Electronic Supplementary Information

for

Binary DNA hairpin-based colorimetric biochip for simultaneous detection of Pb^{2+} and Hg^{2+} ions in real-world samples

Xiaoli Shi,[†] Xiaoyi Gao,[†] Lingling Zhang,[†] Yunchao Li,[†,*] Louzhen Fan,[†] and Hua-Zhong Yu[‡,*]

[†]Department of Chemistry, Beijing Normal University, Beijing 100875, P. R. China

[‡]Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A1S6, Canada

[*] Corresponding authors: liyc@bnu.edu.cn (Y.L.); hogan_yu@sfu.ca (H.Y.)

Part I: General Experimental Information

1. Materials and reagents: Lead nitrate, mercuric nitrate, sodium citrate, citric acid, sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), magnesium chloride, sodium chloride and hydrochloric acid were purchased from Beijing Chemical Reagent Co. (Beijing, China). Silver acetate, hydroquinone, 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS), tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), bovine serum albumin (BSA, globulin-free, molecular biology grade), tween-20, glycerol and sodium azide were ordered from Sigma-Aldrich (Saint Louis, MO, USA). Horseradish peroxidase-labeled streptavidin (Streptavidin-HRP) and 1-component 3, 3′, 5, 5′-tetramethylbenzidine (TMB) membrane substrate were purchased from KPL Inc. (Gaithersburg, MD, USA). Tyramide signal amplification (TSA) biotin kit (including biotin tyramide and streptavidin-HRP) was purchased from PerkinElmer Inc. (Boston, MA, USA). Human serum and human plasma (from healthy people) were provided by the Affiliated Hospital of Beijing Normal University. The lake and industrial waste water samples

were taken from Aohai Lake in Beijing and an electroplating factory in Shenyang, respectively. All oligonucleotides (sequences listed in **Table S1**) were of HPLC-purified grade and obtained from Sangon Biotechnology Inc. (Shanghai, China). Nanogold-streptavidin (1.4 nm diameter) conjugates were ordered from Nanoprobes Inc. (New York, USA). All buffer solutions were prepared in deionized water produced from a Barnstead Easypure System (Thermo Scientific Inc., Dubuque, Iowa, USA). Polycarbonate (PC) substrates were obtained from Bayer Material Science AG (Leverkusen, Germany).

2. Apparatus: A UV/ozone cleaner (Model PSD-UV, Novascan Technologies Inc.) was used to activate the plastic substrates. A UV-2450 UV-Vis spectrophotometer (Shimadzu Corporation) was employed to record the absorption spectra and a JASCO J-810 spectropolarimeter to collect the CD spectra. The optical images of the plastic biochips were scanned with a flatbed scanner (Scanmaker i700, Microtek) and presented in either grayscale or color mode. The concentrations of Pb^{2+} and Hg^{2+} in waste water samples were determined on an inductively coupled plasma mass spectrometer (Elan DRC-e, PerkinElmer).

Table S1. Oligonucleotide sequences of DNA hairpin probes for recognizing Pb^{2+} and Hg^{2+}.

DNA probe strands		**Sequence/modification**
Pb^{2+}	GG-5	5’ -NH_2-$(CH_2)_6$-TTTCCAACT TGG TTG GTG TGGTTGGTC T-biotin -3’
	GG-6a	5’-NH_2-$(CH_2)_6$-TTTCCAACT TGG TTG GTG TGGTTGGAC T -biotin -3’
	GG-6b	5’-NH_2-$(CH_2)_6$-TAGCCA ACA AGG TTG GTG TGG TTG GCA T -biotin -3’
	GG-7	5’ -NH_2-$(CH_2)_6$-TTTCCAACT TGG TTG GTG TGG TTG GAA T-biotin-3’
Hg^{2+}	TT-6a	5’-NH_2-$(CH_2)_6$-CCAAGGAAC TTT GGTTT CCCTTTTCCTTTT -biotin-3’
	TT-6b	5’ -NH_2-$(CH_2)_6$-CA AAGGACC TTT GGTTT CCC TTTTCCTTTT-biotin -3’
	TT-7a	5’-NH_2-$(CH_2)_6$-CAAAGGA ACT TTG GTT TC CCTT TTCCTTTT-biotin-3’
	TT-7b	5’-NH_2-$(CH_2)_6$-CAAATGA ACT TTG GTT TC CCTT TTCATTTT-biotin-3’

Part II: Supplementary Data

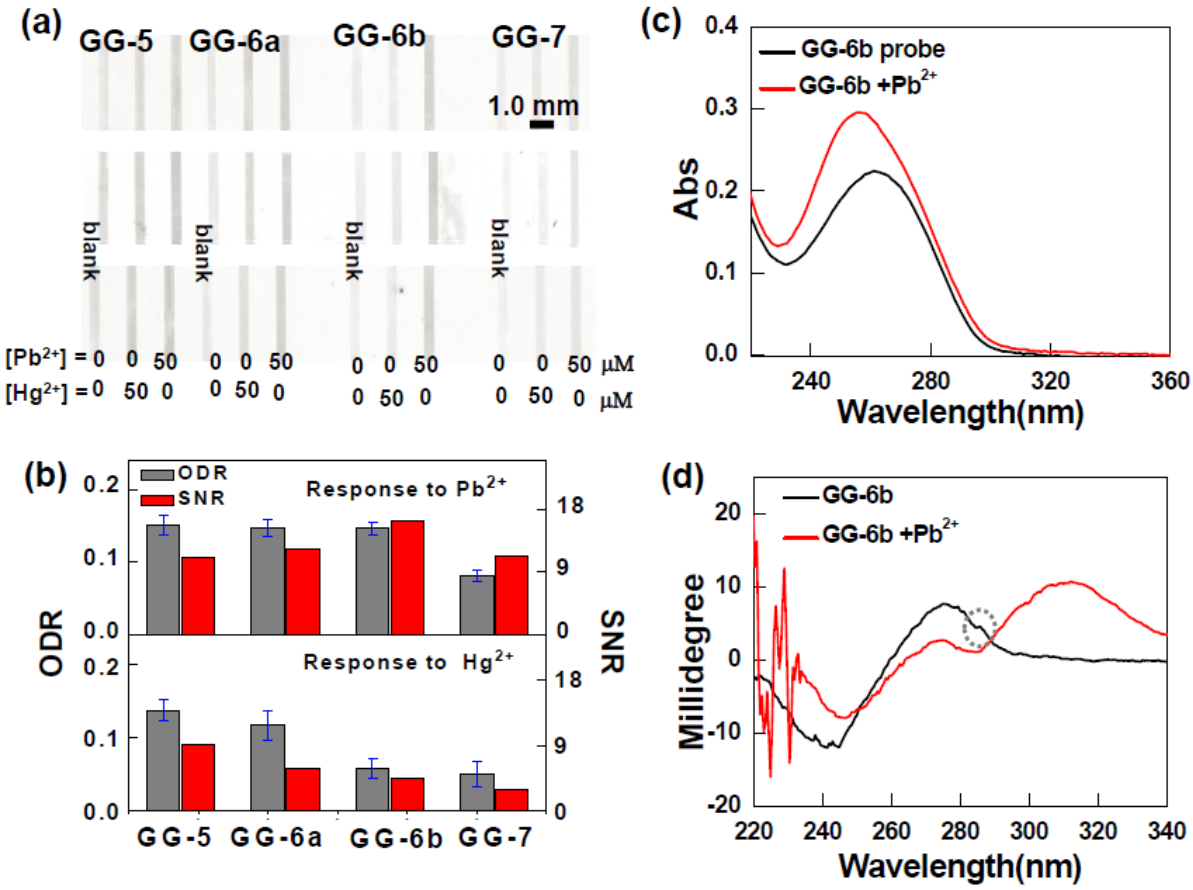

Fig. S1 Optimization of the DNA hairpin probes for Pb^{2+} detection. (a) Three representative optical images of the DNA hairpin-based biochip with four G-rich DNA hairpin probe strands consisting of different stem lengths or base sequences. For each probe, a blank and two samples containing 50 µM Pb^{2+} or 50 µM Hg^{2+} were tested simultaneously. The concentration of the DNA hairpin probes to prepare these chips was 10 µM for all cases, and the sliver staining time was kept at 18 min; (b) Correlation between the assay signal (ODR / SNR) with the probe sequences. (c-d) UV-Vis and CD spectra of GG-6b probes in solution with/ without the presence of Pb^{2+}. The hairpin GG-6b probe has a sharp absorption peak at 260 nm (the characteristic absorption from exposed bases) in its UV/Vis spectrum; a clear increase in the peak intensity was observed with the introduction of Pb^{2+} to the solution. The increased absorbance can be attributed to the fact that more unpaired bases in the probe strand are released upon Pb^{2+} binding, indicating that the hairpin probe is "opened" by such a binding event. In the CD spectra, the hairpin GG-6b probes shows a negative band around 240 nm, a positive band around 275 nm and a positive shoulder peak around 287 nm, confirming its intramolecular hairpin structure.[S1] Upon adding Pb^{2+}, a positive band near 312 nm can be observed, suggesting that the probe transforms into a G-quadruplex conformation.[S2]

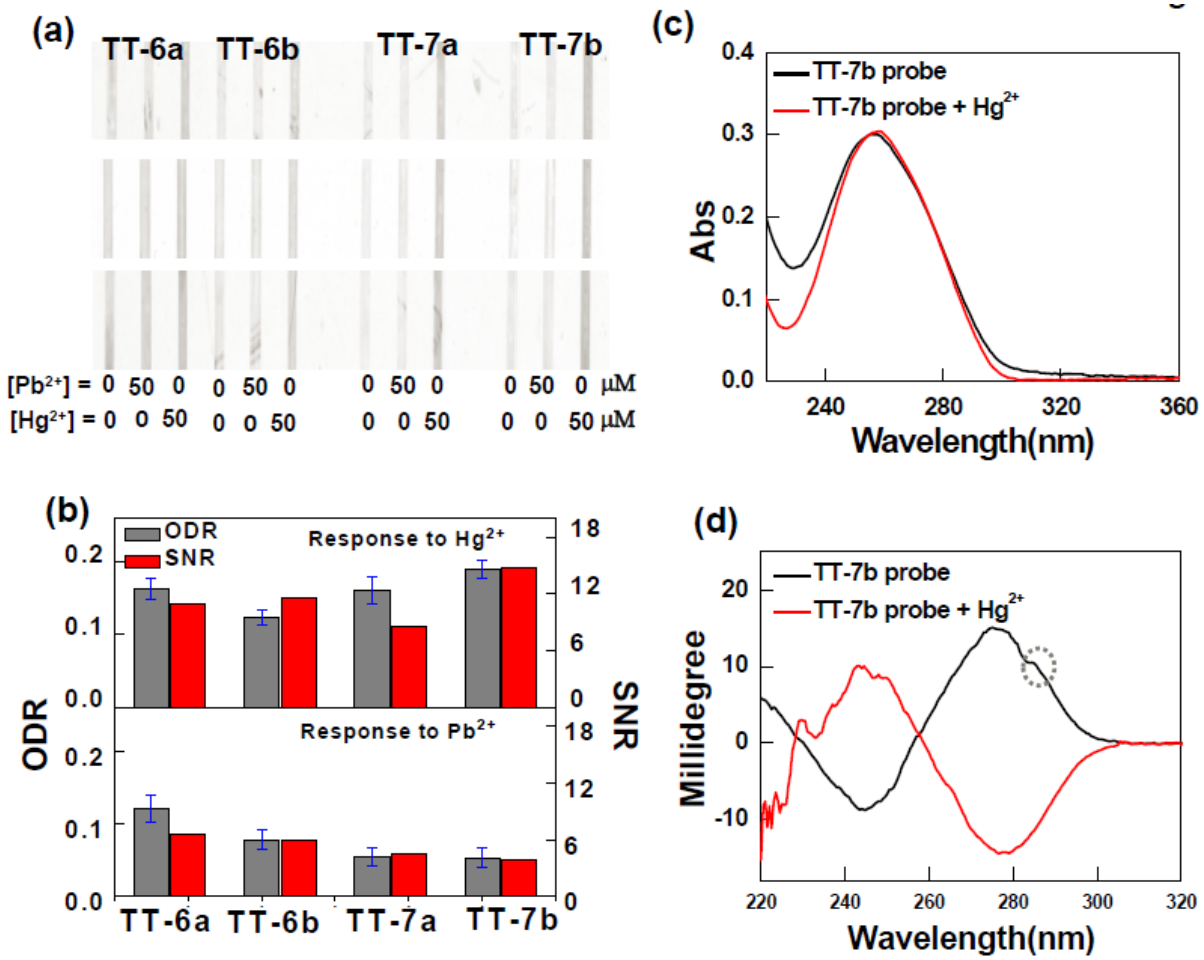

Fig. S2 Optimization of the DNA hairpin probes for Hg^{2+} detection. (a) Three representative optical images of the DNA hairpin-based biochip immobilized with four T-rich DNA hairpin probe strands consisting of different stem lengths or base sequences. For each probe, a blank and two samples containing 50 μM Pb^{2+} or 50 μM Hg^{2+} were tested simultaneously. The concentration of the DNA hairpin probes to prepare these chips was 10 μM for all cases, and the sliver staining time was kept at 18 min; (b) Correlation between the assay signal (ODR and SNR) with the probe sequences. (c-d) UV-Vis spectra and CD spectra of TT-7b probes in solution with/ without the presence of Hg^{2+}. As shown in (c), no obvious change in the absorption peak at 260 nm was observed upon adding Hg^{2+} ions into the solution containing TT probes, suggesting that the probes have a similar number of exposed bases in both cases. The number of matching base pairs (i.e., stem base pairs) in the TT probe of hairpin conformation is equal to that (i.e., T-Hg^{2+}-T number) in its "S-like" conformation. In the CD spectra shown in (d), upon adding Hg^{2+} the characteristic bands at 245, 275 and 285 nm disappear and a dominant negative peak at 280 nm becomes evident, indicating the formation of T-Hg^{2+}-T complexes in the probe.[S3]

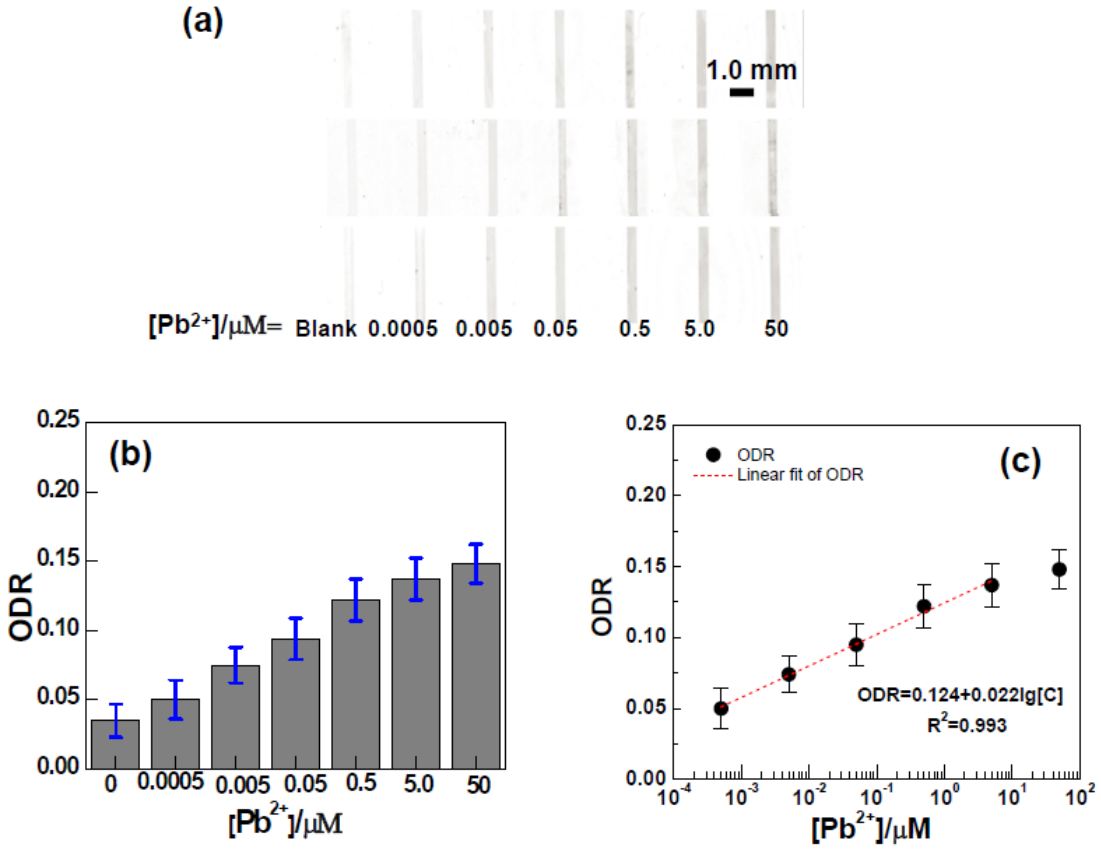

Fig. S3 DNA hairpin biochips for colorimetric detection of Pb^{2+} in buffer. (a) Three representative images showing the responses of the chip (formed with 10 μM GG-6b probe) to different concentrations of Pb^{2+} in buffer; (b) dependence of the average signal (ODR value) on Pb^{2+} concentration represented in a 2D histogram; (c) dependence of the signal (ODR value) on logarithmic concentration of Pb^{2+} represented in a linear graph. The results in (b) and (c) are mean values ± SD obtained from the three parallel chips shown in (a); the dashed line in (c) is the fitting curve for the data points located in the low concentration range.

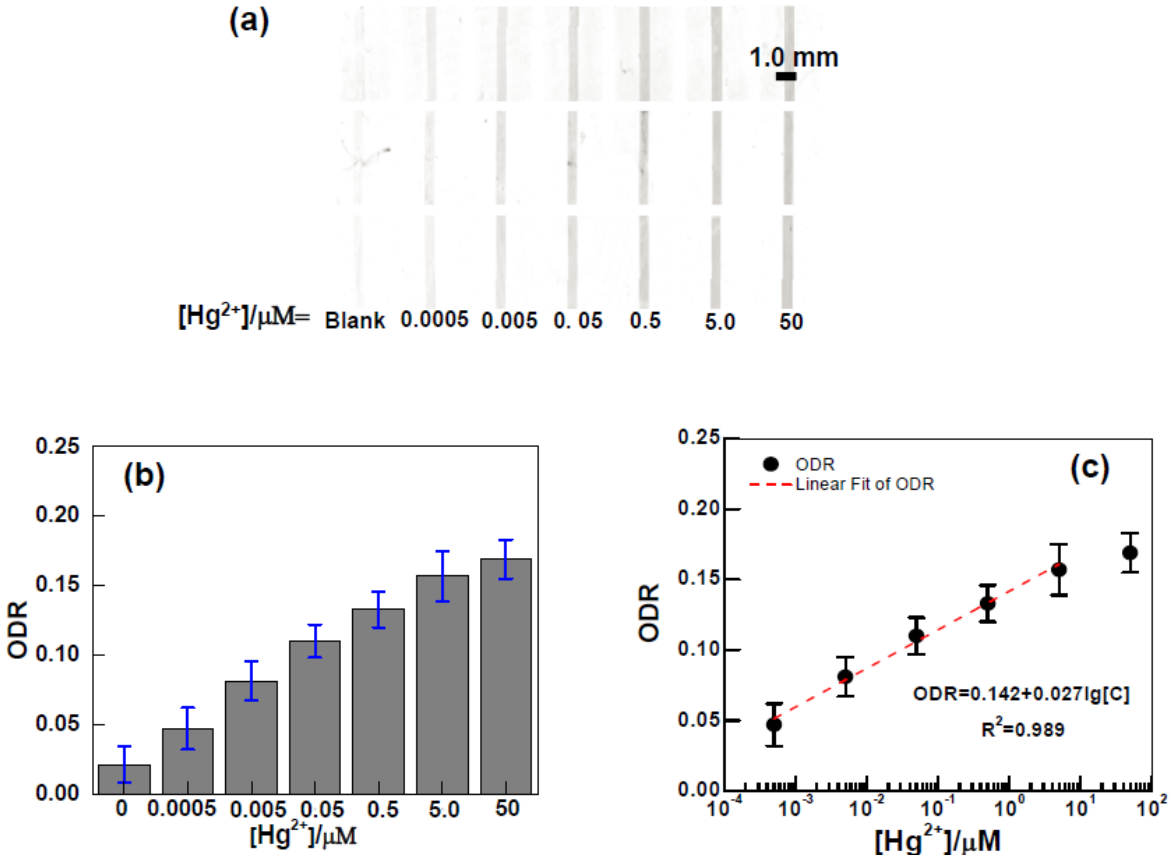

Fig. S4 DNA hairpin-biochips for scanometric detection of Hg^{2+} in buffer. (a) Three representative images showing the responses of the chip (formed with 10 μM TT-7b probe) to different concentrations of Hg^{2+} in buffer; (b) dependence of the average signal (ODR value) on Hg^{2+} concentration represented in a 2D histogram; (c) dependence of the signal (ODR value) on logarithmic concentration of Hg^{2+} represented in a linear graph. The results in (b) and (c) are mean values ± SD obtained from the three parallel chips shown in (a); the dashed line in (c) is the fitting curve for the data points located in the low concentration range.

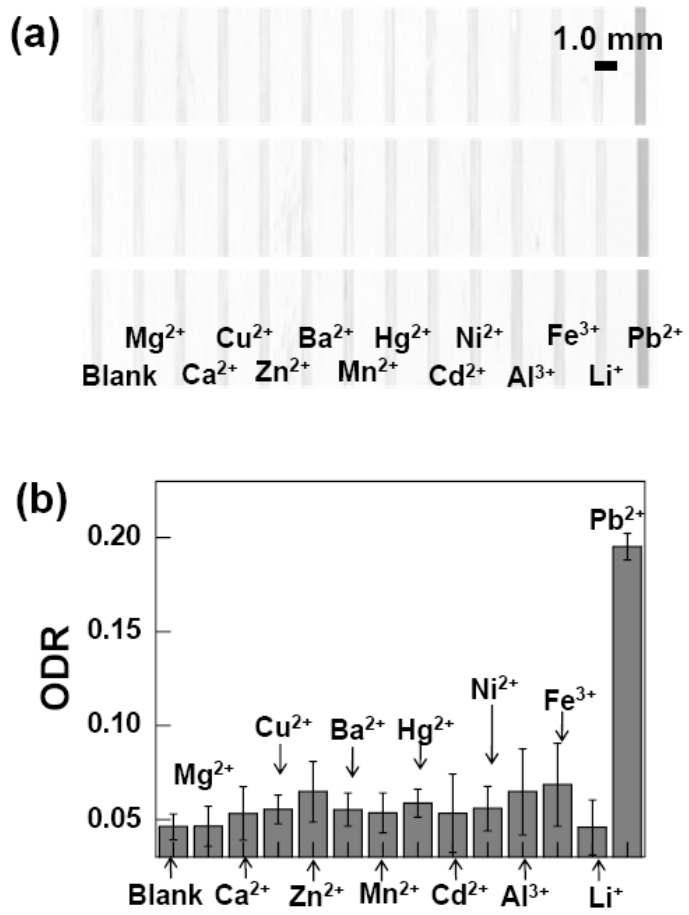

Fig. S5 The selectivity of DNA hairpin-biochips for colorimetric detection of Pb^{2+} in buffer. (a) Three representative chip images showing the responses of the chip (formed with 10 μM GG-6b probe and undergone ca.22 min silver staining) to different metal cations of 1.0 μM in all cases, (b) ODR values of the biochip upon interacting with different metal cations. The ODR values were determined based on the three repeated chips shown in (a).

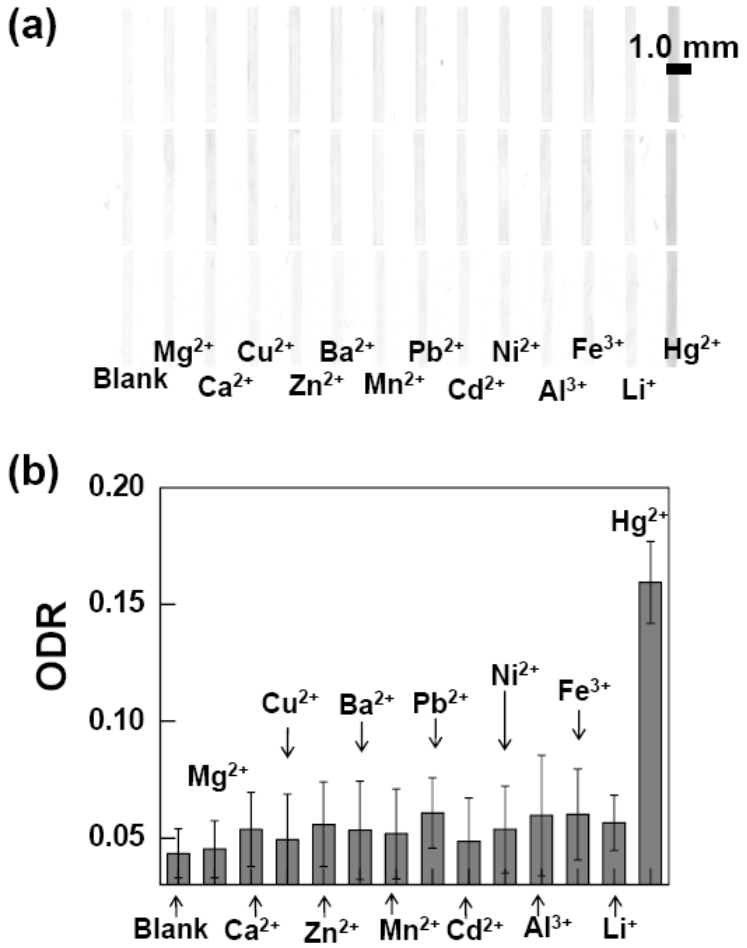

Fig. S6. The selectivity of DNA hairpin-based biochips for the detection of Hg^{2+} in buffer. (a) Three representative images showing the responses of the chip (formed with 10 μM TT-7b probe and undergone ca.22 min silver staining) to different metal cations of 1.0 μM in all cases; (b) ODR values of the biochip upon interacting with different metal cations. The ODR results were determined based on the three repeated chips shown in (a).

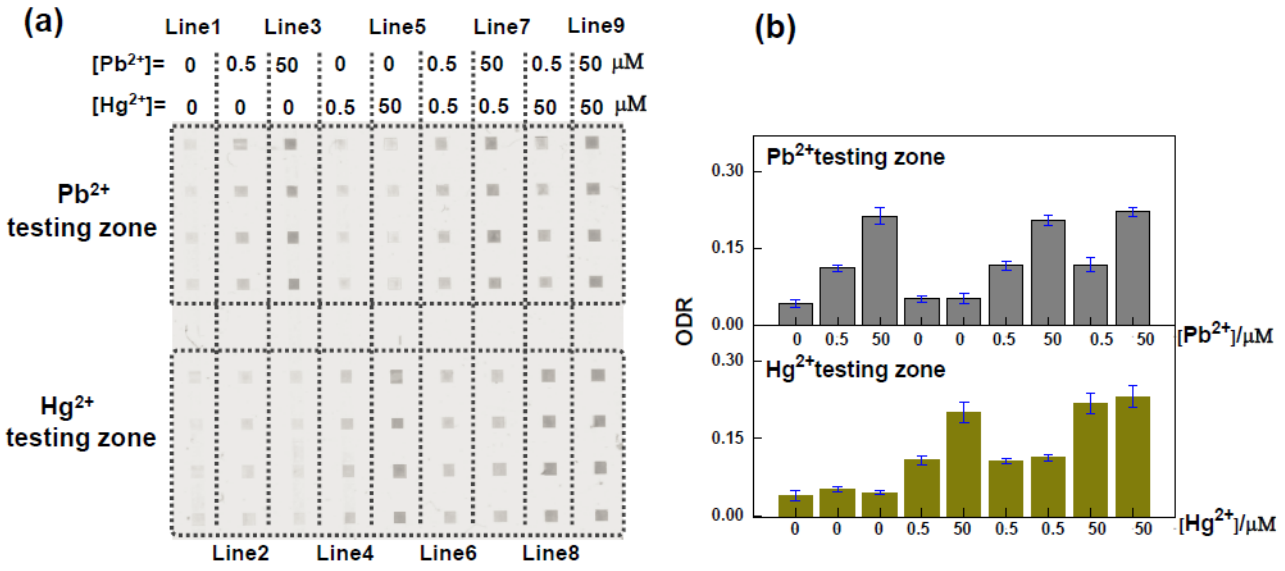

Fig. S7 DNA hairpin-based colorimetric biochip for simultaneous detection of Pb^{2+} and Hg^{2+} in lake water. (a) Representative optical image showing the colorimetric responses (represented by the gray binding spots with different darknesses) of surface-immobilized GG-6b probes (in the Pb^{2+} testing zone) and TT-6b probes (in the Hg^{2+} testing zone) to nine lake water samples containing different concentrations of Pb^{2+} and Hg^{2+} (20 min silver staining); (b) a 2D histogram representing the mean ODR ± its SD corresponding to the spots in each lane of the two testing zone in (a).

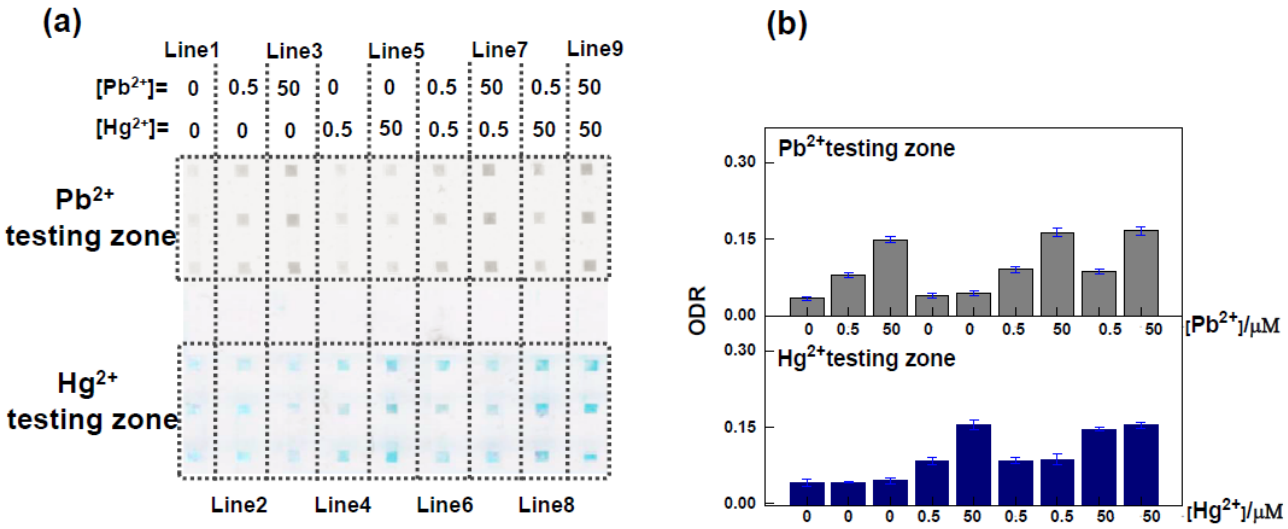

Fig. S8 DNA hairpin-based biochip for simultaneous detection of Pb^{2+} and Hg^{2+} by using a two-color staining (silver staining and TMB staining) protocol. (a) Representative optical image showing the responses of surface-immobilized GG-6a probes (in the Pb^{2+} testing zone) and TT-6b probes (in the Hg^{2+} testing zone) to nine lake water samples containing different concentrations of Pb^{2+} and Hg^{2+}; (b) a 2D histogram representing the mean ODR ± its SD corresponding to the spots in each lane of the two testing zone in (a).

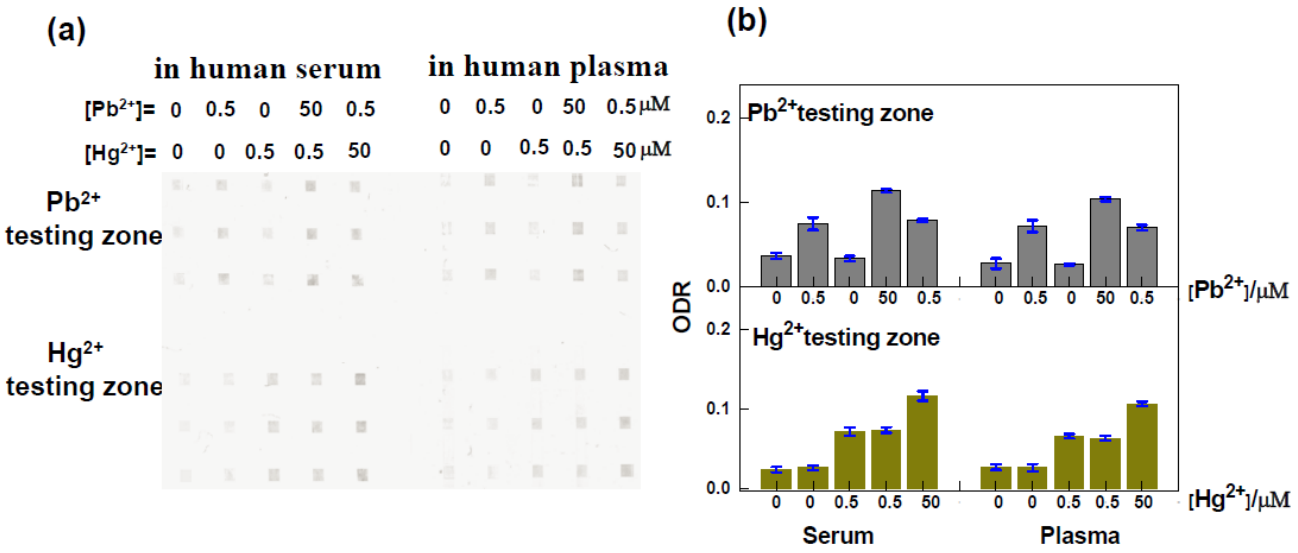

Fig. S9 DNA hairpin-based biochip for simultaneous detection of Pb^{2+} and Hg^{2+} in human serum and plasma. (a) Representative optical image showing the colorimetric responses (represented by the gray binding spots) of surface-immobilized GG-6b probes (in the Pb^{2+} testing zone) and TT-6b probes (in the Hg^{2+} testing zone) to the human serum and plasma samples spiked with different concentrations of Pb^{2+} and Hg^{2+} (20-min silver staining); (b) a 2D histogram representing the mean ODR ± its SD corresponding to the spots in each lane of the two testing zone in (a).

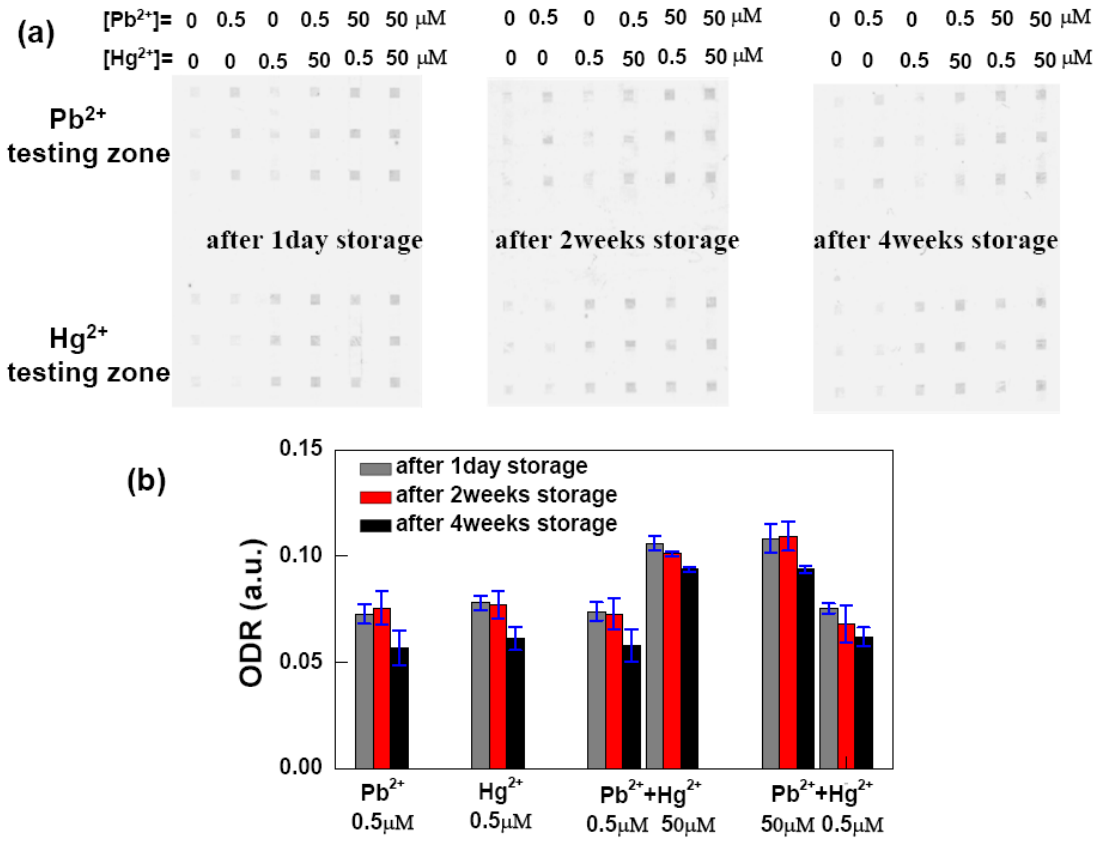

Fig. S10. Influence of the storage time on the response of the DNA hairpin-based biochips to Pb^{2+} and Hg^{2+}. (a) Three representative images showing the responses of the biochips that were stored at 4 °C for different periods of time; (b) dependence of the signal (ODR value) on the storage time derived from (a).

Table S2. Determination of the Pb^{2+} and Hg^{2+} concentrations in two electroplating waste water samples using the present method and ICP-MS.*

Detection method	$[Pb^{2+}]/\mu M$		$[Hg^{2+}]/\mu M$	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
ICP-MS	2.05±0.07	0.055 ± 0.003	3.06 ± 0.04	0.421 ± 0.005
This study	2.3 ± 0.4	0.06 ± 0.01	2.8 ± 0.1	0.47 ± 0.05

* Analysis of three replicates of each sample.

References

[S1] J. Kypr, I. Kejnovska, D. Renciuk, K. M.Vorlıc, *Nucleic. Acids. Res.,* 2009, **37***,* 1713.

[S2] I. Smirnov, R. H. Shafer, *J. Mol. Biol.,* 2000, **296**, 1-5.

[S3] Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, *J. Am. Chem. Soc.,* 2006, **128**, 2172.