Supplementary Information

Copper-mediated On-Off-On gold nanocluster for endogenous GSH sensing to drive cancer cell recognition

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SUPPLEMENTARY METHODS

EXPERIMENTAL SECTION

Microwave-assisted synthesis of AuNCs@Tf@VC.

A mixed aqueous solution of $HAuCl_4$ (2.5 mM, 1 mL) and Tf (5 mg/mL, 1 mL) was stirred at room temperature for 5 minutes. Then, a freshly prepared ascorbic acid solution (0.35 mg/mL, 5 μL) was added dropwise to the mixed solution. Adjusting the the mixed solution to pH 12 with NaOH (1 M , 40μL), then the mixed solution was placed in a closed microwave reactor. The microwave parameters were set to 80 °C, 60 minutes, 150 W, 250 Psi, premix for 120 seconds, high agitation rate. A yellow aqueous solution of AuNCs@Tf@VC is formed which is strongly red-emitting.

Detection of target TfR for AuNCs@Tf.

The cells were incubated with 1 mg/mL AuNCs@Tf for 1 h with or without 1 mg/mL of free Tf for 30 min. The cells were washed 3 times with PBS to remove free AuNCs@Tf, and analyzed by laser scanning confocal microscope (LSCM) and flow cytometer. AuNCs@Tf is excited at a wavelength of 405 nm and the fluorescence emission was collected at 600-700 nm for LSCM. AuNCs@Tf is excited at a wavelength of 405 nm and the fluorescence emission range is 670±15 nm at flow cytometer. A total of 10,000 cells were analyzed as a cell population to calculate intracellular average fluorescence intensity.

Targeted lysosome for AuNCs@Tf-Cu^{2+}

The HeLa cells were seeded at a density of 1.0×10^5 cells into a 35 mm culture dish, and after 20 h of culture, AuNCs@Tf-Cu^{2+} (1mg/mL) was added to the culture dish.

After 2 h, it was washed 3 times with PBS to remove free AuNCs@Tf-Cu^{2+}. The green lysosomal probe was then added to the cells and incubation continued for 30 min. Thereafter, it was washed 3 times to remove the free lysosomal probe. Finally, the cells were observed with a laser confocal microscope. AuNCs@Tf-Cu^{2+} was excited at 405 nm and the emission was collected at 600-700 nm. The green lysosomal probe was excited at 488 nm and the emission was collected at 500-600 nm.

Supplementary figures and Tables

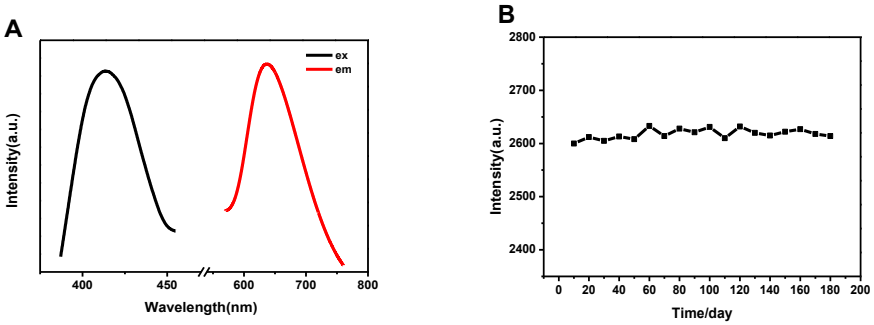

Fig. S1 (A) Fluorescence excitation (black line) and emission (red line) spectra of the AuNCs@Tf. (B) Determination of fluorescence intensity with time.

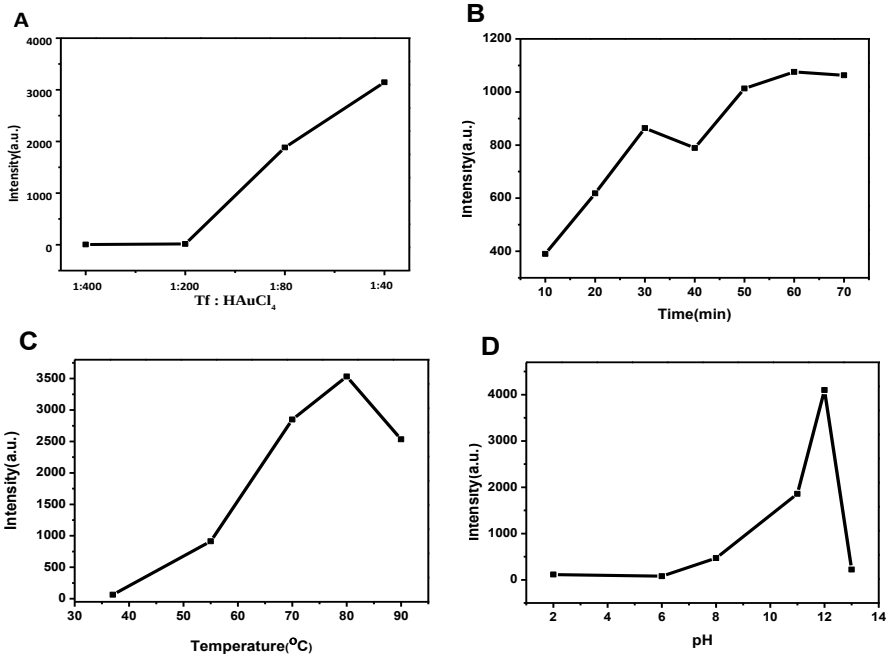

Fig. S2 Optimization of experimental conditions of as-AuNCs@Tf by a microwave apparatus. (A) The reaction molar ratio of the Tf and $HAuCl_4$. (B) Reaction time. (C) Reaction temperature. (D) The influence of pH.

Table S1 The XPS analysis of the elemental composition of AuNCs@Tf.

Element	Position BE(eV)	Atomic Mass	Mass Conc(%)	Atomic Conc(%)
S 2p	167.90	32.065	1.94	0.85
O 1s	531.50	15.999	37.12	32.58
N 1s	400.15	14.007	6.54	6.56
C 1s	284.95	12.001	51.12	59.77
Au 4f	84.30	196.967	3.28	0.23

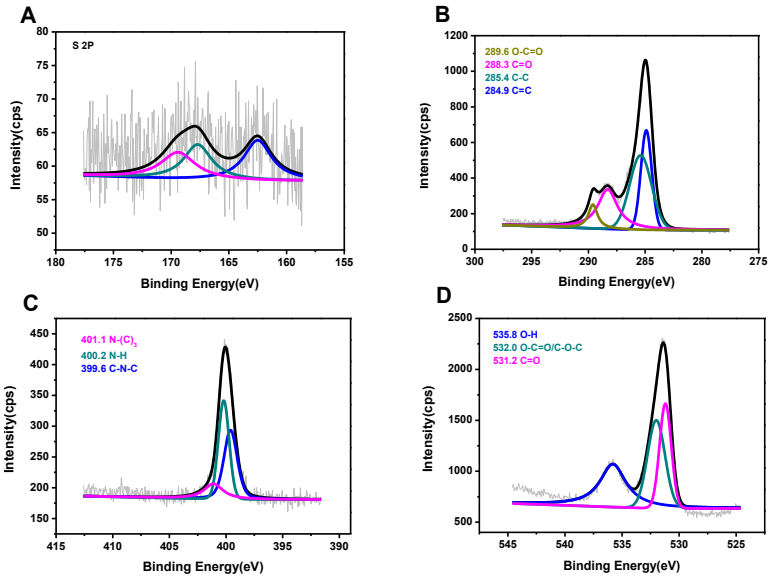

Fig. S3 (A) S 2p, (B) C 1s, (C) N 1s and (D) O 1s spectra of AuNCs@Tf.

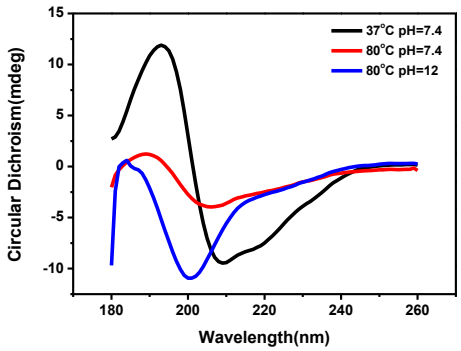

Fig. S4 CD spectra of Tf at different temperatures and pH conditions.

Table S2 Changes of secondary structure of transferrin under different conditions by analyzing software CDNN of circular dichroism.

	Helix(%)	Beta(%)	Beta-Turn(%)	Rndm.Coil(%)
37 °C, pH=7.4	31.0	43.7	18.7	25.3
80 °C, pH=7.4	11.9	61	23.4	27.1
80 °C, pH=12	4.2	55.4	30.0	40.4

As is well known that transferrin receptors (TfR) expression on cancer cells can be 100 times higher than the average expression of normal cells,[1-3] and according to this feature, probes containing transferrin (Tf) can be used to target cancer cells. During the preparation process, a relative high pH value (12) and temperature (80 °C) are necessarily to trigger the disruption of disulfde bonds of Tf into active RS- and the subsequent conversion of RS-Au so that the proteins would undergo a conformation change. The circular dichroism (CD) of Tf under different temperatures and pH conditions were performed. As shown in Figure S4 and Table S2, the α-helix content markedly decreases with an increase in temperature and pH, conversely, the ratio of β-sheet, β-turn and random coil increases accordingly. Due to the change in the secondary structure of the Tf molecule, we think that Tf lost its affinity for TfR at 80 °C and pH 12.

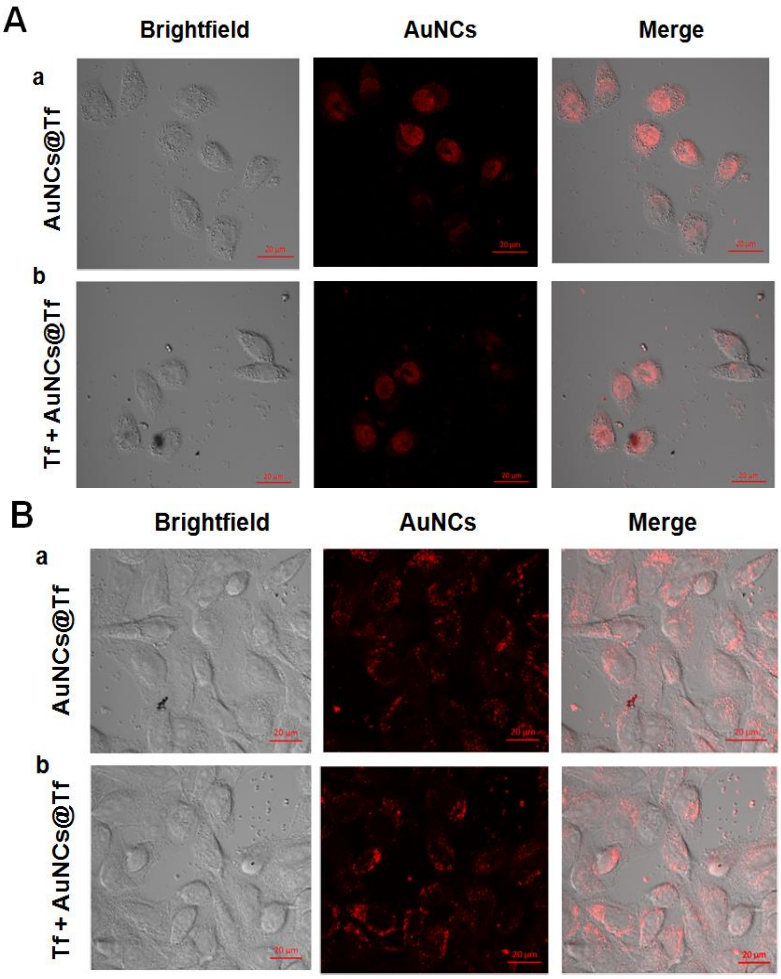

Fig. S5 (A) LSCM of HepG2 cells after incubation with AuNCs@Tf (1mg/mL) for 1.0 h with or without free 1mg/mL Tf pretreatment for 0.5h. (B) LSCM of HeLa cells after incubation with AuNCs@Tf (0.5mg/mL) for 1.0 h with or without free 1mg/mL Tf pretreatment for 0.5h.

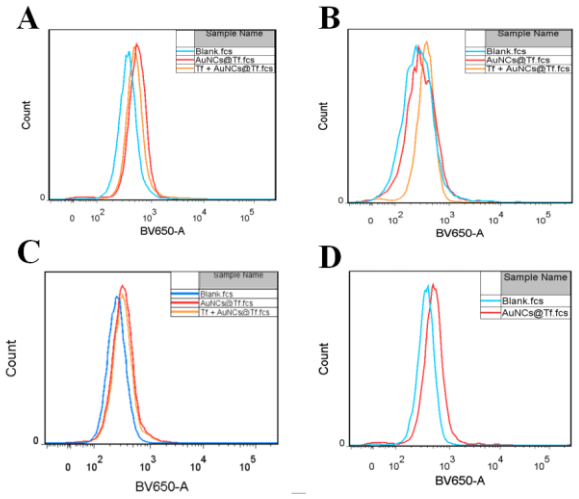

Fig. S6 Flow cytometry analysis of the amount of endocytosed AuNCs@Tf. (A) HepG2 Cells. (B) HCT 116 Cells. (C) SMMC-7721 Cells. (D) 3T3 Cells as normal cells.

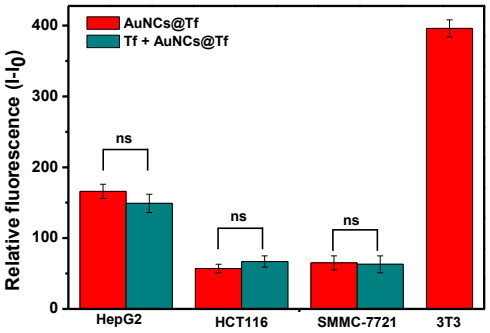

Fig. S7 Relative fluorescence intensity histogram of endocytic AuNCs@Tf in various cells measured by flow cytometry ($I-I_0$, where I and I_0 are the fluorescence intensities of AuNCs@Tf and Blank, respectively). (ns represents not significant difference of cellular uptake AuNCs@Tf between with or without 1mg/mL Tf.)

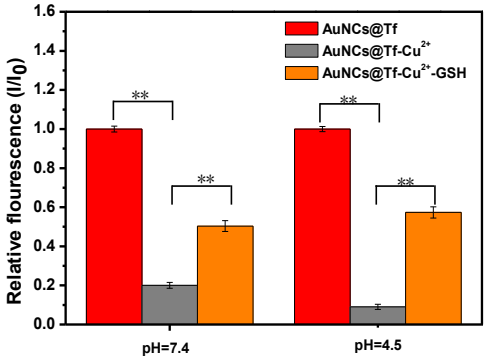

Fig. S8 Changes of relative fluorescence intensity after addition of Cu^{2+} and GSH in AuNCs@Tf under different conditions. (** represents $p < 0.001$)

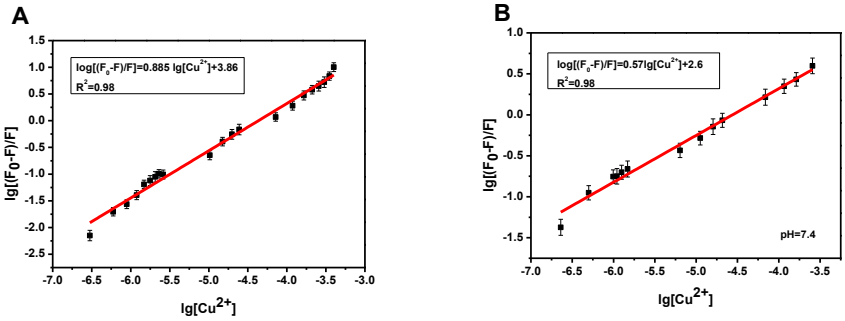

Fig. S9 Double logarithmic plot of Cu^{2+} to AuNCs@Tf. (A) pH4.5. (B) pH 7.4. (F_0 and F refer to the fluorescence intensities of AuNCs@Tf in the absence and presence of ion, respectively. K_A is the binding constant and n is the number of binding sites. The equation is: $lg(F_0-F)/F = lgK_A + nlg[Q]$.

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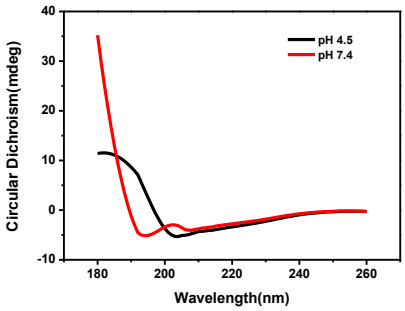

Fig. S10 CD spectra of AuNCs@Tf-Cu^{2+} at pH4.5 and pH7.4.

Table S3 Changes of secondary structure of AuNCs@Tf-Cu^{2+} under different pH conditions by analyzing software CDNN of circular dichroism.

	Helix(%)	Beta(%)	Beta-Turn(%)	Rndm.Coil(%)
pH 4.5	**17.1**	**36.6**	**20.9**	**46.3**
pH 7.4	**50.8**	**29.2**	**15.6**	**20.8**

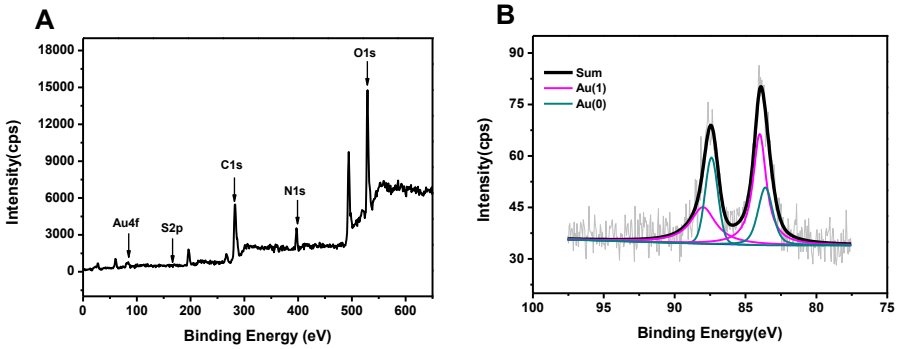

Fig. S11 (A) XPS spectrum of AuNCs@Tf@VC. (B) XPS spectrum of Au 4f.

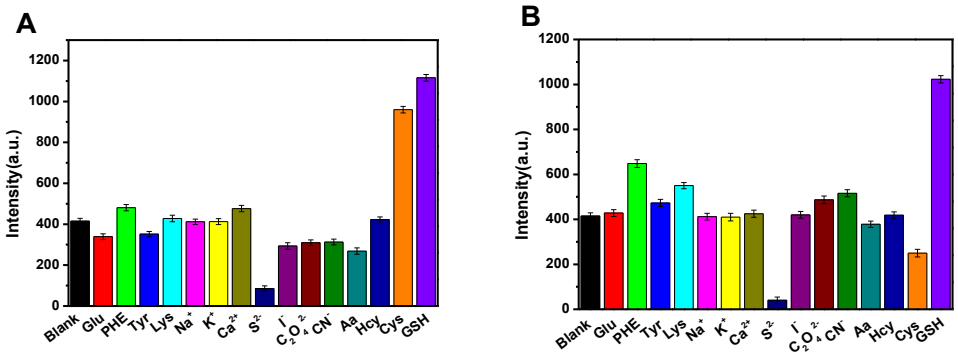

Fig. S12 (A) Fluorescence responses of the AuNCs@Tf@Tf-Cu^{2+} (pH=7.4) system to GSH and 13 other biological molecules or ion. (B) Fluorescence responses of the AuNCs@Tf-Cu^{2+} (pH=7.4) system to GSH and 13 other biological molecules or ion.

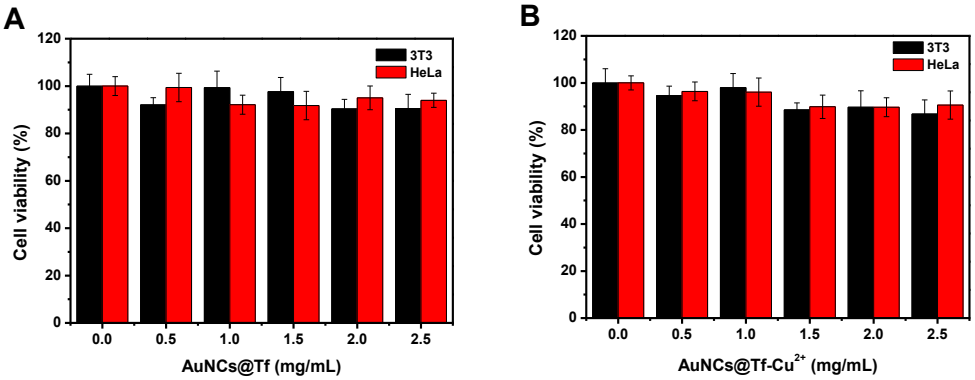

Fig. S13 Viability of 3T3 and HeLa cells after 5 h of incubation with different concentrations of AuNCs@Tf and AuNCs@Tf-Cu^{2+} by an MTT assay. (A) Viability of cells by AuNCs@Tf treatment. (B) Viability of cells by AuNCs@Tf-Cu^{2+} treatment.

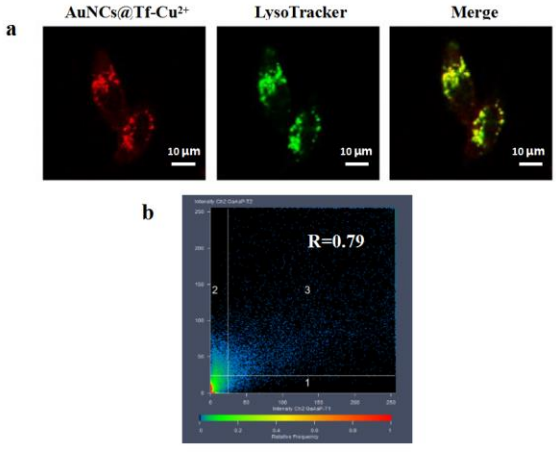

Fig. S14 Localization of AuNCs@Tf-Cu^{2+} (1mg/mL) in HeLa cells for 2h.

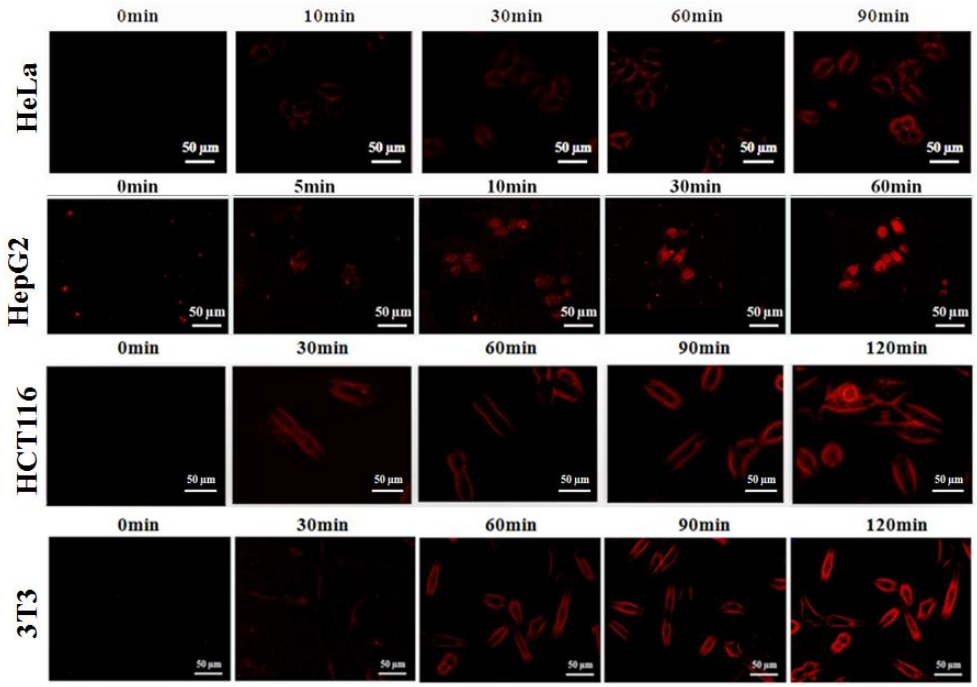

Fig. S15 Fluorescence microscopy imaging of different cells after incubation with AuNCs@Tf for different times.

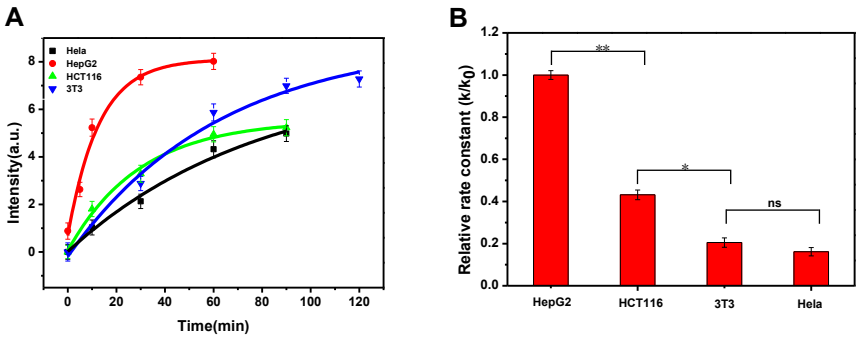

Fig. S16 (A) Fluorescence intensity changes with time, where data come from the corresponding imagines shown in Fig. S15. (B) Relative rate constant of AuNCs@Tf to different cells, where data were fitted according to the data in Fig. S16A (ns represents non-significance, * represents $p < 0.05$ and ** represents $p < 0.001$).

Table S4 Rate constant of AuNCs@Tf-Cu^{2+} and AuNCs@Tf to different cells.

	$k_{(HepG2)}/min^{-1}$	$k_{(HeLa)}/min^{-1}$	$k_{(HCT116)}/min^{-1}$	$k_{(3T3)}/min^{-1}$
AuNCs@Tf-Cu^{2+}	**0.0408**	**0.0146**	**0.0082**	**0**
AuNCs@Tf	**0.078**	**0.016**	**0.03365**	**0.0126**

References:

1. Y. Zhao, J. Peng, Y. J. Niu, X. N. Zhang, N. Jiang, R. R. Jia, J. Li, Z. Q. Shang, S. M. Zhu, L. B. Sun, *RSC Adv.*, 2015, **5**, 64076-64082.
2. T. Zhao, X. W. He, W. Y. Li, Y. K. Zhang, *J. Mater. Chem. B*, 2015, **3**, 2388-2394.
3. Y. Wang, J. T. Chen, X. P. Yan, *Anal. Chem.*, 2013, **85**, 2529-2535.