Supporting Information

A Robust Probe for Lighting up Intracellular Telomerase via Primer Extension to Open A Nicked Molecular Beacon

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Experimental Section

Materials and Reagents. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Trisodium citrate was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS), epigallocatechin gallate (EGCG), ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma-Aldrich Inc. (USA). Telomerase ELISA kit was from Innovation Beyond Limits (Germany). DNase I endonuclease, 3-(4,5-dimethylthiazol-2-yl)-2-diphenyltetrazolium bromide (MTT), dNTPs, HeLa cells, BGC-823 cells, MCF-7 cells and Bel-7402 cells were from KeyGen Biotech. Co. Ltd. (Nanjing, China). QSG-7701 cells were from K&KM Biotech. Co. Ltd. (Wuxi, China). Gel electrophoresis loading buffer and ladder DNA were purchased from Solarbio. Co. Ltd. (Beijing, China). Phosphate buffer saline (PBS, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4, and 1.41 mM KH_2PO_4. Aqua regia was prepared by mixing HCl and HNO_3 with the volume ratio of 3:1. All other reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water (≥ 18 MΩ, Milli-Q, Millipore).

The DNA sequences were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) with the following sequences:

The longer sequence of the molecular beacon (l-MB): 5'-Cy5-AGG GTT $(AAA)_7$ AAC CCT AAC TCT GCT CGA CGG ATT-SH-3';

Telomerase primer (TSP): 5'- AAT CCG TCG AGC AGA GTT-3'.

Apparatus. The transmission electron microscopic (TEM) images were obtained on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). Dynamic light scattering (DLS) was obtained on a 90 Plus/ BI-MAS equipment (Brook haven, USA). Zeta potential analysis was performed on a Zetasizer (Nano-Z, Malvern, UK). The UV-vis absorption spectra were obtained with a UV-vis spectrophotometer (Nanodrop-2000C, Nanodrop, USA). Gel electrophoresis was performed on a DYCP-31 BN electrophoresis analyser (Liuyi Instrument Company, China) and imaged on the Bio-Rad ChemDoc XRS (USA). Flow cytometric analysis was gained on a Coulter FC-500 flow cytometer (Beckman-Coulter). The fluorescence spectra were obtained on a spectrofluorophotometer (RF-5301PC, Shimadzu, Japan). The cell images were gained on a TCS SP5 laser scanning confocal microscope (Leica, Germany). MTT assay was performed on a microplate reader (680, Bio-Rad, USA). Inductively coupled plasma atomic emission spectra (ICP-AES) was detected with an ICP-AES instrument (Optima 5300DV, USA).

Preparation of Nicked MB functionalized probe. Gold Nanoparticles (AuNPs) were prepared according to the previous report.[S1] After heating 200 mL $HAuCl_4$ solution (0.01%) to 100 ^oC, 5.0 mL trisodium citrate (1%) was added quickly to the boiling solution under continuous stirring. The reaction mixture was stirred at 100 ^oC for 1 h until the color turned deep red and then stored at 4 ^oC. 10 μL l-MB (100 μM) and 10 μL TSP (100 μM) were then added to 1 mL AuNP solution and stirred at room temperature overnight. Afterward, 0.1 mL PBS solution containing 2 M NaCl was added to the mixture stepwise for stabilizing the obtained probe, which was centrifuged and washed with PBS twice, finally resuspensed in 1 mL PBS. The supernatant containing excess Cy5-tagged l-MB was collected for determining the amount of MB on each probe.

Demonstration of Telomerase-triggered Fluorescence Switch. The fluorescence intensity of the probe (1 mL) containing dNTPs (10 μL, 10 mM each) in the presence or absence of telomerase (10 μL, 40 IU L^{-1}) was detected after different incubation times. Under excitation at 600 nm, the fluorescence of Cy5 was recorded from 600-750 nm.

Polyacrylamide Hydrogel Electrophoresis. The mixtures of 10 μL l-MB (100 μM) and TSP solution (100 μM), 10 μL l-MB (100 μM), TSP solution (100 μM), 10 μL dNTPs (10 mM each) and 10 μL cell extract, and 10 μL l-MB (100 μM), TSP solution (100 μM), 10 μL dNTPs (10 mM each) and 10 μL telomerase (40 IU L^{-1}) were incubated at 37 ^oC for 1 h. The gel electrophoresis was performed by adding 7 μL l-MB (100 μM), the three mixtures and ladder DNA (10 μM) as indicator in 1.5 μL loading

buffer containing 1.5 μL GelRed, respectively, and then injecting them into polyacrylamide hydrogel in tris-borate-EDTA (TBE) buffer. Electrophoresis was carried out at 100 V in TBE buffer for 1 h. The resulting board was observed under UV irradiation.

Cell Culture. HeLa cells were cultured in a flask in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. BGC, MCF, BEL and QSG cells were respectively cultured in RPMI-1640 (GIBCO) supplemented with 10% fetal calf serum, penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Cell number was determined with a Petroff-Hausser cell counter (USA).

Detection of Telomerase Activity in Cell Extract. Cells were collected in the exponential phase of growth, and 5×10^7 cells were dispensed in a 1.5-mL EP tube, washed twice with ice-cold PBS (0.1 M, pH 7.4), and resuspended in 200 μL of ice-cold CHAPS lysis buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 0.5% CHAPS and 10% glycerol. The mixture was incubated for 30 min on ice and centrifuged at 16000 rpm and 4 ^{o}C for 20 min. The supernatant was collected and diluted to 200 μL as cell extract for detection or storage at -80 ^{o}C.

A standard addition method was used for quantification of telomerase activity in HeLa cell extract. After incubating the mixtures of 10 μL cell extract (corresponding to 2.5×10^6 cells), 10 μL dNTPs (10 mM each) and 1 mL probe in the absence and presence of 10 μL spiked telomerase (20 IU L^{-1} from telomerase ELISA kit) at 37 ^{o}C for 1 h, the fluorescent intensity was detected, respectively. The telomerase in 10 μL cell extract was calculated to be 7.8×10^{-5} IU, i.e. single HeLa cell averagely contained 3.1×10^{-11} IU telomerase.

In Situ Imaging of Telomerase Activity with the Probe. 0.5 mL HeLa cells (or BGC, MCF, BEL, QSG cells) of 1×10^6 mL^{-1} were seeded in each confocal dish for 24 h, and 25 μL probe was then added into each cell-adhered dish. After incubation at 37 ^{o}C for different times, the cells were sent for fluorescent confocal imaging or flow cytometric detection.

The TEM image was taken after HeLa cells (0.5 mL, 1×10^6 mL^{-1}) were incubated with 25 μL probe at 37 ^{o}C for 1.5 h and fixed on a copper grid with 10% methanol for 1 h at 4 ^{o}C.

Quantification of Intracellular Telomerase Activity. The calibration curve for quantification of intracellular telomerase activity was obtained by treating HeLa cells with different amounts of EGCG for 48 h, and then detecting the telomerase-triggered fluorescence intensity (*FI*) with the probe through con-

focal imaging (Figure 2b) and the telomerase activity with ELISA kit analysis. The former was performed with 25 μL probe to obtain the confocal images and then read the average red channel intensity in cell area with Adobe Photoshop software. The ELISA kit analysis was performed with the extracts of EGCG-treated cells through a standard curve method. 50 μL of cell extracts were firstly added in the wells of ELISA plate followed by incubation at 37 °C for 30 min. The plate was then washed with PBS and added with 50 μL labeling reagent (from the kit) to incubate at 37 °C for 30 min. Afterward, the medium was removed, and 50 μL of color development agent A and 50 μL of color development agent B (from the kit) were added to vibrate for 10 min at 37 °C. 50 μL of stop buffer (from the kit) was finally added to each well to stop the color reaction, and the absorbance was measured at 450 nm on a microplate reader to obtain the telomerase activity in the extracts. With the calibration curve and the *FI* of the probe-treated cells, the intracellular telomerase activity could be detected.

Evaluation of Amount of l-MB Assembled on the Probe

To measure the amount of l-MB assembled on the probe, a standard curve was obtained with a series of l-MB solutions (Figure S1). From the fluorescence intensity of the supernatant containing excess l-MB collected after the preparation of probe (Figure S1, inset), the amount of l-MB on each probe was estimated to be around 90.

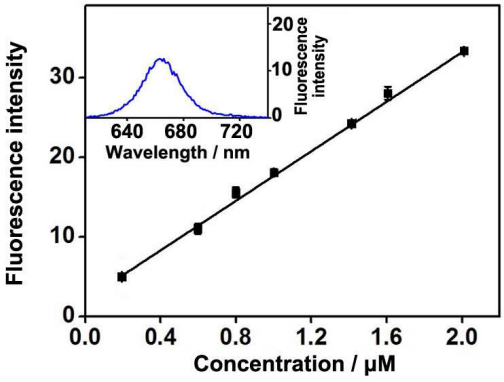

Figure S1. Plot of fluorescence intensity *vs.* l-MB concentration. Inset: fluorescence spectrum of the supernatant containing excess l-MB collected after the preparation of probe.

Fluorescence Recovery and stability of the Probe

The fluorescence switch and its stability were examined in different media such as PBS, DMEM, RPMI 1640 and pure water, respectively (Figure S2). In these media, the fluorescence intensity of the

probe (1 mL) and dNTPs (10 μL, 10 mM each) in the presence or absence of telomerase (10 μL, 40 IU L^{-1}) was observed after incubation for different times (Figure S2a-d). In the presence of telomerase the fluorescence intensity increased and treaded to the maximum value with the increasing incubation time in PBS, DMEM and RPMI 1640 (Figure S2a-c, curve B), while no change of fluorescence intensity was observed in the absence of telomerase (Figure S2a-c, curve A), indicating the switch feature and good stability of the probe. Furthermore, the extension reaction did not also cause the coagulation of the probe (Figure S2e). In the absence of salts the intensity increase was very weak due to the very slow primer extension (Figure S2d).

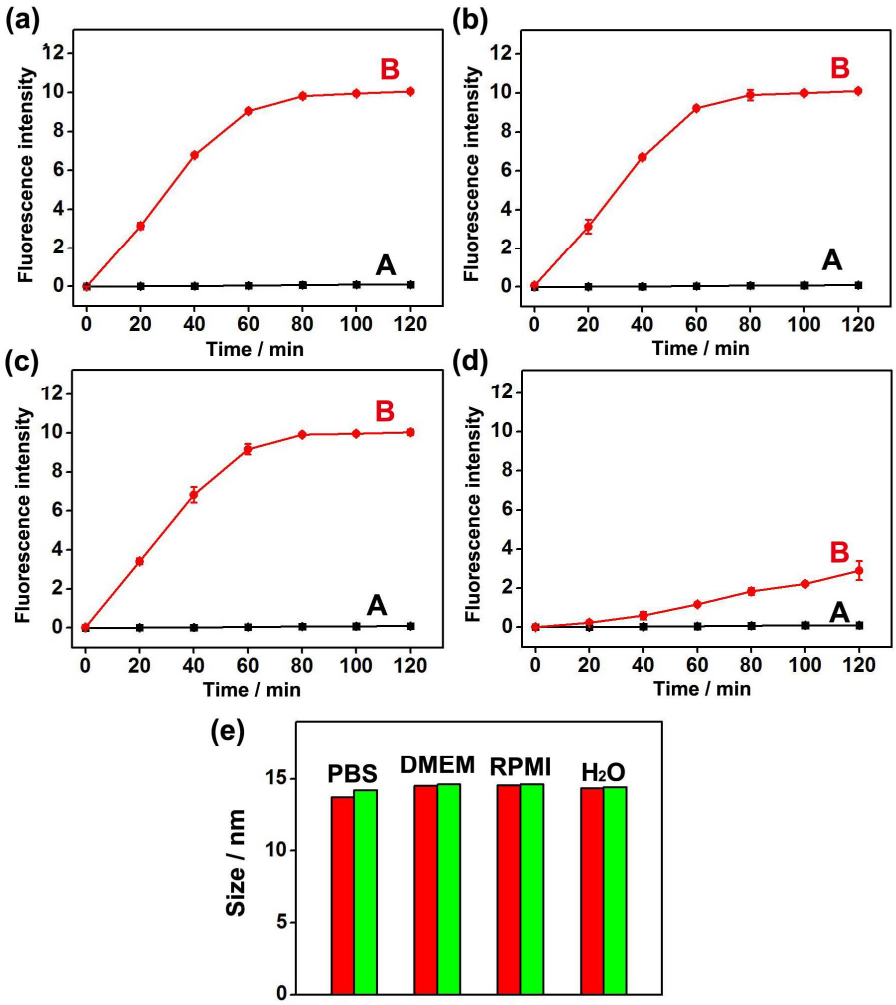

Figure S2. Plots of fluorescence intensity of the probe solution (1 mL) containing dNTPs (10 μL, 10 mM each) *vs*. incubation time in the (A) absence and (B) presence of telomerase (10 μL, 40 IU L^{-1}) in (a) PBS, (b) DMEM, (c) RPMI1640 and (d) water. (e) DLS characterization of the probe before (red column) and after (green column) 2-h incubation with telomerase and dNTPs in marked media.

Telomerase Kinetics

From the increasing fluorescence intensity in PBS (Figure 1e and Figure S2a), the concentrations of opened MB, [P], at different incubation times could be obtained. The plot of [P] *vs.* reaction time was shown in Figure S3a, which could give the reaction rate (V) at different times and the maximum [P] value to be 0.45 μM. Thus the concentration of nicked MB as the substrate of telomerase, [S], could be expressed as [S] = 0.45 – [P], which led to the plot of V *vs.* [S] (Figure S3b), with a linear regression equation of $V = 7.0\times10^{-4} + 0.026$ [S]. This result indicated that the telomerase-triggered fluorescence recovery was a pseudo-first-order reaction, and the reaction constant was 0.026 min^{-1}.

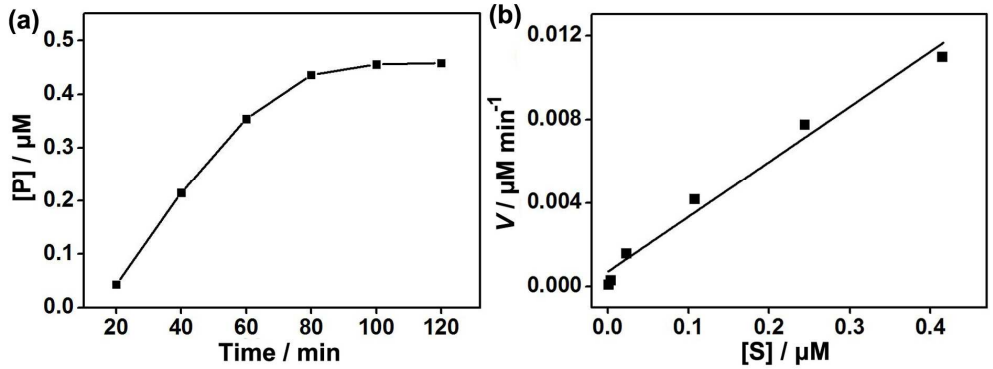

Figure S3. Plots of (a) [P] *vs.* reaction time and (b) V *vs.* [S].

Detection of Telomerase in Cell Extract

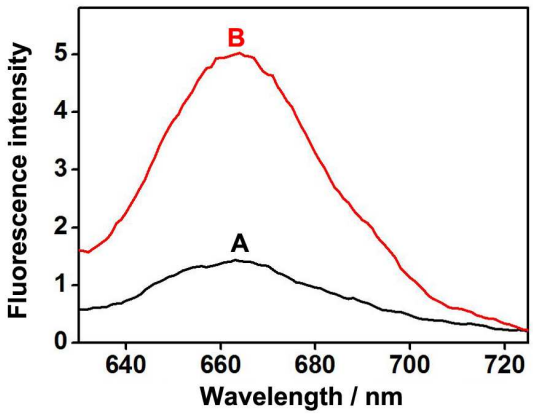

Figure S4. Fluorescence spectra of 1 mL probe containing dNTPs (10 μL, 10 mM each) and 10 μL cell extract in the (A) absence and (B) presence of spiked telomerase (10 μL, 20 IU L^{-1}) after incubation for 1 hour.

Protection Ability of AuNPs to Nicked MB against Nuclease in Acidic pH

After 2 μg DNase I endonuclease was added to 1 mL probe (dispersed in pH 6.0 PBS), the fluorescence intensity of the solution was recorded along with incubation time, which showed negligible fluorescence recovery (Figure S5), indicating the protective ability of AuNP and good probe stability.

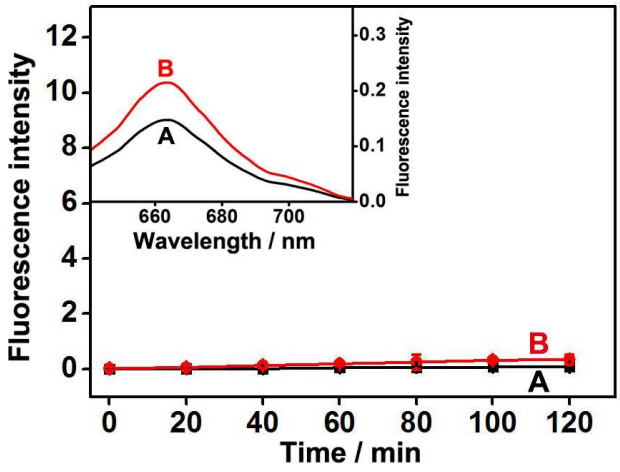

Figure S5. Plots of fluorescence intensity of the probe (1 mL) in pH 6.0 PBS in absence (A) and presence (B) of 2 μg Dnase I *vs.* incubation time. Inset: fluorescence spectra corresponding to A and B at 120 min.

Probe Localization by TEM

The internalization and localization of the probe was characterized by TEM (Figure S6).

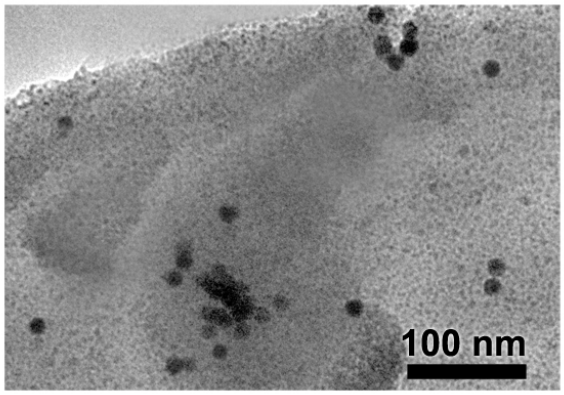

Figure S6. TEM image of probe-internalized HeLa cell.

Optimization of Probe Amount for Incubation with HeLa Cells

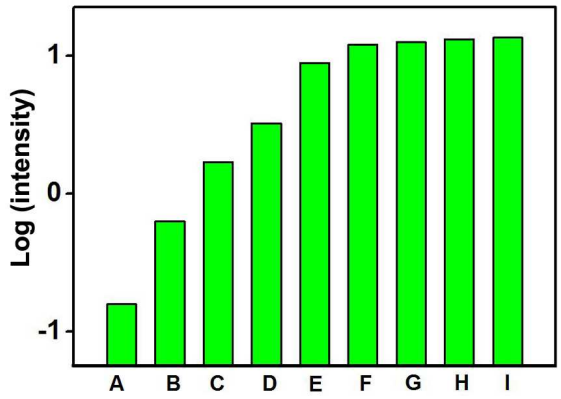

Figure S7. Flow cytometric detection of HeLa cells (0.5 mL, 1×10^6 mL^{-1}) after 90-min incubation with 0, 5, 10, 15, 20, 25, 30, 35 and 40 μL probe (from A to I) at 37 ^oC.

Evaluation of Cytotoxicity of the Probe

The cytotoxicity of probe was tested with HeLa cells by MTT assay. After the cells were incubated with 100 μL culture medium containing 25 μL probe for different times, MTT (50 μL, 1 mg mL^{-1}) was added to the well and incubated at 37 °C for 4 h. Then 100 μL of dimethyl sulphoxide was added to each well and vibrated for 15 min to dissolve the crystals formed by the living cells, and the absorbance at 490 nm was measured to obtain the relative cell viability (%) by $(A_{test}/A_{control})\times100\%$.

Figure S8. Viability of HeLa cells (100 μL, 1.0×10^6 mL^{-1}) after incubation with 25 μL probe for different times.

Demonstration of the Specific Opening of the Nicked MB in Living Cells

HeLa cells (0.5 mL, 1×10^6 mL^{-1}) were seeded in a 20-mm confocal dish for 24 h. After adding 25 μL l-MB-functionalized AuNPs (prepared with the same procedure as the probe) in the dish, the cells were incubated for 1.5 h at 37 ^oC for confocal observation.

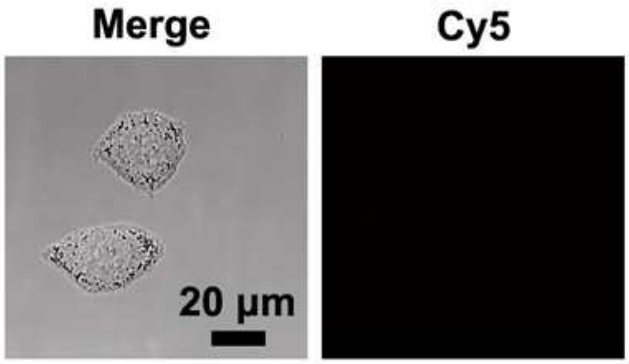

Figure S9. Confocal microscopic images of HeLa cells after incubation with 25 μL l-MB-functionalized AuNP for 1.5 h at 37 ^oC.

Flow Cytometric Analysis of Different Cells After Incubation with the Probe

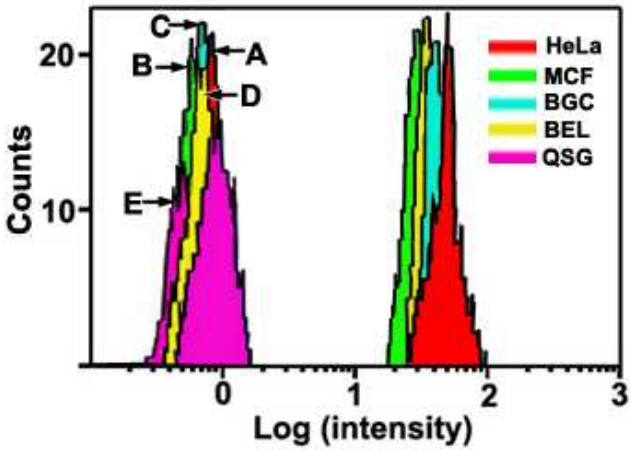

Figure S10. Flow cytometric detection of HeLa, MCF, BGC, BEL and QSG cells (0.5 mL, 1×10^6 mL^{-1}) after incubation with 25 μL probe for 1.5 h at 37 ^oC. A-E corresponded to the control cells of HeLa, MCF, BGC, BEL and QSG, respectively.

ICP-AES Detection of Cellular Uptake Amount of the Probe.

EGCG-treated HeLa cells and different types of cells (0.5 mL, 1×10^6 mL^{-1}) were seeded in 20-mm confocal dishes for 24 h, respectively. After adding 25 μL probe solution to each dish, the cells were incubated for 1.5 h at 37 ^oC. Afterwards, the cells were detached from the dish and dissolved in aqua regia (200 μL) overnight, and the solution was diluted to 1 mL with pure water for ICP-AES detection. From the average diameter of 13 nm, the number of gold atoms (N) corresponding to each AuNP could be calculated.[S2] Thus the amount of probe taken in per cell (*n*) could be determined with the number of gold atoms measured with ICP-AES. The results were shown in Figure S11. The difference between the maximum and minimum probe numbers was less than 10%, which was much smaller than the difference of fluorescence intensity observed in Figure 2e or Figure 3a.

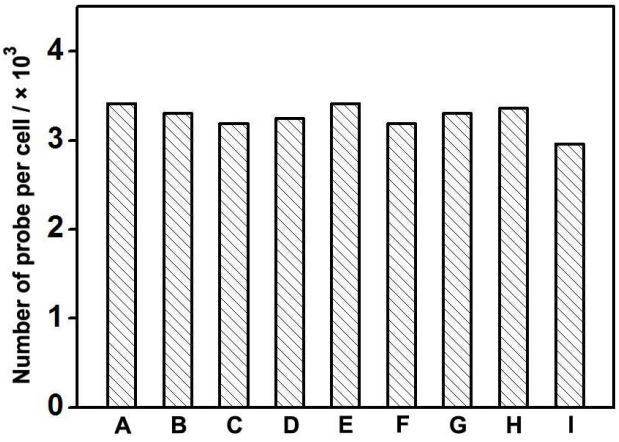

Figure S11. The uptake amounts of probe in different types of cells. From A-D: HeLa cells treated with 0, 60, 120, 250 μg mL^{-1} EGCG followed by incubation with probe; from E-I: HeLa, MCF, BGC, BEL and QSG cells after incubation with probe.

Supporting Reference

(S1) Grabar, K. C.; Freeman, R. G.; Hommer, M. B.; Natan, M. J. *Anal. Chem.* **1995**, *67*, 735.

(S2) Lewis, D. J.; Day, T. M.; MacPherson, J. V.; Pikramenou Z. *Chem. Commun.* **2006**, 1433.