Supporting Information

Experimental

Materials: Graphite and hydrogen peroxide solution (30 wt %) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Single-walled carbon nanotubes (SWNT) were purchased from Nanotech Port Co. Ltd. (Shenzhen, China). Tetramethylbenzidine (TMB) and N-Hydroxysulfosuccinimide sodium (sulfo-NHS) were purchased from BBI (Ontario, Canada). H₂O₂ was obtained from Beijing Chemicals Inc (Beijing, China). Poly (allyl amine hydrochloride) (PAH, Mw = 60000), 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and hemin were purchased from Alfa Aesar (Ward Hill, MA). Tetraethylorthosilicate (TEOS, >98 %) and Folic acid (FA) was purchased from Sigma-Aldrich. All other reagents were of analytical reagent grade. Aqueous solutions were prepared with double-distilled water (ddH₂O) from a Millipore system (>18 MΩ cm). SWNT-COOH and mesoporous silica were prepared according to the previous approaches.¹

Synthesis of PAH-Functionalized Graphene (GP): Graphite oxides (GO) were synthesized from graphite powder by a modified Hummers method.² The graphene-PAH (GP, as shown in Scheme 1A) was prepared by vigorously stirring a solution of 20 mg of the graphene oxides, 100 mg of PAH, and 100 mg of KOH in 50 mL of H₂O at 70 °C for 24 h.³ Then 10 mL of 1M NaBH₄ solution was added, and the reaction was kept on at 70 °C for 2 h. After that, the GP was collected and purified by
centrifugation and adequately washed with water several times to remove the impurities and the excess of PAH by physical absorption.

**Synthesis of Folic Acid-Functionalized Graphene (GF):** To conjugate the graphene-PAH with folic acid (FA), free folic acid (10 mg, 0.0227 mmol) was firstly dissolved in 4 mL 50 mM MES buffer (pH 6.0). The solution of FA was then mixed with a 2 mL aqueous solution of (EDC) (0.068 mmol) and sulfo-NHS (0.068 mmol). After agitating overnight at temperature in the dark, the solution of GP (20 mg) was added to the mixture. The resulting solution was stirred at room temperature for 24 h and then centrifuged at 13000 rpm to separate the precipitate. The resulting black precipitate was sonicated and extensively washed eight times with ddH₂O to remove the physisorbed folic acid.

**Synthesis of Folic Acid Conjugated Graphene-Hemin Composite (GFH):** To prepare GFH, hemin (20 mg) was firstly dissolved in 5 mL ddH₂O. Then, 20 mg GF was added to the solution. The resulting mixture was stirred at room temperature in the dark for 72 h and then centrifuged at 10000 rpm to separate the precipitate. The resulting brown precipitate was washed three times to give GFH.

**Characterization:** Atomic-force microscopy (AFM) measurements were performed using Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). Samples for AFM images were prepared by depositing a dispersed GFH/H₂O solution (20 µg mL⁻¹) onto a freshly cleaved mica surface and washed with ddH₂O. Tapping mode was used to acquire the images under ambient conditions. FTIR characterization was carried out on a BRUKE Vertex 70 FTIR spectrometer. UV-vis
absorbance measurements experiments were carried out on a Jasco-V550 UV/Vis spectrophotometer. Thermogravimetric analysis (TGA) was recorded on a PE TGA-7 thermal analyzer at 10 °C·min⁻¹ in an N₂ atmosphere. EDX was carried out using a HITACHI S-4500 instrument. The zeta potential of GFH in solution (~0.05 mg/mL in millipore water) was determined using a Zeta PALS, zeta potential analyzer (Brookhaven Instruments Corp. Holtsville, NY).

**Bioassay:** Kinetic measurements of GFH peroxidase reactions were performed using a Jasco-V550 UV-Vis spectrophotometer in time course mode at 652 nm. Experiments were carried in a reaction volume of 500 μL buffer solution (25 mM Na₂HPO₄, pH 7.0), with 800 μM TMB and 5 mM H₂O₂ as substrate.

**Cell Culture and Treatment:** The human cervical cancer cells (HeLa), human breast cancer cells (MCF-7) and the mouse fibroblasts cells (NIH-3T3, used as negative control) were grown in Iscove’s modified Dulbecco’s medium (Gibco BRL) supplemented with 10% fetal calf serum in a humidified 37 °C incubator with 5% CO₂. For the bright field microscope images, cells were plated in 24 well plate at density of 6000 cells per well and allowed to incubate with 20 μg/mL GFH for 1.5 h. For control experiment, cells in 24 well plate were firstly treated with 200 μg mL⁻¹ FA in IMDM medium for 30 min and then incubate with 20 μg mL⁻¹ GFH for 1.5 h. Afterwards cells were washed with PBS buffer three times and then visualized using an Olympus BX-51 optical system microscope (Tokyo, Japan) equipped with 40×objective lenses and Olympus digital camera. For the colorimetric method, 20 μg/mL⁻¹ of GFH were incubated with different cells lines in IMDM medium at 37 °C.

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for 1.5 h, then harvested by centrifugation at 2000 rpm, and washed three times with phosphate buffered saline (PBS). Finally, cells were dispersed in detection buffer (25 mM Na$_2$HPO$_4$, pH 7.0) for color reaction. Cell numbers were determined by Trypan blue exclusion in a hemocytometer chamber.  

References


Supporting Figures

**Figure S1.** FTIR spectra of (A) GO, (B) GP, (C) GF and (D) FA.
Figure S2. A) AFM image of GFH; B) Height profile taken across the white line in (A).

Figure S3. (A)UV/Vis spectra for GP, GF and GFH in ddH₂O. (B)Thermogravimetric analysis for GP (black), GF (red), GFH (blue) and hemin (cyan) in N₂ atmosphere with a ramp of 10 °C/min.
**Figure S4.** EDX spectra of (A) GF and (B) GFH.

**Figure S5.** The peroxidase-like activity of GFH (black) and hemin (red) is pH dependence. GFH and hemin show the optimal pH of 7.0.
Figure S6. The time-dependent absorbance changes at 652 nm of 20 ng·ml⁻¹ Hemin in the absence (black) or presence of 80 ng·ml⁻¹ GO (red), 80 ng·ml⁻¹ SWNT-COOH (blue), or 80 ng·ml⁻¹ mesoporous silica (MSN, cyan) in phosphate buffer (25 mM Na₂HPO₄, pH 7.0) at room temperature.
**Figure S7** Bright field images of three cell lines that were incubated with GFH in vitro. (A), (C), (E) GFH bind to NIH-3T3 cells, HeLa cells or MCF-7 cells; (B), (D), (F) The binding of GFH to NIH-3T3 cells, HeLa cells or MCF-7 cells can be blocked by folic acid; GFH was visualized as dark spots under conventional bright field microscopy (arrow points to GFH). Dark counts (I) were determined manually using cell count plugins and Image J software.