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Electronic Supporting Information

for

Rapid Synthesis of Highly Luminescent and Stable Au_{20} Nanoclusters for Active Tumor-Targeted Imaging *in vitro* and *in vivo*

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Experimental Details

Preparation of cell lysate. Hep-2 cells were grown in RPMI-1640 supplemented with 10 % fetal bovine serum in an incubator (37 ^{o}C, 5% CO_2). The cells were washed twice by PBS and then repeated to freeze and thaw three times to make sure the cells were completely lysated. In order to remove the cell debris and proteins, the product was subsequently centrifuged at 14000 rpm for 10 min and filtered by 0.22 μm microporous membrane. The lysate was collected and stored at 4 ^{o}C.

Preparation of human serum samples. The healthy human serum samples were obtained from the local hospital. Firstly, they were kept in the centrifuge cups and incubated at 37 ^{o}C for clotting without adding any anticoagulant. After the blood clotting, the serum with yellow color was obtained immediately by the centrifugation (3000 rpm, 15 min). The supernatant was carefully collected and diluted by PB buffer (pH 7.4), and then kept in 4 ^{o}C.

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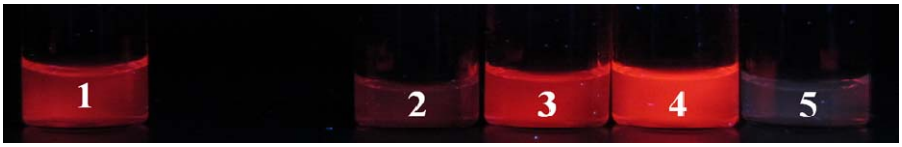
Tables and Figures

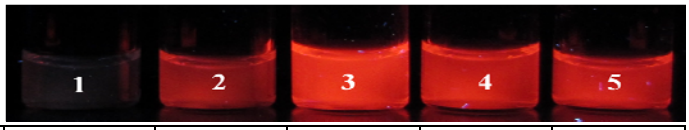
Table S1. Synthesis of Au_{20}NCs with different concentrations of $HAuCl_4$.



Sample	1	2	3	4	5
c_{HAuCl4}	Ref. 1	3.8 mM	4.8 mM	5.8 mM	7.7 mM
QY(%, λ_{ex}:470 nm)	6.3	2.8	8.2	15.2	1.8

c_{BSA}: 19.2 mg/mL, c_{NaOH}: 38 mM, changed the concentration of $HAuCl_4$.

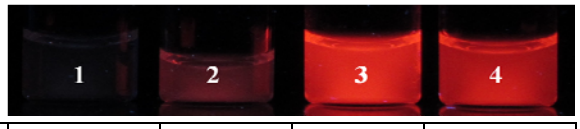
Table S2. Synthesis of Au_{20}NCs with different reaction times.



Sample	1	2	3	4	5
time (min)	20	40	60	80	120
QY(%,λ_{ex}:470 nm)	0.7	4.8	14.5	11.0	8.9

c_{BSA}: 19.2 mg/mL, c_{HAuCl4}: 5.8 mM, c_{NaOH}: 38 mM, changed the reation time.

Table S3. Synthesis of Au_{20}NCs at different temperatures.



Sample	1	2	3	4
T (^{0}C)	60	80	100	120
QY(%,λ_{ex}:470 nm)	0.98	2.7	14.8	11.8

c_{BSA}: 19.2 mg/mL, c_{HAuCl4}: 5.8 mM, c_{NaOH}: 38 mM, changed the reaction temperature.

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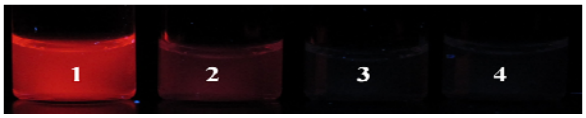
Table S4. Synthesis of Au_{20}NCs with different concentrations of NaOH.



Sample	1	2	3	4
NaOH (mM)	38	58	77	123
QY(%,λ_{ex}: 470 nm)	13.9	2.1	0.08	0.1

c_{BSA}: 19.2 mg/mL, c_{HAuCl4}: 5.8 mM, changed the concentration of NaOH.

Table S5. Comparison of the present work with previously reported methods for the synthesis of AuNCs.

Method	Type of AuNCs	Condition	Time	QY (%)	Reference
Chemical reduction	BSA- Au_{20}NCs	100 ^{o}C, pH 10	1 h	~15	This work
Chemical reduction	BSA-Au_{25}NCs	37 ^{o}C, pH 12	12 h	~6	[1]
Chemical reduction	BSA-$Au_{8,13,25}$NCs	37 ^{o}C, alkaline solution, reduced by ascorbic acid	6 h	~5.7	[2]
Chemical reduction	BSA-Au_{25}NCs	37 ^{o}C, pH 11, reduced by ascorbic acid	5 h	~5.5	[3]
Chemical reduction	PAMAM-Au_{23}NCs	37 °C, reduced by ascorbic acid	3 d	9	[4]
Phase transfer	Thiolated peptides-Au_{38}NCs	Complicated method	> 3 h	Not mention	[5]
Galvanic replacement	glutathione-AuNCs	Using silver dots as template at 80 ^{o}C	> 52 h	10	[6]
Chemical reduction	PhC_2S -Au_{25}NCs	ice/acetone bath	5-10 h	0.8	[7]

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Chemical reduction	Insulin-AuNCs	4 °C, in Na_3PO_4 buffer	12 h	7	[8]
Chemical reduction	Transferrin family protein-AuNCs	Alkaline solution	24 h	1.2-6	[9]
Chemical reduction	Apoferritin protein-AuNCs	Alkaline solution	36 h	8.2	[10]
Photochemical synthesis	pentaerythritol tetrakis 3-mercaptopropionate-AuNCs	Light source (8 W, λ:365 nm)	4 h	5.3	[11]
Chemical reduction	L-3,4-dihydroxyphenylalanine-AuNCs	Room temperature	15 min	1.7	[12]
Phase-transfer	GSH-AuNCs	$NaBH_4$, NaOH, CTAB	> 1 d	5.4	[13]
Core eduction/ligand exchange	GSH-Au_{22}NCs	Complicated method	> 3 d	0.19	[14]

Table S6. Fluorescence lifetimes (τ) of BSA and the as-prepared BSA-Au_{20}NCs.

Sample	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)
BSA-Au_{20}NCs	1.26 (1.16%)	10.4 (1.66%)	453 (97.2%)
BSA	4.12 (100%)	/	/

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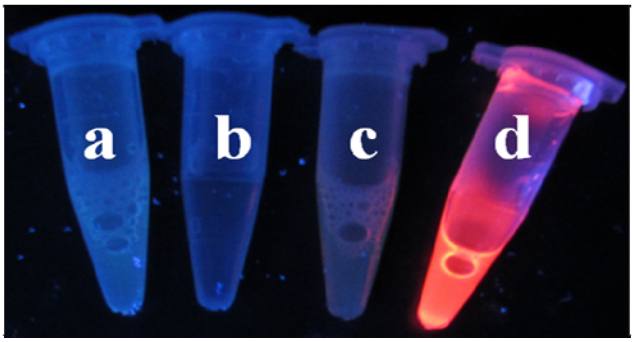

Figure S1. The picture showing the samples under a UV lamp irradiation (λ_{ex}: 365 nm). The samples were prepared using the standard procedure, except for: (a) without adding $HAuCl_4$; (b) without adding BSA; (c) without adding NaOH. The product as shown in (d) was prepared with a standard protocol.

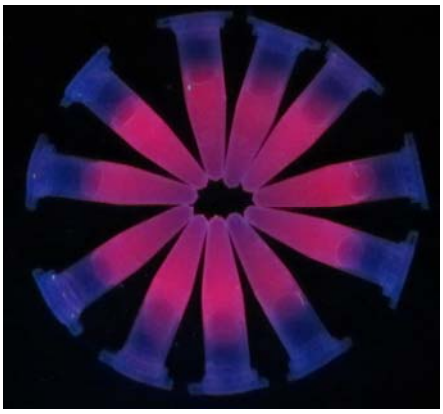

Figure S2. Reproducibility of the present method for the synthesis of BSA-Au_{20}NCs. 11 batches of the products with essentially the same fluorescent intensity and wavelength were obtained.

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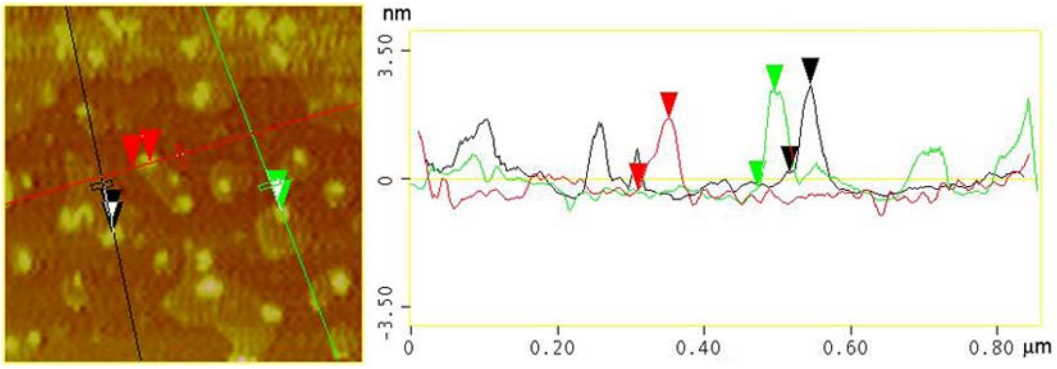

Figure S3. AFM image and the corresponding height measurement of the Au_{20}NCs.

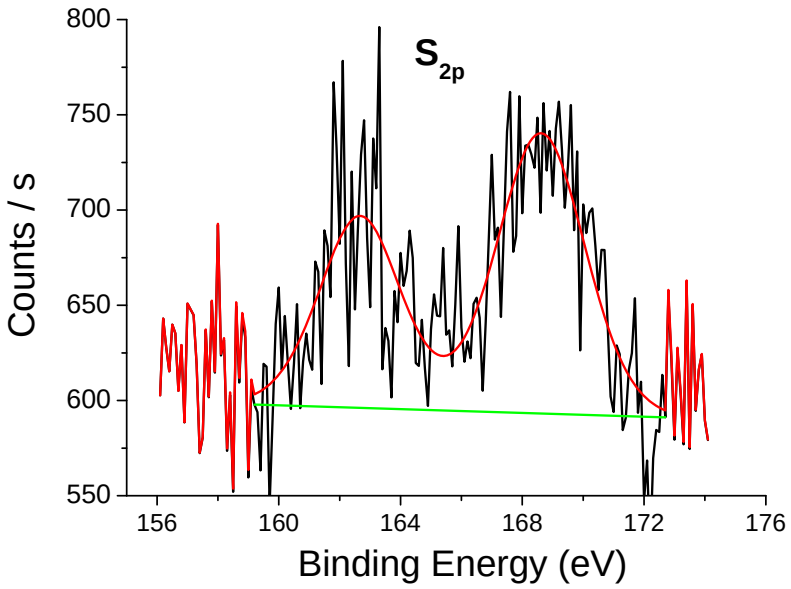

Figure S4. XPS spectrum of the BSA-Au_{20}NCs showing the binding energy of S_{2p}.

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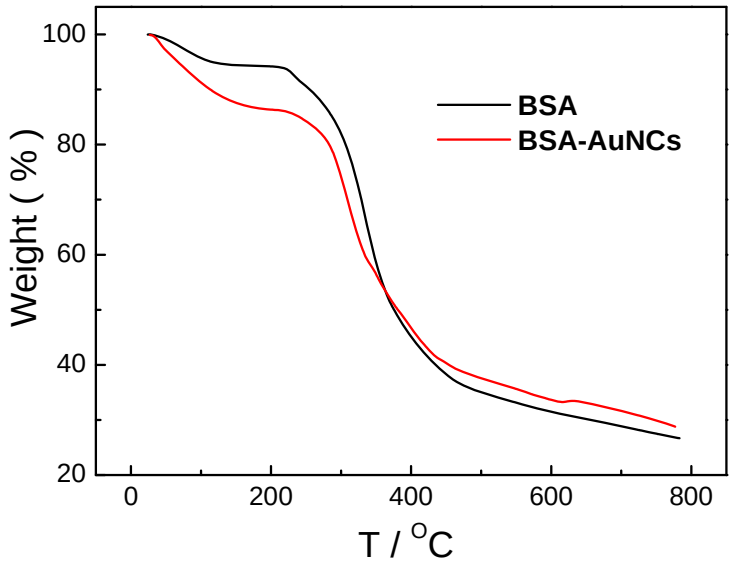

Figure S5. TGA analysis of the as-prepared BSA-Au_{20}NCs and pure BSA.

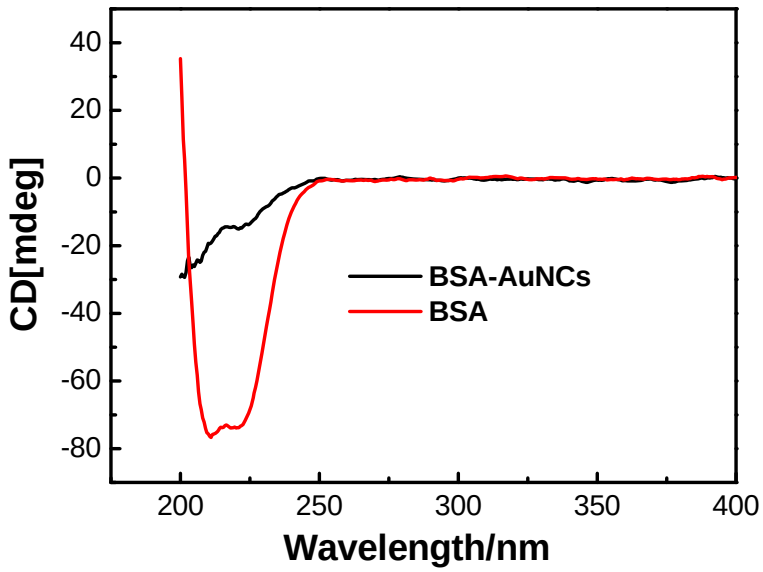

Figure S6. CD spectra of BSA-Au_{20}NCs and pure BSA.

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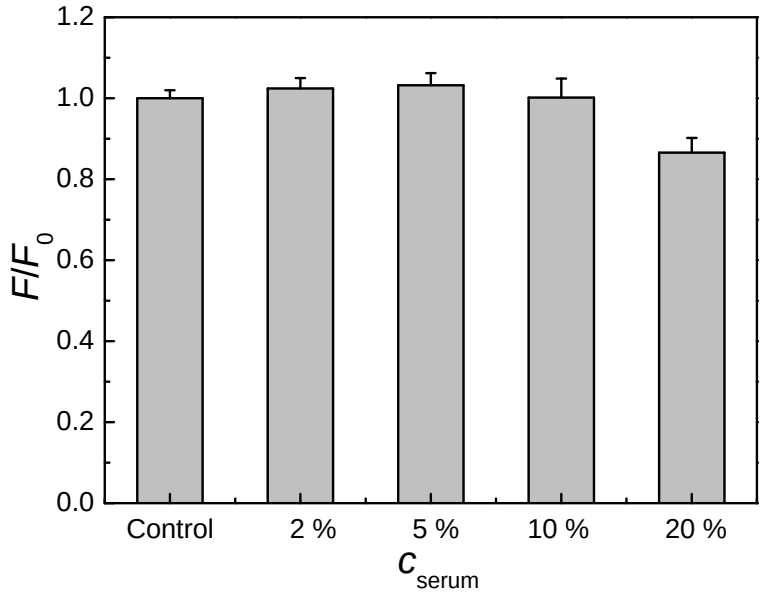

Figure S7. Fluorescence stability of the Au_{20}NCs in different concentrations of serum.

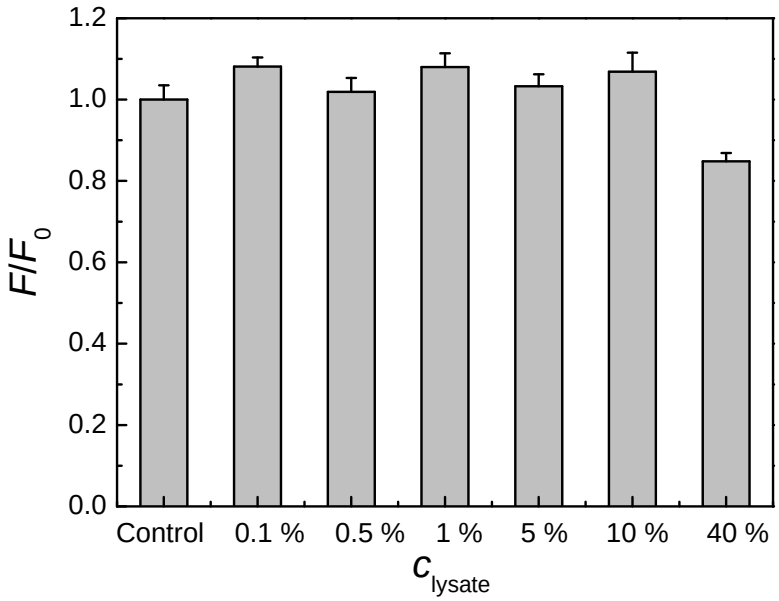

Figure S8. Fluorescence stability of Au_{20}NCs in different concentrations of cell lysate.

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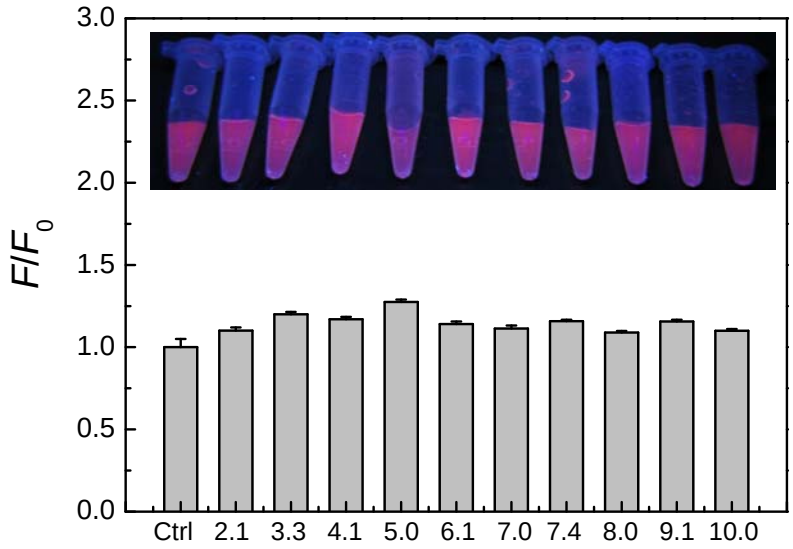

Figure S9. Fluorescence intensity of the as-obtained Au_{20}NCs at different pH values. The inset shows the corresponding photographs of the samples at different pH values under UV light irradiation.

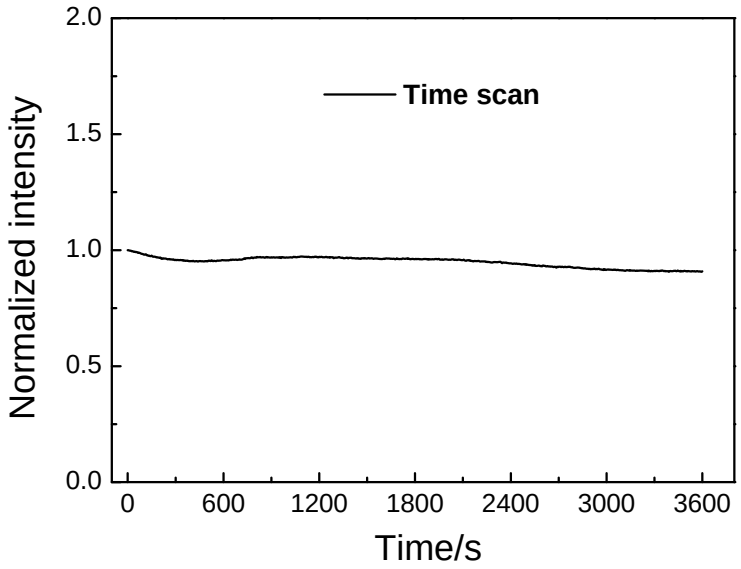

Figure S10. Study on the photostability of the Au_{20}NCs by continuous irradiation for 1 h with a UV light.

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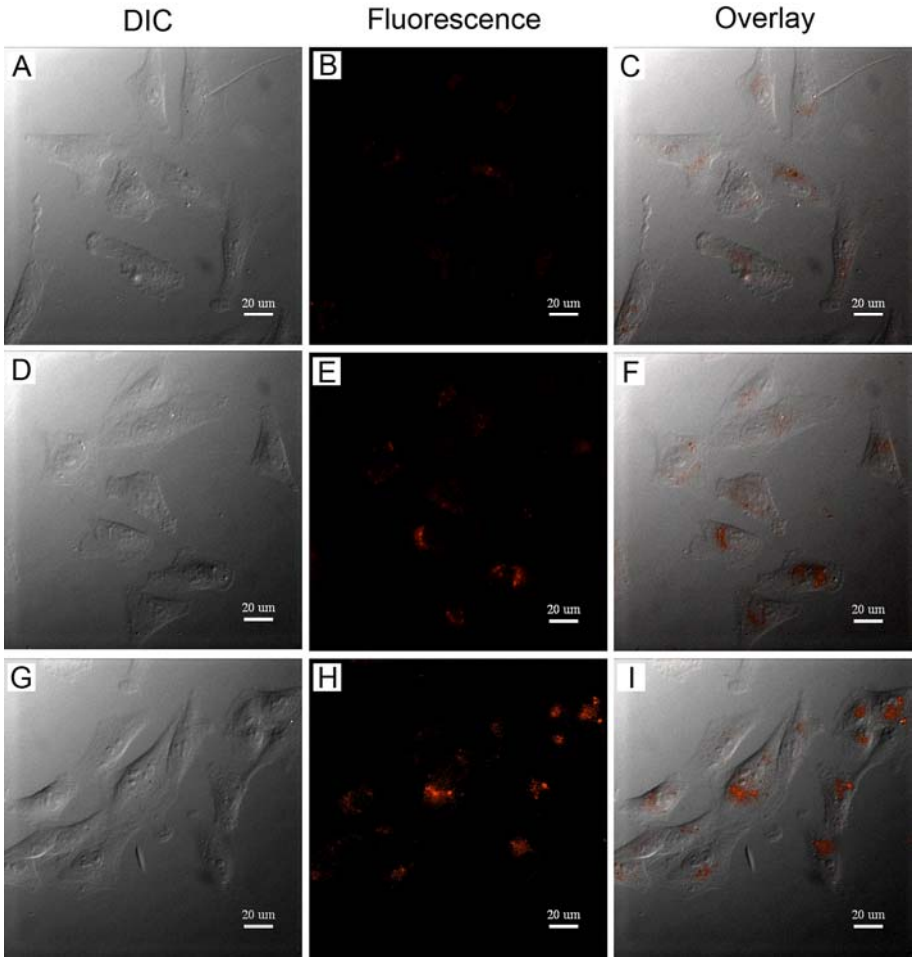

Figure S11. Delivery of Au_{20}NCs with different concentrations into the living Hep-2 cells: (A-C) 6 mg/mL, (B-F) 8 mg/mL, and (G-I) 10 mg/mL. Differential interference contrast (DIC) (A, D, G), fluorescence (B, E, H), as well as the overlay of DIC and fluorescence mode (C, F, I) were given. Scale bars, 20 μm.

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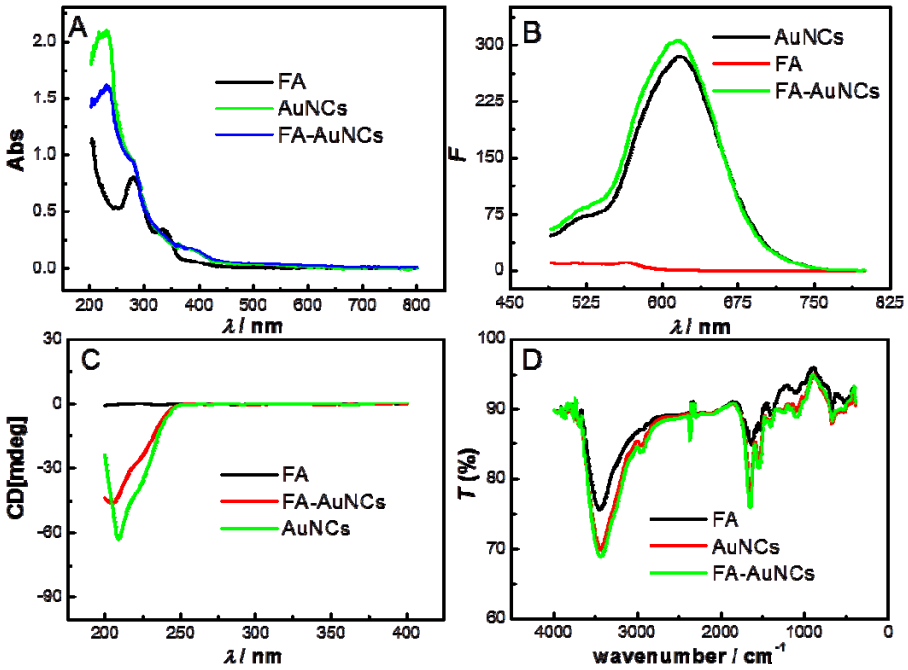

Figure S12. Characterization of the conjugation of Au_{20}NCs with FA: (a) UV-Vis spectra; (b) fluorescence spectra; (c) CD spectra; (d) FT-IR spectra.

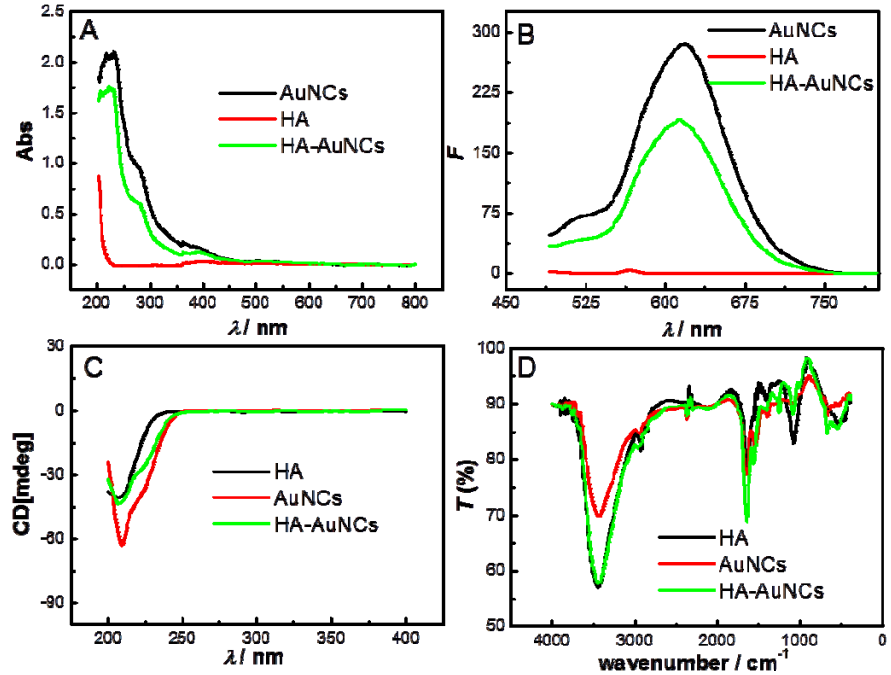

Figure S13. Characterization of the conjugation of Au_{20}NCs with HA: (a) UV-Vis spectra; (b) fluorescence spectra; (c) CD spectra; (d) FT-IR spectra.

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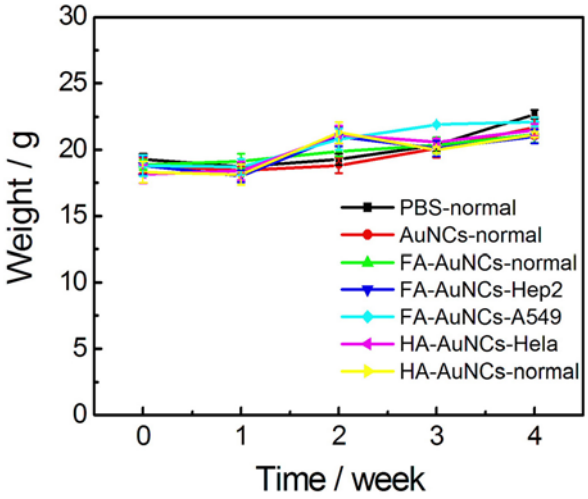

Figure S14. Change in body mass of different nude mice (tumor-bearing or not) injected with 200 μL (60 mg mL^{-1}) Au_{20}NCs, FA-Au_{20}NCs, and HA-Au_{20}NCs, respectively (n=3). The mice injected with 200 μL PBS were used as a control group for comparison.

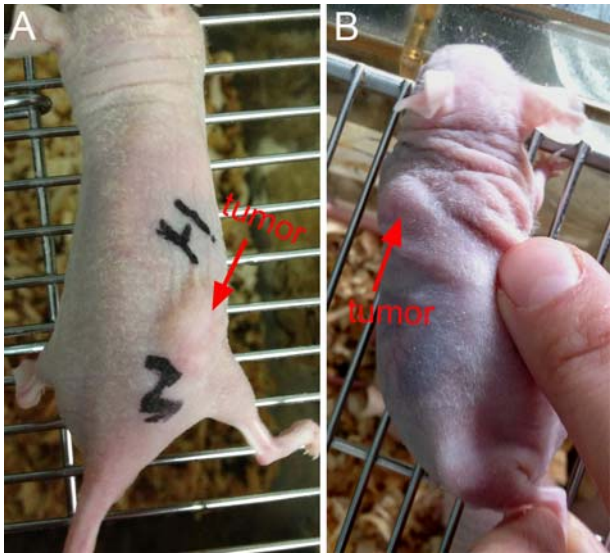

Figure S15. Pictures of the mice bearing a (A) HeLa and (B) Hep-2 tumor, respectively, as marked with the arrows.

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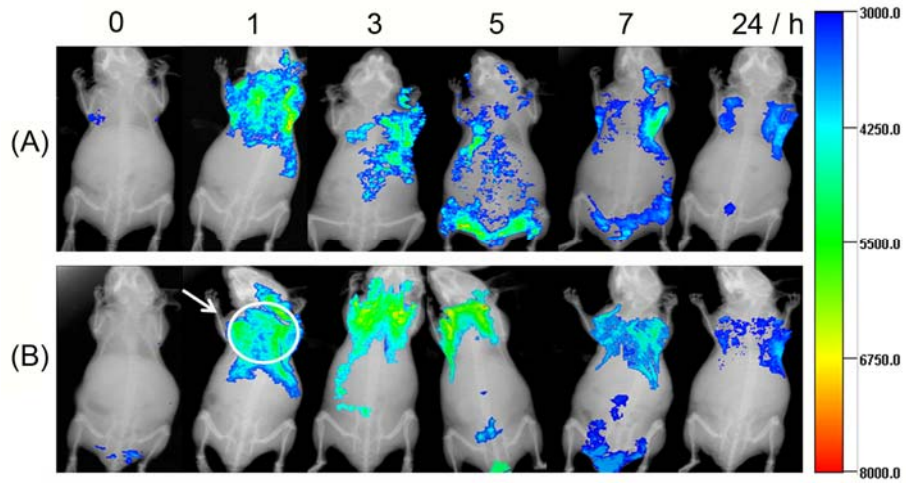

Figure S16. Dynamic distributions of Au_{20}NCs (60 mg/mL) in (A) a normal nude mouse and (B) a Hep-2 tumor-bearing nude mouse, respectively. The images were captured by a NIR fluorescence imaging system after the Au_{20}NCs has been circulated for different time intervals. The white arrow indicates the location of the tumor.

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