Supporting Information

EXPERIMENTAL SECTION

Instrumentation

UV absorbance measurements were carried out on a JASCO V-550 UV-vis spectrophotometer, equipped with a Peltier temperature control accessory. Fluorescence spectra were measured on a JASCO FP-6500 spectrofluorometer equipped with a temperature-controlled water bath. All spectra were recorded in a 1.0 cm path length cell. FT-IR characterization was carried out on a BRUKE Vertex 70 FT-IR spectrometer. The sample was thoroughly ground with exhaustively dried KBr. AFM measurements were performed using Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). TEM images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. TGA was performed with a Pyres 1 TGA apparatus (Perkin Elmer, MA) at a heating rate of 10 ℃ min^{-1} from 50 to 900 ℃ under nitrogen atmosphere. Electrochemical measurements were performed with a CHI 660B Electrochemistry Workstation (CHI, USA). A three-electrode setup was used with a common Ag/AgCl reference and a Pt wire auxiliary electrodes placed in the cencral buffer solution. Differential-pulse voltammetry (DPV) measurements were carried out with a CHI 660B electrochemistry workstation (CHI, USA) in a 0.1 M NaCl solution with 0.1 M $NaNO_3$ as an electrolyte for the electrochemical measurement from -0.6 V to 0.3V versus a Ag/AgCl (3 M KCl) reference electrode. EIS was performed using Solartron instrument with a S11255 HF Frequency response analyzer in 100 mM PBS containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture with 1 M KCl as the supporting electrolyte. The impedance spectra were recorded within the frequency range of 10^{-2}-10^5 Hz. The amplitude of the applied sine wave potential in each case was 5 mV.

Materials

Graphite was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Meso-tetra(4-carboxphenyl)-porphrine (TCPP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino) ethanesulfonic acid (MES), Tween 20 solution was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrazine solution (85 %) and ammonia solution (25 wt%) were provided by Beijing Chemicals Inc (Beijing, China). Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Alfa Aesar. Other chemicals including amino acids and GSH were obtained from Sigma-Aldrich. All aqueous solutions were prepared using ultrapure water (18.2 MΩ, Milli-Q, Millipore). The oligonucleotides used in this work were offered by Biotechnology Inc. (Shanghai, China).

Preparation of GO, TCPP/CCG and CCG: GO was synthesized from graphite by a modified Hummers method.[1] TCPP/CCG was prepared by following the procedure: 1.5 mL GO solution (2.7 mg mL^{-1}) and 15.8 mg TCPP power were mixed, add H_2O to 20 mL. After 0.5 h ultrasonication, the whole solution was stirred at 70 ℃ overnight. Subsequently, 3.2 μL hydrazine solution and 64 μL ammonia solution were added to the above solution and the resulting mixture was held at 95 ℃ for 1 h under vigorous agitation. The product was subsequently filtered through a Nylon membrane with 0.22 μm pores thoroughly washed with water and then dried under vacuum at room temperature. CCG solution was obtained by the similar methods, just no TCPP as stabilizer during the reduction process.

Cell Culture and Cell Extract Preparation: The cell lines: A549 cells, HeLa cells and NIH3T3 cells were cultured in flasks in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum, penicillin, and streptomycin (100 μg mL^{-1}) in an 5 % CO_2-humidified chamber at 37 ℃. NMM-treated A549 cells were cultured in the medium containing 0.25 μM NMM. All of the cells were cultured for 72 h. The cells were collected and separated from the medium by centrifugation at 3000 rpm for 5 min, then washed twice with a sterile PBS (pH 7.2). Cell lysates were generated by lysing cells with sonication (30 % amplitude, 3 pulse) for 1 min.

MTT Assay: The toxicity of TCPP/CCG to cells was measured by MTT assay. Briefly, A549 cells were plated at a density of 1×10^4 cells per well in 100 μL of RPMI medium in 96-well plates and grown for 24 h. The cells were then exposed to a series of concentrations of TCPP/CCG composite for 24 h, and the viability of the cells was measured using the methylthiazoletetrazolium method. Controls were cultivated under the same conditions without the addition of the nanocomposites. Then 5 μL of MTT (5 mg mL^{-1}) was added into the wells and further incubated for an additional 4 h. Subsequently, the supernatant was discarded, followed by the additional of 100 μL DMSO into each well and incubation in the shaker incubator with gentle shakes. Then the optical density (OD) was read at a wavelength of 570 nm. Relative inhibition of cell growth was expressed as follows: $\%= (1–[OD]_{test}/[OD]_{control})\times100$.

Electrochemical Detection of GSH: The glassy carbon electrodes (GCE, Φ=3 mm, CHI) were polished successively with 1.0, 0.3, and 0.05 μm alumina (Buhler) and sonicated for 3 min before modification. 10 μL of TCPP/CCG suspension (50 μg mL^{-1}) was casted on the pretreated GCE and dried under an infrared lamp. The activation solution (200 mM EDC and 50 mM NHS in MES buffer) was then dropped on the surface to activate the carboxyl group for 0.5 h. After rinsing with 10 mM phosphate buffer solution (PBS, pH=7.4), 10 μL hDNA (1 μM) in buffer (10 mM Tris-HCl, 1 mM EDTA, 0.1 M NaCl, pH 7.4) was immediately dropped onto the surface and then incubated for 4 h. The surface was then washed by PBS buffer and subsequently immersed into 1 M ethanolamine (pH 8.5) solution for 5 min to to passivate the unreacted NHS ester, then washed by PBS buffer again. The modified electrodes were stored in air prior to use.

For the in situ formation of hDNA-templated AgNPs, the modified electrodes were immersed in 800 μM $AgNO_3$ in buffer (20 mM HEPES, 100 mM $NaNO_3$, pH 7.4) for 1 h and following washed with buffer. Subsequently, the modified electrode were dipped into a freshly prepared $NaBH_4$ (10 mM in HEPES buffer) for 10 min and washed with buffer.

For electrochemical detection, the prepared electrode was incubated with different concentration of GSH solution. After reacted 1 h and rinsed briefly using PBS buffer,

the electrodes were incubated with 10 μL prepared mixture solution containing 10 μM H_1 and 10 μM H_2. Finally, the prepared electrode was incubated in 20 μM MB solution for 20 min, followed by being washed with distilled water. The electrode was then placed in a 0.1 M NaCl solution with 0.1 M $NaNO_3$ as an electrolyte for the electrochemical measurement from -0.6 V to 0.3 V versus Ag/AgCl (3 M KCl) reference electrode.

GSH kit: Total GSH assay kit (Beyotime, China) was used as an alternative to evaluate the cellular GSH levels. The GSH kit was based on the use of glutathione reductase for the quantification of GSH. The principle is as follows: 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent) reacts with the sulfhydryl group of GSH to produce a yellow-colored 5-thio-2-nitrobenzoic acid (TNB) and GSSG. Then, GSSH is reduced by glutathione reductase to recycle the GSH and produce TNB. Measurement of the absorbance of TNB at 412 nm can be employed to evaluate the GSH level of the sample.

Table S1: DNA sequences used in the work.

Name	Sequence
hDNA	5'-$NH_2(CH_2)_6$TTTTTTAGCACAGGATTCCGCGTCGCTTAA-3'
H1	5'-GGATTCCGCGTCGCTTAACAAAGTTTAAGCGACGCGGAATCCTGTGCT-3'
H2	5'-ACTTTGTTAAGCGACGCGGAATCCAGCACAGGATTCCGCGTCGCTTAA-3'

Table S2. Comparison between the current method and other reported techniques for direct analysis of GSH.

Analytical method	Mechanisms of the strategy	Sensitivity data
MnO_2 nanosheet based electrochemiluminescence assay[2]	GSH reduced the MnO_2 nanosheets to Mn^{2+}, inhibiting the electrochemiluminescence of lucigenin	3.7 nM
Hg(II)-mediated "signal-on'' electrochemical assay[3]	Complexation between GSH and Hg(II) resulted in an increase in probe flexibility and increased MB signal	5 nM
MnO_2 nanosheet-modified upconversion nanoparticles based fluorescence assay[4]	MnO_2-induced quenching effect could be reversed by adding a small amount of GSH	0.9 μM
Au naoparticles (AuNPs) based colorimetric assay[5]	The introduction of GSH competed with Arg-AuNPs for Hg^{2+}, preventing the aggregation of AuNPs	10.9 nM
Ratiometric fluorescence assay by coupling a DNA-ligand ensemble with Ag cluster formation[6]	Acridine orange was chosen as a reference and the fluorescence of Ag clusters decreased due to the stronger Ag-S interaction	63 nM
Gold nanoparticles (GNPs) based high-performance liquid chromatography (HPLC) assay[7]	Using nonionic surfactant-capped GNPs as postcolumn reagents for HPLC assay of biothiols due to biothiols induced the aggregation of GNPs	2.0 μM
Ratiometric electrochemical assay by DNA metallization-mediated hybridization chain reaction amplification (This work)	The introduction of GSH liberated AgNPs from DNA, then the released DNA triggered hybridization chain reaction and MB could intercalate into the double strand DNA polymer, resulting in the suppression of Ag signal and the enhancement of MB signal.	103 pM

Characterization of the Synthesized TCPP/CCG

The prepared TCPP/CCG suspension was stable for a few months at ambient temperature, while the nonfunctionalized CCG formed insoluble aggregates within a week. Furthermore, atomic force microscopy (AFM) demonstrated that the average topographic height of TCPP/CCG sample was about 2 nm, indicating the well-separated single sheet of composite TCPP/CCG (Fig. S1b).

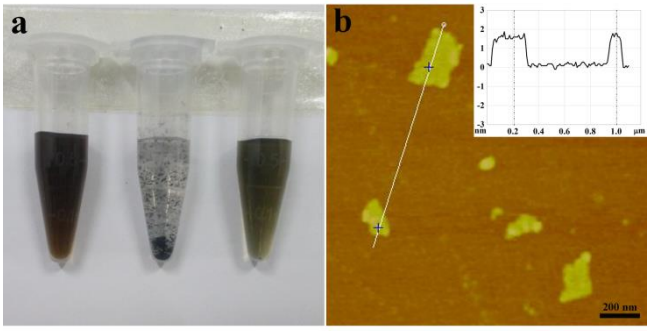

Figure S1. (a) Images (from left to right) of water dispersions of GO, CCG and TCPP/CCG. (b) Tapping mode (Inset: height analysis) of the AFM image of TCPP/CCG.

The fabrication process of TCPP/CCG was characterized by the absorption spectrum (Fig. S2a). The 230 nm UV absorption peak of GO shifted to 270 nm after using hydrazine as a reducing agent. The absorption spectrum of TCPP showed a strong absorbance at 413 nm as well as a couple of weak peaks in the longer wavelength, which were ascribed to the Soret band and Q-bands of TCPP, respectively. Upon adsorbed on graphene surface, the Soret band of TCPP shifted to 448 nm and the Q-bands red-shifted to 668 nm owing to the π-π stacking between graphene and porphyrin. Meanwhile, the fluorescence of TCPP was significantly quenched by graphene (Fig. S2b), indicating the occurrence of electron transfer between TCPP and CCG. Additionally, Fourier-transform infrared spectroscopy (FT-IR) also demonstrated that TCPP could strongly adsorb on the surface of graphene. As displayed in Fig. S2c, the emergence of absorption band at 1697 cm^{-1} in TCPP was assigned to C=O vibration of carboxyl groups, while it shifted to 1714 cm^{-1} in

TCPP/CCG, which might be ascribed to the hydrophobic and π-π stacking interactions between TCPP and CCG. The spectrum of TCPP/CCG also showed a broad band at 3414 cm^{-1} that corresponded to O-H stretching vibration of carboxyl groups. Thermogravimetric analysis (TGA) in Fig. S2d revealed 48.8 % mass loss between TCPP/CCG and CCG, which was the content of TCPP in the TCPP/CCG composite.

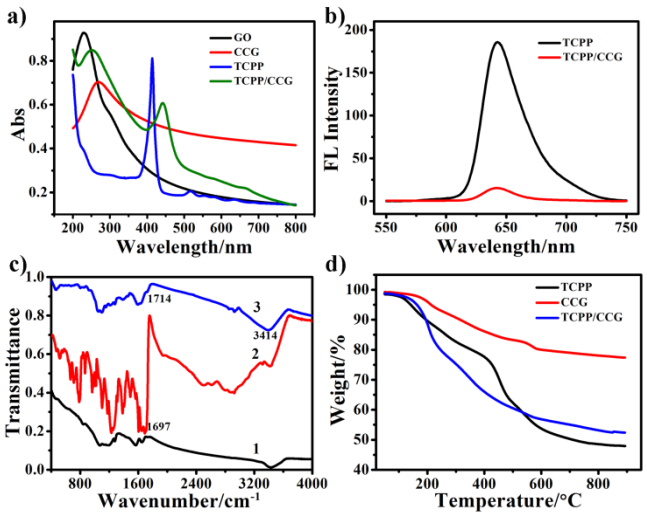

Figure S2. (a) Absorption spectra of GO, CCG, TCPP/CCG and TCPP solutions. (b) Fluorescence (λ_{ex}=430 nm) spectra of TCPP and TCPP/CCG in water. (c) FT-IR spectra of (1) CCG; (2) TCPP; (3) TCPP/CCG composites. (d) TGA curves of CCG, TCPP/CCG and TCPP composites.

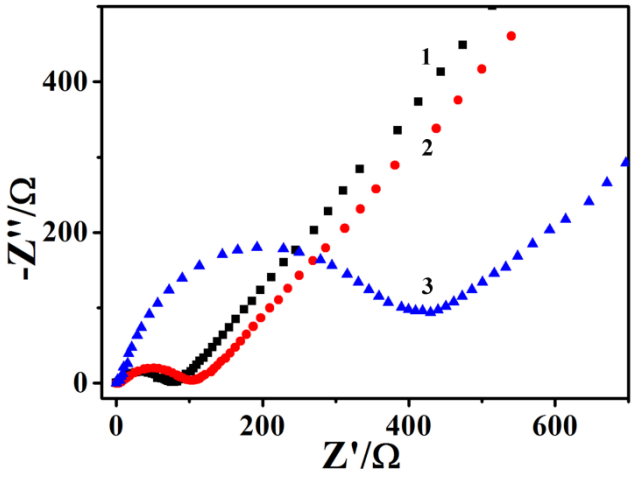

Figure S3. EIS data of (1) bare GCE; (2) TCPP/CCG modified GCE; (3) GO modified GCE.

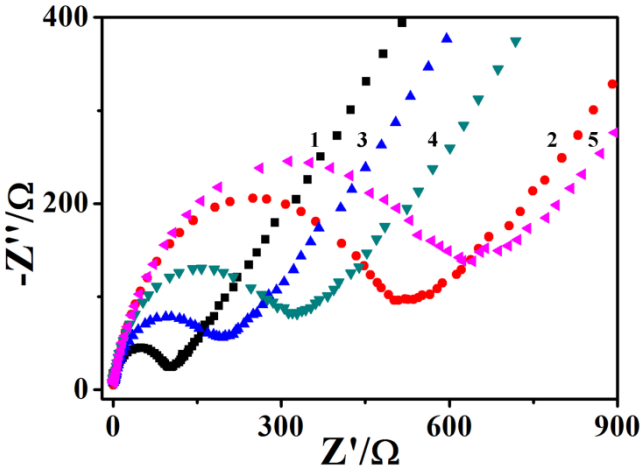

Figure S4. Electrochemical impedance spectroscopy of (1) TCPP/CCG modified electrode; (2) hDNA conjugated onto as-fabricated electrode in (1); (3) the in situ growth of AgNPs on electrode (2); (4) incubated (3) in GSH solution; (5) the HCR dsDNA polymers modified electrode.

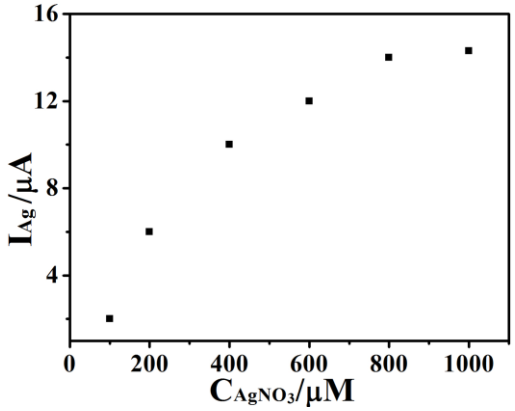

Figure S5. Optimize concentration of $AgNO_3$ used in the sensor construction.

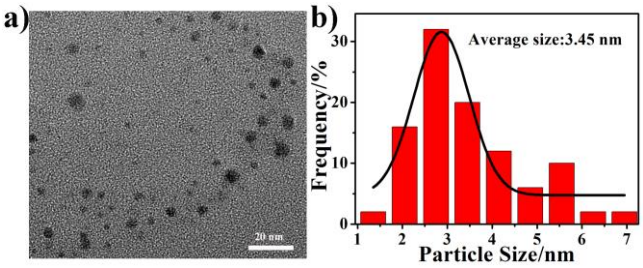

Figure S6. (a) TEM image of hDNA-templated silver deposition. (b) Size distribution analysis of AgNPs.

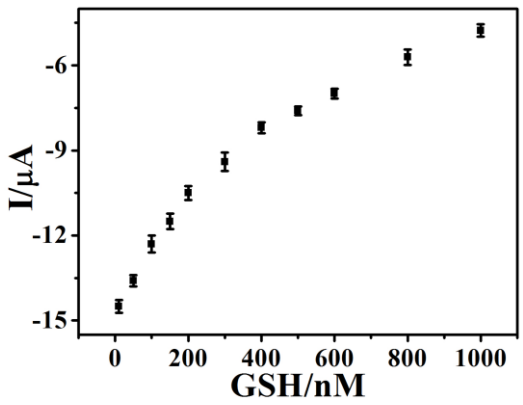

Figure S7. The relationship between the current I_{Ag} and the concentration of GSH.

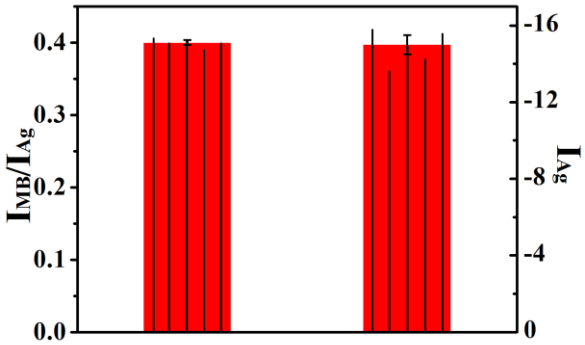

Figure S8. The reproducibility of the as-fabricated ratiometric electrochemical biosensor.

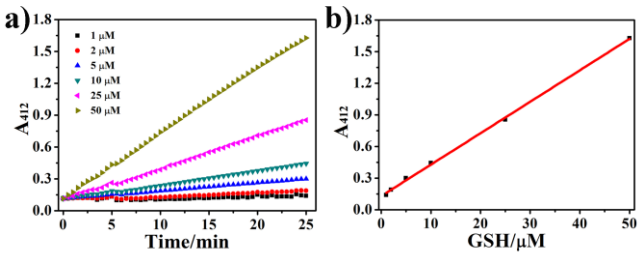

Figure S9. (a) Time-dependent absorbance changes at 412 nm of the commercial GSH kit at different concentrations of GSH. (b) The calibration curve of the commercial GSH kit for accurate quantification.

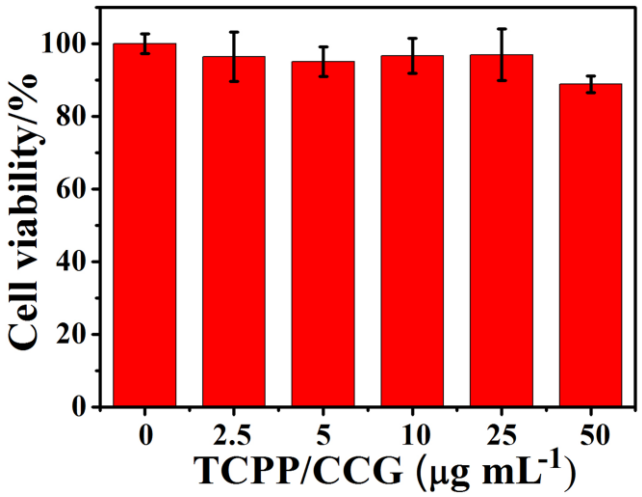

Figure S10. MTT array of TCPP/CCG composite.

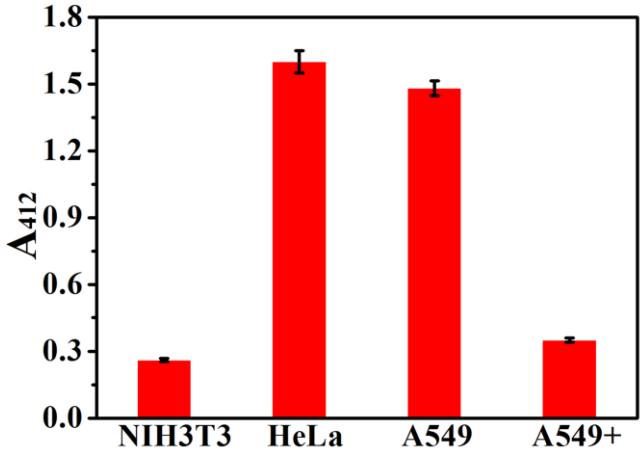

Figure S11. Cellular GSH levels evaluated by the commercial GSH kit.

References

(1). W. S. Hummers, R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339-1339.

(2). W. Gao, Z. Liu, L. Qi, J. Lai, S. A. Kitte, G. Xu, *Anal. Chem.*, 2016, **88**, 7654-7659.

(3). H. R. L. Z. Zhad, R. Y. Lai, *Chem. Commun.*, 2014, **50**, 8385-8387.

(4). R. Deng, X. Xie, M. Vendrell, Y.-T. Chang, X. Liu, *J. Am. Chem. Soc.*, 2011, **133**, 20168-20171

(5). Y. Xianyu, Y. Xie, N. Wang, Z. Wang, X. Jiang, Small, 2015, 11, 5510-5514.

(6). F. Cao, E. Ju, C. Liu, F. Pu, J. Ren, X. Qu, *Chem. Commun.*, 2016, **52**, 5167-5170.

(7). C. Lu, Y. Zu, V. W.-W. Yam, *J. Chromatogr. A*, 2007, **1163**, 328-332.