Supporting Information

DNAzyme recognition triggered cascade signal amplification for rapid and highly sensitive visual detection of uranyl ions

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Table S1. Modeling approach for evaluation of the amount of DNAzyme/subtrate strand immobilized on the microbeads.

$$D_{DNA/MBs} = \frac{C_{molar\_DNA} \times V_{DNA} \times (F_0 - F)}{C_{mass\_MB} \times V_{MB} \times F_0} \quad (1)$$

$$D_{DNA/1\_MB} = D_{DNA/MBs} \times V_{1\_MB} \times \rho_{1\_MB} \quad (2)$$

parameters

$D_{DNA/MBs}$	Density of DNAzyme/primer strand immobilized on the agarose microbeads (nmol mg^{-1})
$D_{DNA/1\_MB}$	Density of DNAzyme/primer strand immobilized on each microbead (nmol per bead)
C_{molar_DNA}	Concentration of DNAzyme/primer strand in the DNA stock solution before immobilization (nmol mL^{-1})
V_{DNA}	Volume of the DNA stock solution for immobilization (mL)
C_{mass_MB}	Concentration of microbeads in the MB stock solution before immobilization (mg mL^{-1})
V_{DNA}	Volume of the MB stock solution for immobilization (mL)
V_{1_MB}	Average volume of one microbead (mm^3)
ρ_{1_MB}	Density of agarose microbead (g mm^{-3})
F_0	Fluorescent intensity of the DNA solution without MB immobilization (a.u.)
F	Fluorescent intensity of the DNA in the supernatant after MB immobilization (a.u.)

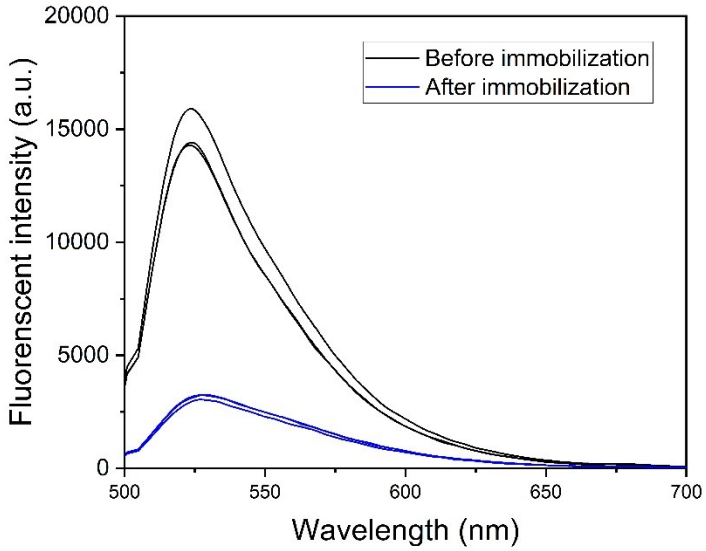

Figure S1. Evaluation of the immobilized amount of the DNAzyme/substrate-primer strands on the agarose microbeads. As discribed in the Experimental Section, a DNAzyme/substrate-primer stock solution (containing 20 nmol mL^{-1} DNA probes) and a MB stock solution (containing 100 mg mL^{-1} agarose microbeads) were prepared for MB immobilization. 0.5 mL DNAzyme/primer stock solution was diluted into 1 mL with PBS buffer, stained with Sybr Green I and measured by fluorescence spectroscopy (black curve, before immobilization). Another 0.5 mL DNAzyme/primer stock solution was mixed with 0.5 mL MB stock buffer, incubated for 30 min at RT for the surface immobilization of MBs via specific binding between avidin and biotin. After that, the MBs were removed by centrifugation, and the supernatant were collected, stained with Sybr Green I, and measured by fluorescence spectroscopy (blue curve, after immobilization). The emission fluorescent intensities at the wavelength of 520 nm were recorded.

Evaluation of the amount of DNA strands immobilized on MBs

The density of DNAzyme/substrate-primer decorated on MBs ($D_{DNA/MBs}$, nmol DNA strands per mg MBs) was calculated according to the equation (1) in Table S1. The fluorescent intensities F_0 and F were obtained from the measurement of DNA solution/supernatant before and after immobilization, respectively, with Sybr Green I

as the fluorescent indicator. It was observed that 80% of the DNA probes in the solution were bounded on the surface of MBs via 30-min incubation. The density $D_{DNA/MBs}$ was calculated to be 0.16 nmol mg^{-1}.

To assess the amount of DNA strands on each bead, equation (2) was used for calculation. The average diameter of the avidin-agarose microbeads is 105 μm. The mass density (ρ_{1_MB}) of the 6% agarose bead is 0.994 g cm^{-1}. The density of DNAzyme/substrate-primer decorated on each MB ($D_{DNA/1\_MB}$) was calculated to be 9.6 pmol per MB (i.e. 5.8×10^{10} DNA probes per MB).

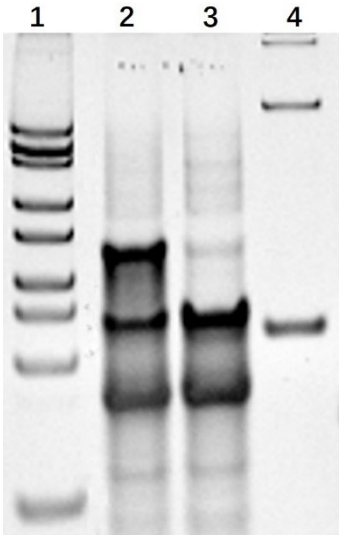

Figure S2. The polyacrylamide gel electrophoresis analysis of the DNAzyme cleavage reaction and the triggered RCA products. Lane 1, DNA marker containing a mix of 9 individual DNA fragments: 500, 400, 300, 200, 150, 100, 75, 50, 25 bp (the bands from up to down). Lane 2 and 3, DNAzyme/substrate-primer probes after interation with 200 nM UO_2^{2+} for 5 and 30 min, respectively. The upper band in lane 2 is the DNAzyme/substrate-primer complex. The middle bands are the cleaved DNA strand containing primer. Lane 4, DNAzyme/primer/MBs recognition with 200 nM UO_2^{2+} generated RCA product (the upper band).

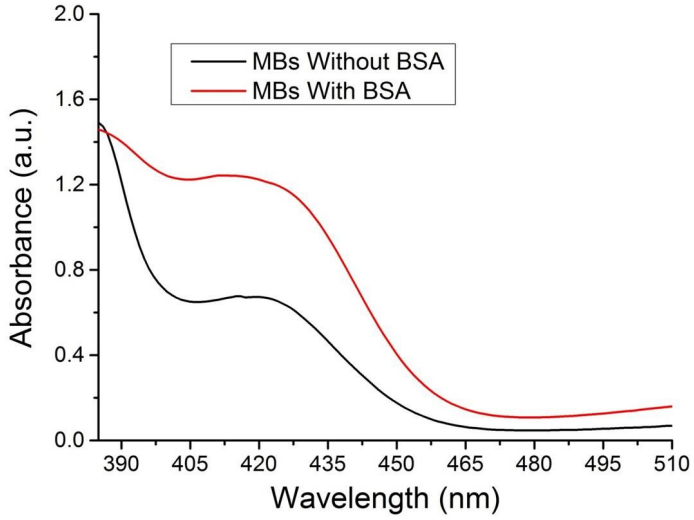

Figure S3. Evaluation of the BSA blocking effects on the colorimetric signaling. UV-Vis absorbance of BSA-blocked (red curve) and unblocked (black curve) DNAzyme/substrate-primer/MBs triggered colorimetric reaction with cascade amplification, in presence of 200 nM uranyl ions.

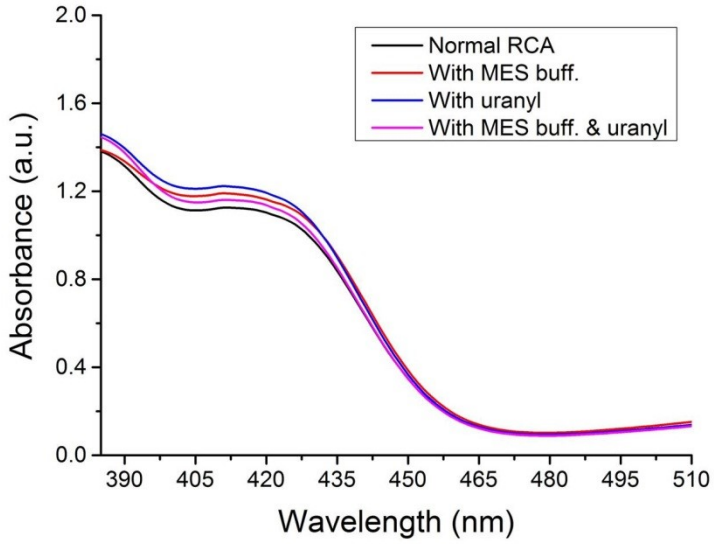

Figure S4. Effect of the buffer conditions on RCA reaction. All RCA reactions were performed with 5 μL Ligation DNA (2 μM) as the primer, for 30 min. 5 μL ultrapure H2O (black), 5 μL MES buffer (red), 5 uL 1 μM uranyl in ultrapure H2O (blue), and 5 uL 1 μM uranyl in MES buffer (purple) were added RCA reaction solutions (final volume: 50 μL), respectively. The RCA products were tested with the

same protocol of the colorimetric assay. RCA kepts the same high efficiency in all conditions.