Electronic Supplementary Information

Label-free fluorescent sensor for detection of Pb²⁺ and Hg²⁺

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Method	Targets	Linear ranges	LOD	Sensing system	reference
Fluorescent	Pb ²⁺	0-386.4 nM	~10 nM	Modified T30695,	Current
	Hg ²⁺	0-299.4 nM	~5.7 nM	SYBR Green I	work
Fluorescent	Hg ²⁺	0-100 nM	5 nM	DNA, Ag	1
	Cu ²⁺	0-2 μΜ	10 nM	nanoclusters	
Fluorescent	Hg ²⁺	20-150 nM	15 nM	FAM-labeled DNA,	2
	Ag ⁺	20-150 nM	18 nM	ROX-labeled DNA,	
	Pb ²⁺	20-150 nM	20 nM	Cy5-labeled DNA,	
				Carbon nanotubes	
Fluorescent	Hg ²⁺	0-20 nM	1.8 nM	TAMRA-labeled	3
	Ag+	0-20 nM	2.5 nM	ssDNA, Cy5-labeled	
				ssDNA, CdTe QDs	
Electrochemical	Pb ²⁺	10 pM-10 μM	10 pM	DNA (1+2+3),	4
	Ag ⁺	100 nM-800 nM	10 nM	G-rich DNA,	
	Hg ²⁺	0.1 nM-10μM	0.1 nM	C-rich DNA,	
				EDTA, cysteine	
Electrochemical	Pb ²⁺	1 pM-100 μM	0.1 pM	Pb ²⁺ -specific	5
	Hg ²⁺	1 pM-100 μM	1 pM	DNAzyme, mercury-	
				specific	
				oligonucleotide	
Surface-enhanced	Hg ²⁺	0-1 μΜ	500 pM	Six DNA, DTT, NAP-	6
Raman scattering	Ag+	0-10 μΜ	1 nM	5 column, Au NWs	
	Pb ²⁺	0-100 μΜ	50 nM		
Colorimetric	Hg ²⁺	200-800 nM	0.1 µM	Tween 20-AuNPs,	7
	Ag+	400-1000 nM	0.1 μΜ	EDTA, NaCl	

Table S1 Comparison of performances of different sensors for detection of two or more heavy metal ions

Supplementary Materials and Methods

Determination of pH

When investigated the influence of pH on the Hg²⁺ and Pb²⁺ testing, 50 nM modified T30695 was firstly added into Tris-acetate buffer (10 mM, pH varying from 5 to 11) of appropriate volume, and then Hg²⁺ or Pb²⁺ of 250 ppb were respectively introduced into the above solution. After incubated at room temperature and 30 min, 4 μ L of 50× SG were added into Hg²⁺ samples and 6 μ L of 400× SG were added into Pb²⁺ samples. The fluorescence intensity of each sample was monitored after kept in darkness at room temperature and 15 min.

Determination of temperature

In this study, the optimization of temperature included two parts: the incubation temperature between Pb²⁺ or Hg²⁺ and modified T30695, and the reaction temperature of the reaction between SG and G-quaduplex or T-Hg(II)-T. For optimization of the reaction temperature, the incubation temperature was set at room temperature (25 °C), and SG were introduced into the sensing system to react at varying temperature (from 15 to 75 °C). For optimization of the incubation temperature, modified T30695, Pb²⁺ or Hg²⁺ and Tris-acetate buffer were incubated at varying temperature, and SG were added to react at the optimal reaction temperature. Control experiments were carried out by replacing Pb²⁺ or Hg²⁺ with ultrapure water.

Determination of time

In this study, the analysis time included two parts: the incubation time of Pb²⁺ or Hg²⁺ with modified T30695, and the reaction time of the reaction between SG and G-quaduplex or T-Hg(II)-T mixing solution. For optimization of the reaction time, 50 nM modified T30695, Pb²⁺ or Hg²⁺ and appropriate volume of Tris-acetate buffer was incubated at 30 min, and SG were added to react at varying time. For optimization of the incubation time, modified T30695, Pb²⁺ or Hg²⁺ and Tris-acetate buffer were incubated at varying time, and SG were added to react at the optimal reaction time. Control experiments were carried out by replacing Pb²⁺ or Hg²⁺ with ultrapure water.

Supplementary Results and Discussion

Determination of pH

As shown in Fig. S1, the influence of pH was studied. It could be seen that for the Pb^{2+} detection the relative fluorescence intensity increased as the pH changed from 5 to 8, after which the relative fluorescence intensity decreased dramatically. As for the Hg^{2+} detection, the relative fluorescence intensity reached the maximum when pH=8. Therefore, pH=8 was chose in all the following study.



Fig. S1 Determination of pH on the relative fluorescence intensity that caused by 250 ppb Pb2+ (A) and Hg2+ (B) separately in different sensing systems that use Tris-acetate buffer (10 mM) of different pH.

Determination of temperature

As a key parameter to this sensing system, the analysis temperature was also investigated. As shown in Fig. S2, the relative fluorescence intensity decreases with the increase of temperature (from 25 °C to 75 °C) for Hg^{2+} detection and the relative florescence intensity changes a little with the increase of temperature (from 15 °C to 75 °C) for Pb^{2+} detection. Therefore, 25 °C was chose as the incubation temperature and reaction temperature. Moreover, 25 °C suit the potential for real-time detection of this sensor.



Fig. S2 Determination of reaction temperature (A) and incubation temperature (B) on relative fluorescence intensity. The solution contains 50 nM modified T30695, 250 ppb Pb2+ or Hg2+ and optimal concentration of SG. **Determination of time**

In order to obtain a highly sensitive sensor, it is necessary to optimize the analysis time. The results were shown in Fig. S3 (A), when the reaction time is changed, the fluorescence intensity reaches its peak at ~15 min for Hg^{2+} detection and the fluorescence intensity have almost no change for Pb^{2+} detection. Fig. S3 (B) shows that at varying incubation time, the fluorescence intensity achieves maximum at 30 min for Hg^{2+} and Pb^{2+} detection. Therefore, by taking into account the performances of the both systems, 15 min was selected as the reaction time and 30 min was selected as the incubation time.



Fig. S3 Determination of reaction time (A) and incubation time (B) on relative fluorescence intensity. The solution contains 50 nM modified T30695, 250 ppb Pb^{2+} or Hg^{2+} and optimal concentration of SG.

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