

Sporting Information

Experimental Section

Materials: Graphite was purchased from Sinopharm Chemical Reagent (Shanghai, China). Doxorubicin (DOX), Tetraethylorthosilicate (TEOS), (3-aminopropyl) trimethoxysilane (APTES), Rhodamine B, succinic anhydride (SAA) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used as received. N-cetyltrimethylammonium bromide (CTAB) was obtained from Alfa Aesar. Hydrochloric acid, Hydrazine solution (85%) and ammonia solution (25 wt %) were provided by Beijing Chemicals Inc. (Beijing, China). PEGlyted P₀ (RWIMYF) was synthesized with the standard Fmoc chemistry on a solid support, and used as the probe for sensing cyclin A₂.¹ Human cyclin A₂ was prepared as described in our previous work.² All other proteins (including BSA, cytochrome C, IgG and lysozyme) were obtained from Sigma-Aldrich. All aqueous solutions were prepared using ultrapure water (18.2 MΩ, Milli-Q, Millipore).

General techniques: Fluorescence measurements were carried out on a Jasco FP-6500 spectrofluorometer. AFM images were performed using a Nanoscope Atomic force microscopy (AFM) measurements were performed using Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). TEM images were recorded using a FEI TECNAI G2 F20 high-resolution transmission electron microscope operating at 200 KV. SEM images were obtained with a Hitachi S-4800 FE-SEM. The ζ-potential of the nanoparticles was measured in a Zetasizer 3000HS analyzer. FT-IR characterization was carried out on a BRUKE Vertex 70 FTIR spectrometer. The samples were thoroughly ground with exhaustively dried KBr. Fluorescence images were captured using an Olympus BX-51 optical equipped with a CCD camera. Flow cytometric analysis was performed on a BD LSRFortessa flow cytometer.

Preparations: Graphene oxide (GO) was synthesized from graphite by the modified Hummers method.³ The procedure of synthesize GS was according to our previous report.⁴

Briefly, 20 mg as-synthesized GO aqueous solution was added into 50 mL water containing 0.5 g CTAB and 20 mg NaOH, and ultrasonically treated for 1 h. Afterward, TEOS (400 μ L dissolved in 1.6 mL ethanol) was slowly dropwised to the above mixture to reaction for 12 h after magnetic stirring for 2 h at 40 °C. Then, 80 μ L of hydrazine was additionally introduced into the above mixture, heated at 70 °C for 5 h and washed with warm ethanol for three times. The product dispersed in 50 mL acetone stirred at 40 °C for 24 h, centrifugation and washed by warm ethanol for three times. Subsequently, the product was mixed with 200 μ L APTES in 50 mL ethanol and stirred for 12 h at 80 °C under reflux. Finally, the obtained dry GS-NH₂ 50 mg reacted with 0.5 g succinic anhydride in 20 mL DMF under N₂ for 8 h with continuous stirring. By doing so, carboxyl groups were formed onto the GS surface for conjugation of P₀. The product GS-COO⁻ was washed and dissolved in the proper amount of water.

Preparation of GS probe: The GS-COO⁻ (20 mg) were soaked in a solution of RhB (20 mg) for 24 h. The nanoparticles were then centrifuged and washed thoroughly with pure water to remove unloaded and adsorbed molecules. Then, 5 mg of RhB@GS-COO⁻ was dispersed in 5 mL PBS (pH 6.0), and mixed with 0.1 mM P₀, which was stirred vigorously for 1 h and added with ultrapure water to 5 mL after washed thoroughly.

Characterization of GS Probe

FT-IR: The existence of mesoporous silica on graphene surface was validated by the bending vibration of SiO-H bonds (958 cm⁻¹), the stretching vibration of SiO-H bonds (3418 cm⁻¹), and the stretching vibration of Si-O-Si bonds (as: 1077 cm⁻¹; s: 803 cm⁻¹). The amination of GS was supported by the stretching vibration of -CH₂ (as: 2925 cm⁻¹, s: 2854 cm⁻¹) and the bending vibration of N-H (1584 cm⁻¹), which belong to the attached APTES. The band at 1718 cm⁻¹ could be assigned to the C=O stretching vibrations from the carboxyl groups, which indicated that GS converted to carboxylated formation GS-COO⁻ by succinic anhydride. The efficient adsorb of P₀ onto the GS-COO⁻ was validated by the band at 1697 cm⁻¹, which was characteristic of acylamide vibration.

ζ potential: As shown in Figure S3, the ζ potential of GS-NH₂ (17.9 mV) was much positive than that of GO (-53.4 mV) due to the deoxidization of electronegative groups on GO such as hydroxyl and carboxyl during silica coating and electropositive amino functionalize. After carboxylation, the ζ potential changed to -28.3 mV and then decreased to -8.82 mV after electrostatic adsorption of electropositive P₀.

Cell Culture and Cell Extract Preparation: K562, HeLa, MCF-7, MDA 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) at 37 °C in a humidified atmosphere containing 5% CO₂. K562⁺ cells were cultured in the medium containing 0.25 μM DOX. The starved, normal and DOX-treated K562 cells all cultured for 72 h.⁵ The cells were collected by centrifugation at 3000 rpm for 5 min and washed twice with a sterile PBS (pH 7.2). Cell lysates were generated by lysing 1×10⁷ cells in 500 μL radio immune precipitation assay (RIPA) buffer (PBS containing 1% NP40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 4% aprotinin, 1% sodium orthovanadate and 0.5% PMSF) on ice, centrifuge for 30 min at 4 °C and discarding the deposition. Protein concentrations were determined using the Bradford assay.

MTT Assay: The toxicity of GS-COO⁻ to cells was measured by MTT assay. Briefly, K562 cells were plated at a density of 1×10⁴ cells per well in 100 μL of RPMI medium in 96-well plates and grown for 24 h. Then, the cells were exposed to a series of concentrations of GS-COO⁻ 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. Subsequently, 10 μL of MTT (5 mg mL⁻¹) was added into the wells and incubated for an additional 4 h. Finally, the supernatant was discarded, followed by the additional of 100 μL DMSO into each well and incubation with gentle shakes in the shaker incubator. The optical density (OD) was read at a wavelength of 570 nm, and relative inhibition of cell growth was calculated as follows: % = (1 - [OD]_{test}/

$[\text{OD}]_{\text{control}} \times 100$. Three replicates were done for each treatment group. Statistical comparisons were performed using the Student's *t*-test. *P*-values <0.05 were considered statistically significant.

Detection of cyclin A₂: The cuvette was first filled with 200 μL of GS probe (in 50 mM phosphate solution, pH 6.0 or in 5 mg mL⁻¹ starved K562 cell extracts). Then, 200 μL of cyclin A₂ solution of appropriate concentrations was added and mixed thoroughly. Fluorescence spectrum was measured with excitation at 550 nm after shake gently on the oscillator for 60 min. Control experiments with non-specific proteins including BSA, CC, IgG, lysozyme and starved cell extract were carried out under the same conditions.

Cellular uptake and location: The 2 mg RhB was activated using EDC (10 mg mL⁻¹, 15 mL) and NHS (10 mg mL⁻¹, 15 mL) in a MES buffer (pH 6.0) for 2 h at room temperature with continuous stirring. Then the pH value of the solution was adjusted to 8.0 by NaOH, followed by the addition of GS-NH₂ 10 mg and continuous stirring for another 12 h. The result product was then centrifuged at 12 000 rpm for 15 min and washed 3 times with deionized water to remove unreacted RhB. For flow cytometry, K562 cells (1 $\times 10^6$ cells/well) were treated with RhB labeled GS (100 $\mu\text{g mL}^{-1}$) for different time at 37 °C. A single cell suspension was prepared consecutively by trypsinization, washing with PBS, and filtration through 35 mm nylon mesh. After that, the cells were lifted using a cell stripper and analyzed using flow cytometer for RhB. For observation by fluorescence microscopy, K562 cells (1 $\times 10^6$ cells) were co-cultured with RhB labeled GS for 2 h in DMEM. Then, the cells were washed three times with PBS and treated with lysotracker for 20 min for lysosome staining. After washed twice with PBS, the cells were observed using an Olympus BX-51 optical equipped with a CCD camera.

Fluorescence microscopy: For in situ imaging, K562 and NIH3T3 cells were seeded in 24-well assay plates separately and cultured for 24 h. GS probe (100 $\mu\text{g mL}^{-1}$) was added to the cells, the mixture was incubated at 37 °C for 2 h. The cells were then washed twice with

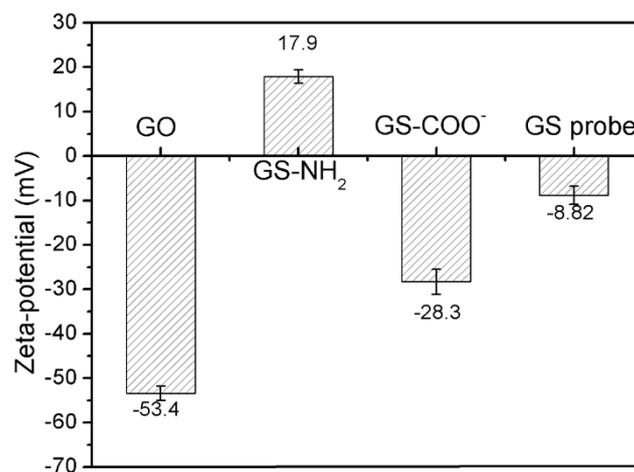


Figure S3. The corresponding zeta potential measured at each step of the GS probe preparation process in deionized water.

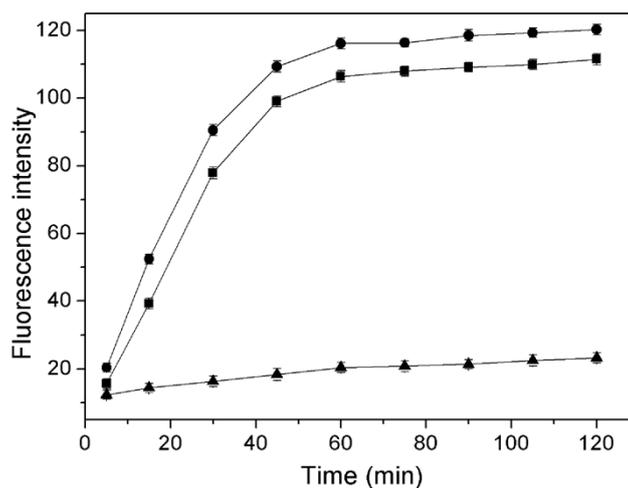


Figure S4. Time courses of fluorescence intensity for GS probe (triangles), GS probe suspension containing 100 nM cyclin A₂ (squares), and supernatant collected by centrifugation at 12000 rpm for 20 min after incubating GS probe with 100 nM cyclin A₂ (circles). Excitation at 550 nm; emission at 572 nm.

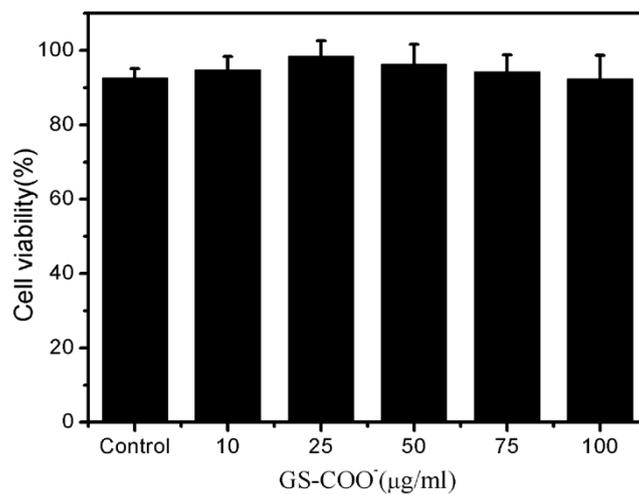


Figure S5. MTT assay after incubated with different concentration of GS-COO⁻ in K562 cells. Results are expressed as means \pm the standard error (n= 3).

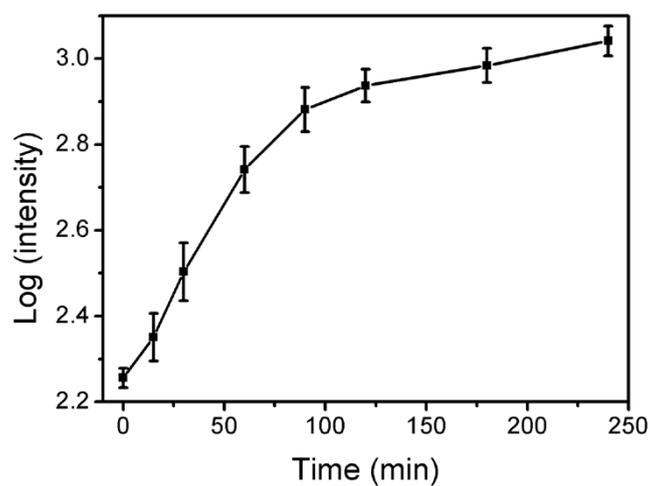


Figure S6. Flow cytometric detection of K562 cells (0.5 mL, 1×10^6 mL⁻¹) after incubated with 10 μ L RhB labeled GS (100 μ g mL⁻¹) for different time.

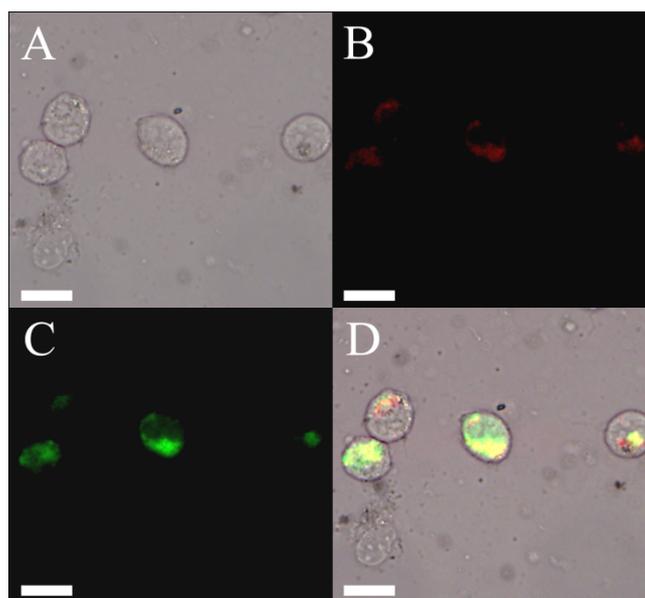


Figure S7. Fluorescence microscopy images of K562 cells after incubated with 10 μL RhB labeled GS ($100 \mu\text{g mL}^{-1}$) for 90 min. (A) brightfield, (B) RhB labeled GS, (C) lysotracker, and (D) merge. Scale bar = 20 μm .

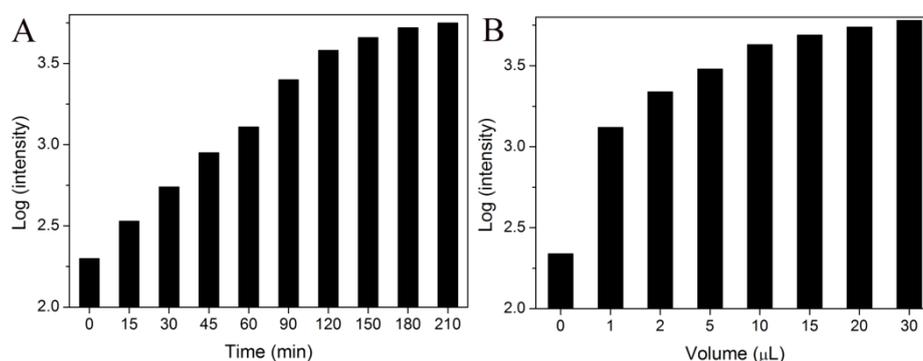


Figure S8. Flow cytometric detection of K562 cells (0.5 mL , $1 \times 10^6 \text{ mL}^{-1}$) after incubated with 10 μL GS probe ($100 \mu\text{g mL}^{-1}$) for different time (A) and various GS probe volumes for 2 h (B).

References

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