Supporting Information for

Encapsulating Single Nanoprobe in a Multifunctional Nanogel for High-Fidelity Imaging of Caspase Activity in Vivo

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1. Supplementary Results and Discussion

1.1. Supplementary characterization data for the AuNP@gel probes

After grafting the peptides onto AuNPs, a multifunctional nanogel shell was produced \textit{in situ} by linking the vinyl groups on the pep-AuNPs with vinyl-bearing crosslinkers and other functional monomers. Careful selection of the monomers endowed the AuNP@gel probes with specific, desired functionalities. The chemical composition of the as-obtained AuNP@gel probes was characterized by Fourier transform infrared (FTIR) spectroscopy. The presence of two newly-generated characteristic peaks at 1715 and 1057 cm\(^{-1}\) are attributed to the carbonyl (C=O) and ether bonds (C-O-C) in the crosslinkers, respectively, confirming the successful polymerization on the surface of the AuNPs (Figure S4). The surface modification of AuNPs with peptides and subsequent nanogel formation was characterized by dynamic light scattering (DLS) (Figure S5). The average hydrodynamic sizes of the as-prepared citrate-AuNPs and the resulting pep-AuNPs were determined to be 37.82 and 45.75 nm, respectively. In contrast, the \textit{in-situ} formation of nanogels resulted in a significantly increased hydrodynamic size of 72.06 nm. TEM images revealed that the AuNP@gels have a typical core-shell nanostructure, wherein the AuNP core has a uniform diameter of \(~20\) nm, and the average shell thickness is \(~3\) nm (Figure S6).

1.2. Biological properties of the AuNP@gel probes

The biological properties of the as-obtained AuNP@gels were evaluated and compared against conventional AuNPs prepared by the co-modification strategy. The conventional AuNP probes were prepared with different ratios of peptides to PEG (1:1, 1:10, 1:100). A higher density of PEGs provides higher stealth effect against protein adsorption, but also hinders the interactions between the co-modified peptides and their target caspase enzymes. To validate this hypothesis, a set of AuNP probes (0.5 nM) were treated with Cas-3 (4 nM), with the resulting activated fluorescence of the cleaved peptides measured by a fluorescent imaging system (Azure Biosystems C600). These AuNP probes include pep-AuNPs, pep/PEG (1:1)-AuNPs, pep/PEG (1:10)-AuNPs, pep/PEG (1:100)-
AuNPs, and AuNP@gels at a pH of 7.4 and 5.5, respectively. Dithiothreitol (DTT) was employed to cut off Au-S bonds to remove the surface ligands from AuNP surfaces.\textsuperscript{1} As illustrated in Figure S7, increases in PEG concentration gradually weaken the fluorescence (see samples 1-4, up). When excessive DTT (100 mM) was added into the samples, strong fluorescence was recovered with similar intensity (bottom), indicating that the amounts of peptides anchored on different AuNPs are equivalent. As a result, the difference in Cas-3-mediated fluorescence recovery can be attributed to the different densities of PEG on the AuNPs. These results suggest the dichotomy between avoiding nonspecific protein adsorption and maintaining probe functionality when determining the ratio of PEGs to ligands on the conventional co-modification nanoprobes.

With regards to the AuNP@gel probes in pH 7.4 buffer solution, the treatment of either Cas-3 or DTT was unable to cause any fluorescence recovery (sample 5), as the nanogels are capable of shielding the peptide probes from interaction with external Cas-3. Furthermore, DTT was unable to remove the peptide probes from the AuNP surface due to the crosslinking of the peptide tails within the nanogels. However, nanogels degraded rapidly when the AuNP@gel probes were dissolved in a buffer solution with pH of 5.5 as they released pep-AuNPs into the solution. When the pep-AuNPs were exposed to either Cas-3 or DTT, fluorescence was activated with high intensities (sample 6), similar to conventional AuNP probes.

The roles of the monomers in the nanogels were systematically investigated. We demonstrated the tendency of the nanogels to degrade in acidic conditions. 0.5 nM of AuNP@gel probes were dissolved in both pH 7.4 and pH 5.5 buffer solutions, with their resulting size changes at varying incubation times monitored by DLS. The average hydrodynamic diameters of the AuNP@gel probes decreased from 67 to 48 nm in the pH 5.5 solution within 1 h (Figure S9). In the neutral solution, negligible changes were observed in the size of AuNP@gel probes, suggesting that the nanogels of the AuNP@gel probes degrade rapidly in acidic solutions. Then, we tested the protonation of the tertiary amines (TAs) on the AuNP@gel probes. As a comparison, AuNP@gel probes wherein the ionizable monomers were replaced with neutral monomers that could not be protonated were also prepared. The probes were dissolved in buffer solutions with different pH values,
and their zeta potentials were measured immediately after mixing. With decreasing pH values, the average zeta potentials of the TA-incorporated AuNP@gels were observed to increase correspondingly from –16.2 to +5 mV due to ionization of the TA groups in acidic solutions (Figure S10). However, the zeta potentials of the neutral amide-incorporated AuNP@gels show only subtle changes when immersed in different pH solutions.

Finally, the TA-initiated proton sponge effect of the AuNP@gel probes occurring in endosomes was investigated. As previously discussed, the AuNP@gel probes enter into the HeLa cells through FA receptor-mediated endocytosis. Though endocytosis is thought to be the most efficient pathway to deliver nanoparticles into cells, this pathway usually leads to a subsequent endosomal trapping of the nanoparticles. It has been well accepted that the trapped nanoparticles may stay in the lysosomes for more than 12 h. Lysosomes are characteristic of their harsh conditions including acidic environments, high-level GSH, and an abundant enzymatic degradation system, which often alter the physiochemical properties of nanoparticles, thus, deactivating their functionality. Therefore, numerous strategies have been developed to assist with the escape of nanoparticles from endosomes as quickly as possible. The labeling of ionizable groups onto nanoparticles has proven to be one such method to achieve an endosomal escape function. In this study, we explored the endosomal escape of the AuNP@gel probes and compared it to that of FA-conjugated pep/PEG (1:1)-AuNPs. To avoid the possible removal of peptides from AuNP surfaces caused by intracellular GSH, the HeLa cells were cultured in GSH-free media before and after incubation with AuNP probes. Since the fluorescence of the Cy5 dyes in the anchored peptides was quenched by the AuNP cores, a relatively high-power laser (19.84 mW, shutter: 80%) was used to stimulate the excitation, with the emission signals subsequently amplified by a photomultiplier tube (PMT). The lysosomes were specifically labeled with lysosome trackers as indicated by the green dots (Figure S11). Upon incubation with the labeled cells, the AuNP probes were tracked by time-dependent colocalization experiments. For the AuNP@gel probe-treated cells, the red signals were completely mismatched with the green signals (Figure S11a) throughout the whole tracking process, indicating the rapid escape of the probes from endosomes. We reasoned that the nanogel shells of the AuNP@gel probes could be degraded in endosomes to
release pep-AuNPs, which were transferred into cytoplasm instantly due to a TA-initiated proton sponge effect. For the cells treated with FA-conjugated pep/PEG (1:1)-AuNPs, the red signals largely overlapped with the green signals (Figure S11b), indicating that the AuNPs were trapped into lysosomes.

The cellular colocalization experiments were confirmed by TEM imaging. After incubation of the AuNP@gel probes with HeLa cells for 20 min, the probes interacted with the FA receptors on cell surfaces, leading to the formation of endocytosis vesicles (red arrows, Figure S12); at 2 h, the endocytosis vesicles fused with early endosomes where the pH value is around 5.5. During this process, the acid-responsive nanogels coating on the AuNP probes were degraded to instantly destroy the endosomal membranes via a proton sponge effect. Yellow arrows represent the endosomes with intact membrane structure; while green arrow shows the endosomes with broken membrane structure. At 4 h, all the AuNP probes have escaped from endosomes and entered into cytoplasm to interact with caspase -3/-7. The exposed AuNP probes in cytoplasm were indicated by the white arrows. The whole process was illustrated in the schematic picture. The scissors represent the active caspase -3/-7 enzymes. In contrast, the PEG/pep-AuNP probes were trapped in endosomes at 4 h (Figure S13), agreeing well with the fluorescent colocalization experiments.

These colocalization experiments and TEM characterizations demonstrate that upon degradation of the nanogels in the acidic endosomes, the released TA moieties experience a proton sponge effect, which destroys the endosomal membranes and instantly delivers the exposed pep-AuNPs into cytoplasm to realize their functions.

1.3. In vitro detection of Cas -3/-7 using the AuNP@gel probes

The AuNP@gel probes were first evaluated for in vitro detection of Cas -3/-7. Because the encapsulated pep-AuNP probes are unable to interact with the Cas -3/-7 in solutions, the AuNP@gel probes were first dispersed in pH 5.5 buffer solution to degrade the protective nanogels. The released pep-AuNP probes (0.5 nM) were subsequently transferred in Cas reaction buffer and incubated with 8 nM of Cas -3 or Cas -7 at 37 °C for 2 h, with their emissions measured by a fluorescent spectrometer. The results show that
the Cy5 fluorescence of the pep-AuNP probes can be activated by Cas-3 or Cas-7 with different intensities (Figure S14). This phenomenon is due to the fact that Cas-3 and Cas-7 share the same cleavage site in the peptides. However, Cas-3 shows higher activity in cutting off the peptides than does Cas-7. When the pep-AuNP probes were incubated with a mixture of Cas-3 (8 nM) and its inhibitors Z-VAD-fmk (20 µM), the fluorescence was suppressed significantly. This suggests that the fluorescence activation is derived from the specific Cas-3/-7-mediated cleavage.

The specificity of the probes was further investigated by incubating the pep-AuNPs (0.5 nM) with equal concentrations (8 nM) of recombinant human Cas-3, Cas-7, Cas-9, legumain, cathepsin B or 10% FBS. The enzymatic reaction kinetics for the samples were monitored by a microplate reader. Only Cas-3 or Cas-7 were observed to exhibit continuous time-dependent fluorescence activation within a period of 2 h.

The sensitivity of the pep-AuNP probes for Cas-3 and Cas-7 was also evaluated. 0.5 nM of pep-AuNPs was incubated with various concentrations (0, 1, 2, 4, 8, and 16 nM) of Cas-3 or Cas-7 at 37 °C for 2 h. The resulting fluorescence intensities at approximately 670 nm are highly correlated with the concentrations of Cas-3/-7 (Figures S15, S16), with higher concentrations of Cas-3/-7 leading to stronger fluorescence recovery. Based on two independent calibration lines, the limits of detection for Cas-3 and Cas-7 were determined to be 0.23 and 0.65 nM, respectively.

1.4. In vivo biodistribution studies of the AuNP@gel probes

To evaluate the efficacy of the AuNP@gel probes for in vivo caspase imaging, in vivo biodistribution of the AuNP@gel probes was explored and compared with that of pep-AuNP probes in the absence of nanogels. Quantitative biodistribution analysis using inductively coupled plasma-mass spectrometry (ICP-MS) revealed that the AuNP@gel probes showed 5.7 times higher accumulation in the tumor than the pep-AuNP probes (Figure S18). The ICP-MS data also showed that fewer AuNP@gel probes (relative to pep-AuNP probes) accumulated in the liver and spleen, two major macrophage-rich organs in the MPS. These results align well with the abovementioned results, i.e., the AuNP@gel probes possess a higher delivery efficiency to tumors, and lower macrophage uptake than
conventional pep-AuNP probes.

2. Experimental section

Materials and Instrumentation. Tetrachloroauric acid (HAuCl₄), sodium citrate, folic acid (FA), cysteine, glutathione, N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), N-acryloxysuccinimide, 2-dimethylaminoethyl methacrylate, acrylamide and N, N'-methylene bisacrylamide, glycerol dimethacrylate, N-acryloxysuccinimide, 2-methacryloyloxyethyl phosphorylcholine, ammonium persulphate, N, N, N', N'-tetramethylethylenediamine, N, N-diisopropylethylamine, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. DAPI, Hoechst 33258, Lyso-Tracker Green, small molecular Cas -3/-7 fluorescence probe (Ac-DEVD-AMC) and Cas -3/-7 inhibitor (Z-VAD-fmk) were purchased from Life Technologies (Shanghai, China). Mouse Anti-Human-Cas 3-Antibody, Goat Anti-Mouse IgG Antibody (FITC binding) were purchased from Abcam company (Shanghai, China). Cas -3/-7 -responsive peptides (SH-Cys-Pro-Ala-Asp-Val-Glu-Asp-Lys-NH₂, CPADVEDK) were purchased from GL Biochem Ltd. (Shanghai, China). Sulfo-Cyanine5 NHS esters were purchased from Lumiprobe Corporation (USA). All recombinant human proteins were purchased from Sino Biological Inc. (Beijing, China). HeLa cells were supplied by American Type Culture Collection. The 96-well polystyrene plate was purchased from R&D Systems (Shanghai, China). All solutions were prepared by using de-ionized water (Milli-Q grade, Millipore) with a resistivity of 18.2 MΩ-cm (at 25°C). RPMI 1640 and fetal bovine serum (FBS) were from GIBCO (Shanghai, China). TEM Imaging was obtained by FEI Tecnai G2 at 200 kV. AFM imaging was obtained by Bruker Dimension Icon with tapping mode. UV-Vis absorption and fluorescence spectra were collected with U-3900 spectrophotometer and F-4600 (Hitachi), respectively. Dynamic light scattering (DLS) and zeta potential (ζ) were performed on a Zeta Sizer Nano ZS (Malvern Zetasizer 3000HS and He/Ne laser at 632.8 nm at scattering angles of 90 at 25 °C). IR spectra were collected by FTIR Spectrometer (Bio-rad, FTS6000). MTT results and enzyme kinetics were collected by a microplate reader (BioTek, Synergy S3 2/SLFPAD). The contents of gold in cells, major organs, and
tumor tissues were measured on an X series inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Elemental, UK). The cell imaging was carried on a TCS SP8 laser confocal scanning microscope (Leica, Germany). The confocal images were captured by a Nikon (A1+) confocal microscope. The flow cytometry data was carried on a LSR Fortessa (BD, USA). *In vivo* fluorescence imaging was performed on a photolIMAGER™ Optima (Biospace, France).

**Preparation of 20 nm AuNPs.** 12.5 mL of sodium citrate (40 mM) was added into 125 mL of HAuCl₄·3H₂O (0.2 mM) aqueous solution under vigorous stirring at 135 °C. The solution rapidly turned red, indicating the formation of 20 nm AuNPs (0.5 nM). The UV-Vis spectrum was collected and the maximal absorption peak appeared at around 520 nm.

**Calculation of quenching efficiency (QE).** After grafting the Cy5/vinyl-labeled CPADVEDK peptides onto AuNPs (20 nm) through the high affinity between the thiol group in the head cysteine and Au, the fluorescence of Cy5 dyes was significantly quenched via nanomaterial surface energy transfer (NSET). The quenching efficiency (QE) of the probes was calculated by the equal,

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QE = \left(1 - \frac{F_{\text{quenching}} - F_0}{F_{\text{activating}} - F_0}\right) \times 100\%
\]

\(F_{\text{quenching}}\) and \(F_{\text{activating}}\) represent the fluorescence intensities of Cy5/vinyl-labeled CPADVEDK peptides (1 µM) in the presence and absence of AuNP quenchers (2.5 nM), respectively. \(F_0\) represents the fluorescence intensity of the as-prepared citrate-covered AuNPs (2.5 nM) under the same test conditions. The QE was calculated to be 98.46%.

**Quantitation of the average peptide number on each AuNP.** 2.5 nM of pep-AuNPs were incubated with 100 mM of DTT at 37 °C for 2 h, the solution was centrifuged at 10,000 rpm for 10 min. The fluorescence intensity of the released peptides in the supernatants was measured by a fluorescence spectrometer. The Cy5 dyes were excited at 620 nm and the emissions were collected at 640 nm. The fluorescence of the solution was recovered by roughly 50-fold enhancement (*Figure S3*), due to the release of the Cy5/vinyl-labeled...
CPADVEDK peptides from the AuNP surfaces. With the assistance of a calibration curve that was created by different concentrations of free Cy5/vinyl-labeled CPADVEDK peptides, the concentration of the released Cy5/vinyl-labeled CPADVEDK peptides were quantified to be 375 nM, corresponding to 150 peptides per AuNP.

**Synthesis of SH-PEG 2000-FA**. 27.7 mg of NHS-FA was dissolved in 5 mL DMF and the solution was mixed with 88 mg of SH-PEG 2000 NH2. The mixture was allowed to react at room temperature for 2 h and the products were precipitated by recrystallization. The products were rinsed with cold diethyl ether to yield SH-PEG 2000-FA.

**Preparation of pep/PEG-AuNPs**. 5 mL of the as-prepared pep-AuNPs (2.5 nM) were added with varied concentrations of SH-PEG 2000-FA (1 µM, 10 µM, and 100 µM), corresponding to the ratios of peptide to PEG added: 1:1, 1:10, 1:100. The solutions were stirred at room temperature for 1 h to produce pep/PEG-AuNPs with different ratios of peptide to PEG. The pep/PEG-AuNPs were purified by centrifugation (10 min, 12000 rpm) and PBS rinsing alternatively, and were finally re-suspended by 5 mL of 0.01 M phosphate buffer for further use.

**Synthesis of vinyl-labeled folic acids**. 22.7 mg of folic acid (FA) was dissolved in 5 mL of DMF and then mixed with 29.6 mg of NHS and 50 mg of EDC-HCl. The mixture was stirred at room temperature for 24 h. After that, 30 mL of 30% acetone in diethyl ether was added under stirring to precipitate yellow products, which were collected by a filter; after rinsing with cold ether three times, the precipitates (NHS-FA) were yielded. 27.7 mg of NHS-FA was dissolved in 5 mL DMF and the solution was added with 6.8 mg of N-(3-aminopropyl) methacrylamide. The mixture was allowed to react at room temperature for 2 h to yield vinyl-labeled FAs, which were collected by recrystallization and purified with cold diethyl ether.

**Isolation of peritoneal macrophage cells**. Bal/bc mice (8 weeks) were sacrificed according to institutional guidelines. Then, RPMI 1640 medium was injected into the
abdomen of each mice. Peritoneal cells were collected from the abdomen and isolated by centrifugation at 200 g for 10 min, which were subsequently cultured in a 12-well dish (3 x 10^5 /mL) with RPMI 1640 medium containing 10% FBS in a humidified incubator with 5% CO₂ at 37 °C. The adherent cells were used in our experiments.

**Cell culture.** HeLa cells were cultured in RPMI-1640 medium containing 10% (v/v) FBS, and 1% (w/v) penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO₂.

**MTT assay.** HeLa cells (2 × 10^4 cells/well) were grown in 96-well plate in 100 µL of RPMI 1640 supplemented with FBS. After 24 h seeding, the cells were incubated with various concentrations (from 0.5 to 32 nM) of the pep/PEG-AuNP or AuNP@gel probes for another 24 h. The cell viability was measured by directly adding 10 µL of 5 mg/mL MTT solution to the incubated cells in each well. After 4 h incubation at 37 °C, the media were removed. DMSO (50 µL) was added to each well to dissolve the produced formazan. The absorbance at 490 nm was recorded for each well by a microplate reader. All the experiments were repeated three times.

**Colocalization analysis of AuNP@gel and pep-AuNP probes.** HeLa cells were cultured in confocal culture dishes with GSH free RPMI-1640 medium containing 10% (v/v) FBS, and 1% (w/v) penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO₂. After 24 h of incubation, HeLa cells were stained with 300 nM Lysotracker Green and 100 nM Hoechst 33258 for 30 min and then washed with PBS three times. Then, HeLa cells were co-incubated with 0.5 nM of AuNP@gel or pep-AuNP probes. The stained cells were observed with the laser scanning confocal microscope. The excited wavelengths were 488 nm for Lysotracker Green and 633 nm for the probes.

**Chemotherapy mouse model.** Two million HeLa cells suspended in 50 µL PBS were infected subcutaneously in the left hind leg of the six week old female nu/nu mice. Tumors were grown ~0.8 cm and then prepared for next experiments. The protocols were approved by the Institutional Animal Care and Use Committee of Nankai University.
Tissue distribution of pep-AuNP and AuNP@gel probes. Two groups of tumor-bearing mice received 5 pmol of pep-AuNP and AuNP@gel probes, respectively, by tail vein injection. After 24 h, the mice were killed, and the tumors and major organs including heart, lung, spleen, liver, and kidney were collected for acid digestion. After treatment with aqua regia for 24 h, the samples were filtered and the solutions were diluted 25-fold for measurement by ICP-MS. Each experiment was carried out by three parallel samples for statistical analysis.

Isolation of hepatocytes and Kupffer cells. Female nu/nu mice were first intravenously treated with 5 pmol of pep-AuNP or AuNP@gel probes. After 24 h, 72 h or 144 h, the hepatocytes and Kupffer cells were isolated from the mice by perfusion and differential centrifugation method. Briefly, the mice were anesthetized and their livers were perfused initially through hepatic portal vein with 20 mL of PBS containing 50 mM EDTA to eliminate red blood cell, and continually perfused with 20 mL of PBS, and then 10 mL of 0.05% collagenase V in RPMI 1640 medium through hepatic portal vein. After perfusion, the liver was dissected and treated with 10 mL of 0.05% collagenase V in RPMI 1640 medium repeatedly. The cell suspension was filtered through a 75 µm cell strainer, and the hepatocytes were collected by centrifugations at 150 rcf for 5 min, and the supernatant was further centrifuged at 500 rcf to obtain Kupffer cells.
3. Supplementary Figures

Figure S1. HPLC spectrum of Cy5/vinyl-labeled CPADVEDK peptides.

Figure S2. The complete molecular weight of Cy5/vinyl-labeled CPADVEDK peptides; MS:
calc. for: C_{70}H_{94}N_{11}O_{23}S_{3}, [M-H] 1568.8, found 1567.1.
Figure S3. Fluorescence spectra of pep-AuNP probes (2.5 nM) before and after incubation with 100 mM of DTT in PBS buffer. (Excitation wavelength: 620 nm; Excitation slit: 5 nm; Emission slit: 10 nm).

Figure S4. FT-IR spectra of Citrate-AuNP, pep-AuNP, and AuNP@gel probes. The dashed lines at 1715 and 1057 cm\(^{-1}\) correspond to the carbonyl (C=O) and ether bonds (C-O-C) respectively in the nanogel.
Figure S5. Dynamic light scattering (DLS) data show the hydrodynamic diameters of citrate-AuNPs (a), pep-AuNPs (b), and AuNP@gels (c).

Figure S6. Zeta potential data show the surface charge of citrate-AuNPs, pep-AuNPs, and AuNP@gels.
Figure S7. TEM image of the AuNP@gel probes structures. Inset is a typical magnified image of AuNP@gel.

Figure S8. Fluorescence recovery of different AuNP probes (0.5 nM) by incubating with Cas- 3 (4 nM) and DTT (1 mM), respectively. 1, Pep-AuNP probes; 2, pep/PEG (1:1)-AuNPs; 3, pep/PEG (1:10)-AuNPs; 4, pep/PEG (1:100)-AuNPs; 5, AuNP@gel probes at pH 7.4; and 6, AuNP@gel probes at pH 5.5.
**Figure S9.** The intracellular Au contents in HeLa cells treated with different kinds of AuNP probes. Folic acid (FA)-pretreated HeLa cells were set as the controls. The error bars represent the standard deviations of three independent measurements.

**Figure S10.** Hydrodynamic diameters of AuNP@gel probes that were dissolved in pH 5.5 and pH 7.4 buffered solutions for different times. The error bars represent the standard deviations of three independent measurements.
Figure S11. Zeta potentials of ionizable AuNP@gels and neutral AuNP@gels (without ionizable monomer: 2-dimethylamine ethyl methacrylate) in different pH. The error bars represent the standard deviations of three independent measurements.
Figure S12. Confocal microscopic images of HeLa cells incubated with AuNP@gel (a) or pep/PEG (1:1)-AuNP (b) probes for 30 min, 60 min, 120 min, and 180 min. The images in
the green fluorescent channels indicate the stained lysosomes. The images in the red fluorescent channels indicate the Cy5-labeled AuNP@gel or pep/PEG (1:1)-AuNP probes. The fluorescence intensity profiles indicate the degree of overlap between different AuNP probes and endosomes as highlighted by the short white lines. Scale bar, 10 μm. The laser power of fluorescence microscope is 19.84 mW, the shutter of blue and green channels were opened to 8%, while the shutter of Cy5 channel was opened to 80 %. A photomultiplier tube (PMT) was used to amplify the Cy5 signals of the AuNP probes.

Figure S13. TEM images of the AuNP@gel probes after incubating with HeLa cells for (a) 20 min, (b) 2 h, and (c) 4 h. The red arrows in (a) indicate the formation of endocytosis vesicles; the two yellow arrows and one green arrow in (b) indicate AuNPs in the endosomes with intact membrane structure and broken membrane structure, respectively; and the white arrows in (c) indicate the released AuNPs in cytoplasm. We propose these dynamic processes in the below schematic picture. The scissors represent the active caspase -3/-7 enzymes.
Figure S14. TEM image of the PEG/pep-AuNP probes that were incubated with HeLa cells for 4 h. The PEG/pep-AuNP probes are still trapped in the endosomes, whose intact membrane structure is clearly seen, as indicated by the blue arrows. The endocytosis process was illustrated by a schematic picture (right).

Figure S15. Fluorescent emission spectra of pep-AuNP probes in the presence of various substances including Cas -3, Cas -7, and the mixture of Cas -3 and its inhibitors Z-VAD-fmk. The concentration of Cas -3 and Cas -7 was fixed to be 8 nM, while the concentration of Z-VAD-fmk was 20 µM. The solutions were incubated at 37 °C for 2 h before recording the spectra.
Figure S16. Fluorescence emission spectra of pep-AuNP probes in the presence of various concentrations of Cas -3 following incubation at 37 °C for 2 h. Inset: a standard curve for Cas -3.

Figure S17. Fluorescence emission spectra of pep-AuNP probes in the presence of various concentrations of Cas -7 following incubation at 37 °C for 2 h. Inset: a standard curve for Cas -7.
Figure S18. The fluorescence release ratio study of the 40 mM Ac-DEVD-AMC and 0.5 nM AuNP@gel probes were incubated with equal concentration (8 nM) of recombinant human Cas -3.

Figure S19. Fluorescent microscopy images of DOX-induced Cas -3/-7 activation of the Ac-DEVD-AMC and AuNP@gel probes in HeLa cells. The same sample pictures were respectively showed by directly captured under microscopy (original image) and post-removed background interference (background subtraction). Scale bars, 20 μm.
Figure S20. Real-time imaging of apoptosis in tumor-bearing mice treated with PBS. 5 pmol of AuNP@gel probes in PBS was injected by tail vein. After 4 h, 100 μL of PBS was administered intravenously and real-time whole-body fluorescence was monitored by photoIMAGER™ fluorescence imager at different times.

Figure S21. In vivo biodistribution of the pep-AuNP and AuNP@gel probes in major organs at 24 h after tail vein injection based on ICP-MS analysis (data expressed as percentage of the injected dose per gram of tissue (% ID/g)). The error bars indicate standard deviations calculated on the basis of three mice.
Figure S22. The biological characterization of initial mice liver hepatocytes and Kupffer cells. (a) Light microscopy show that hepatocytes are large and often rough-surfaced. (b) Kupffer cells are much smaller and often attached to debris. Scale bars, 10 μm. Flow cytometry data for the forward scatter (FSC) and side scatter (SSC) show the different size and complexity of hepatocytes (c) and Kupffer cells (d).

Figure S23. Quantification of the pep-AuNP and AuNP@gel probes accumulation in the Kupffer cells (a) and hepatocytes (b) of mice after injected with pep-AuNP or AuNP@gel probes 4, 72 and 168 h.
Figure S24. Immunohistochemical analysis of tumors resected from mouse injected with 5 pmol of AuNP@gel (red) probes 24 h after treated with PBS or DOX. Tissue sections were co-stained for active Cas-3 (green). Scale bars, 50 μm.

Figure S25. The MTT assay of HeLa cells with different concentrations of pep-AuNP and AuNP@gel probes for 24 h. The error bars indicate standard deviations of three independent replicates.
Figure S26. H&E histology of major organs from control group (no probe treatment) and experimental group that was intravenously injected with 5 pmol of AuNP@gels. Scale bars, 100 μm.
4. Supplementary References


