Supporting Information

X-Ray Responsive Nanoparticles with Triggered Release of Nitrite, a Precursor of Reactive Nitrogen Species, for Enhanced Cancer Radiosensitization

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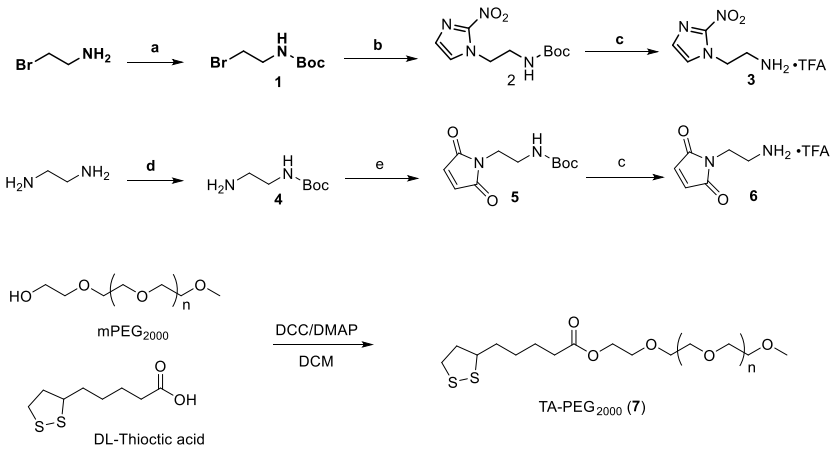
Materials and Methods.

$HAuCl_4.3H_2O$, 2-nitroimdazole were purchased from Sigma-Aldrich (USA). All other chemicals and organic solvents were supplied by Thermal fisher (USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin and trypsin-EDTA were obtained from Invitrogen (Carlsbad, CA, USA). Cell penetrating peptide (CPP) NH2-CGRKKRRQRRR-COOH was purchased from GL BIOCHEM LTD (Shanghai, China). All the chemicals obtained from commercial sources were used as received unless otherwise noted. UV-Vis absorption spectra were measured on a Genesys 10S UV-Vis meter (Thermo Scientific). Transmission electron microscope (TEM) images were recorded using a JEOL JEM1400 operated at 120 kV. Dynamic light scattering (DLS) and zeta potential measurements were performed by Brookhaven 90 Plus Nanoparticle Size Analyzer. Fluorescence images were collected using a Leica confocal microscope by scanning with a depth of 0.3 μm. ICP analysis was done by a XSERIES 2 ICP-MS (Thermo Scientific). LC-MS was performed on a Waters® e2695 Separations Module with an ACQUITY QDa Detector.

Ligand synthesis



Scheme S1. a) TEA, DCM, rt., 3h; b) K_2CO_3, DMF, 80 °C overnight; c) 30% TFA in DCM, rt., 1 h); d) TEA, DCM, rt., overnight; e) Ac_2O, Et_2O, 0 °C 2h, then 60 °C, 0.5 h.

2-(Boc-amino)ethyl bromide **(1)**

Di-tert-butyl dicarbonate (1.1 g, 5.4 mmol) was dissolved in 20 mL dichloromethane (DCM), then 2-bromoethylamine hydrobromide (1.1g, 5.4mmol) was added at 0 °C. 1.0 mL triethylamine (TEA) was then added dropwise at 0 °C. The reaction was warmed to rt. and then kept at rt. for 3 h under stirring. 30 mL water was added, DCM layer was then collected and washed with water, acetic acid solution （1.0 N） and then brine. After drying over Na_2SO_4, DCM was removed to give an oil like product 1.15 g (yield 95%). ESI-MS: m/z 223.02 (calcd); 224.06 $[M+H]^+$ (found).

tert-butyl (2-(2-nitro-1H-imidazol-1-yl)ethyl)carbamate (**2)**

2-nitroimidazole (300 mg, 2.65 mmol) and **1** (593 mg, 2.65 mmol) were dissolved in 3 mL DMF. After addition of K_2CO_3 (365mg, 2.65 mmol), the reaction mixture was stirred at 80 °C overnight. The solvent was removed, the residue was then redissolved in 20 mL ethyl acetate (EA). The solution was washed with water twice and brine once, dried over Na_2SO_4. The solvent was removed under reduced reassure, and the residue was purified by silica gel chromatography (MeOH/DCM, 1:20~1:10) to give a white solid **2** (530 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.71 (S, 1H), 6.70 (S, 1H), 4.76 (brs, 1H), 3.68 (quintet, , *J*= 1.2, 4.2, 7.2 Hz, 2H); 3.35 (dd, *J*= 6, 11.2 Hz, 2H), 1.40 (s, 9H); ESI-MS: m/z 256.12 (calcd); 257.06 $[M+H]^+$, (found).

2-(2-nitro-1H-imidazol-1-yl) ethan-1-amine (**3)**

400 mg compound **2** (1.56 mmol) was dissolved by 5 mL TFA solution (30% in DCM) at 0 °C. The reaction was then warmed up to rt. and kept at rt. for 1 h under stirring. The solvent was then removed and the residues were precipitated out by addition of 10 mL diethyl ether. The white solid was filtered and washed with diethyl ether twice to give nIm **3** (420 mg, quantitatively). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.53 (d, *J* = 1.2 Hz, 1H), 7.21 (d, *J* = 1.2 Hz 1H), 4.77 (t, *J*= 6.0 Hz, 2H), 3.50 (t, *J*= 6.0 Hz, 2H), ^{13}C NMR (75 MHz, CD_3OD) δ=128.0, 127.3, 46.9, 39.4; ESI-MS: m/z 156.06 (calcd); 157.02 $[M+H]^+$, (found).

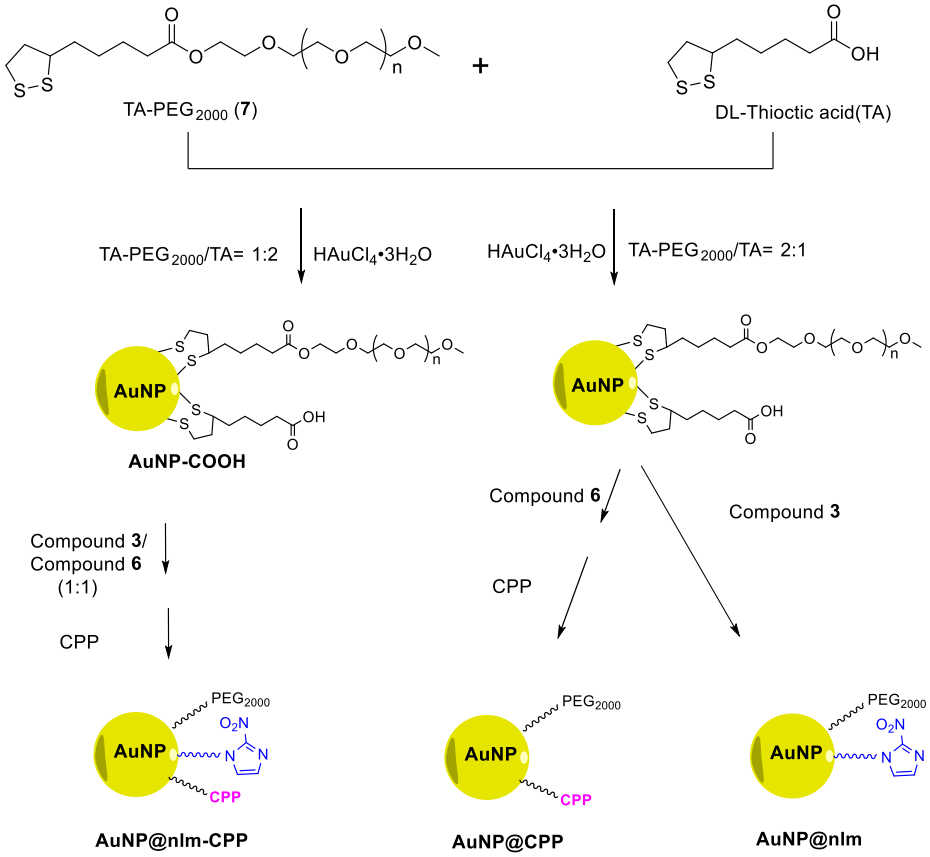
1-(2-Aminoethyl) maleimide (**6)**

6 was synthesized according to a reported methods.[1] ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.90 (d, *J*= 0.9 Hz, 2H), 3.82 (t, *J*= 5.7, 2H), 3.16 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ=171.2, 134.6, 38.7, 35.1; ESI-MS: m/z 140.06 (calcd); 140.96 $[M+H]^+$, (found).

TA-PEG_{2000} (**7**)

$mPEG_{2000}$ (2.0 g, 1 mmol), DMAP (50 mg, 0.41 mmol) and thioctic acid (515 mg, 2.5 mmol) were dissolved in 20 mL DCM. DCC (458 mg, 2.4 mmol) was then added at 0 °C. The mixture was stirred at rt. for 12 h. The reaction mixture was filtrated, and the filtrate was concentrated to give a crude product. The crude product was recrystallized twice in 2-propanol to give a pure white solid **7**. (1.6 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.21 (m, 2H), 3.86 (m, 1H), 3.59-3.71 (m), 3.54 (m, 2H), 3.40 (m, 1H), 3.37(s, 3H), 3.05-3.22 (m, 2H), 2.45(m, 1H), 2.34 (t, *J*= 7.2 Hz, 2H), 1.90 (m, 1H), 1.66(m,4H), 1.47(m,2H).

Nanoparticle synthesis



Scheme S2: Preparation of AuNPs with different functionalities.

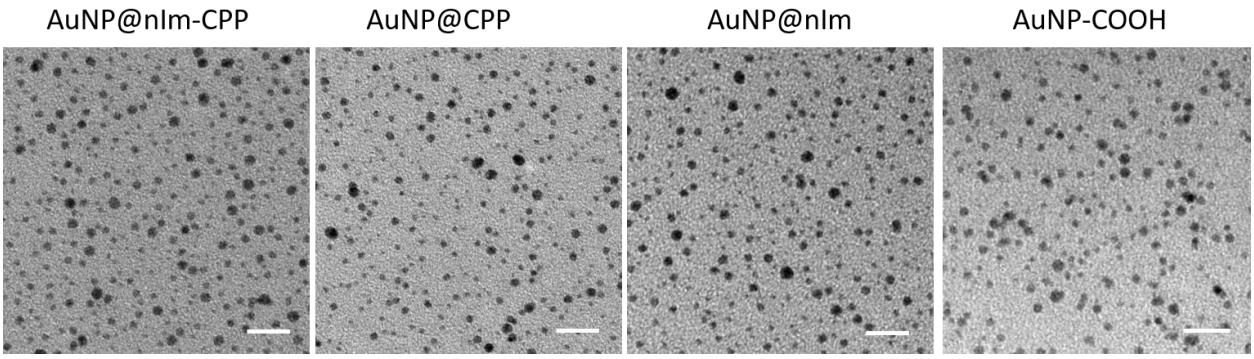


Fig. S1. TEM images of AuNPs with different functionalities. All scale bars represent 20 nm.

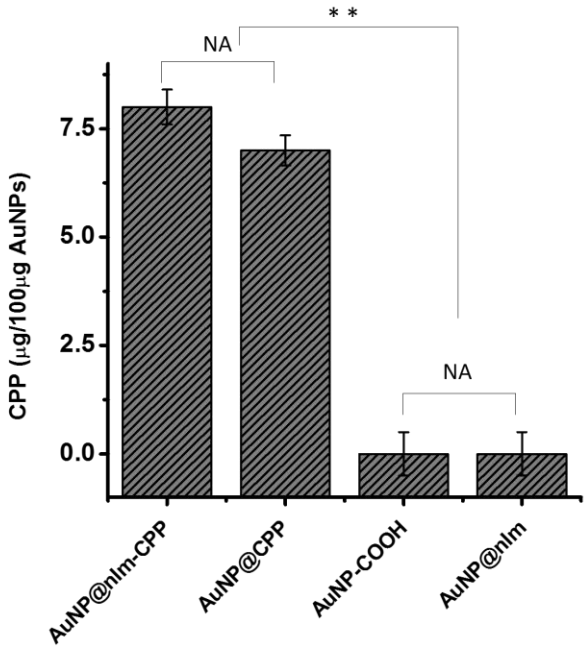
Table S1: Zeta potential of synthesized hybrid nanoparticles

Measurement	Zeta Potential at 25 °C /mV				
	1	2	3	Ave	Stdev
AuNP@nIm-CPP	8.18	7.33	9.43	8.31	1.05
AuNP@CPP	6.85	8.07	6.82	7.24	0.71
AuNP-COOH	-12.50	-12.50	-12.60	-12.53	0.05
AuNP@nIm	0.92	0.60	-	0.76	0.22

Particle size determination was performed by Brookhaven 90 Plus Nanoparticle Size Analyzer. Nanoparticles were suspended in water at 100 µg/mL. Zeta potential was measured on the same machine by loading the sample into disposable capillary cells (DTS1070) from Malvern Instruments, Inc. (USA). Average zeta potential was calculated based on three or two data points.

CPP quantification on nanoparticles.

The amount of cell-penetrating peptide CPP on the prepared AuNPs was quantified by ninhydrin based assay.[2] Briefly, a ninhydrin ethanol solution 0.35% (w/v) was freshly prepared. CPP or CPP functionalized AuNPs in ethanol were added into ninhydrin solution. These mixtures were then incubated at 60°C for 30 min followed by the measurement UV/Vis absorption. Standard CPP ethanol solutions with concentration ranging from 0 to 125 µg/mL were freshly prepared to build the calibration curve. The typical absorbance at 570 nm from the additive product of ninhydrin and free amino groups was plotted versus the concentration of CPP to make the standard calibration curve. CPP quantification was based on the absorption at 570 nm according to the



calibration curve.

Fig. S2. The amount of CPP on AuNPs quantified by ninhydrin based assay. The bars represent mean ± SD, n = 3. **P < 0.01, NA: No significance (t-test).

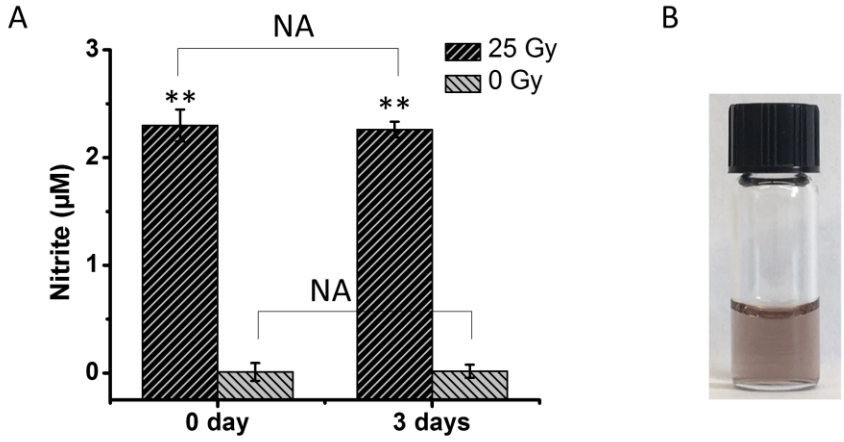


Fig. S3. A) Nitrite release from freshly prepared AuNP@nIm-CPP (250 µg/mL) DMEM solution (0 day) and from solution prepared 3 days earlier (3 days); B) A photo shows a homogeneous AuNP@nIm-CPP (250 µg/mL) DMEM solution after 3 days at room temperature under dark, which indicates no precipitate formation in this solution. The bars represent mean ± SD, n = 5, **P < 0.01, NA: No significance (t-test).

Nanoparticle stability study was performed by adding AuNP@nIm-CPP (250 µg/mL) into cell culture medium DEME (phenol red free). After 3 days incubation at room temperature, a picture was taken to check if precipitates formed. Nitrite release properties from this solution have been done in comparison with freshly prepared AuNP@nIm-CPP DMEM solution at the same concentration.

Cell uptake of AuNPs with different functionalities.

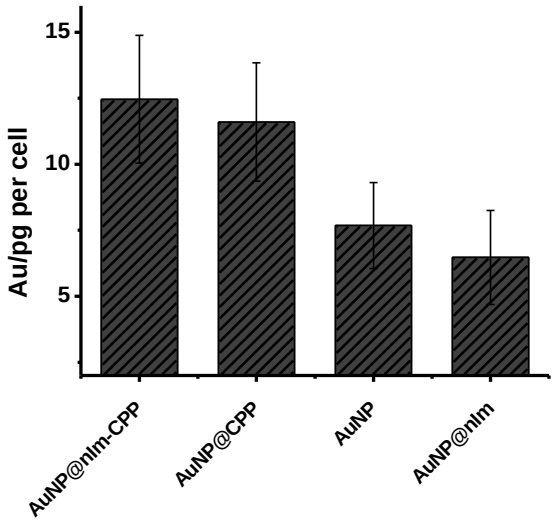
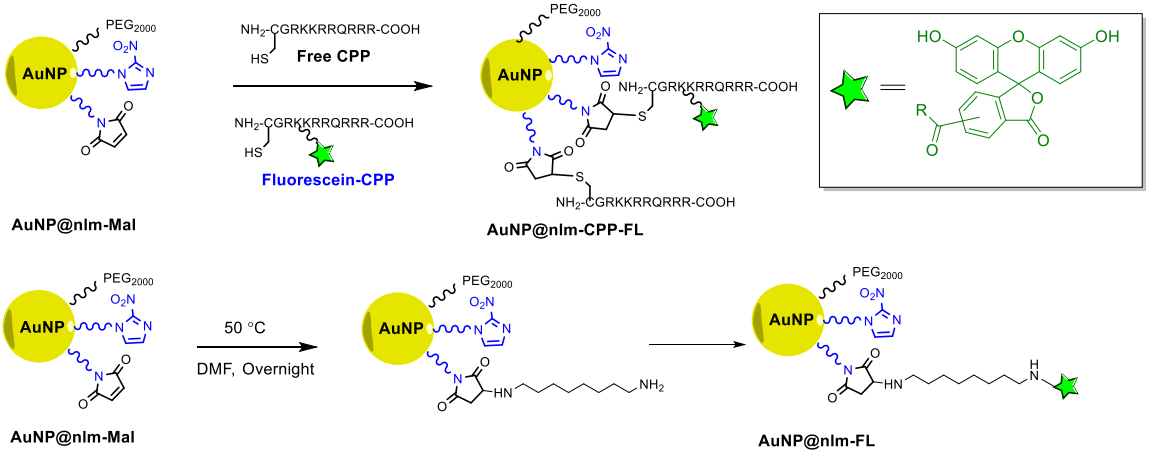


Fig. S4. A) The cellular level of Au after 8 h incubation in the presence of different AuNPs (250 µg/mL)

The bars represent mean ± SD, n = 2.



Scheme S3. Preparation of fluorescein labeled AuNPs.

For control, the nanoparticle without CPP (AuNP@nlm-FL) was prepared. To facilitate fluorescein labeling, free amine groups was firstly introduced onto maleimide functionalized AuNPs by incubation with excess amount of 1,8-Octanediamine at 50 °C overnight . The AuNPs with free amine groups were then reacted with 5(6)-carboxyfluorescein-NHS ester to form AuNP@nlm-FL.

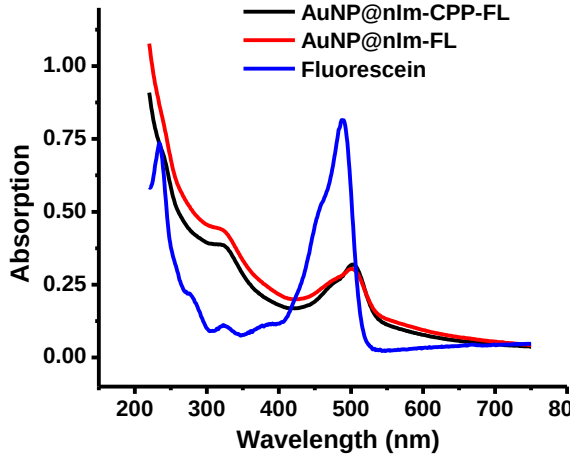


Fig. S5. UV-Vis absorption spectra of free 5(6)-carboxyfluorescein (10 µM) and fluorescein labeled AuNPs (100 µg/mL

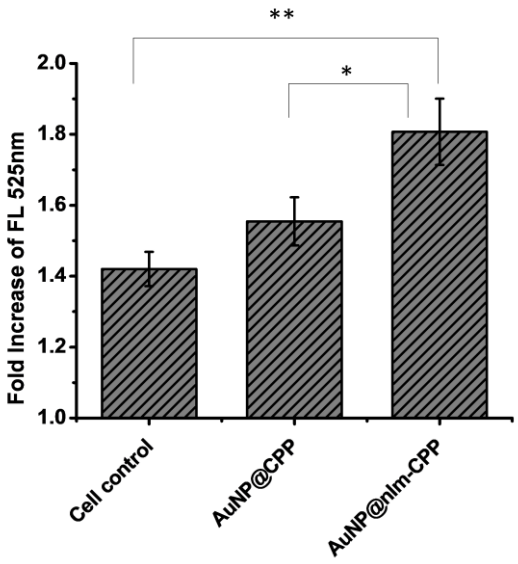


Fig. S6. Intracellular ROS and RNS determination based on the fluorescence of the oxidative product from 2,7-Dichlorodihydrofluorescein (λex= 490 nm, λem= 525 nm). Fold increase was calculated by dividing the fluorescence intensity at 525 nm (λex= 490 nm) from the wells with 10 Gy radiation by the value from the wells without radiation.The bars represented mean ± SD, n = 3, $*P < 0.05$, $**P < 0.01$ (t-test).

References:

1. F. Liu, A. Soh Yan Ni, Y. Lim, H. Mohanram, S. Bhattacharjya and B. Xing, *Bioconjugate Chem*, 2012, **23**, 1639.
2. E. Soto-Cantu, R. Cueto, J. Koch and P. S. Russo, *Langmuir*, 2012, **28**, 5562.