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Electronic Supplementary Information for:

Gold nanoparticles functionalized with cresyl violet and porphyrin *via* hyaluronic acid for targeted cell image and phototherapy

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1. Reagents and apparatus

Hyaluronic acid (HA; M_w = 3500 Da) was obtained from Yangzhou Zhongfu New Materials Co., Ltd. Hyaluronidase, L-ascorbic acid palmitate, cresyl violet (CV), cystamine dihydrochloride, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC), L-cysteine, glutathione, tetra(4-aminophenyl)porphyrin (referred to porphyrin), bovine serum albumin, and human serum albumin were obtained from Sigma-Aldrich. $HAuCl_4$, trisodium citrate, $CaCl_2$, NaCl, KCl, $MgCl_2$, vitamin C, and glutamine were purchased from Beijing Chemicals, Ltd. Dimethylsulfoxide, N-hydroxysuccinimide (NHS), dithiothreitol and morpholinoethanesulfonic acid (MES) were purchased from J & K Chemical Ltd. Dulbecco's modified Eagle's media (DMEM), fetal bovine serum, penicillin (100 μg/mL), streptomycin (100 μg/mL), and phosphate buffered saline (PBS) solution were obtained from Invitrogen corporation. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Serva Electrophoresis GmbH (Germany), and 2',7'-dichlorofluorescin diacetate (DCFH-DA) from Biyuntian Co. (China). Ultrapure water (over 18 MΩ·cm) from a Milli-Q reference system (Millipore) was used throughout.

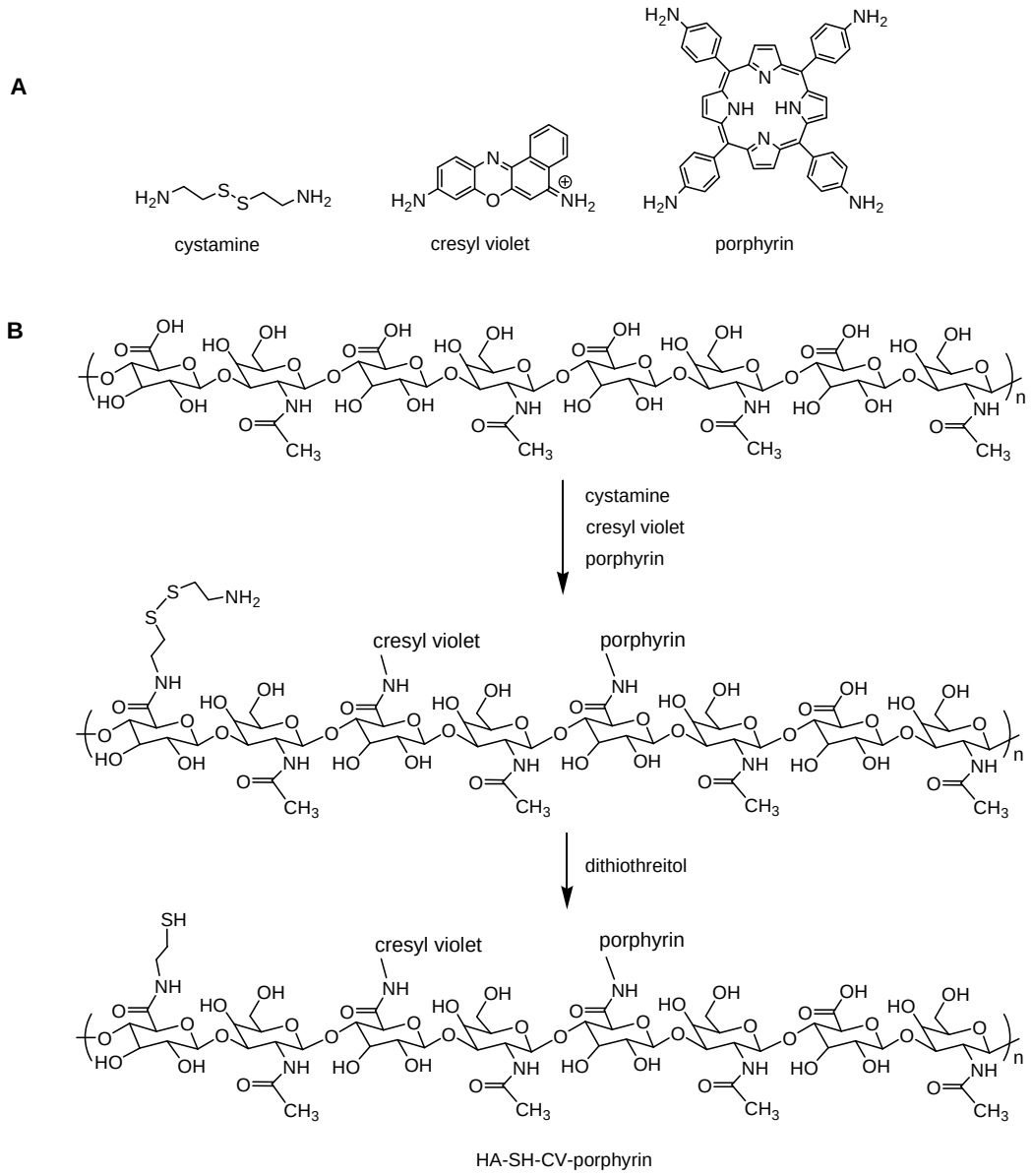
UV-vis spectra were recorded in 1-cm quartz cells with a TU-1900 spectrophotometer (Beijing, China). Fluorescence measurements were made on a Hitachi F-2500 fluorescence spectrophotometer (Tokyo, Japan). The incubation was carried out in Shaker incubator (SKY-100C, Shanghai Sukun Industry & Commerce Co., Ltd). Transmission electron microscopy (TEM) images were taken on a JEM-1011 instrument. The absorbance for MTT analysis was recorded on a microplate reader

(BIO-TEK Synergy HT, USA) at 490 nm. Fluorescence imaging experiments were performed on a FV 1000-IX81 confocal laser scanning microscope (Olympus, Japan).

2. Preparation of nanoprobe

The nanoprobe was prepared *via* the following three steps. Firstly, gold nanoparticles (AuNPs) were prepared as described previously (Song et al, *Talanta*, 2013, 116, 237), and their solution was stored in a refrigerator at 4 °C for future use.

Secondly, HA containing free thiol groups as well as CV and porphyrin moieties, i.e., HA-SH-CV-porphyrin, was prepared (Scheme S1). In brief, HA was dissolved in water and dialyzed (a membrane with molecular weight cutoff of approximately 1000) for 1 day to remove the sodium salt and HA oligomers. The purified HA was prepared as solid form *via* lyophilization. Then, in 20 mL of MES buffer solution (pH 5.5), HA



Scheme S1 (A) Structures of components. (B) Synthesis of HA-SH-CV-porphyrin.

(100 mg), EDC (20 mg), and NHS (4.8 mg) were added. After the resulting mixture was stirred for 2 h, CV (0.24 mg), porphyrin (0.28 mg) and cystamine dihydrochloride (120 mg) were added, followed by further stirring for 5 h. Then, the reaction mixture was treated with dithiothreitol (100 mg) for 12 h to generate HA that contains free thiol groups, followed by dialysis (a membrane with molecular weight cutoff of approximately 1000) against water for 1 day to remove unreacted chemicals. The purified solution was lyophilized, yielding a yellow power product of HA-SH-CV-porphyrin, which was stored in a refrigerator at -20 °C for future use.

Finally, the nanoprobe (AuNPs-HA-CV-porphyrin) was prepared by treating HA-SH-CV-porphyrin with AuNPs. Briefly, HA-SH-CV-porphyrin (12 mg) was dissolved in 3 ml of water, followed by addition of 10 mL AuNPs solution (3 nM). The mixture was stirred for 24 h to form a stable Au-S bond. Then, the mixture was centrifuged and washed three times with water to remove the unbound HA-SH-CV-porphyrin.

3. TEM image of the nanoprobe

The size and monodispersity of the nanoprobe were determined by TEM analysis. Samples for TEM analysis were prepared by placing about 10 μL of the nanoprobe solution on the carbon-coated copper grid and then drying at room temperature.

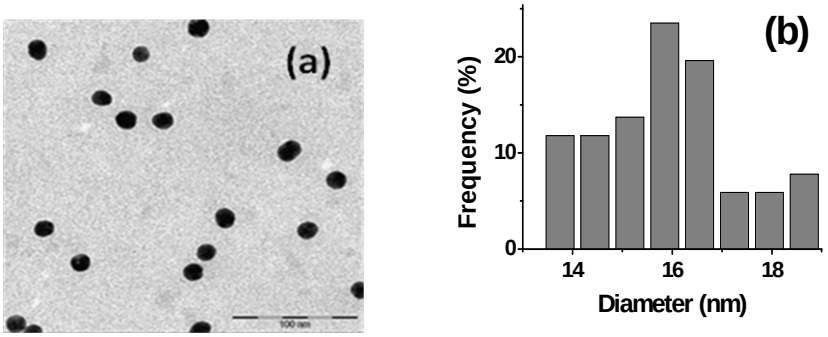


Fig. S1 TEM image (a) and diameter distribution (b) of nanoprobe. Scale bar, 100 nm.

4. UV-vis absorption spectra of different substances

The amounts of porphyrin and CV conjugated to the AuNPs were calculated based on the following equation set and the additivity of absorbance of porphyrin, CV and AuNPs, which show the characteristic absorption bands at 420, 580 and 520 nm (Fig. S2), respectively.

$$A_{420\ nm} = \varepsilon_{porphyrin420} \times C_{porphyrin} + \varepsilon_{CV420} \times C_{CV} + \varepsilon_{AuNPs420} \times C_{\ AuNPs}$$

$$A_{520\ nm} = \varepsilon_{porphyrin520} \times C_{porphyrin} + \varepsilon_{CV520} \times C_{CV} + \varepsilon_{AuNPs520} \times C_{\ AuNPs}$$

$$A_{580\ nm} = \varepsilon_{porphyrin580} \times C_{porphyrin} + \varepsilon_{CV580} \times C_{CV} + \varepsilon_{AuNPs580} \times C_{\ AuNPs}$$

where A is the absorbance of the nanoprobe at different wavelengths; ε and C are the molar absorptivity and the concentration of a related substance, respectively. All the absorbance measurements were performed in a quartz cell with an optical length of 1 cm. At 420, 520 and 580 nm, the molar absorptivities of porphyrin are 1.1×10^5, 1.3×10^4 and 1.6×10^4 M^{-1} cm^{-1}, those of CV are 4.1×10^3, 2.1×10^4 and 4.5×10^4 M^{-1} cm^{-1}, and those of AuNPs are 1.8×10^8, 2.7×10^8 and 7.9×10^7 M^{-1} cm^{-1}, respectively. Similarly, the amounts of porphyrin and CV conjugated to per gram of the HA backbone can be calculated to be 0.5 and 0.2 μmol, respectively, based on the spectrum of HA-SH-CV-porphyrin (curve e).

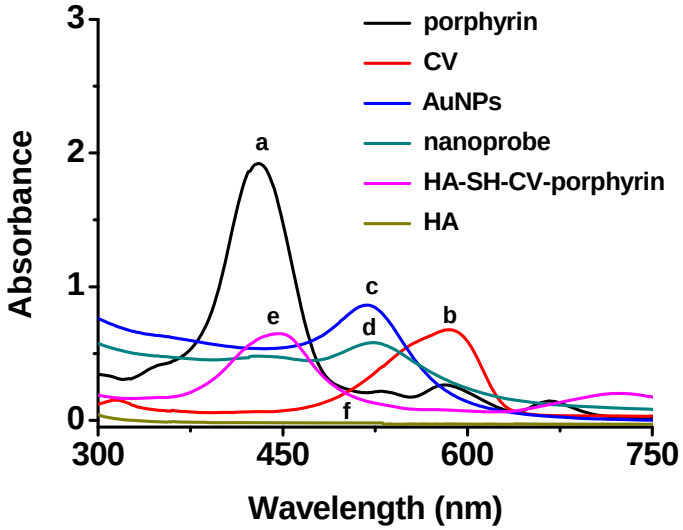


Fig. S2 UV-vis absorption spectra of different substances in water. (a) Porphyrin (16 μM); (b) CV (15 μM); (c) AuNPs (3 nM); (d) nanoprobe (25 μg/mL); (e) HA-SH-CV-porphyrin (2.6 mg/mL); (f) HA (10 mg/mL)

5. The colour of bare AuNPs and nanoprobe in different solutions

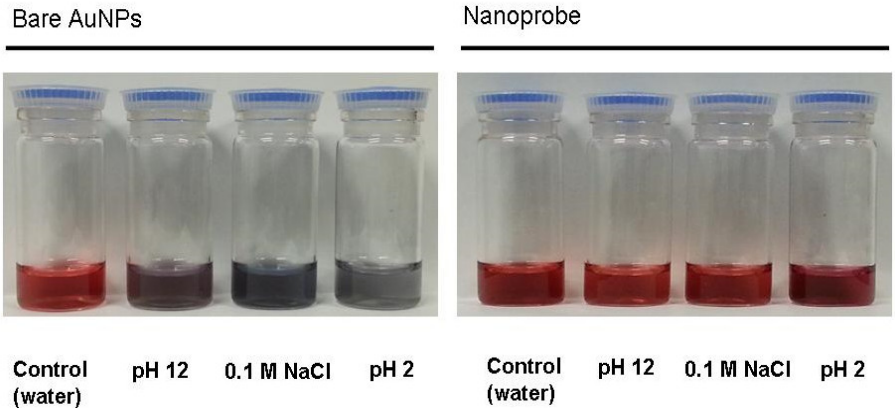


Fig. S3 The color changes of bare AuNPs and nanoprobe in different media: water (control), pH 12 (basic medium adjusted by adding dilute NaOH to water), 0.1 M NaCl (about pH 7), and pH 2 (acidic medium adjusted by adding dilute HCl to water). As is

seen, the color of bare AuNPs is easier to change with the media than that of the nanoprobe, indicating that the nanoprobe has higher stability.

6. Optimization of reaction time

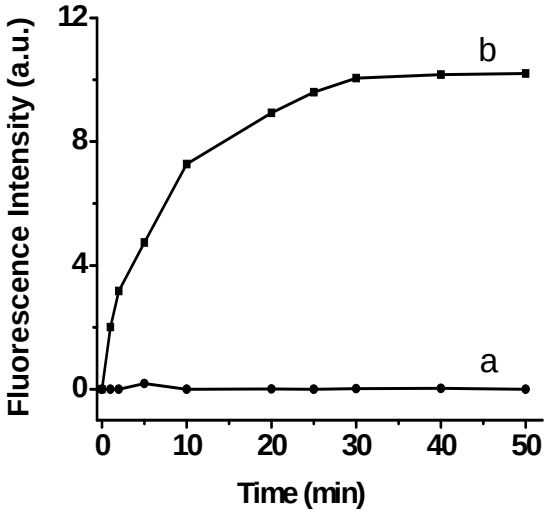


Fig. S4 Effect of reaction time on the fluorescence of the nanoprobe (50 μg/mL) in 5 mM PBS of pH 7.4 in the absence (a) and presence (b) of hyaluronidase (20 μg/mL) at 37 °C. $\lambda_{ex/em}$ = 550/620 nm.

7. UV-vis absorption spectra of the reaction system

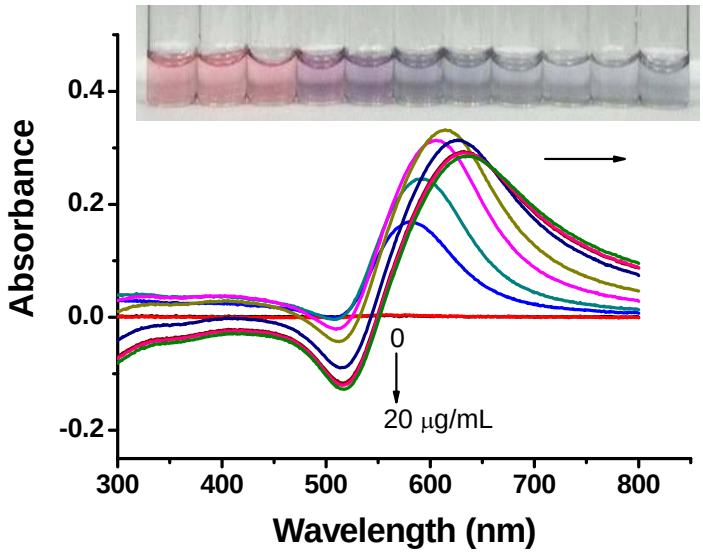


Fig. S5 Absorption spectra of the nanoprobe (50 μg/mL) with hyaluronidase at different concentrations (0, 1, 2, 4, 8, 10, 12, 14, 16, 18, 20 μg/mL). The spectra were recorded against the corresponding reagent blank (see Ma et al, *Mikrochim. Acta,* 1998, 128, 181) without hyaluronidase. The inset (from left to right) shows the corresponding color change.

8. Inhibitor effect

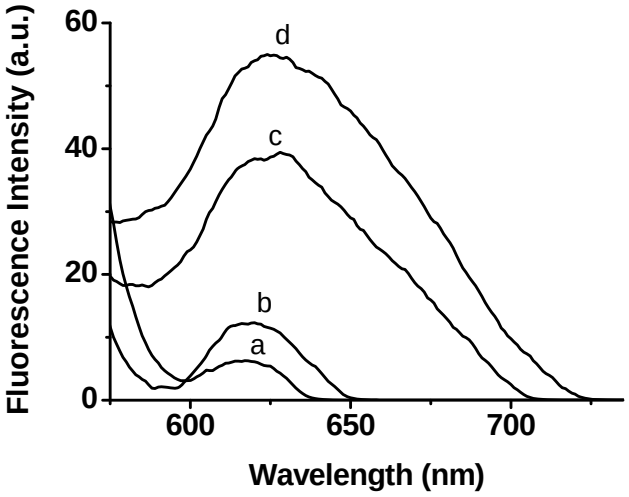


Fig. S6 Fluorescence spectra of different reaction systems. (a) The nanoprobe (50 μg/mL) in pH 7.4 PBS (control); (b) system (a) + hyaluronidase (60 μg/mL) + inhibitor (40 μg/mL); (c) system (a) + hyaluronidase (60 μg/mL) + inhibitor (20 μg/mL); (d) system (a) + hyaluronidase (60 μg/mL). λ_{ex} = 550 nm.

9. Selectivity study

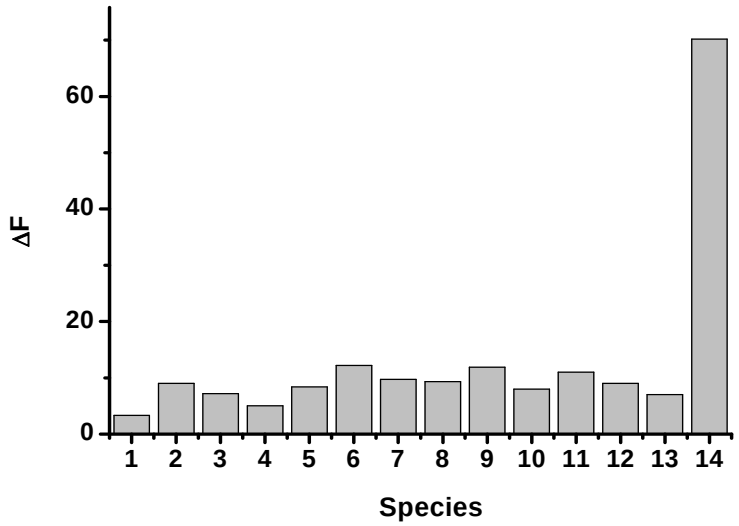


Fig. S7 Effects of common cellular species on the fluorescence of the nanoprobe (50 μg/mL): 1) 100 nM bovine serum albumin; 2) 100 nM human serum albumin; 3) 1 mM L-cysteine; 4) 5 mM glutathione; 5) 2.5 mM $MgCl_2$; 6) 100 μM $CuCl_2$; 7) 1 mM $CaCl_2$; 8) 1 mM vitamin B1; 9) 10 mM glucose; 10) 50 μM matrix metalloproteinase 2; 11) 100 μM reduced nicotinamide adenine dinucleotide; 12) 100 μM carboxylesterase; 13) 100 μM cytochrome c; 14) 40 μg/mL hyaluronidase. $\Delta F = F - F_0$, where F_0 and F are the fluorescence intensity before and after the species is added to the nanoprobe solution, respectively. $\lambda_{ex/em}$ = 550/620 nm.

10. Fluorescence of the nanoprobe under ultraviolet irradiation

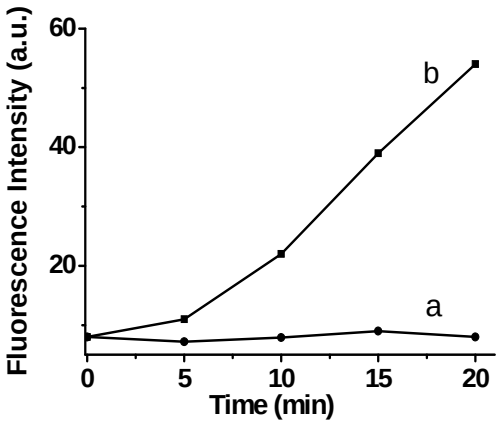


Fig. S8 Change of fluorescence intensity of the nanoprobe (50 μg/mL) (a) without and (b) with ultraviolet irradiation of 365 nm for different periods of time. $\lambda_{ex/em}$ = 550/620 nm.

11. The singlet oxygen detection

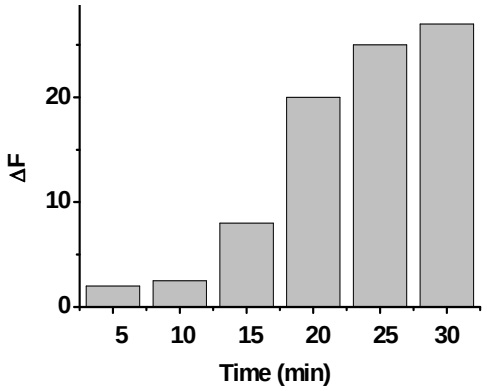


Fig. S9 Fluorescence change of DCFH-DA (5 μM) in the solution of the nanoprobe (50 μg/mL) under ultraviolet irradiation of 365 nm for different periods of time. $\Delta F = F - F_0$, where F_0 and F are the fluorescence intensity before and after ultraviolet irradiation, respectively. As is seen, the fluorescence of DCFH-DA is gradually increased under ultraviolet irradiation, suggesting the generation of singlet oxygen. $\lambda_{ex/em}$ = 488/525 nm.

12. Cell imaging

Unless otherwise stated, U-87, HeLa, and NIH-3T3 cells used in this study were cultured in DMEM containing 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin at 37 °C in a 5% CO_2 incubator. For fluorescence imaging, the adherent cells grown on glass-bottom culture dishes (MatTek Co.) containing 1 mL of culture media were first incubated with the nanoprobe (50 μg/mL) or CV (5 μM) at 37 °C, and then washed thoroughly with 0.1 M PBS (pH 7.4). Fluorescence imaging

experiments were performed on a FV 1000-IX81 confocal laser scanning microscope (Olympus, Japan) with FV5-LAMAR for excitation at 559 nm and a variable bandpass emission filter set to 580-680 nm through a 100 × 1.4 NA objective. Optical sections were acquired at 0.8 μm.

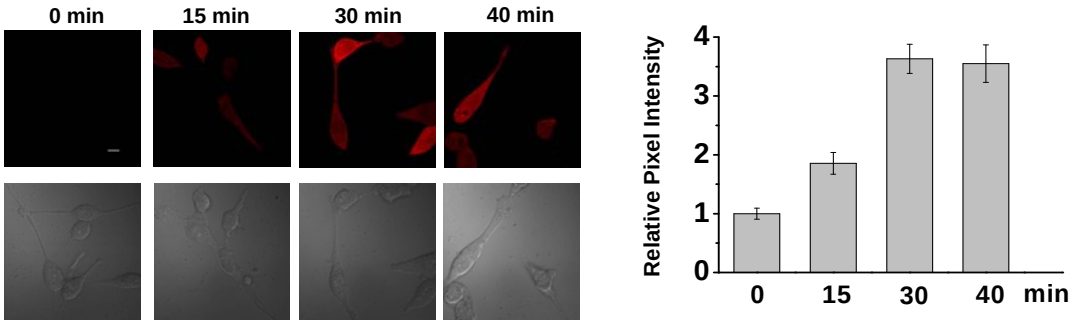


Fig. S10 The fluorescence images of U-87 cells treated with the nanoprobe (50 μg/mL) for different periods of time. The differential interference contrast (DIC) images of the corresponding samples are shown at the bottom. Scale bar, 10 μm. The relative pixel intensities of the corresponding fluorescence images are shown on the right (the intensity at 0 min is defined as 1.0).

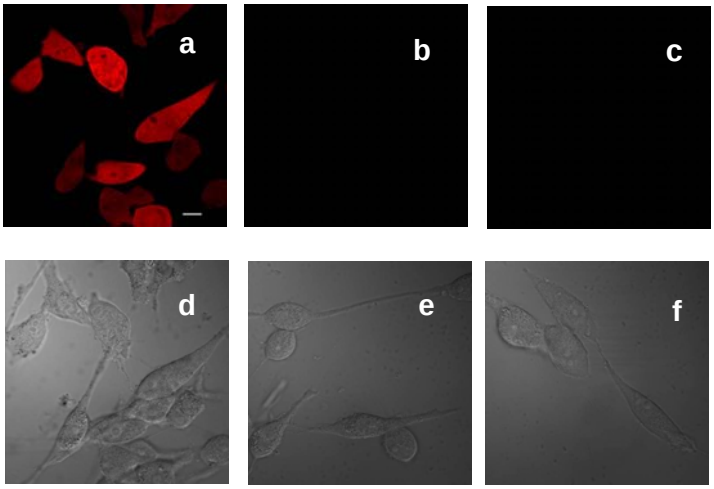


Fig. S11 Confocal fluorescence images of U-87 cells. (a) U-87 cells were incubated with 50 μg/mL nanoprobe for 30 min; (b) U-87 cells were pre-treated with 100 μL of saturated HA solution (1 g/mL) for 1 h, and then incubated with nanoprobe (50 μg/mL) for 30 min; (c) U-87 cells were pre-treated with 100 μL of 6-O-palmitoyl-L-ascorbic acid (50 μg/mL) for 1 h, and then incubated with nanoprobe (50 μg/mL) for 30 min. The DIC images of the corresponding samples are shown below (panels d-f). Scale bar, 10 μm.

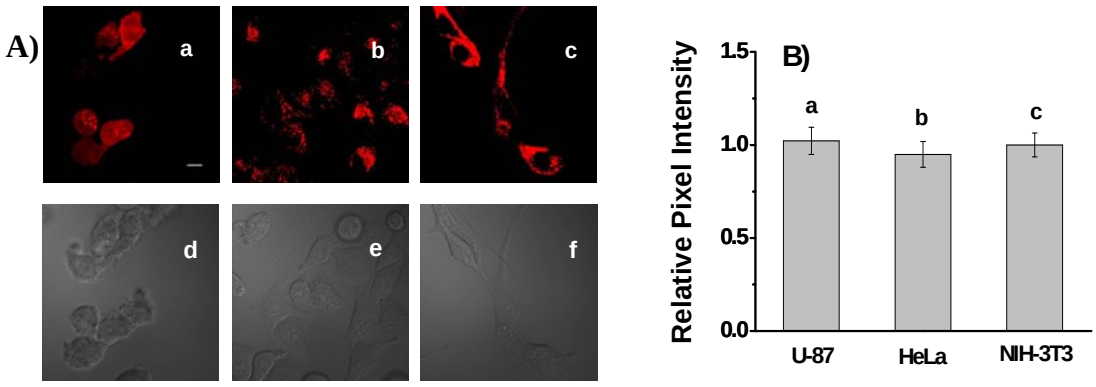


Fig. S12 A) Confocal fluorescence images of different cells: (a) U-87, (b) HeLa, and (c) NIH-3T3 cells. The cells were incubated with CV (5 μM) at 37 °C for 30 min (interestingly, less CV enters the nucleus of NIH-3T3 cells; the reason for this is unclear). The DIC images of the corresponding samples are shown below (panels d-f). Scale bar, 10 μm. B) Relative pixel intensity obtained from the corresponding fluorescence images with ImageJ software (the pixel intensity from NIH-3T3 cells is defined as 1.0).

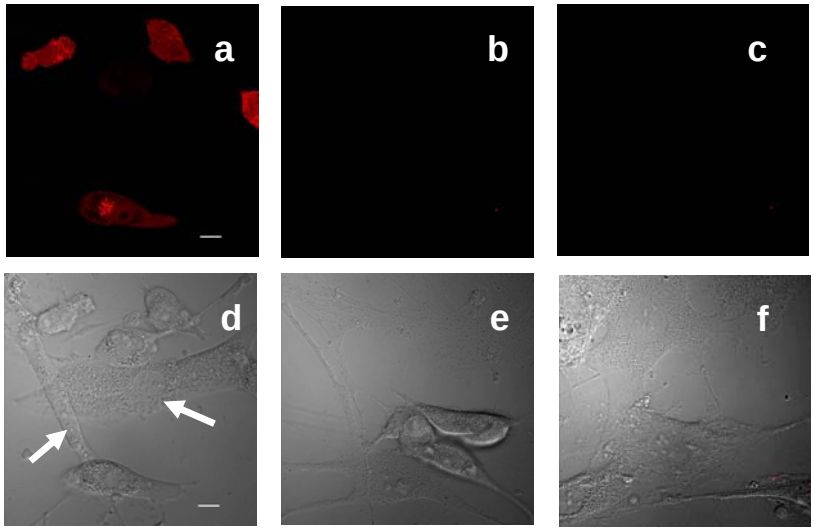


Fig. S13 Fluorescence images of different cell samples. (a) The cell mixture of NIH-3T3 and U-87 cells after incubation with the nanoprobe (50 μg/mL) at 37 °C for 30 min. (b) The cell mixture of NIH-3T3 and U-87 cells in the absence of the nanoprobe (control). (c) Only NIH-3T3 cells incubated with the nanoprobe (50 μg/mL) at 37 °C for 30 min (another control). The DIC images of the corresponding samples are shown below (panels d-f). In image d, the white arrows indicate the NIH-3T3 cells, which scarcely show fluorescence in image a. Scale bar, 10 μm.

13. Cell viability assays

The toxic effects of the nanoprobe itself on the three kinds of cells (U-87, HeLa, and NIH-3T3) were examined in the absence of ultraviolet radiation. In brief, U-87, HeLa, or NIH-3T3 cells were seeded onto 96-well plates at a density of 7000 cells/well. After incubation for 24 h, the culture medium was replaced with 100 μL of serum-free medium containing different concentrations of the nanoprobe (0-4.0 mg/mL), followed by incubation for 24 h at 37 °C. The cells were then washed twice with serum-free culture medium, and the cell viability was evaluated by the MTT assay (Song et al, *J. Mater. Chem.*, 2012, 22, 12568).

To determine the cell viability after ultraviolet irradiation, cells (U-87, HeLa, or NIH-3T3) were seeded in 96-well U-bottom plates at a density of 7000 cells/well, and then treated as described above with serum-free medium containing different concentrations of the nanoprobe (0-4.0 mg/mL) or free porphyrin (0-25 μM). After the cells were washed twice with serum-free medium, they were irradiated under a UV lamp (8 W) of 365 nm for 5 min. Then the cells were incubated in serum-free DMEM at 37 °C for 6 h, and the viability of the irradiated cells was evaluated by the MTT assay.

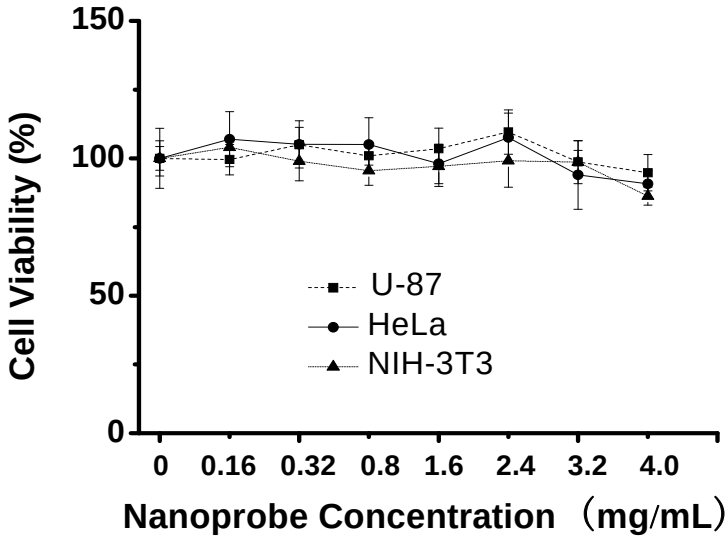


Fig. S14 Cell viability changes after 24 h of incubation with the nanoprobe at varied concentrations of 0-4.0 mg/mL. The cell survival without treatment by the nanoprobe is defined as 100%; the results are the mean ± SD of 5 separate measurements.