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Graphene−DNAzyme Based Biosensor for Amplified Fluorescence "Turn-On" Detection of Pb^{2+} with a High Selectivity

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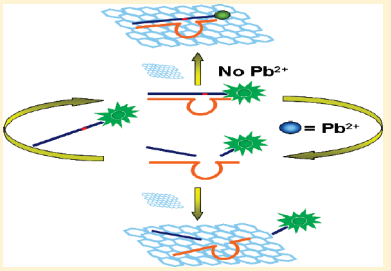
Supporting Information

ABSTRACT: On the basis of the remarkable difference in affinity of graphene (GO) with ssDNA containing a different number of bases in length, we for the first time report a GO−DNAzyme based biosensor for amplified fluorescence "turn-on" detection of Pb^{2+}. A FAM-labeled DNAzyme−substrate hybrid acted as both a molecular recognition module and signal reporter and GO as a superquencher. By taking advantage of the super fluorescence quenching efficiency of GO, our proposed biosensor exhibits a high sensitivity toward the target with a detection limit of 300 pM for Pb^{2+}, which is lower than previously reported for catalytic beacons. Moreover, with the choice of a classic Pb^{2+}-dependent GR-5 DNAzyme instead of 8-17 DNAzyme as the catalytic unit, the newly designed sensing system also shows an obviously improved selectivity than previously reported methods. Moreover, the sensing system was used for the determination of Pb^{2+} in river water samples with satisfying results.



Nanomaterials possess unique optical, electronic, magnetic, and catalytic properties, which make them ideal candidates for signal generation and transduction in developing novel sensing systems with advanced and powerful functions. Graphene, a 2D carbon nanocrystal with only one atom thickness, has been widely used in nanoelectronics,[1] nanocomposites,[2] as well as bioanalysis.[3] Particularly, graphene oxide (GO), which is a water-soluble derivative of graphene, has attracted increasing interest in biological applications because of its unique characteristics such as good water dispersibility,[4] facile surface modification,[5] and high mechanical strength.[6] Furthermore, GO could bind single-stranded DNA via hydrophobic and π-stacking interactions between the nucleobases and GO. Very recently, GO was reported to be a fluorescence superquencher with the long-range nanoscale energy transfer property, which, in combination with the unique GO/DNA interactions, has been employed to develop sensing systems for detection of DNA, proteins, and other small molecules by using complementary oligonucleotides or aptamers as recognition units.[7] Most of such sensing systems are based on the fact that the ssDNA and its duplex with target exhibit different affinity to GO.[8] Few studies are focused on the interaction between GO and ssDNA containing a different number of bases in length. Aptamers and DNAzymes are two main members of functional nucleic acids which can either bind to a target molecule or perform catalytic reactions with the ability to recognize a broad range of targets. Although aptamers have been extensively investigated in constructing GO based fluorescence "turn-on" biosensors based on the conformation change from ssDNA to the duplex with its target, no DNAzyme−GO based biosensor has been reported. Since the presence of target will induce a conformation change from the duplex of DNAzyme with substrate to ssDNA (cleaved substrate), it is difficult to use the above-mentioned strategy to design a DNAzyme−GO based fluorescence "turn-on" biosensor.

Lead is a major environmental pollutant. It is quite harmful to human health, especially to children,[9] and a very small amount of lead ion could cause serious damage to the brain and central nervous system.[10] Accordingly, the development of sensors to detect Pb^{2+} in environmental and biological samples is of considerable significance and has become the important subject of current chemical research. Many analytical methods have been developed to detect Pb^{2+}, including atomic absorption spectrometry, inductively coupled plasma-mass spectrometry, inductively coupled plasma-atomic emission spectrometry, and UV−vis spectrometry.[11] Though these techniques are sensitive and accurate for Pb^{2+} assay, most of them necessitate the use of costly apparatus and require complicated pretreatment procedures and so are inappropriate for on-site and real-time detection. Fluorescence Pb^{2+} sensors have attracted great attention in this field due to their high sensitivity, facile operation, nonsample-destructing or less cell-damaging, and the ability to provide in situ and real-time information for a variety of applications.[12] As a result, various Pb^{2+}-sensitive fluorescence sensors employing peptides, proteins, nucleic acids, and small organic ligands[13] as recognition modules have been developed in the past decades. Among them, DNAzymes based fluorescence sensors (known as catalytic beacons)

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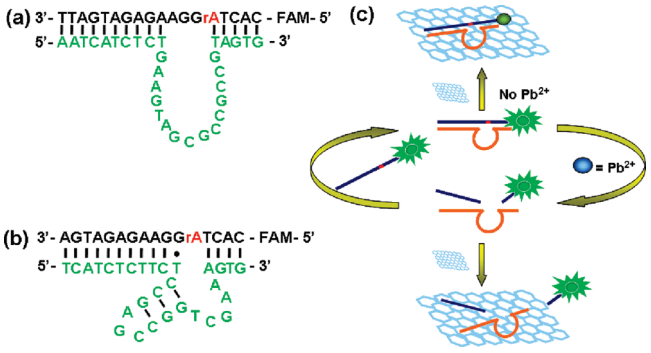

Figure 1. (a) Secondary structure of the GR-5 DNAzyme hybridized with the FAM-labeled substrate strand (named DS); (b) secondary structure of the 8-17 DNAzyme hybridized with the FAM-labeled substrate strand (named DS2); the "rA" in the middle of the substrate represents adenosine ribonucleotide; (c) schematic illustration of the DNAzyme−GO based fluorescence sensor for Pb^{2+}.

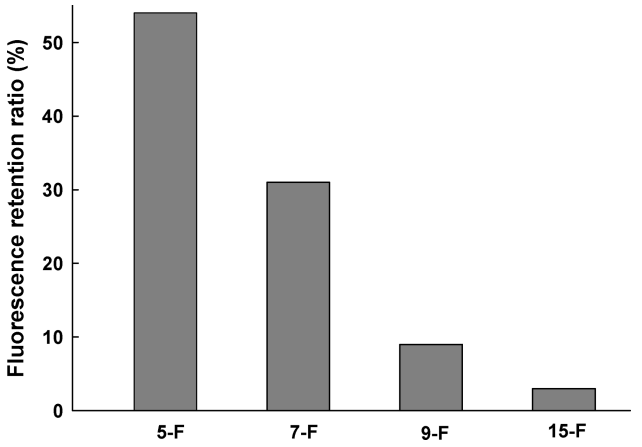

Figure 2. Comparison of the fluorescence retention ratio of GO to ssDNA with different lengths. The concentration of GO was fixed at 100 μg/mL. The 5-F, 7-F, 9-F, and 15-F denote FAM-labeled ssDNA containing 5, 7, 9, and 15 bases, respectively.

have been extensively investigated due to their high metal ion specificity.[14] While most of these sensors exhibit satisfying sensitivity with detection limits below the maximum contamination levels in water, an even higher sensitivity is desired if these sensors are to be used in complex biological or environmental samples.[15] Moreover, almost all of them adopt 8-17 DNAzyme as catalytic units, which suffered from interferences from several other metal ions such as Zn^{2+}, Co^{2+}, Mn^{2+}, and Cd^{2+}, especially at high concentrations.[16] In this paper, on the basis of the remarkable difference in the affinity of GO with ssDNA containing a different number of bases in length, we constructed a graphene−DNAzyme based sensing system for amplified fluorescence "turn-on" detection of Pb^{2+}. By taking advantage of the super fluorescence quenching efficiency of GO, our proposed biosensor exhibits a high sensitivity toward the target with a detection limit of 300 pM for Pb^{2+}, which is lower than previously reported catalytic beacons. Interestingly, with the choice of a classic Pb^{2+}-dependent GR-5 DNAzyme instead of 8-17 DNAzyme as the catalytic unit, the new designed sensing system also shows an obviously improved selectivity than previously reported methods[16] with no interference from Zn^{2+} and other common metal ions. To the best of our knowledge, this is the first example of the use of the graphene−DNAzyme nanoplatform for an amplified assay.

The design strategy of the graphene−DNAzyme based sensing system is depicted in Figure 1. The 5′ end of the substrate strand is labeled with a fluorophore carboxyfluorescein (FAM), which is hybridized with DNAzyme strand to form a DNAzyme−substrate hybrid containing a large ssDNA loop (containing 15 bases) to bind with the GO. The DNAzyme strand is hybridized with the substrate strand in an asymmetric manner. The 5′ end of the substrate strand is hybridized with the 3′ end of the enzyme strand through only 5 base pairs, which make the fluorophore close to the GO surface to the greatest extent to afford a high quenching efficiency and a low background fluorescence and also make the catalytic cleavage-induced FAM-linked oligonuleotide moiety difficult to bind to the GO to guarantee a high signal-to-background ratio (SBR) for the assay of Pb^{2+}. To keep an efficient hybridization between the enzyme strand and the substrate strand to maintain the catalytic activity of the DNAzyme, the 3′ end of the substrate strand is hybridized with the 5′ end of the enzyme strand through 10 base pairs. In the absence of Pb^{2+}, the introduction of GO with the DNAzyme−substrate solution would result in strong binding between the large ssDNA loop of DNAzyme−substrate and GO and bring the labeled FAM and superquencher GO into close proximity, thus significantly quenching the fluorescence of FAM. Besides, the fluorescence of unhybridized FAM-labeled substrate strand could also be quenched by GO through a previously reported classic GO/ssDNA interaction. Upon the addition of Pb^{2+}, the DNAzyme was activated and cleaved the substrate strand at the RNA site into two parts, releasing a short FAM-linked oligonuleotide fragment, a related long oligonuleotide fragment, and the DNAzyme strand. The DNAzyme strand can hybridize with another substrate strand and then induce the second cycle of cleavage by binding Pb^{2+}, providing an amplified detection signal for Pb^{2+}. The amount of produced short FAM-linked oligonuleotide fragments are positively related to the concentrations of Pb^{2+}. The introduction of GO into the sensing solution will result in weak quenching of the fluorescence of FAM due to the weak affinity of the short FAM-linked oligonuleotide fragment to GO, and the fluorescence intensity should gradually increase with increasing Pb^{2+}concentration added.

On the basis of the fact that the ssDNA and duplex with its target exhibit different affinity to GO, several aptamers−GO based fluorescence "turn-on" biosensors have been developed for various targets. However, this strategy is not suitable for constructing DNAzyme−GO fluorescence "turn-on" biosensors, as a reversed conformation change from the duplex of DNAzyme with substrate to ssDNA (cleaved substrate) was involved in the DNAzyme−target reaction. To solve this problem, we turn our attention to the difference in affinity of GO with ssDNA containing different numbers of bases in length. It could be expected that short ssDNA will exhibit weaker affinity to GO than that of long ssDNA. To verify this hypothesis, we first investigate the fluorescence retention ratio of GO to ssDNA with different lengths. As shown in Figure 2, the fluorescence retention ratio of GO to ssDNA decreased with the increasing base number of ssDNA. When the concentration of GO was fixed at 100 μg/mL and oligonucleotide fixed at 50 nM, one observed that the fluorescence retention ratio of GO to FAM-labeled ssDNA containing 15 bases (named 15-F) was 3%, in another word, a fluorescence quenching efficiency of 97% was obtained in this system. In contrast, for the similar ssDNA containing 5 bases (named 5-F),

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a 55% fluorescence retention ratio was achieved. This phenomenon indicated that the affinity of the short ssDNA to GO is significantly weaker than that of the long ssDNA, which forms the basis for the design of DNAzyme−GO based fluorescence "turn-on" biosensors. Given that too few bases might result in poor hybridization efficiency of DNAzyme with substrate to reduce its catalytic activity, a FAM-labeled ssDNA containing 5 bases was chosen as a building block for the substrate strand to construct the DNAzyme−GO sensing system.

To demonstrate the feasibility of our new strategy in constructing the DNAzyme−GO sensing platform, the Pb^{2+}-stimulated fluorescence response of the DNAzyme−GO based biosensors with different length base pairs on the FAM-labeled end away from the cleavage site was recorded, respectively (see the Supporting Information, Figure S1). GR-5 DNAzyme is hybridized with DS (containing 5 bases), and GR-5 DNAzyme1 is hybridized with DS1 (containing 9 bases). Results showed that an obviously larger SBR was observed for a DNAzyme−GO sensing system containing 5 bases on the FAM-labeled end away from the cleavage site than that containing 9 bases, indicating the success of our new design by using the difference in affinity of GO with ssDNA containing a different number of bases in length to construct DNAzyme−GO based fluorescence "turn-on" biosensors. This strategy provides a general platform for design of DNAzyme−GO based sensing systems for amplified detection of various cofactors.

Two kinds of Pb^{2+}-dependent DNAzymes have been adopted to develop catalytic DNA biosensors for lead ions. One is the well-known "8-17 DNAzyme" which was isolated by several groups under different conditions.[17] The other is the classic "GR-5 DNAzyme" which was selected by Break and Joyce.[18] For optimization purpose, both of the two DNAzymes were employed to build the DNAzyme−GO nanosensor and recorded their fluorescent response induced by Pb^{2+}, respectively (see the Supporting Information, Figure S2). The 8-17 DNAzyme is hybridized with DS2 in this experiment. A significantly larger SBR was observed for the GR-5 DNAzyme involved nanoplatform than that of 8-17 DNAzyme, which could be ascribed to the difference in the number of free bases in the loop section of the DNAzyme−substrate hybrid. The GR-5 DNAzyme−substrate hybrid possesses 15 free bases in its loop section. While the 8-17 DNAzyme−substrate hybrid contains only 8 unhybridized bases, which show obviously weaker affinity to GO than that of GR-5 DNAzyme and, therefore, cause higher background fluorescence with a lower SBR. Moreover, as proved by the Lu group, GR-5 DNAzyme demonstrates remarkably high selectivity to Pb^{2+} than that of 8-17 DNAzyme[16] and is therefore chosen for further investigation.

In order to achieve the best sensing performance, the concentration for GO and the ratio of DNAzyme strand to substrate were optimized. The concentrations for both DNAzyme and substrate were fixed at 50 nM. Since the introduction of more GO could afford lower background fluorescence for the DNAzyme−GO nanoplatfrom, the SBR of the fluorescence signal increased notably with the addition of increasing concentrations of GO (see the Supporting Information, Figure S3). However, with the concentration of GO higher than 100 μg/mL, the SBR decreased with a further increasing concentration of GO, which might be ascribed to the excessive quenching effect of the GO at a high concentration on the cleavage-produced short FAM-labeled oligonucleotide. Different from previously reported catalytic beacons, in our design, the DNAzyme strand is free from labeling

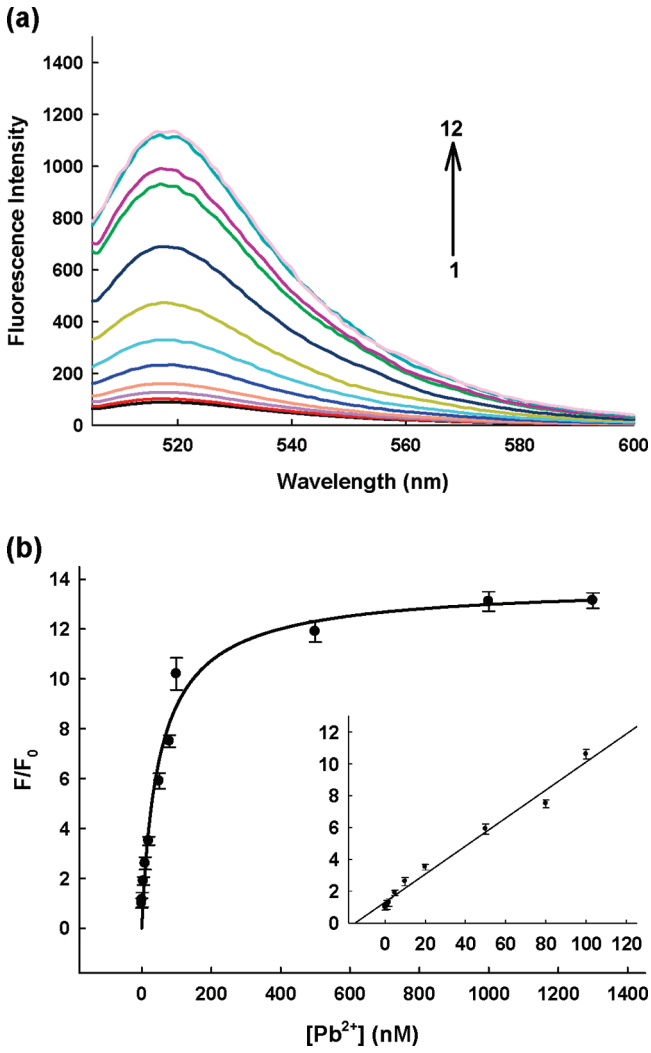

Figure 3. (a) Fluorescence emission spectra of the sensing system on exposure to Pb^{2+} solutions of different concentrations and then mixed with GO: (1) 0, (2) 1 nM, (3) 2 nM, (4) 5 nM, (5) 10 nM, (6) 20 nM, (7) 50 nM, (8) 80 nM, (9) 100 nM, (10) 500 nM, (11) 1 μM, and (12) 1.3 μM. (b) Calibration curve of the sensing system for Pb^{2+}. The curve was plotted with the fluorescence enhancement vs Pb^{2+} concentration. Inset shows the linear responses at low Pb^{2+} concentrations. The buffer containes 50 mM HEPES (pH 7.26), 50 mM NaCl, and 5 mM $MgCl_2$. The concentrations for DNAzyme and substrate are 30 and 50 nM, respectively. The metal ion treatment reaction was performed for 20 min.

of a quencher and enables the use of excess substrates over the DNAzyme strands. The effect of the ratio of DNAzyme strand to substrate on the fluorescence response of the sensing system is then investigated (see the Supporting Information, Figure S4). An increasing ratio of DNAzyme strand to substrate results in the increase of the SBR of the fluorescence signal for the sensing system until it reached 0.6. However, when the ratio exceeded 0.6, the SBR of the fluorescence signal decreased with an increasing ratio of DNAzyme to substrate. On the basis of these results, a concentration of GO at 100 μg/mL and a ratio of DNAzyme strand to substrate at 0.6 were chosen for further Pb^{2+} sensing experiments. In the optimized condition, more than 1.5 equiv of substrates are employed for 1 equiv of DNAzyme strand, which make it possible to take advantage of DNAzymes as catalysts for amplified sensing through multiple turnover reactions.

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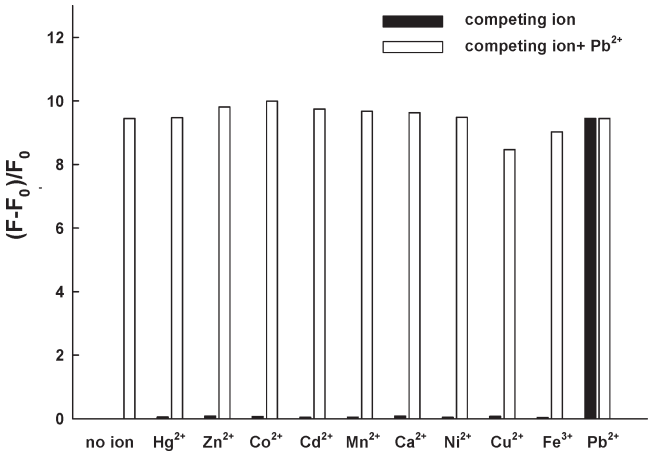

Figure 4. Selectivity of the DNAzyme−GO based sensor for Pb^{2+} over other competing metal ions. Black bars: fluorescence response of the sensing system to different metal ions; the concentration was 100 nM for Pb^{2+} and 10 μM for other metal ions. White bars: Fluorescence response of the sensing system to Pb^{2+} in the presence of other metal ions; the concentration was 100 nM for Pb^{2+}, 1 μM for Cu^{2+}, and 10 μM for other metal ions. The metal ion treatment reaction was performed for 20 min.

Figure 3a showed the fluorescence spectra of the sensing system upon incubation with different Pb^{2+} concentrations and then mixed with GO under optimization conditions. A dramatic increase in the fluorescence intensity was observed with the increasing concentrations of Pb^{2+}. An approximately 14-fold fluorescence enhancement was observed when the Pb^{2+} concentration reached 1 μM, which is much higher than the 6-fold increase of the catalytic beacon with a dual quencher design.[19] The large fluorescence enhancement of the sensing system might arise from the super quenching efficiency of GO to lower the background fluorescence and the significant difference in affinity of GO with ssDNA containing a different number of bases in length, which could improve the sensitivity of the new designed GO-based sensing system. The calibration curve for the biosensor was shown in Figure 3b, and a dynamic range from 1 nM to 1 μM for Pb^{2+} was achieved with a linear relationship range until 100 nM. A detection limit of 300 pM for Pb^{2+} was estimated based on the 3δ/slope rule, which was much lower than almost all of previously reported DNAzyme based fluorescence sensors for Pb^{2+}.[16,19,20] In addition to the high quenching efficiency of GO, the use of GR-5 DNAzyme instead of 8-17 DNAzyme as the catalytic unit might also play an important role in lowering the background fluorescence of the sensing system to afford a high sensitivity. As demonstrated by the Lu group, although being selective for Pb^{2+}, the 8-17 DNAzyme is still active in the presence of other divalent metal ions and even some monovalent metal ions, which might cause enhanced background fluorescence for the sensing system even in the blank buffer solutions, and result in a reduced sensitivity. However, GR-5 DNAzyme shows ignorable activity to other metal ions compared to that of Pb^{2+}, which could afford low background fluorescence for the sensor and a high sensitivity for Pb^{2+}. These results suggested that this sensing system was appropriate for quantitative determination of Pb^{2+} at a low concentration.

Besides sensitivity, selectivity was another important issue to assess the performance of a new proposed sensor. Although the 8-17 DNAzyme was shown to be selective for Pb^{2+}, the Lu group found that this DNAzyme also shows catalytic activity with other metal ions such as Zn^{2+}, Co^{2+}, Mn^{2+}, or Cd^{2+} as cofactor, especially at high concentrations. The GR-5 DNAzyme based sensor shows a remarkably high selectivity to Pb^{2+}. To investigate the selectivity of the GR-5 DNAzyme−GO based sensing system for Pb^{2+}, the fluorescence responses of the sensor to various metal ions were recorded. As shown in Figure 4 (black bars), 100 nM of Pb^{2+} could induce a significant fluorescence enhancement of the sensing system, while all other metal ions at concentrations of 10 μM did not give an obvious fluorescence increase, indicating that our sensing system exhibits a high selectivity to Pb^{2+} over other competing metal ions. To test the practical applicability of our fluorescent sensing system for Pb^{2+}, competition experiments were also carried out by recording the fluorescent intensity changes of the sensing system toward 100 nM of Pb^{2+} in the presence of competing metal ions (white bars in Figure 4). Experimental results indicated that 1 μM of Cu^{2+} and 10 μM of other metal ions showed no obvious interference for Pb^{2+} detection. Cu^{2+} present at a concentration larger than 1 μM induced a slight fluorescent intensity decrease, which might be a result of its quenching effect on the fluorophore. All these selective results indicate that our developed sensor possesses a remarkably high selectivity to Pb^{2+}, which is consistent with the original GR-5 DNAzyme sensor and could meet the selective requirements for environmental and biomedical applications.

Table 1. Recovery Experiments of Pb^{2+} in River Water Samples

river water	Pb^{2+} spiked (nM)	Pb^{2+} recovered (nM)	recovery (%)
1	5.0	$4.7^a \pm 0.4^b$	94
2	20.0	$21.3^a \pm 2.6^b$	106.5
3	100.0	$98.7^a \pm 4.5^b$	98.7

[a] Mean values of four determinations. [b] Standard deviation.

In order to evaluate the practical application of the new sensor for detection of Pb^{2+}, the recovery experiments with spiked Pb^{2+} in river water samples were carried out. The river water samples were obtained from the Xiang River (Changsha, China). The samples collected were simply filtered and showed that no Pb^{2+} was present. The analytical results are shown in Table 1. One observed that the results obtained in real water samples showed good recovery values, which confirmed that the proposed sensor was applicable for practical Pb^{2+} detection in real samples with other potentially competing species coexisting.

In summary, we have developed a graphene−DNAzyme based biosensor for fluorescence "turn-on" detection of Pb^{2+} based on the remarkable difference in affinity of GO with ssDNA containing a different number of bases in length. By taking advantage of the super fluorescence quenching efficiency of GO, our proposed sensor exhibits a high sensitivity toward target with a detection limit of 300 pM for Pb^{2+}, which is lower than previously reported catalytic beacons. Moreover, with the choice of a classic GR-5 DNAzyme as the catalytic unit, the new designed sensing system also shows an obviously improved selectivity to Pb^{2+} than previously reported methods. The sensing system was used for the determination of Pb^{2+} in river water samples with satisfying results. Since numerous DNAzymes have been selected to bind a wide range of targets, the DNAzyme−GO system provides a new general platform for sensitive detection of various targets and could find wide applications in the environmental and biomedical fields.

■ ASSOCIATED CONTENT

Supporting Information. Apparatus, experimental procedures, and supplementary spectra data. This material is available free of charge via the Internet at http://pubs.acs.org.

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