Highly Sensitive Multiplexed Heavy Metal Detection Using Quantum-Dot-Labeled DNAzymes

Chung-Shieh Wu,[†,‡] Maung Kyaw Khaing Oo,[†] and Xudong Fan[†,*]

[†]Biomedical Engineering Department, University of Michigan, 1101 Beal Avenue, Ann Arbor, Michigan 48109, United States, and [‡]Department of Biological Engineering, Bond Life Sciences Center, University of Missouri, Columbia, Missouri 65211, United States

ABSTRACT We developed highly sensitive and specific nanosensors based on quantum dots (QDs) and DNAzyme for multiplexed detection of heavy metal ions in liquid. The QDs were coated with a thin silica layer for increased stability and higher quantum yield while maintaining a relatively small size for highly efficient energy transfer. The QD—DNAzyme nanosensors were constructed by conjugating quencher-labeled DNAzymes onto the surface of carboxyl-silanized QDs. In the presence of metal ions, the emission is restored due to the cleavage of DNAzymes. The detection could be completed within 25 min with a single laser excitation source. The detection limit of 0.2 and 0.5 nM was experimentally achieved for Pb^{2+} and Cu^{2+}, respectively, which is a 50- and 70-fold improvement over the recent results obtained with dye molecules. Multiplexed detection was also demonstrated using two different colors of QDs, showing negligible cross-talk between the Pb^{2+} detection and Cu^{2+} detection.

KEYWORDS: quantum dot · heavy metal · FRET · sensor

Heavy metal contamination is one of the most serious concerns to human health because these substances are toxic and retained by the ecological system.[1] The presence of heavy metals in ambient environment has been increased dramatically during the last few decades.[2] The combustion of immense quantities of coal and other fossil fuels introduces a large amount of industrial heavy metals into atmosphere and the aquatic environment. Although some of the heavy metals such as copper (Cu),[3,4] iron (Fe),[4,5] and zinc (Zn)[6] are biologically essential and required by some organisms,[4] they can lead to toxicity at higher concentrations. On the other hand, some other heavy metals such as lead (Pb),[7] mercury (Hg),[8] and cadmium (Cd)[9] are not biologically essential and are harmful to organisms even at very low concentrations because they lead to excessive free-radical proliferation.[10]

To minimize the pollution of heavy metals, one way is to develop analytical tools for heavy metal detection and prevent the damaging effects of pollution in the very beginning stage. Conventional analytical techniques for heavy metals, such as atomic absorption spectrometry,[11] inductively coupled plasma mass spectrometry,[12] capillary electrophoresis,[13] X-ray fluorescence spectrometry,[14] and microprobes,[15] have routinely been used for metal ion analysis with high sensitivity. However, most of these techniques suffer from the disadvantages of high costs, sophisticated instruments, and requirements for trained personnel and sample pretreatment, making it difficult for real-time and on-site monitoring of metal ions.[16] Therefore, the development of a highly sensitive and portable sensor system to detect toxic heavy metal ions has long been a focus of research.

DNAzymes, a new category of enzymes that are highly specific and sensitive for metal ions such as Pb(II), Cu(II), and Zn(II), have increasingly been used as metal sensors.[17,18] Recent studies by Lu *et al.* have demonstrated that the combinatorial selection of DNAzymes can be employed for heavy metal detection.[18] For instance, they reported functional DNAzyme biosensors labeled with fluorophores and quenchers that used DNAzyme-catalyzed ligation reaction for Pb^{2+} detection with a detection limit of 10 nM.[19] By using *in vitro* selection methods,[18] DNAzymes with high specificity for any metal ion such as Pb^{2+}, Hg^{2+}, Cu^{2+}, and UO_2^{2+} can be obtained, and by modifying fluorophore/quencher pairs attached to those DNAzymes, different types of heavy metal sensors can be developed.[18−22] However, applications of the organic fluorophore have been limited due to several problems such as low photostability, low quantum yield, and the requirement for multiple excitation sources.[23]

*Address correspondence to xsfan@umich.edu.

Received for review August 29, 2010 and accepted September 24, 2010.

10.1021/nn1021988

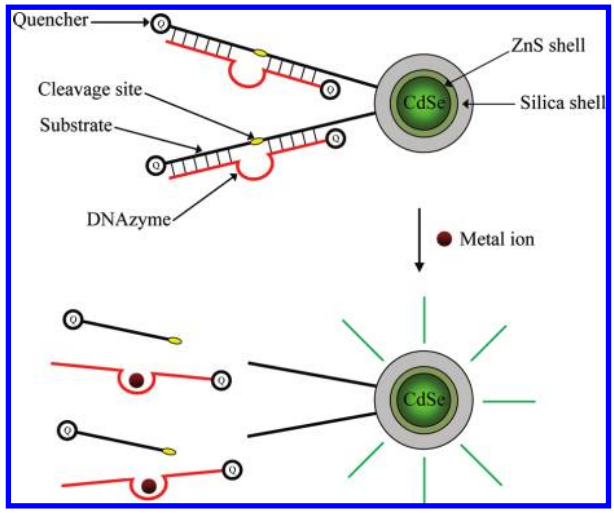

Figure 1. Schematic of QD-based catalytic DNAzymes. The ZnS-capped CdSe QD is embedded in a siloxane shell and covalently coupled to DNAzymes. Each DNAzyme is composed of two quenchers. In the absence of the target metal ions, the fluorescence from the QD is quenched. Once the target metal ion binds to the DNAzyme, the fluorescence from the QD is restored due to the cleavage of the DNAzyme substrate.

Semiconductor quantum dots (QDs) represent a revolution in the application of fluorophores in fluorescent labeling due to their high photo- and chemical stability and high quantum yield, as compared to traditional organic dyes. In addition, different QD fluorescence emissions can be controlled by simply adjusting the QD size. Furthermore, all colors of QDs can be excited by a single excitation source, which makes it much easier to achieve multiplexed detection than traditional dyes that require the excitation light source be tuned into their respective narrow absorption bands.[24−26] Therefore, QDs are increasingly adopted as an alternative for organic dyes in biosensing,[27,28] imaging applications,[3,27,29,30] and ion detections.[31,32] In this study, we combined the benefits of DNAzymes and QDs to develop a highly sensitive and selective sensor that is capable of detecting sub-nanomolar heavy metal ions in less than half an hour in a multiplexed format with a single excitation source.

RESULTS AND DISCUSSION

As illustrated in Figure 1, the DNAzyme heavy metal sensor relies on fluorescence resonance energy transfer (FRET) to carry out the detection. To reach the highest possible quenching efficiency, the dual quenchers on both substrate and DNAzyme segments were designed and introduced in this study.[19] In the absence of the target metal ions, DNAzymes (and hence quenchers) are bound to the QD surface. The quencher on the DNAzyme can efficiently quench fluorescence of the QDs due to its close proximity. The quencher attached to the substrate is used to reduce the background fluorescence from the free QDs in the solution when the DNAzyme is detached from the substrate due to the low stability of the DNAzyme−substrate complex at room temperature. In the presence of the target metal ions, the substrate and enzyme are cleaved off the QD and the QD emission is restored.

FRET is highly dependent upon the distance between the donor (QD) and the acceptor (quencher). In order to maximize the quenching efficiency, the smallest possible QDs are preferred. In our previous work, we have successfully synthesized a very compact QD−DNA probe (2.8 nm for green QDs and 3.3 nm for red QDs) that enabled highly efficient energy transfer.[3] Therefore, they seem to be promising candidates for the development of QD−DNAzyme-based heavy metal sensors. However, we found that while the QD emission was initially restored upon the addition of the target metal ion and the subsequent cleavage of the DNAzymes, the emission dropped ~50% in 5 min, which prevented us from carrying out sensitive and quantitative measurement. This quenching effect of metal ions on the fluorescence of QDs was also reported recently by other groups[33−35] and was attributed to the metal ions nonspecifically bound to the QD surface that facilitate the nonradiative electron/hole recombination.[36−38]

To overcome the quenching effect, we introduced surface silanization onto QDs to isolate and protect the QDs, which has previously been applied to generate higher quantum yield and more stable QDs in aqueous solution.[39,40] The thickness of the silica shell could be controlled by adjusting the reaction time and the silane compound concentration. The method for synthesis of carboxyl-silanized QDs is shown in Figure 2a. The hydroxyl group was first introduced using 2-mercaptoethanol to replace the TOPO Trioctylphosphine oxide group on the QDs' surface. Then robust QDs could easily be established *via* a silanization reaction in organic solvent. In this article, 3-APS 3-aminopropyltrimethoxysilane was used to form a silica shell and introduce the primary amine group to the QDs, which was subsequently converted to the carboxyl group. The carboxyl-silanized QDs could readily be coupled to DNAzymes by using well-established cross-linking methods and commercially available cross-linkers.

In Figure 2b−e, we used dynamic laser scattering (DLS) to characterize the size of the before and after silanization of OH-capped QDs and found that the thickness of the silane layer on the QDs was 3.1 nm (3.3 nm) for QD530 (QD625), corresponding to the final QD size of approximately 9.6 nm (12.7 nm) in diameter. Since the Förster distance for QD and dye is approximately 7 nm,[41] the above size ensures the efficient energy transfer between the QD and the quencher, as shown later. To test their stability, the silanized QDs were challenged with high concentrations of Pb^{2+} and Cu^{2+} solution. As shown in Figure 3, the QD fluorescence intensity shows virtually no change even after 30 min of incubation, attesting to high resistance of the silanized QDs to the metal quenching effect.

To form the QD−DNAzyme complex, we used the zero-length cross-linkers, EDC and sulfo-NHS, to attach

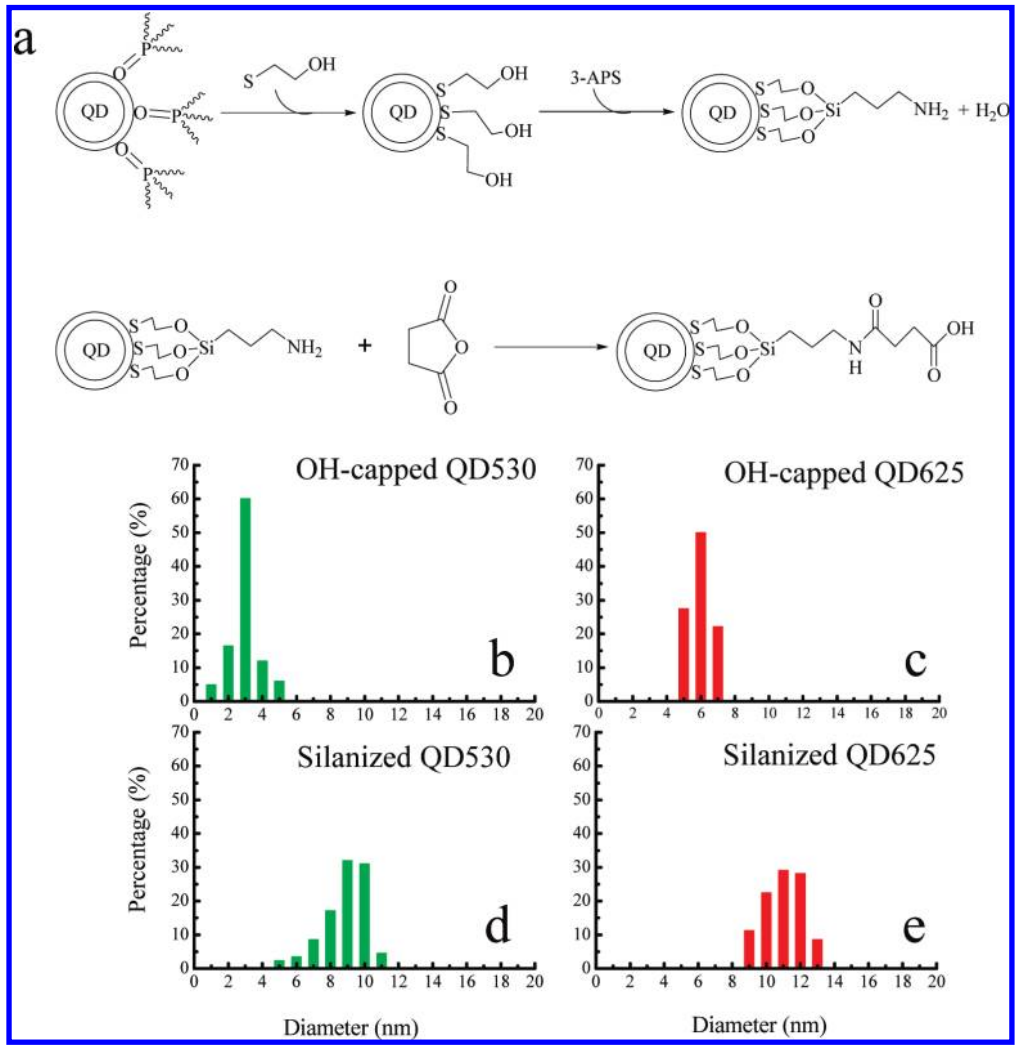

Figure 2. (a) Reaction scheme of the silanization and functionalization of QDs. Chemically synthesized TOPO-capped QDs were replaced by mercaptoethanol using thiol exchange reaction to introduce hydroxyl groups. Then, the QDs were silanized with 3-APS. The ethoxysilane (Si-OCH_2CH_3) groups hydrolyze into hydroxyl groups and release water molecules. Finally, functional exchange reaction of the amine functions by ring opening of succinic anhydride. (b−e) Size distributions of OH-capped QDs and silanized QDs measured by DLS. After silanization, the QD size is ~9.6 nm (12.7 nm) in diameter for QD530 (QD625).

the aminated quencher-labeled DNAzyme to the carboxyl-silanized QD surface. The quenching effect of the DNAzyme was investigated in Figure 4a,b by incubating 500 μL of QDs at 50 nM with 500 μL of DNAzymes with different concentrations. A drastic increase in the QD emission quenching efficiency from 64 to 97.5% for the Pb^{2+}-specific QD530−DNAzyme and from 65 to 95% for the Cu^{2+}-specific QD625−DNAzyme was observed when the DNAzyme-to-QD molar ratio was increased from 8:1 to 16:1. To further verify that the DNAzymes were successfully conjugated with carboxyl-silanized QDs and that the decreased emission was indeed from the quenchers on the DNAzymes, we used negative control DNAzymes without any quenchers attached and attached them to the corresponding QDs under the same cross-linking reaction conditions. Figure 4c (4d) shows the emission of the silanized QD530 (QD625) linked with nonquencher DNAzymes, control DNAzyme1 (2), prepared with NPb-Sub (NCu-Sub) and NPb-Enz (NCu-Enz) (see Table 1). The nearly identical spectrum was observed as compared to the original QDs before DNAzyme attachment, thus confirming the successful assembly of QD−DNAzyme sensors. In the subsequent experiments, we used QD−DNAzyme sensors prepared with the DNAzyme-to-QD molar ratio of 16:1. Under this condition, the size of QD530−DNAzyme and QD625−DNAzyme became 15.8 and 16.2 nm in diameter, respectively, according to DLS results, indicating that there were on average 11 DNAzymes on each QD530 and 10 DNAzymes on each QD625.

To characterize the performance of the QD−DNAzyme sensors, various concentrations of Pb^{2+} and Cu^{2+} were added to the QD−DNAzyme solutions. Figure 5 shows the time-dependent fluorescence with various concentrations of metal ions. The higher reaction rate is associated with the higher ion concentration. Saturation was reached in approximately 25 min,

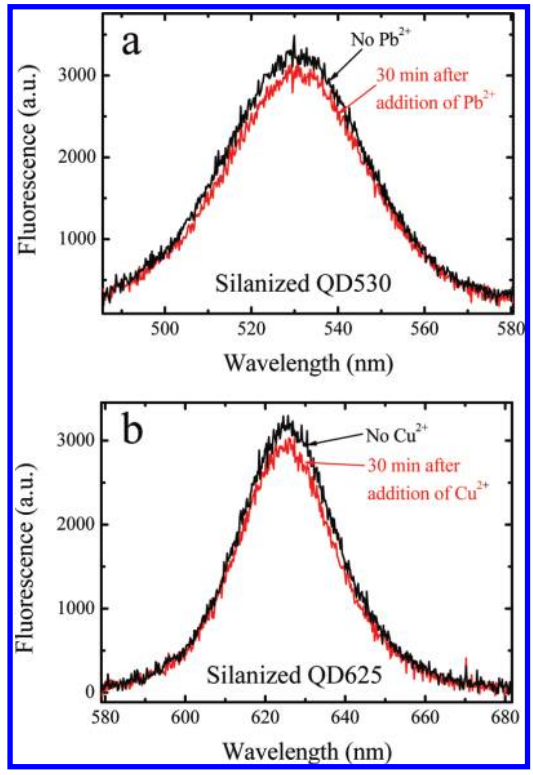

Figure 3. Stability test of the silanized QDs with high concentrations of heavy metal ions. The control spectra were obtained by diluting the 25 nM silanized QD solutions with deionized H_2O at the ratio of 1:1. The 30 min spectra were obtained 30 min after the addition of 50 μL of 3 μM metal ion solution to 50 μL of 25 nM silanized QDs.

TABLE 1. Sample Names and DNA Sequences

sample name	sequence
Pb-Sub	NH_2C_6-5′-ACTCACTAT**rA**GGAAGAGATG-3′-Iowa black FQ
Pb-Enz	5′-CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-3′-Iowa black FQ
Cu-Sub	Iowa black RQ-5′-AGCTTCTTTCTAATACGGCTTACC-3′-C_6NH_2
Cu-Enz	Iowa black RQ-5′-GGTAAGCCTGGGCCTCTTTCTTTTTAAGAAAGAAC-3′
NPb-Sub	NH_2C_6-5′-ACTCACTAT**rA**GGAAGAGATG-3′
NPb-Enz	5′-CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-3′
NCu-Sub	5′-AGCTTCTTTCTAATACGGCTTACC-3′-C_6NH_2
NCu-Enz	5′-GGTAAGCCTGGGCCTCTTTCTTTTTAAGAAAGAAC-3′

and no degradation in fluorescence was observed thereafter. Figure 6a,c shows the fluorescence spectra taken 25 min after sample injection. The corresponding fluorescence intensity increase with respect to the background is presented in Figure 6b,d and is found to be linearly proportional to the heavy metal ion concentration in the range of 1 to 50 nM. The lowest concentration experimentally obtained is 0.2 and 0.5 nM for Pb^{2+} and Cu^{2+}, respectively, which is a 50- and 70-fold improvement as compared to the recently reported results using DNAzymes.[19,20]

One of the advantages of using QDs in replacement of conventional dye molecules is the multiplexed detection capability with a single laser excitation. To demonstrate such capability, Pb^{2+}-specific QD530−DNAzymes were mixed with Cu^{2+}-specific QD625−DNAzymes before the mixture of heavy metal

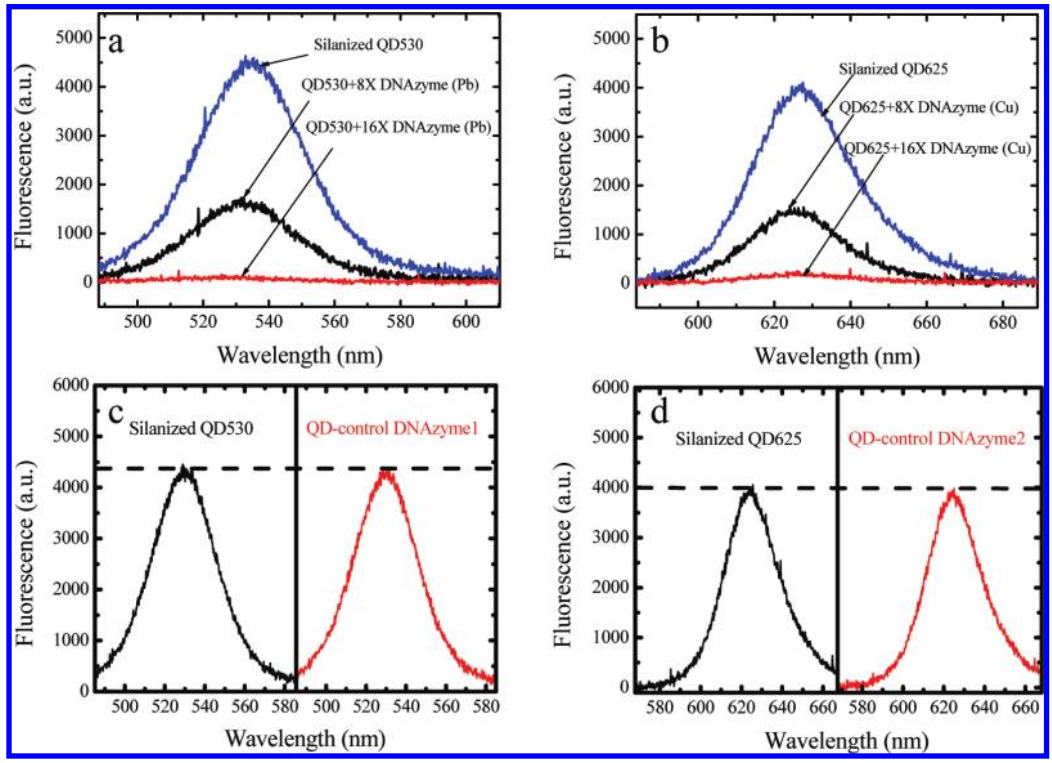

Figure 4. Fluorescence emission spectra evolution of the QD−DNAzymes. (a) Pb^{2+}-specific QD530−DNAzyme complex at a DNAzyme-to-QD molar ratio of 0:1, 8:1, and 16:1. (b) Cu^{2+}-specific QD625−DNAzyme complex at a DNAzyme-to-QD molar ratio of 0:1, 8:1, and 16:1. (c) Negative control experiment. The DNAzyme1-to-QD530 molar ratio was 16:1. (d) Negative control experiment. The DNAzyme2-to-QD625 molar ratio was 16:1.

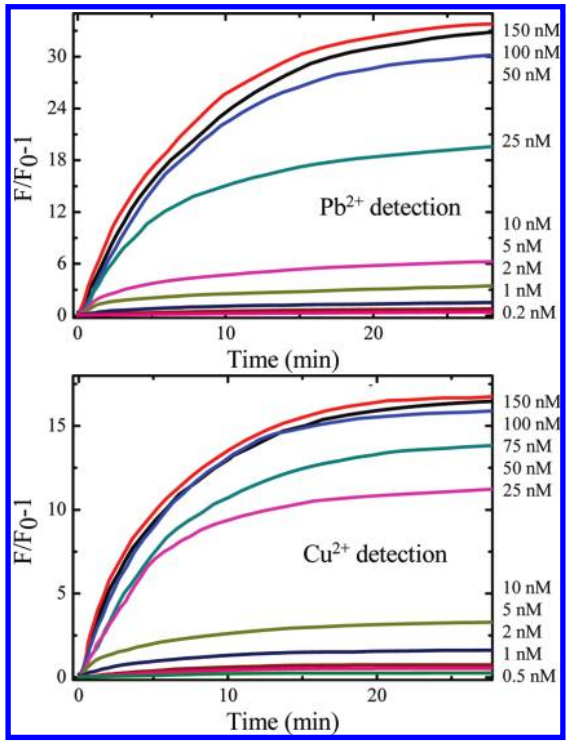

Figure 5. Time-dependent spectrally integrated emission intensity of QD−DNAzyme sensors over the background at different metal ion concentrations. F_0 (F) is the QD emission intensity in the absence (presence) of target heavy metal ions. (Top) Pb^{2+}-specific QD530−DNAzyme. Spectral integration takes place from 490 to 600 nm. (Bottom) Cu^{2+}-specific QD625−DNAzyme. Spectral integration takes place from 580 to 700 nm.

ions was added. The excitation was set at 480 nm, and emission centered around 530 nm for Pb^{2+} and 625 nm for Cu^{2+} was monitored. The increase of the fluorescence intensity in the 530 nm channel and 625 nm channel relative to the respective background reflects the concentration of the corresponding metal ions. Figure 7 shows that the Pb^{2+} and Cu^{2+} can be simultaneously and quantitatively detected with virtually no cross-talk between the 530 nm channel and the 625 nm channel.

CONCLUSION

We have developed the QD−DNAzyme sensors for ultrasensitive and selective and multiplexed detection of heavy metal ions in liquid by combining the highly stable silanized QDs with high ion-specific DNAzymes. The detection could be completed within half an hour, and the sub-nanomolar detection limit was achieved for Pb^{2+} and Cu^{2+}, nearly 2 orders of magnitude better than the state-of-the-art. Future work will involve using other types of quenchers such as gold nanoparticles and optimizing the salinization method to better control the saline layer thickness, both of which will further improve the quenching efficiency and result in an even better detection limit. Our sensors will have broad applications in monitoring of waste treatment, industrial drain control, medical diagnostics, and homeland security.

EXPERIMENTAL SECTION

Materials. 2-Mercaptoethanol (99.0%), sodium hydroxide (NaOH, 98%), methanol (99.8%), ethanol (99.8%), 3-aminopropyltrimethoxysilane (3-APS, 97%), chlorotrimethylsilane (99%), TMAH pentahydrate (97%), chloroform (99%), succinic anhydride (99%), tetrahydrofuran (THF, 99.9%), sodium ascorbate, phosphate buffered saline (PBS), isacetate buffer, 0.2 μm syringe filter, 0.45 μm syringe filter, 30 000 MWCO Nanosep centrifugal filter, and 100 000 MWCO Nanosep centrifugal filter were obtained from Sigma-Aldrich (St. Louis, MO). Sodium chloride (NaCl), lead(II) acetate trihydrate (99.999%), cupric chloride (1 M), HEPES, pH 7.0 buffer, and phosphate buffer (50 mM, pH 6.5) were obtained from Fisher Scientific (Pittsburgh, PA). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (Sulfo-NHS) were obtained from PIERCE (Rockford, IL). NAP-25 columns were obtained from GE Healthcare (South Burlington, VT).

All DNAs used in experiments were HPLC grade and were obtained from IDT (Coralville, IA). The DNA sample names and the corresponding sequences are given in Table 1. Briefly, Pb-Enz (33-base labeled with Iowa black FQ) and Cu-Enz (35-base labeled with Iowa black RQ) served as the Pb^{2+} and Cu^{2+} enzyme sequence. Pb-Sub with a ribo-adenosine (rA) (19-base labeled with Iowa black FQ) and Cu-Sub (24-base labeled with Iowa black RQ) served as the substrates for Pb^{2+} and Cu^{2+} enzyme sequences, respectively. NPb-Enz and NCu-Enz were Pb^{2+} and Cu^{2+} enzyme sequences without quenchers; NPb-Sub and NCu-Sub were Pb^{2+} and Cu^{2+} substrate sequences without quenchers. They were used to form the negative control DNAzymes.

Preparation of OH-Capped QDs. TOPO-capped QD synthesis was carried out by following the protocol that we developed previously.[3] To obtain OH-capped QDs, TOPO-capped QDs were dried by evaporation, then 100 mg of the QDs in 1 mL of 2-mercaptoethanol and 0.5 mL of methanol was added and pH was adjusted to 9 with 0.2 M NaOH. This mixture was stirred overnight at 85 °C, and the resulting OH-capped water-soluble QDs were then precipitated by adding 3 mL of ethanol. The residue was washed three times with ethanol, and then the resulting suspension was centrifuged at 14 000 rpm for 5 min. The supernatant was discarded, and the QDs were dissolved in 1 mL of deionized H_2O and filtered through a 0.2 μm syringe filter to obtain OH-capped QDs in clear solution. During the experiment, we prepared two types of OH-capped QDs with the emission peak at 525 and 619 nm, respectively.

Silanization of QDs and Surface Functional Modification. To obtain the silanized QDs, 0.5 mL of 3-aminopropyltrimethoxysilane (3-APS) diluted with 2 mL of methanol was added to 1 mL of OH-capped QDs (15 μM) slowly at 0 °C with vigorous mixing. This mixture became cloudy immediately and then turned clear within a few minutes. After 10 min of stirring, the solution was heated to ~60 °C for 30 min and then cooled to ~30 °C. To quench the silanization reaction, a mixture of 15 mL of methanol and 1.5 mL of chlorotrimethylsilane basified with 2.3 g of solid TMAH pentahydrate was added at room temperature. After ~2 h of stirring, the solution was heated to ~60 °C for 30 min and then stirred slowly under argon at room temperature for 2 days. Methanol was removed *in vacuo* for 24 h, and the solution was filtered through a 0.45 μm syringe filter and free silane was removed *via* size-exclusion chromatography using a NAP-25 column with deionized H_2O. Then the solution was concentrated twice with a centrifuge (8000 rpm) using a 30 000 MWCO Nanosep centrifugal filter. Finally, the silanized QD solution was reduced to ~1 mL and stored in a 4 °C dark room. The emission peak of the amino-silanized QDs was found to slightly red shift to 530 nm (QD530) and 625 nm (QD625) with respect to their un-

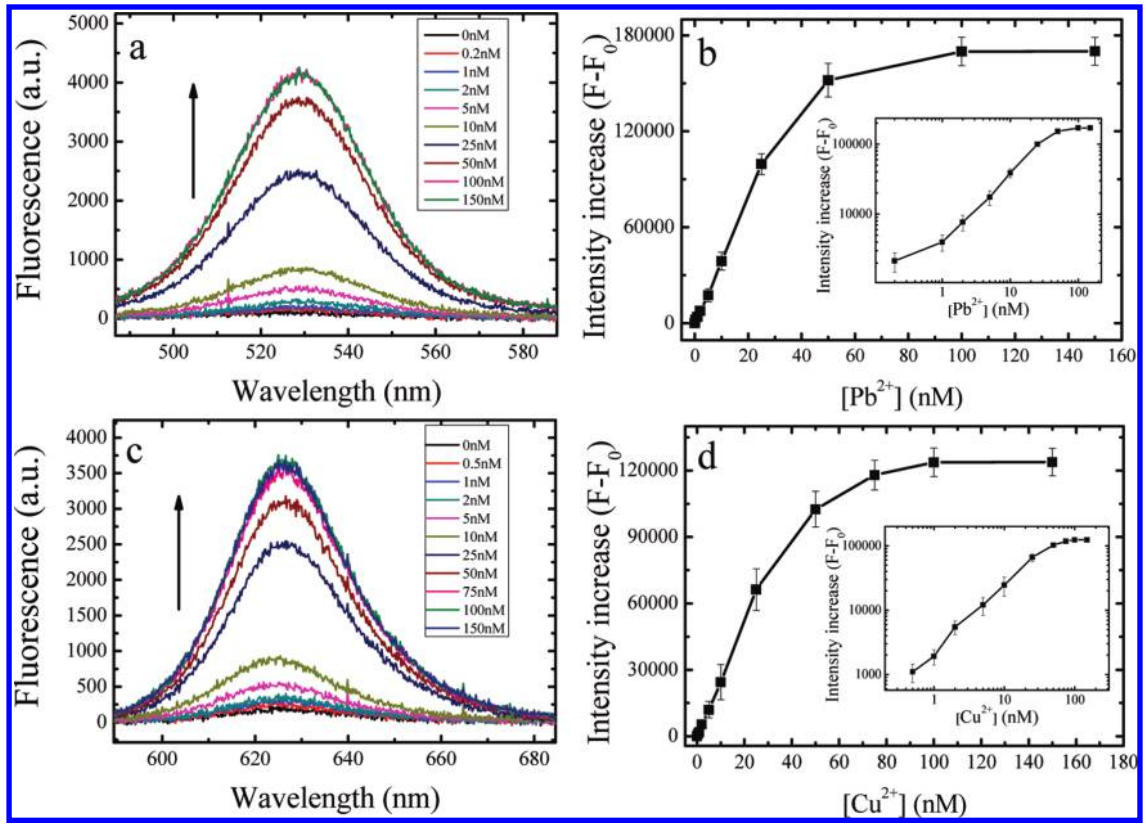

Figure 6. (a) Fluorescence spectra of Pb^{2+}-specific QD530−DNAzyme at various Pb^{2+} concentrations taken after 25 min of sample incubation. (b) QD530 emission intensity increase ($F-F_0$) based on the spectra in (a) as a function of the Pb^{2+} concentration. Error bars indicate variations from three measurements. Inset: the corresponding log−log plot. (c) Fluorescence spectra of Cu^{2+}-specific QD625−DNAzyme at various Cu^{2+} concentrations taken after 25 min of sample incubation. (d) QD625 emission intensity increase ($F-F_0$) based on the spectra in (c) as a function of the Cu^{2+} concentration. Error bars indicate variations from three measurements. Inset: the corresponding log−log plot.

salinized counterparts (*i.e.*, OH-capped QDs). The quantum yield was approximately 30% using rhodamine 6G in ethanol as a standard.

Carboxyl-silanized QDs were generated by the following procedures described in the literature.[42] The amino-silanized QD solution was precipitated using anhydrous chloroform. The wet precipitate (100 mg) was suspended in 5 mL of THF, and 10 mg of succinic anhydride was added under sonification. The suspension was stirred for 16 h at room temperature, and then THF was removed by a rotary evaporator. The residue was dissolved in 2 mL of deionized H_2O and then filtered through a 0.2 μm syringe filter. The excessive succinic anhydride was removed by a NAP-25 column with deionized H_2O. The carboxyl-silanized QDs were concentrated with a centrifuge (8000 rpm) using a 100 000 MWCO Nanosep centrifugal filter.

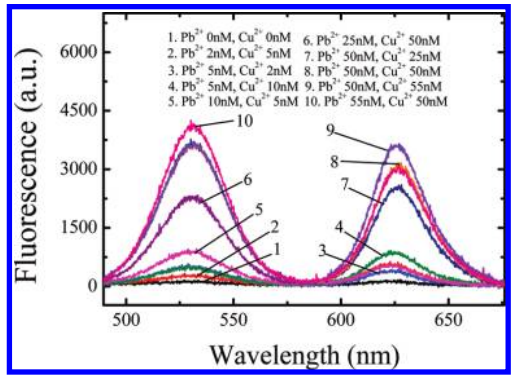

Figure 7. Multiplexed detection of heavy metal ions using QD−DNAzyme sensors.

Preparation of DNAzymes and QD−DNAzyme Sensors. The names and the modifications of the DNA sequences are given in Table 1. The sequence selection was based on the work by Lu *et al.*[19,20] Each DNAzyme was designed with two quenchers to efficiently quench the QD fluorescence. For the Pb^{2+}-specific DNAzyme, the substrate strand was 5′-end labeled with the amino group to couple with the carboxyl group on QDs and the 3′-end was labeled with Iowa black FQ. The enzyme strand was 3′-end labeled with Iowa black FQ. For the Cu^{2+}-specific DNAzyme, the substrate strand was 5′-end labeled with Iowa black RQ and the 3′-end was labeled with the amino group. The enzyme strand was 5′-end labeled with Iowa black RQ.

To form the Pb^{2+}-specific DNAzyme, a total of 0.8 μM enzyme (Pb-Enz) and 0.8 μM corresponding substrate (Pb-Sub) were annealed in 50 mM trisacetate buffer, pH 7.2 with 25 mM NaCl, in a 95 °C water bath for 5 min and subsequently cooled to 4 °C slowly. For Cu^{2+}-specific DNAzyme, 0.8 μM enzyme (Pb-Enz) and 0.8 μM corresponding substrate (Pb-Sub) were annealed in 50 mM HEPES, pH 7.0 buffer, with 1.5 M NaCl by heating in a 95 °C water bath for 5 min and then cooled to 4 °C slowly. The annealed DNAzyme (0.8 μM, 500 μL) was mixed with carboxyl-silanized QDs (50 nM, 500 μL), and then EDC (0.2 mg) and sulfo-NHS (0.1 mg) dissolved in 50 μL of phosphate buffer (25 mM, pH 6.5) were added. After 12 h of incubation at 4 °C, unreacted EDC/sulfo-NHS and excessive DNA were removed by NAP-25 column with 1× PBS, and the purified QD−DNAzyme conjugates were stored in a 4 °C dark room. The QD−DNAzyme was concentrated with a centrifuge (8000 rpm) using a 100 000 MWCO Nanosep centrifugal filter with the final concentration of 25 nM.

Spectroscopy of Heavy Metal Detection. Detection of heavy metal ions took place by mixing 90 μL of the 25 nM QD−DNAzyme and 10 μL of different concentrations of metal ion stock solutions in a microquartz cuvette at room temperature. The metal

ion solutions were prepared by diluting lead(II) acetate trihydrate and cupric chloride with deionized H_2O. For the multiplexed heavy metal ion detection, 180 μL of the mixture of Pb^{2+}-specific QD530−DNAzyme and Cu^{2+}-specific QD625-DNAzyme (25 nM each) was mixed with a 20 μL mixture of Pb^{2+} and Cu^{2+} solutions in a quartz cuvette at room temperature. For the Cu^{2+} and multiplexed ion detection, ascorbate was added to a final concentration of 50 μM. A 480 nm laser was used for QD excitation. The fluorescence spectra were recorded by a USB 4000 miniature fiber optic spectrometer (Ocean Optics, FL) at a scan rate of 300 ms for post-analysis.

Acknowledgment. Our work is supported by the National Science Foundation (CBET-0747398).

REFERENCES AND NOTES

1. Verma, N.; Singh, M. Biosensors for Heavy Metals. *BioMetals* **2005**, *18*, 121–129.
2. Panov, V.; Zykova, I.; Chekrenev, S. Heavy Metals: The Industry and Environmental Protection. *Fibre Chem.* **2008**, *40*, 241–245.
3. Wu, C.-S.; Cupps, J. M.; Fan, X. Compact Quantum Dot Probes for Rapid and Sensitive DNA Detection Using Highly Efficient Fluorescence Resonant Energy Transfer. *Nanotechnology* **2009**, *20*, 305502.
4. Brewer, G. J. Risks of Copper and Iron Toxicity during Aging in Humans. *Chem. Res. Toxicol.* **2010**, *23*, 319–326.
5. Bruins, M. R.; Kapil, S.; Oehme, F. W. Microbial Resistance to Metals in the Environment. *Ecotoxicol. Environ. Safety* **2000**, *45*, 198–207.
6. Todd, M. D.; Lee, M. J.; Williams, J. L.; Nalezny, J. M.; Gee, P.; Benjamin, M. B.; Farr, S. B. The CAT-Tox (L) Assay: A Sensitive and Specific Measure of Stress-Induced Transcription in Transformed Human Liver Cells. *Fundam. Appl. Toxicol.* **1995**, *28*, 118–128.
7. Rogers, J. T.; Richards, J. G.; Wood, C. M. Ionoregulatory Disruption as the Acute Toxic Mechanism for Lead in the Rainbow Trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **2003**, *64*, 215–234.
8. Miyake, Y.; Togashi, H.; Tashiro, M.; Yamaguchi, H.; Oda, S.; Kudo, M.; Tanaka, Y.; Kondo, Y.; Sawa, R.; Fujimoto, T.; *et al.* MercuryII-Mediated Formation of Thymine-HgII-Thymine Base Pairs in DNA Duplexes. *J. Am. Chem. Soc.* **2006**, *128*, 2172–2173.
9. Kim, H.-K.; Rasnik, I.; Liu, J.; Ha, T.; Lu, Y. Dissecting Metal Ion-Dependent Folding and Catalysis of a Single DNAzyme. *Nat. Chem. Biol.* **2007**, *3*, 763–768.
10. Hussein, H.; Farag, S.; Kandil, K.; Moawad, H. Tolerance and Uptake of Heavy Metals by Pseudomonads. *Process Biochem.* **2005**, *40*, 955–961.
11. Kunkel, R.; Manahan, S. E. Atomic Absorption Analysis of Strong Heavy Metal Chelating Agents in Water and Waste Water. *Anal. Chem.* **1973**, *45*, 1465–1468.
12. Bings, N. H.; Bogaerts, A.; Broekaert, J. A. C. Atomic Spectroscopy. *Anal. Chem.* **2006**, *78*, 3917–3946.
13. Tanyanyiwa, J.; Hauser, P. C. High-Voltage Contactless Conductivity Detection of Metal Ions in Capillary Electrophoresis. *Electrophoresis* **2002**, *23*, 3781–3786.
14. Lorber, K. E. Monitoring of Heavy Metals by Energy Dispersive X-ray Fluorescence Spectrometry. *Waste Manage. Res.* **1986**, *4*, 3–13.
15. Bertsch, P. M.; Hunter, D. B. Applications of Synchrotron-Based X-ray Microprobes. *Chem. Rev.* **2001**, *101*, 1809–1842.
16. Czarnik, A. W. Desperately Seeking Sensors. *Chem. Biol.* **1995**, *2*, 423–428.
17. Lu, Y. New Transition-Metal-Dependent DNAzymes as Efficient Endonucleases and as Selective Metal Biosensors. *Chem.—Eur. J.* **2002**, *8*, 4588–4596.
18. Liu, J.; Cao, Z.; Lu, Y. Functional Nucleic Acid Sensors. *Chem. Rev.* **2009**, *109*, 1948–1998.
19. Liu, J.; Lu, Y. Improving Fluorescent DNAzyme Biosensors by Combining Inter- and Intramolecular Quenchers. *Anal. Chem.* **2003**, *75*, 6666–6672.
20. Liu, J.; Lu, Y. A DNAzyme Catalytic Beacon Sensor for Paramagnetic Cu^{2+} Ions in Aqueous Solution with High Sensitivity and Selectivity. *J. Am. Chem. Soc.* **2007**, *129*, 9838–9839.
21. Liu, J.; Brown, A. K.; Meng, X.; Cropek, D. M.; Istok, J. D.; Watson, D. B.; Lu, Y. A Catalytic Beacon Sensor for Uranium with Parts-Per-Trillion Sensitivity and Millionfold Selectivity. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2056–2061.
22. Wang, Z.; Lee, J. H.; Lu, Y. Highly Sensitive "Turn-On" Fluorescent Sensor for Hg^{2+} in Aqueous Solution Based on Structure-Switching DNA. *Chem. Commun.* **2008**, 6005–6007.
23. Eggeling, C.; Widengren, J.; Rigler, R.; Seidel, C. A. M. Photobleaching of Fluorescent Dyes under Conditions Used for Single-Molecule Detection: Evidence of Two-Step Photolysis. *Anal. Chem.* **1998**, *70*, 2651–2659.
24. Han, M.; Gao, X.; Su, J. Z.; Nie, S. Quantum-Dot-Tagged Microbeads for Multiplexed Optical Coding of Biomolecules. *Nat. Biotechnol.* **2001**, *19*, 631–635.
25. Hohng, S.; Ha, T. Single-Molecule Quantum-Dot Fluorescence Resonance Energy Transfer. *ChemPhysChem* **2005**, *6*, 956–960.
26. Willard, D. M.; Van Orden, A. Quantum Dots: Resonant Energy-Transfer Sensor. *Nat. Mater.* **2003**, *2*, 575–576.
27. Gill, R.; Zayats, M.; Willner, I. Semiconductor Quantum Dots for Bioanalysis. *Angew. Chem., Int. Ed.* **2008**, *47*, 7602–7625.
28. Sharon, E.; Freeman, R.; Willner, I. CdSe/ZnS Quantum Dots-G-Quadruplex/Hemin Hybrids as Optical DNA Sensors and Aptasensors. *Anal. Chem.* **2010**, *82*, 7073–7077.
29. Wang, L.-Y.; Wang, L.; Gao, F.; Yu, Z.-Y.; Wu, Z.-M. Application of Functionalized CdS Nanoparticles as Fluorescence Probe in the Determination of Nucleic Acids. *Analyst* **2002**, *127*, 977–980.
30. Chan, W. C. W.; Nie, S. Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection. *Science* **1998**, *281*, 2016–2018.
31. Moshe, M.; Elbaz, J.; Willner, I. Sensing of UO_2^{2+} and Design of Logic Gates by the Application of Supramolecular Constructs of Ion-Dependent DNAzymes. *Nano Lett.* **2009**, *9*, 1196–1200.
32. Freeman, R.; Finder, T.; Willner, I. Multiplexed Analysis of Hg^{2+} and Ag^{+} Ions by Nucleic Acid Functionalized CdSe/ZnS Quantum Dots and Their Use for Logic Gate Operations. *Angew. Chem., Int. Ed.* **2009**, *48*, 7818–7821.
33. Liang, J.-G.; Ai, X.-P.; He, Z.-K.; Pang, D.-W. Functionalized CdSe Quantum Dots as Selective Silver Ion Chemodosimeter. *Analyst* **2004**, *129*, 619–622.
34. Chen, Y.; Rosenzweig, Z. Luminescent CdS Quantum Dots as Selective Ion Probes. *Anal. Chem.* **2002**, *74*, 5132–5138.
35. Wu, P.; Li, Y.; Yan, X.-P. CdTe Quantum Dots (QDs) Based Kinetic Discrimination of Fe^{2+} and Fe^{3+}, and CdTe QDs-Fenton Hybrid System for Sensitive Photoluminescent Detection of Fe^{2+}. *Anal. Chem.* **2009**, *81*, 6252–6257.
36. Xia, Y.-S.; Zhu, C.-Q. Use of Surface-Modified CdTe Quantum Dots as Fluorescent Probes in Sensing Mercury(II). *Talanta* **2008**, *75*, 215–221.
37. Susha, A. S.; Javier, A. M.; Parak, W. J.; Rogach, A. L. Luminescent CdTe Nanocrystals as Ion Probes and pH Sensors in Aqueous Solutions. *Colloids Surf., A* **2006**, *281*, 40–43.
38. Isarov, A. V.; Chrysochoos, J. Optical and Photochemical Properties of Nonstoichiometric Cadmium Sulfide Nanoparticles: Surface Modification with Copper(II) Ions. *Langmuir* **1997**, *13*, 3142–3149.
39. Bruchez, M., Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. Semiconductor Nanocrystals as Fluorescent Biological Labels. *Science* **1998**, *281*, 2013–2016.
40. Gerion, D.; Pinaud, F.; Williams, S. C.; Parak, W. J.; Zanchet, D.; Weiss, S.; Alivisatos, A. P. Synthesis and Properties of Biocompatible Water-Soluble Silica-Coated CdSe/ZnS Semiconductor Quantum Dots. *J. Phys. Chem. B* **2001**, *105*, 8861–8871.
41. Clapp, A. R.; Medintz, I. L.; Mauro, J. M.; Fisher, B. R.;

Bawendi, M. G.; Mattoussi, H. Fluorescence Resonance Energy Transfer between Quantum Dot Donors and Dye-Labeled Protein Acceptors. *J. Am. Chem. Soc.* **2004**, *126*, 301–310.

42. Thomas, S.; Herwig, B.; Günter, E. M. T. Controlled Surface Functionalization of Silica Nanospheres by Covalent Conjugation Reactions and Preparation of High Density Streptavidin Nanoparticles. *J. Nano. Nanotechnol.* **2004**, *4*, 504–511.