Supplementary Information:

A sensitive biosensor with a DNAzyme for lead (II) detection based on fluorescence turn-on

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Supplementary Figures



Fig. S1. The fluorescence emission spectrum of 17E-17S-FAM and the absorption spectrum of 17E-17S/EB complexes. 17S, 5'-ACT CAC TAT rAG GAA GAG ATG. The fluorescence emission spectra were excited at 490 nm. The spectral overlap was used to calculate the Förster distance (R_0). To simply predict the R_0 and FRET efficiency (E), we used the classical equations given in the literature.¹

$$R_0 = 0.211 (\kappa^2 n^{-4} Q_D J(\lambda))^{1/6} \qquad (\text{in Å}) \qquad (1)$$

$$E = 1 - \frac{F_{DA}}{F_D} \tag{2}$$

 $J(\lambda)$ is the overlap integral between the donor emission and the acceptor absorption. The quantum yield of the donor was taken as 0.95. The κ^2 value was assumed to be 2/3. The refractive index (n) was taken as 1.4. These assumed values led to an R₀ of 45.6 Å. F_{DA} and F_D mean the relative fluorescence intensity of the donor (17E-17S-FAM) in the presence (F_{DA}) and absence (F_D) of acceptor (EB). According to the measured values (F_{DA}: 518; F_D: 6868), the FRET efficiency can be roughly evaluated as 0.91.



Fig. S2. (a) Fluorescence spectra of 17E/17S-FAM after addition of different concentration of EB. (b) Ratio of fluorescence (I_{585}/I_{520}) as a function of EB concentrations. [17S-FAM] = 2 x 10⁻⁸ M, [17E] = 2 x 10⁻⁸ M, [EB] = 1~5 x 10⁻⁶ M. The error bars represent the standard deviations of three parallel measurements.The excitation wavelength is 490 nm.



Fig. S3. Increased ratio of FAM fluorescence intensity at 520 nm as a function of Pb²⁺ concentration. [17S-FAM] = 2×10^{-8} M, [17E] = 2×10^{-8} M, [EB] = 4×10^{-6} M. The error bars represent the standard deviations of three parallel measurements. The excitation wavelength is 490 nm.



Fig. S4. Application of the biosensor to the analysis of Pb^{2+} in river water samples. [17S-FAM] = 2 x 10⁻⁸ M, [17E] = 2 x 10⁻⁸ M, [EB] = 4 x 10⁻⁶ M. The excitation wavelength is 490 nm.

River water	Pb ²⁺ spiked (nM)	Pb ²⁺ recovered (nM)	Recovery (%)
1	10.0	$10.7^{a} \pm 1.3\%^{b}$	107.0
2	30.0	$30.3^a \pm 3.8\%^b$	101.1
3	80.0	$82.4^{a} \pm 1.7\%^{b}$	103.1

Table S1. Recovery Experiments of Pb²⁺ in River Water Samples

^a Mean values of three determinations. ^b Relative standard derivation.

Reference:

¹ J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3nd ed., Kluwer cademic/ Plenum Publishers: New York, 2006.