figure 1. CL intensity of the AE-H_2O_2 system in the (a) absence and (b) presence of 0.5 mg/mL GO. The inset shows the Stern−Volmer plot of AE quenched by GO. [AE] = 0.2 nM, [H_2O_2] = 2 mM, [NaOH] = 0.05 M, and [GO] = 50−1000 ng/mL.

The change in I_0/I of AE with GO concentration is shown in the inset of Figure 1.

At a low GO concentration range (50−1000 ng/mL), I_0/I is in linearly proportional to the GO concentration. K_{sv} is calculated to be 1.0×10^{11} mL/g, which is remarkably greater than the quenching constant produced by a fluorescent-conjugated oligoelectrolyte−GO pair.[21] From the above observation, it can be concluded that the CL signal of the AE-H_2O_2 system can be effectively quenched by GO. Similar to the fluorescence quenching mechanism of GO and excited-state organic dyes,[21] the quenching effect of GO could be partly contributed to by either electron transfer or resonance energy transfer. In this CL system, electron transfer or resonance energy transfer would occur between GO and the excited state *N*-methylacridone (NMA*, the chemiluminescent luminophor of the AE-H_2O_2 system).

To validate our assumption, fluorescence spectra were employed to investigate the interactions between GO and NMA*. The AE is a trifluoromethanesulfonate in this study (its structure is shown in Figure S6), which can be ionized in aqueous solution, producing AE^+ and $CF_3SO_3^-$. The positively charged AE^+ will be absorbed on the negatively charged surface of GO by electrostatic interactions and $\pi-\pi$ stacking (AE^+ has an aromatic ring). Once AE^+ is linked to the edges or basal planes of GO, the fluorescence of AE quenched immediately because of photoinduced electron transfer or long-range resonance energy transfer.[22]

The fluorescence spectra of AE in the absence and presence of GO (Figure 2A) reveal the interactions of exited-state AE and GO. Significant quenching of the AE solution in the presence of GO is observed, suggesting a close proximity of AE to GO,[23] demonstrating the assumption that AE molecules were adsorbed on the surface of GO. It is noted that there is a strong background for the GO solution in Figure 2A, trace b, which is probably from the intrinsic fluorescence emission of GO.[24,25] Information about the CL reaction products of AE could be inferred from the result of fluorescence measurement by the addition of alkaline hydrogen peroxide to convert AE to NMA. As shown in Figure 2B, trace a corresponds to the fluorescence emission spectrum of NMA[26] and exhibits a strong fluorescence emission. However, the addition of GO cause an extreme quenching of the fluorescence emission (trace b) owing to the long-range resonance energy transfer or

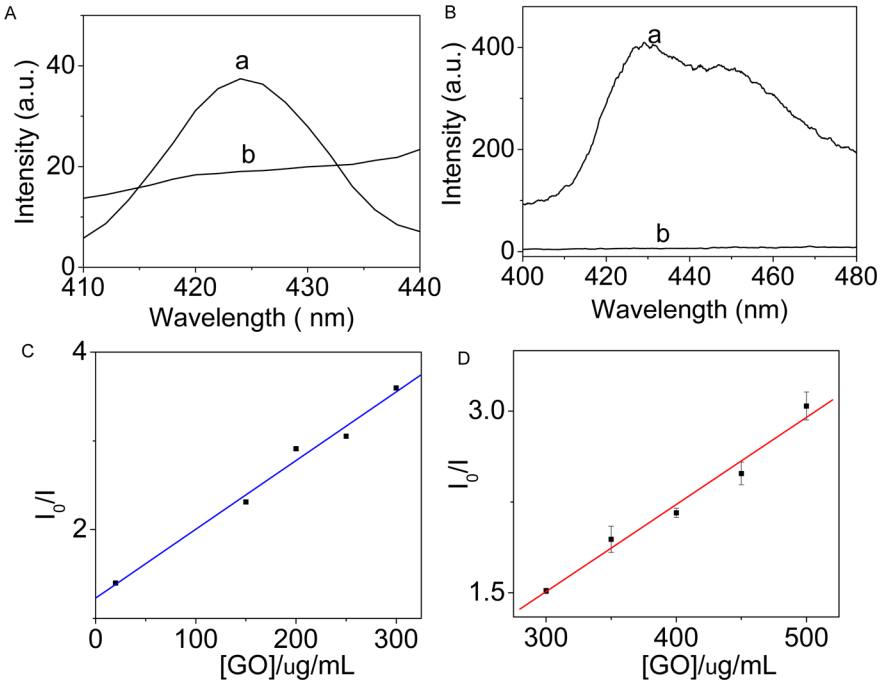

Figure 2. (A) Fluorescence emission spectra of (a) AE and (b) AE + GO aqueous solutions. Excitation wavelength (λ_{ex}) = 370 nm. (B) Fluorescence emission spectra of chemiluminescent products of (a) AE and (b) AE + GO aqueous solutions, λ_{ex} = 250 nm. [AE] = 15 nM and [GO] = 0.1 mg/mL. Stern–Volmer plot for the fluorescence quenching of (C) AE and (D) NMA by GO.

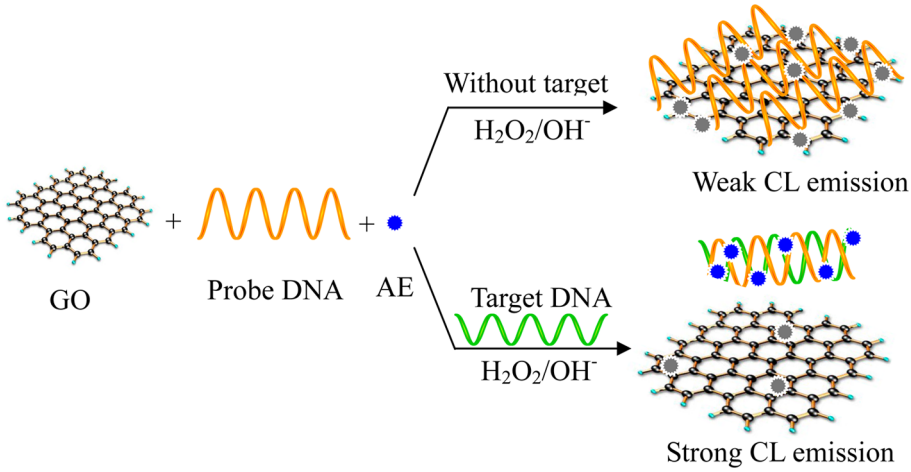

Figure 3. Schematic representation of the label-free CL DNA detection on the basis of the quenching effect of GO as well as the discrimination ability of GO toward single-stranded DNA/double-stranded DNA.

photoinduced electron transfer between GO and NMA*.[21] It also should be noted that the values of K_{sv} for AE and NMA are 8×10^9 and 4×10^9 mL/g, respectively (Figure 2C,D). The results indicated that the fluorescence quenching of AE by GO is more effective than that of NMA, presumably because of the shorter distances caused by the electrostatic interactions of AE and GO. The CL K_{sv} for AE by GO is higher than the fluorescence K_{sv} for AE and NMA, demonstrating that GO has a better CL-quenching capacity than that of fluorescence, partially owing to high sensitivity of CL. Likewise, the chemiluminescent luminophor of the AE-H_2O_2 system is also NMA*. The fluorescence experiments strongly support that the deactivation of light emission of NMA* by GO from its excited state to ground state occurred. Thus, the resonance energy transfer or photoinduced electron transfer between NMA* and GO may induce the CL quenching of AE.

Principle of DNA Detection Based on the Quenching Effect of GO. The GO-based CL quenching may serve as a sensing platform for biomolecules analysis. As a proof of concept, a label-free and homogeneous DNA sensor was developed in this study, which is based on the competitive binding between negatively charged DNA and GO toward positively charged AE.

Figure 3 shows a schematic representation of the GO-based CL quenching label-free DNA sensor. In the absence of target DNA, probe DNA (single stranded) is absorbed by $\pi-\pi$ stacking on the surface of GO as well as AE by $\pi-\pi$ stacking and electrostatic interactions. According to our results above, the CL of absorbed AE is quenched by GO. However, in the presence of target DNA (single stranded), a double-stranded structure of DNA is generated, resulting in the release of the oligonucleotide from the GO surface because of the

conformation change.[3] As a large and supercharged molecule, double-stranded DNA shows greater ionic attraction for positively charged AE than the weakly ionizable GO.[22,23] Thus, AE favors binding with the double-stranded DNA, which will be released from the GO surface. Once AE leaves the GO surface, the quenching effect of GO will be no longer effective, and a strong CL signal of the AE-H_2O_2 system can be observed. The binding affinity of double-stranded DNA and AE is demonstrated by control experiments. When the double-stranded DNA (20 nM) was directly added into the mixtures solution (GO-AE-H_2O_2 system), a remarkable increase in the CL signal was observed, as shown in Figure S7, indicating that AE favored binding with double-stranded DNA and was indeed released from the GO surface.

Detection of DNA with GO-Based CL Assay. To test the performance of the label-free and homogeneous CL DNA detection method, *M. tuberculosis* DNA was chosen as a model analyte. The ionic strength plays an important role in this sensing system. When the concentration of salt (sodium chloride) is lower than 0.2 M, the analytical performance of this sensing system is not satisfactory (data not shown). Therefore, 0.2 M NaCl was chosen.

The buffer condition is also very important. It was found that AE was not stable in alkaline or neutral solution and thus PBS (20 mM, pH 5.2) was employed. As shown in Figure 4, with an

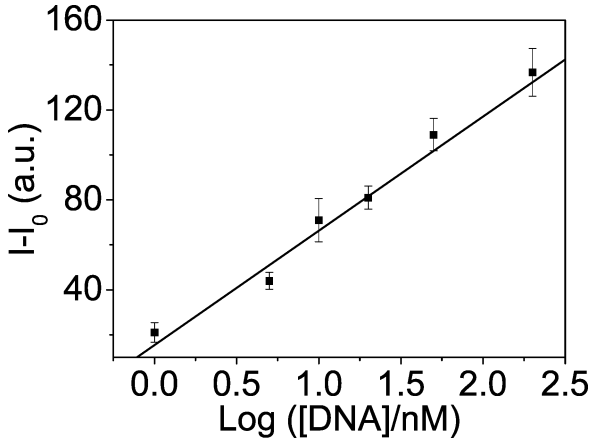

Figure 4. Calibration plot of log C versus $I - I_0$ for the target DNA over 1 to 200 nM, where I_0 and I are the CL intensity without and with *M. tuberculosis* DNA, respectively.

increased concentration of target DNA, the CL signal intensities increased accordingly with a dynamic detection range from 1 to 200 nM. The linear regression equation is $I - I_0 = 15.4 + 50.8 \log C_{DNA}$ with a correlation coefficient of 0.98 (I_0, CL intensity in the absence of target DNA; I, CL intensity in the presence of target DNA; C_{DNA}, target DNA concentration). A detection limit of 0.65 nM was obtained (signal-to-noise ratio = 3), which is lower than that of previously reported electrochemical methods for the detection of *M. tuberculosis* DNA.[20] The reproducibility of the proposed assay was also investigated. A series of five repetitive measurements of the 10 nM *M. tuberculosis* DNA solution yielded reproducible CL response with coefficients of variation of 6.20%, indicating that the reproducibility of the proposed method was acceptable.

Selectivity and Real Sample Analysis. The selectivity of the present detection system for *M. tuberculosis* DNA was further evaluated by detecting the CL response of perfectly complementary targets containing one-, three-, five-, and seven-base mismatched strands as well as noncomplementary strands at the same concentration of 200 nM. As shown in Figure 5, a

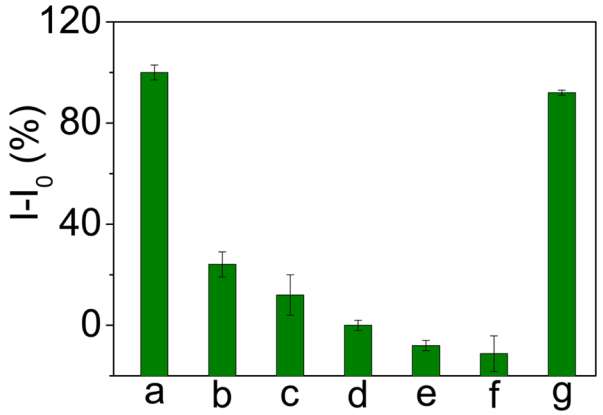

Figure 5. Relative CL response from probe DNA hybridized with different target DNA. (a) Complementary sequence, (b) one-base mismatched sequence, (c) three-base mismatched sequence, (d) five-base mismatched sequence, (e) seven-base mismatched sequence, (f) noncomplementary sequence, and (g) mixture containing all of the sequences mentioned.

strong CL response is observed for the complementary target DNA, whereas the mismatched strands yields a relative weak CL signal, and the noncomplementary DNA strand produced a negative signal. A possible explanation for the negative signal is as follows. The background signal (I_0) was from the CL emission of few AE molecules that were not adsorbed on the surface of GO. The presence of noncomplementary DNA with negative charge will be adsorbed on the surface of GO, which can further absorb positively charged AE, leading to a low CL signal (I) and negative value of $I - I_0$. Moreover, the selectivity of this assay for *M. tuberculosis* DNA in the presence of all possible interference strands was evaluated by considering the cross reactivity (Figure 5).

Obviously, the present assay can still detect the *M. tuberculosis* DNA in the presence of all possible interference stands. The results show the hybridization assay has a high specificity, which is essential for the detection of biologically important single-nucleotide polymorphisms, by changing the sequence of the probe DNA to a specific target.

To evaluate the practicality of the proposed assay to real samples, detection of *M. tuberculosis* DNA in human serum was performed. It was found that it did not obtain good recovery values in a high concentration of human serum (>1%) because this assay was homogeneous and serum with high concentration would affect the CL reaction of AE and H_2O_2. Thus, four concentrations of *M. tuberculosis* DNA were spiked into 1% human serum and detected by CL measurement. The recovery values ranged from 98% to 104% (Table 1). These results revealed that the proposed assay could potentially be applied to realistic biological samples, such as diluted human serum.

CONCLUSIONS

We have demonstrated for the first time that GO can directly quench the CL emission of an AE-H_2O_2 system effectively. By taking advantage of the different ionic attraction of double-stranded DNA and GO toward AE, a novel label-free and

Table 1. Recovery of *M. tuberculosis* DNA in 1% Human Serum[a]

sample	added DNA (nM)	found DNA (nM)	recovery (%)[b]
1	1.0	1.04 ± 0.02	104
2	5.0	5.19 ± 0.05	103
3	10.0	10.4 ± 0.03	104
4	50.0	49.4 ± 0.01	98

[a]The standard deviations of measurements were calculated from three parallel experiments. [b]The recovery is obtained according to the ratio between the amount of target predicted from the linear calibration curve and the amount that was actually added.

homogeneous DNA sensor for *M. tuberculosis* was designed. The quenching ability for the AE-H_2O_2 CL system, the unique interactions with biomolecules, and the biocompatibility of GO provide great potential for CL-sensor design and for various applications such as the detection of ultrasensitive pathogen DNA, antigen, antibody, metallic ion, and small important biosubstances.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Chemical structure of GO; AFM height image of GO sheets deposited on mica substrates; UV−vis absorption spectrum of a 0.2 mg/mL GO aqueous solution; effect of the concentration of H_2O_2 and NaOH on quenching AE CL by GO in aqueous solution; molecular structure of acridinium ester; and CL intensity of AE-H_2O_2-GO system in the presence and absence of 20 nM double-stranded DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Myers, M. J.; Farrell, D. E.; Deaver, C. M.; Mason, J.; Swaim, H. L.; Yancy, H. F. *J. Food. Prot.* **2010**, *73*, 1090.
(2) Freeman, R.; Liu, X.; Willner, I. *J. Am. Chem. Soc.* **2011**, *133*, 11597.
(3) He, S.; Song, B.; Li, D.; Zhu, C.; Qi, W.; Wen, Y. *Adv. Fun. Mater.* **2010**, *20*, 453.
(4) Han, W. H.; Liao, J. M.; Chen, K. L.; Wu, S. M.; Chiang, Y. W.; Lo, S. T. *Anal. Chem.* **2010**, *82*, 2395.
(5) Homola, J. *Chem. Rev.* **2008**, *108*, 462.
(6) Hu, L.; Xu, G. *Chem. Soc. Rev.* **2010**, *39*, 3275.
(7) Cosnier, S.; Mailley, P. *Analyst* **2008**, *133*, 984.
(8) King, D. W.; Cooper, W. J.; Rusak, S. A .; Peake, B. M.; Rusak, S. A.; James, J. K.; Daniel, W. O.; Megan, L. M.; Chris, R. M.; Stephen, M. T. *Anal. Chem.* **2007**, *79*, 4169.
(9) He, Y.; Zhang, H.; Chai, Y.; Cui, H. *Anal. Bioanal. Chem.* **2011**, *399*, 3451.
(10) Arnold, L. J; Hammond, P .W.; Wiese, W. A.; Nelson, N. C. *Clin. Chem.* **1989**, *35*, 1588.
(11) Lu, C. H.; Yang, H. H.; Zhu, C. L.; Chen, X.; Chen, G. N. *Angew. Chem., Int. Ed* **2009**, *48*, 4785.
(12) Dong, H.; Gao, W.; Yan, F.; Ji, H.; Ju, H. *Anal. Chem.* **2010**, *82*, 5511.
(13) Zhang, C.; Yuan, Y.; Zhang, S.; Wang, Y.; Liu, Z. *Angew. Chem., Int. Ed.* **2011**, *50*, 6851.
(14) Yang, W.; Ratinac, K. R.; Ringer, S. P.; Thordarson, P.; Gooding, J. J.; Braet, F. *Angew. Chem., Int. Ed.* **2010**, *49*, 2114.
(15) Bi, S.; Zhao, T.; Luo, B. *Chem. Commun.* **2012**, *48*, 106.
(16) Luo, M.; Chen, X.; Zhou, G.; Xiang, X.; Chen, L.; Ji, X. *Chem. Commun.* **2012**, *48*, 1126.
(17) Xu, Q.; Liu, J.; He, Z. *Chem. Commun.* **2010**, *46*, 8800.
(18) Brown, R. C.; Li, Z.; Rutter, A. J.; Mu, X.; Weeks, O. H.; Smith, K. *Org. Biomol . Chem.* **2008**, *7*, 386.
(19) Das, M.; Dhand, C.; Sumana, G.; Srivastava, A.; Nagarajan, R.; Nain, L. *Biomacromolecules* **2011**, *12*, 540.
(20) Li, Y.; Wu, Y. *J. Am. Chem. Soc.* **2009**, *131*, 5851.
(21) Cai, L.; Zhan, R.; Pu, K. Y.; Qi, X.; Zhang, H.; Huang, W. *Anal. Chem.* **2011**, *83*, 7849.
(22) Liu, M.; Zhao, H.; Quan, X.; Chen, S.; Fan, X. *Chem. Commun.* **2010**, *46*, 7909.
(23) Balapanuru, J.; Yang, J. X.; Xiao, S.; Bao, Q.; Jahan, M.; Polavarapu, L. *Angew. Chem., Int. Ed.* **2010**, *122*, 6699.
(24) Eda, G.; Lin, Y. Y.; Mattevi, C.; Yamaguchi, H.; Chen, H. A.; Chen, I.; Chen, C. W; Chhowalla, M. *Adv. Mater.* **2010**, *22*, 505.
(25) Jung, J. H.; Cheon, D. S.; Liu, F.; Lee, K. B.; Seo, T. S. *Angew. Chem., Int. Ed.* **2010**, *49*, 5708.
(26) Lai, Y.; Qi, Y.; Wang, J.; Chen, G. *Analyst* **2008**, *134*, 131.