

Experimental:

MTT assay:

To determine cell viability the colorimetric MTT metabolic activity assay was used. HeLa cells (1×10^4 cells/well) were cultured in a 96-well plate at 37 °C, and exposed to varying concentrations of Au@IPN-pNIPAAm nanogels for 24 h. Cells treated with medium only served as a negative control group. After removing the supernatant of each well and washing twice by PBS, 20 μ l of MTT solution (5 mg ml⁻¹ in PBS) and 100 μ l of medium were then introduced. After incubation for another 4 h, the resultant formazan crystals were dissolved in dimethyl sulfoxide (100 μ l) and the absorbance intensity measured by a microplate reader (Bio-RAD 680, USA) at 490 nm with a reference wavelength of 620 nm. All experiments were performed in quadruplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

Apoptosis assay:

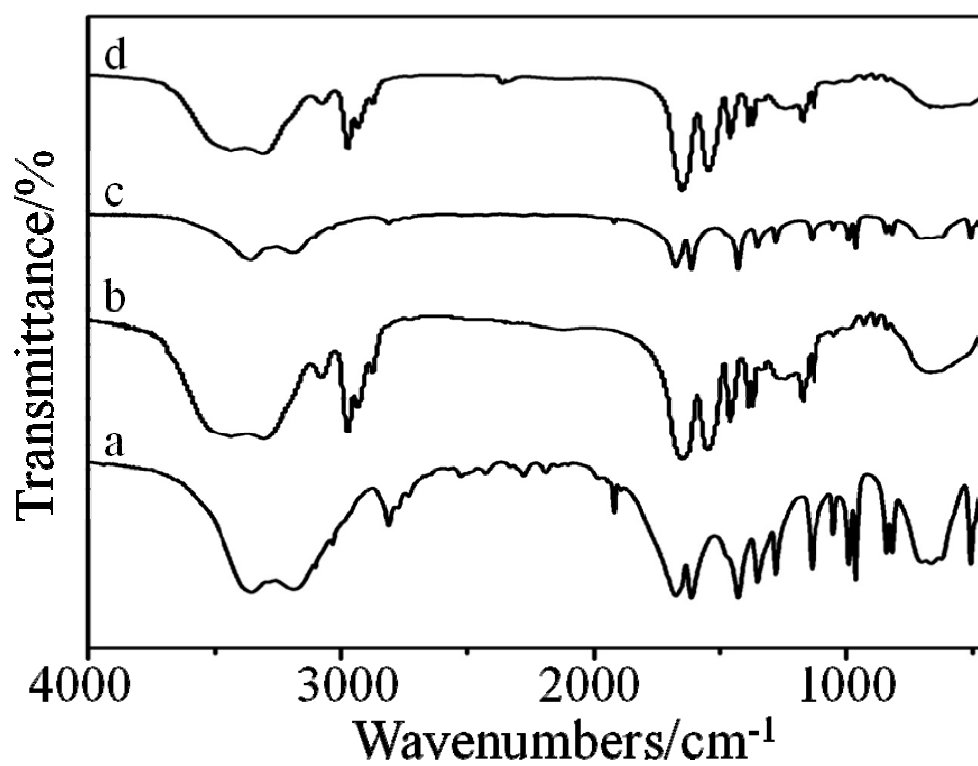
Apoptosis/necrosis of HeLa cells induced by Au@IPN-pNIPAAm nanogels was assayed by an annexin V-FITC/PI apoptosis detection kit, which comprises two individual stains: (1) annexin V has a strong Ca²⁺-dependent affinity for phosphatidylserine which might translocate from the cytoplasmic face of the plasma membrane to the cell surface during apoptosis, and (2) PI is membrane-impermeant and generally excluded from viable cells, but could intercalate into double-stranded nucleic acids. After co-culturing with Au@IPN-pNIPAAm nanogels for 12 h, HeLa cells were harvested and incubated with 200 μ l of binding buffer (HEPES buffered PBS supplemented with 2.5 mM CaCl₂), 2 μ l of annexin V-FITC and 5 μ l of PI at room temperature for 15 min in the dark, following the manufacturer's instructions. After a final wash in PBS, HeLa cells were immediately analyzed on an EPICS XL flow cytometer (Beckman Coulter, USA), gated on live cells by FSC/SSC. Samples were excited by the light wavelength of 488 nm with barrier filters of 525 nm and 575 nm for FITC fluorescence and PI detection, respectively. Data were analyzed by EPICS XL flow cytometer software and plotted for annexin V-FITC and PI in a

two-way dot plot. Live, early apoptotic and late apoptotic/necrotic cells are designed as annexin-/PI-, annexin +/PI-, and annexin+/PI+, respectively.

FACS assay for cellular uptake:

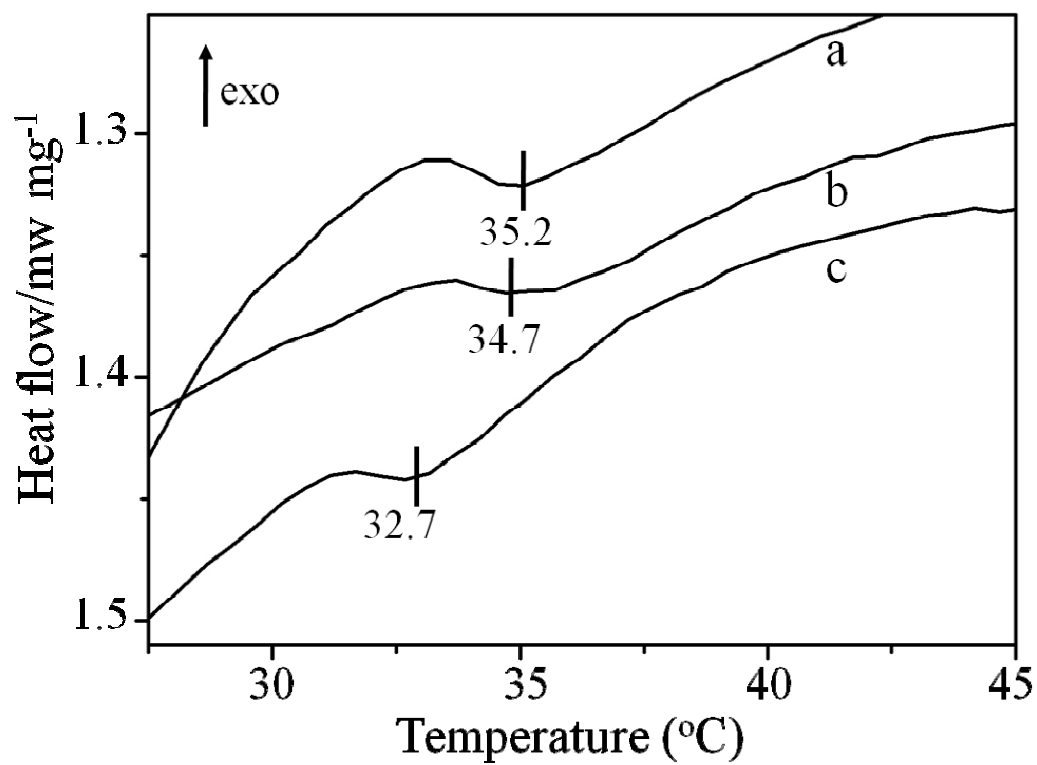
To analyze the internalization of FITC-labeled Au@IPN-pNIPAAm nanogels by fluorescence-activated cell sorting (FACS) analysis, 6×10^5 Hela cells were firstly plated and cultured overnight on 60 mm dishes. After being washed with PBS, the cell monolayers were then incubated with different concentrations of FITC-labeled Au@IPN-pNIPAAm nanogels for 6 h at 37 °C to analyze dose-response of uptake. To examine the effects of exposure time, the cells were incubated with $200 \mu\text{g ml}^{-1}$ of FITC-labeled Au@IPN-pNIPAAm nanogels for different periods. The cells were then resuspended in ice-cold PBS buffer solution, and the fluorescent cells with FITC-labeled nanogels were then measured from 20,000 cells by using an EPICS XL flow cytometer (Beckman Coulter, USA) in the FL1 channel. Cytometry analyses were performed using EPICS XL flow cytometer software, and analytical gates were chosen as 1% of control cells falling within the positive region.

FT-IR Results



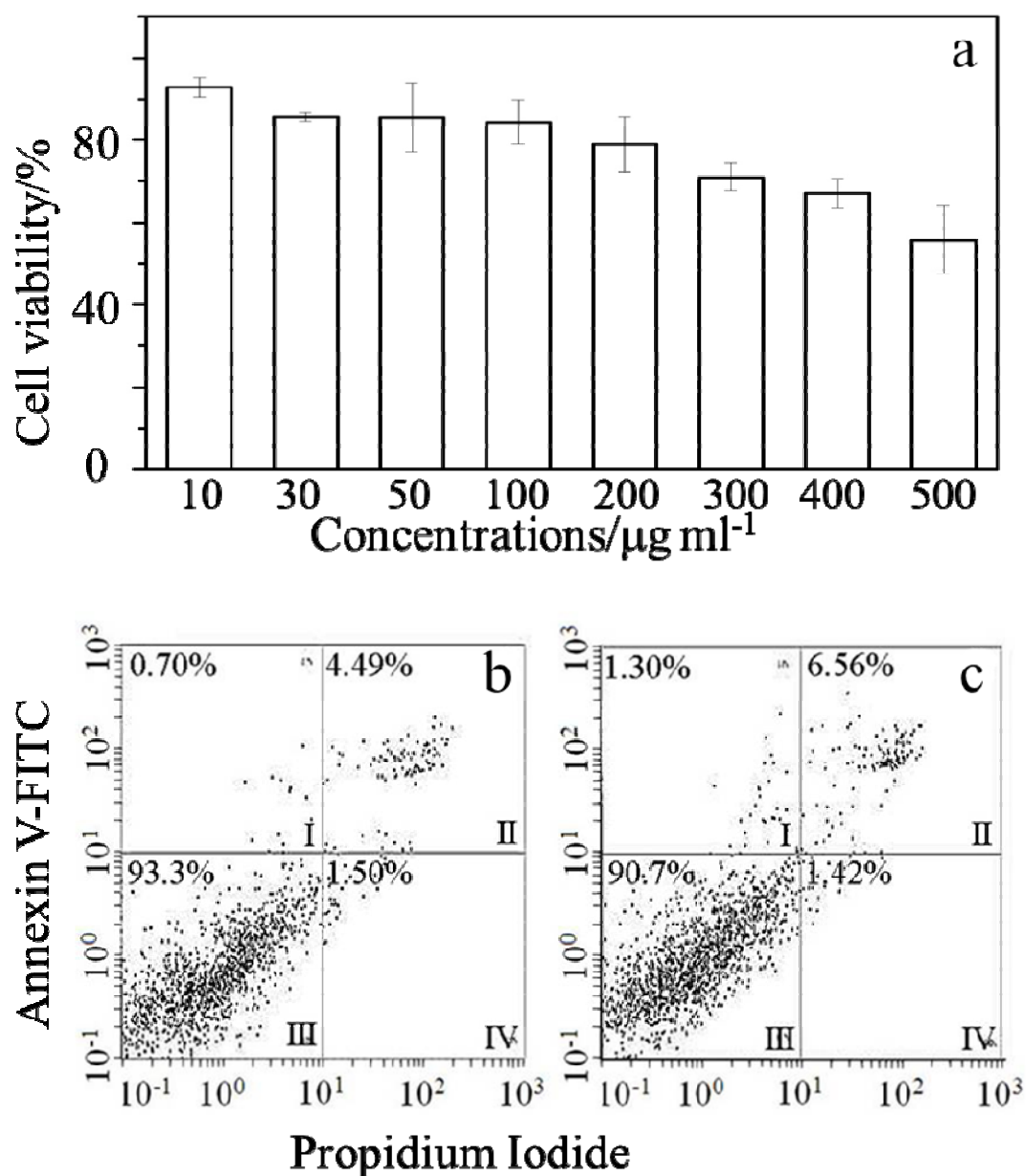
ESI 1 FT-IR spectra of (a) Au@AAM NPs, (b) AAm, (c) IPN hydrogels of p(NIPAAm-co-AAm) and pNIPAAm, and (d) Au@IPN-pNIPAAm nanogels.

DSC Results



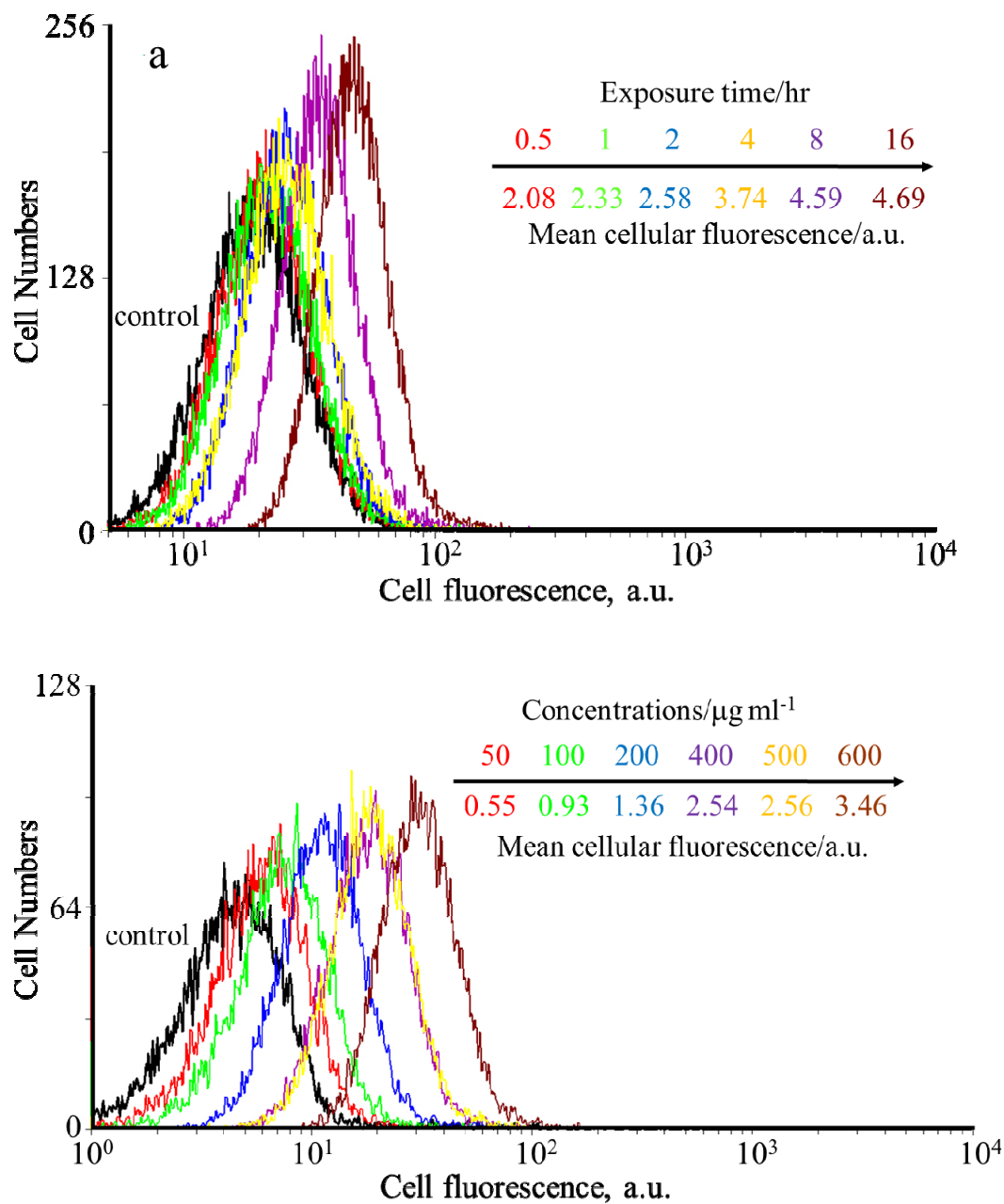
ESI 2 LCST of the hybridAu@IPN-pNIPAAm nanogels detected by DSC. The molar of AAm to NIPAAm: (a) 1:9; (b) 1:30; and (c) 0.

In vitro toxicity



ESI 3 (a) Dose-response cell viability detected by MTT assay. Bars represent the corresponding standard deviations ($n = 3$). (b, c) Quantitative evaluation of cell apoptosis/necrosis caused by Au@IPN-pNIPAAm nanogels. The flow cytometric scatter plots for 20 000 Hela cells were obtained using (b) non-treatment (control), and (c) 200 $\mu\text{g ml}^{-1}$ Au@IPN-pNIPAAm nanogels, respectively. I: early apoptotic cells; II: late apoptotic cells; III: healthy cells; IV: necrotic cells.

FACS Results for cellular uptake



ESI 4 FACS analyses of the effects of (a) exposure time, and (b) dose (represented by concentration between 50 and 600 $\mu\text{g ml}^{-1}$) on cellular uptake of Au@IPN-pNIPAAm nanogels.