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Fluorescent Aptaswitch for Detection of Lead Ions

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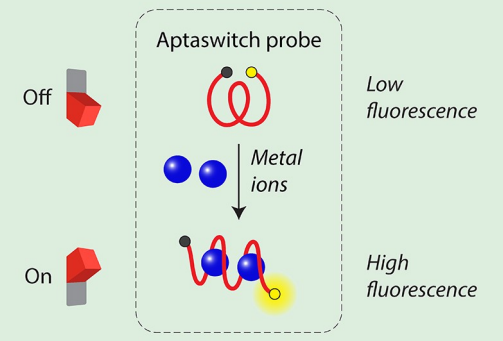
ABSTRACT: Detection of metal ions has essential roles in biology, food industry, and environmental sciences. In this work, we developed a Pb^{2+} detection strategy based on a fluorophore-tagged Pb^{2+}-binding aptamer. The DNA aptamer changes its conformation on binding Pb^{2+}, switching from an "off" state (low fluorescence) to an "on" state (high fluorescence). This method provides a quantitative readout with a detection limit of 468 nM, is highly specific to Pb^{2+} when tested against other metal ions, and is functional in complex biofluids. Such metal sensing DNA aptamers could be coupled with other biomolecules for sense-and-actuate mechanisms in biomedical and environmental applications.



KEYWORDS: *metal sensing, aptamers, lead detection, heavy metals, DNA switches, biosensing*

In the popular TV drama *Grey's Anatomy*, Dr. Richard Webber is diagnosed with cobalt poisoning. In real life, metal ions play critical structural and functional roles in biology. Still, specific metal ions are known to be poisonous at certain levels and can be contaminants in food and water sources. Detection of metal ions thus has implications in varied fields such as biology (cell signaling, therapeutics, and enzyme catalysis), food quality control, and environmental science (pollutants in drinking water and surface soil).[1−3] For example, Pb^{2+} is one of the most toxic pollutants in drinking water and surface soil and may be accumulated in the human body as a potential neurotoxin that causes bone and kidney damage.[4] Several strategies have been developed for the detection of metal ions. Standard techniques for metal analysis include atomic absorption spectroscopy,[5] mass spectroscopy,[6] and potentiometric methods.[7] While these techniques are highly accurate and sensitive with industrial standards, they are costly; available only in large, centralized laboratories; and require extensive sample pretreatment. More recent techniques such as colorimetry,[8] fluorimetry,[9] and voltammetry[10] have shown adaptability for miniaturization and on-site applications. These methods are developed on the basis of different materials such as metal−organic frameworks,[11] gold nanoparticles,[12] graphene,[13] carbon nanotubes,[14] organic polymers,[15] and biomolecules such as proteins,[16] peptides,[17] and nucleic acids.[18]

DNA has emerged as an often-used material for sensing metal ions due to its many desirable properties.[18] DNA is a polyanion allowing electrostatic attraction with metal ions, is highly stable, and can be renatured after denaturation without losing binding affinity to metals. Any sequence of DNA can be synthesized at a low cost and is amenable to in vitro selection for metal-binding sequences.[19] On the basis of these properties, several single stranded and structured DNA sequences such as DNAzymes,[20] molecular beacons,[21] G-quadruplexes,[22] and hydrogels[23] have been explored for metal sensing. Beyond these properties, DNA is also highly programmable and has been used to create various nanostructures that are useful in biosensing.[24] Typically, metal ion sensors based on DNA molecules use T−T or C−C mismatches and G-quadruplexes.[25] Here, we develop a DNA aptamer-based strategy for detecting Pb^{2+} ions, where DNA aptamer reconfiguration is controlled by the presence or absence of metal ions, with a fluorescence-based readout.

In our recent work, we obtained the X-ray crystal structure of a Pb^{2+}-binding DNA aptamer sequence.[26] The crystal structure of this DNA sequence revealed that Pb^{2+} binds to the DNA strand when folded into a tetraplex structure (Figure 1a). On the basis of this work, we first designed a hairpin-like DNA motif that incorporates the aptamer sequence (Figure S1). We modified the DNA strand to contain a fluorophore and a quencher, hypothesizing that Pb^{2+} binding would bring the fluorophore and the quencher in close proximity and thus decrease the fluorescent signal. However, we observed an increase in the signal of the fluorophore on addition of Pb^{2+}. This result indicated that the crystal structure of the sequence

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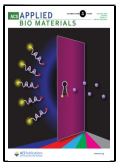

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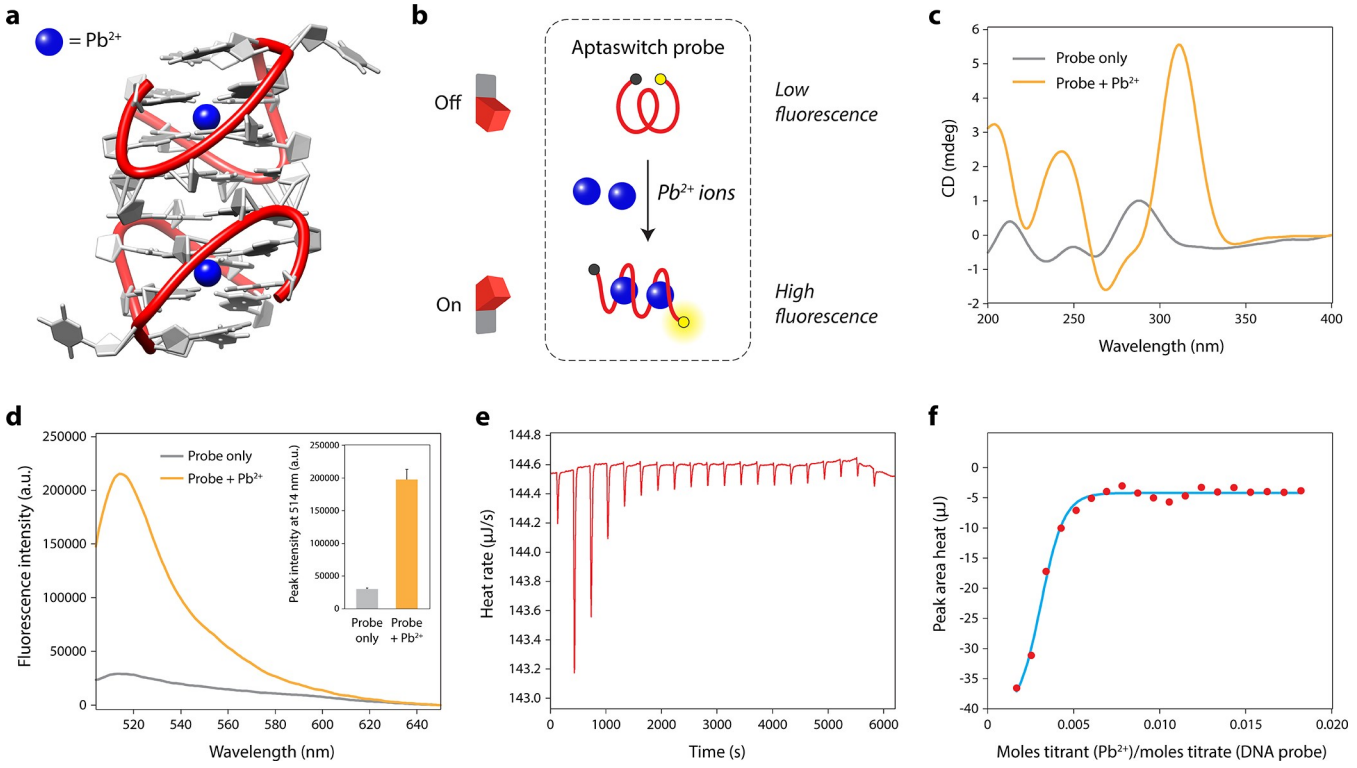

Figure 1. Design and characterization of a Pb^{2+}-detecting aptaswitch. (a) Crystal structure of a Pb^{2+}-binding aptamer sequence (PDB: 7D31, ref 26). (b) Concept of Pb^{2+} detection using a fluorescent signal-on aptamer sequence (aptaswitch). (c) CD spectra of the DNA probe in the presence and absence of Pb^{2+}. (d) Fluorescence spectra of the DNA probe in the absence of Pb^{2+} (gray) and presence of Pb^{2+} (orange). Peak intensity at 514 nm for each is shown in the inset. (e) Trace of calorimetric titration of Pb^{2+} ions to DNA probe and (f) binding curve of DNA probe with Pb^{2+}.

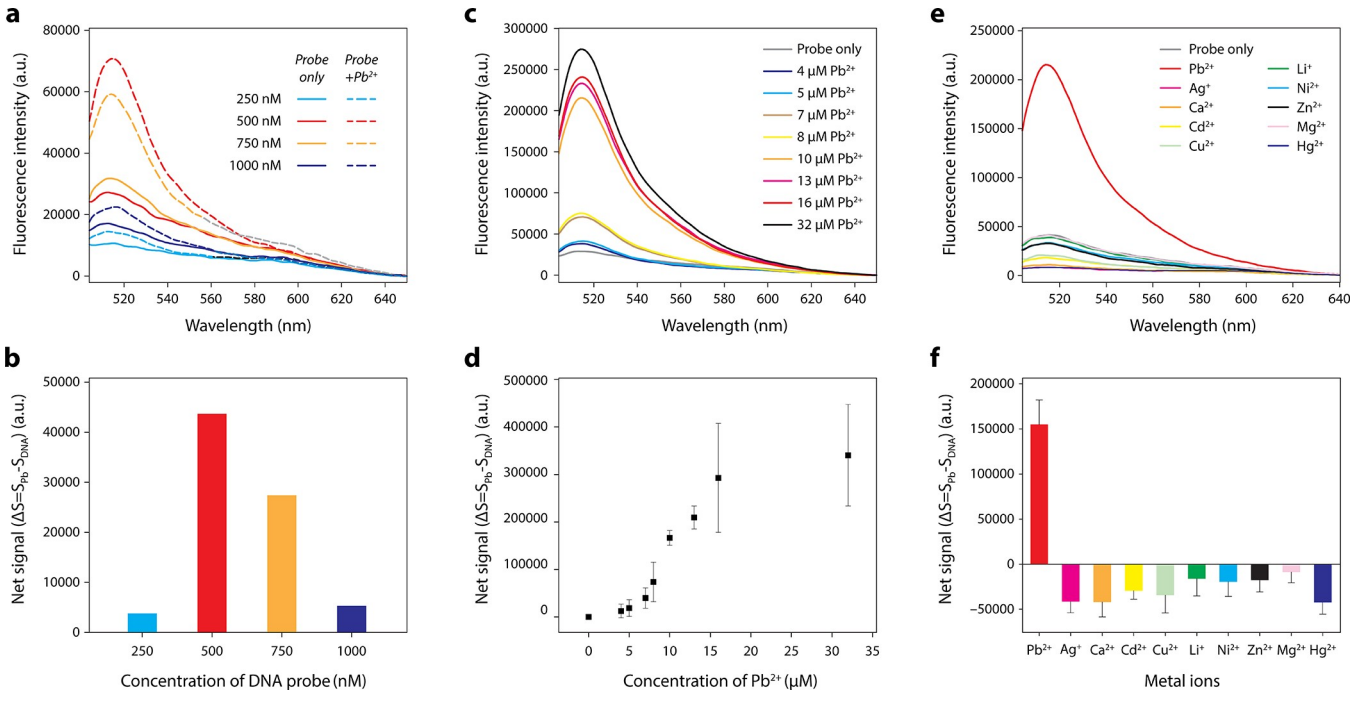

Figure 2. Sensing properties of the Pb^{2+}-detecting aptaswitch. (a) Fluorescence spectra of different concentrations of DNA probe without Pb^{2+} (—) and with Pb^{2+} (---). (b) Signal for detection of 7 μM Pb^{2+} using different concentrations of the DNA probe. (c) Fluorescence spectra for 500 nM DNA probe with different concentrations of Pb^{2+} and (d) corresponding signals at each concentration. (e) Fluorescence spectra of DNA probe with different metal ions and (f) corresponding detection signals for each metal ion.

in the presence of Pb^{2+} is perhaps different from conformational changes that happen in solution. On the basis of this result, we then designed a DNA aptamer probe that contained this guanine-rich sequence but without the stem-loop region (Figure 1b). To confirm that the Pb^{2+} binding observed in the crystal structure is also true in solution for this DNA aptamer

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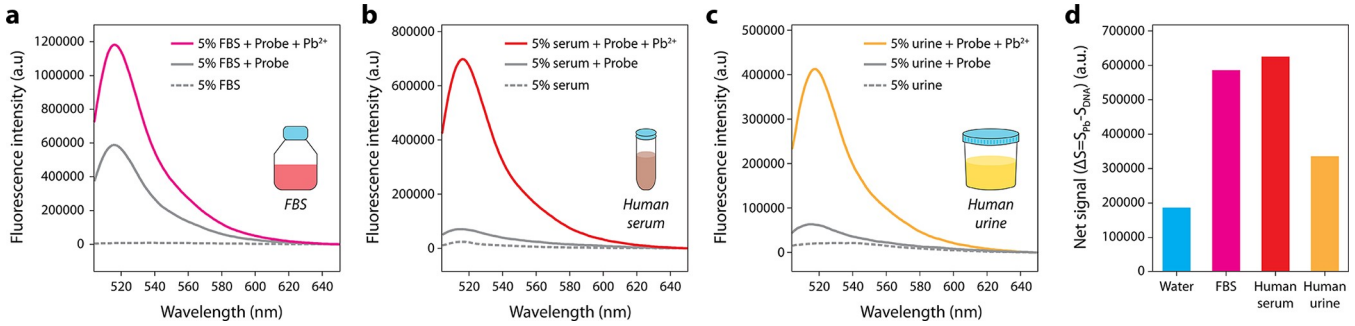

Figure 3. Detection in biological fluids. (a–c) Fluorescence spectra of biofluids only (gray ---), DNA probe in biofluids (gray —), and DNA probe in biofluids in the presence of Pb^{2+} (colored —) in 5% solutions of (a) FBS, (b) human serum, and (c) human urine. (d) Signal of Pb^{2+} detection in different biofluids.

sequence (DNA probe), we tested the binding of Pb^{2+} ions to the DNA probe in solution using circular dichroism (CD) spectroscopy (Figure 1c). Compared to the CD spectrum of the DNA probe, the addition of Pb^{2+} ions caused the appearance of a strong positive peak at 312 nm which is characteristic of Pb^{2+}-stabilized quadruplexes.[26] This result indicated that the DNA aptamer sequence can be used for sensing Pb^{2+} ions based on a conformational change. To read out this conformational change, we modified the DNA probe to contain a fluorophore (FAM) at the 3′ end and a quencher (Iowa black) at the 5′ end. To confirm the working of the assay, we first tested the DNA probe (500 nM) with a high concentration (50 μM) of Pb^{2+} and observed a change in the fluorescence spectra (Figure 1d). We used the peak intensity at 514 nm (corresponding to the emission wavelength of FAM) to calculate the signal difference for the detection of Pb^{2+} ions as the DNA probe switches from the off to the on state (Figure 1d, inset). We also characterized the binding of Pb^{2+} ions to the DNA probe using isothermal titration calorimetry (ITC) and found a binding constant (K_d) of 50 nM (Figure 1e,f).

To optimize the detection assay, we first tested different concentrations of the DNA probe (250–1000 nM) with a fixed concentration of Pb^{2+} ions (7 μM). We mixed the DNA probe with Pb^{2+} ions, heated it at 90 °C for 5 min, and allowed it to cool to room temperature over 2 h. For each concentration of the DNA probe, we recorded the fluorescence spectra with and without Pb^{2+} ions (Figure 2a). We recorded the peak intensity at 514 nm in each case to obtain the signal for the DNA probe in the absence of Pb^{2+} ions (S_{DNA}) and in the presence of Pb^{2+} ions (S_{Pb}). We then calculated the increase in peak intensity ($\Delta S = S_{Pb} - S_{DNA}$) and observed that the highest signal difference between the control (DNA probe only) and Pb^{2+} added samples occurred when using 500 nM DNA probe concentration (Figure 2b). We thus chose 500 nM as our DNA probe concentration for further experiments.

Next, we performed sensitivity experiments to obtain the limit of detection of Pb^{2+} ions. We incubated 500 nM DNA probe with different concentrations of Pb^{2+} ions and recorded the fluorescence spectrum for each case (Figure 2c). We performed the experiment in triplicate and plotted the peak intensity signal (ΔS) at 514 nm for each concentration of Pb^{2+} (Figure 2d). We calculated the limit of detection (LOD) (defined as the concentration of analyte that yields a signal exceeding the mean background by 3 SDs) using a linear range and determined an LOD value of 468 nM (Figure S2). Next, we tested the specificity of the assay to different metal ions. For this, we incubated the DNA probe with different metal ions (at 10 μM concentration) and recorded the fluorescence spectra (Figure 2e). We monitored the change in fluorescent signal at 514 nm to check the response of the DNA probe to different metal ions. Results showed varied levels of specificity for these metal ions, with the assay showing high specificity to the correct metal ion (Pb^{2+}) when tested against Ag^{+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Li^{+}, Ni^{2+}, Zn^{2+}, Mg^{2+}, and Hg^{2+} ions (Figure 2f). This result shows that the reconfiguration of the DNA aptamer probe is specific to Pb^{2+} ions. CD spectra of different metal ions with the DNA probe also confirmed these results, with only the Pb^{2+} ions causing a change in the spectrum (Figure S3).

To demonstrate the potential utility of our assay with real samples, we tested the efficiency of the DNA probe to detect Pb^{2+} ions in various complex fluids. We spiked 10 μM Pb^{2+} ions in 5% solutions of fetal bovine serum (FBS), human serum, and human urine and incubated them with the DNA probe (Figure 3a–c). Results showed that, even in these complex fluids, our detection worked well, as seen by the increase in signal in the presence of Pb^{2+} ions in each of the complex fluids (Figure 3d). The difference in the signal intensities between the complex fluids could be attributed to the interaction of the DNA probe and the Pb^{2+} ions with other molecules in the biofluids.

In this work, we demonstrated the use of a metal-binding aptamer as a fluorophore-tagged aptaswitch for detecting Pb^{2+} ions in solution. Our method shows comparable detection limits with other recent studies[22,27−29] and is highly specific to the detection of Pb^{2+} ions (Table S1). Detection of metal ions spiked into complex fluids shows the promise of this strategy toward point-of-care testing. Our strategy adds to the existing tool kit of aptamer-based biosensors for detecting Pb^{2+} ions.[30] With further optimization, the sensitivity of the assay could be improved for real-life detection of Pb^{2+} ions from biological samples and industry effluents. In the future, such metal ion responsive sequences can be incorporated into nucleic acid nanostructures, allowing dynamic reconfigurations for biosensing and therapeutic delivery applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsabm.2c00368.

Materials used, experimental methods, and additional results (PDF)

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Author Contributions

J.M. performed experiments, analyzed data, and edited the manuscript. H.L. and J.G. provided structural support, analyzed data, and edited the manuscript. A.R.C supervised the project, analyzed data, and wrote the manuscript. J.S. conceived and supervised the project and edited the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Anastassopoulou, J.; Theophanides, T. The Role of Metal Ions in Biological Systems and Medicine. In *Bioinorganic Chemistry: An Inorganic Perspective of Life*; Kessissoglou, D. P., Ed.; NATO ASI Series; Springer Netherlands: Dordrecht, 1995; pp 209−218. DOI: 10.1007/978-94-011-0255-1_17.

(2) Mrvčić, J.; Stanzer, D.; Šolić, E.; Stehlik-Tomas, V. Interaction of Lactic Acid Bacteria with Metal Ions: Opportunities for Improving Food Safety and Quality. *World J. Microbiol. Biotechnol.* **2012**, *28* (9), 2771−2782.

(3) Tchounwou, P. B.; Yedjou, C. G.; Patlolla, A. K.; Sutton, D. J. Heavy Metal Toxicity and the Environment. In *Molecular, Clinical and Environmental Toxicology: Vol. 3: Environmental Toxicology*; Luch, A., Ed.; Experientia Supplementum; Springer: Basel, 2012; pp 133−164. DOI: 10.1007/978-3-7643-8340-4_6.

(4) Needleman, H. Lead Poisoning. *Annu. Rev. Med.* **2004**, *55* (1), 209−222.

(5) Pohl, P. Determination of Metal Content in Honey by Atomic Absorption and Emission Spectrometries. *TrAC Trends Anal. Chem.* **2009**, *28* (1), 117−128.

(6) Townsend, A. T.; Miller, K. A.; McLean, S.; Aldous, S. The Determination of Copper, Zinc, Cadmium and Lead in Urine by High Resolution ICP-MS. *J. Anal. At. Spectrom.* **1998**, *13* (11), 1213−1219.

(7) Mimendia, A.; Legin, A.; Merkoçi, A.; del Valle, M. Use of Sequential Injection Analysis to Construct a Potentiometric Electronic Tongue: Application to the Multidetermination of Heavy Metals. *Sens. Actuators B Chem.* **2010**, *146* (2), 420−426.

(8) Kaur, B.; Kaur, N.; Kumar, S. Colorimetric Metal Ion Sensors - A Comprehensive Review of the Years 2011−2016. *Coord. Chem. Rev.* **2018**, *358*, 13−69.

(9) Formica, M.; Fusi, V.; Giorgi, L.; Micheloni, M. New Fluorescent Chemosensors for Metal Ions in Solution. *Coord. Chem. Rev.* **2012**, *256* (1), 170−192.

(10) Lu, Y.; Liang, X.; Niyungeko, C.; Zhou, J.; Xu, J.; Tian, G. A Review of the Identification and Detection of Heavy Metal Ions in the Environment by Voltammetry. *Talanta* **2018**, *178*, 324−338.

(11) Rudd, N. D.; Wang, H.; Fuentes-Fernandez, E. M. A.; Teat, S. J.; Chen, F.; Hall, G.; Chabal, Y. J.; Li, J. Highly Efficient Luminescent Metal-Organic Framework for the Simultaneous Detection and Removal of Heavy Metals from Water. *ACS Appl. Mater. Interfaces* **2016**, *8* (44), 30294−30303.

(12) Shi, X.; Gu, W.; Zhang, C.; Zhao, L.; Li, L.; Peng, W.; Xian, Y. Construction of a Graphene/Au-Nanoparticles/Cucurbit[7]Uril-Based Sensor for Pb2+ Sensing. *Chem. - Eur. J.* **2016**, *22* (16), 5643−5648.

(13) Xuan, X.; Hossain, M. F.; Park, J. Y. A Fully Integrated and Miniaturized Heavy-Metal-Detection Sensor Based on Micro-Patterned Reduced Graphene Oxide. *Sci. Rep.* **2016**, *6* (1), 33125.

(14) Huang, H.; Chen, T.; Liu, X.; Ma, H. Ultrasensitive and Simultaneous Detection of Heavy Metal Ions Based on Three-Dimensional Graphene-Carbon Nanotubes Hybrid Electrode Materials. *Anal. Chim. Acta* **2014**, *852*, 45−54.

(15) Thomas, S. W.; Joly, G. D.; Swager, T. M. Chemical Sensors Based on Amplifying Fluorescent Conjugated Polymers. *Chem. Rev.* **2007**, *107* (4), 1339−1386.

(16) Chen, P. R.; He, C. Selective Recognition of Metal Ions by Metalloregulatory Proteins. *Curr. Opin. Chem. Biol.* **2008**, *12* (2), 214−221.

(17) Liu, Q.; Wang, J.; Boyd, B. J. Peptide-Based Biosensors. *Talanta* **2015**, *136*, 114−127.

(18) Zhou, W.; Saran, R.; Liu, J. Metal Sensing by DNA. *Chem. Rev.* **2017**, *117* (12), 8272−8325.

(19) Guo, W.; Zhang, C.; Ma, T.; Liu, X.; Chen, Z.; Li, S.; Deng, Y. Advances in Aptamer Screening and Aptasensors' Detection of Heavy Metal Ions. *J. Nanobiotechnology* **2021**, *19* (1), 166.

(20) Zhang, Q.; Cai, Y.; Li, H.; Kong, D.-M.; Shen, H.-X. Sensitive Dual DNAzymes-Based Sensors Designed by Grafting Self-Blocked G-Quadruplex DNAzymes to the Substrates of Metal Ion-Triggered DNA/RNA-Cleaving DNAzymes. *Biosens. Bioelectron.* **2012**, *38* (1), 331−336.

(21) Lu, Z.; Xiong, W.; Wang, P.; Li, X.; Zhai, K.; Shi, R.; Xiang, D. Simultaneous Detection of Lead (II) and Mercury (II) Ions Using Nucleic Acid Aptamer Molecular Beacons. *Int. J. Environ. Anal. Chem.* **2019**, *0* (0), 1−13.

(22) Li, T.; Dong, S.; Wang, E. A Lead(II)-Driven DNA Molecular Device for Turn-On Fluorescence Detection of Lead(II) Ion with High Selectivity and Sensitivity. *J. Am. Chem. Soc.* **2010**, *132* (38), 13156−13157.

(23) Zhong, R.; Xiao, M.; Zhu, C.; Shen, X.; Tang, Q.; Zhang, W.; Wang, L.; Song, S.; Qu, X.; Pei, H.; Wang, C.; Li, L. Logic Catalytic Interconversion of G-Molecular Hydrogel. *ACS Appl. Mater. Interfaces* **2018**, *10* (5), 4512−4518.

(24) Xiao, M.; Lai, W.; Man, T.; Chang, B.; Li, L.; Chandrasekaran, A. R.; Pei, H. Rationally Engineered Nucleic Acid Architectures for Biosensing Applications. *Chem. Rev.* **2019**, *119* (22), 11631−11717.
(25) Zhang, X.-B.; Kong, R.-M.; Lu, Y. Metal Ion Sensors Based on DNAzymes and Related DNA Molecules. *Annu. Rev. Anal. Chem.* **2011**, *4* (1), 105−128.
(26) Liu, H.; Gao, Y.; Mathivanan, J.; Shen, F.; Chen, X.; Li, Y.; Shao, Z.; Zhang, Y.; Shao, Q.; Sheng, J.; Gan, J. Structure-Guided Development of Pb2+-Binding DNA Aptamers. *Sci. Rep.* **2022**, *12* (1), 460.
(27) Wen, Y.; Wang, L.; Li, L.; Xu, L.; Liu, G. A Sensitive and Label-Free Pb(II) Fluorescence Sensor Based on a DNAzyme Controlled G-Quadruplex/Thioflavin T Conformation. *Sensors* **2016**, *16* (12), 2155.
(28) Swearingen, C. B.; Wernette, D. P.; Cropek, D. M.; Lu, Y.; Sweedler, J. V.; Bohn, P. W. Immobilization of a Catalytic DNA Molecular Beacon on Au for Pb(II) Detection. *Anal. Chem.* **2005**, *77* (2), 442−448.
(29) Chen, Y.; Li, H.; Gao, T.; Zhang, T.; Xu, L.; Wang, B.; Wang, J.; Pei, R. Selection of DNA Aptamers for the Development of Light-up Biosensor to Detect Pb(II). *Sens. Actuators B Chem.* **2018**, *254*, 214−221.
(30) Yang, D.; Liu, X.; Zhou, Y.; Luo, L.; Zhang, J.; Huang, A.; Mao, Q.; Chen, X.; Tang, L. Aptamer-Based Biosensors for Detection of Lead(II) Ion: A Review. *Anal. Methods* **2017**, *9* (13), 1976−1990.

https://doi.org/10.1021/acsabm.2c00368
ACS Appl. Bio Mater. 2022, 5, 5089−5093