Electronic Supplementary Information

Label-free and Enzyme-free Colorimetric Detection of Pb^{2+} Based on RNA-Cleavage and Annealing-Accelerated Hybridization Chain Reaction

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Experimental Section

Oligonucleotides and reagents

All of the DNA oligonucleotides (Table S1) were synthesized by Shanghai Sangon Biotechnological Co., Ltd. (Shanghai, China), dissolved and directly used without further purification. The gold chloride hydrates ($HAuCl_4 \cdot 3H_2O$) was purchased from Sigma-Aldrich. Other chemical reagents were of analytical grade, and ultrapure water with resistivity of 18.25 MΩ·cm was used throughout the work.

Apparatus

The UV-vis absorption spectra were recorded on a Lambda 25 UV-vis spectrophotometer (PerkinElmer, USA) at room temperature, with a scanning speed of 960 nm/min and slit of 1 nm, and the quartz cell with 10 mm path lengths of 100 μl volume were used all through the work. Transmission electron microscopy (TEM) measurements were carried out on a FEI Tecnai G2 F20 S-T WIN instrument (FEI, USA). Incubation or annealing was carried out on a SimpliAmp Thermal Cycler (ABI, USA). Polyacrylamide gel electrophoresis analysis (PAGE) gel imaging was performed by using an Azure c300 Biosystem (Azure Biosystems, USA).

Detection of Actual water samples

For the Pb^{2+} detection in the actual water samples, the actual water samples were centrifuged at 5000 G for 10 min and filtered with 0.22 μM filter to remove the insoluble impurities at first. The detection procedure was the same as the typical Pb^{2+} detection procedure, except that 50 μL of ultrapure water in solution A was replaced by equal volume of real water samples.

For comparison, we also constructed a Pb^{2+} colorimetric sensor without the stage of HCR signal amplification.

Colorimetric sensor without the stage of HCR signal amplification

For the Pb^{2+} detection without HCR, solution C (the mixed solution of 10 μL of 1M NaAc, 10 μL of 100 mM Tris-HAc (pH 7.5), 5 μL of 10 μM GR5, 5 μL of 10 μM S_{TM}, and 64 μL of H_2O) was kept at 95 ℃ for 5 min, and annealed to 25 ℃ with a rate of 0.1 ℃/s. Then, 2.5 μL of Pb^{2+} stock solution was added into 96.5 μL of solution C and incubated at 37 ℃ for 30 min. After 1 μL 100 mM EDTA was added to chelate the Pb^{2+} and terminate reaction, 7.5 μL of the reaction mixture and 3.5 μL of 100

mM TrisHAc (pH7.5) were added into 100 μL of AuNPs and waited for 10 min. The UV-vis absorption spectra were also tested by Lambda 25 UV-vis spectrophotometer. (Since $PbCl_2$ is a kind of slightly soluble precipitate, NaAc and TrisHAc rather than NaCl and TrisHCl were used in this work in order to reduce the interference during Pb^{2+} detection. More time was spent in the reaction of DNAzyme mediated RNA-cleavage, because more substrate strand (S_{TM}) were needed to be split to produce significant DNA conformational changes and obvious color changes.)

Polyacrylamide gel electrophoresis analysis

The PAGE was carried out in 10% gel and the denatured PAGE analysis was carried out in 15% denatured gel (8% urea), in which DNA samples were run at 120 V for 45 min, followed by staining and gel imaging with the Azure c300 Biosystem.

Table S1. DNA Sequences used in this work.

Name	Sequence (from 5' to 3')
GR5	TTTCGCCATCTGAAGTAGCGCCGCCGTATAGTGACTCGTGAC
cGR5	GTCACGAGTCACTATACGGCGGCGCTACTTCAGATGGCGAAA
S_{TM}	*GTCACGAGTCACTATrA***GGAAGATG**GCGAAA
HA_6	**TCTTCC**TATAGTGACTCGTGACGGTAGGGTCACGAGTCACTATA
HB_6	GTCACGAGTCACTATAGGAAGATATAGTGACTCGTGAC**CCTACC**
HA_8	**CATCTTCC**TATAGTGACTCGTGACGGTAGGGTCACGAGTCACTATA
HB_8	GTCACGAGTCACTATAGGAAGATGTATAGTGACTCGTGAC**CCTACC**
S_T	**GGAAGATG**GCGAAA
S_M	*GTCACGAGTCACTATrA*

The cGR5 is completely complementary to GR5, which can hybridize with GR5 and help the detachment of split or unsplit S_{TM}. The rA denotes adenosine ribonucleotide, which can be split by GR5 in the presence of Pb^{2+}. Sequence

in italic represents the DNA migration domain. Sequence in bold represents the toehold domain of the trigger DNA or DNA hairpins. Sequence underline represents the stem domain of the DNA hairpins. S_T and S_M represent the two short DNA fragments that split by DNAzyme GR5 in the presence of target ion Pb^{2+}.

Table S2. Comparison results of different biosensors for Pb^{2+} detection.

Detection mode	Detection time	Linear range	LOD	Reference
Colorimetric	10 min	3 nM~100 nM	3 nM	1
Colorimetric	28 min	Not mentioned	500 nM	2
Colorimetric	30 min	0.05 nM~5 nM	20 pM	3
Fluorescence Anisotropy	No mentioned	10 nM~10 μM	24.5 nM	4
Fluorescence		4.83 nM~4.83 mM	4.83 nM	5
Fluorescence	20 min	3 nM~200 nM	0.6 nM	6
Fluorescence	20 min	1 nM~100 nM	0.3 nM	7
Fluorescence	No mentioned	1 nM~1 μM	0.3 nM	8
Dual Polarization Interferometry	25 min	1 μM ~400 μM	1.83 nM	9
Colorimetric and Electrochemiluminescence	70 min/2 h	0.5 nM~2 μM	0.14 nM	10
Electrochemical	30 min	0.1 nM~5 μM	45.8 pM	11
Colorimetric	30 min	0.1 nM~15 nM	59.39 pM	This work

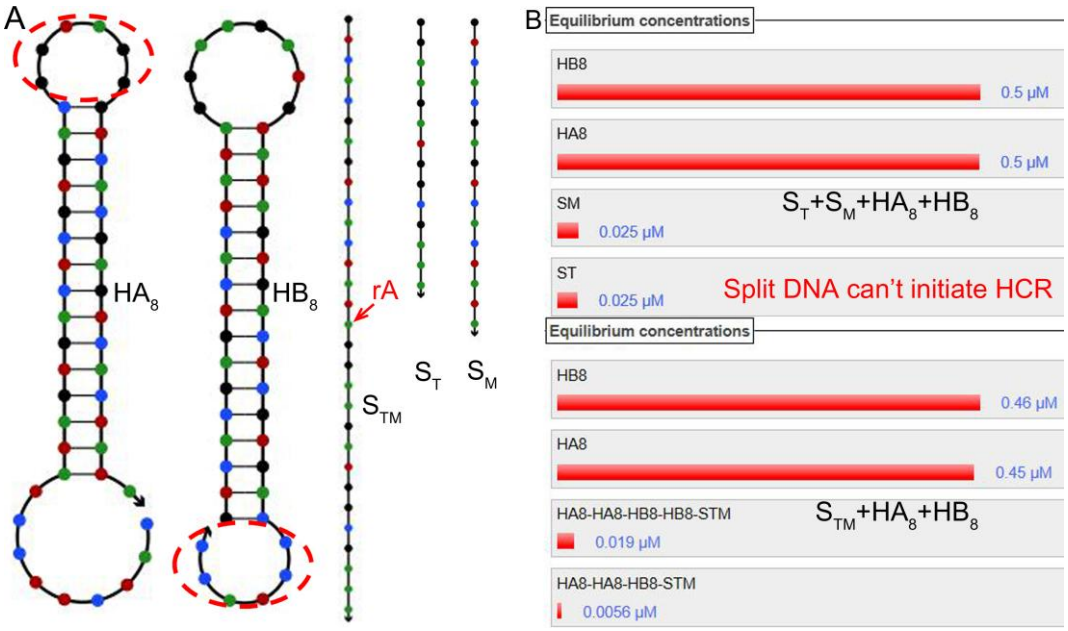


Figure S1 (A) Structure of DNA hairpins, trigger DNA S_{TM}, S_T and S_M. DNA bases in dotted box are variable, and the arrow indicates the ribonucleotide site. (B) DNA assembly results simulated by online software NUPACK (0.1 M Na^+, 500 nM HA_8, 500 nM HB_8, 25 nM S_{TM} or S_T+S_M). Maximum complex of 5 DNA strands is used for simulation, therefore, largest complex contains only 5 DNA strands, and little DNA hairpins are consumed. (Simulating larger DNA complex will consume time in the order of hours or days). Simulation results indicated that split DNA can't initiate HCR.

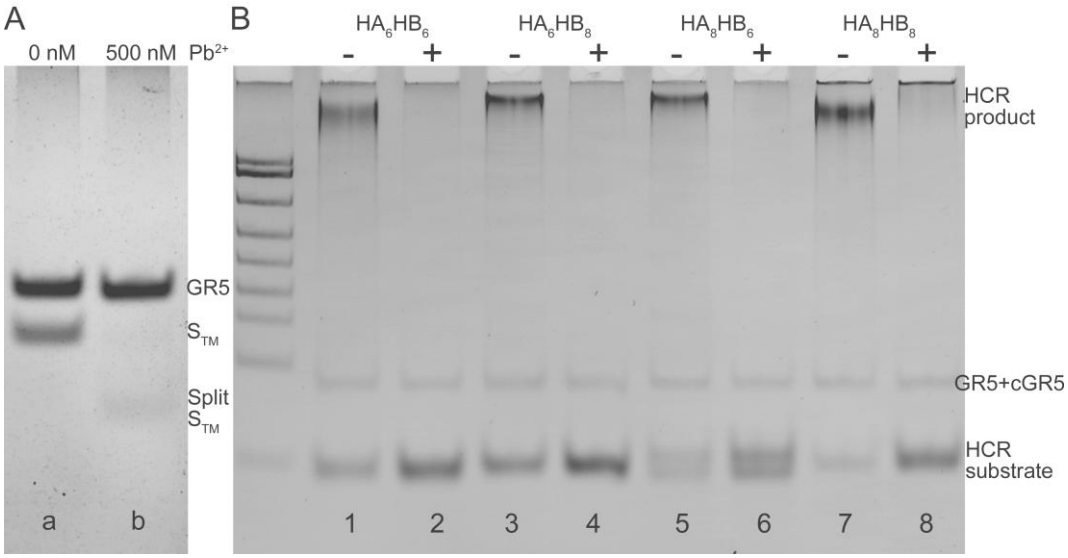
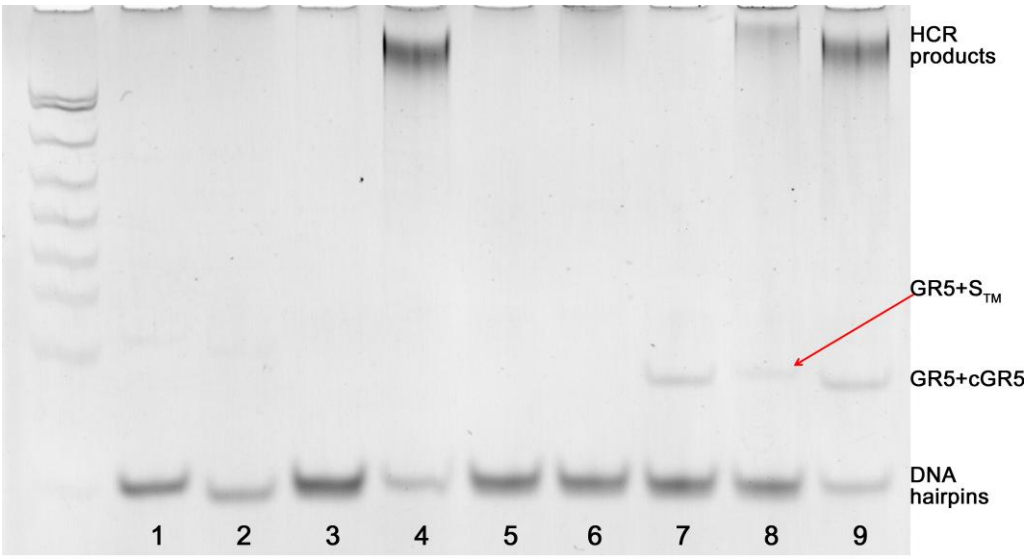


Figure S2 (A) The denatured PAGE results show that S_{TM} is cleaved by GR5 in the presence of 500 nM Pb^{2+} (100 mM NaAc, 10 mM TrisHAc, pH 7.5, 500 nM GR5, 500 nM S_{TM}, 0/500 nM Pb^{2+}). (B) Optimization of HCR substrate sequences. Different combinations of DNA hairpins are assembled in

the absence (-) or in the presence (+) of 500 nM Pb^{2+}. (100 mM NaAc, 10 mM TrisHAc, pH 7.5, 1 mM EDTA, 200 nM HA_6/HA_8, 200 nM HB_6/HB_8, and 25 nM of S_{TM} were annealing from 95 ℃ to 25 ℃ to accelerate the HCR process.)



	1	2	3	4	5	6	7	8	9
HA_8	+	-	+	+	+	+	+	+	+
HB_8	-	+	+	+	+	+	+	+	+
S_{TM}	-	-	-	+	-	-	-	+	+
GR5	-	-	-	-	+	-	+	+	+
cGR5	-	-	-	-	-	+	+	-	+

Figure S3 The PAGE results show the DNA assembly of relevant DNA strands in the reaction system. (-) indicates the reaction system does not contain the corresponding DNA strands, while (+) indicates the reaction system contains the corresponding DNA strands. (100 mM NaAc, 10 mM TrisHAc, pH 7.5, 1 mM EDTA, 200 nM HA_8/HB_8, 25 nM GR5, 25 nM cGR5 or 25 nM S_{TM}. DNA mixture are annealing from 95 ℃ to 25 ℃ to accelerate the HCR process.)

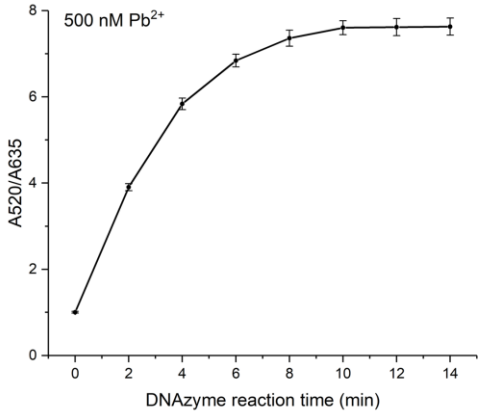


Figure S4 Reaction time of DNAzyme mediated RNA-cleavage. (The reaction conditions are the same as the typical Pb^{2+} detection procedure described in the experimental section, except that the incubation time was 0-14 min at 37 ℃, respectively.)

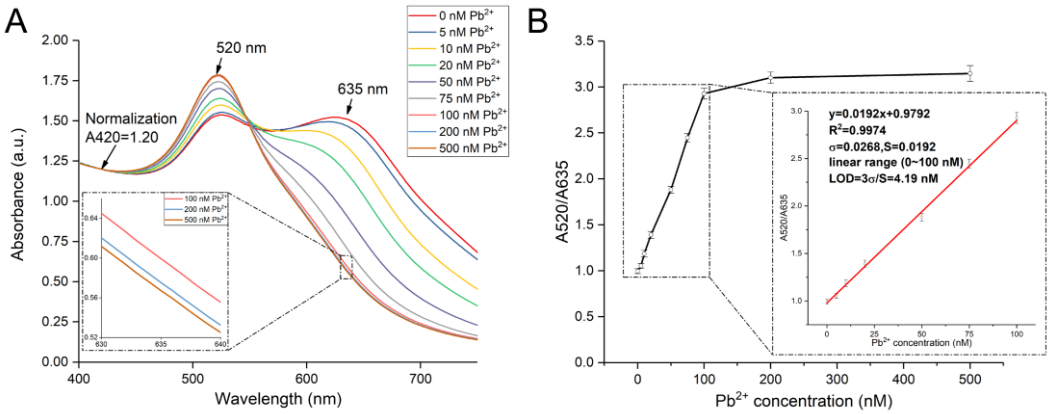


Figure S5 (A) UV-vis absorption spectra when different concentrations of Pb^{2+} are detected without the stage of HCR. (B) The relationship between the UV-vis absorance ratio A520/A635 and detected Pb^{2+} concentration without the stage of HCR signal amplification, the inset showed their linear relationship.

References

1. Wang, Z. D.; Lee, J. H.; Lu, Y., Label-Free Colorimetric Detection of Lead Ions with a Nanomolar Detection Limit and Tunable Dynamic Range by using Gold Nanoparticles and DNAzyme. *Adv. Mater.* **2008,** *20* (17), 3263-3267.

2. Wei, H.; Li, B. L.; Li, J.; Dong, S. J.; Wang, E. K., DNAzyme-based colorimetric sensing of lead (Pb(2+)) using unmodified gold nanoparticle probes. *Nanotechnology* **2008,** *19* (9), 095501.

3. Yun, W.; Cai, D. Z.; Jiang, J. L.; Zhao, P. X.; Huang, Y.; Sang, G., Enzyme-free and label-free ultra-sensitive colorimetric detection of Pb2+ using molecular beacon and DNAzyme based amplification strategy. *Biosens. Bioelectron.* **2016,** *80*, 187-193.
4. Wang, Z. X.; Pei, X. J.; Li, N.; Tang, X. J., Phosphate-perylene modified G-quadruplex probes for the detection of Pb2+ using fluorescence anisotropy. *J Mater Chem B.* **2016,** *4* (24), 4330-4336.
5. Huang, Y. F.; Yan, J.; Fang, Z. Z.; Zhang, C. H.; Bai, W. H.; Yan, M. M.; Zhu, C.; Gao, C. G.; Chen, A. L., Highly sensitive and selective optical detection of lead(II) using a label-free fluorescent aptasensor. *Rsc Adv.* **2016,** *6* (93), 90300-90304.
6. Zhang, X. B.; Wang, Z. D.; Xing, H.; Xiang, Y.; Lu, Y., Catalytic and Molecular Beacons for Amplified Detection of Metal Ions and Organic Molecules with High Sensitivity. *Anal. Chem.* **2010,** *82* (12), 5005-5011.
7. Zhao, X. H.; Kong, R. M.; Zhang, X. B.; Meng, H. M.; Liu, W. N.; Tan, W.; Shen, G. L.; Yu, R. Q., Graphene-DNAzyme based biosensor for amplified fluorescence "turn-on" detection of Pb2+ with a high selectivity. *Anal. Chem.* **2011,** *83* (13), 5062-6.
8. Wen, Z. B.; Liang, W. B.; Zhuo, Y.; Xiong, C. Y.; Zheng, Y. N.; Yuan, R.; Chai, Y. Q., An efficient target-intermediate recycling amplification strategy for ultrasensitive fluorescence assay of intracellular lead ions. *Chem. Commun.* **2017,** *53* (54), 7525-7528.
9. Wang, S.; Lu, S. S.; Zhao, J. H.; Huang, J. S.; Yang, X. R., Real-Time Study of the Interaction between G-Rich DNA Oligonucleotides and Lead Ion on DNA Tetrahedron-Functionalized Sensing Platform by Dual Polarization Interferometry. *ACS Appl. Mater. Interfaces.* **2017,** *9* (47), 41568-41576.
10. Xu, J. M.; Zhang, Y.; Li, L.; Kong, Q. K.; Zhang, L.; Ge, S. G.; Yu, J. H., Colorimetric and Electrochemiluminescence Dual-Mode Sensing of Lead Ion Based on Integrated Lab-on-Paper Device. *ACS Appl. Mater. Interfaces.* **2018,** *10* (4), 3431-3440.
11. Ma, R. N.; Wang, L. L.; Zhang, M.; Jia, L. P.; Zhang, W.; Shang, L.; Jia, W. L.; Wang, H. S., A novel one-step triggered "signal-on/off" electrochemical sensing platform for lead based on the dual-signal ratiometric output and electrode-bound DNAzyme assembly. *Sensor Actuat B-Chem.* **2018,** *257*, 678-684.