Supporting Information

Experimental Section

Materials and Methods:

Tetraethylorthosilicate (TEOS), Tris-(2-carbozyethyl) phosphine hydrochloride (TCEP) and (3-aminopropyl) trimethoxysilane (APTES) were purchased from Sigma-Aldrich and used as received without further purification. Graphite powder was obtained from Sinopharm Chemical Reagent (Shanghai, China). 4-Maleimidobutyric acid N-hydroxysuccinimide ester (GMBS) (97%) was obtained from Acros Organics and N-cetyltrimethylammonium bromide (CTAB) was obtained from Alfa Aesar. Hydrazine (85%) and hydrochloric acid were received from Beijing Chemicals Inc. (Beijing, China). All other reagents were all of analytical reagent grade and used as received. The aqueous solutions used in this work were prepared with nanopure water (18.2 M cm, Milli-Q, Millipore). The oligonucleotide used in this paper were offered by Biotechnology Inc. (Shanghai, China). The sequences of the DNA probes and targets used in this work:

15-mer-Assistant-Probe: 5′-SH-$(CH_2)_6$-ATG GAG GAC GTG TGC-3′

26-mer-Target Recognize-Probe: 5′-ACC AGG CGG CCG CAC ACG TCC TCC AT-3′

26-mer-target 0 mismatch: 5′-ATG GAG GAC GTG TGC GGC CGC CTG GT-3′

26-mer-target 1 mismatch: 5′-ATG GAG GAC GTG CGC GGC CGC CTG GT-3′

26-mer-noncomplementary target: 5′-AAA AAA AAA AAA AAA AAA AAA AAA

AA-3′

Transmission electron microscopic (TEM) images were obtained using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 KV. Scanning electronic microscopy (SEM) measurement was performed with a Hitachi S-4800 FE-SEM. AFM measurements were performed using a Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). FT-IR characterization was carried out on a BRUKE Vertex 70 FTIR spectrometer. The samples were thoroughly ground with exhaustively dried KBr. N_2 adsorptiondesorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. The samples were degassed at 150°C for 5 h. The pore size was determined following the BarrettJoiner-Halenda (BJH) method. Electrochemical measurements were performed with a CHI 660B Electrochemistry Workstation (CHI, USA). A three-electrode setup was used with a common Ag/AgCl reference electrode, a glassy carbon working electrode and a Pt wire auxiliary electrode placed in the central buffer solution. The glassy carbon electrodes (GCE, ø = 3 mm, CHI) were first polished successively with 1.0, 0.3 and 0.05 m alumina (Buhler) and sonicated for 3 min before modification.

Preparation of Fc-GSHs: The preparation of GSHs was followed by the literature with some modifications.[1,2] Briefly, 5.8 mL graphene oxide (GO) (3.8 mg/mL) aqueous solution was injected into 44.2 mL water mixed with 0.5 g CTAB and 20 mg NaOH, and then ultrasonically treated for 1 h. After magnetic stirring for 2 h at 40°C,

tetraethylor-thosilicate (TEOS, 400 μL dissolved in 1.6 mL ethanol) was slowly added to the above mixture and kept at 40°C for 12h. Then, 80 μL of hydrazine was additionally introduced into the above mixture, and then heated at 70 °C for 5 h. The obtained product was centrifuged and washed with warm ethanol for three times. The product was then mixed with 200 μL APTES in 50 mL ethanol and stirred for 12 h at 80 °C under reflux before centrifugation. Finally, the product was dispersed in 50 mL acetone stirred at 40 °C for 24 h. The product was collected by centrifugation and washed by warm ethanol for three times. The product GSHs-NH_2 was then placed under high vacuum to remove the remaining solvent in the mesopores. Ferrocenecarboxylic acid (Fc) was covalently conjugated onto amine-functionalized GSHs by using the cross-linking reagents EDC and Sulfo-NHS. In this work, 0.2 mmol Fc was dissolved in MES buffer (pH 6.3) followed by the addition of 0.2 mmol EDC and 0.5 mmol Sulfo-NHS. The mixture was then stirred at room temperature for 30 min to activate the carboxylic group of Fc. Subsequently, the pH was adjusted to 8.0 with concentrated PBS solution, and amine-functionalized GSHs solution (1 mg mL^{-1}) was added to the above solution, and the mixture was stirred for 4 h at room temperature. Fc-GSHs was collected by centrifugation to remove excess EDC, Sulfo-NHS and Fc. Fc-GSHs was dispersed in water and stored at 4 °C.

Fabrication of ratiometric electrochemical DNA sensor: A bifunctional cross-linker GMBS was used to functionalize Fc-GSHs with DNA probe, the procedure was according to the previous report.[3] Briefly, Fc-GSHs (10 mg) was well suspended in a mixture solution of phosphate buffered saline (PBS) buffer (100 mM

PBS, 150 mM NaCl, pH 7.3) and N,N-Dimethylformamide (DMF) (7:3) containing excess GMBS (4 mg) for several hours. The resulting particles were collected by centrifugation, extensively washed with DMF and PBS buffer. Further modification of DNA was performed by mixing TCEP pretreated DNA (20 nmol) with maleimide-modified Fc-GSHs in conjugation buffer (100 mM PBS, 1 M NaCl, pH 7.3) and stirred overnight at room temperature. The Fc-GSHs-DNA was recovered by centrifugation and washing with the conjugation buffer. The obtained Fc-GSHs-DNA nanohybrids were then dispersed in a solution containing redox-active molecules MB (1mg) in PBS solution (100 mM, pH 7.4) and stirred overnight to allow the guest molecules to diffuse into the pores of Fc-GSHs-DNA. The nanoparticles were then centrifuged and washed thoroughly with buffer to remove adsorbed molecules. The resulted nanomaterials were dissolved in hybridize solution (100 mM PBS, 1 M NaCl, pH 7.3) and mixed with the target recognize probe DNA, culturing for 2h at room temperature. The nanoparticles were then collected by centrifugation and washing.

Electrochemical detection: Fc-GSHs-DNA probes were redispersed in 20 μL buffer and subsequently dropped on the pretreated glassy carbon electrode and dried at room temperature. The surface was then washed by PBS solution and cultured with target DNA in 20 μL hybridize solution (100 mM PBS, 1 M NaCl, pH 7.3) for 1h. After carefully washed, the above electrodes were applied for electrochemical detection. For electrochemical detection, the modified electrodes were scanned in a common running PBS buffer (25 mM, 25 mM NaCl). DPV signals were obtained with a potential step of 5 mV, pulse amplitude of 50 mV, pulse width of 50 ms and a pulse

period of 200 ms.

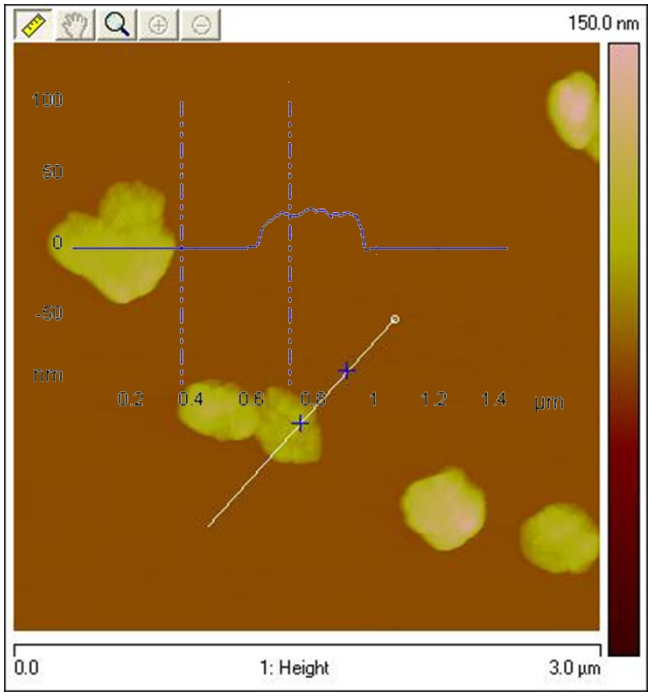

Fig. S1 Representative AFM images of the as-synthesized hybridized nanomaterials GSHs and the corresponding height profiles along the line in AFM images.

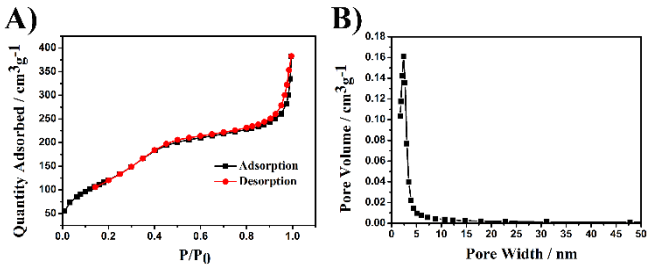

Fig. S2 BET nitrogen adsorption−desorption isotherm (A) and BJH pore size distribution curve (B) of GSHs.

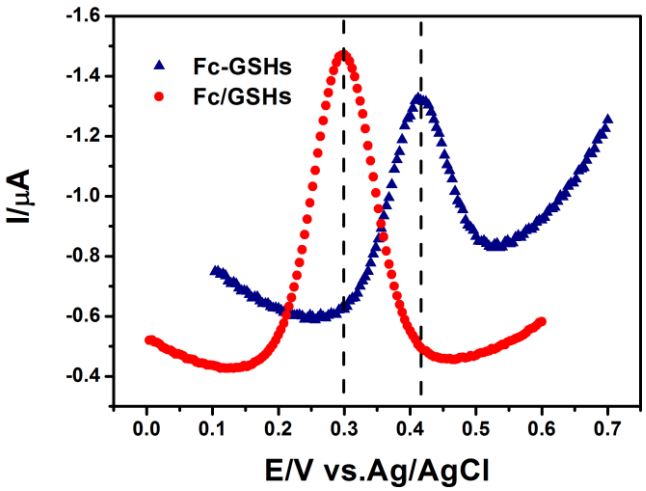

Fig. S3 DPV of Fc conjugated and Fc loaded GSHs modified glassy carbon electrodes.

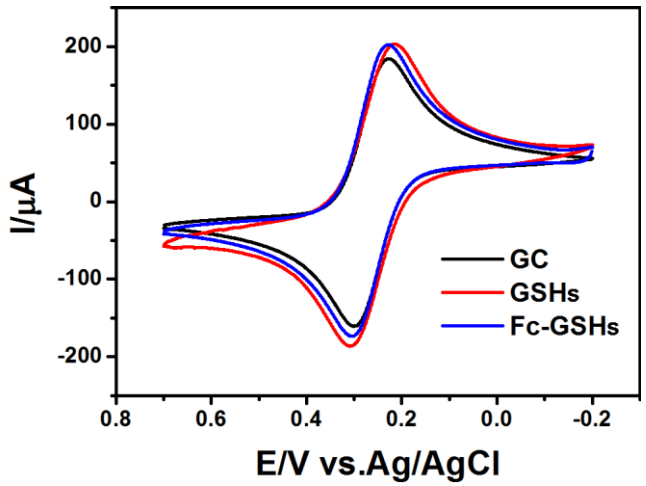

Fig. S4 Cyclic voltammograms of bare glassy carbon (GC) electrode, GSHs and Fc-GSHs modified electrode in 10 mM $K_3[Fe(CN)_6]$ containing 0.1M KCl.

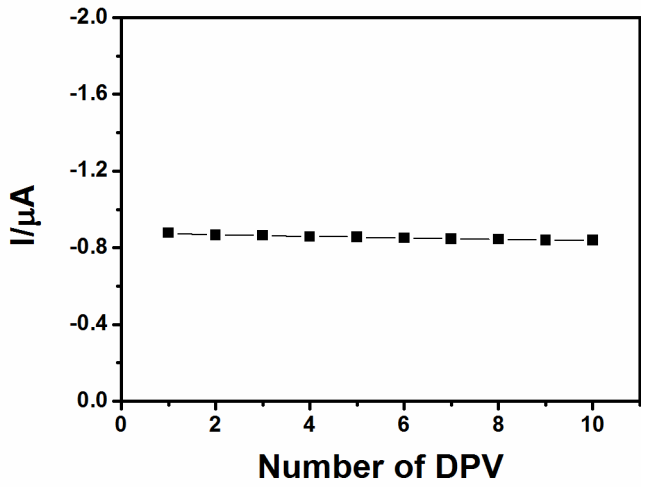

Fig. S5 The stability of modified electrode surface for 10 measurements. DPV peak current of Fc in Fc-GSHs was recorded as the readout signal.

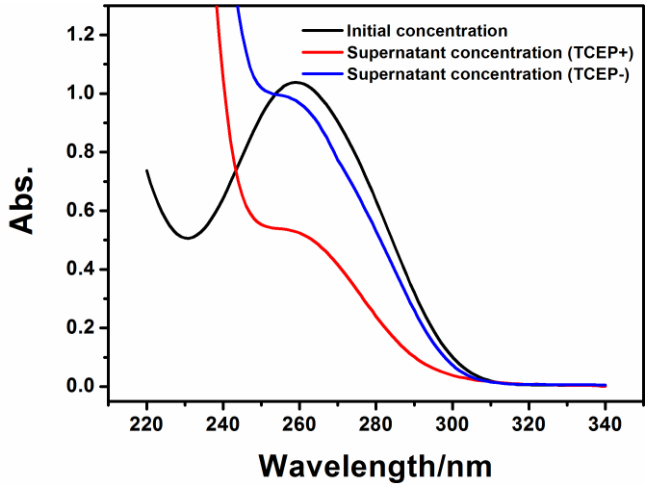

Fig. S6 The UV/Vis spectrum of the assistant DNA probe solution before reaction (black line), after cleavage by TCEP (red line) and then react with maleimide-functionalized GSHs, and after reaction with maleimidefunctionalized GSHs (without cleavage by TCEP) (blue line).

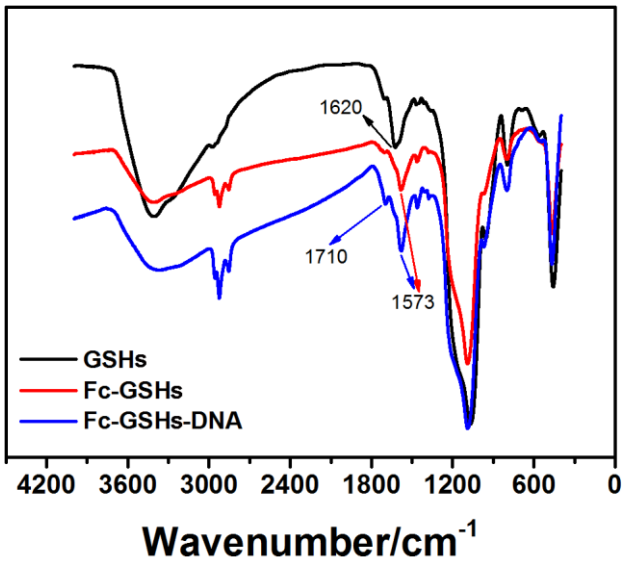

Fig. S7 FTIR spectra of GSHs, Fc-GSHs, and Fc-GSHs-DNA.

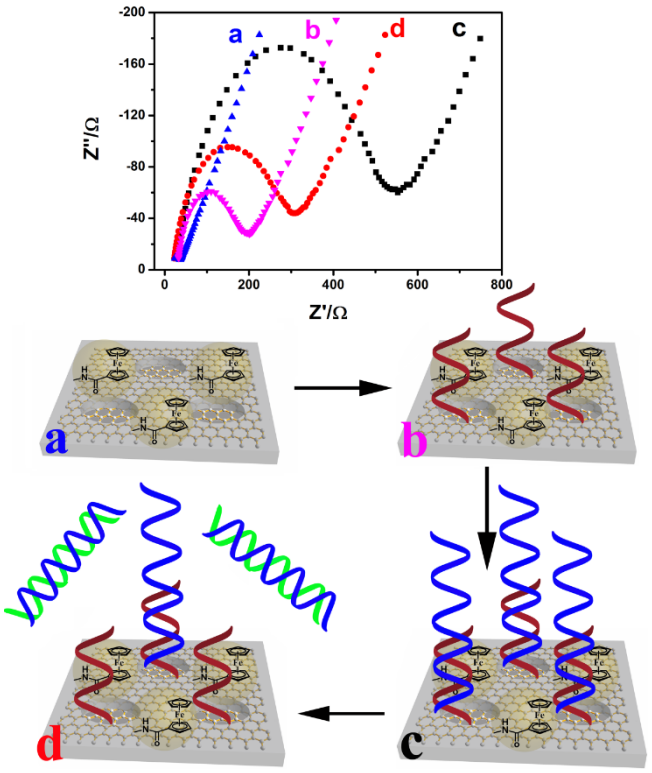

Fig. S8 Electrochemical impedance spectroscopy of a) bare Fc-GSHs modified electrode; b) single-strand assistant DNA probe conjugated onto as-fabricated electrode in a); c) electrode b) treated with recognition DNA probe to form duplex DNA on GSHs surface; d) electrode c) cultured with target DNA.

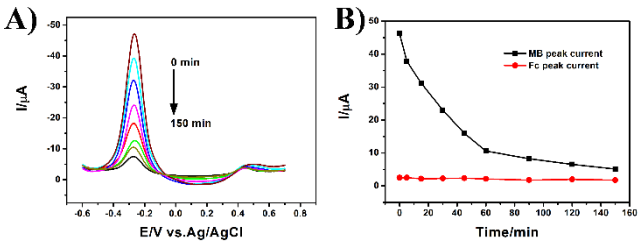

Fig. S9 Release profiles of electro-active molecules MB from single-strand assistant DNA probe modified GSHs: A) DPV spectra; B) linear plot. The optimized culture time is 60 min according to the linear plot.

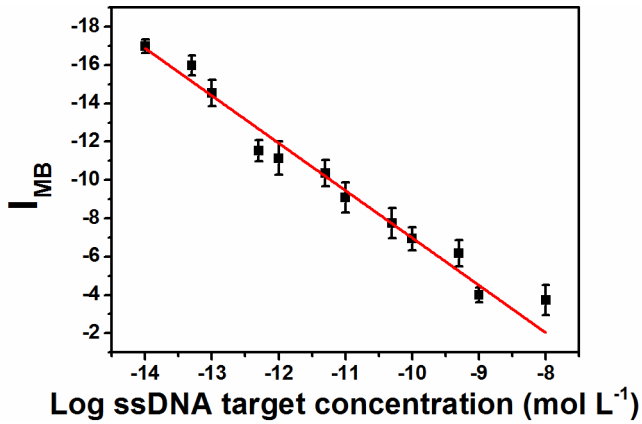

Fig. S10 The linear relationship between the current I_{MB} and logarithm of target DNA concentration.

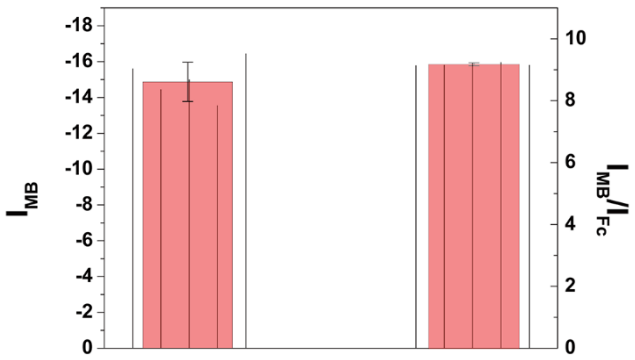

Fig. S11 The reproducibility of the as-fabricated ratiometric electrochemical biosensor.

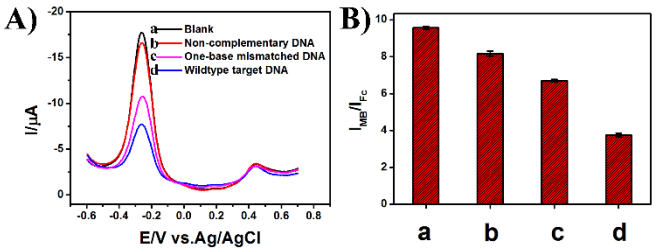

Fig. S12 The specificity of the ratiometric electrochemical sensor for the discrimination of target DNA, one-base mismatched DNA and noncomplementary random DNA, the concentration of DNA is 50 pM; A) DPV spectra; B) histogram.

References:

1. S. Yang, X. Feng, L. Wang, K. Tang, J. Maier and K. Müllen, *Angew. Chem. Int. Ed.*, 2010, **49**, 4795-4799.
2. L. Wu, J. Ren and X. Qu, *Nucleic Acids Res.*, 2014, **42**, e160.
3. L.-L. Li, Q. Yin, J. Cheng and Y. Lu, *Adv. Healthcare Mater.*, 2012, **1**, 567-572.