

<Supporting Information>

Microwave-assisted one-step rapid synthesis of folic acid modified gold nanoparticles for cancer cells targeting and detection

Experimental Section

Materials

HAuCl₄·3H₂O, NaOH and HCl were purchased from Xilong Chemical (Guangdong, China). Folic acid, trypsin, penicillin and streptomycin were obtained from Sigma Aldrich Chemicals (Scotland, UK). Dulbecco Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL/Life Technologies (Paisley, UK). WST-1 Cell Proliferation and Cytotoxicity Assay Kit were supplied by Beyotime insititute of biotechnology (Haimen, China). All reagents were of analytical grade. High purity water (18.2 MΩ) was used in all of the experimental processes.

Preparation of FA-GNPs

All the used glasswares in the following procedures were cleaned in a bath of freshly prepared aqua regia, rinsed thoroughly in ultrapure water and dried in cabinet drier. FA solution (0.2 mL, 1.0 mM) was added drop by drop to the HAuCl₄ (5 mL, 0.8 mM) solution under magnetic stirring. 1 mol/L NaOH was subsequently added to obtain a clear orange solution (pH=10.60). The solution was stirred for 30 min and

immediately placed in a sealed vessel and reacted in a **single-mode** microwave reactor (ChemPower, Shanghai Zhizhun Scientific Instrument Co., Ltd.), **operating at a frequency of 2.45 GHz**. The reactions were kept for 2 h at 100 °C. FA functionalized gold nanoparticles (FA-GNPs) were thus obtained in this solution. After cooling to room temperature, the solution was dialyzed overnight to remove the extra free small molecules for further characterization.

Characterization

The morphology of particles were characterized by a transmission electron microscope (JEM-2100 HC, JEOL, Japan) operated at an accelerating voltage of 120 kV. The samples were prepared by dropping GNPs solution (5 uL) onto a carbon-coated copper grid. All UV-visible spectra were collected on a Beckman H800 UV-vis spectrophotometer with 1 nm resolution. **Stability of FA-GNPs in different concentrations of PBS solution (0.6–1 mM) was measured by UV-vis spectrometry.** Fourier transform infrared (FTIR) spectra were obtained from a Nicolet Avatar FTIR model 330 spectrometer (Thermo, America). The range of spectra was from 400 cm^{-1} to 4000 cm^{-1} . The reported values were based on 10 measurements with 15 cycles for each sample. For X-ray diffractometer (XRD) and X-ray photoelectron spectroscopy (XPS) experiments, samples were prepared as follows: GNPs were precipitated by centrifugation at 15 000 rpm for 30 min. The pellet could easily be redispersed in ddH₂O (1 mL). The solution was centrifuged again and redispersed in ddH₂O for XPS experiments, or dried at room temperature for XRD experiments. For XPS experiments, a few drops of the GNPs solution were put on a silicon substrate and

dried in air. The surface composition of **FA-GNPs** was investigated in an PHI Quantum 2000 Scanning ESCA Microprobe (PHI, America) using Al K α excitation source. The crystal structure of the nanoparticles was studied using X-ray diffractometer with CuK α radiation ($\lambda=1.54059$ Å) operated at 40 kV and 40 mA (PANalytical X'Pert Pro, Almelo, the Netherlands).

Cytotoxicity assay

Human adenocarcinoma HeLa cells were cultured in DMEM at 37 °C for 12 h in a humidified incubator containing 5% CO₂. The culture media were supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin. Cells in the exponential phase were added to each well of 96-well tissue culture plates at a density of 5×10^3 cells/mL and incubated for 12 h, respectively. Then 200 μ L of FA-GNPs nanoparticles were added to each well at different concentrations (10, 25, 50 and 100 μ g/mL), incubated for 12 h, 24 h and 36 h at 37 °C, respectively. Cells without nanoparticles were used as the control group. 10 μ L of WST-1 solution was added to each well and incubated for 2 h at 37 °C. OD values at a wavelength of 450 nm were measured with a microplate reader (Bio-Rad Laboratories, USA). For each concentration of FA-GNPs, five wells of cells were analyzed and the final values were expressed as a percentage of the control.

Cancer cells targeting of FA-GNPs

The determination of the intracellular uptake of Au was carried out by inductively coupled plasma mass spectroscopy (ICP-MS) (Sciex Elan DRC-e, Perkin Elmer, USA). The detached HeLa cells were dissolved in freshly prepared aqua regia, and

diluted to a final volume of 50 mL for ICP measurements. Au content per cell was reported based on the number of cells in each sample. The calibration solutions were prepared from a stock standard solution from Perkin-Elmer in the calibration range of 0.25 to 1000 ppb Au. Samples were measured using an integration time of 4.5 s for three replicates, and the averaged results with standard deviation were given. HeLa cells were cultured for intervals of 1, 2, 3, 4, and 5 h. At the end of each interval, the cells were washed with phosphate buffer solution (PBS) three times to remove free and physically absorbed GNPs, detached by trypsin, and resuspended in PBS (100 mL). Cell density was evaluated using a hemocytometer. The competitive inhibition assays were also performed by culturing cells with both FA-Au NPs and free FA of varying concentrations. At the end of the incubation time, cells were washed, detached, resuspended in PBS, and counted as described above. The cells were diluted to a final volume of 10 mL for ICP measurements. The Au content per cell was reported based on the number of cells in each sample. Each sample was conducted with three replicates and the averaged results with standard deviation were given.

Detection of cancer cells

HeLa cells and fibroblasts were analysed. For each assay, 300 μL of FA-GNPs (10 nmol) were added to 30 μL of PBS with different amount of cells (750, 3000, 7500, 15000, 22500, 30000 and 45000 cells), then incubated for 30 min at 4 °C in a 500 μL microcentrifuge tube (Beckman), and centrifugated at 1000 rpm for 5 min. The supernatant was withdrawn and the absorbance was measured. PBS with no cells but containing the same amount of nanoparticles was taken as the blank. $\Delta\text{Absorbance}$

(ΔA) was calculated according to the following formula: $\Delta A = A_0 - A_1$ (A_0 = Absorbance of blank, A_1 = Absorbance of supernatant). The absorbance values were obtained with the Beckman H800 UV-vis spectrophotometer at 529 nm. Each experiment was repeated at least five times.

Fig. S1 The XPS of the FA-GNPs presenting (A) Au 4f, (B) C 1s, (C) N 1s, (D) O 1s spectra.

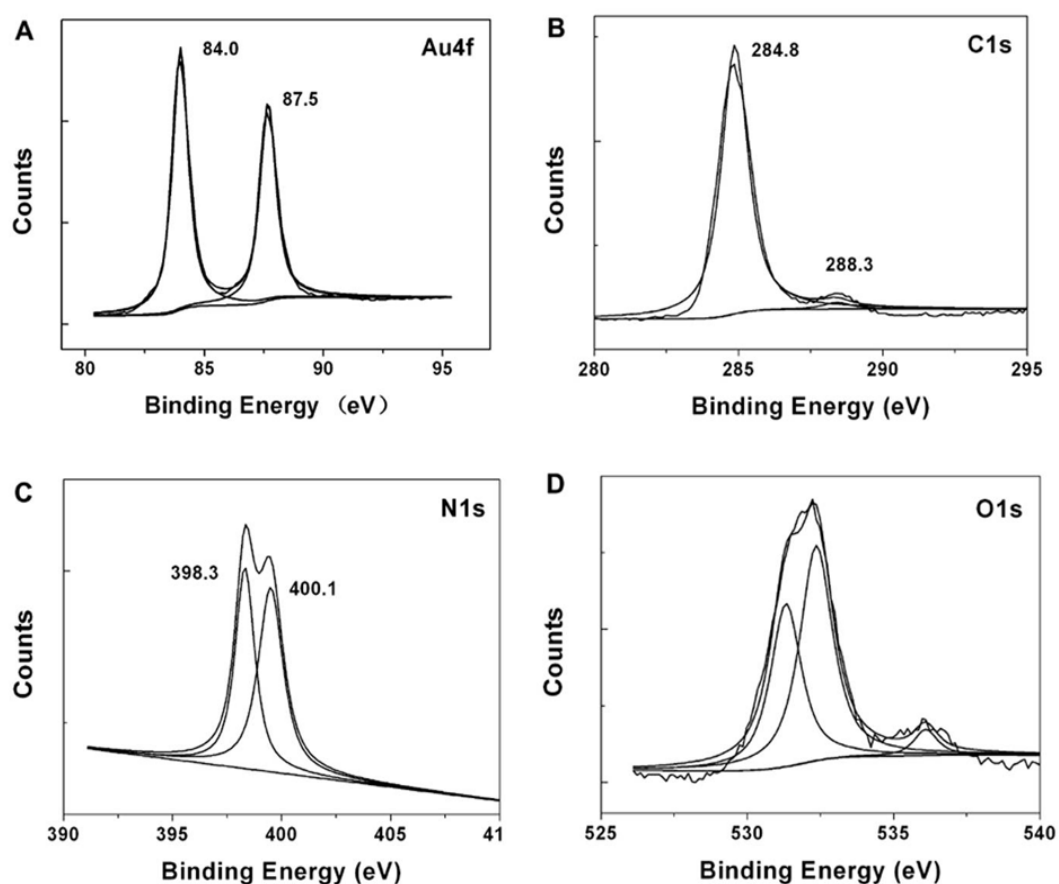


Fig. S2 Stability of FA-GNPs in PBS buffer.

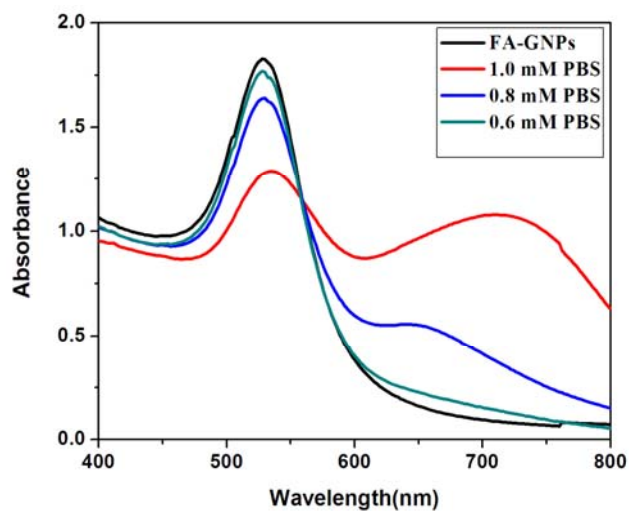


Fig. S3 The XRD pattern of FA-GNPs. The spectrum included five peaks at 38.2° , 44.6° , 64.7° , 77.5° and 82.4° that can be assigned to the (111), (200), (220), (311) and (222) planes.

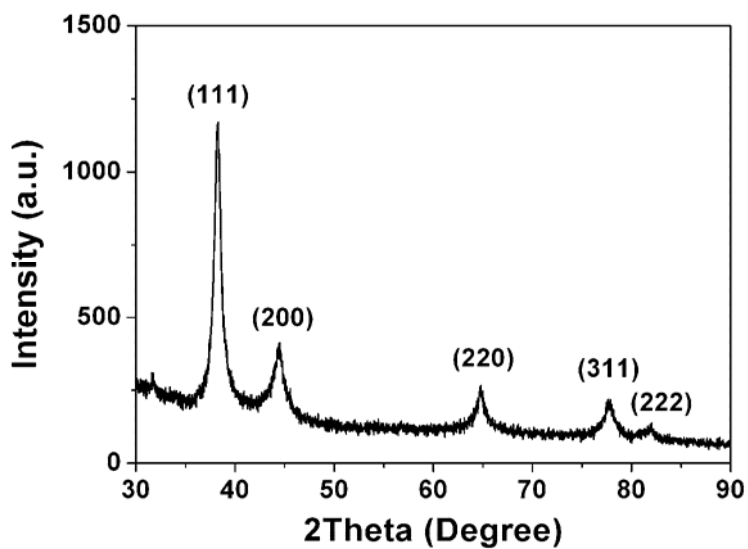


Fig. S4 WST-1 assay of HeLa cells viability after treatment with FA-GNPs at different concentration for 12 h, 24 h, 36 h, respectively.

