Supporting information for

Ratiometric fluorescent monitoring of cerebral Cu^{2+} based on coumarin-labeled DNA coupled with Cu^{2+}-induced oxidation of o-phenylenediamine

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Contents:

1. Experimental Section

Chemicals and Materials. Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), o-phenylenediamine (OPD), hydrogen peroxide (H_2O_2) and all metal salts were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 5-hydroxytryptamine (5-HT), lactate (Lact), glucose, glycine (Gly), alanine (Ala), proline (Pro), phenylalanine (Phe), isoleucine (Ile), tyrosine (Tyr), valine (Val), serine (Ser), leucine (Leu), tryptophan (Trp), threonine (Thr), methionine (Met), asparagine (Asn), glutarnine (Gln), aspartic acid (Asp), glutamic acid (Glu), lysine (Lys), arginine (Arg), 3,4-dihydroxyphenylacetic acid (DOPAC), histidine (His), cysteine (Cys), reduced glutathione (GSH), ascorbic acid (AA), dopamine hydrochloride (DA), N-ethylmaleimide (NEM) and ascorbate oxidase (AAOx) were purchased from Sigma-Aldrich (St. Louis, MO). Coumarin-labeled ssDNA (C-ssDNA: 5'-coumarin 120-AACCTGGGGGAGTAT-3') was ordered from Sangon Inc. (Shanghai, China). Artificial cerebrospinal fluid (aCSF) was prepared by mixing KCl (2.4 mM), KH_2PO_4 (0.5 mM), NaCl (126 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM), $MgCl_2$ (0.85 mM) and $CaCl_2$ (1.1 mM) into distilled water, then the pH of solution was adjusted to 7.4. Tris-HCl buffer (10 mM, pH 7.4) was prepared using metal-free reagents in distilled water.

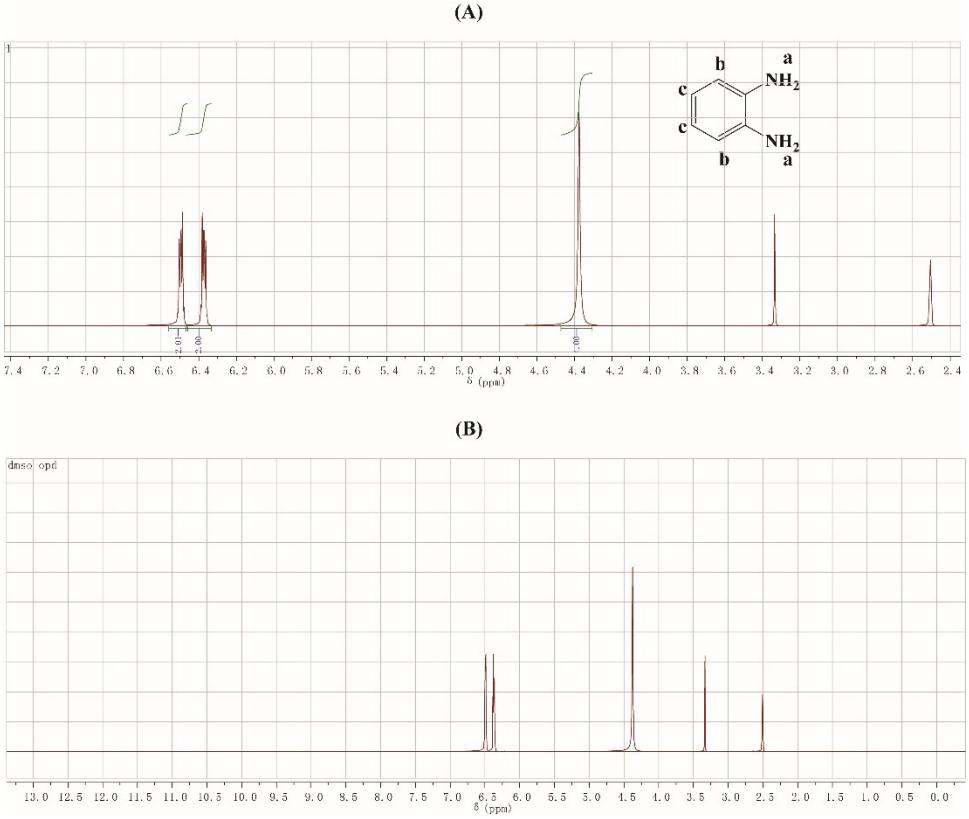
Apparatus and measurements. Nuclear magnetic resonance (NMR) measurements were performed on a Bruker Biospin AVANCE Ⅲ 500MHz NMR spectrometer, with DMSO-d6 as the solvent and the temperature was 298K. Mass spectrometry (MS) was carried out on a high-resolution time of flight mass spectrometer (maXis Impact + 1290 infinity, Bruker Daltonics Inc.). Zeta potential measurements were obtained by a Nano-ZS Zetzsozer ZEN3600 (Malvern Instruments Ltd., U.K.). Transmission electron microscope (TEM) image was recorded using a Transmission Electron Microscope (JEM-2100F, Japan) operated at 200 kV. Scanning electron microscopy (SEM) images and Energy-dispersive X-ray (EDX) spectra were recorded using an S-4800 scanning electron microscope (Hitachi, Japan). X-ray photoelectron spectroscopy (XPS) was obtained by a Thermo ESCALAB 250 X-ray photoelectron spectrometer (Shimadzu Kratos Corporation, Japan). Fluorescence spectroscopy was measured in a microplate reader (infinite M200 pro, TECAN, Switzerland) using a black 384-well microplate (Corning, U.S.A.) under 380 nm excitation. UV-vis absorption spectroscopy of oxOPD was also measured in a microplate reader (infinite M200 pro, TECAN, Switzerland) using a transparent 96-well plate (Corning, U.S.A.). Atomic absorption spectroscopy was performed on a TAS-990 atomic absorption spectrophotometer (Purkinje General Instrument Ltd., Beijing).

Gel Electrophoresis. A solution of OPD (10 mM) and Cu^{2+} (10 mM) was prepared as a reaction mixture. After incubated at 37 °C for 2h, the reaction mixture was subject to centrifugation at the speed of 13000 rpm for 15 min. The supernatant was collected, while the CuNPs precipitate, after washed three times, was redispersed in the same volume of distilled water. Then, we mixed the same concentration of C-ssDNA with the reaction mixture, the supernatant and CuNPs, respectively. 25 μL of the samples were mixed with 5 μL 6×DNA loading buffer for electrophoresis on 3% agarose gel matrix with Gelred nucleic acid dye in TBE buffer. Gel matrixes were imaged by using 2500 Tanon automatic digital gel image analysis system.

Detection Procedures. For Cu^{2+} assay, 10 μL of 1.5 μM C-ssDNA, 10 μL of 10 mM OPD aqueous solution, 10 μL of Tris-HCl buffer (100 mM, pH 7.4) and 10 μL of Cu^{2+} solution with varied concentrations (0 – 200 μM) were sequentially added to a 1 mL calibrated test tube. The mixture was diluted to volume (100 μL) with distilled water and then further incubated at 37 °C for 20 min. After that, the mixture was measured by fluorescence spectroscopy at an excitation wavelength of 380 nm. The two fluorescence emission spectra and fluorescence intensities at 452 nm and 576 nm were obtained respectively. For the determination of the level of striatum Cu^{2+} in rat brain microdialysates, the samples of microdialysates were added to the sensing system (total volume: 100 μL) and after incubating at 37 °C for 20 min, the fluorescence intensities were measured.

Animals and Surgery. All surgeries involving animals were conducted with approval of the Animal Ethics Committee in East China Normal University, China. *In vivo* microdialysis experiments were carried out as reported previously. Briefly, adult male Sprague-Dawley normal rats (250-300 g, Shanghai SLAC Laboratory Animal Co. Ltd., China) were housed under a constant 12-hour light-dark cycle with ad libitum access to standard laboratory food and water. The normal rats were anaesthetized with chloral hydrate (initial dose of 350 mg/kg, (i.p.). The anaesthetic rat were positioned onto a stereotaxic frame and the body temperature of the animal was maintained at 37°C with a heating pad during the process of the whole operation. The microdialysis probe was implanted in the striatum at the site of 2.5 mm anterior to bregma, 2.5 mm lateral from midline, and 7.0 mm below dura within 30 min slowly and carefully to reduce injury to the rat. The prepared aCSF was externally perfused with pump at 2 μL/min into inlet for at least 60 min for equilibration. Finally, at least 200 μL solution was obtained and used without further treatments.

2. Supplementary Figures



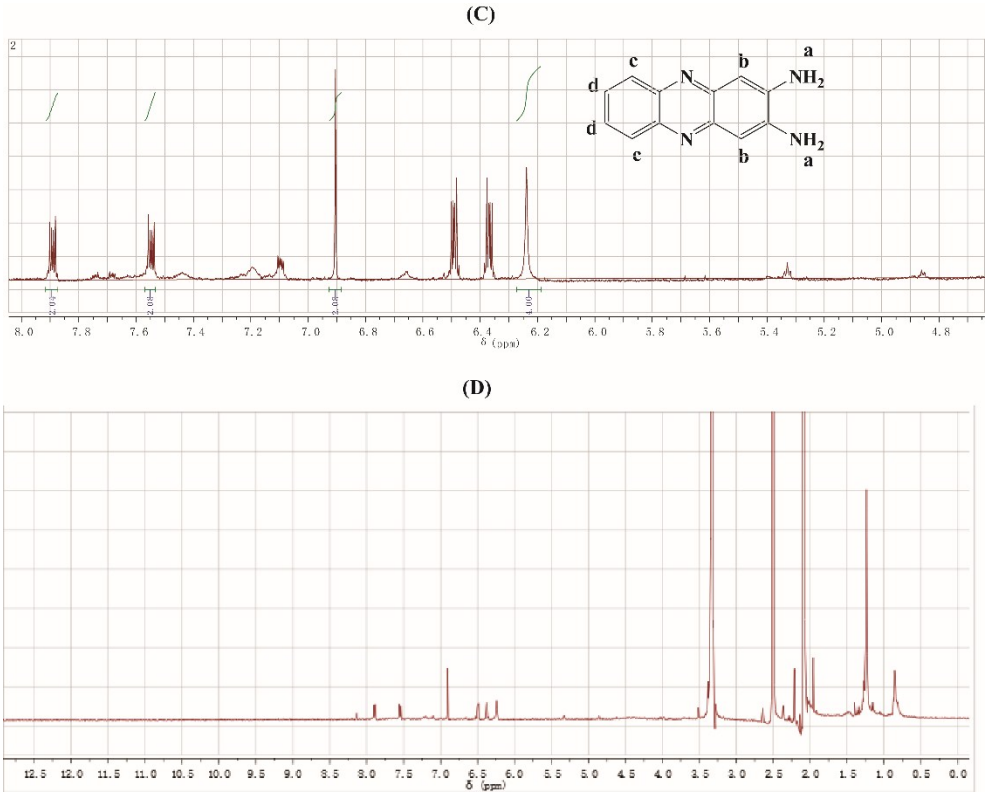


Figure S1. The ^{1}H NMR (A) OPD and (C) the resultant oxOPD; and the full spectra spectrometry of of ^{1}H NMR (B) OPD and (D) the resultant oxOPD from the incubated mixture solution of Cu^{2+} and OPD.

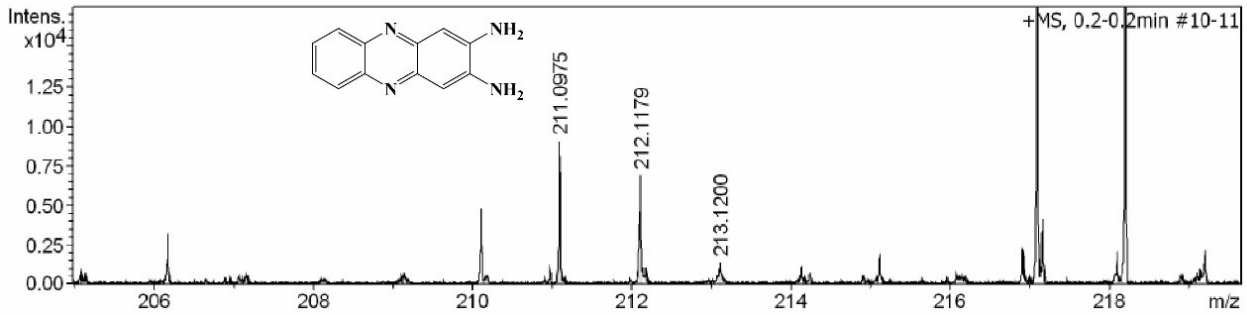


Figure S2. The MS spectrometry of the resultant oxOPD from the incubated mixture solution of Cu^{2+} and OPD.

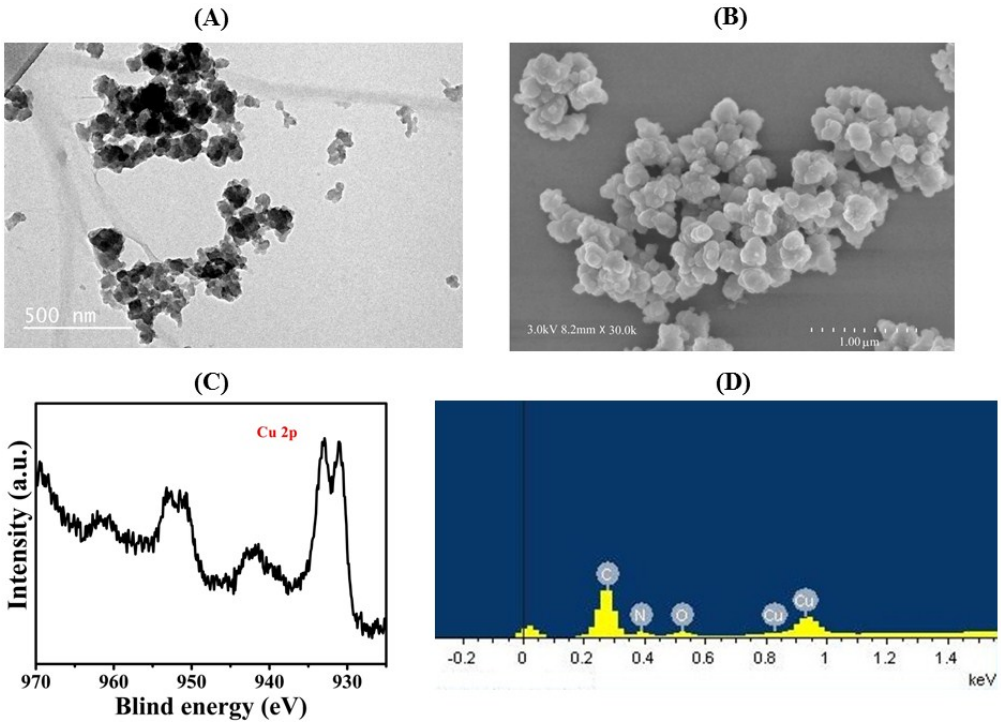


Figure S3. (A) TEM image of copper nanoparticles (CuNPs). (B) The SEM image of CuNPs. (C) XPS spectrum of Cu 2p electrons of CuNPs. (D) Energy-dispersive X-ray (EDX) spectra of CuNPs.

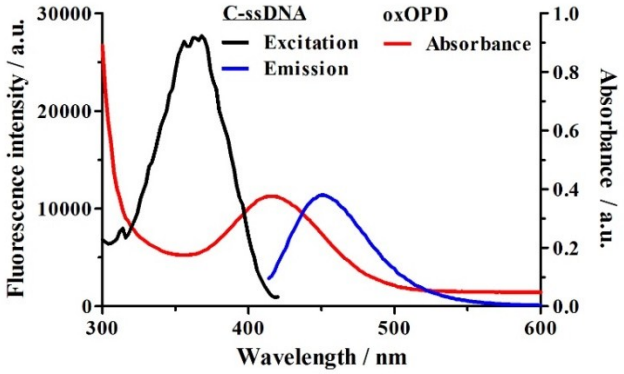


Figure S4. Excitation and emission spectra of C-ssDNA and UV-vis absorption spectra of the oxOPD in Tris-HCl buffer (10 mM, pH 7.4)

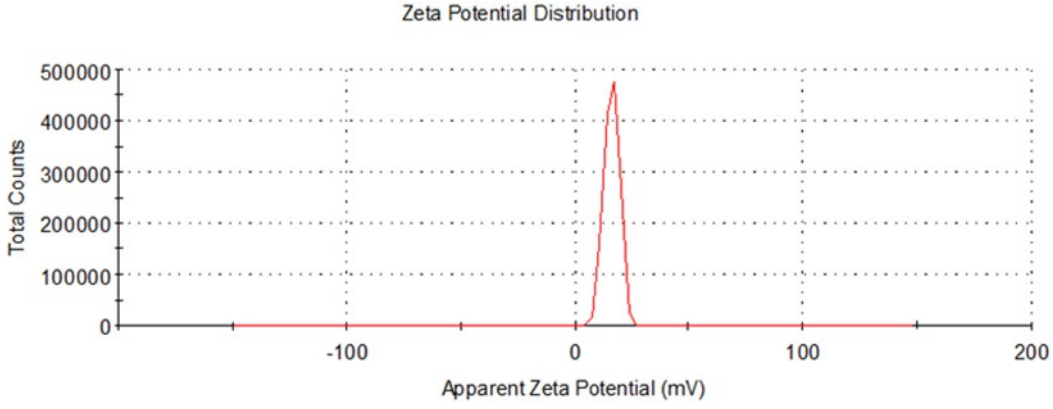


Figure S5. The Zeta potential of the final product of the incubated mixture solution of Cu^{2+} and OPD.

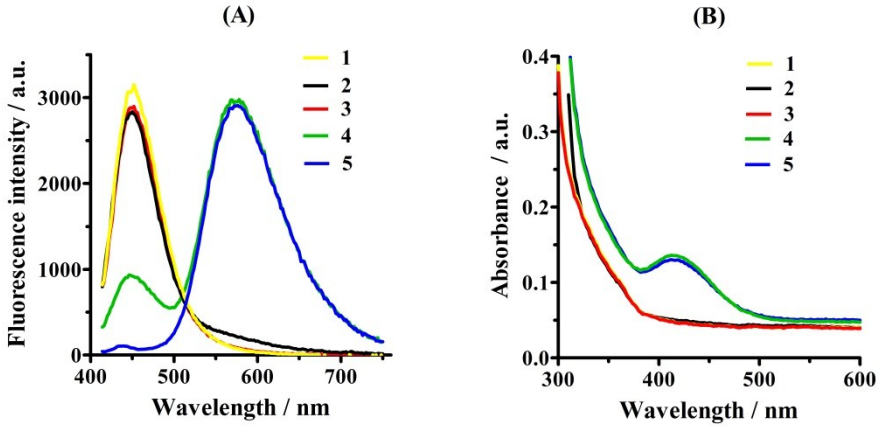


Figure S6. (A) Fluorescence emission spectra and (B) absorbance of the C-ssDNA (curve 1), C-ssDNA+Cu^{2+} (curve 2), C-ssDNA+OPD (curve 3), C-ssDNA+OPD+Cu^{2+} (curve 4) and OPD+Cu^{2+} (curve 5) in 10 mM Tris-HCl buffer at pH 7.4 for 37 °C for 20 min . The final concentrations or activity of the C-ssDNA, Cu^{2+}, and OPD are 0.15 μM, 10 μM, and 1 mM, respectively.

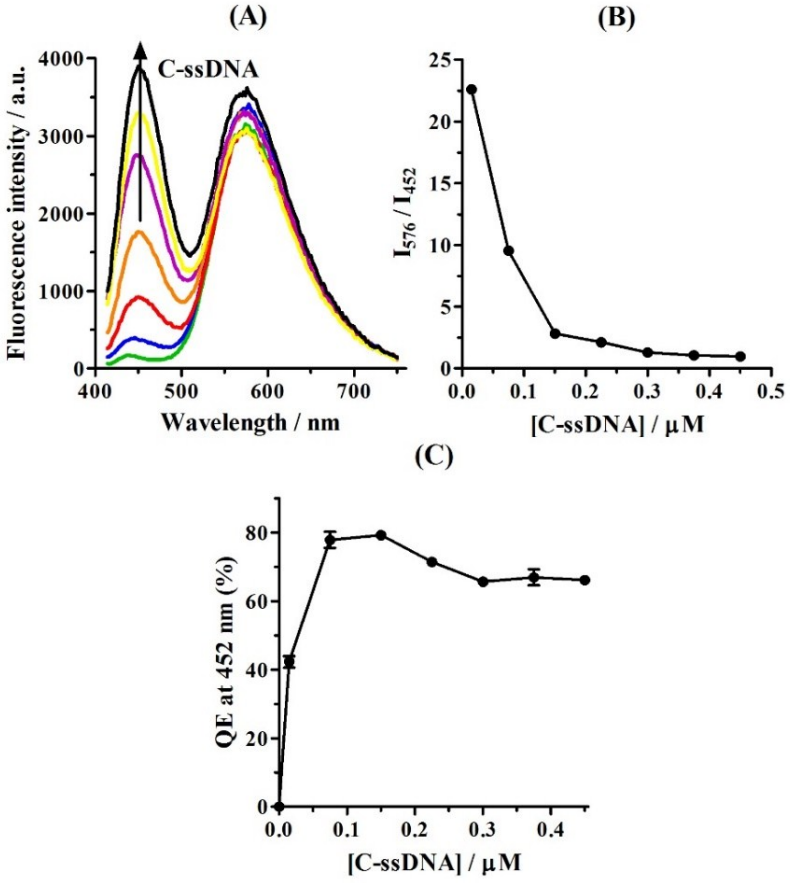


Figure S7. (A) Fluorescence emission spectra of different concentrations of C-ssDNA (0.015, 0.075, 0.150, 0.225, 0.300, 0.375 and 0.450 μM) incubated with OPD and Cu^{2+} after 20 min at 37 °C. (B) Plot of the fluorescence intensity ratio against the C-ssDNA concentrations. (C) Quenching efficiency at 452 nm upon the addition of different concentrations of C-ssDNA (0.015, 0.075, 0.150,

0.225, 0.300, 0.375 and 0.450 μM) into the OPD (1 mM) and Cu^{2+} (10 μM) at 37 °C for 20 min.

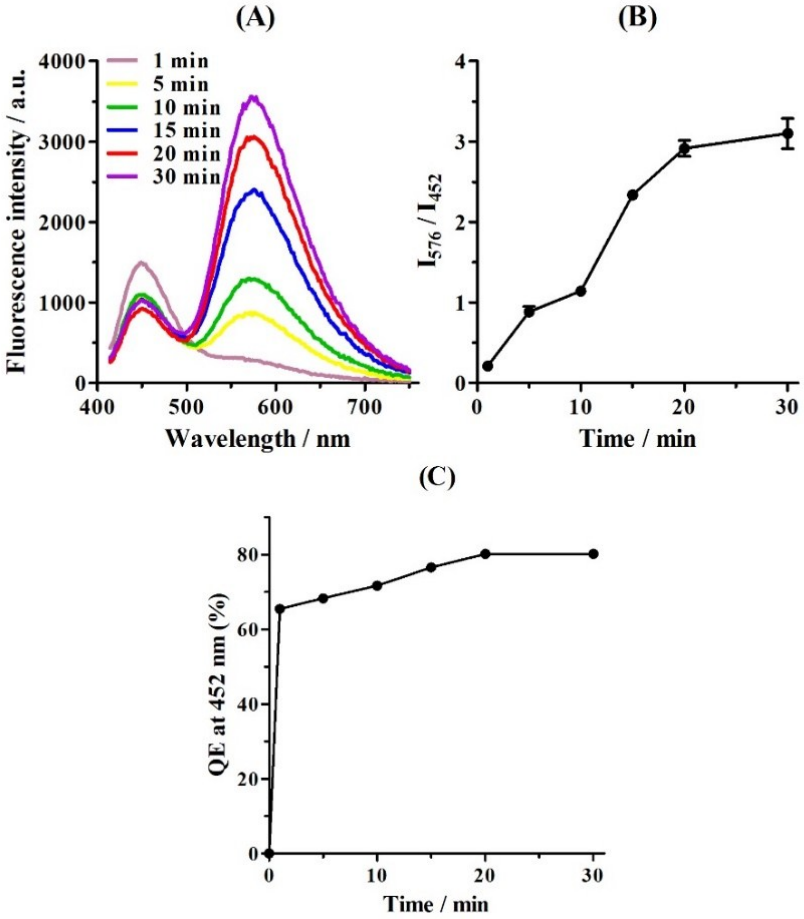


Figure S8. (A) Time-dependent changes of fluorescence emission spectra of the sensing system and (B) Effect of reaction time on the fluorescence intensity ratio (I_{576}/I_{452}) of the C-ssDNA based biosensors system to Cu^{2+} detection. (C) Effect of reaction time on the quenching efficiency at 452 nm.

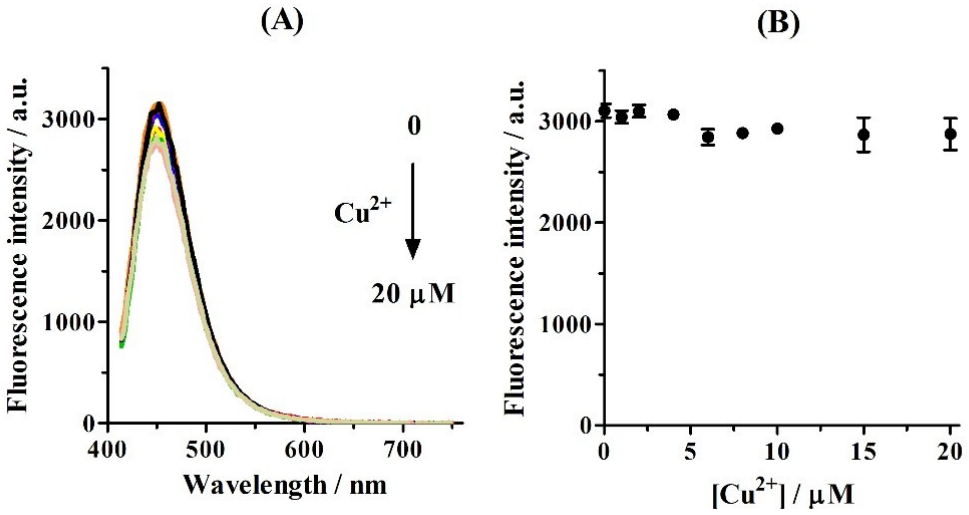


Figure S9. (A) Fluorescence emission of the C-ssDNA solution in the presence of different concentration of Cu^{2+} (0, 1, 2, 4, 6, 8, 10, 15 and 20 μM). (B) Quenching efficiency at 452 nm upon the addition of different concentrations of Cu^{2+} (0, 1, 2, 4, 6, 8, 10, 15 and 20 μM) into C-ssDNA at 37 °C for 20 min.

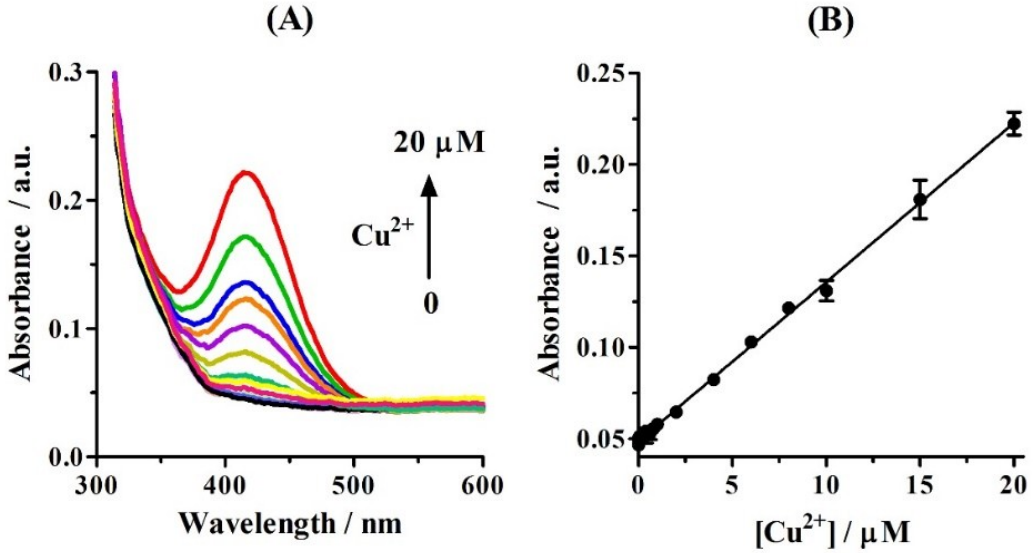


Figure S10. (A) UV-visible spectral change observed for C-ssDNA and OPD upon the addition of different concentrations of Cu^{2+} from bottom to top were 0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 4, 6, 8, 10, 15 and 20 μM respectively. (B) The linear relationship between the absorption peak around 418 nm and the concentration of Cu^{2+}. The regression equation could be described as $Y = 0.0087X + 0.0486$ ($R^2 = 0.9947$) with the detection limit of 10 nM.

Table S1. The analytical performance of various methods for Cu^{2+} sensing.

Methods	Reagents	Linear range (μM)	LOD (μM)	Ref.
Colorimetric	Fluorescein hydrazone	0.25-14	0.25	1
Colorimetric	Rhodamine derivative	1.0-10	0.17	2
Electrochemical	Gold nanoparticles	0.30-1.40	0.30	3
Electrochemical	[PAH–GO]n/GCE	0.50-50	0.35	4
Atomic absorption spectrometric	DDTC	0.40-4.69	0.052	5
Photoelectrochemical	Z-scheme BiOI-CdS	0.10-100	0.020	6
Raman spectrometric	Cys-AuNSs	8.50-40	10	7
Fluorescent	DNAzyme	0.08-30	0.021	8
Fluorescent	C-ssDNA/OPD	0.01-20	0.0043	This work

The *t* test and the statistical comparison.

The AAS method was taken as a standard method to evaluate the accuracy of our method. A *t* test was made to determine whether the means obtained between our method and the AAS method are distinct. The *t* value was calculated according to equation 1:

$$t = \frac{\left|\overline{X}_1 - \overline{X}_2\right|}{S}\sqrt{\frac{n_1 n_2}{n_1 + n_2}} \qquad (1)$$

in which $s = \sqrt{\frac{s_1^2(n_1-1)+s_2^2(n_2-1)}{(n_1-1)+(n_2-1)}}$, s_1 and s_2 represent the standard deviation of determined results by the present method and the AAS method, respectively. n_1 and n_2 are sample numbers of our method and the AAS method (herein, $n_1 = 3$, $n_2 = 3$). If the *t* value is smaller than standard *t* value, 2.92 (a = 0.1), the result obtained from the present method was considered to be in agreement with that obtained from the AAS method.

3. References

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