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.\(^1\)

\[
R_0 = 0.211(\kappa^2 n^{-4} Q_D J(\lambda))^{1/6} \quad \text{(in Å)} \quad (1)
\]
\[ E = 1 - \frac{F_{DA}}{F_D} \]  

(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 \( \kappa^2 \) value was assumed to be 2/3. The refractive index \( (n) \) was taken as 1.4. These assumed values led to an \( R_0 \) 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. \([17S-FAM] = 2 \times 10^{-8} \text{ M}, [17E] = 2 \times 10^{-8} \text{ M}, [EB] = 1-5 \times 10^{-6} \text{ M}. \)

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 Pb$^{2+}$ concentration. [17S-FAM] = 2 x 10$^{-8}$ M, [17E] = 2 x 10$^{-8}$ M, [EB] = 4 x 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.
Table S1. Recovery Experiments of Pb\(^{2+}\) in River Water Samples

<table>
<thead>
<tr>
<th>River water</th>
<th>Pb(^{2+}) spiked (nM)</th>
<th>Pb(^{2+}) recovered (nM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.0</td>
<td>10.7(^a) ± 1.3(^b)</td>
<td>107.0</td>
</tr>
<tr>
<td>2</td>
<td>30.0</td>
<td>30.3(^a) ± 3.8(^b)</td>
<td>101.1</td>
</tr>
<tr>
<td>3</td>
<td>80.0</td>
<td>82.4(^a) ± 1.7(^b)</td>
<td>103.1</td>
</tr>
</tbody>
</table>

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

Reference: