Sensing Parts-per-Trillion Cd^{2+}, Hg^{2+} and Pb^{2+} Collectively and Individually Using Phosphorothioate DNAzymes

*Po-Jung Jimmy Huang and Juewen Liu **

Department of Chemistry, Waterloo Institute for Nanotechnology

University of Waterloo

200 University Ave West, Waterloo, Ontario, Canada, N2L 3G1

Fax: (+1) 519-746-0435

E-mail: liujw@uwaterloo.ca

This document is the Accepted Manuscript version of a Published Work that appeared in final form in Analytical Chemistry, copyright © American Chemical Society after peer review and technical editing by publisher. To access the final edited and published work see Huang, P.-J. J., & Liu, J. (2014). Sensing Parts-per-Trillion Cd 2+ , Hg 2+ , and Pb 2+ Collectively and Individually Using Phosphorothioate DNAzymes. Analytical Chemistry, 86(12), 5999–6005. https://doi.org/10.1021/ac501070a

Abstract: Cadmium, mercury and lead are collectively banned by many countries and regions in electronic devices due to their extremely high toxicity. To date, no sensing method can detect them as a group and also individually with sufficient sensitivity and selectivity. An RNA-cleaving DNAzyme (Ce13d) was recently reported to be active with trivalent lanthanides, which are hard Lewis acids. In this work, phosphorothioate (PS) modifications were systematically made on Ce13d. A single PS at the substrate cleavage site shifts the activity from being dependent on lanthanide to soft thiophilic metals. By incorporating the PS modification to a few other DNAzymes, a sensor array was prepared to detect each metal. Individual sensors have excellent sensitivity (limit of detection = 4.8 nM Cd^{2+}, 2.0 nM Hg^{2+}, and 0.1 nM Pb^{2+}). This study provides a new route to obtain metal-specific DNAzymes by atomic replacement and also offers important mechanistic insights into metal binding and DNAzyme catalysis.

Introduction

Cadmium, mercury and lead are the most common heavy metal contaminants. These metals are bioaccumulative, imposing serious organ damages and can lead to cancer and even death.[1, 2] For this reason, they are collectively banned by European Union according to the Restriction of Hazardous Substances Directive since 2006. California has also taken similar regulations. To enforce such regulations and to manage their adverse environmental and health effects, convenient analytical strategies are critical. The current standard method is inductive-coupled plasmon-mass spectrometry (ICP-MS). Being highly reliable, it is available only in centralized labs with a high cost and long turnaround time. In order to provide on-site analysis, a number of metal sensing platforms have been developed.[2-11]

DNAzymes are DNA-based catalysts obtained through in vitro selection.[12-16] Owing to their high catalytic efficiency and versatility in sensor design, RNA-cleaving DNAzymes have emerged as a unique metal sensing platform.[14, 17-21] Since DNAzymes require metal cofactors, by using specific metals during selection, RNA-cleaving DNAzymes selective for Mg^{2+},[13] Pb^{2+},[22, 23] UO_2^{2+}[24] and lanthanides[25] have been reported. These metals are hard or borderline Lewis acids.

High thiophilicity is a common feature of many toxic metals including cadmium and mercury, but natural DNA does not contain sulfur, which might be a reason for the lack of unmodified DNAzymes for them. By incorporating modified bases with soft base ligands (e.g. imidazole group), Zn^{2+} and Hg^{2+} dependent DNAzymes were also isolated.[26, 27] Since these modified bases are not commercially available, their analytical applications have not been widely pursued. Using modified bases also complicates in vitro selection since DNA polymerase may not incorporate such bases. Phosphorothioate (PS) DNA refers to replacement of one of the non-bridging oxygen atoms in the phosphate backbone by sulfur (Figure 1A). The PS modification is often used in the antisense technology to increase DNA stability against nuclease degradation.[28] It is also useful for studying the

mechanism of (deoxy)ribozyme catalysis,[29-32] assembling nanoparticles,[33] and forming DNA structures.[34] However, PS-modified RNA-cleaving DNAzymes have not yet been studied.

We recently reported a lanthanide-dependent DNAzyme (named Ce13d).[25] Ce13d has a similar activity in the presence of trivalent lanthanides and Y^{3+}. Pb^{2+} also shows moderate activity, while other metals are inactive. Since lanthanides are hard Lewis acids that prefer oxygen-based ligands, we hypothesize that it might be converted to a thiophilic-metal-dependent enzyme by PS modification. Herein, we report the first PS-modified DNAzymes that detect Hg^{2+}, Cd^{2+} and Pb^{2+} as a group and individually with ultrahigh sensitivity.

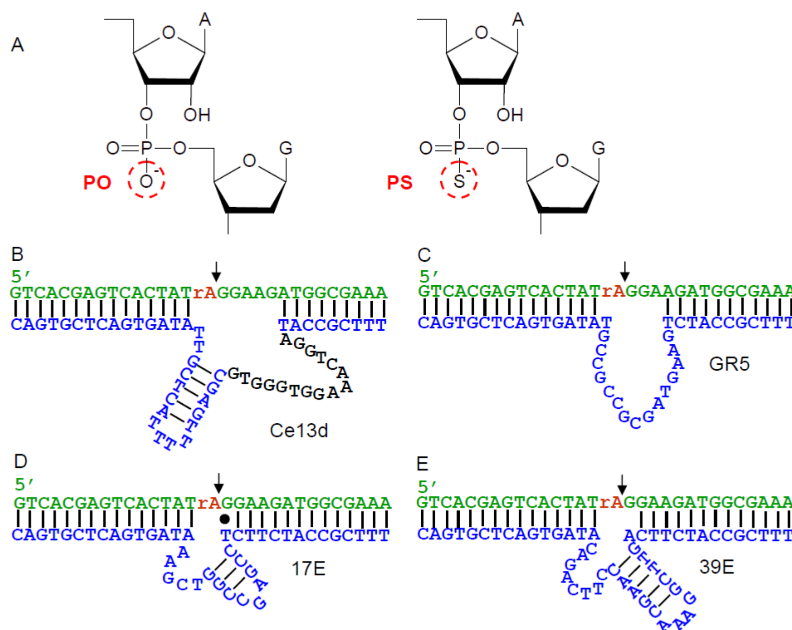

Figure 1. (A) Structure of the normal phosphodiester (PO) linkage and the phosphorothioate (PS) modification at the cleavage junction (rA-G). Secondary structures of the four DNAzymes used in this work: (B) Ce13d; (C) GR5; (D) 17E; and (E) 39E.

Materials and Methods

Chemicals. The fluorophore/quencher-modified DNAs were purchased from Integrated DNA Technologies (IDT, Coralville, IA). The unmodified and phosphorothioate (PS) modified enzyme

strands were from Eurofins (Huntsville, AL). The DNA sequences used in this study are listed in Table 1. Cerium chloride heptahydrate, magnesium sulfate, manganese chloride tetrahydrate, iron chloride tetrahydrate, cobalt chloride hexahydrate, nickel chloride, copper chloride dehydrate, zinc chloride, cadmium chloride hydrate, mercury perchlorate, and lead acetate were purchased from Sigma-Aldrich except the iron salt was from Alfa Aesar. The solutions were made by directly dissolving their salts in water. 2-(N-morpholino)ethanesulfonic acid (MES), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), EDTA disodium salt dehydrate, and sodium chloride were purchased from Mandel Scientific Inc (Guelph, Ontario, Canada). Acrylamide/bisacrylamide 40% solution (29:1), urea, and 10× TBE solution were purchased from Bio Basic Inc.

Gel electrophoresis. The DNAzyme complexes were formed by annealing the FAM-labeled substrate and the enzyme at a molar ratio of 1:1.5 in buffer A (25 mM NaCl, 50 mM MES, pH 6). For a typical gel-based activity assay, a final of 10 μM metal ions were incubated with 5 μL of 1 μM DNAzyme complex in buffer A for 30 min to 1 h. The samples were then quenched with 1× loading dye with 8 M urea and 2 mM EDTA and run in 15% dPAGE at 120V for 80 min. The gel images were taken with a ChemiDoc MP imaging system (Bio-Rad).

DNAzyme beacon assay. The sensor kinetic studies were carried out with 96 well plates and monitored with SpectraMax M3 microplate reader. The stock complex was formed by annealing the FAM-labeled substrate and the quencher-labeled enzyme with a molar ratio of 1:1.5 in buffer A. The stock complex was stored in -20 °C overnight before use. Each complex was further diluted with 25 mM HEPES buffer (pH 7.6). For each well, 100 μL of 50 nM FAM-Q DNAzyme was used. 1 μL of metal ion was added after 5 min of background reading to initiate cleavage. The samples were continuously monitored after addition for at least 30 min with 25 s intervals.

Table 1. DNAzyme and substrate sequences used in this work. rA = riboadenosine; Q = Iowa Black ® FQ; FAM = carboxyfluorescein; *= PS modification.

DNA Name	Sequences and modifications (from 5′-terminus)
PO substrate	CGTTCGCCTCATGACGTTGAAGGATCCAGACT-FAM
PS1	GTCACGAGTCACTAT*rAGGAAGATGGCGAAA-FAM
PS2	GTCACGAGTCACTATrA*GGAAGATGGCGAAA-FAM
PS3	GTCACGAGTCACTAT*rA*GGAAGATGGCGAAA-FAM
Ce13d	TTTCGCCATAGGTCAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
17E	CGCCATCTTCTCCGAGCCGGTCGAAATAGTGACTCGTGAC
GR5	TTTCGCCATCTGAAGTAGCGCCGCCGTATAGTGACTCGTGAC
39E	TTTCGCCATCTTCAGTTCGGAAACGAACCTTCAGACATAGTGACTCGTGAC
Ce13d-Q	Q-TTTCGCCATAGGTCAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
GR5-Q	Q-TTTCGCCATCTGAAGTAGCGCCGCCGTATAGTGACTCGTGAC
Ce13d-A1*	TTTCGCCATA*GGTCAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G2*	TTTCGCCATAG*GTCAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G3*	TTTCGCCATAGG*TCAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -T4*	TTTCGCCATAGGT*CAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -C5*	TTTCGCCATAGGTC*AAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -A6*	TTTCGCCATAGGTCA*AAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -A7*	TTTCGCCATAGGTCAA*AGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -A8*	TTTCGCCATAGGTCAAA*GGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G9*	TTTCGCCATAGGTCAAAG*GTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G10*	TTTCGCCATAGGTCAAAGG*TGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -T11*	TTTCGCCATAGGTCAAAGGT*GGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G12*	TTTCGCCATAGGTCAAAGGTG*GGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G13*	TTTCGCCATAGGTCAAAGGTGG*GTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G14*	TTTCGCCATAGGTCAAAGGTGGG*TGCGAGTTTTTACTCGTTATAGTGACTCGTGAC
Ce13d -G15*	TTTCGCCATAGGTCAAAGGTGGGT*GCGAGTTTTTACTCGTTATAGTGACTCGTGAC

Results and Discussion

PS-modified substrate. The Ce13d DNAzyme (Figure 1B) contains a substrate strand with the ribo-adenosine (rA) being the cleavage site. The bottom strand in blue/black is the enzyme. With a lanthanide (Ln^{3+}), the substrate is cleaved into two pieces. To measure its cleavage activity, the 3′-end of the substrate was labeled with a FAM (carboxyfluorescein). A gel-based assay was performed with

the first row divalent transition metal, group 2B ions, Mg^{2+} and Pb^{2+}. Ce^{3+} was also included to represent Ln^{3+}. With the normal phosphate oxygen (PO) substrate, Ce13d was cleaved only with Ce^{3+} and to a lesser extent with Pb^{2+}. With a single PS in the substrate at the linkage between rA and G (Figure 1A), the Ce^{3+}-dependent activity was significantly suppressed, cleaving only ~5%. At the same time, high activity appeared with thiophilic metals (Cu^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+}) while very low activity was observed with Fe^{2+} and Zn^{2+}. All the other metals remained inactive. We clearly see the influence of the PS modification on shifting the metal preference.

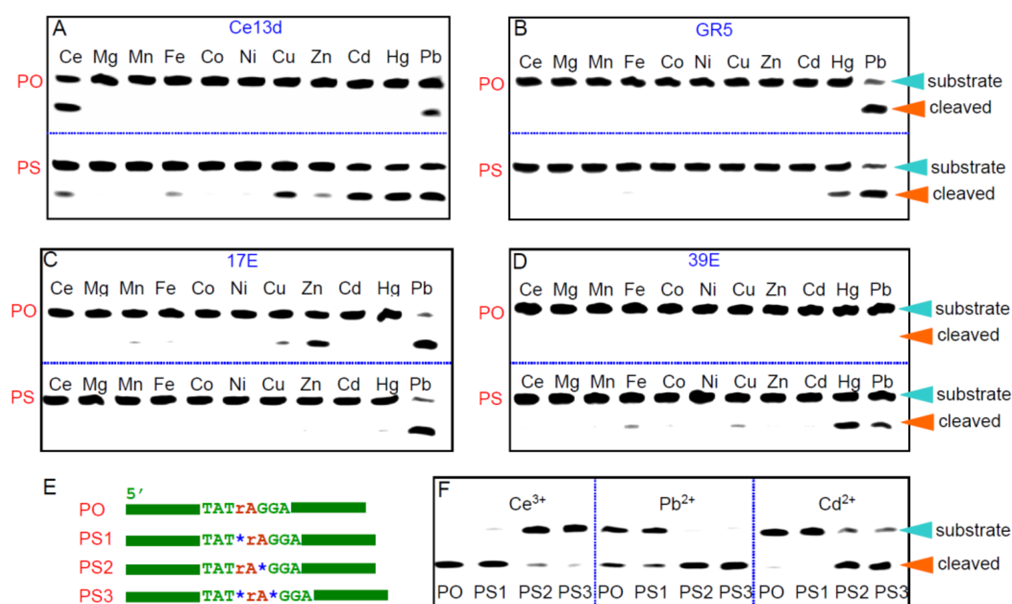

Figure 2. (A-D) Gel images of the four DNAzymes with different metal ions and PO or PS (PS2 in (E)) substrate. (E) Schemes of the substrate with different sites of the PS modification (denoted by the blue stars). (F) Gel image of the four substrates with the Ce13d DNAzyme in the presence of different metal ions. For all the gels, the DNAzyme concentration was 1 µM and metal concentration was 10 µM. Reaction time was 30 min.

The above tested substrate was named PS2. To confirm specificity of the PS modification, a few control substrates were also tested (Figure 2E), where the PS was placed on the neighboring linkage (PS1) or dual PS modifications were introduced (PS3). Three metals were tested (Figure 2F). PS1 behaved very similarly to the original PO substrate, indicating that the PS modification at this site has no effect. PS3 behaved similarly to PS2, therefore implying that metal coordination to the phosphate at the cleavage site (Figure 1A) is crucial. The Ce^{3+} activity was suppressed with the PS2 substrate, which could be rescued by using thiophilic metals. From the analytical standpoint, Ce13d/PS2 is useful for detecting these toxic metals as a group.

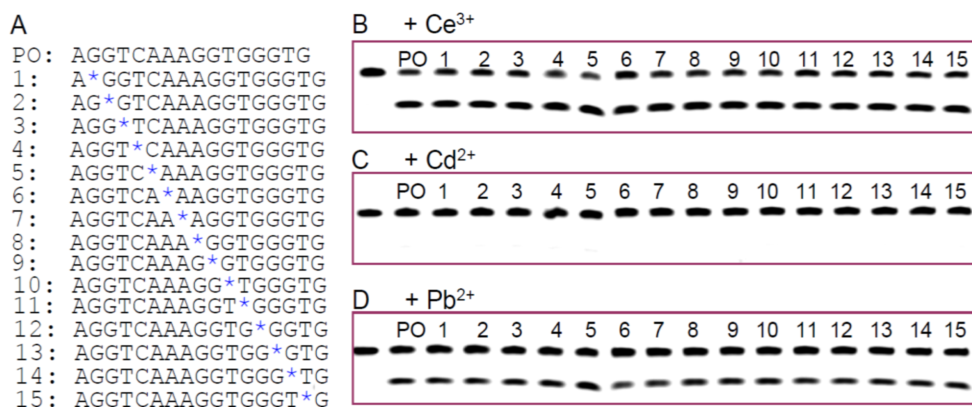

Figure 3. (A) The enzyme loop sequence of Ce13d (the nucleotides in black of Figure 1B) and the sites of PS modification (blue stars). In this assay, the normal PO substrate was used for reference. Gel images of the 16 Ce13d-based enzyme assays (the first lanes are the substrate alone, the second lanes are with the normal all PO enzyme and the rest are the PS modified) with (B) Ce^{3+}, (C) Cd^{2+} or (D) Pb^{2+}. The reaction time was 1 h.

PS-modified enzymes. Previous studies indicate that the nucleotides in the Ce13d enzyme loop (Figure 1B, in black) are highly conserved and crucial for activity.[25] To test whether their phosphates are involved in metal binding, we systematically modified each linkage (Figure 3A). Together with the

unmodified, a total of 16 enzymes were tested. Interestingly, in all the cases, Ce^{3+} and Pb^{2+} showed similar activity (Figure 3B, D), while Cd^{2+} was completely inactive (Figure 3C). Quantification of cleavage is shown in Figure S1. Therefore, the phosphates in the enzyme loop are unlikely to bind the metal and the additional ligands may be from the nucleobases in the loop. This enzyme loop is rich in guanine and adenine; both are good ligands for lanthanides.[35, 36] Overall, the PS modification at the cleavage junction has the largest effect in shifting metal preference.

Other PS-modified DNAzymes. Ce13d detects thiophilic metals as a group; it is also desirable to achieve selectivity within this group. Cd13d is a newer member of the DNAzyme family and a few other metal-specific DNAzymes are already known. PS modifications on these DNAzymes may produce different metal binding patterns to improve selectivity. The first ever reported DNAzyme is called GR5 (Figure 1C),[22] which is highly specific for Pb^{2+}.[37] Then there are the famous 17E (Figure 1C) and 10-23 DNAzymes. The 10-23 DNAzyme was recently suggested to be one of the 17E mutants.[38, 39] The 17E DNAzyme has been selected by a number of different labs under different selection conditions.[13, 40-43] The 39E DNAzyme is highly specific for UO_2^{2+} (Figure 1D).[24, 44, 45] The four examples in Figure 1 represent the main *independent* and well characterized metal-specific DNAzymes reported so far.[46, 47] Note that many other DNAzymes contain required fluorophore/quencher or base modifications for activity; they are not studied here.[19, 48]

For the GR5 DNAzyme, indeed only Pb^{2+} cleaved the normal PO substrate (Figure 2B). When the PS2 substrate was used, Hg^{2+} was also slightly active. Therefore, with this pair of DNAzymes, we can already identify Pb^{2+} and Hg^{2+} with high confidence. The 17E DNAzyme is the most active with low concentration of Pb^{2+} and Zn^{2+} can also assist cleavage (Figure 2C).[23, 40, 49] Interestingly, it becomes slightly more selective for Pb^{2+} over Zn^{2+} with the PS substrate. Since 39E is highly selective for UO_2^{2+}, none of the tested metals was active with the normal PO substrate, but Hg^{2+} and Pb^{2+} were active with the PS substrate.

Therefore, Ce13d is a unique DNAzyme that can be activated by all thiophilic metals with the PS substrate. All the other DNAzymes are only active with Pb^{2+} and Hg^{2+} under the same conditions. It is likely that Ce13d has a general metal binding site that is not available in other DNAzyme.

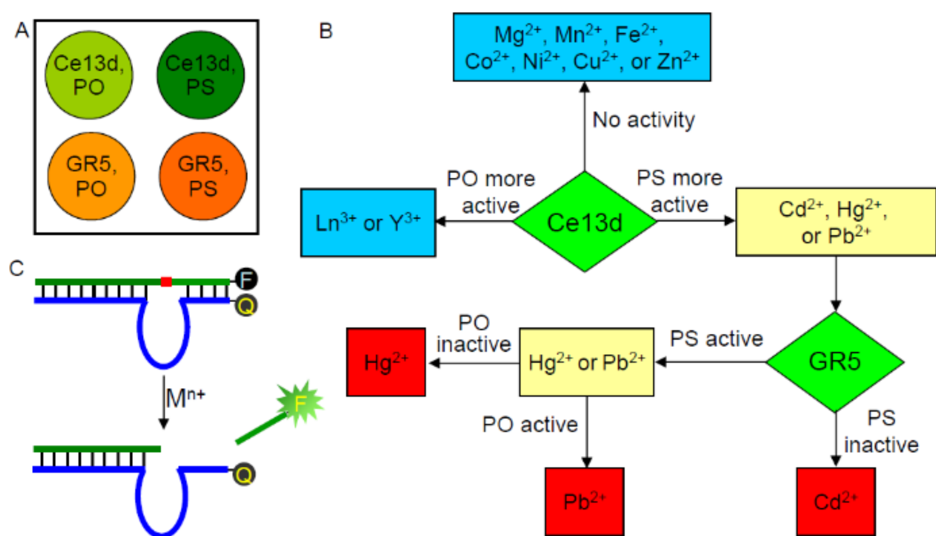

Figure 4. (A) A scheme of the four-component sensor array. (B) A flow chart of detecting Hg^{2+}, Pb^{2+} and Cd^{2+} based on the Ce13d and GR5 DNAzymes and the PO and PS substrates. The metal ions are categorized based on their sensor response. (C) Schematics showing the DNAzyme beacon sensor design.

Metal sensor array. With the above results, we made a sensor array (Figure 4A) to individually detect Cd^{2+}, Hg^{2+} and Pb^{2+} (i.e. toxic heavy metals). Based on the activity of Ce13d (Figure 4A), the metal ions are separated in three groups. If it is inactive with either the PO or PS substrate, the sample might contain no divalent metals or only the first row transition metals. We included Cu^{2+} and Zn^{2+} in this group based on the subsequent biosensor assays (Figure 4C, *vide infra*). On the other hand, if it is more active with the PO substrate, the metal is Ln^{3+} or Y^{3+}. Otherwise, if it is more active with the PS substrate, the sample contains the three toxic metals. Among these three, Pb^{2+} and Hg^{2+} can be

identified based on the response of the GR5 DNAzyme. After ruling out these two, the only one left is Cd^{2+}. We did not include 17E or 39E in the array since their information is redundant. The key component is Ce13d, which can narrow down the metals to a group of only three.

The above assays are based on gel electrophoresis using 10 μM metal ions and the next step is to design biosensors. Since all the DNAzymes share the same substrate sequence, we employed a common DNAzyme beacon strategy for signaling. The beacons were made by hybridizing a quencher labeled enzyme with the FAM-labeled substrate (Figure 4C). Enhanced fluorescence is observed after cleavage. With 500 nM metal, the response of the Ce13d/PO sensor is shown in Figure 5A, where only Ce^{3+} and Pb^{2+} showed activity, consistent with the gel-based assay. The initial slope of the kinetic trace is plotted in Figure 5C. To have a complete understanding, we tested three metal concentrations from 50 nM to 5 μM. Using a rate of 0.05 unit as cut-off, only Ce^{3+} and Pb^{2+} showed response. With the Ce13d/PS sensor, Cd^{2+}, Hg^{2+} and Pb^{2+} showed the highest response (Figure 5B), which is also consistent with the gel-based assay. On the other hand, Cu^{2+} was more active in the gel assay than Ce^{3+} but in the sensor their responses were similar. This is attributed to fluorescence quenching effect of Cu^{2+}. With this sensor, only Cd^{2+}, Hg^{2+} and Pb^{2+} were active with a cut-off value of 0.05. Figure 5E is obtained by subtracting the PO response from the PS data, where a clear separation of the three groups can be observed: Ce^{3+} as one group, Cd^{2+}, Hg^{2+} and Pb^{2+} as the second group and the rest being the third, supporting our classification in Figure 4B.

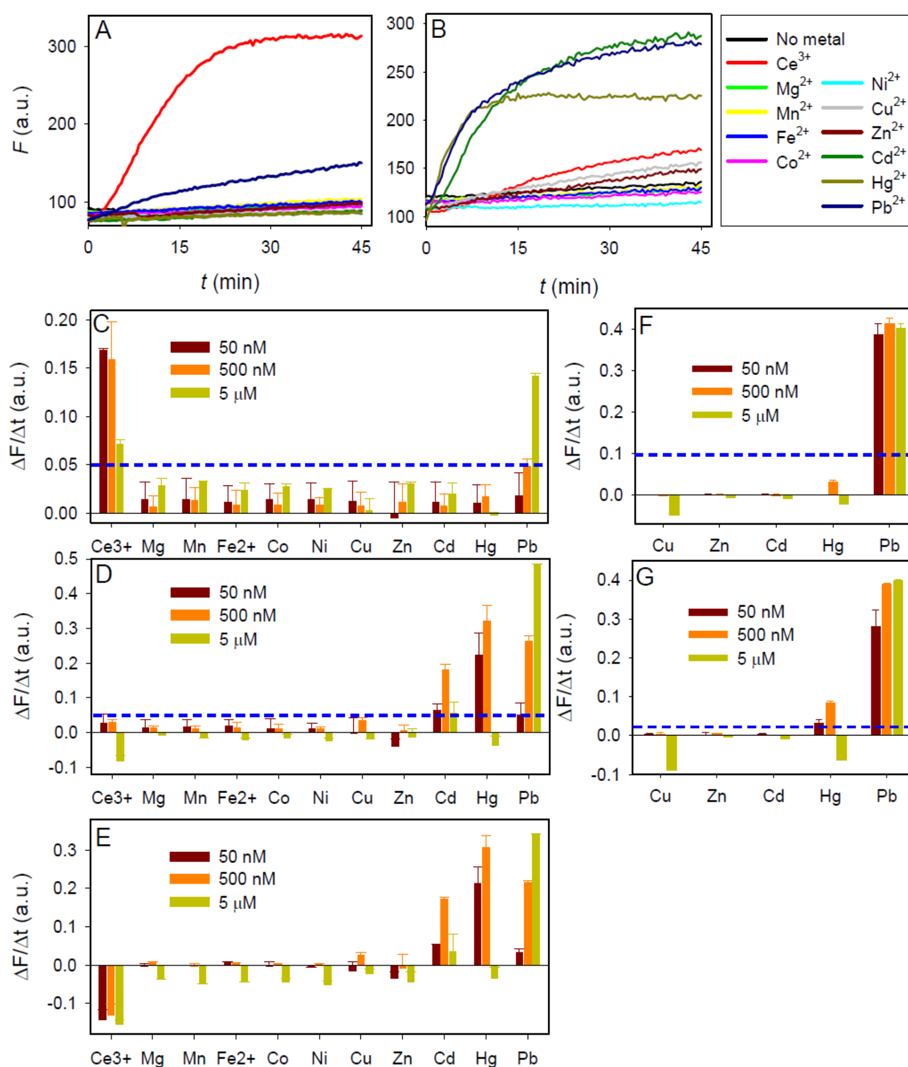

Figure 5. Sensor signaling kinetic traces with different metal ions using the Ce13d/PO (A) or Ce13d/PS (B) as sensor. Quantification of the rate of fluorescence increase with various concentrations of different metal ions with the Ce13d/PO sensor (C) or Ce13d/PS sensor (D), and their difference (E). Rate of fluorescence increase with various concentrations of different metal ions with the GR5/PO (F) or GR5/PS (G) sensor.

Since the other component of this test is GR5, the same assay was performed with it. With the GR5/PO sensor, only Pb^{2+} was active using 0.1 unit as the cut-off (Figure 5F). With the GR5/PS sensor

and 0.02 as the cut-off (Figure 5G), Hg^{2+} and Pb^{2+} are the active ones. Hg^{2+} induced significant quenching at 5 μM and appeared inactive from the sensors (see Figure S2 for the kinetic traces).

Individual sensor performance. Once a metal is identified, we can use one of the sensors for quantification. By far, GR5 is the best sensor for Pb^{2+} and a previously reported detection limit was 3.7 nM (in pH 7.0 HEPES buffer).[37] We observed significantly improved activity at pH 7.6.[50] The Pb^{2+}-dependent fluorescence kinetic traces are in Figure 6A, where even 0.2 nM Pb^{2+} can be clearly distinguished from the background. With 5 nM Pb^{2+}, full cleavage was observed in 30 min. Since our DNAzyme concentration was 50 nM, each Pb^{2+} turned over 10 sensor molecules in 30 min to amplify the signal, highlighting the advantage of using DNAzyme for metal detection.

The calibration curve is shown in Figure 6B; an apparent dissociation constant (K_d) of 4.2 nM Pb^{2+} is obtained. This is the tightest metal binding in DNAzymes reported till date. The detection limit was 0.1 nM Pb^{2+} from 3σ/slope, where σ is the standard deviation of background variation. Next Cd^{2+} detection was carried out using the Ce13d/PS sensor (Figure 6C, D). It has an apparent K_d of 154 nM Cd^{2+}, and the detection limit was 4.8 nM Cd^{2+}. Finally, the Ce13d/PS sensor was also tested for Hg^{2+} (Figure 6E, F) and the detection limit was determined as 2 nM. The US Environmental Protection Agency (EPA) maximal contamination limits are 15 ppb (72 nM) for Pb^{2+}, 5 ppb (45 nM) for Cd^{2+}, and 2 ppb (10 nM) for Hg^{2+}. All the three sensors can meet these limits and detect the targets down to parts-per-trillion level.

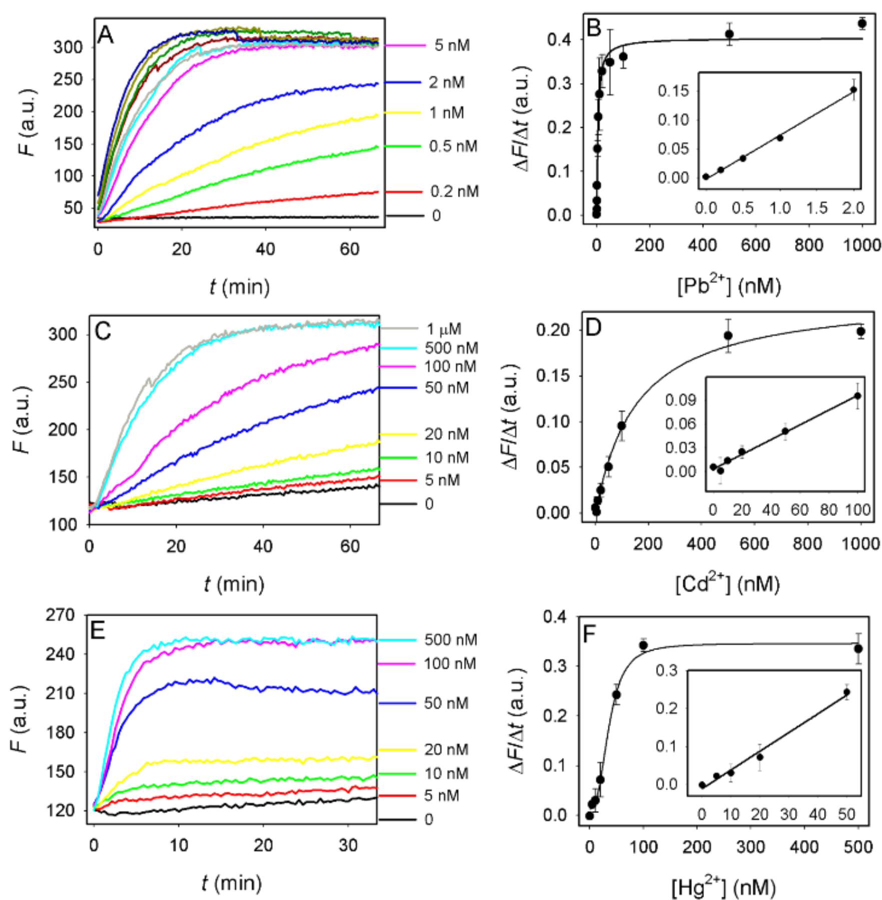

Figure 6. Kinetics of sensor fluorescence increase with the GR5/PO DNAzyme for Pb^{2+} (A), Ce13d/PS for Cd^{2+} (C) and Ce13d/PS for Hg^{2+} (E). The initial rates of fluorescence increase as a function of Pb^{2+} (B), Cd^{2+} (D) and Hg^{2+} (F) concentration. Insets: the responses to low metal concentrations. For all the tests, the DNAzyme concentration was 50 nM in pH 7.6 HEPES buffer.

Modified DNAzymes have been extensively reported for various purposes. This work highlights the advantages of the PS modification. First, the single O-to-S change confers minimal perturbation of the structure of the original DNAzyme. New active enzymes are obtained without performing additional selection experiments. Second, it is cost effective to produce (e.g. less than $3 per PS modification), while modified bases cost much more and may not be commercially available. Third, the chemical effects of such modifications are readily predictable. Finally, it provides important mechanistic insights for fundamental studies.

Conclusions

In summary, we systematically studied the effect of PS modification on the lanthanide-dependent Ce13d DNAzyme, where the phosphate at the cleavage site determines its metal preference. This enzyme can be activated using lanthanide or thiophilic metals based on a single PS modification, which is not observed in any other tested DNAzymes. This suggests a well-defined metal binding site that can tolerate a diverse range of metals. This will be a useful model system for studying DNAzyme bioinorganic chemistry. From the analytical chemistry standpoint, Ce13d/PS detects Cd^{2+}, Hg^{2+}, and Pb^{2+} below their toxic levels in drinking water. These are the most popular toxic heavy metals that are collectively banned by the European Union in electronic devices. Therefore, it is important to detect them as a group. We also used the concept of flow-chart-based metal analysis. This is a classic method of solution inorganic chemistry for metal separation and detection. With the accumulation of metal-specific DNAzymes, this method will find more applications in detecting multiple metals simultaneously.

Supporting Information. DNAzyme kinetics and cleavage quantification. “This material is available free of charge via the Internet at http://pubs.acs.org.”

Acknowledgment

Funding for this work is from the University of Waterloo, the Ontario Early Researcher Award, and the NSERC (Discovery Grant and Strategic Project Grant) of Canada.

References

(1) Jarup, L. *Brit. Med. Bull.* **2003**, *68*, 167-182.

(2) Kim, H. N.; Ren, W. X.; Kim, J. S.; Yoon, J. *Chem. Soc. Rev.* **2012**, *41*, 3210-3244.

(3) Zhang, X.-B.; Kong, R.-M.; Lu, Y. *Annu. Rev. Anal. Chem.* **2011**, *4*, 105-128.

(4) Nolan, E. M.; Lippard, S. J. *Chem. Rev.* **2008**, *108*, 3443-3480.

(5) Domaille, D. W.; Que, E. L.; Chang, C. J. *Nat. Chem. Biol.* **2008**, *4*, 168-175.

(6) Liu, J.; Cao, Z.; Lu, Y. *Chem. Rev.* **2009**, *109*, 1948–1998.

(7) Li, D.; Song, S. P.; Fan, C. H. *Acc. Chem. Res.* **2010**, *43*, 631-641.

(8) Wang, H.; Yang, R. H.; Yang, L.; Tan, W. H. *ACS Nano* **2009**, *3*, 2451-2460.

(9) Lin, Y. W.; Huang, C. C.; Chang, H. T. *Analyst* **2011**, *136*, 863-871.

(10) Wegner, S. V.; Okesli, A.; Chen, P.; He, C. *J. Am. Chem. Soc.* **2007**, *129*, 3474-3475.

(11) Du, Y.; Li, B. L.; Wang, E. K. *Acc. Chem. Res.* **2013**, *46*, 203-213.

(12) Breaker, R. R. *Nat. Biotechnol.* **1997**, *15*, 427-431.

(13) Santoro, S. W.; Joyce, G. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4262-4266.

(14) Lu, Y. *Chem. Eur. J.* **2002**, *8*, 4588-4596.

(15) Navani, N. K.; Li, Y. *Curr. Opin. Chem. Biol.* **2006**, *10*, 272-281.

(16) Silverman, S. K. *Angew. Chem. Int. Ed.* **2010**, *49*, 7180-7201.

(17) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642-6643.

(18) Xiao, Y.; Rowe, A. A.; Plaxco, K. W. *J. Am. Chem. Soc.* **2007**, *129*, 262.

(19) Liu, Z.; Mei, S. H. J.; Brennan, J. D.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 7539-7545.

(20) Lu, L. M.; Zhang, X. B.; Kong, R. M.; Yang, B.; Tan, W. H. *J. Am. Chem. Soc.* **2011**, *133*, 11686-11691.

(21) Wang, H.; Kim, Y.; Liu, H.; Zhu, Z.; Bamrungsap, S.; Tan, W. *J. Am. Chem. Soc.* **2009**, *131*, 8221-8226.

(22) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223-229.

(23) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466-10467.

(24) Liu, J.; Brown, A. K.; Meng, X.; Cropek, D. M.; Istok, J. D.; Watson, D. B.; Lu, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2056-2061.

(25) Huang, P.-J. J.; Lin, J.; Cao, J.; Vazin, M.; Liu, J. *Anal. Chem.* **2014**, *86*, 1816-1821.

(26) Santoro, S. W.; Joyce, G. F.; Sakthivel, K.; Gramatikova, S.; Barbas, C. F., III *J. Am. Chem. Soc.* **2000**, *122*, 2433-2439.

(27) Hollenstein, M.; Hipolito, C.; Lam, C.; Dietrich, D.; Perrin, D. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4346 - 4350.

(28) Deleavey, Glen F.; Damha, M. J. *Chem. Biol.* **2012**, *19*, 937-954.

(29) Wang, S.; Karbstein, K.; Peracchi, A.; Beigelman, L.; Herschlag, D. *Biochemistry* **1999**, *38*, 14363-14378.

(30) Nawrot, B.; Widera, K.; Wojcik, M.; Rebowska, B.; Nowak, G.; Stec, W. J. *Febs Journal* **2007**, *274*, 1062-1072.

(31) Cunningham, L. A.; Li, J.; Lu, Y. *J. Am. Chem. Soc.* **1998**, *120*, 4518-4519.

(32) Sekhon, G. S.; Sen, D. *Biochemistry* **2010**, *49*, 9072-9077.

(33) Lee, J. H.; Wernette, D. P.; Yigit, M. V.; Liu, J.; Wang, Z.; Lu, Y. *Angew. Chem. Int. Ed.* **2007**, *46*, 9006-9010.

(34) Zhang, D.; Deng, M.; Xu, L.; Zhou, Y.; Yuwen, J.; Zhou, X. *Chem. Eur. J* **2009**, *15*, 8117-8120.

(35) Nishiyabu, R.; Hashimoto, N.; Cho, T.; Watanabe, K.; Yasunaga, T.; Endo, A.; Kaneko, K.; Niidome, T.; Murata, M.; Adachi, C.; Katayama, Y.; Hashizume, M.; Kimizuka, N. *J. Am. Chem. Soc.* **2009**, *131*, 2151-2158.

(36) Wang, F.; Liu, B.; Huang, P.-J. J.; Liu, J. *Anal. Chem.* **2013**, *85*, 12144-12151.

(37) Lan, T.; Furuya, K.; Lu, Y. *Chem. Comm.* **2010**, *46*, 3896-3898.

(38) Wang, B.; Cao, L.; Chiuman, W.; Li, Y.; Xi, Z. *Biochemistry* **2010**, *49*, 7553-7562.

(39) Schlosser, K.; Gu, J.; Sule, L.; Li, Y. F. *Nucleic Acids Res.* **2008**, *36*, 1472-1481.

(40) Li, J.; Zheng, W.; Kwon, A. H.; Lu, Y. *Nucleic Acids Res.* **2000**, *28*, 481-488.

(41) Faulhammer, D.; Famulok, M. *Angew. Chem., Int. Ed.* **1996**, *35*, 2837-2841.

(42) Cruz, R. P. G.; Withers, J. B.; Li, Y. *Chem. Biol.* **2004**, *11*, 57-67.

(43) Schlosser, K.; Li, Y. F. *Chem. Biol.* **2009**, *16*, 311-322.

(44) Brown, A. K.; Liu, J. W.; He, Y.; Lu, Y. *ChemBioChem* **2009**, *10*, 486-492.

(45) Cepeda-Plaza, M.; Null, E. L.; Lu, Y. *Nucleic Acids Res.* **2013**, *41*, 9361-9370.

(46) Schlosser, K.; Gu, J.; Lam, J. C. F.; Li, Y. F. *Nucleic Acids Res.* **2008**, *36*, 4768-4777.

(47) Schlosser, K.; Li, Y. *Nucleic Acids Res.* **2009**, *37*, 413-420.

(48) Mei, S. H. J.; Liu, Z.; Brennan, J. D.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 412-420.

(49) Brown, A. K.; Li, J.; Pavot, C. M. B.; Lu, Y. *Biochemistry* **2003**, *42*, 7152-7161.

(50) Huang, P.-J. J.; Liu, J. *Chem. Comm.* **2014**, *50*, 4442-4444.