Supporting information

Folic acid-functionalized fluorescent gold nanoclusters with polymer as linker for cancer cells imaging

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Experimental section

Chemicals

Ovalbumin (Ova) was purchased from Sigma-Aldrich (St. Louis, MO). Tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 4H_2O$), azo-bis-isobutryonitrile (AIBN), N-Acryloxysuccinimide (NAS), folic acid (FA) and other chemicals were of analytical reagent grade purity and obtained from Beijing Chemical Corporation (Beijing, China). *S, S'*-bis (α, α'-dimethylacetic acid) trithiocarbonate (DATB) were synthesized according to the reference.[1] Water used throughout the experiments was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Polymerization of PNAS

PNAS was synthesized by RAFT methods. NAS (5.0 mmol, 0.845 g), DATB (50.0 μmol, 14.0 mg) and AIBN (25.0 μmol, 4.0 mg) were added into 8.0 mL dioxane. Then the flask was sealed under nitrogen after three freeze-evacuate-thaw cycles, and then placed in an oil bath thermostatted for RAFT polymerization at 60 °C for different times (4-20 h). The final polymer was obtained by pouring the reaction mixture into excess diethyl ether while stirring, recovered by filtration, washed several times with the same solvent and finally dried under vacuum at room temperature overnight.

Synthesis of FA-PNAS

Three different kinds of PNAS (100.0 mg) and FA (10.0 mg) were added into DMSO (8.0 mL) solution, respectively. Then the flask was sealed under nitrogen and reacted for 24 h at room temperature. The final PNAS-FA was obtained by pouring the reaction mixture into excess diethyl ether while stirring, recovered by filtration, washed several times with the same solvent and finally dried under vacuum at room temperature overnight. The effect of different kinds of PNAS for FA binding was determined by fluorescent spectrum.

Synthesis of Ova-AuNCs

All glassware was washed with Aqua Regia ($HCl:HNO_3$ = 3:1, v/v), and rinsed with ethanol and ultrapure water. In a typical synthesis, Ova solution (10.0 mL, 10.0 mg/mL, 37 °C) was added to aqueous $HAuCl_4$ solution (10.0 mL, 1.5 mM, 37 °C) under vigorous stirring. Two minutes later, NaOH solution (0.9 mL, 1.0 M) was introduced, and the mixture was vigorously stirred for 2 h at 37 °C. The color of the solution changed from light yellow to light brown, and then to deep brown. The reaction was completed in 2 h, as confirmed by time-course measurements of the fluorescence evolution.

Preparation of FA-Ova-AuNCs

FA-PNAS solution (10.0 mg/mL, 0.2 mL) and Ova-AuNCs (2.0 mL, 141.4 μg/mL) were mixed and reacted under vigorous stirring for 24 h at room temperature. The dialysis procedure using water for 24 h was followed for avoiding the residual unreacted species by using a membrane with a molecular weight cutoff of 6.0-8.0 kDa, and the as-obtained light-brown FA-Ova-AuNCs solution was kept at 4 °C in the dark for further use.

Characterization

Molecular weight

We determined the molecular weight and molecular weight distribution of the polymers using GPC. The molecular weight measured by GPC was carried on a Hitachi L-2130 pump with a Waters 2410 refractive index detector, and a Waters 2487 ultraviolet detector with the combination of Hersteller MZ-Gel SDplus 5 μm, and the DMF was used as eluent at a flow rate of 1.0 mL/min.

TEM and scattering mean diameters

The TEM images of Ova-AuNCs were acquired on a JEM-2010 (Jeol Ltd, Japan) at an accelerating voltage of 200 kV. The TEM samples were prepared by dropping the colloidal solution of Ova-AuNCs in water onto 50 Å thick carbon coated copper grids with the excess solution being immediately wicked away. The scattering mean diameters were estimated by TEM images using a software Nano measure 1.2.

Zeta potential

The zeta potential (ζ) values of the FA-PNAS, Ova-AuNCs and FA-Ova-AuNCs were measured at 25 ˚C by the Zetasizer Nano ZS (Malvern Instruments, Southborough, UK). The samples for the zeta potential determination were exhaustive dialyzed by water for 24 h.

UV-vis absorbance

A UV-visible spectrophotometer (Beijing purkinje general instrument Co. LTD, Beijing) was used for the UV-visible absorption analysis of the samples.

Loading efficiency of FA on the Ova-AuNCs

The loading efficiency of FA on the Ova-AuNCs was determined using a fluorescence measurements were performed using an F-4500 fluorescence spectrophotometer (Hitachi, Japan). The calibration curves were setup by detecting the fluorescent emission at 455 nm of a series of FA standard solutions at different concentrations. And the FA-Ova-AuNCs fluorescent emission at 455 nm was detected to calculate the concentration of FA loading on the Ova-AuNCs.

Incubation FA-Ova-AuNCs with Hela cells and human keratinocytes cells

A line of Hela cells and a line of human keratinocytes cells that grow readily in culture and form confluent monolayers with relatively low trans-monolayer permeability were exposed, for 3 h, to a 1: 9 dilution of the FA-Ova-AuNCs solution (141.4 μg/mL) in serum-free medium. The cells were then washed intensively for 3 times to remove all extracellular FA-Ova-AuNCs before CLSM observation.

Cytotoxicity assay

In order to evaluate the biocompatibility of the FA-Ova-AuNCs, a commercial kit

(CCK-8 Kit), which can produce soluble purple formazan in the presence of viable cells, was used for the cytotoxicity assay. The cells (1× 10^4 cells/well) were plated in the 96-well plates in 5% CO_2 atmosphere at 37 ^oC for 24 h. Then different amount of FA-Ova-AuNCs were dissolved in the serum-free medium and incubated with the cells for 24 h. The cells were washed with PBS for three times and incubated in serum-free medium with 10 % CCK-8 solution for 2 h. The optical absorbance of the cells was measured at 450 nm by a microplate reader (Model SpectraMax M5). Control experiment was done by detecting the growth culture medium without the FA-Ova-AuNCs.

Cell imaging

Confocal laser scanning microscopy (CLSM) characterization was conducted by a confocal laser scanning biological microscope (FV1000-IX81, Olympus, Japan) with FV5-LAMAR for excitation at 488 nm and a variable bandpass emission filter set to 570-670 nm. Fluorescence micrographs were recorded with a Zeiss Apotome inverted microscope combined with a 100x oil immersion objective. The cells grown on glass-bottom culture dishes containing 0.4 mL of culture medium were first incubated with FA-Ova-AuNCs (40 μL, 14.1 μg/mL) for 3 h at 37 ^oC, and then washed thoroughly with PBS solution (pH 7.4). To confirm the receptor-mediated uptake, competition experiments were conducted where the cell culture was pre-treated with saturated FA solution for 1.0 h prior to FA-Ova-AuNCs treatment.

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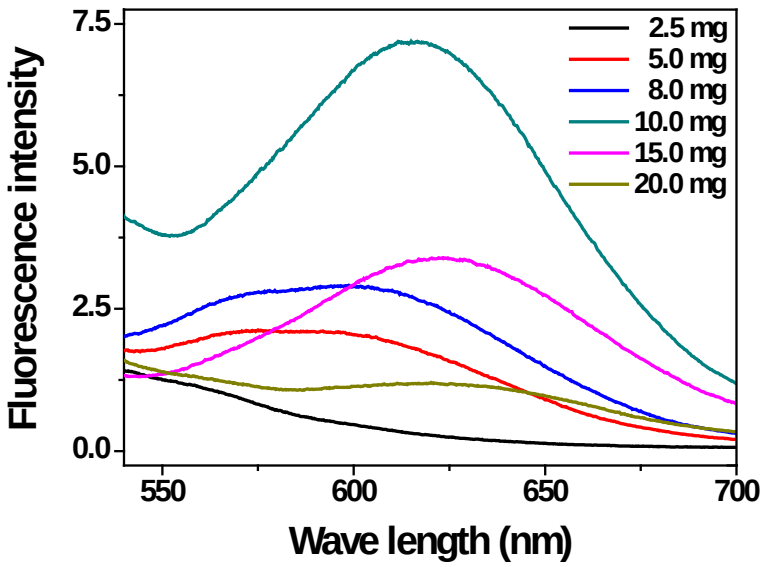


Figure S1. The effect of Ova concentration on the photoemission spectrum (λ_{ex} = 488 nm) for the reaction solution of $HAuCl_4$ and Ova at 37 °C.

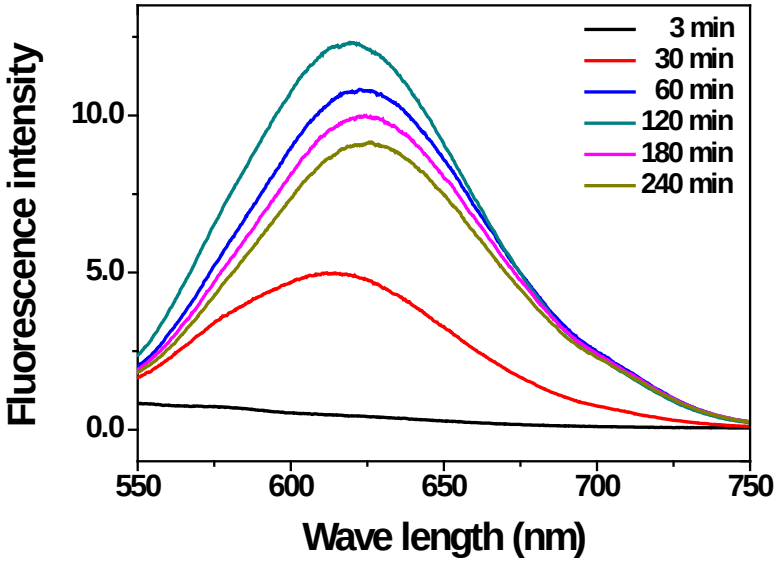


Figure S2. Time evolution of the photoemission spectrum (λ_{ex} = 488 nm) for the reaction solution of $HAuCl_4$ and Ova at 37 °C.

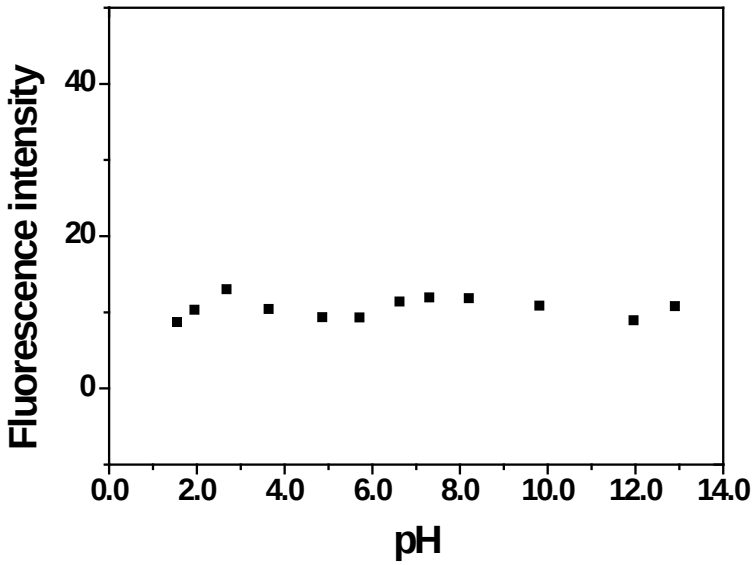


Figure S3 Effect of pH on the fluorescence intensities of aqueous Ova-AuNCs solution (λ_{ex}= 488 nm).

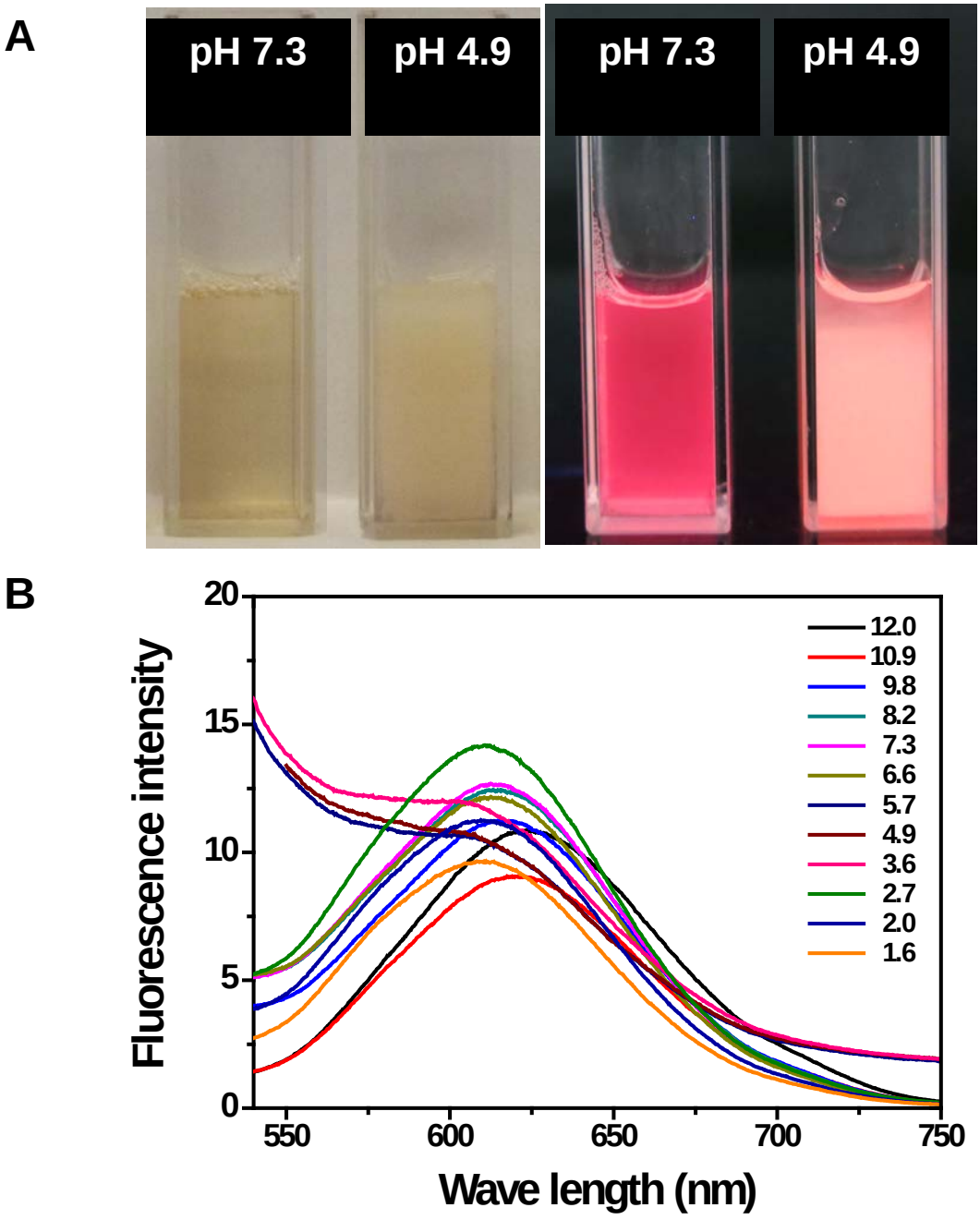
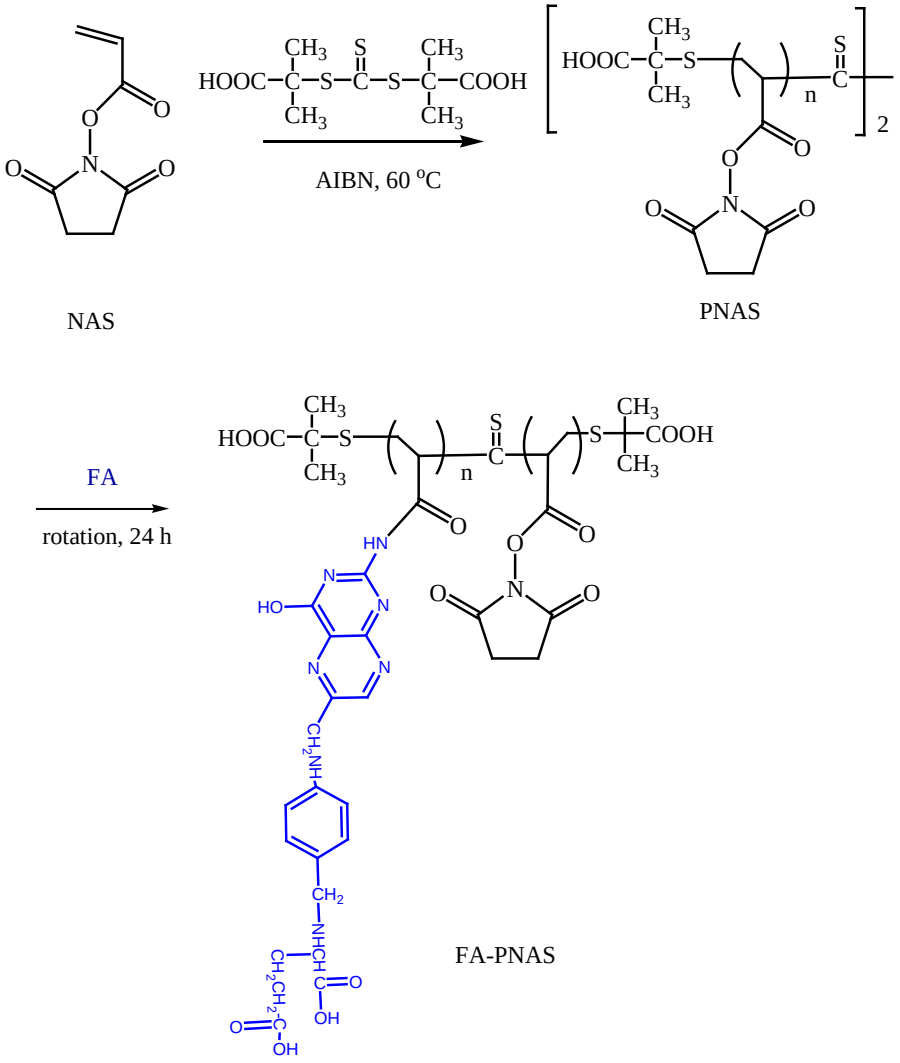


Figure S4 The photographs of aqueous Ova-AuNCs at pH 7.3 and 4.9 taken in visible and UV light, respectively (A). The fluorescence emission of aqueous Ova-AuNCs solution at different pH (λ_{ex}= 488 nm, B).



Scheme S1 Schematic diagram of synthetic route for FA-PNAS.

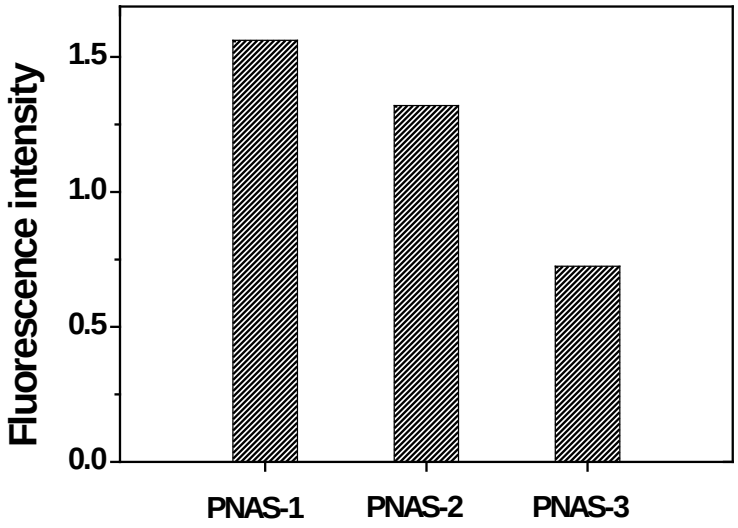


Figure S5 FA reacted with different molecular weight of PNAS.

Table S1 Physical properties of the PNAS

Polymers	Polymerization time (h)	Polymerization temperature (^{o}C)	Mn	PDI
PNAS-1	4.0	60	20564 [a]	1.1
PNAS-2	5.0	60	24139 [a]	1.2
PNAS-3	20.0	60	42618 [a]	1.3

[a] Mn obtained by GPC

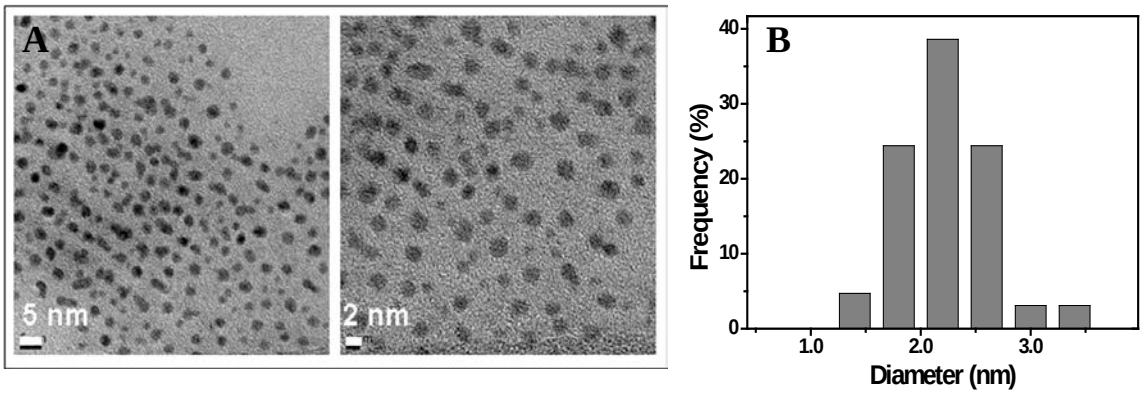


Figure S6 HRTEM images of FA-Ova-AuNCs (A) and diameter distribution of the FA-Ova-AuNCs estimated by HRTEM images (B).

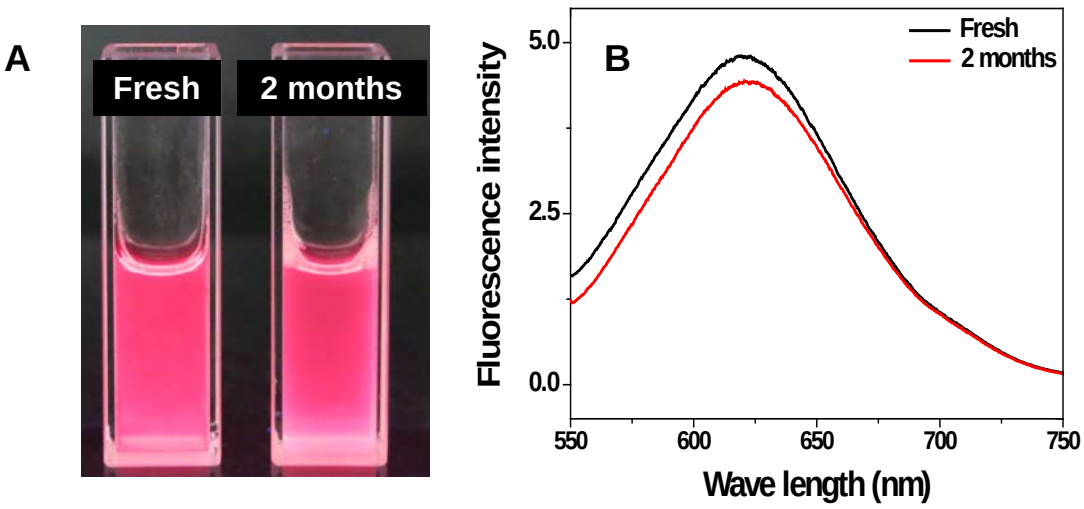


Figure S7 (A) Photographs of FA-Ova-AuNCs freshly prepared and after 2 months of storage taken under UV light (365 nm). (B) Fluorescence spectra of FA-Ova-AuNCs freshly prepared and after 2 months of storage.

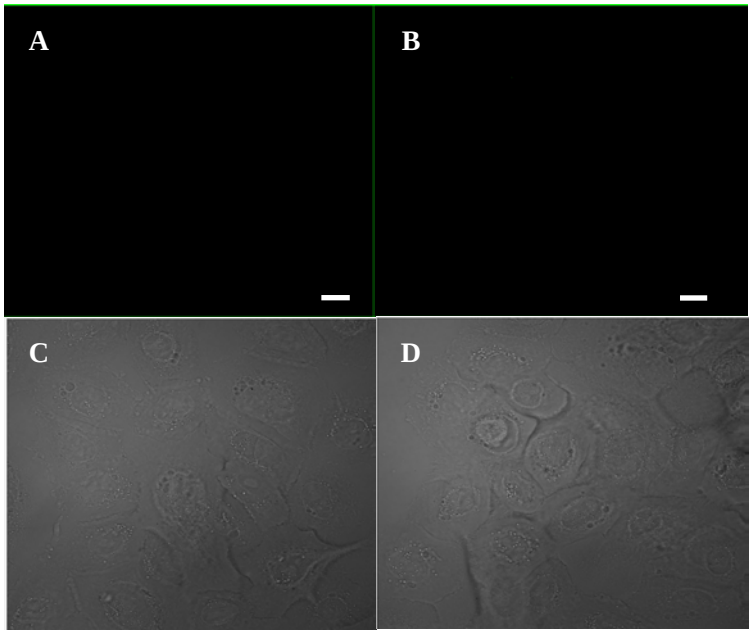


Figure S8 Fluorescence images of human keratinocytes cells under different conditions. (A) Human keratinocytes cells (control). (B) Human keratinocytes cells incubated with FA-Ova-AuNCs solution for 3 h. (C, D) The differential interference contrast (DIC) images of the corresponding samples. Scale bar, 10 μm.

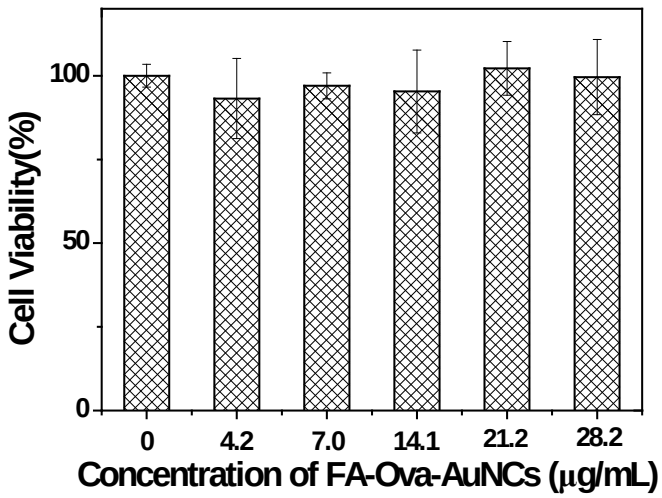


Figure S9 Effects of FA-Ova-AuNCs with varied concentrations on the viability of HeLa cells (the viability of the cells without FA-Ova-AuNCs is defined as 100%). The results are the mean of five measurements.