Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

Simple and sensitive detection of uracil-DNA glycosylase activity using dsDNA-templated copper nanoclusters as fluorescent probes

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Reagent and apparatus

The DNA oligonucleotides used in this work were synthesized and purified by Sangon Inc. (Shanghai, China) and the sequences of the sequences were listed in Table S1. Uracil-DNA glycosylase (UDG), uracil glycosylase inhibitor (UGI) and 8-oxoguanine DNA glycosylase (hOGG1) were obtained from New England Biolabs Ltd. (Beijing, China). One unit of UGI activity was defined as the amount of protein required to inhibit one unit of E.coli UDG in 1h at 37 °C in a total reaction volume of 50uL. One unit of UDG was the amount of enzyme which would catalyze the release of 60 pmol of uracil per minute from the double stranded, uracil-containing DNA. BSA was purchased from Sigma-Aldrich. All other chemicals were of analytical reagent grade and used without further purification. Ultrapure water (18 $M\Omega \cdot cm^{-1}$) was obtained through a Millipore Milli Q water purification system (Billerica, MA), and was used in all experiments.

Procedure for UDG activity detection

To prepare duplex DNA, ssDNA1 and ssDNA2 were dissolved in the reaction buffer (20 mM Tris–HCl, pH 8.0 at 25.0 °C, 10 mM NaCl, 2mM $MgCl_2$) and diluted with the reaction buffer to the desired concentration. 50 μL ssDNA1 (100 μM) and 50 μL ssDNA1 (100 μM) were mixed and annealed for 10 min at 90 °C and then gradually cooled to room

temperature (ca. 20 °C), and was further incubated for 30 min at room temperature to ensure that the stable dsDNA was obtained. Finally, the obtained dsDNA solution was stored at 4 °C for further use. 20 μL dsDNA (5 μM) was mixed with 25 μL UDG，10 μL 10×UDG Reaction Buffer (200 mM Tris-HCl，10 mM EDTA，10 mM DTT，pH 8.0 at 25.0 °C) and 45μL ultrapure water to give a total volume of 100 μL，and the whole mixture was incubated at 37 °C for 90 min to allow the reaction to take place. Finally,100 μL MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.6) and 10 μL sodium ascorbate (0.1 M) were added and vibrated for 2 min, then 30 μL $CuSO_4$ (10 mM) solution was added to the mixture solution. The mixed solution was kept for 10 min at room temperature, and then the fluorescent CuNCs were formed. The fluorescence spectra of the final solution were measured by using a Hitachi F-4600 Fluorometer (Tokyo, Japan) at the excitation wavelength of 340 nm. The fluorescence emission intensity at 575 nm was recorded. The UDG activity was quantified by the fluorescence intensity. In the inhibition experiment, to evaluate the effects of inhibitor on UDG liberation process, different concentrations UDG were added to 5 μM dsDNA solutions and incubated for a certain time. The following procedures were similar as above.

Table S1. The used sequences for the selection of numbers of uracils on the dsDNA strand

name	dsDNA sequence
dsDNA-1	5′- ATATATATAA***U***TATATATATAT -3′ 3′- TATATATATT AATATATATATA -5 ′
dsDNA-2	5′- ATATATA***U***AAATATA***U***ATATAT -3′ 3′- TATATAT ATTT ATAT ATATATA -5′
dsDNA-3	5′- ATATA***U***ATAAA***U***ATATA***U***ATAT -3′ 3′- TATAT ATA TTT A TATATATATA-5′
dsDNA-4	5′- ATA***U***ATAT ***U***TATA***U***ATAT***U***TAT -3′ 3′- TAT ATATA AATATA TATAAATA-5 ′
dsDNA-5	5′- AT***U***TAT ***U***TAA***U***TAT***U***AAT***U***TAT -3′ 3′- TAAATAAATT AATAATTAAATA-5′

Table S2. The used sequences for the selection of location of uracils on the dsDNA strand

name	sequence
dsDNA-3	5′- ATATA***U***ATAAA***U***ATATA***U***ATAT -3′ 3′- TATAT ATA TTT A TATATATATA-5′
dsDNA-3a	5′- ATA TAT***UU***T ATA TA***U***TATA TAT -3′ 3′- TATATA AAATAT ATAATAT ATA-5′
dsDNA-3b	5′- ATATAT ATA***UUU***ATA TAT ATAT -3′ 3′- TAT ATATATAAAT ATA TAT ATA-5 ′

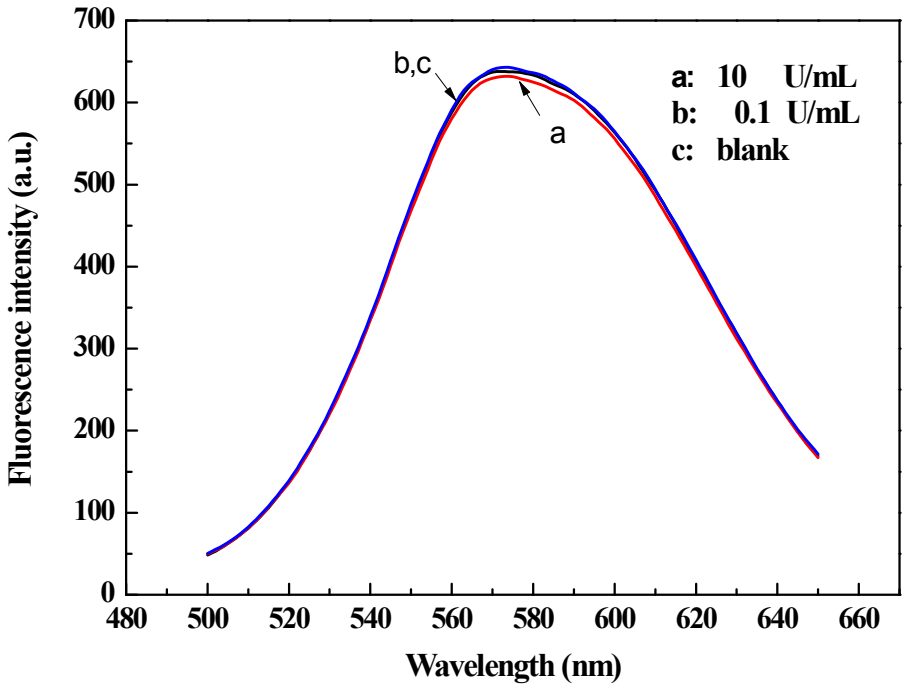

Fig. S1 Fluorescence emission spectra of CuNCs in the presence or absence of UDG. (a) dsDNA strand (5′-ATA TAT ATA TAT ATA TAT ATA TAT -3′ + 3′-TAT ATA TAT ATA TAT ATA TAT ATA -5′) + UDG (10 U/mL), (b) dsDNA strand (5′-ATA TAT ATA TAT ATA TAT ATA TAT -3′ + 3′-TAT ATA TAT ATA TAT ATA TAT ATA -5′) + UDG (0.1 U/mL), (c) dsDNA strand (5′-ATA TAT ATA TAT ATA TAT ATA TAT -3′ + 3′-TAT ATA TAT ATA TAT ATA TAT ATA -5′). Conditions: 5×10^{-6} M dsDNA, 0.05 M sodium ascorbate, 0.01M Cu^{2+};

Tris–HCl 20 mM, pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$.

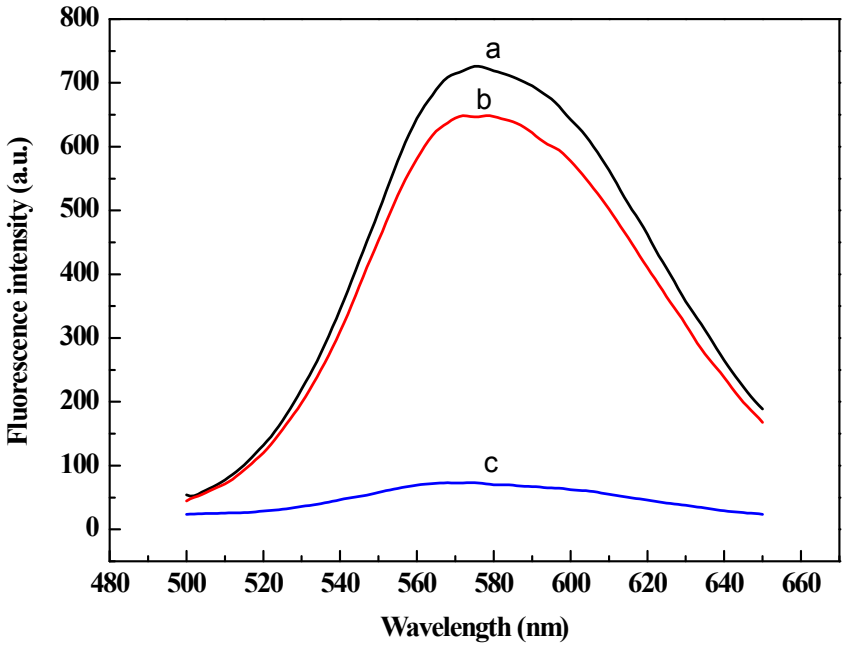

Fig. S2 Fluorescence spectra of obtained CuNCs under different conditions:(a) DNA1 strand (5′-ATA TAU ATA AAU ATA TAU ATA T-3′) + DNA2 strand (3′-TAT ATA TAT TTA TAT ATA TAT A-5′), (b) DNA1 strand+DNA2 strand + denatured UDG, (c) DNA1 strand+DNA2 strand + UDG . Conditions: 5×10^{-6} M dsDNA, 5 U/mL UDG, 0.05M ascorbate, 0.01 M Cu^{2+}, 20 mM Tris–HCl (pH

8.0 at 25.0 °C).

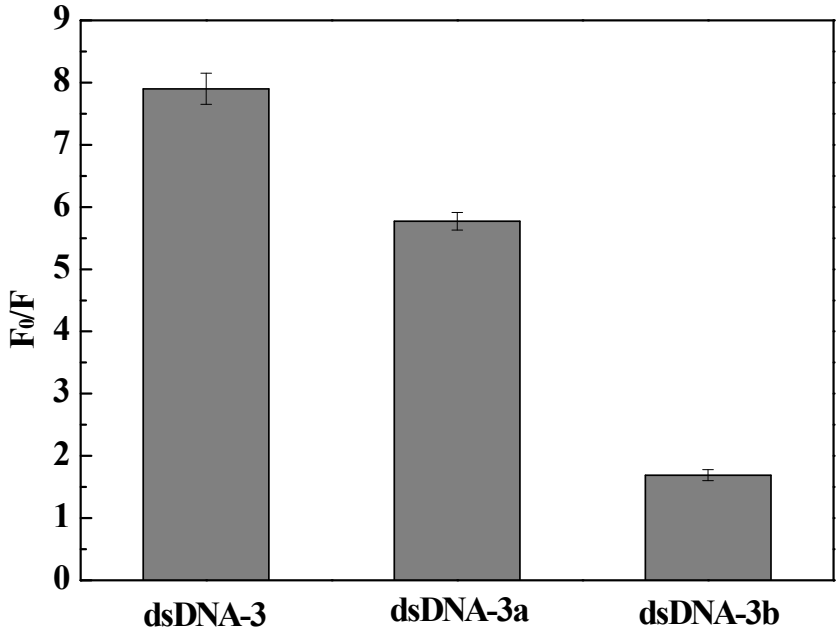

Fig. S3 Effect of the location of uracils on the DNA on the formation of dsDNA-templated fluorescent CuNCs. Conditions: 5×10^{-6} M dsDNA; 5 U/mL UDG, 0.05M sodium ascorbate, 0.01M Cu^{2+}; Tris–HCl 20 mM, pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$.

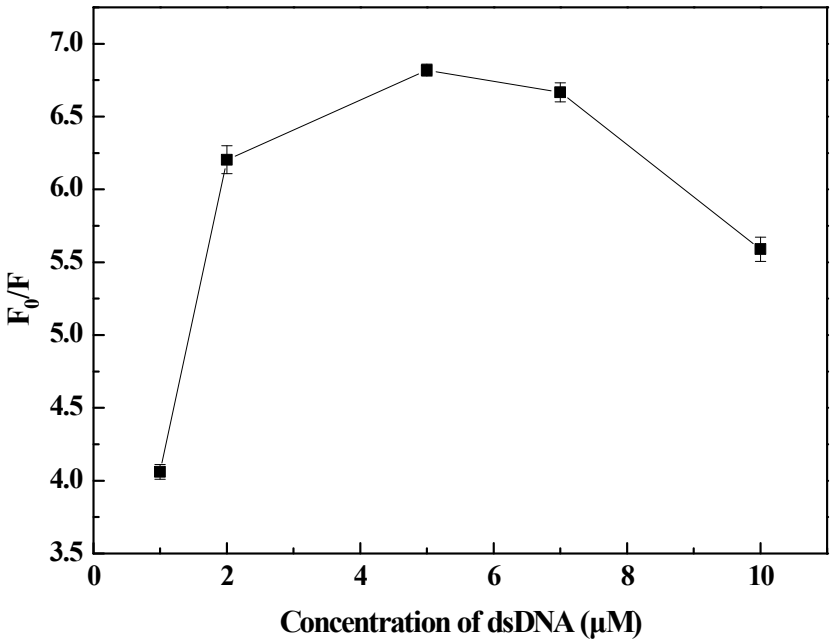

Fig. S4 Effect of dsDNA concentration on assay sensitivity. Conditions: 5 U/mL UDG, 0.05M sodium ascorbate, 0.01M Cu^{2+}; Tris–HCl 20 mM, pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$.

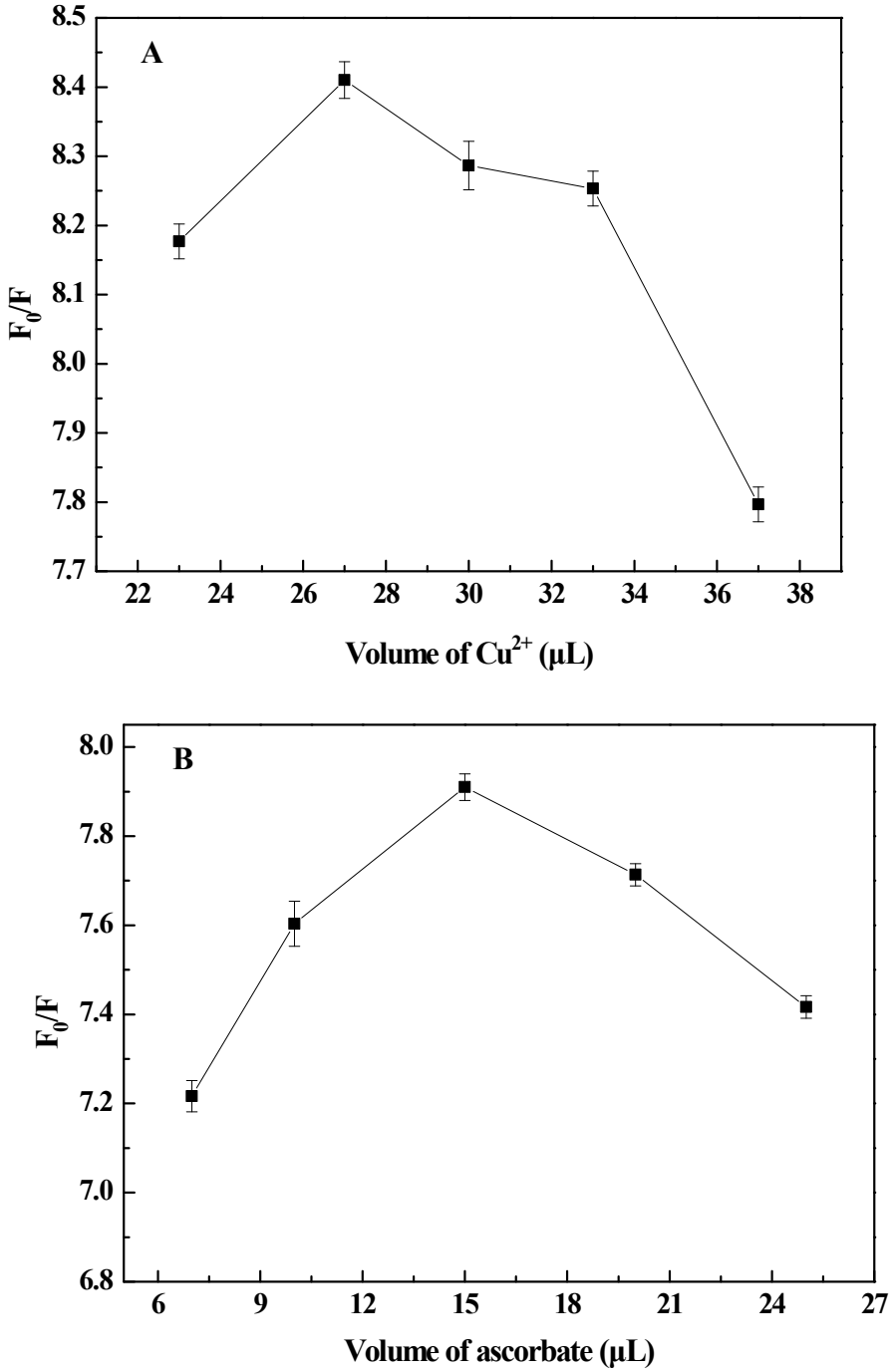

Fig. S5 Effect of Cu^{2+} amount (A) and ascorbate amount (B) on the fluorescence intensity of dsDNA-CuNCs without the addition of UDG. Conditions: 5×10^{-6} M dsDNA; 5 U/mL UDG, 0.05 M sodium ascorbate,

0.01M Cu^{2+}; Tris–HCl 20 mM, pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$.

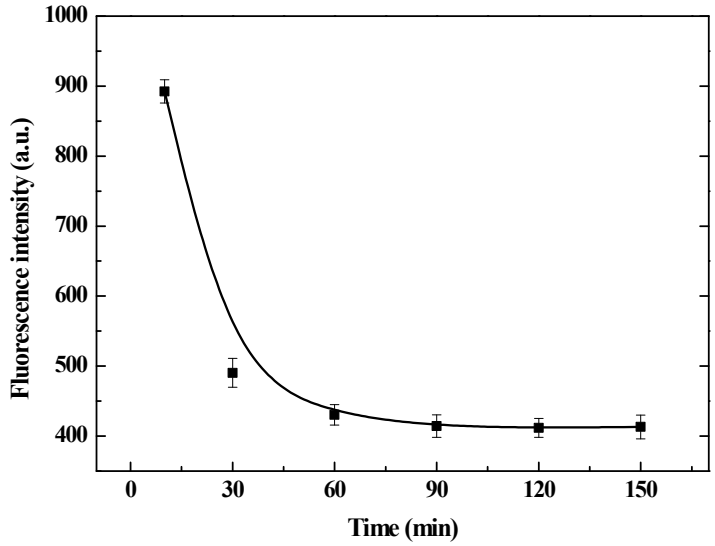

Fig. S6 Plot for the kinetics of the enzymatic reaction. Conditions: 5×10^{-6} M dsDNA; 0.05 M sodium ascorbate, 0.01 M Cu^{2+}; UDG: 5 U/mL; Tris-HCl 20 mM, pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$.

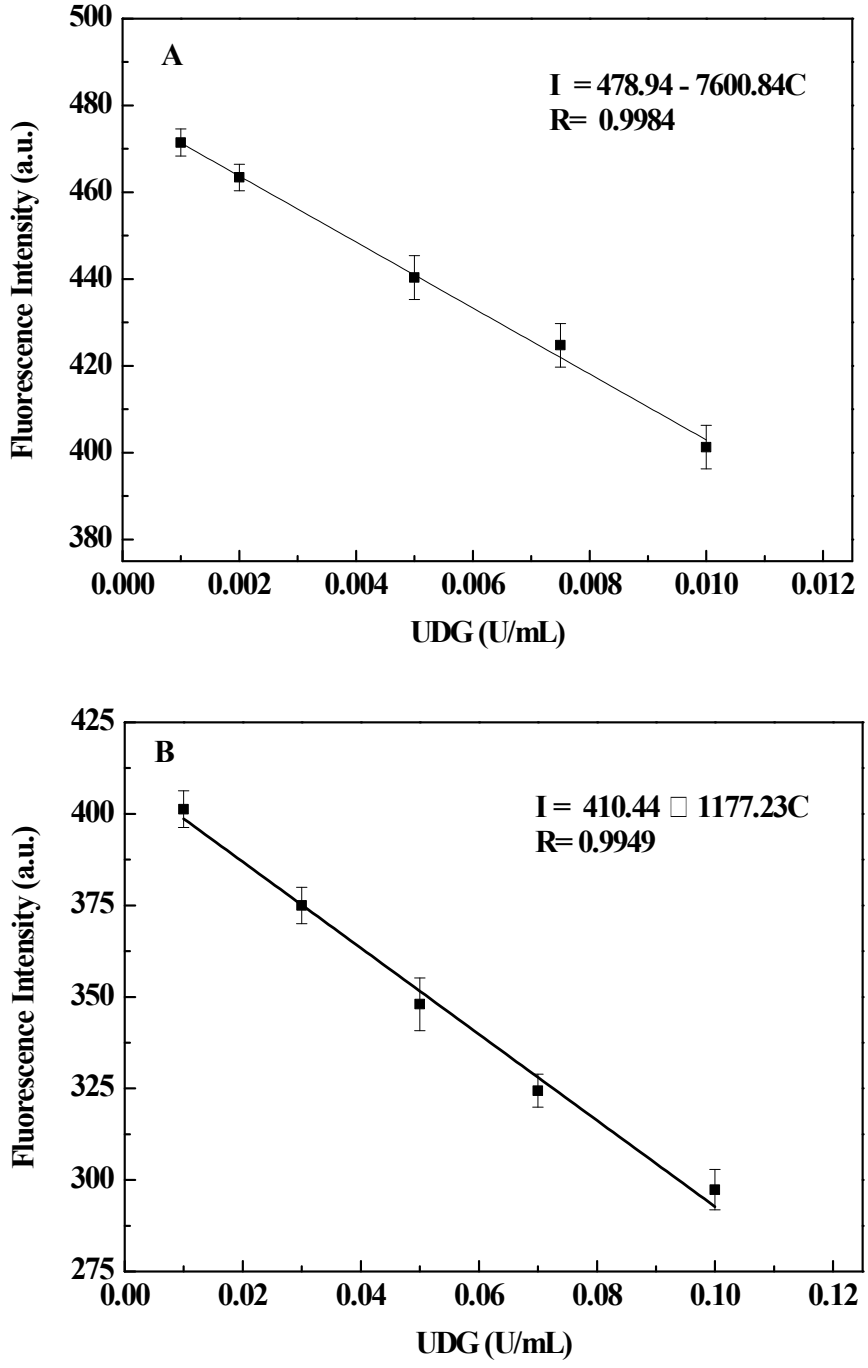

Fig. S7 Linear relationship between the fluorescence intensity and UDG concentration from 0.001 U/mL to 0.1 U/mL. Error bars represent the

standard deviations of three independent measurements.

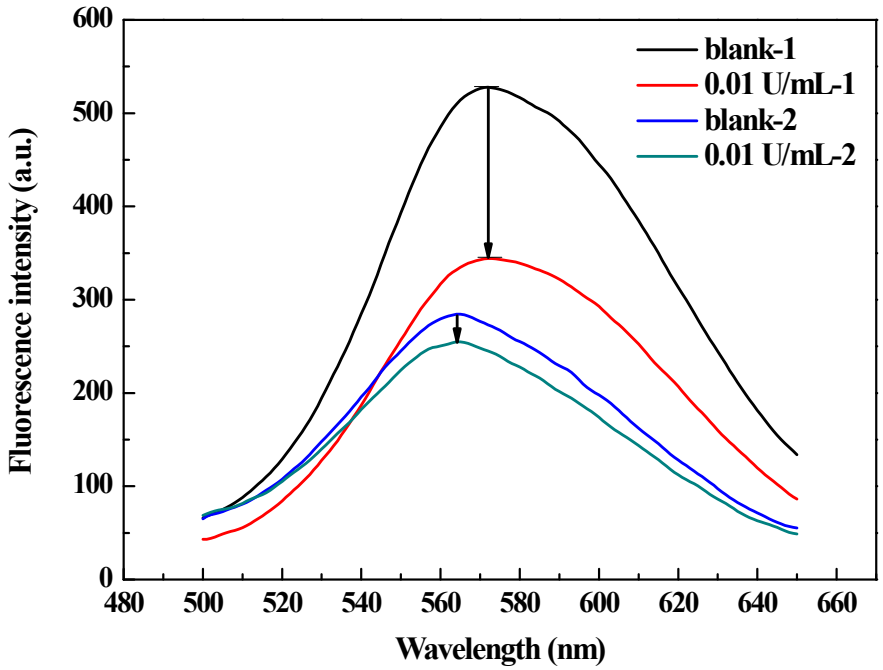

Fig. S8 Fluorescence spectra of the obtained CuNCs under different conditions. Black line: poly(AT-TA) dsDNA in the absence of UDG; red line: poly(AT-TA) dsDNA in the presence of UDG; blue line: the common dsDNA (5′-CGTAC**U**TAGAAUTATCTUGACA-3′; 3′-GCATGAATC TTAATAGAACTGT -5′) in the absence of UDG; green line: the common dsDNA in the presence of UDG. Conditions: 5×10^{-6} M dsDNA , 0.05M sodium ascorbate, 0.01M Cu^{2+}; Tris–HCl 20 mM, pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$.

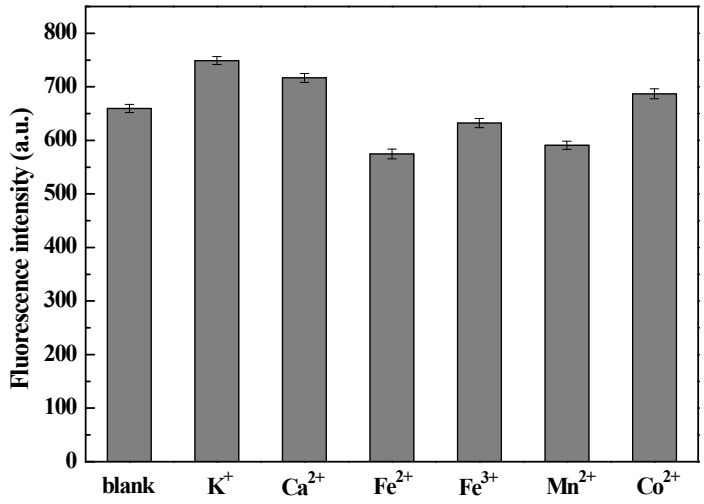

Fig. S9. The influence of common metal ions on the fluorescence response. Conditions: 5×10^{-6} M dsDNA; 0.05 M sodium ascorbate, 0.01 M Cu^{2+}; Tris-HCl 20 mM, pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$.

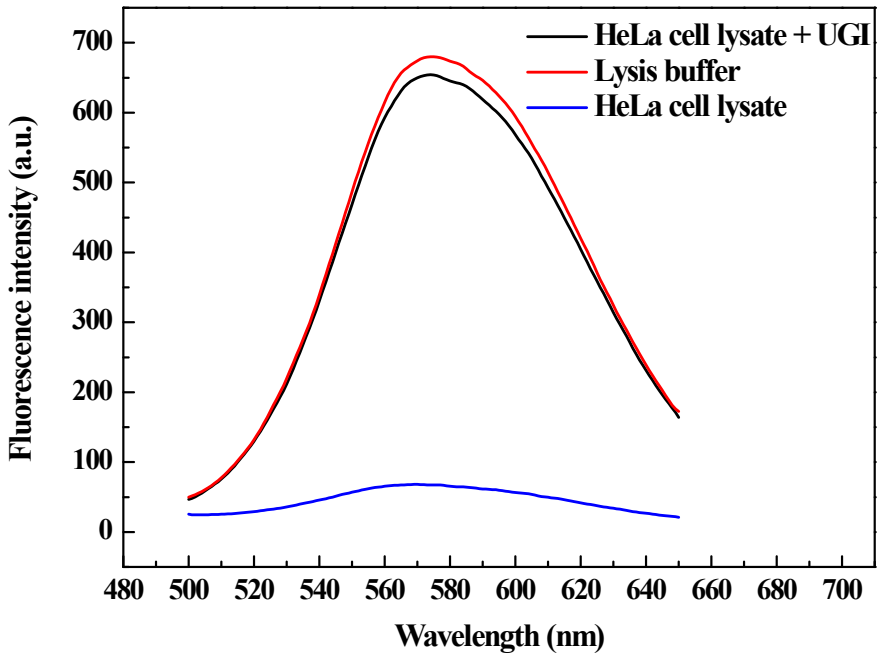

Fig. S10 Fluorescence emission spectra in the absence and presence of 1% HeLa cells lysate, and the inhibitory effect of 2.0 U/mL UGI on the UDG activity in the HeLa cells lysate. Conditions: 5×10^{-6} M dsDNA, 0.05 M sodium ascorbate, 0.01 M Cu^{2+}, 20 mM Tris–HCl (pH 8.0 at 25.0 °C, 10 mM NaCl, 2 mM $MgCl_2$).