Supporting Information

In Situ Decrypting Plasmonic Nanoparticle Size-controlled Phosphorylation of Epidermal Growth Factor Receptor in living cells

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Supporting Figure

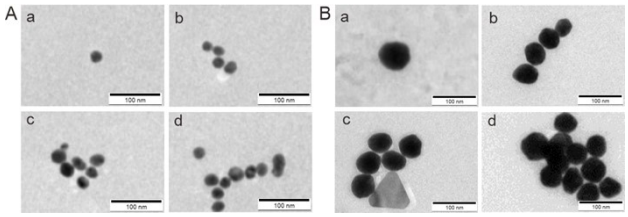

Figure S1. TEM images of the various cluster states of two different size GNPs. (A) TEM images of the various cluster states of G_{16}NPs. (B) TEM images of the various cluster states of G_{73}NPs. The scale bar in A-B is 100 nm.

Figure S2. Schematic diagram of probe synthesis that PEG and EGF gradually coupled to the surface of GNPs. Briefly, with the help of EDC/NHS, EGF was coupled on the surface of GNPs through condensation reaction with the linker molecules, HS-PEG-COOH (MW=3400) fixed the GNPs surface by Au-S bond. The application of HS-PEG (MW=2000) can ensure the monodisperse stable of particles in the solution; also prevent nonspecific adsorption of other molecules.

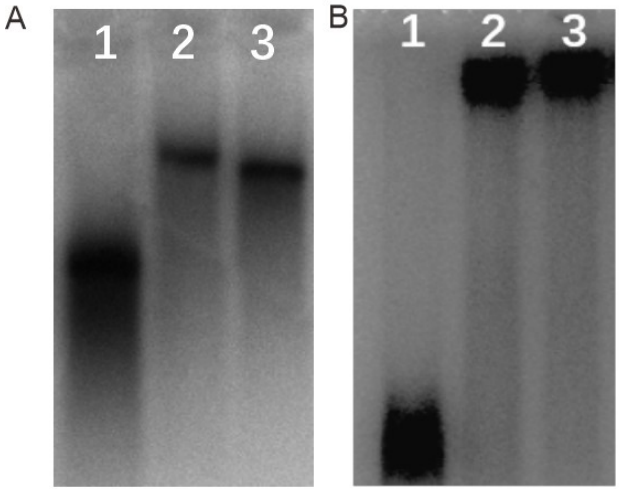

Figure S3. Agarose gel electrophoresis data of G_{16}NPs, G_{16}@PEG and G_{16}@EGF ($A_{1\text{-}3}$); and G_{73}NPs, G_{73}@PEG and G_{73}@EGF ($B_{1\text{-}3}$)[1].

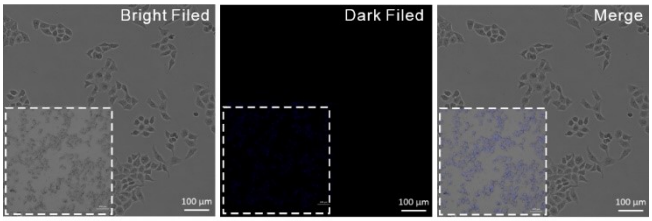

Figure S4. Laser confocal images for native HeLa cells and HeLa cells stained by DAPI (the insert).

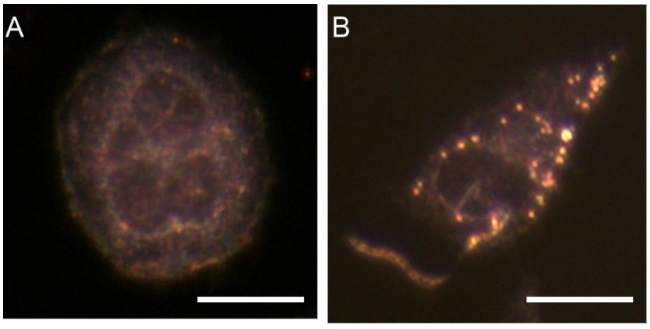

Figure S5. Dark-Filed images for HeLa cells incubation with G_{16}@PEG (A), G_{16}@EGF (B) for 30 min. The scale bar in **A-B** is 40 μm.

Methods and Materials

Chemicals and Instruments.

Gold chloride trihydrate ($HAuCl_4$), trisodium citrate (Na_3-citrate), 1-ethyl-3-(3-(dimethylamino) propyl) carodiimide (EDC), N-hydroxysuccinimide (NHS), thiol-PEG-COOH (HS-PEGCOOH, MW=3400), poly(ethylene glycol) methyl ether thiol (HSPEG, MW = 2000), phosphate-buffered saline (PBS) solution, and casein blocking agent were purchased from Sigma-Aldrich (St. Louis, MO, USA), UV−vis spectrometer, Electrophoresis apparatus(obtained from being liuyi), DAPI reagent, tween 20, malvern laser granulometer, BCA Protein Assay, phosphor-EGFR (Tyr1068) antibody; EGF Receptor Antibody, goat anti-rabbit IgG-HRP, Dark field microscope, PEG. All glassware would

be cleaned with aqua regia before use.

Synthesis and characterization of GNPs.

GNPs with diameters of 16 nm and 73 nm were used in our experiments. First, the classical Fren's method was adapted to synthesize the G_{16}NPs,[2] specifically, the solution of $HAuCl_4$ was added to a sodium citrate solution which was in a boiling state and a wine-red solution was prepared after vigorous stirring for 7 min. And for G_{73}NPs ,1 mL of the above synthesized G_{16}NPs solution was taken out as the seed solution, mixed with Na_3-citrate and hydroxylamine hydrochloride and stirred for 5 min. the state that the color of the whole solution turned into coffee after dropped into $HAuCl_4$ solution indicated that the particles were growing. The size of GNPs was measured by transmission electron microscopy (TEM) (JEM 1230, JEOL, Japan) and dynamic light scattering (DLS) analysis (Zetasizer Nano ZS, Malvern).[3]

Synthesis and characterization of targeted nanoprobes.

To prepare the targeted nanoprobes, 10 μM HS-PEG-COOH，the linker molecule，was added into 0.1 nM GNPs that synthesized above and incubated for 1 h. The PEG modified GNPs were obtained after reacting with 10 μM HS-PEG for 3 h and were purified by centrifugation, three times for removing the excessive reagents. The hetero functional linker HS-PEG-COOH that were activated by addition of 0.1 mM EDC/NHS at 37 °C for 1 h, connected with EGF through amide bond at 4 °C overnight in PBS. After that, the nanoparticles were washed with PBS twice and redispersed in 1 mL of DMEM and stored in 4 °C. GNPs, GNPs@PEG particles and GNPs@EGF were detected by UV-vis spectra, the hydrodynamic radius, zeta potential and agarose gel electrophoresis, respectively. Furthermore, the nanoprobe suspended in the cell culture medium for 0, 4, and 24 h and then were detected by UV-vis spectra, respectively. The number of coupled protein molecules on the surface of GNPs were determined by using BCA assay.

Culture and imaging of HeLa cells under DFM.

HeLa cells were cultured in advanced DMEM supplemented with 10% fetal bovine serum (FBS), contained 1% of two different types of antibiotics which are streptomycin and penicillin, in an incubator at 37°C, 5% CO_2 and 95% relative humidity. Then, cells were seeded in a six well plate with the density about 1×10^4 cells/mL. Next, before experiments, cells were starved in serum-free DMEM medium for 24 h. The probes prepared in advance were incubated with cells for 10 min and cells were washed with 1× PBS buffer for three times. Before imaging under DFM, HeLa cells were fixed with 4% (w/v) paraformaldehyde for 20 min and washed with 1× PBS buffer.

DAPI staining and imaging of cells.

The fresh prepared cell suspension 200 μL was plated in a 24 well culture dish that sterile cell climbing tablets were placed in advanced for the cells spreading evenly on its surface and cultured in the incubator until the cell density was about 60 -- 70%. Next, the cells climbing slides were taking out gently, placed on the surface of the clean glass slides after the cells washed by 1X PBS

twice. And then, the 4% (w/v) paraformaldehyde used to fix the cells was dripped to the surface of the cell creeper for 20 min. After PBS washing for three times, to prevent nonspecific combination, the freshly prepared 5 % skimmed milk was added to immersion these creepers for 30 min and then the dye of DAPI 100 μL incubated with the cells in the environment of dark for 20 min. Last, removed the redundant dye with PBS and observed with a confocal laser microscope away from light.

Western blot analysis.

Cells were cultured in a 6-well dish with a confluency of approx. 90 % and serum-starved for 12 h as described above. Then the cells were stimulated with a fresh medium containing EGF, GNPs@PEG and GNPs@EGF for 10 min, respectively. The cells were washed third times with ice-cold PBS after the medium removed. The scraped cell suspension that harvested in lysis buffer (Cell lysate: PMSF: protease inhibitor cocktail=100:1:2) on ice for 30 min was centrifuged at 12,000 g for 20 min at 4 °C) and the total protein concentration was determined with BCA Protein Assay using BSA (Beyotime) as a protein standard. The mixed solution that the lysate and a quarter volume of 4× SDS sample buffer containing 20 mM 2-mercaptoethanol boiled at 95 °C for 5 min. Next, proteins were separated by SDS-PAGE with 5 -- 8% Tris-Acetate Protein Gels and transferred to a polyvinylidene difluoride (PVDF) membrane for 2 h.

The membranes that tailored according to protein marker after transfer were blocked with 5% milk in TBST for 1 h at room temperature. The membranes were washed with TBST and incubated with the appropriate primary antibodies: phosphor-EGFR (Tyr1068) antibody (abcam; 1:1000); EGF Receptor Antibody (abcam; 1:1000) at 4 °C overnight and HRP-conjugated secondary antibodies (zsbio; 1:10000) for 2 h at 25°C. Chemiluminescence was used to detected the HRP signal with Tanon-5200 multi and the intensity of chemiluminescent image was quantified by Image J.

References

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2.X. Ji, X. Song, J. Li, Y. Bai, W. Yang and X. Peng, *J. Am. Chem. Soc.* 2007, 129, 13939.

3. L. F. Liang, *College Physics*, 1996, 15, 2.