Electronic Supporting Information

A reversible metal ions fueled DNA molecular device for "turn-on and -off" fluorescence detection of mercury ions (II) and biothiols respectively with high selectivity and sensitivity

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Experimental Section

1. Materials and sample preparations

All DNA were chemically synthesised and purified by Generay Biotech Co., Ltd (China). Tris (hydroxymethyl)-aminomethane (Tris), glutathione and 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 3WJ solution was heated at 95°C for 5 min (with a molar ratio of 1:1:1 for ssDNA) and gradually cooled down to room temperature overnight. 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#).

2. Fluorescent Measurements

Steady-state fluorescence spectra of HEX-labeled DNA were measured using a fluorescence spectrophotometer F-7000 (Hitachi) with a slit of 5 nm for excitation (535 nm) and a slit of 10 nm for emission. The scan speed and response time were set

to 30 nm/min and 0.1 s respectively. The working solution for fluorescence assay was in the buffer containing 3.3 mM Tris-HCl buffer (pH 7.4), 3.3 mM Magnesium chloride and 85 mM sodium chloride. All samples were incubated at 37°C in water bath for 40 min before measurement. The 555 nm emission of HEX-labeled DNA was used for curve plotting. For the real time fluorescent spectra, the excitation and emission were set to 535 nm (5 nm slit) and 555 nm (10 nm slit).

3. CD

The circular dichroism (CD) measurements were performed on a MOS-450 CD spectrometer (Bio-Logic, France) at ambient temperature. The scan step was set to 0.125 nm.The concentration of DNA samples used for measurement was 10 μM, Hg^{2+} was added where necessary. The ellipticity was recorded in the wavelength from 190-360 nm. All CD measurements were conducted at ambient temperature.

4. Optimization of conditions

a. Effect of incubate time

The working solution of the assay was 5 nM DNA 3WJ buffered with 3.3 mM Tris-HCl buffer (pH 7.4), 3.3 mM magnesium chloride and 85 mM sodium chloride. The concentration of Hg^{2+} used for measurement was 500 μM. Samples were incubated at 37°C in water bath. While samples were measured at 0 min, 5 min, 10 min, 20 min, 30 min, 40 min, 60 min, 90 min respectively.

b. Effect of temperature

The influences of temperature was tested as follows: the system DNA 3WJ was 5 nM buffered with 3.3 mM Tris-HCl buffer (pH 7.4), 3.3 mM magnesium chloride and 85 mM sodium chloride in the presence of 500 μM Hg^{2+}. The mixture were incubated at 25°C, 37°C, 50°C, 65°C for 40 min separately. Finally, all the samples were measured by fluorescence spectroscopy.

5. The utility of the molecular device for sensing Hg^{2+}, glutathione and cysteine.

In order to sense Hg^{2+}, 10 nM 3WJ was added different concentrations of Hg^{2+} (from 5 to 1500 nM). Each sample was incubated at 37°C for 40 min and then cooled down to ambient temperature for fluorescence measurement. For sensing glutathione and cysteine, 10 nM 3WJ was mixed with 500 nM Hg^{2+}, then different concentrations of

glutathione or cysteine (from 20 to 1000 nM) was incubated at 37°C for 40 min and then cooled down to ambient temperature for fluorescence measurement.

6. Metal ions selectivity

The selectivity for Hg^{2+} is evaluated over a variety of environmentally relevant metal ions, including Al^{3+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, K^{+}, Li^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} Pb^{2+} and Cd^{2+}. The concentrations of Hg^{2+} and other metal ions used were 100 μM and 500 μM respectively. The 3WJ used was 5 nM. FL against different metal ions species was plotted. Note: FL was calculated by subtraction of the fluorescence of 3WJ in the presence of metal ions from the fluorescence of 3WJ in the absence of metal ions.

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

Five 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, 10, 30, 300 and 400 nM). The mean value, standard deviation and recovery of each sample were calculated accordingly.

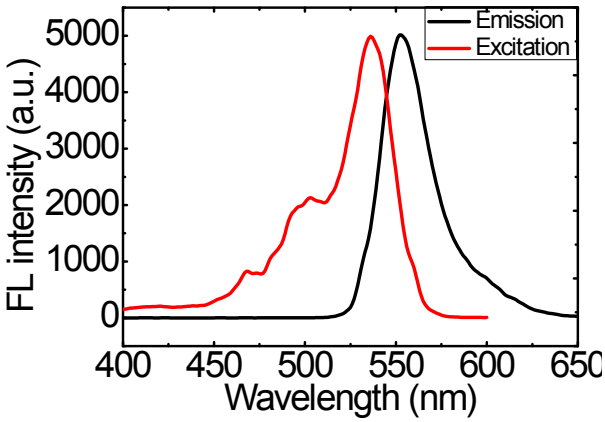

Fig S1. Fluorescence excitation and emission spectra of HEX-labeled DNA.

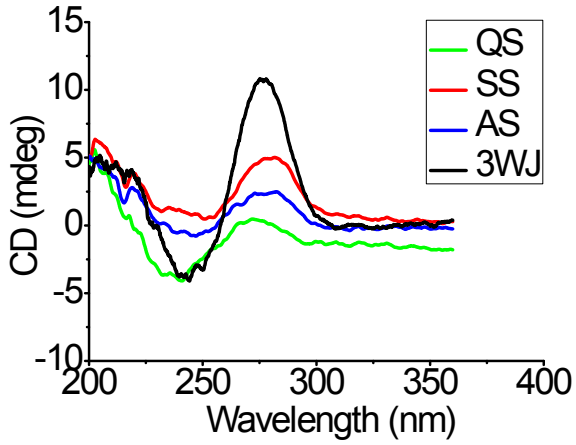

Fig. S2. CD spectra for three single-stranded oligonucleotides (OS, SS, AS) composed of 3WJ and 3WJ itself.

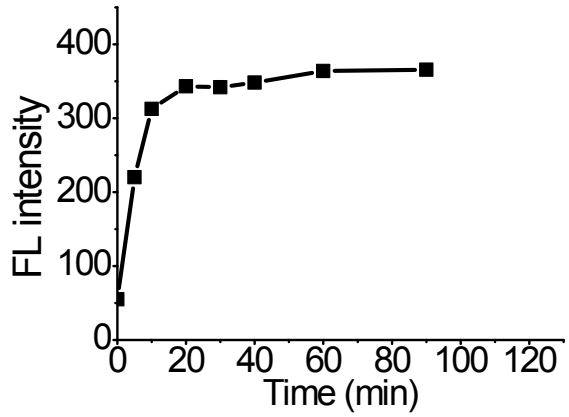

Fig. S3. The optimisation of incubation time of 3WJ in the presence of Hg^{2+}.

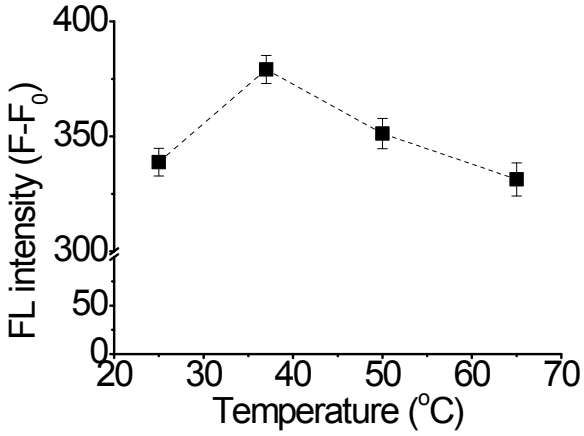

Fig. S4. The optimisation of incubation time of 3WJ in the presence of Hg^{2+}. Triplicate independent experiments have been performed for each temperature point and error bars are give accordingly.

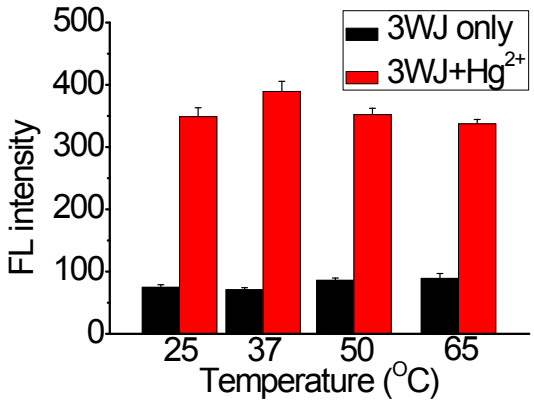

Fig. S5. Fluorescence intensity for 3WJ in the presence and absence of Hg^{2+} at different temperatures. Triplicate independent experiments have been performed for each temperature point and error bars are give accordingly.

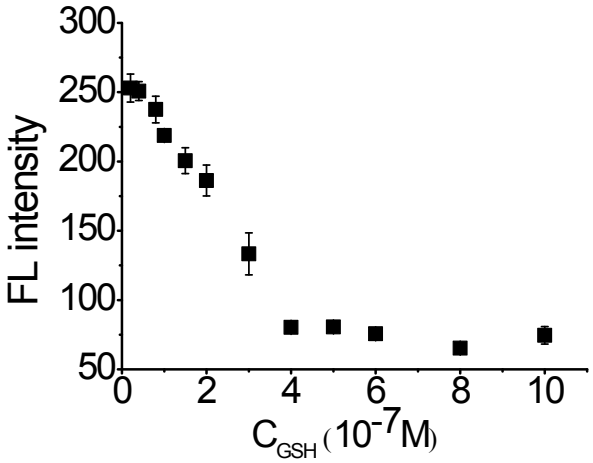

Fig. S6. Fluorescence intensity *vs*. concentration of glutathione (20 to 1000 nM)

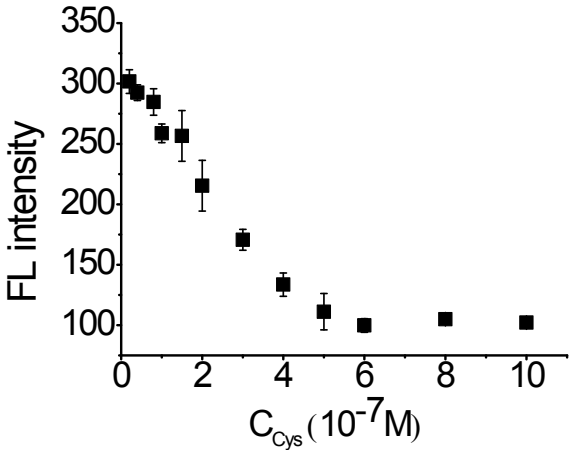

Fig. S7. Fluorescence intensity *vs*. concentration of cysteine (20 to 1000 nM).

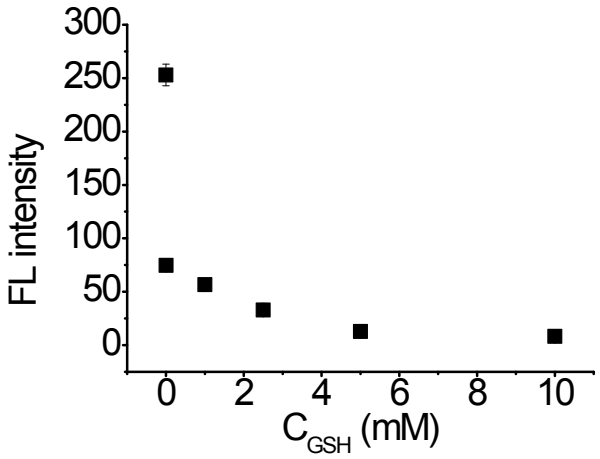

Fig. S8. Fluorescence intensity *vs.* concentration of cysteine (up to 10 mM).

	Hg^{2+} added (nM)	Measured (nM) mean[a] ± SD	Recovery (%)
Sample 1	5	5.15 ± 0.28	103.0
Sample 2	10	10.2 ± 0.50	102.3
Sample 3	30	30.8 ± 1.47	102.8
Sample 4	300	314.9 ± 5.96	104.9
Sample 5	400	389.5 ± 11.90	97.4

a: Mean obtained from four 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 nM-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**	**This Work**
Colorimetric	DNA, nanoparticle	Hg^{2+}	100 nM-2000 nM	100 nM	—	13
	DNA, nanoparticle	Hg^{2+}	0-600 nM	30 nM	drinking water	14
	DNA, nanoparticle	Hg^{2+}	0-5 μM	0.5 μM	—	15
	DNA, nanoparticle	Hg^{2+}	0.5-5.0 μM	250 nM	—	16
CD	DNA, nanorod	Hg^{2+}	0.2-50 nM	0.1 nM	tap water	17
Electrochemical	DNA-RGO, $[Ru(NH_3)_6]^{3+}$	Hg^{2+}	8-100 nM	5 nM	lake water	18
	DNA, $Ru(bpy)^2(dppz)^{2+}$	Hg^{2+}	0.1-10 nM	20 pM	tap water lake water	19

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

References:

1. J. L. Rodrigues, S. S. de Souza, V. C. de Oliveira Souza and F. Barbosa, Jr., *Talanta*, 2010, **80**, 1158-1163.
2. M. M. Santoyo, J. A. Figueroa, K. Wrobel and K. Wrobel, *Talanta*, 2009, **79**, 706-711.
3. J. Chen, H. Chen, X. Jin and H. Chen, *Talanta*, 2009, **77**, 1381-1387.
4. Y. W. Lin and H. T. Chang, *Analyst*, 2011, **136**, 3323-3328.
5. P. Hou, Y. Long, J. Zhao, J. Wang and F. Zhou, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2012, **86**, 76-79.
6. M. Zhang, H. N. Le, X. Q. Jiang and B. C. Ye, *Chem. Commun.*, 2013, **49**, 2133-2135.
7. S. Li, H. Deng, W. Cao, C. Zhang, S. Jin, X. Xue, J. Zhang, F. Li, G. Zou and X. J. Liang, *Analyst*, 2014, **139**, 3369-3372.
8. A. Wang, Y. Yang, F. Yu, L. Xue, B. Hu, W. Fan and Y. Dong, *Talanta*, 2015, **132**, 864-870.

9. Y. Zhang, H. Jiang and X. Wang, *Anal. Chim. Acta*, 2015, **870**, 1-7.
10. W. Guo, J. Yuan and E. Wang, *Chem. Commun.*, 2009, 3395-3397.
11. D. M. Kong, N. Wang, X. X. Guo and H. X. Shen, *Analyst*, 2010, **135**, 545-549.
12. L. Qi, Y. Zhao, H. Yuan, K. Bai, Y. Zhao, F. Chen, Y. Dong and Y. Wu, *Analyst*, 2012, **137**, 2799-2805.
13. J. S. Lee, M. S. Han and C. A. Mirkin, *Angew. Chem. Int. Ed.*, 2007, **46**, 4093-4096.
14. D. Liu, W. Qu, W. Chen, W. Zhang, Z. Wang and X. Jiang, *Anal. Chem*., 2010, **82**, 9606-9610.
15. X. Xu, J. Wang, K. Jiao and X. Yang, *Biosens. Bioelectron*., 2009, **24**, 3153-3158.
16. C. W. Liu, Y. T. Hsieh, C. C. Huang, Z. H. Lin and H. T. Chang, *Chem. Commun.*, 2008, 2242-2244.
17. Y. Zhu, L. Xu, W. Ma, Z. Xu, H. Kuang, L. Wang and C. Xu, *Chem. commun.*, 2012, **48**, 11889-11891.
18. Y. Zhang, H. Zhao, Z. Wu, Y. Xue, X. Zhang, Y. He, X. Li and Z. Yuan, *Biosens. Bioelectron*., 2013, **48**, 180-187.
19. B. Zhang and L. H. Guo, *Biosens. Bioelectron*., 2012, **37**, 112-115.