Supplementary Material

A novel sensitive DNAzyme- based optical fiber evanescent wave biosensor for rapid detection of Pb^{2+} in human serum

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§ These authors have equal contribution to this work.

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1. Experimental section

1.1 The optical fiber evanescent wave biosensor platform

The photo of OFEWB were shown Fig. S1.

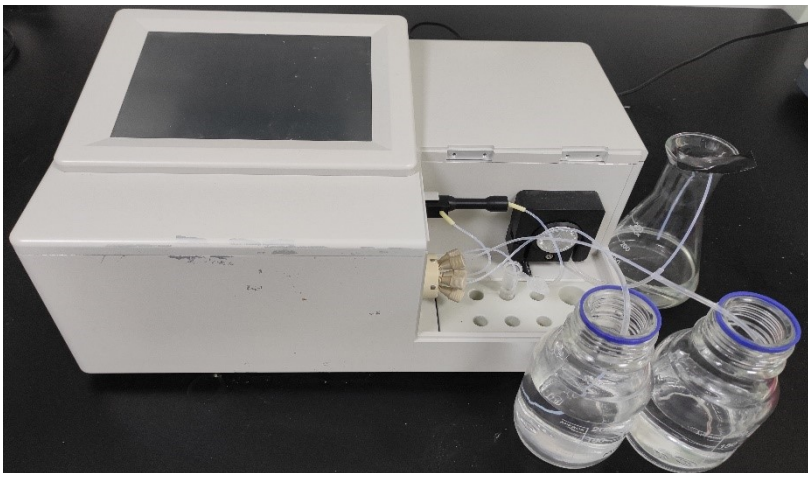

Fig. S1. The photo of the OFEWB

1.2 The preparation steps of combined tapered optical fiber

2.5 cm cladding of the optical fiber was first removed to form the sensing region, then the sensing region was etched by hydrofluoric acid (30%) for 3h to form a combined tapered configuration (600 μm cone waist diameter, 220 μm cone tip diameter, 0.37 cone angle).

1.3 Optimization of ionic strength

Table S1. Ionic strength under different $NaNO_3$ concentrations

$NaNO_3$ concentration	Ionic strength
50 mM	0.05 mol/kg
150 mM	0.15 mol/kg
250 mM	0.25 mol/kg
500 mM	0.50 mol/kg

The calculating formula of ionic strength is as follows:

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 \quad , \quad I = \frac{1}{2}[c_{(Na^+)} z_{(Na^+)}^{\ 2} + c_{(NO_3^-)} z_{(NO_3^-)}^{\ 2}] \quad (1)$$

2. Result and discussion

2.1 The Pb^{2+} detection mechanism

To prove the hybridization between GR-5S-Cy3 and GR-5E-BHQ2 could lead to the fluorescence quenched by the quencher based on the FRET effect. The signal intensity corresponding to the incubation time between GR-5S-Cy3 and GR-5E-BHQ2 were compared and the results were shown in Fig. S2. It can be seen from the Fig. S2 that the fluorescence could be effectively quenched even the incubation time was only 2 min. The fluorescence intensities during the whole test range were still lower than 18 a.u. which proved the feasibility of our principle.

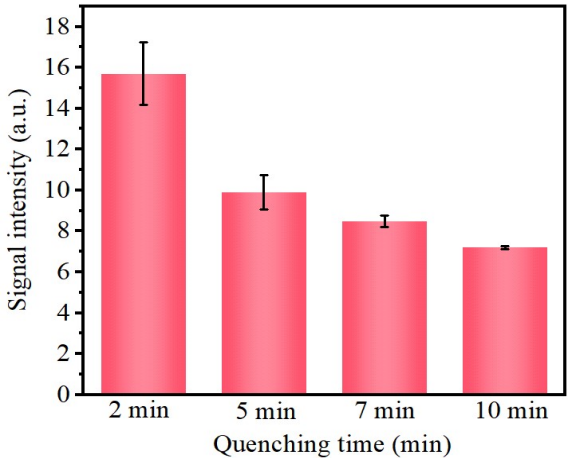

Fig. S2. The effect of quenching time on signal intensity; test conditions: 160 nM GR-5S-Cy3, 200 nM GR-5E-BHQ2 The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

To evaluate the DNA structure (single strand, duplex strand) and DNA length (full length substrate, the cleaved shorter substrate fragment) on the sensing sensitivity of the method, A GR-5 DNAzyme sequence without BHQ2 (denoted as GR-5E), a substrate DNA sequence without Cy3

(denoted as GR-5S) and a cleaved DNA sequence labelled with Cy3 (denoted as PD-GR-5S-Cy3) were designed, respectively.

Firstly, the effect of DNA structure on the Pb^{2+} detection using the OFEWB was evaluated. We fixed the concentration of GR-5S-Cy3, GR-5S-Cy3+GR-5E at 50 nM, 100 nM, 160 nM and then detected the corresponding signal intensity using the OFEWB. The results shown in Fig S2 indicated the signal intensity of GR-5S-Cy3 and GR-5S-Cy3+GR-5E were almost same at the corresponding concentration, indicating the effect of DNA structure has less effect on our proposed method.

Secondly, the effect of DNA length on Pb^{2+} detection using the OFEWB was evaluated. Similarly, we also fixed the concentration of PD-GR-5S-Cy3, PD-GR-5S-Cy3+GR-5S +GR-5E at 50 nM, 100 nM, 160 nM and then detected the corresponding signal intensity using the OFEWB. According to the results in Fig S3, it could be observed the intensity of PD-GR-5S-Cy3 and PD-GR-5S-Cy3+GR-5S +GR-5E were almost same at the corresponding concentration, indicating the DNA length has less effect on our proposed method.

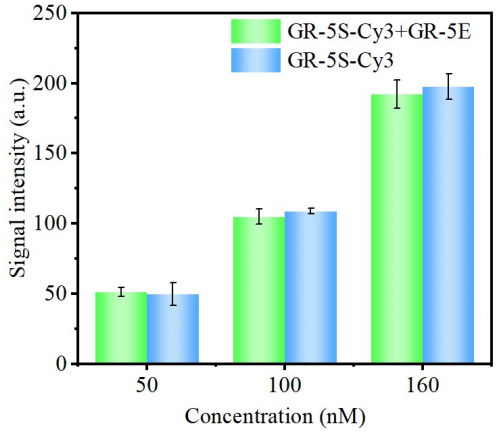

Fig. S3 The effects of the DNA structure on Pb^{2+} detection using OFEWB. The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

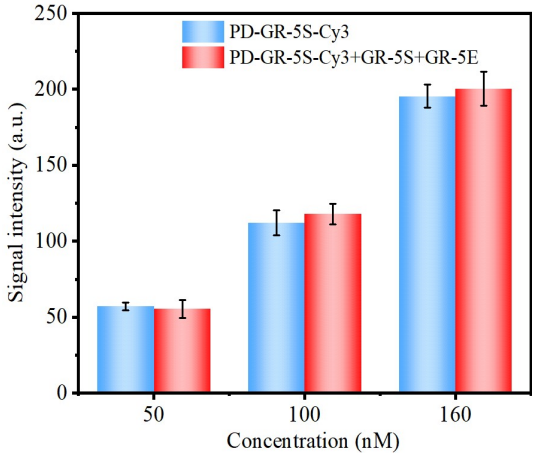

Fig. S4 The effects of the DNA length on Pb^{2+} detection using OFEWB. The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

2.2 Measurement of Pb^{2+} in different water samples

Table S2 Detection results of Pb^{2+} spiked in water samples

Samples	Spiked/nM	Detected/nM	Recovery/%	RSD/%
Tap water	20	16.40	82.00	2.91
	50	41.43	82.86	1.96
	80	65.50	81.88	2.92
Bottle water	20	17.16	85.79	5.28
	50	47.01	94.02	3.95
	80	95.62	119.53	1.01
Underground water	20	19.54	97.69	3.04
	50	54.35	108.69	3.63
	80	82.11	105.64	2.87

2.3 Detection of Pb^{2+} in human serum

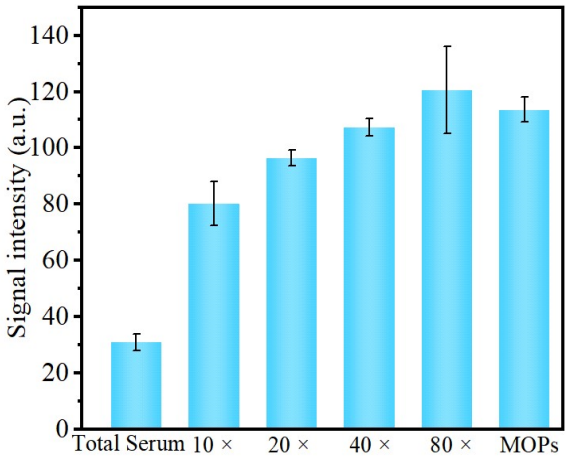

Fig. S5 Matrix effect of serum on the Pb^{2+} detection using the OFEWB; Testing condition: 30°C, pH=7, 0.15 mol/kg ionic strength, 120 nM Pb^{2+}, 160 nM GR-5S, 200 nM GR-5E. The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

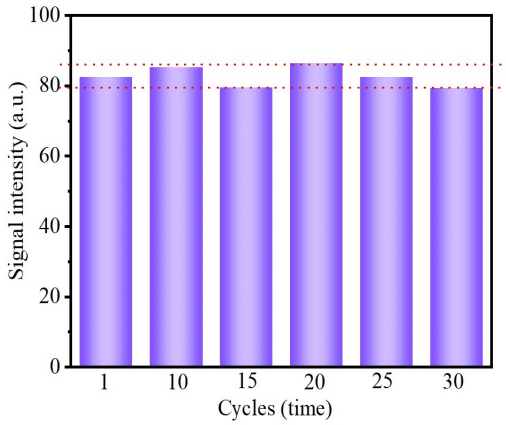

Fig. S6 The reusability of the OFEWB for the detection of Pb^{2+} in diluted serum. Testing condition: 30°C, pH=7, 0.15 mol/kg ionic strength, 160 nM GR-5S-Cy3, 200 nM GR-5E-BHQ2, 80 nM Pb^{2+}. The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

Table S3 Performance comparation between OFEWB and fluorescence spectrometer

Equipment	DNA amount	LOD
OFEWB	300 μL	9.34 nM
Fluorescence spectrometer	30 μL	77.21 nM