Supporting Information

Luminescent Gold Nanoparticles with pH-Dependent Membrane Adsorption

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Materials and Equipment

Organic dye DiR for staining phospholipid bilayer membrane was purchased from Invitrogen. Citrate-coated 20 nm gold nanoparticles were purchased from Ted Pella. All the other chemicals were purchased from Sigma-Aldrich and used as received unless specified. Particle size and zeta potential of nanoparticles in the aqueous solution were analyzed by a Brookhaven 90Plus Dynamic Light Scattering Particle Size Analyzer (DLS) and a Brookhaven ZetaPALS zeta potential analyzer, respectively. Transmission electron microscopy (TEM) images were obtained using a 200kV Jeol 2100 transmission electron microscope. Absorption spectra were collected using a Varian 50 Bio UV-Vis spectrophotometer. The luminescence spectra were collected by a PTI QuantaMaster^{TM} 30 Fluorescence Spectrophotometer (Birmingham, NJ). FTIR spectra were obtained using a Nicolet Avatar 360 FTIR spectrometer. pH was measured by a Accumet AB15 pH meter and a Accuphast microprobe electrode.

Synthesis of Luminescent Gold Nanoparticles

Glutathione-Coated Luminescent Gold Nanoparticles (G-AuNPs) were synthesized by a self-dissociation of Au(I)-glutathione polymers in an aqueous solution, a method previously reported by our group (Scheme S1).[1] In a typical synthesis, a fresh aqueous solution containing reduced L-glutathione (25 mM) was added into a $HAuCl_4$ aqueous solution (25 mM) at a 1:1 molar ratio of gold to thiolated ligand. The solution was centrifuged at 21,000 g for 1 min. to remove the

insoluble aggregates as well as large NPs. The supernatant was further purified by adding a small amount of ethanol into the aqueous solution (the ratio between water and ethanol is 2:1). Under such condition, the luminescent gold NPs were precipitated out of the solution while the free GSH and gold ions remained in the solution. The precipitates were then resuspended in aqueous solution (DI water, PBS or 10% (v/v) FBS-containing MEM (without phenol red)). The final solution contained G-AuNPs with diameter of ~2 nm. The pH of solution was adjusted by 1 M NaOH or 1 M HCl and then measured by a pH meter.

Glutathione and Cysteamine-Coated Luminescent Gold Nanoparticle (GC-AuNPs) were synthesized by a modified self-dissociation method to create luminescent gold nanoparticles with diversity in surface chemistry (Scheme S1). A fresh aqueous solution containing reduced L-glutathione (12.5 mM) and cysteamine hydrochloride (12.5 mM) was added into a $HAuCl_4$ aqueous solution (25 mM), and macroscopic orange precipitate was formed immediately. After four weeks, the solution was then centrifuged at 21,000 g for 1 min. to remove the insoluble aggregates as well as large NPs. The supernatant was further purified by adding a small amount of ethanol into the aqueous solution (the ratio between water and ethanol is 2:1). Under this condition, GC-AuNPs were precipitated out of the solution. The precipitates were then resuspended in aqueous solution (DI water, PBS or 10% (v/v) FBS-containing MEM (without phenol red)). The final solution contained GC-AuNPs with diameter of ~3 nm. The pH value of the solution was adjusted by 1 M NaOH or 1 M HCl and then measured by a pH meter.

Synthesis of Cysteamine-Coated Plasmonic Gold Nanoparticles (Non-luminescent)

Cysteamine-coated plasmonic AuNPs were prepared by the $NaBH_4$ reduction of $HAuCl_4$. Briefly, a fresh aqueous solution (150 µL) containing cysteamine hydrochloride (4.7 mM) was added to a $HAuCl_4$ aqueous solution (7.5 mM, 100 µL). The color of the solution turned from yellowish to cloudy orange, suggesting the formation of Au(I)-thiolate polymers. Aqueous solution containing $NaBH_4$ (10 mM, 20 µL) was quickly added to fully reduce the gold polymers until the color of

solution turned from orange to reddish, indicating the formation of plasmonic AuNPs. The AuNPs were purified by Sephadex-20 column.

Characterization of Surface Ligands on Glutathione and Cysteamine-coated Luminescent Gold Nanoparticles (GC-AuNPs)

The ratio of glutathione (**GSH**) to cysteamine (**CA**) on GC-AuNPs was calculated by analysis of FTIR spectra of GSH, CA, the mixture of GSH and CA at a 1:1 molar ratio and GC-AuNPs. To make a well mixed sample for FTIR spectrum measurement, GSH and CA were dissolved in aqueous solution at a 1:1 molar ratio and then freeze dried. The luminescent gold NPs were freeze dried for 24 hours.

The FTIR spectrum of CA was found to be different from that of GSH in a range of 1380-1900 cm^{-1} (Figure S4 A). GSH showed an obvious absorbance peak at 1714 cm^{-1}, originating from the C=O vibration of COOH group in GSH, while the peak at 1714 cm^{-1} is absent from the spectrum of CA. On the other hand, CA exhibited strong absorbance at 1492 cm^{-1}, originating from the N-H vibration of NH_2 group in CA, and GSH showed low absorbance at 1492 cm^{-1} (~ 19.1% of the absorbance at 1714 cm^{-1}). Therefore, the peak at 1714 cm^{-1} can be considered as a characteristic peak for GSH, while CA has a characteristic peak at 1492 cm^{-1}.

In the FTIR spectra of mixture of GSH and CA (Figure S4 B), the absorbance of the sample at 1714 cm^{-1} can be completely attributed to GSH, and the absorbance of sample at 1492 cm^{-1} was mainly contributed by CA and partially contributed by GSH. Thus, the molar ratio of GSH to CA ($R_{GSH:CA}$) in a mixed sample (either in the mixture of GSH and CA or on the nanoparticle surface) can be calculated using the following equation.

$$R_{GSH:CA} = \frac{Abs_{1714,GSH}}{Abs_{1492,CA}} \times F = \frac{Abs_{1714,sample}}{Abs_{1492,sample} - Abs_{1714,sample} \times 19.1\%} \times F$$

Where $Abs_{1714,GSH}$ denotes absorbance of sample at 1714 cm^{-1} contributed by GSH, which equal to the total absorbance of the mixed sample at 1714 cm^{-1} ($Abs_{1714,sample}$); $Abs_{1492,CA}$ is absorbance of sample at 1492 cm^{-1} contributed by CA, which can be obtained by subtracting the absorbance of

GSH at 1492 cm^{-1} (19.1% of $Abs_{1714,GSH}$, namely 19.1% of $Abs_{1714,sample}$) from the total absorbance of sample at 1492 cm-1 ($Abs_{1492,sample}$). F stands for a constant factor.

In the mixture of GSH and CA at a 1:1 molar ratio, $R_{GSH:CA}$ was known as 1, $Abs_{1714,sample}$ was 0.0269, $Abs_{1492,sample}$ = 0.0171 (Figure S4 A), and thus F was calculated to be 0.446.

For the GC-AuNPs, $Abs_{1714,sample}$ was 0.00146, $Abs_{1492,sample}$ = 0.00125, F was 0.446 (Figure S4 B). Therefore, $R_{GSH:CA}$ was determined to be 0.67, corresponding to the ratio of CA to GSH at 10 to 7.

Agarose Gel Electrophoresis

Britton-Robinson buffer solution at pH 5.3 or pH 7.4 was prepared as follows. Aqueous solution containing 0.04 M phosphoric acid, 0.04 M acetic acid and 0.04 M boric acid was prepared, and then 0.2 M sodium hydroxide was added to adjust the pH to 5.3 and 7.4 respectively.

The luminescent gold nanoparticles, G-AuNPs and GC-AuNPs were dissolved in Britton-Robinson buffer solution at pH 5.3 or at pH 7.4 containing 10% (v/v) fetal bovine serum (FBS) and then incubated in a 37 ºC water bath for 30 min. As control samples, G-AuNPs and GC-AuNPs were dissolved in Britton-Robinson buffer solution at pH 7.4 or pH 5.3 and then incubated in a 37 ºC water bath for 30 min.

Solution (30 μL) of each sample (1 and 1': G-AuNPs; 2 and 2': G-AuNPs + FBS; 3 and 3': GC-AuNPs; 4 and 4': GC-AuNPs + FBS) was mixed with 3 μL 75% glycerol and then analyzed by 2% agarose gel electrophoresis using the Mini-sub cell GT Gel electrophoresis system (Bio-Rad Laboratories Inc.) and Britton-Robinson buffer solution at pH 5.3 or pH 7.4 with 7.5 $V \cdot cm^{-1}$ for 30 min. Pictures were taken under the irradiation of 365 nm UV excitation (Figure 2A).

In order to identify the protein band, FBS was stained by Coomassie brilliant blue 250 (CBB250). Briefly, after G-AuNPs and GC-AuNPs were incubated with or without 10% (v/v) FBS at 37 ºC for 30 min, 10% (v/v) CBB250 (1 mg/mL) was added to the samples. Solution (30 μL) of each sample (5 and 5': G-AuNPs + CBB250; 6 and 6': G-AuNPs + FBS + CBB250; 7 and 7': GC-AuNPs +

CBB250; 8 and 8': GC-AuNPs + FBS + CBB250) was mixed with 3 µL 75% glycerol and then analyzed by agarose gel electrophoresis at pH 5.3 or pH 7.4 with 7.5 $V \cdot cm^{-1}$ for 30 min (Figure 2B).

Cell Culture

The cell line HeLa was purchased from ATCC (USA) and cultured in minimum essential medium (MEM) with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin (PS) at 5% CO_2, 37 ºC. Cells were plated in cell dishes with 14 mm glass coverslips and allowed to adhere for 48 h.

Microscopy Imaging

Interaction of Cysteamine-Coated Plasmonic AuNPs with Cell Membrane

Bright field live cell images were obtained by a BX-51 upright microscope (Olympus) with a 20X/0.46BD objective and an 18.2 Color Mosaic CCD camera (Diagnostic Instruments). Since the cysteamine-coated plasmonic AuNPs were not stable in PBS buffer, their interaction with cell membrane was performed by incubating the fixed HeLa cells with cysteamine-coated AuNPs in DI water at pH 7.4, pH 6.0 or pH 5.3 for 20 min at 25°C (Figure S2 [SI]). The cells were rinsed with water before imaging.

Interaction of Luminescent AuNPs with Cell Membrane

Fluorescence and bright field live cell images were obtained by an IX-71 inverted fluorescence microscope (Olympus) with a 1.3NA 100X oil-immersion objective under Hg-lamp excitation (for fluorescence imaging, Ex: 532-587 nm; Em: 605-682 nm; 30 W/cm^2; 2 s exposure time) and a Photon Max 512 CCD camera (Princeton Instrument). Live HeLa cells were rinsed with PBS buffer and incubated with G-AuNPs or GC-AuNPs in PBS or in 10% (v/v) FBS-containing MEM (without phenol red) at pHs 7.4, 6.0 or 5.3 at 25°C (Figures 3A-D, S1, S7 and S12 [SI]).

To obtain the curve of pH-dependent adsorption of GC-AuNPs onto a live cell membrane (Figures 4, S11 and S14 [SI]), live cell imaging was performed at 25°C in PBS at pHs 8.0, 7.4, 6.8, 6.3, 6.0, 5.8, 5.3, 4.8, or in 10% (v/v) FBS-containing MEM (without phenol red) at pHs 7.9, 7.4, 6.8, 6.5, 6.0, 5.8, 5.5, 5.3, 4.8. At each pH, cells were washed by PBS to remove cell culture medium and then rinsed with PBS or 10% (v/v) FBS-containing MEM at the specific pH to adjust the extracellular pH value. Fluorescence images were taken in such solution without GC-AuNPs but with the specific pH, and the intensity of cell membrane was measured as the autofluorescence background at this pH in PBS or in 10% (v/v) FBS-containing MEM. Subsequently, the solution was replaced by GC-AuNPs solution, and live cell images were taken after 5 min.

For each case (PBS or 10% (v/v) FBS-containing MEM with or without GC-AuNPs at a specific pH), 6 groups of fluorescence and bright-field images were taken. For each fluorescence image, the luminescent intensity of the cell membrane was measured from 6 different regions (autofluorescence background was subtracted) to give a mean value as the "intensity of cell membrane". The final result of "intensity of cell membrane" for each case was obtained from 6 fluorescence images and presented as mean±SD (n=6).

For colocalization experiment, the phosphorlipid bilayer membrane of live HeLa cells was first stained with DiR (2 μM, 37°C, 10 min) and then fluorescence image was taken in PBS (pH 5.3). After the fluorescence of DiR was completely photobleached by exposuring to excitation light for 2 min, GC-AuNPs (in PBS, pH 5.3) was added on the cells, and then fluorescence image was taken subsequently (Scheme S2 and Figure 3E).

To obtain three-dimensional luminescence imaging of live cells, HeLa cells were rinsed with PBS buffer and incubated with GC-AuNPs in PBS at pH 5.3 at 25°C, and then Z stack imaging was performed by an Applied Precision DeltaVision RT Deconvolution microscope (Applied Precision) with a 60X oil-immersion objective under Hg-lamp excitation (for fluorescence imaging, Ex: 541-569 nm; Em: 580-653 nm; 0.5 s exposure time). 45 *xy* sections were obtained at different z height and the distance between each *xy* section was 0.3 μm (Figure S8).

References

[1] C. Zhou, C. Sun, M. X. Yu, Y. P. Qin, J. G. Wang, M. Kim, J. Zheng, J. Phys. Chem. C 2010, 114, 7727.

Supplementary Figures

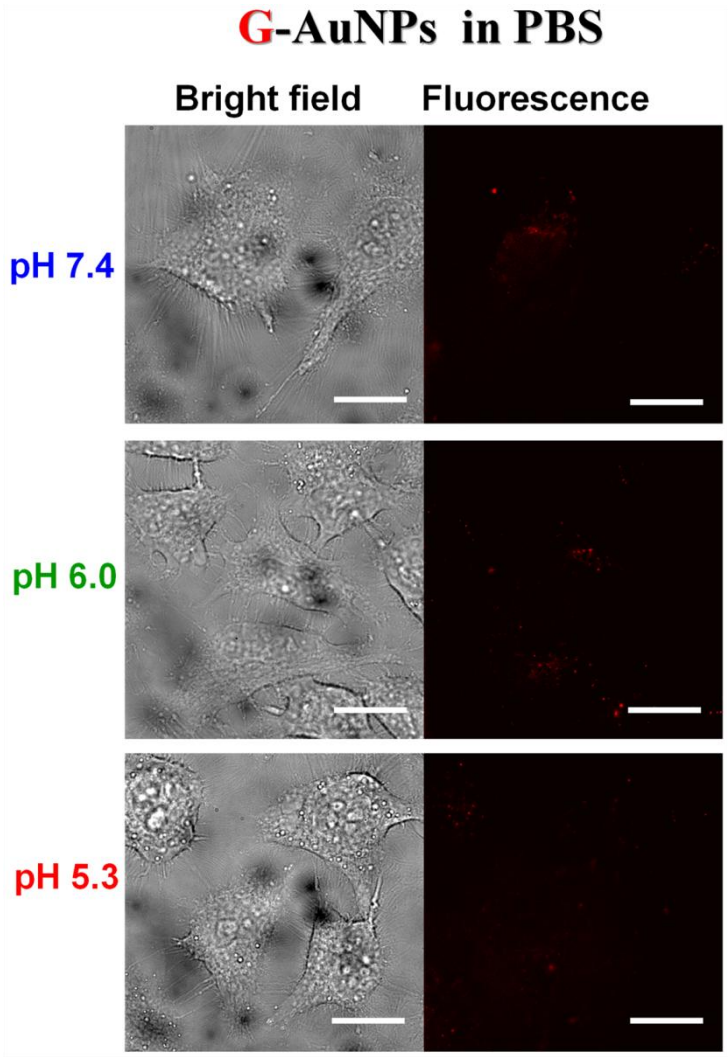


Figure S1. Fluorescence microscopy images of live HeLa cells incubated with G-AuNPs in PBS at pHs 7.4, 6.0 and 5.3 at 25°C (scale bar, 20 μm).

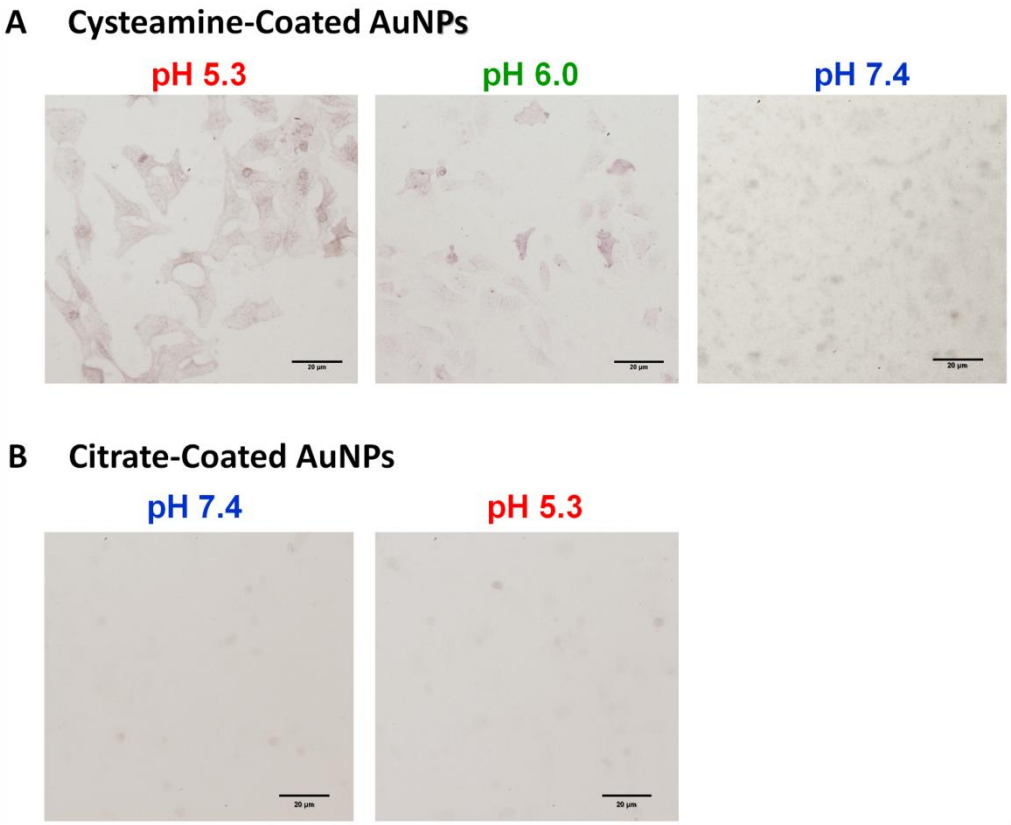
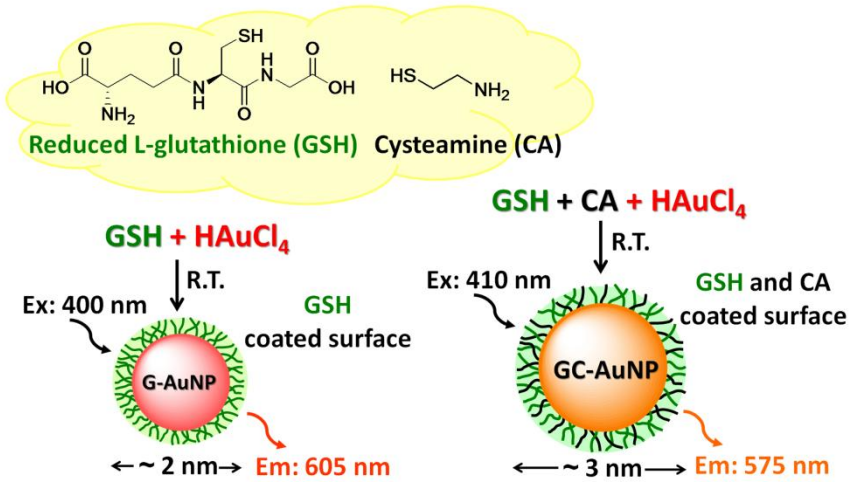


Figure S2. (A) Bright filed microscopy images of fixed HeLa cells incubated with cysteamine-coated plasmonic AuNPs at pH 7.4, 6.0 and 5.3 for 20 min at 25°C, indicating the pH-dependent adsorption of cysteamine-coated AuNPs on cell membrane (scale bar, 20 µm). At pH 5.3, cysteamine-coated AuNPs showed positively charged surface (zeta potential: +31.24±2.41 mV) which facilitated their interaction with the negatively charged cell surface. Because of adsorption of these plasmonic AuNPs, cells were stained brownish and thus clearly visualized under the brightfiled microscope. At pH 6.0, positive charge of the particle surface decreased (zeta potential: +13.95±2.75 mV), corresponding to the largely reduced binding of particles on the cells. At pH 7.4, the particles were not stable; they formed aggregates and precipitated on the bottom of cell culture dish. This phenomenon should be ascribed to the largely reduced surface charge that is close to neutral and thus failed to stabilize the particles. (B) As control, citrated-coated 20 nm AuNPs were incubated with live HeLa cells under the same conditions. Due to the negatively charged surface at pH 7.4 and pH 5.3 (zeta potential: -43.16±2.13 mV and -39.95±4.08 mV, respectively), the citrate-coated AuNPs exhibited very weak interaction with cells (scale bar, 20 µm).



Scheme S1. Scheme for synthesis of glutathione (GSH)-coated luminescent gold nanoparticles (G-AuNPs) and GSH/cysteamine (CA)-coated luminescent gold nanoparticles (GC-AuNPs).

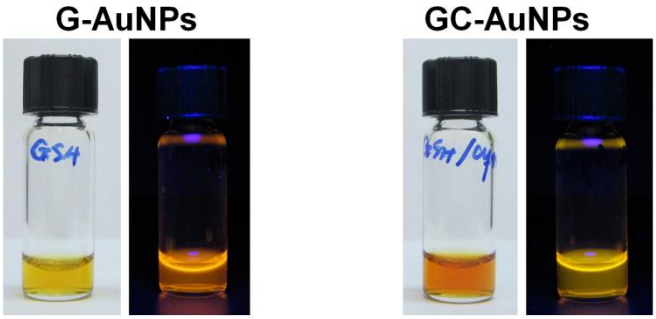


Figure S3. Photographs of G-AuNPs and GC-AuNPs taken with or without 365 nm UV excitation.

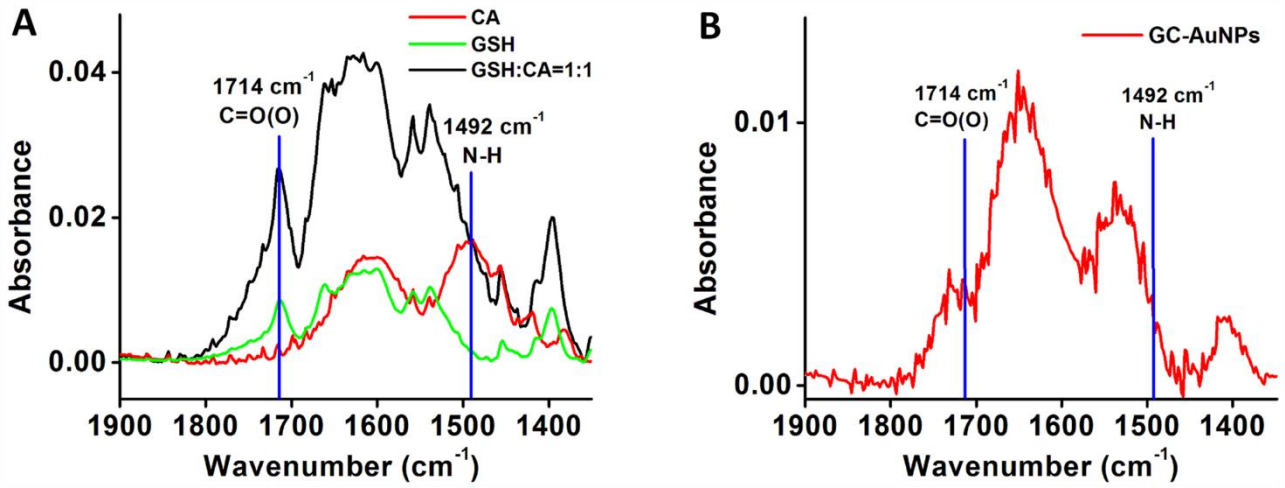


Figure S4. FTIR spectra of (A) reduced L-glutathione (GSH), cysteamine (CA) and the mixture of GSH and CA (at a 1:1 molar ration of GSH to CA) and (B) GC-AuNPs.

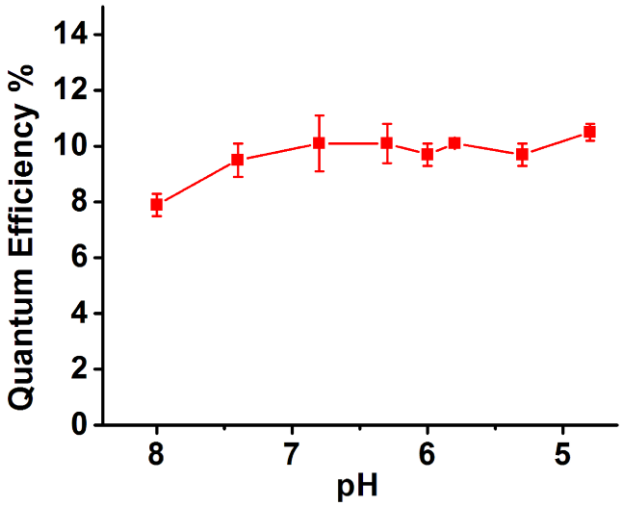


Figure S5. Quantum efficiencies of GC-AuNPs in a range of pH 4.8~8.0 in aerated aqueous solution at room temperature. Rhodamine 6G with quantum efficiency of 0.98 in aqueous solution was used as a reference. Results were presented as mean±SD (n = 3).

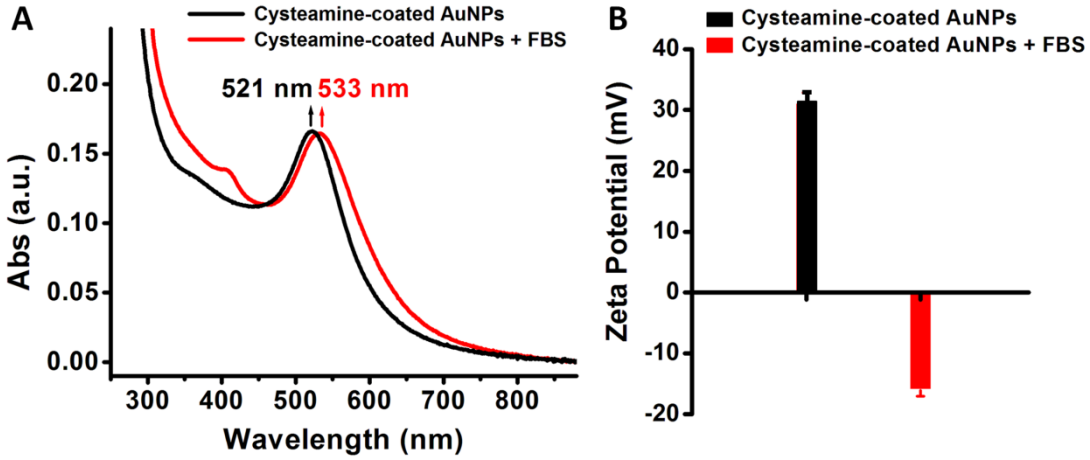


Figure S6. Serum protein adsorption on cysteamine-coated AuNPs after incubation with 10% (v/v) fetal bovine serum (FBS) at 37 ºC for 30 min revealed by (A) adsorption spectra and (B) zeta potential measurements. (A) The surface plasmon peak of AuNPs shifted from 521 nm to 533 nm after incubation with FBS. The 12 nm red shift of surface plasmon peak indicated the slight aggregation of cysteamine-coated AuNPs in such serum protein-containing solution. (B) Zeta potentials of cysteamine-coated AuNPs were +31.24±1.56 mV (at pH 5.3) and -16.04±1.00 mV (after incubation with FBS), indicating the adsorption of proteins with negative charge on the particle surface.

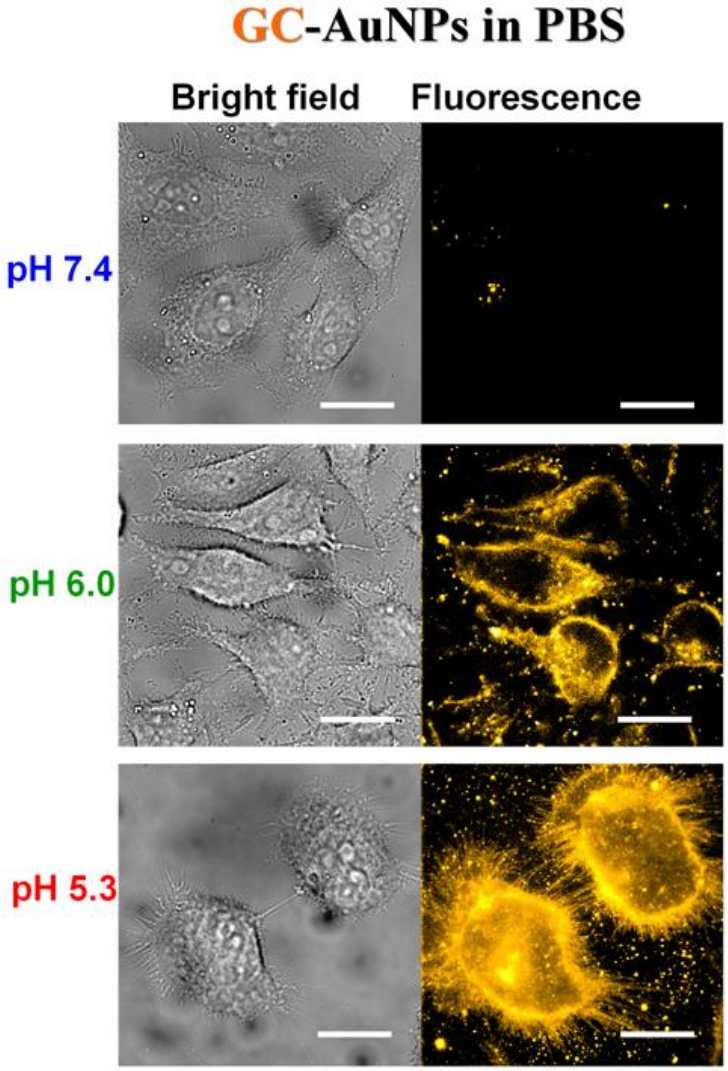


Figure S7. Fluorescence microscopy images of live HeLa cells incubated with GC-AuNPs in PBS at pHs 7.4, 6.0 and 5.3 at 25°C (scale bar, 20 μm).

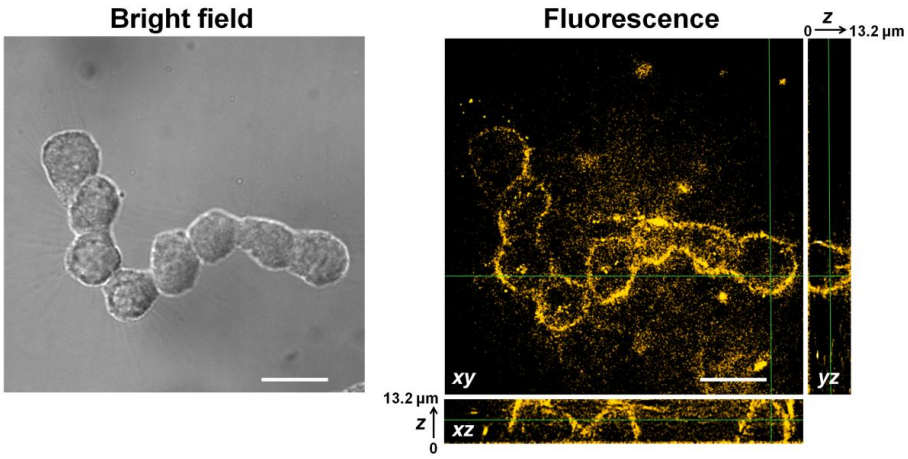
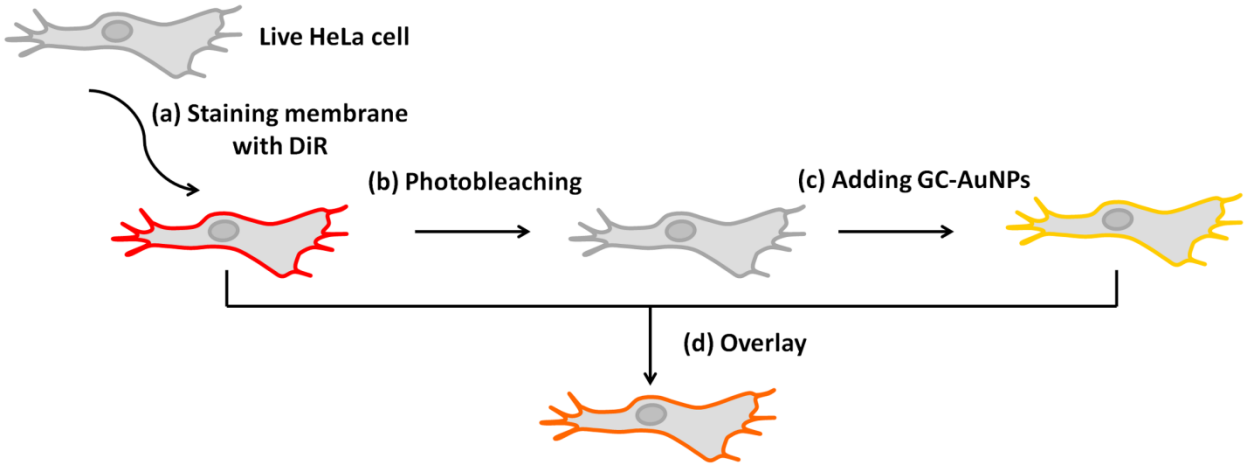


Figure S8. Three-dimensional luminescence images of live HeLa cells incubated with GC-AuNPs in PBS at pH 5.3 at 25 °C (scale bar, 20 µm). The yz and xz cross sections taken at the lines shown in the xy cross section were displayed respectively. The distribution of luminescence on the cell membrane indicated the particle binding on the cell surface. No luminescence was detected in the intracellular area, suggesting GC-AuNPs were not internalized by cells.



Scheme S2. Scheme of co-localization experiment procedure. (a) The phospholipid bilayer membrane of the live HeLa cell was stained with DiR by incubating cells with 2 μM DiR in PBS at 37 ºC for 10 min. The fluorescence image was taken in PBS (pH 5.3) to show the subcellular distribution of DiR (picture labeled as "**DiR**" in Figure 3E). (b) After exposure to the excitation light for 2 min, the fluorescence of DiR was completely photobleached. (c) The solution of GC-AuNPs in PBS (pH 5.3) was added onto the cell, fluorescence imaging of the cell was obtained subsequently to analyze the subcellular distribution of GC-AuNPs (picture labeled as "**GC-AuNPs**" in Figure 3E). (d) The cell images labeled as "**DiR**" and "**GC-AuNPs**" were merged to show the co-localization result (picture labeled as "Overlay" in Figure 3E).

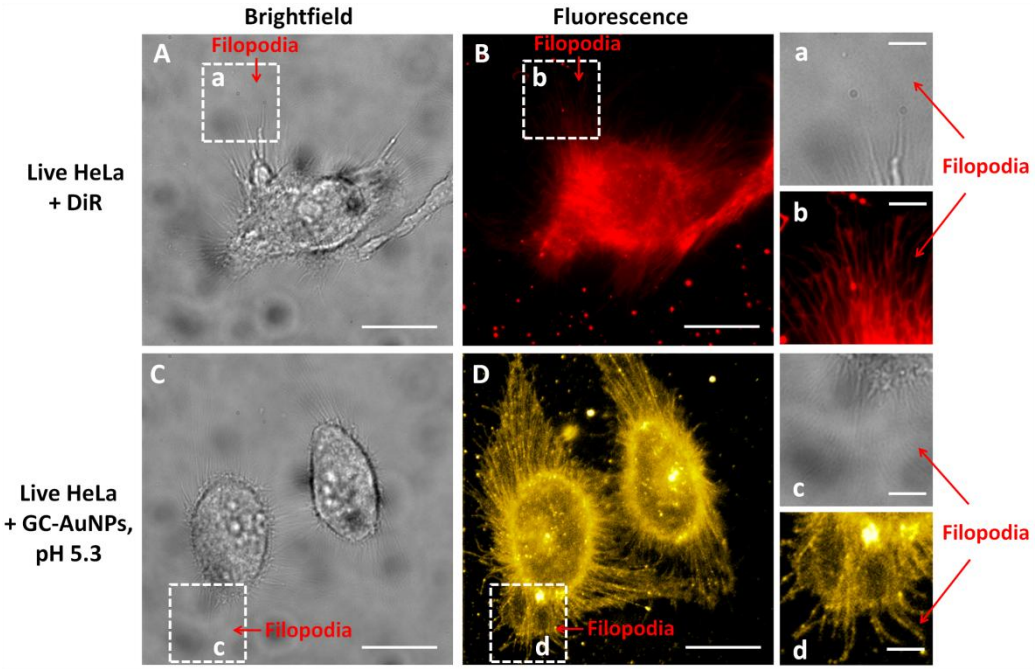


Figure S9. Brightfield and fluorescence images of live HeLa cells incubated with (A, B, a, b) 2 μM DiR in PBS at 37°C for 10 min or (C, D, c, d) 0.2 mg/mL GC-AuNPs at pH 5.3 in PBS at 25°C for 10 min (A-D: scale bar, 20 μm; a-d: scale bar, 5 μm). Filopodia was clearly visualized by DiR staining (a, b) or membrane adsorption of GC-AuNPs (c, d).

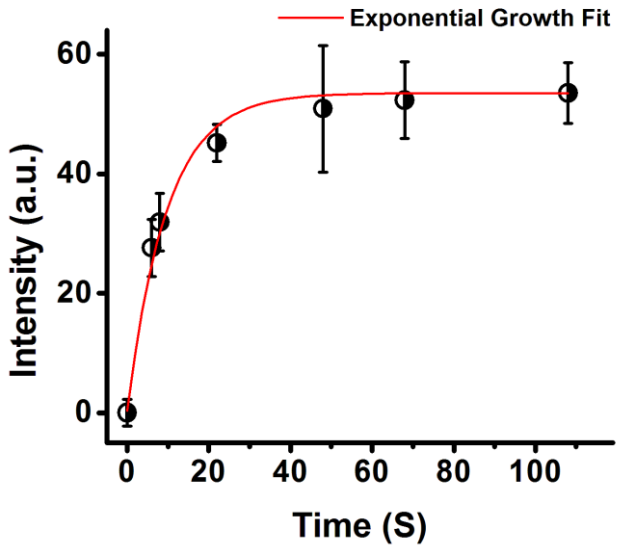


Figure S10. Time course of luminescence intensity enhancement on the cell membrane. Live HeLa cells were incubated with GC-AuNPs in PBS at pH 5.3 at 25 °C. Results were presented as mean±SD (n = 4).

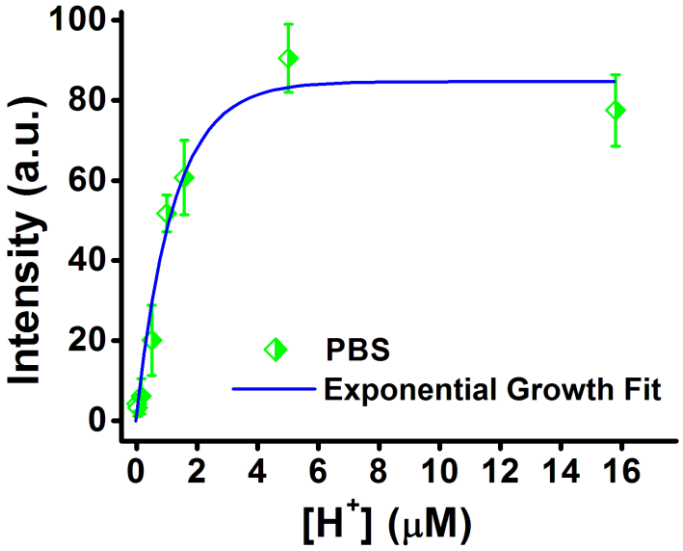


Figure S11. (A) Luminescence intensity of the live cell membrane incubated with GC-AuNPs versus H^+ concentration in PBS. Results were presented as mean±SD (n = 6).

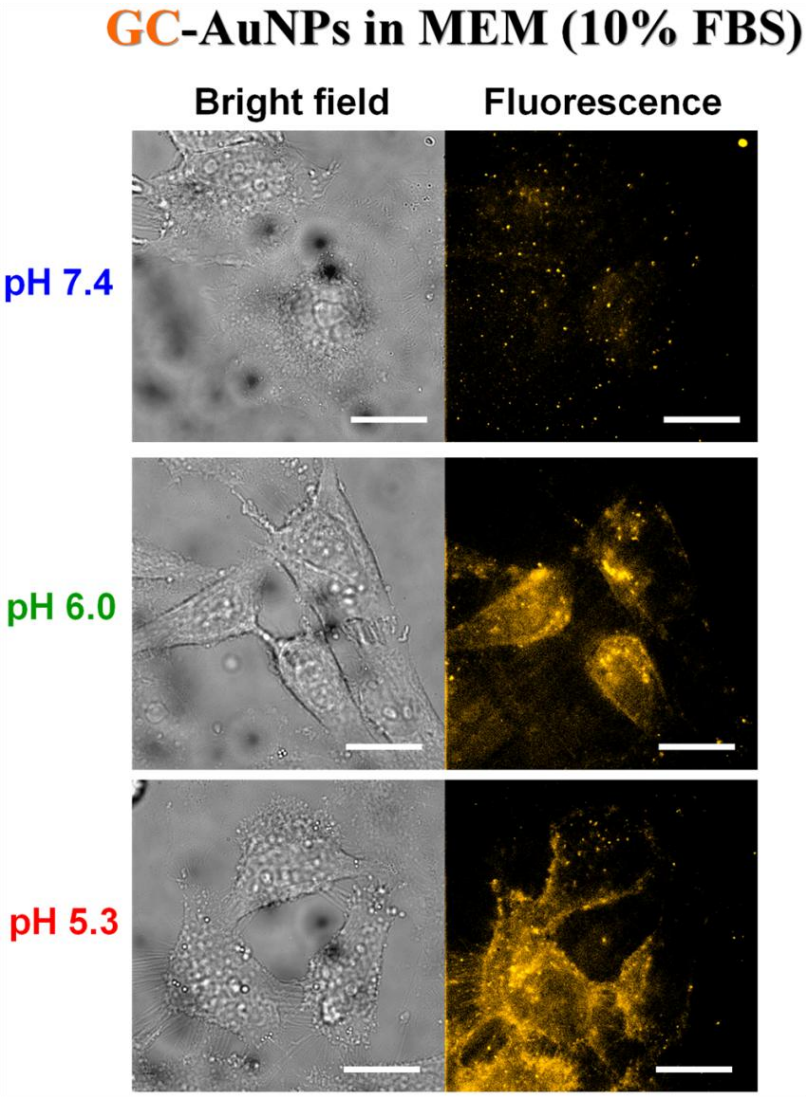


Figure S12. Fluorescence microscopy images of live HeLa cells incubated with GC-AuNPs in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) at pHs 7.4, 6.0 and 5.3 at 25°C, suggesting the pH-dependent adsorption of GC-AuNPs on live cell membrane in serum protein-containing medium (scale bar, 20 μm).

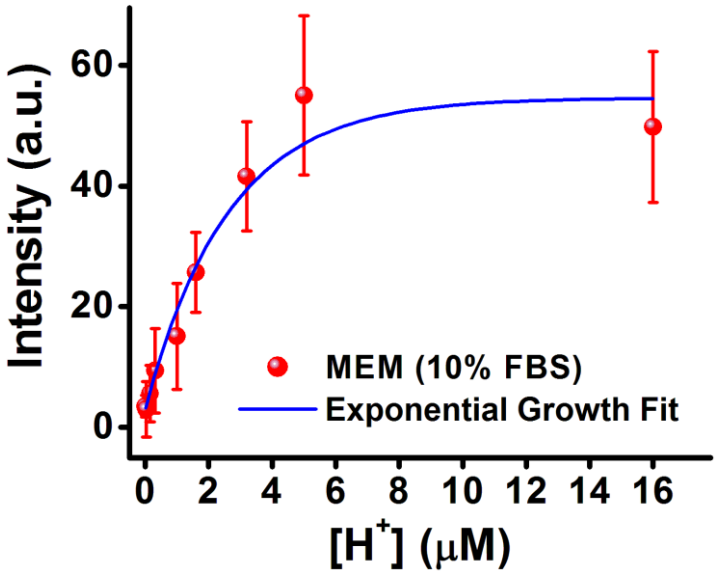


Figure S13. Luminescence intensity of the live cell membrane incubated with GC-AuNPs versus H^+ concentration in minimum essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum (FBS). Results were presented as mean±SD (n = 6).

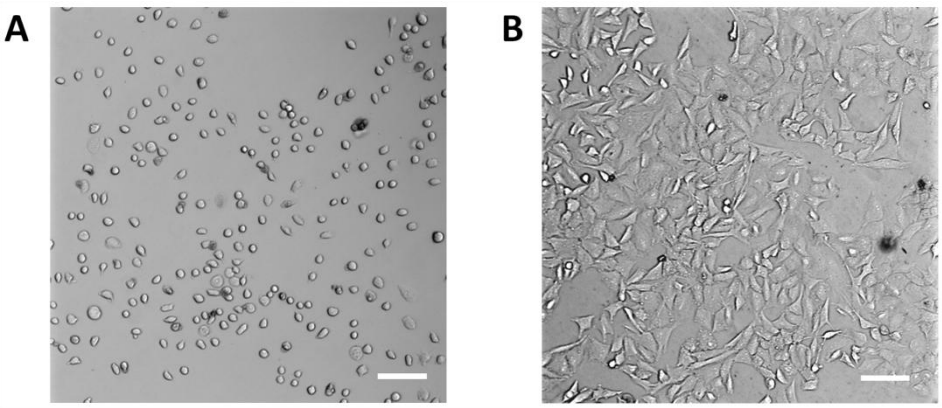


Figure S14. Bright field image of live HeLa cells incubated with GC-AuNPs in (A) PBS or in (B) MEM supplemented with 10% (v/v) FBS at pH 5.3 for 1 h and then stained with 0.4% trypan blue for 2 min (scale bar, 100 μm). The cells exhibited a resistance to trypan blue (a membrane impermeable dye) staining, indicating the membrane integrity was retained.