Supporting Information

Development of split G-quadruplex and DAPI-based fluorescent probe for Hg(II) and Pb(II) ions detection

Youyang Xu¹, Yuxin Liu¹, Xiangxiang Li, Yule Cai, Zihan Gao, Jieqiong

Qiu*

¹ These authors contributed equally to the work and can be considered co-

first authors.

*Corresponding author:

Jieqiong Qiu

Zhejiang Key Laboratory of Silkworm Bioreactor and Biomedicine,

College of Life Sciences and Medicine, Zhejiang Sci-Tech University,

Hangzhou 310018, People's Republic of China

Email: <u>qiujieqiongqjq@163.com;</u> <u>qiujieqiong@zstu.edu.cn</u>

Tel: 0571-86843192

Table of contents

Table S1. The sequences of oligonucleotides involved in this study.

Fig. S1 Fluorescence emission spectra of ODN-7, ODN-8, ODN-9 and

ODN-10 in the presence or absence of Hg(II) and Pb(II).

Fig. S2 Selectivity of the ODN-7 probe for Hg(II) detection.

Fig. S3 Fluorescence emission spectra of ODN-7 in the presence of 1200 nM Hg(II) and mixed with 50 μ M Pb(II) or 5 μ M Ag(I).

Fig. S4 Fluorescence intensity changes (F-F0) of ODN-7 in the mixed buffer (pH 8.5) with different concentration of Na(I).

Fig. S5 Fluorescence emission spectra of ODN-7 with Pb(II) concentrations from 0 to 50 μ M in 25 mM Tris-HCl (200 mM NaCl, pH 8.5).

Fig. S6 Selectivity of ODN-7 for the detection of Pb(II) in 25 mM Tris-HCl (200 mM NaCl, pH 8.5).

Table 2S Average recovery rates of Hg(II) detection in tap water (n=3). Table 3S Average recovery rates of Pb(II) detection in tap water (n=3).

Name	Sequence
ODN-1	5'-TTTGCGTCCCCTCGCTTT-3'
ODN-2	5'-GTTTGCGTCCCCTCGCTTTC-3'
ODN-3	5'-CGTTTGCGTCCCCTCGCTTTCG-3'
ODN-4	5'-GGGTGGGTTTGCGTCCCCTCGCTTTGGGTGGG-3'
ODN-5	5'-GGGTGGGGTTTGCGTCCCCTCGCTTTCGGGTGGG-3'
ODN-6	5'-GGGTGGGCGTTTGCGTCCCCTCGCTTTCGGGGTGGG-3'
ODN-7	5'-GGGTGGGCGTTTGCGTCCCCTCGCTTTCGGGTGGG-3'
ODN-8	5'-GGGTGGGAGTTTGCGTCCCCTCGCTTTCGGGTGGG-3'
ODN-9	5'-GGGTGGGTGTTTGCGTCCCCTCGCTTTCGGGTGGG-3'
ODN-10	5'-GGGTGGGGGTTTGCGTCCCCTCGCTTTCGGGTGGG-3'
ODN-7 ODN-8 ODN-9 ODN-10	5'-GGGTGGGAGTTTGCGTCCCCTCGCTTTCGGGTGGG-3' 5'-GGGTGGGTGTTTGCGTCCCCTCGCTTTCGGGTGGG-3' 5'-GGGTGGGGGGTTTGCGTCCCCTCGCTTTCGGGTGGG-3'

Table S1. The sequences of oligonucleotides involved in this study.



Fig. S1 Fluorescence emission spectra of ODN-7(a), ODN-8(b), ODN-9(c) and ODN-10(d) in the presence or absence of 1200 nM Hg(II) and 50 μ M Pb(II) by adding 125 nM DAPI at Ex =360 nm. The incubation time was 20 min at RT, and Tris-HCl (25 mM, 200 mM NaCl, pH 8.5) was used.



Fig. S2 (a) Selectivity of the ODN-7 probe for Hg(II) detection. Fluorescence intensity changes (F-F0) of the ODN-7 probe in the presence of metal ions (Hg(II), K(I), Mg(II), Al(III), Cu(II), Co(II), Fe(II), Mn(II), Fe(III), Sn(II), Ni(II), Ag(I)). (b) Fluorescence intensity changes (F-F0) of the ODN-7 probe in the presence of Hg(II) mixed with other metal ions (K(I), Mg(II), Al(III), Cu(II), Co(II), Fe(II), Mn(II), Sn(II), Ni(II), Ag(I)). (b) Fluorescence intensity changes (F-F0) of the ODN-7 probe in the presence of Hg(II) mixed with other metal ions (K(I), Mg(II), Al(III), Cu(II), Co(II), Fe(II), Mn(II), Fe(III), Sn(II), Ni(II), Ag(I)). 1200 nM Hg(II), 10 μM Ag(I) and 12 μM other metal ions were used, 125 nM DAPI, 100 nM ODN-7 in 25 mM Tris-HAc (200 mM NaAc) were used for each reactions.



Fig. S3 Fluorescence emission spectra of ODN-7 in the presence of 1200 nM Hg(II) and mixed with 50 μ M Pb(II) or 5 μ M Ag(I) by adding 125 nM DAPI at Ex = 360 nm in 25 mM Tris-HAc buffer solution (200 mM NaAc, pH 8.5) and 25 mM Tris-HCl buffer solution (200 mM NaCl, pH 8.5) in a volume ratio of 1:1(a), 3:2(b), and 3:1(c). The incubation time was 20 min at RT.



Fig. S4 Fluorescence intensity changes (F-F0) of ODN-7 in the mixed buffer (pH 8.5) with different concentration of Na(I). Each reaction was performed using 1200 nM Hg(II), 125 nM DAPI and 100 nM ODN-7 in the mixed buffer, including 25 mM Tris-HAc (pH 8.5) with NaAc (0, 12.5, 50, 100, 200 mM) and 25 mM Tris-HCl (pH 8.5) with NaCl (0, 12.5, 50, 100, 200 mM) in a volume of 3:1.



Fig. S5 (a). Fluorescence emission spectra of ODN-7 with Pb(II) concentrations from 0 nM to 50 μ M in 25 mM Tris-HCl (pH 8.5) containing 200 mM NaCl buffer. The Pb(II) concentrations used in the experiment were: 0, 0.01, 0.1, 0.5, 5, 10, 15, 20, 30, 40, 45 and 50 μ M. (b). Linear response of relationship between the fluorescence change (F-F0) and the Pb(II) concentrations in the 4.57 nM-30 μ M_range. 1200 nM Hg(II), 125 nM DAPI, and 100 nM ODN-7 in Tris-HCl (25 mM, 200 mM NaCl, pH 8.5) buffer were used for each reaction.



Fig. S6 (a) Selectivity of ODN-7 for the detection of Pb(II). Fluorescence intensity changes (F-F0) of the ODN-7 probe by adding the metal ions (Hg(II), Mn(II), Ni(II), Ca(II), Cu(II), Fe(II), Al(III), Mg(II), Co(II), K(I), Ag(I)). (b) Fluorescence intensity changes (F-F0) of the ODN-7 probe by adding Hg(II) and other metal ions (Mn(II), Ni(II), Ca(II), Cu(II), Fe(II), Al(III), Mg(II), Co(II), K(I), Ag(I)). 1200 nM Hg(II), 50 μM Pb(II), and 50 μM other metal ions were used, 125 nM DAPI, 100 nM ODN-7 in Tris-HCl (25 mM, 200 mM NaCl, pH 8.5) were used for each reaction.

Sample	Detected ion	Hg(II) (nM)	Pb(II) (µM)	Repeats	Found (nM)	RSD (%)	Recovery (%)
1		50	/	1	52.7	2.542	99.4%
				2	50.0		
				3	46.5		
		200	/	1	207.1	2.755	103.9%
2	Hg(II)			2	211.5		
				3	204.8		
		400	/	1	401.8	5.715	102.4%
3				2	410.9		
				3	415.6		
4		50	0.5	1	53.5	2.501	100.3%
				2	49.5		
				3	47.5		
				1	188.4		
5	Hg(II)	200	5	2	205.0	7.182	99.2%
				3	201.8		
6		400	10	1	416.1	3.536	102.9%
				2	411.1		
				3	407.4		

Table 2S Average recovery rates of Hg(II) detection in tap water (n=3).

Sample	Detected ion	Hg(II) (nM)	Pb(II) (μM)	Repeats	Found (nM)	RSD (%)	Recovery (%)
1		/	0.5	1	498.6	1.738	97.4%
				2	483.4		
				3	478.8		
			1	1	1023.7	1.095	101.5%
2	Pb(II)	/		2	1022.2		
				3	999.4		
3		/	2	1	1924.8	1.110	97.0%
				2	1924.8		
				3	1970.5		
4	Pb(II)	50	0.5	1	507.8	2.695	98.6%
				2	475.8		
				3	497.1		
5		200	1	1	950.7	1.547	97.2%
				2	978.1		
				3	985.7		
6		400	2	1	2096.8	0.720	104.0%
				2	2081.6		
				3	2060.3		

Table 3S Average recovery rates of Pb(II) determination in tap water (n=3).