Electronic Supplementary Information

DNAzyme-induced disposable strip biosensor for visual detection of trace levels of UO_2^{2+} in remote areas

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Materials and methods

Chemicals and materials

Sodium phosphate (Na_3PO_4), bovine serum albumin (BSA), Tween-20, Triton X-100, tris-(hydroxymethyl)aminomethane (Tris), bovine serum albumin (BSA) and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, Mo). Glass fiber (CFSP001700) and nitrocellulose membrane (HF24002XSS) were purchased from Kinbio (Shanghai, China). Other reagents and chemicals were of analytical grade and used directly without further purification. All solution was prepared with ultrapure water (18.2 MΩ·cm) from the Millipore water purification system.

DNA oligonucleotides used in this work were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China) and listed in **Tables S1**. After synthesis, the DNA strands were purified using high - performance liquid chromatography (HPLC) to remove any unreacted monomers and other impurities. Gold nanoparticles (AuNPs, 20 nm) were purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of AuNPs-DNA conjugate

Conjugation reactions were carried out by adding 100 μL of thiolated DNA (DNA probe I, 1.0 OD) to 900 μL of the 10-fold concentrated AuNPs solution. After standing at 4 ℃ for 24 h, the solution was subjected to "aging" by the addition of NaCl up to a concentration of 150 mM, and a certain quantity of 1% sodium dodecyl sulfate (SDS) was added to reach a final concentration of 0.01%. The solution was allowed to stand for another 24 h at 4 ℃, and the excess of reagents was removed by centrifugation for 20 min at 12000 rpm. The supernatant was discarded, and the red pellets were resuspended in 1 mL of buffer (pH 7.6) containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20, and 10% sucrose. The resulting AuNPs-DNA probe I conjugate solution was stored at 4 ℃ before further use.

UO_2^{2+} assay procedure

The DNA solutions were heated at 90 ℃ for 10 min on a battery-operated heating block to dissociate intermolecular interactions, followed by gradual cooling to room

temperature. 50 nM DNAzyme substrate stand (S) was first incubated with 100 nM DNAzyme stand (E) in 50 mM MES buffer (300 mM NaCl, pH 5.5) at room temperature for 30 min to form the DNAzymes. The UO_2^{2+} solution was then added into the resulting DNAzymes and incubated for 30 min which led to the maximum cleavage of the DNAzyme substrate strand (S) at the rA position. Finally, the mixed sample solution was applied to the sample pad of the strip for assay. After waiting for 5 min, another Tris-Ac buffer was added to the sample pad to wash the strip. Accumulation of AuNPs on the test and control zones produced the characteristic red bands. Visual detection of UO_2^{2+} was simply realized by observing the color on the test zone of the strip. The red bands were visualized within 10 min.

To evaluate the specificity of the assay, other metal ions (Sr^{2+}, Cs^{2+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, and Pb^{2+}) at 1 μM were tested in the same way.

Preparation of the Strip Biosensor

The strip structure comprises four key components: sample pad, conjugate pad, nitrocellulose membrane, and absorption pad, all affixed to a common inert plastic backing layer. The sample pad (20 mm × 15 cm), constructed from glass fiber, was impregnated with a pH 8.0 buffer solution containing 0.25% Triton X-100, 20 mM Tris-HCl, and 150 mM NaCl. Following saturation, the pad underwent drying at 37 °C for 2 hours and was subsequently stored in desiccators at room temperature.

For the conjugate pad (16 mm × 15 cm), 5 μL of AuNPs-DNA probe a conjugate solution was dispensed onto a glass fiber pad using an HM3030 dispenser (Shanghai Kinbio Tech. Co., Ltd., Shanghai, China). This pad was air-dried at room temperature and preserved in a desiccator at 4 °C.

The nitrocellulose membrane (25 mm × 15 cm) featured distinct test and control zones, prepared by dispensing 1.5 mg/mL solutions of streptavidin (SA)-biotinylated DNA probe II and SA-biotinylated DNA probe III, respectively. The SA-biotin conjugation strategy was employed to facilitate immobilization of DNA probes on the membrane, with the test and control zones spaced approximately 5 mm apart. The membrane was dried at 37 °C for 1 hour and stored in a dry state at 4 °C.

Final assembly involved laminating the sample pad, conjugate pad, nitrocellulose

membrane, and absorption pad onto a 60 mm × 15 cm plastic adhesive backing, with each component overlapping by 2 mm to ensure solution migration during assays. Strips of 4 mm width were precision-cut using a ZQ2000 programmable strip cutter (Shanghai Kinbio Tech. Co., Ltd., Shanghai, China).

UV-vis absorption determination

The wavelength scanning range was set to 250-650 nm, and the scanning interval was 10 nm. The ultrapure water was used for calibration, and the baseline absorbance was recorded without solute absorption. S stand, E stand, and UO_2^{2+} was added into the prepared conjugate solution containing AuNPs-I, probe-II and probe-III. After thorough shaking and incubating for about 30 min at room temperature, the corresponding UV-vis absorption spectra were recorded.

DLS measurement

A Malvern Zetasizer Nano ZS DLS system (Malvern Instruments Ltd., Worcestershire, UK) was used to conduct all DLS measurements. The DLS system is equipped with a 633 nm He-Ne laser and an avalanche photodiode detector configured to collect backscattered light at 173°. The sample was held at 25 °C by a temperature controlled sample holder and allowed to equilibrate for 60 s prior to analysis. Each size measurement was determined from 10 runs, 10 s each. Each sample was analyzed in triplicate to calculate an average and standard deviation. All reported mean particle hydrodynamic diameters (DH) are calculated from the intensity based particle size distributions.

Spiked samples assay

For spiked samples analysis, tap water was collected from the laboratory (Hengyang, Hunan, China), river water was taken from the Xiangjiang River (Hengyang, Hunan, China), and pond water was collected from our college. Those water samples were filtered through a 0.22 μm membrane to remove the insoluble impurities. The river water samples were spiked with different concentrations of UO_2^{2+} for recovery studies. Subsequently, tap water, river water, and pond water samples were individually subjected to analysis using a UV-vis spectrophotometer following the established protocol.

Table S1. DNA sequences used for UO_2^{2+} detection.

Name	Sequence（5’-3’）
S	TCACTATrA GGAATAGATG GACGTGCAAC
E	CACGTCCATCT CAGCAGTCGGG TAGTTAAACCGA CCTTCAGAC ATAGTGA
I	SH-AAAAAA GTTGCACGTC AAAAAA
II	CATCTATTCC-Biotin
III	Biotin-GACGTGCAAC AAAAAA

S is the substrate strand of the UO_2^{2+}-specific DNAzyme and rA in S denotes riboadenosine. E is the enzyme strand of the UO_2^{2+}-specific DNAzyme. DNA probe I was used to conjugate with AuNPs and dispensed on the conjugate pad of the strip. DNA probe II and DNA probe III were dispensed on the test and control zones of the strip, respectively.

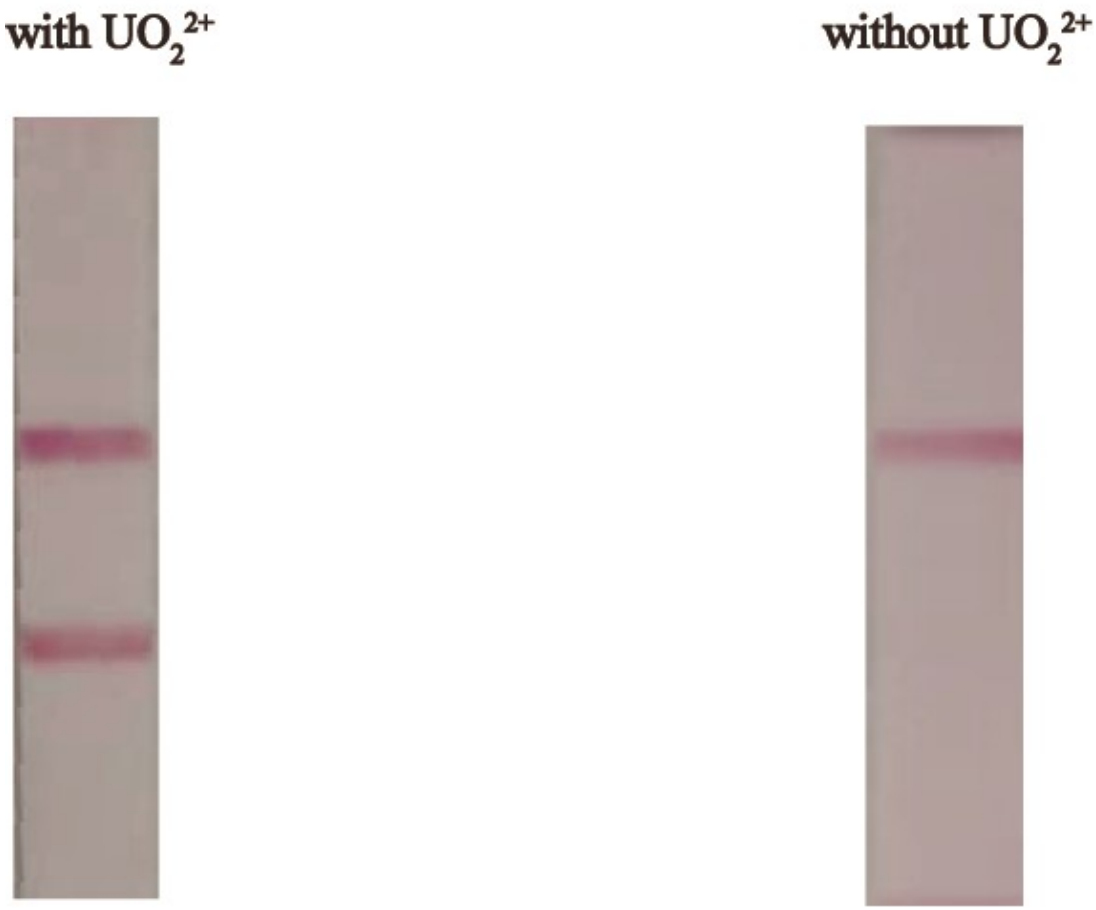

Fig. S1. Photo images of the strip biosensor with and without UO_2^{2+}.

The efficiency of DNA fixation

As shown in **Fig. S2**, a strong absorbance spectrum of the solution was observed without AuNPs, which is characteristic of the nucleic acid base absorption. After the DNA was immobilized on AuNPs, a significant decreased absorbance at 260 nm was detected. which may indicate DNA fixation on AuNPs surfaces. In **Fig. 1C**, the absorbance of the DNA solution at 260 nm was measured before (A_0) and after (A_1) incubation with AuNPs and subsequent centrifugation. The significant decrease in absorbance (A_1) is attributed to the immobilization of DNA onto the AuNPs. The fixation efficiency was then calculated using the formula $(A_0 - A_1)/A_0 \times 100\%$, yielding the value of 82.2%.

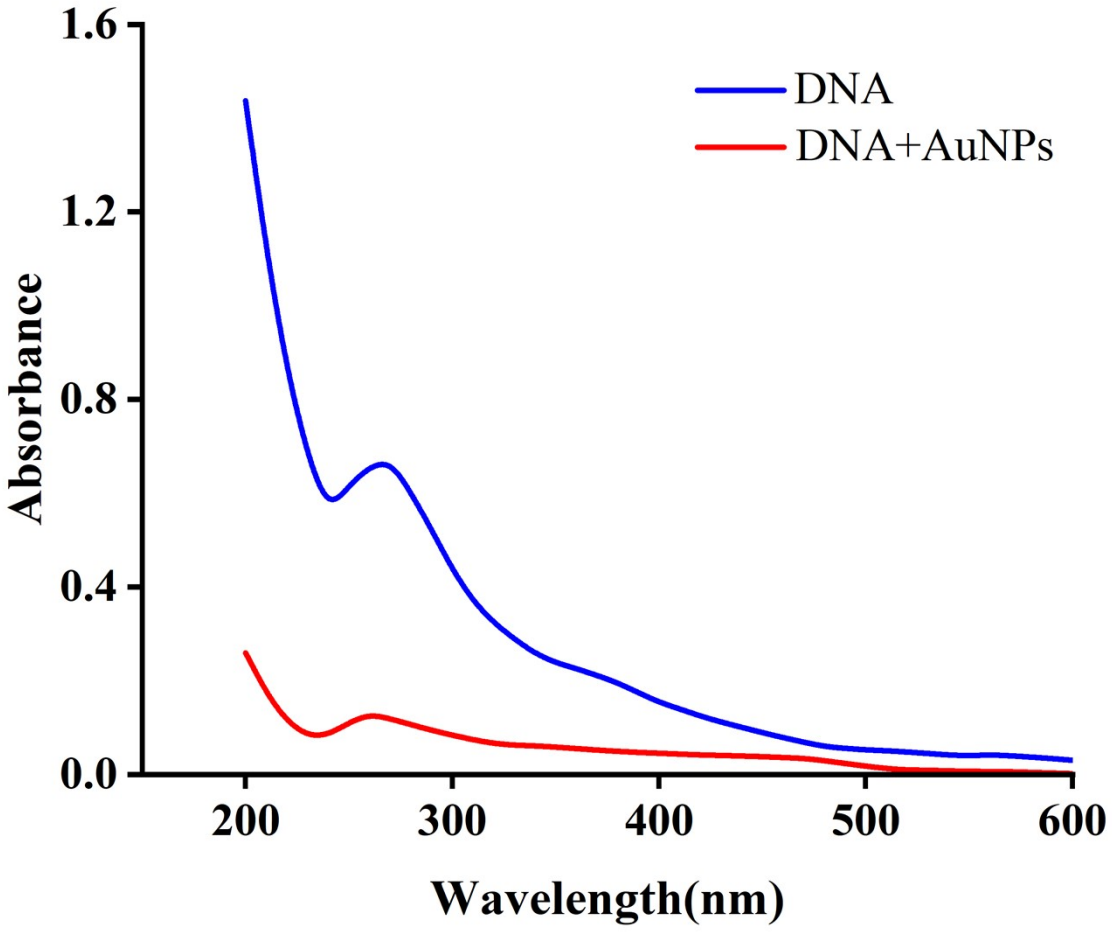

Fig. S2. UV-vis absorption spectra of the DNA in the absence and presence of AuNPs. After centrifugtion, the supernatant was measured.

Optimization of the assay parameters

In order to achieve the best detection performance of our proposed biosensor, several assay parameters were optimized, such as the concentration of DNA substrate, temperature, and pH (**Fig. S3-S5**). We first focused on the concentration of DNA substrate that determines the efficiency of the colorimetric sensor. As shown in **Fig. S3,** the signal-to-noise (S/N) ratio constantly increased when the concentration of DNA substrate concentration was raised from 0.5 to 1.5 μM. The optimal S/N ratio was achieved at a substrate concentration of 1.5 μM. A higher concentration could generate a high background signal. While a lower concentration could affect the efficiency of the sensor to detect the target UO_2^{2+}, thus yielding a weak response signal. Therefore, the concentration of DNA substrate was selected as 1.5 μM for target detection.

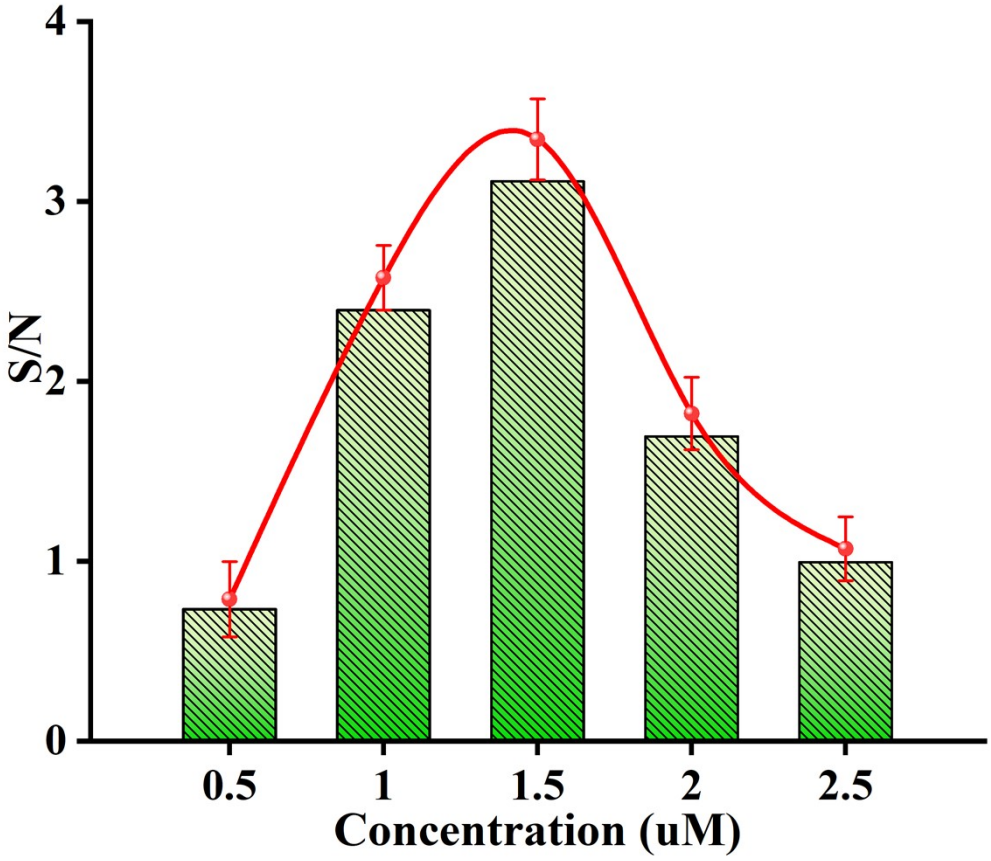

Fig. S3. Effect of the concentration of DNA substrate on the performance of the colorimetric sensor. S: 1 μM, AuNPs-DNA probe I: 1 μM. The experiments were performed at room temperature (25 °C). The error bars represent the standard deviation of three independent measurements.

The influence of reaction temperature on biosensor response was examined by assessing the sensor's performance toward 1 μM UO_2^{2+} at distinct temperatures (4 °C, 25 °C, 37 °C, and 45 °C). As illustrated in **Fig. S4**, the absorption ratio (A650/A520) exhibited a progressive increase with temperature elevation within the 4 - 25 °C range. Conversely, further raising the temperature to 45 °C induced a decline in the A650/A520 ratio. The highest A650/A520 value for the 1 μM UO_2^{2+} solution (blue histogram) was recorded at 25 °C. At elevated temperatures (37 °C and 45 °C), the DNAzyme displayed a significant loss of activity, attributed to the instability of supramolecular DNAzyme assembly via nucleic acid hybridization under these conditions. To optimize the signal-to-noise ratio (red line in **Fig. S4**), 25 °C was determined as the optimal reaction temperature.

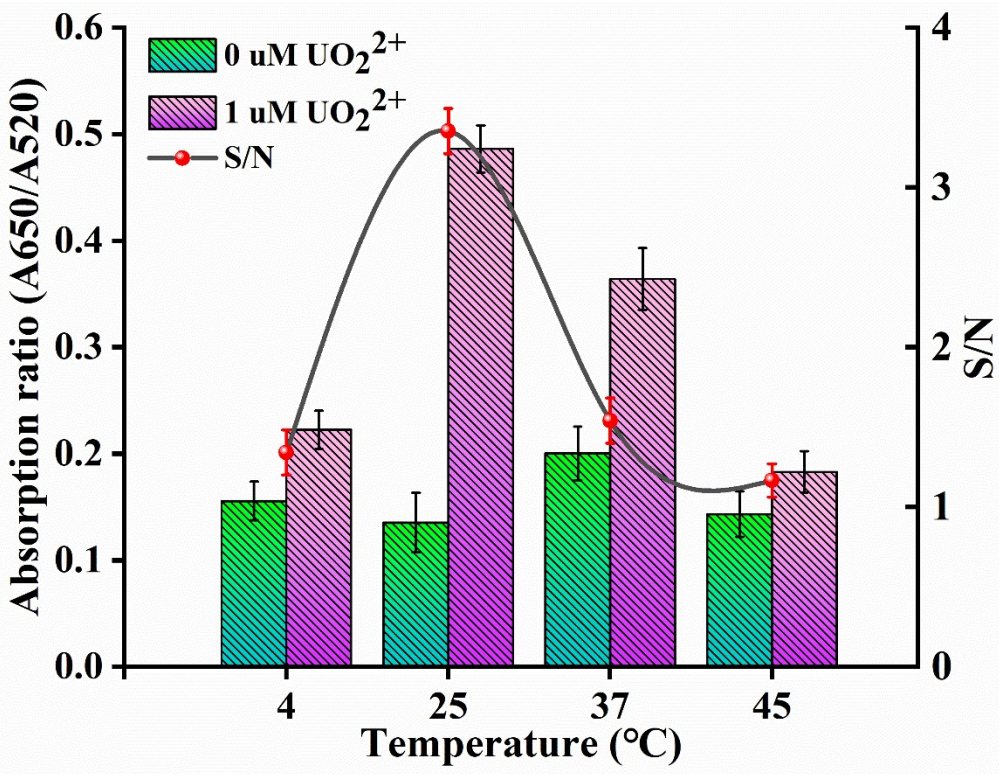

Fig. S4. Effect of the reaction temperature on the performance of the colorimetric sensor. The histograms represent the absorbance ratio of the solution with 1 μM UO_2^{2+} (purple) and without UO_2^{2+} (green). The black line represents the S/N ratio. The error bars represent the standard deviation of three independent measurements.

To optimize the conditions based on the pH - absorbance curve, first, by referring to the literature, the optimal pH range can be initially determined to be approximately 7.0 - 8.0. As shown in **Fig S5**, when the pH of the AuNPs solution was 7.6, the OD value of the AuNPs solution at 520 nm was the largest, that is, the curve reached the highest point. Therefore, it was concluded that the optimal pH for DNA - modified AuNPs was 7.6.

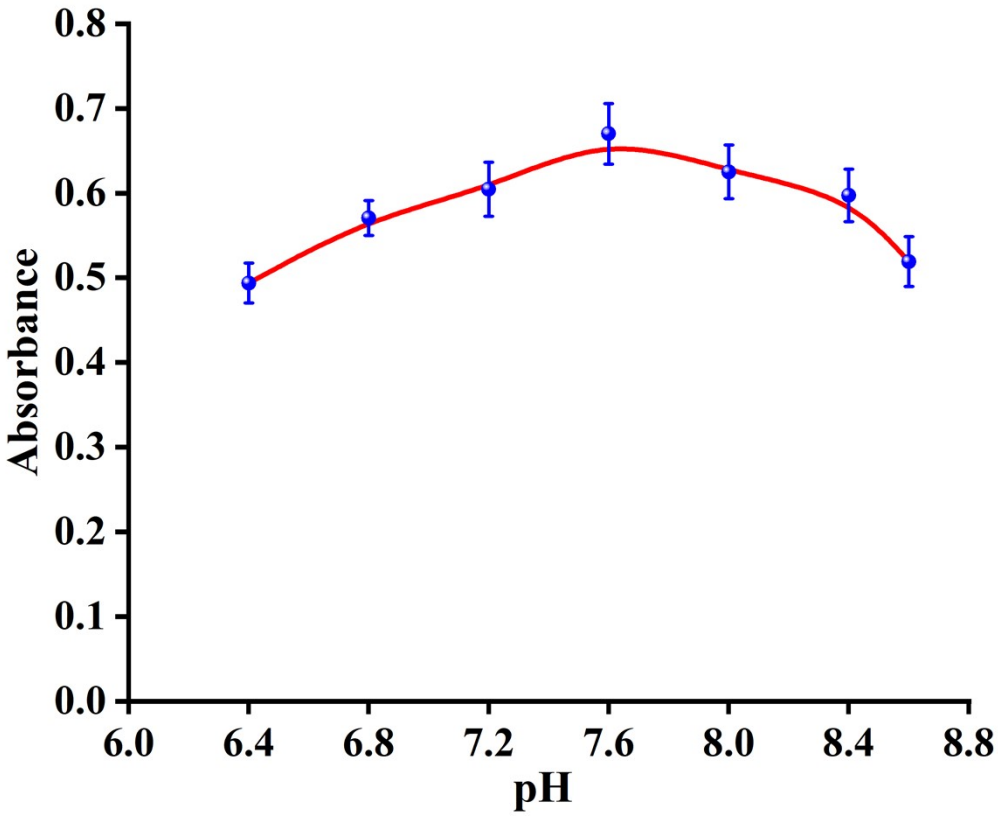

Fig. S5. Optimal pH of AuNPs marking probe I determined by UV - Vis Spectrometry. The graph presents the UV absorption peak values of the AuNPs solution at 520 nm under different pH

The selectivity of the biosensor

As depicted in **Fig. S6**, only UO_2^{2+} and the mixture produce distinct bands in the TZ, indicating specific response, while other metal ions show no TZ bands, demonstrating the strip's specificity for UO_2^{2+} detection. The strip responses to various metal ions are also quantitatively analyzed via Image J, with the corresponding results provided in **Fig. S6** (inset).

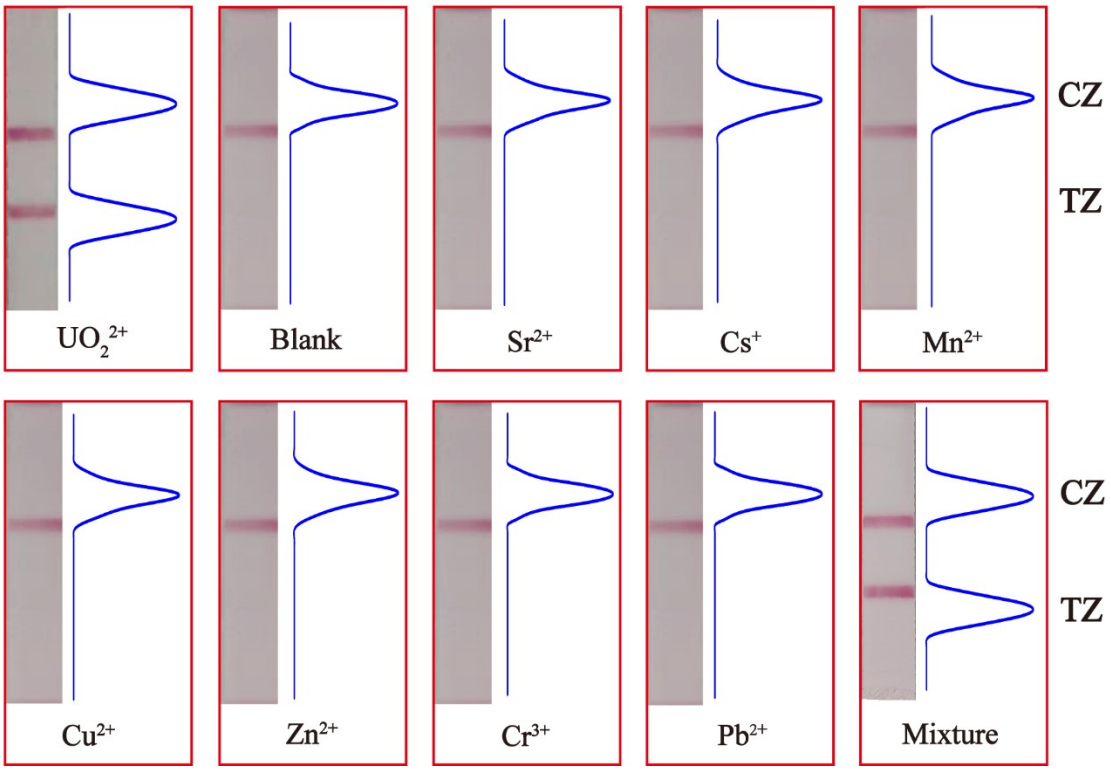

Fig. S6. Photo images and corresponding intensities (inset) of TZ and CZ for testing strip specificity with various metal ions analyzed by Image J.

Table S2. Comparison of detection sensitivity between our UO_2^{2+} detection sensor and some preciously reported methods.

Sensing method	Sigal output	Limit of detection	Reference
Peroxidase-like activity by graphene oxide adsorption	Colorimetric	4.7 μM	1
Near-infrared fluorescent probe cyanine dye IR-780	Fluorescent	93 nM	2
Target-responsive hydrogel	Colorimetric	37 nM	3
UV-induced photocleavage	Fluorescent	6.8 nM	4
Dual-color encoded DNAzyme nanostructures	Fluorescent	3.9nM	5
FeN_5-mediated nanozymes	Colorimetric	3.1 nM	6
Disposable strip biosensor	Colorimetric	160 pM	This work

Table S3. Determination of UO_2^{2+} in spiked samples using the strip biosensor

Sample	Adde (nM)	Found (mean[a] ± SD[b]) (nM)	Recovery (%)
Tap water 1	10	9.5±0.6	95
Tap water 2	100	96.9±2.1	96.9
Tap water 3	500	458.1±3.5	91.6
Pond water 1	10	10.9±0.3	109
Pond water 2	100	105.3±1.0	105.3
Pond water 3	500	481±5.1	96.2
River water 1	10	11.6±0.7	116
River water 2	100	98.5±4.6	98.5
River water 3	500	504±3.8	100.8

[a] Mean of three determinations. [b] SD, standard deviation. Each sample was analyzed using the developed strip biosensor, and all data were reported as the mean of three replicate measurements.

Table S4. Determination of UO_2^{2+} in real samples using the strip biosensor and ICP-MS.

Sample	ICP-MS[a] (nM)	Strip biosensor[b] (nM)	RSD[c] (%)	Relative error[d] (%)
Wastewater 1	230±2.7	235.8±4.1	1.7	2.5
Wastewater 2	31±0.7	33.1±1.4	4.2	6.8
Wastewater 3	4.7±0.6	4.3±0.2	4.6	-8.5

[a] Each sample was analyzed using the standard ICP-MS (Inductively coupled plasma mass spectrometry), and all values were obtained as an average of three repetitive determinations ± standard deviation (mean ± SD). [b] Each sample was analyzed using our proposed strip biosensor, and all values were obtained as an average of three repetitive determinations ± standard deviation (mean ± SD). [c] Relative standard deviation (RSD) of the strip biosensor. [d] Our proposed method vs. ICP-MS.

The stability of the biosensor

When the sensor needs to be stored in the refrigerator at 4 °C, and the stability of our sensor is also an important factor affecting the analytical performance. Therefore, the long-time stability of this biosensor was executed. The detection signal retained 90.3% of its initial value after 15 days, indicating satisfactory long-term storage stability (**Fig. S7**).

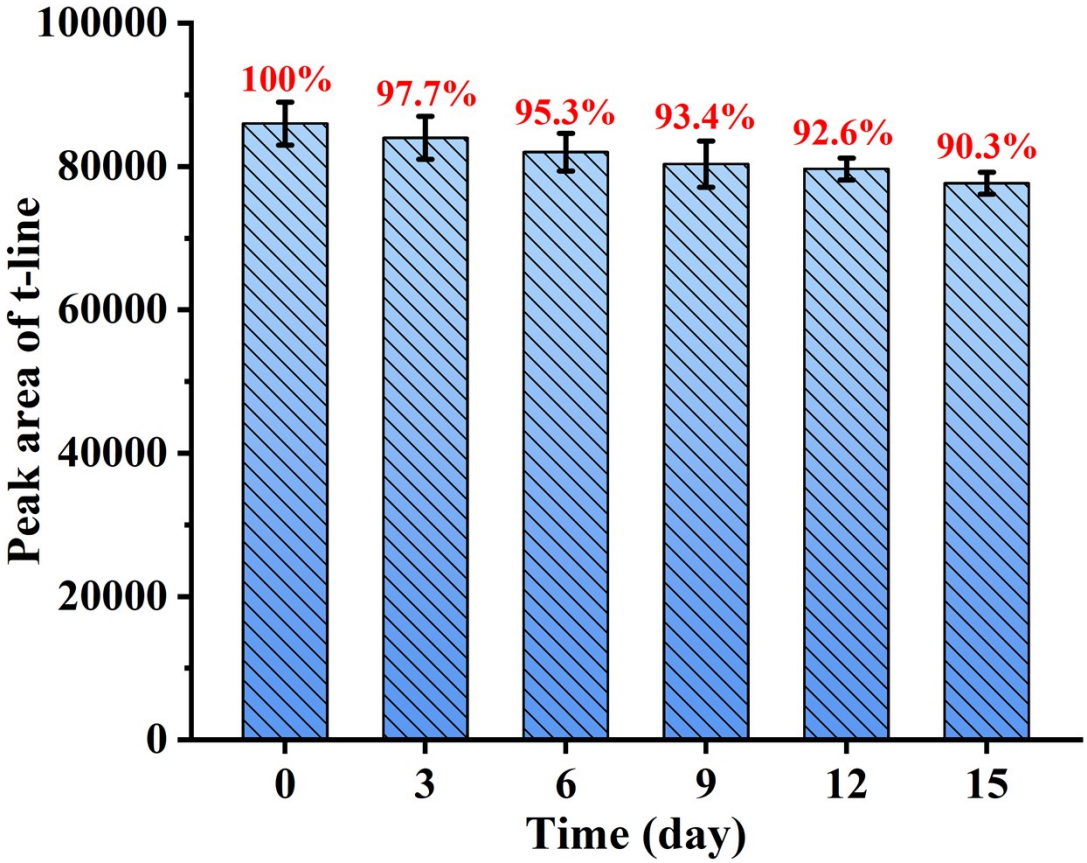

Fig. S7. The stability of the strip biosensor without/with 1 μM UO_2^{2+}. All values were obtained as an average of three repetitive determinations ± standard deviation (mean ± SD).

References

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