Electronic Supporting Information

A dual-channel detection of mercuric ions using a label free G-quadruplex-based DNAzyme

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Experimental Section

1. Materials and sample preparations

All DNA were chemically synthesised and purified by Generay Biotech Co., Ltd (China). Morpholineopropanesulfonic acid (MOPS), cysteine were purchased from Shanghai Sangon Biotechnology (China). Metal salts and other chemicals were were obtained from Shanghai Jingchun Scientifical Co., Ltd. (China). All chemicals were used without further purification. Deionized water was prepared using a Milli-Q® Ultrapure water system. Standard solutions of all metal ions were prepared in deionized water as stock solutions (100 mM). A Hg^{2+} stock solution (100 mM) was prepared by dissolving $HgCl_2$ with 0.5% HNO_3. The DNA concentrations were quantified by 260 nm absorbance by a UV-1800 spectrophotometer (SHIMADZU, Japan) with the extinction coefficients calculated using online IDT OligoAnalyzer 3.1 (http://www.idtdna.com/calc/analyzer#). NMM was purchased from Porphyrin Products. Its concentration was measured by a UV-Vis spectrometer using λ= 379 nm, with an assumption of an extinction coefficient of 1.45×10^5 $M^{-1}cm^{-1}$.

2. Fluorescent Measurements

Steady-state fluorescence spectra were measured using a fluorescence spectrophotometer F-7000 (Hitachi) with a slit of 5 nm for excitation (399 nm) and a slit of 10 nm for emission (570 to 700 nm). The scan speed and response time were set to 60 nm/min and 0.1 s respectively. The 610 nm emission of NMM DNA was

used for curve plotting.

3. UV-Vis spectra Measurements

All UV-Vis spectra were carried out using a Shimadzu UV-1800 spectrometer at ambient temperature. The 421 and 743 nm absorbance was used for curve plotting.

4. Optimization of conditions

a. Concentrations of ABTS and H_2O_2

200 nM HTD1 DNA and 250 nM 'breaker' DNA was buffered with pH 7.4 MOPS buffer (10 mM MOPS, 150 mM $NaNO_3$). The mixture was put into 37°C water bath for 30 min. 10 mM KCl and 1 μM hemin was added subsequently. The reaction was put Into 37°C water bath for 1 h. Different concentrations of ABTS (0.8, 1.6, 3.2, 5, 6, and 8 mM) or H_2O_2 (0.5, 1, 1.5 , 2, 2.5, 3, 5 and 8 mM) were supplemented separately. All samples were put in ambient temperature for 15 min before being scanned from 380 nm-800 nm using a UV-Vis spectrometer.

b. Concentrations of NMM and K^+

200 nM HTD1 DNA and 250 nM 'breaker' DNA was buffered with pH 7.4 MOPS buffer (10 mM MOPS, 150 mM $NaNO_3$). The mixture was put into 37°C water bath for 30 min. 10 mM KCl and 5 μM NMM was added where necessary subsequently. The reaction was put Into 37°C water bath for 20 min, cooled down to room temperature before measurement. Different concentrations of K^+ (0, 2.5, 5, 10, 15, 20 and 30 mM) or NMM (0.2, 0.5, 1 ,2, 3.5, 5, 8 μM) where necessary were supplemented separately. All samples were put in ambient temperature for 15 min before being scanned using a fluorescence spectrometer.

c. Incubation temperature

The influences of temperature was tested as follows: 200 nM HTD1 DNA and 250 nM 'breaker' DNA was put in pH 7.4 MOPS buffer (10 mM MOPS, 150 mM $NaNO_3$). 500 nM Hg^{2+} was used to test the system. 1 μM hemin or 5 μM was added where necessary subsequently. The mixture were incubated at 20, 25, 37, 50, 60°C for 30 min separately. . Finally, all the samples were measured by either a fluorescence spectrometer or a UV-Vis spectrometer.

5. The utility of the bio-sensor for detecting Hg^{2+}

For FL measurements, 200 nM HTD1 and 250 nM 'breaker' DNA was added in pH=7.4 MOPS buffer (10 mM MOPS and 150 mM $NaNO_3$). Different concentrations of Hg^{2+} were added afterwards, all samples were put into 37°C water bath for 30 min. 5 μM NMM into each sample was added and then put into 37°C water bath for 20 min. All samples were recorded subsequently by a fluorometer. For CL measurements, 10 mM KCl , 1 μM hemin were added and all sample were put into 37°C water bath for 1 h. Then 3.2 mM ABTS 2.5 mM H_2O_2 were subsequently added. All samples were put at room temperature for 15 min before CL measurements.

6. Metal ions selectivity

The selectivity for Hg^{2+} is evaluated over a variety of environmentally relevant metal ions, including Al^{3+}, Ca^{2+}, K^{+}, Li^{+}, Mn^{2+}, Ni^{2+}, Zn^{2+} Pb^{2+}, Cd^{2+}. The concentrations of Hg^{2+} and other metal ions used were 100 μM and 500 μM respectively. The HTD1 and 'breaker' DNA used were 200 and 250 nM respectively. For CL measurements, all sample were put in pH 7.4 MOPS buffer (10 mM MOPS and 150 mM $NaNO_3$) at first and incubated at 37°C for 15 min. Then all samples were supplemented with 10 mM KCl, 1 μM hemin and put at 37°C for 1 h. Then 3.2 mM ABTS and 2.5 mM H_2O_2 were added. After being put at room temperature for 15 min, all samples were measured by UV-Vis spectrometer from 380 nm-800 nm. For FL measurements, all samples were added 5 μM NMM, put 37°C water bath for 20 min and cooled down to room temperature. All samples were recorded subsequently by a fluorometer. FL and CL against different metal ions species was plotted. Note: FL and CL was calculated by subtraction of the FL or CL in the presence of metal ions from the control.

7. Analysis of Hg^{2+} in real samples

Four water samples were collected from tap water from Tianjin University of Science and Technology, Tianjin, China. The tap water samples were spiked with Hg^{2+} of a series of concentrations (5, 50, 100, 200 and 400 nM). The mean value, standard deviation and recovery of each sample were calculated accordingly.

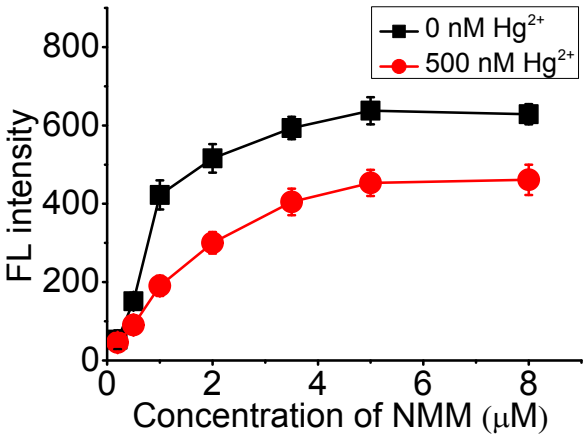

Fig. S1. The optimisation of NMM concentrations of the DNAzyme-based bio-sensor.

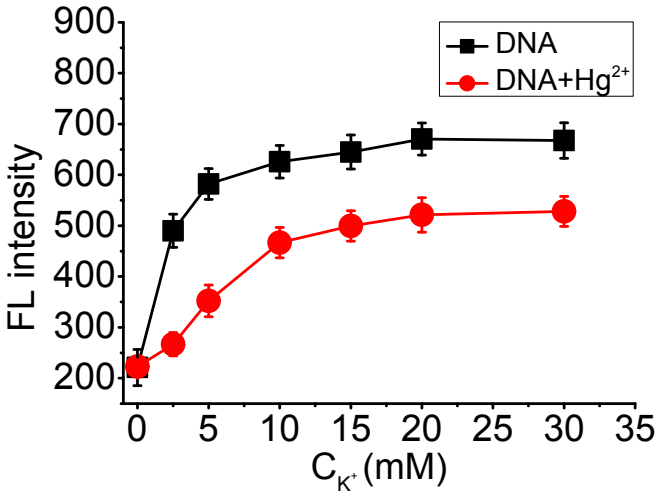

Fig. S2. The optimisation of potassium ion concentrations of the DNAzyme-based bio-sensor.

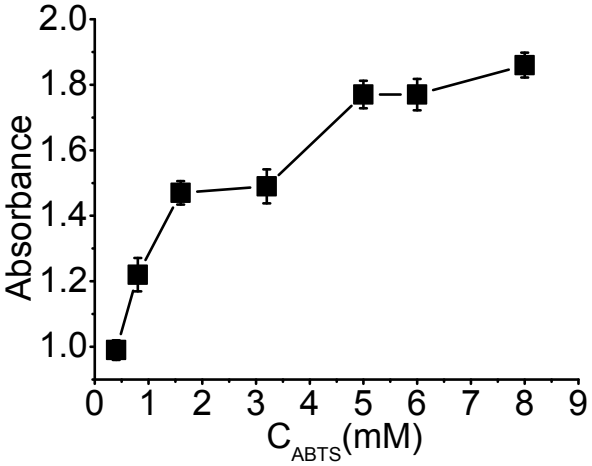

Fig. S3 .The optimisation of ABTS concentrations of the DNAzyme-based bio-sensor.

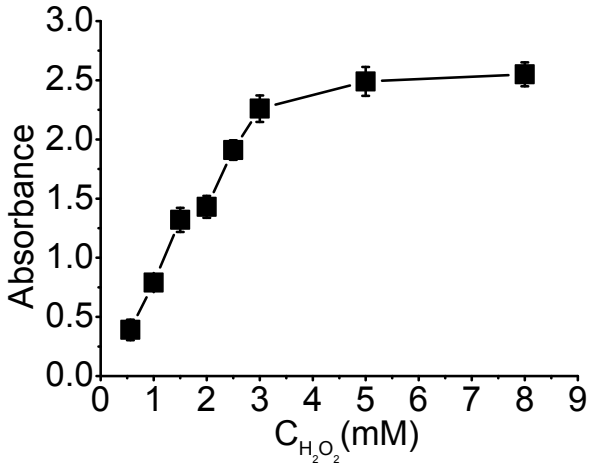

Fig. S4. The optimisation of H_2O_2 concentrations of the DNAzyme-based bio-sensor.

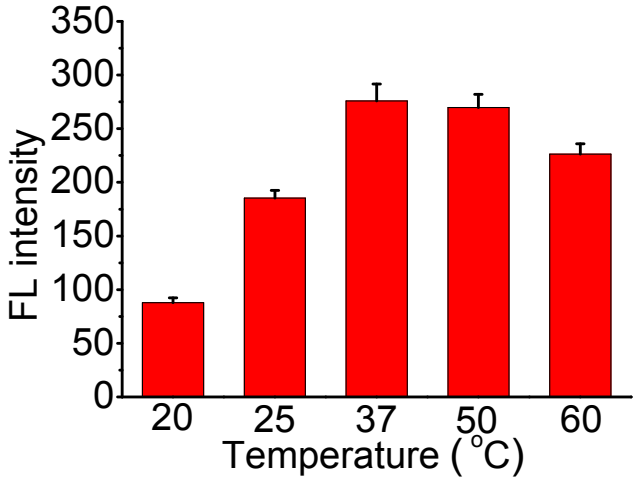

Fig. S5. The optimisation of temperatures of the bio-sensor for FL detection.

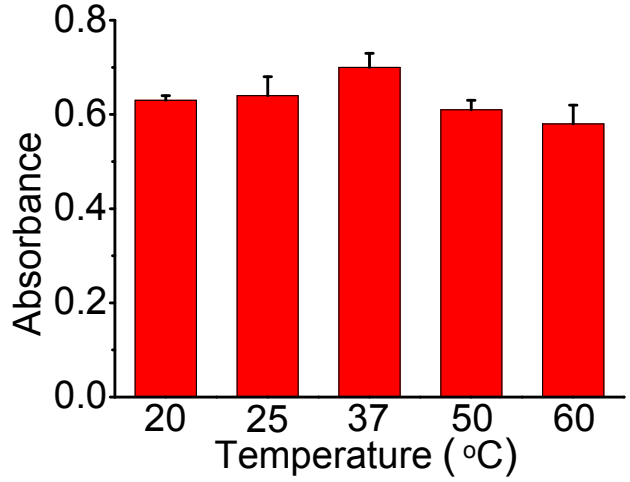

Fig. S6. The optimisation of temperature of the bio-sensor for CL detection.

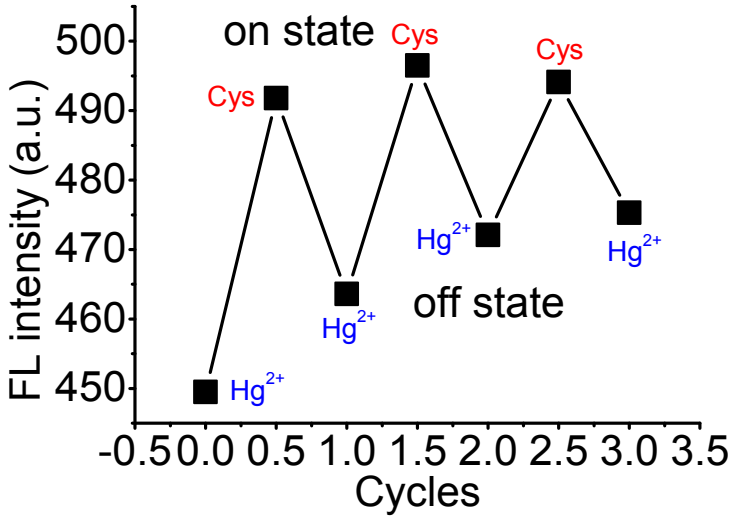

Fig. S7. Reversible FL changes of the bio-sensor with cyclic treatment of Hg^{2+} (500 nM) and cysteine (1 µM).

	CL meaurements			FL meaurements		
	Hg^{2+} added	Hg^{2+} measured Mean[a] ± SD	Recovery (%)	Hg^{2+} added	Hg^{2+} measured Mean[a] ± SD	Recovery (%)
Sample 1	10	10.4 ± 0.24	104.5	10	10.6 ± 0.11	106.0
Sample 2	50	57 ± 0.71	114.0	50	49.5 ± 0.22	99.0
Sample 3	100	95.5 ± 1.86	95.5	100	95.4 ± 0.59	95.4
Sample 4	200	205 ± 5.7	102.3	200	197.4 ± 2.15	98.7
Sample 5	400	410 ± 22.5	102.5	400	401.7 ± 16.3	100.4

a: Mean obtained from three separate measurements

Tab. S1. Detection results of mercury (II) ion spiked in tap water.

Method	Probes	Target	Linear Range	(Limit of Detection) LOD	Real sample	Ref.
HPLC/ICP-MS	—	Hg^{2+}	0-100 nM	1.2 nM	human blood	1
		Hg^{2+}	0-250 nM	4 nM	fish	2
		Hg^{2+}	0.1 nM-10 nM	0.03 nM	hair and fish	3
Fluorescence	FAM-DNA-DABCYL molecular beacon	Hg^{2+}	20-500 nM	10 nM	water and fish	4
	FAM-thymidine-terminated DNA molecular beacon	Hg^{2+}	37-92 nM	3.3 nM	—	5
	molecular beacon, hoechst dye	Hg^{2+}	0-7 μM	5 nM	urine, serum	6
	CWQ-11 dye and T_{33}-containing DNA	Hg^{2+}	0-100 nM	4 nM	tap water lake water	7
	CN-vinyl = (Z)-2-(4-nitrophenyl)-3-(4-(vinyloxy)phenyl) acrylonitrile dye	Hg^{2+}	0-50 μM	37 nM	tap water lake water	8
	DNA stablised nanocluster	Hg^{2+}	30-16000 nM	30 nM	lake water	9
	DNA stablised nanocluster	Hg^{2+}	5-1500 nM	5 nM	—	10
	G-quadruplex-hemin DNAzyme	Hg^{2+}	5-400 nM	19 nM	tap water river water	11
	DNAzyme	Hg^{2+}	1–20 nM	0.2 nM	river water	12
	HEX and BHQ1labeled DNA 3WJ	Hg^{2+}	0-500 nM	3 nM	tap water	13
	NMM binding G-quadruplex-based DNAzyme	**Hg^{2+}**	**5-500 nM**	**5 nM**	**tap water**	**This work**
Colorimetric	DNA, nanoparticle	Hg^{2+}	100 nM-2000 nM	100 nM	—	14
	DNA, nanoparticle	Hg^{2+}	0-600 nM	30 nM	drinking water	15
	DNA, nanoparticle	Hg^{2+}	0-5 μM	0.5 μM	—	16
	DNA, nanoparticle	Hg^{2+}	0.5-5 μM	250 nM	—	17
	G-quadruplex DNAzyme	Hg^{2+}	5-400 nM	19 nM	spring/river/tap/ spring water	18
	G-Quadruplex-based DNAzymes	Hg^{2+}	250-1250 nM	250 nM	lake water	19
	G-quadruplex-based DNAzyme	**Hg^{2}**	**10-500 nM**	**10 nM**	**tap water**	**This work**
Naked eyes	G-quadruplex-based DNAzyme	Hg^{2+}	----	~100 nM	----	20
CD	DNA, nanorod	Hg^{2+}	0.2-50 nM	0.1 nM	tap water	21
Electrochemical	DNA-RGO, $[Ru(NH_3)_6]^{3+}$	Hg^{2+}	8-100 nM	5 nM	lake water	22
	mercaptoacetic acid modified gold microwire electrode	Hg^{2+}	0-37 nM	1 nM	sea water	23
	DNA, $Ru(bpy)^2(dppz)^{2+}$	Hg^{2+}	0.1-10 nM	20 pM	tap water lake water	24

Tab. S2. a summary of our approach with others for the detection of Hg^{2+}.

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