Electronic supplementary material

Exonuclease III-assisted substrate fragment recycling amplification strategy for ultrasensitive detection of uranyl by a multipurpose RCDzyme

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Experimental

Procedure for the control experiments

(1) The demonstration of the cleaved mechanism of DNAzyme by UO_2^{2+} using SGI

Into a 2-mL EP tube, a 7 μL of 5.0 μM S-DNA was mixed with 5 μL of 5.0 μM E-DNA in 10 μL of MES buffer solution (pH 5.5) including 3.0 M $NaNO_3$, and incubated at 95°C for 5 min, then cooled down to room temperature for 1 h. Thereafter, the different concentrations of UO_2^{2+} were added into DNAzyme solution prepared. The scission reaction was performed for 40 min at room temperature. Subsequently, a 50 μL of 5×SGI was added in this solution. The mixture was diluted to a final volume of 450 μL with water and incubated at room temperature for 15 min. Then, the fluorescence spectra were obtained by scanning from 500 to 570 nm at λ_{ex} = 497 nm. The spectral bandwidths of both the excitation and emission slits were set to 5 nm, respectively. The fluorescence intensity of the system was measured at λ_{em} = 524 nm, and represented as ΔF =

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$F - F_0$, here F and F_0 were the fluorescence intensities of the system with and without UO_2^{2+}, respectively.

(2) The demonstration of the cleaved mechanism of DNAzyme by UO_2^{2+} with UV–vis spectra

An aliquot of 50 μL E-DNA (25 μM) was mixed with 50 μL S-DNA (25 μM) in 43 μL of 1 M NaCl solution, followed by incubating in a 90 °C water bath for 3 min. After cooling to room temperature, 8.1 μL of above-mentioned solution and different concentrations of UO_2^{2+} were added in MES buffer of pH 4.5, held at room temperature for 6 min, and diluted to 210 μL by water. Then 50 μL of AuNPs, 140 μL of 200 mM NaCl were added. The final concentration of E-DNA/S-DNA was 0.177 μM. Afterward, the UV–vis absorption spectra were measured at λ_{max} 526 nm, and quantified by $\Delta A = A - A_0$, where A_0 and A were the absorbance of the system without and with UO_2^{2+}, respectively.

Figure Captions:

Fig. S1. Optimization of single-labeled molecular beacon.

$c_{UO_2^{2+}}$ = 75 nM, $c_{S\text{-}DNA}$ = 20 nM, $c_{E\text{-}DNA}$ = 25 nM.

Fig. S2. Effect of the concentration of SSP6 on the biosensing system.

$c_{UO_2^{2+}}$ = 0.63 nM, $c_{S\text{-}DNA}$ = 12.7 nM, $c_{E\text{-}DNA}$ = 15.9 nM, $c_{Exo\ III}$ = 8.9 U mL^{-1}.

Fig. S3. Effect of the concentration of Exo III on the biosensing system.

$c_{S\text{-}DNA}$= 12.7 nM, $c_{E\text{-}DNA}$= 15.9 nM, $c_{UO_2^{2+}}$ = 0.63 nM, c_{SSP6} = 55.6 nM.

Fig. S4. Influence of molar ratio between S-DNA and E-DNA on the biosensing system.

$c_{UO_2^{2+}}$ = 75.0 nM, c_{SSP6} = 31.3 nM, $c_{Exo\ III}$ = 8.9 U mL^{-1}.

Fig. S5. Effect of pH on the scission process for biosensing system.

c_{SSP6} = 11.0 μM, $c_{UO_2^{2+}}$ = 0.2 μM, $c_{S\text{-}DNA}$= 56.0 nM, $c_{E\text{-}DNA}$= 56.0 nM.

Fig. S6. Effect of the amount of NEB buffer on the biosensing system.

c_{SSP6} = 55.6 nM, $c_{UO_2^{2+}}$ = 0.63 nM, $c_{S\text{-}DNA}$= 12.7 nM, $c_{E\text{-}DNA}$= 15.9 nM, $c_{Exo\ III}$ = 8.9 U mL^{-1}.

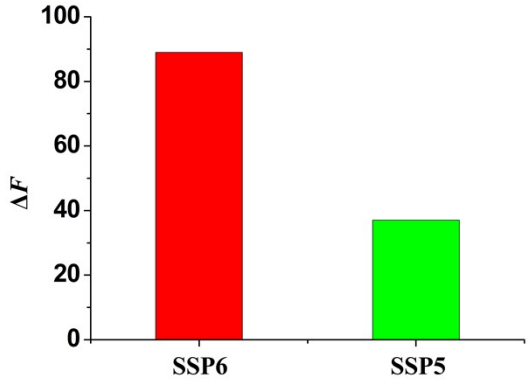


Fig. S1. Optimization of single-labeled hairpin signal probe.

$c_{UO_2^{2+}}$ = 75 nM, $c_{S\text{-}DNA}$ = 20 nM, $c_{E\text{-}DNA}$ = 25 nM, c_{SSP6} = 31.3 nM, c_{SSP5} = 31.3 nM.

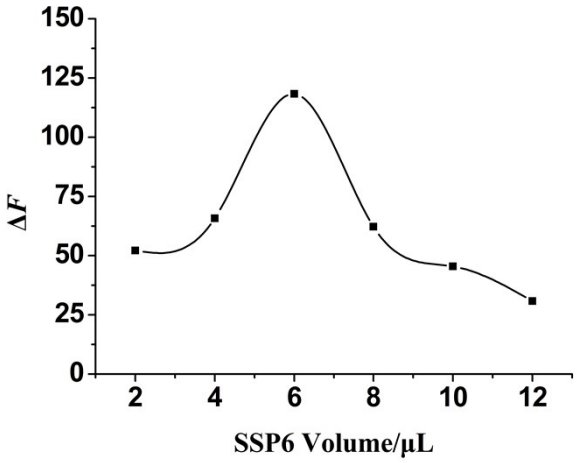


Fig. S2. Effect of the concentration of SSP6 on the biosensing system.

$c_{UO_2^{2+}}$ = 0.63 nM, $c_{S\text{-}DNA}$ = 12.7 nM, $c_{E\text{-}DNA}$ = 15.9 nM, $c_{Exo\ III}$ = 8.9 U mL^{-1}.

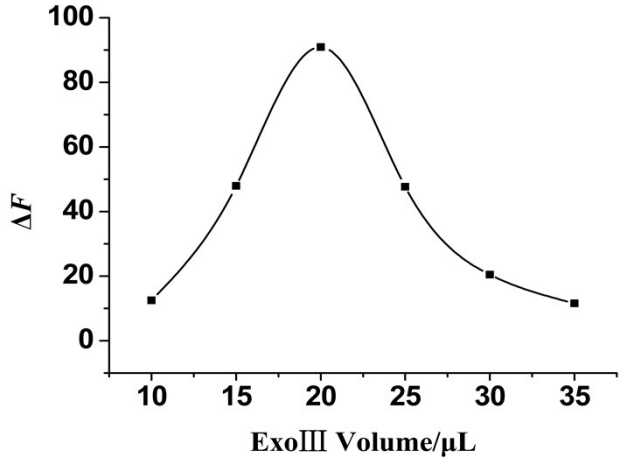


Fig. S3. Effect of the concentrations of Exo III on the biosensing system.

$c_{\text{S-DNA}}$= 12.7 nM, $c_{\text{E-DNA}}$= 15.9 nM, $c_{UO_2^{2+}}$ = 0.63 nM, c_{SSP6} = 55.6 nM.

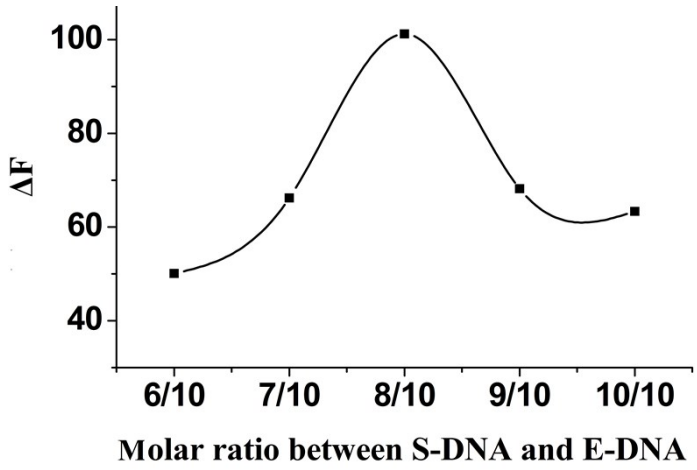
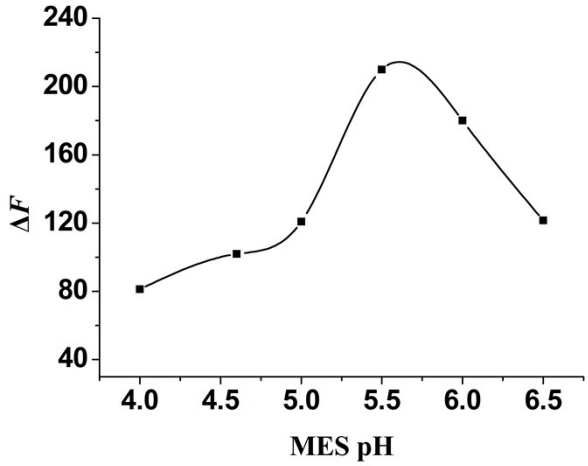


Fig. S4. Influence of molar ratio between S-DNA and E-DNA on the biosensing system.

$c_{UO_2^{2+}}$ = 75.0 nM, c_{SSP6} = 31.3 nM, $c_{\text{Exo III}}$ = 8.9 U mL^{-1}.



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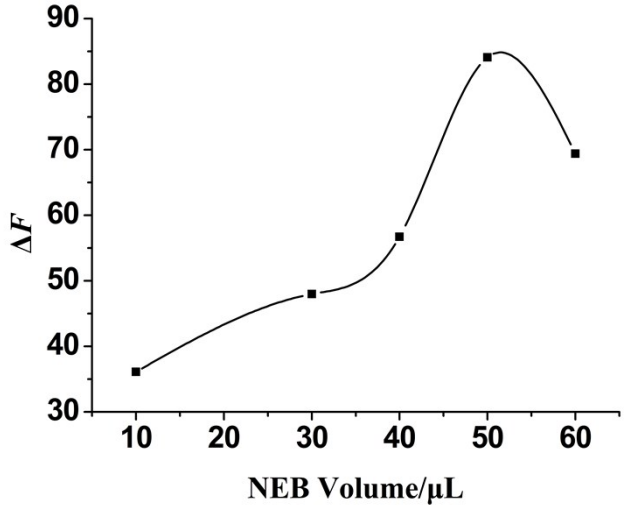
c_{SSP6} = 11.0 μM, $c_{UO_2^{2+}}$ = 0.2 μM, $c_{S\text{-}DNA}$= 56.0 nM, $c_{E\text{-}DNA}$= 56.0 nM.



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c_{SSP6} = 55.6 nM, $c_{UO_2^{2+}}$ = 0.63 nM, $c_{S\text{-}DNA}$= 12.7 nM, $c_{E\text{-}DNA}$= 15.9 nM, $c_{Exo\ III}$ = 8.9 U mL^{-1}.

Table S1. Sequences of SSP used in this work[a]

SSP	sequences
SSP5	5'-FAM-CAG TCC GAT GAG ATA GTG AGT GAC TGG GG-3'
SSP6	5'-FAM-CAG TCA GAT GAG ATA GTG AGT GAC TGG GG-3'

[a] The underlined sequences in SSP represent the stem of SSP.

Table S2. Comparison of this strategy with other methods for detection of UO_2^{2+} [a].

Methods	Samples	Linearity ranges	LOD	Instrumental cost	Ref
CBS	Soil	45pM – 400nM	45 pM	moderate	30
CS		50nM–2μM (Labled)	50nM	cheap	4
		1–100nM(Label-free)	1nM	cheap	4
UApt	water	22 – 550 nM	6.7 nM	moderate	9
LDG	water	13.6 – 150 nM	4.09 nM	moderate	6
GO-RCD	water	0.29 – 30 nM	86 pM	moderate	38
TRLFS	–		0.5 μM	moderate	40
CPSS	water	–	6.14 μg L^{-1}	moderate	41
DBES	–	2 –14 nM	1 nM.	cheap	39
UV–vis	tea leaves	10.0–250 μg L^{-1}	2.7 μg L^{-1}	moderate	42
Exo III	water	8.1 – 95 pM	2.4 pM	moderate	this work

[a]:CBS: Catalytic beacon sensor; CS: Colorimetric Sensors; UApt: Uranyl aptamer-gold nanoparticles; LDG: Uranyl specific DNAzyme–gold nanoparticle; GO-RCD: Graphene oxide and RCDzyme-based signal amplification strategy; TRLFS: Time-resolved laser fluorescence spectroscopy; CPSS: Chelating polymeric sorbent-spectrophotometry; DBES: DNAzyme based electrochemical sensors.