Supplementary Data

Near-Infrared Photothermally Activated DNA nanotweezers for Imaging ATP in Living Cells

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1. Experimental section

1.1 Materials and reagents

All of the chemicals were obtained from commercial suppliers and used without further purification. The labeled DNA listed in Tabel S1 and S2 were synthesized and purified by Sangon Biotech Co. (Shanghai, China). Cetyltrimethylammonium bromide (CTAB), hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), L-ascorbic Acid, trisodium citrate and hydrochloric acid (HCl, 37 wt. % in water) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Sodium oleate (NaOL) was purchased from Bide Pharmatech Ltd. (Shanghai，China). Adenosine 5'-triposphate (ATP) was purchased from Bomei Biotech. Co. Ltd. (Hefei, China). Guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), uridine 5'-triphosphate (UTP), sodium borohydride ($NaBH_4$), and Tris (2-carboxyethyl) phosphine hydrochloride solution (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylthiazolyldiphenyl-tetrazolium bromide (MTT), Silver nitrate ($AgNO_3$), Tween 20 were purchased from Aladdin Holdings Group Co., Ltd (Shanghai, China). Hoechst 33342, phosphate buffered saline (PBS) and dulbecco's modified Eagle's medium (DMEM, serum-free medium with high glucose and penicillin-streptomycin) and human cervical cancer cells (HeLa) were purchased from Keygenbio Co., Ltd. (Nanjing, China). Fetal Bovine Serum (FBS) was purchased from Gibco (USA). The water used throughout the experiments was Millipore water (18.2 MΩ). All experiments were conducted in room temperature unless otherwise mentioned.

Table S1. Sequences of oligonucleotides used for this work.

Name	Sequence (5' to 3')
T1	Cy3-TTACGGCATCCTGCGGTCGGAGGCACCAGGCGTAGCGTG TA-Cy5
T2	*TATATATATATGCGGAGGAA*GGTTTTACACGCTACGCCTGGTGCC Split aptamer 2
T3	CCGACCGCAGGATGCCGTAATTACCTGGGGGAGTAT Split aptamer 1
Lock	*TTCCTCCGCATATATATATATA*TTT-$(CH_2)_6$-SH
ATP aptamer	Cy3-ACCTGGGGGAGTATTGCGGAGGAAGGT-Cy5
Split aptamer 1	Cy3-ACCTGGGGGAGTAT
Split aptamer 2	TGCGGAGGAAGGT-Cy5

Table S2. Sequences of oligonucleotides used to optimize the FRET efficiency of T123.

Name	Sequence (5' to 3')
T2-0bp	TATATATATATGCGGAGGAAGGTTACACGCTACGCCTGGTGCC
T2-1bp	TATATATATATGCGGAGGAAGGT**T**TACACGCTACGCCTGGTGCC
T2-2bp	TATATATATATGCGGAGGAAGGT**TT**TACACGCTACGCCTGGTGCC
T2-3bp	TATATATATATGCGGAGGAAGGT**TTT**TACACGCTACGCCTGGTGCC
T2-4bp	TATATATATATGCGGAGGAAGGT**TTTT**TACACGCTACGCCTGGTGCC
T2-5bp	TATATATATATGCGGAGGAAGGT**TTTTT**TACACGCTACGCCTGGTGCC
T3-0bp	CCGACCGCAGGATGCCGTAAACCTGGGGGAGTAT
T3-1bp	CCGACCGCAGGATGCCGTAA**T**ACCTGGGGGAGTAT
T3-2bp	CCGACCGCAGGATGCCGTAA**TT**ACCTGGGGGAGTAT
T3-3bp	CCGACCGCAGGATGCCGTAA**TTT**ACCTGGGGGAGTAT
T3-4bp	CCGACCGCAGGATGCCGTAA**TTTT**ACCTGGGGGAGTAT
T3-5bp	CCGACCGCAGGATGCCGTAA**TTTTT**ACCTGGGGGAGTAT

1.2 Instruments

The morphologies of AuNR were examined with JEM-1011 and JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The fluorescence experiments were conducted on a fluorescence spectrophotometer (F-7000, Hitachi Ltd., Japan). UV-vis absorption spectra were recorded on a UV-vis spectrophotometer (UV-3600, Shimadzu Co., Japan and Nanodrop-2000C, Nanodrop, USA). The zeta potential analysis was performed using a Malven Zetasizer Nano-Z instrument (Malvern Instruments Ltd., Worcestershire, UK). Thermal images were taken by infrared thermal camera (Fotric 225-1, Fotric, China). The cell viability assay was performed using a Thermo Scientific Varioskan Flash (Thermo Fisher Scientific, U.S.A.). Confocal fluorescence images of cells were acquired with a TCS SP5 confocal microscopy (Leica, Germany) and TCS SP8 confocal microscopy (Leica, Germany).

1.3 Synthesis of gold nanorod (AuNR)

Firstly, we prepared the seed solution from which the AuNR was grown. Briefly, 2.5 mL of 0.5 mM $HAuCl_4$ was mixed with 2.5 mL of 0.2 M CTAB solution in a 10 mL scintillation vial. Then 0.3 mL of 0.01 M $NaBH_4$ which was freshly prepared was diluted to 0.5 mL with water and was injected into the Au (III)-CTAB solution under vigorous stirring (1200 rpm). The color of the

solution immediately changed from yellow to brownish yellow. The solution was stirred for 2 min and then aged at 30 ℃ for 30 min before use.

For the AuNR growth solution, 3.5 g CTAB and 0.617 g sodium oleate (NaOL) were first dissolved in 125 mL warm water (~ 50 °C). When the solution cooled down to 30 ℃, 12 mL of 4 mM $AgNO_3$ was added. After adding 125 mL of 1 mM $HAuCl_4$ solution, the mixture was left undisturbed at 30 °C for 15 min. The solution became colorless after 90 min of stirring (700 rpm), and then 1.8 mL of HCl (37 wt. % in water, 12.1 M) was introduced to adjust the pH of the mixture. After stirring the solution gently (400 rpm) for 15 min, 0.625 mL of 0.064 M L-ascorbic acid (AA) was added with vigorously stirring for 30 s. Then 0.4 mL of seed solution was injected into the growth solution under vigorously stirring for 30 s and the mixture was kept undisturbed at 30 ℃ for 12 h. Finally, the products were isolated by centrifugation at 10,000 rpm for 10 min and the precipitate was resuspended in ultrapure water for subsequent experiments.

1.4 Photothermal performance of AuNR

The laser employed throughout the experiment was 1064 nm. We used a 1.5 mL tube containing 1 mL of the testing solution with the concentration from 0 μg/mL to 100 μg/mL. The laser power density was adjusted to 1 W/cm^2, and the temperature of the solution to be tested was detected over time. The temperature of the solution was measured by a digital thermometer with a thermocouple probe and was recorded every 10 s. Meanwhile, the thermal images were taken by an infrared thermal camera to record the real-time temperature.

To calculate the photothermal conversion efficiency, AuNR with a concentration of 50 μg/mL was irradiated by 1064 nm laser with a power density of 1 W/cm^2 for 10 min. Then the laser was turned off and in the meantime, a digital thermometer was used to measure the temperature change in real time and recorded it every 10 s for a total of 10 min.

The photothermal conversion efficiency of AuNR was measured according to previous report.[1] It can be calculated according to the eq1:

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{1064}})} \qquad \text{(eq1)}$$

Where h ($W \cdot cm^{-2} \cdot K^{-1}$) is the heat transfer coefficient, S (cm^2) means the surface area of the container, T_{max} (K) represents the equilibrium temperature; T_{surr} (K) means the ambient temperature of the surroundings. Q_{dis} (W) is heat loss from light absorbed by the container. I ($W \cdot cm^{-2}$) is the power density of the incident laser, and A_{1064} represents the absorbance of samples at 1064 nm.

The hS can be calculated according to eq2:

$$hS = \frac{m_D c_D}{\tau_s} \quad \text{(eq2)}$$

Where τ_s is the sample system time constant, m_D and c_D are the mass (g) and heat capacity ($J \cdot g^{-1} \cdot °C^{-1}$) of the solvent. τ_s is obtained by eq3, eq4 and Figure S2c.

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad \text{(eq3)}$$

$$t = -\tau_s ln\theta \quad \text{(eq4)}$$

Q_{dis} is calculated by eq5:

$$Q_{dis} = hs(T_{H_2O\ max} - T_{H_2O\ surr}) \quad \text{(eq5)}$$

Finally, the photothermal conversion efficiency of AuNR was calculated to be 27.89 %.

1.5 Gel electrophoresis assays

The formation of the T123 and T123L were demonstrated using a native polyacrylamide gel (10 %). T1 was mixed with T2 and T3 to get T123 and mixed with T2, T3 and Lock strand to get T123L. The DNA oligonucleotides were diluted to 1 μM with PBS buffer and then heated in a metal bath at 95 °C for 5 min. Then, the mixture was cooled naturally and loaded on 0.75 mm thin gel, respectively. Electrophoresis was carried out in 1 × TBE buffer at 80 V for 10 min and followed by

120 V for 40 min at room temperature. Finally, the gel was stained by gel-green dye for 6 min and the results were obtained via a Bio-Rad fluorescence gel imaging system.

1.6 Synthesis and characterization of AuT123L

Firstly, the thiol-functionalized oligonucleotide Lock was mixed with T1, T2, T3 in equal quantities and annealed at 95 ℃ for 5 min to obtain T123L. AuNR solution was centrifuged at 10,000 rpm for 10 min to remove excess ascorbic acid, $AgNO_3$ and small spherical particles. According to published protocols, a 1 mL aliquot of AuNR sample was centrifuged at 10,000 rpm for 10 min, then the supernatant was discarded. 50 μL of 20 μM mPEG-SH (5 kDa) and 1 mL of 0.01 wt % Tween 20 were added to resuspend the AuNR pellet. The solution was centrifuged and resuspended three times in order to thoroughly displace CTAB from the surface of AuNR. Next, the hybridized T123L (50 μM, 50 μL) was activated by TCEP with a ratio of 1:100 for 1 h at room temperature to cleave the disulfide bond. The activated DNA was added to the prepared AuNR solution and then incubated overnight at room temperature under mild shaking. Subsequently, after aging for 12 h with 0.1 M NaCl, the modified AuNR solution was washed by centrifuged with ultrapure water at 10,000 rpm twice for 10 min, and stored in 10 mM PBS buffer (pH 7.4, 137 mM NaCl) at 4 ℃ for subsequent Zeta, DLS and fluorescence characterization.

1.7 Determination of DNA number on AuNRs

Standard calibration curves were obtained by measuring the fluorescence intensity of Cy3 at different concentrations of T123L (Figure S6a). DNA agonists bound to AuNRs were replaced by 10 mM DL-Dithiothreitol (DTT) via a thiol exchange reaction. The sample was incubated for 18 h and centrifuged to obtain the supernatant and measure the fluorescence intensity of Cy3 (Figure S6b). The molar concentration of the AuNR was calculated from its mass concentration, volume and density.

1.8 ATP sensing assay

Firstly, T123 and T123L were diluted to 50 nM with 10 mM PBS (containing 137 mM NaCl and 12.5 mM $MgCl_2$). ATP solution of different concentrations were added, and incubated for 30 min for fluorescence detection. In the selective experiment, ATP was replaced with CTP, GTP and UTP (4 mM) for fluorescence detection experiment. The excitation slit was 10 nm and the emission

slit was 5 nm. The excitation wavelength was 525nm.

For ATP sensing with the assembled probe AuT123L, 200 μL AuT123L was mixed 4 mM ATP and irradiated by 1064 nm laser with a power density of 1 W/cm^2 for 5 min, then incubated for 30 min. In the control experiment, ATP was added and incubated for 30 min without laser irradiation. The excitation slit was 10 nm and the emission slit was 10 nm. The excitation wavelength was 525nm.

1.9 Cell culture

Hela cells (human cervical carcinoma) were used throughout the experiments. The cell lines were grown in Dulbecoo's modification of Eagle's medium (DMEM) supplemented with 10 % Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin and were maintained at 37 °C in an atmosphere containing 5 % CO_2. When the confluence rate of cells reached about 80 %, the cells were separated from the culture flask by trypsin and a certain number of cells were seeded in 96-well plates for subsequent MTT experiments or in confocal dishes for confocal microscopy imaging.

1.10 Cell viability test

The detail of cell culture was displayed in Supporting Information. Hela cells were dispersed on 96-well plates and cultured in cell medium at 37 °C with 5 % CO_2 for 24 h. The cells were divided into three groups. The first group was incubated with AuT123L of different concentrations (0, 50, 75, 100, 125, 150 μg/mL) for 24 h under dark conditions. The second group was irradiated with 1064 nm laser at 1 W/cm^2 for different time to detect the phototoxicity of the laser. Cells in the third group were first incubated with AuT123L of 50 μg/mL, and 1064 nm laser irradiation of 1 W/cm^2 was used for different time after changing the medium. 50 μL solutions of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were added to each well and the cells were incubated at 37 °C for 4 h. Then, the MTT solution was removed and the remaining precipitate was dissolved by 150 μL DMSO. Finally, the absorption intensity at 490 nm of each well was recorded.

1.11 Cell imaging by confocal microscopy

Hela cells were seeded in confocal dishes and cultured in DMEM for 24 h. Then the medium was replaced with fresh DMEM containing 50 μg·mL^{-1} AuT123L, and incubated for 4 h. Fresh DMEM was added after washing with PBS for three times, and then the cells were irradiated with 1064 nm laser for 5 min. Subsequently, the cells were incubated for another 0.5 h to ensure that the tweezers reacted with ATP, and then were used for confocal observation. Lysotracker and Hoechst 33342 were used to specifically stain the lysosomes and nucleus of cells, respectively, according to manufacturer protocol.

2. Supplemental Figures

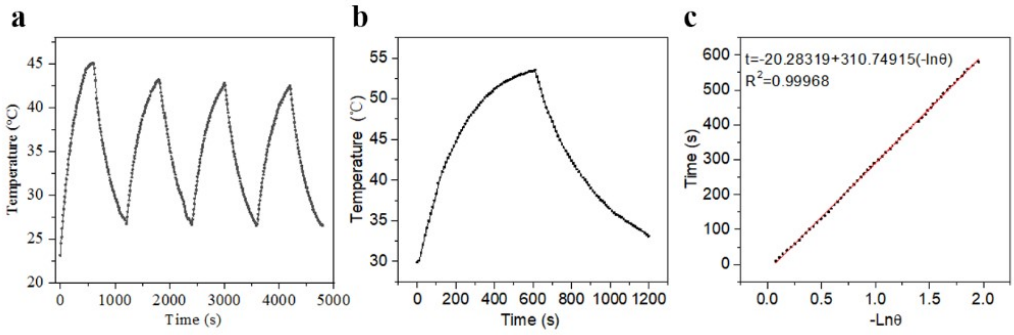

Fig. S1. a) The photothermal conversion cycling test of AuNR (40 μg/mL) aqueous solution under 1 W/cm^2 of 1064 nm laser irradiation (heating 10 min and cooling 10 min for one cycle). b) Photothermal effect of AuNR (50 μg/mL) when irradiated with a 1064 nm laser (1 W/cm^2). The laser was turned off after irradiation for 10 min. c) Plot of cooling time versus negative natural logarithm of the temperature driving force obtained from the cooling stage as shown in (b).

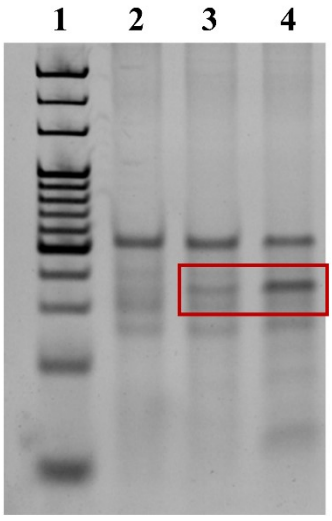

Fig. S2. Gel electrophoresis. Line 1-4: 20 bp marker, 1 μM T123, 1 μM T123 + 5 mM ATP, 1 μM T123 + 20 mM ATP.

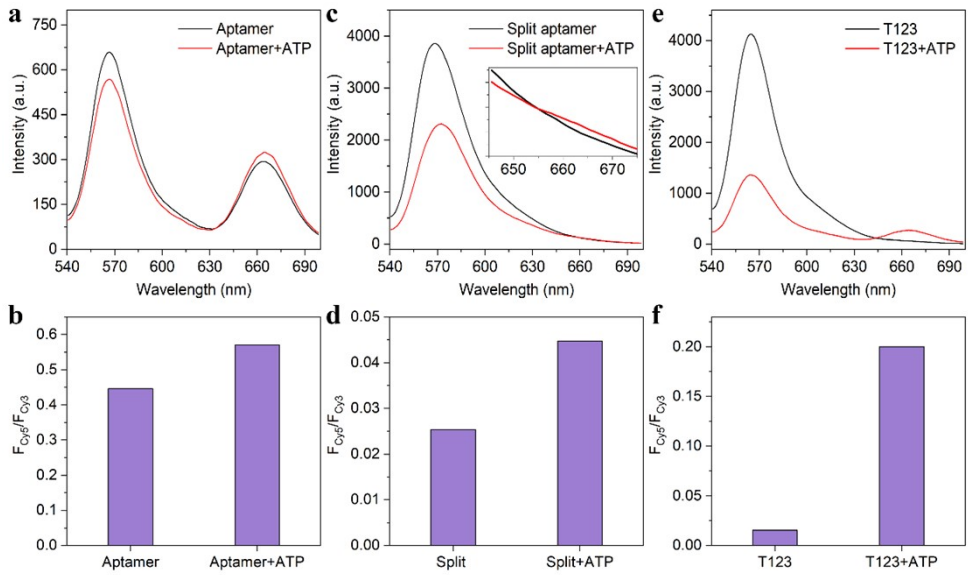

Fig. S3. The response of ATP aptamer a) b), split aptamer c) d) and T123 e) f) to ATP. The concentration of ATP is 2 mM. The concentration of the probe is 100 nM and the samples were excited at 525 nm.

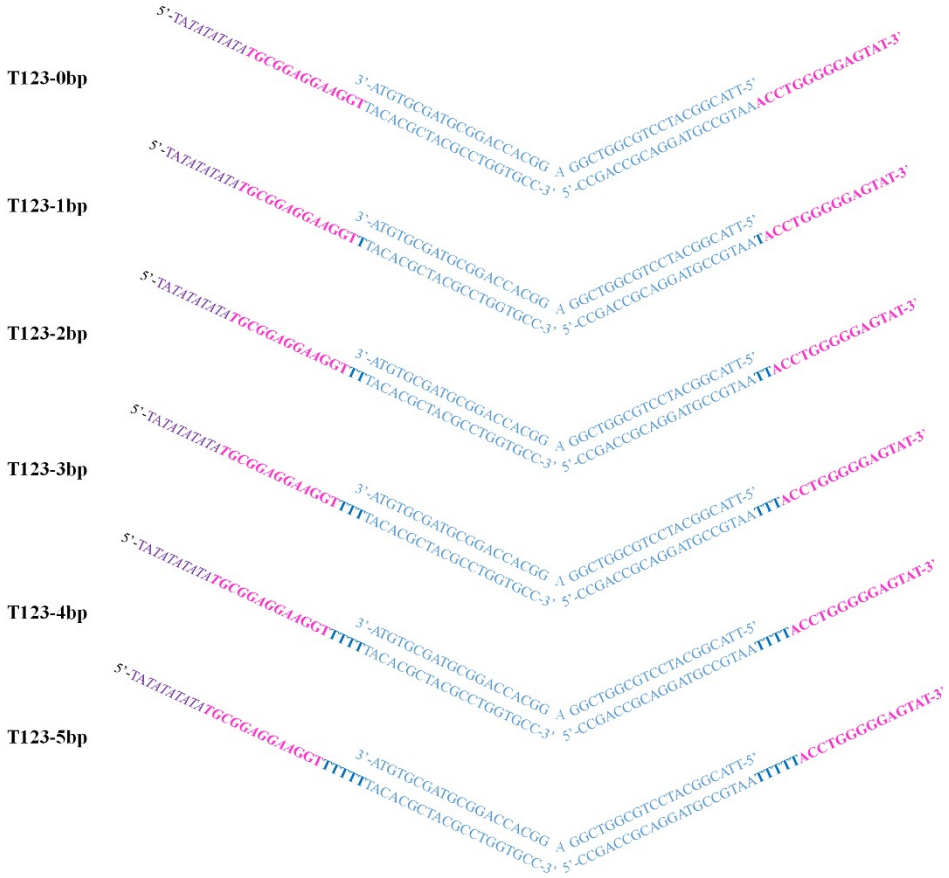

Fig. S4. The DNA sequences of the nanotweezers.

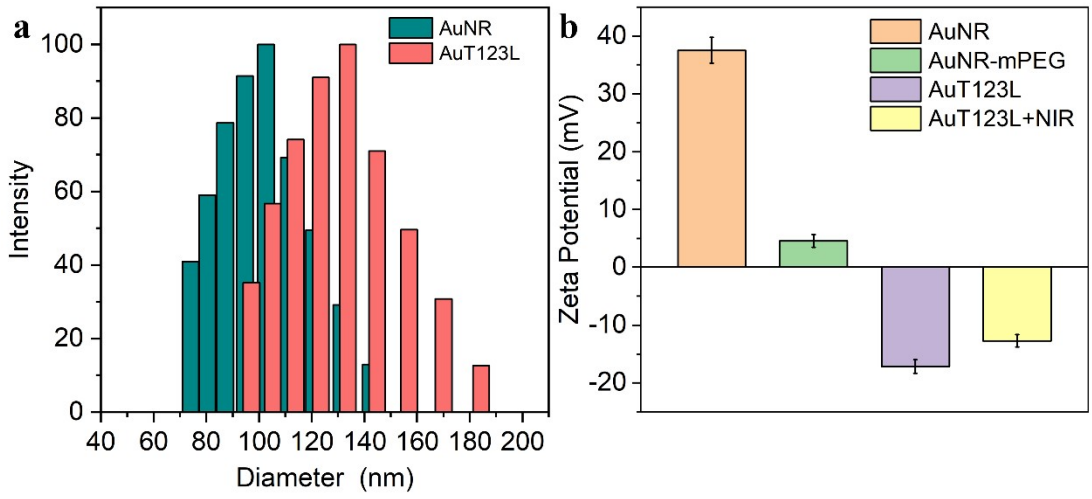

Fig. S5. a) Hydrodynamic diameters distribution of AuNR (green) and AuT123L (red). b) Zeta potentials of AuNR, AuNR-mPEG, AuT123L and AuT123L + NIR.

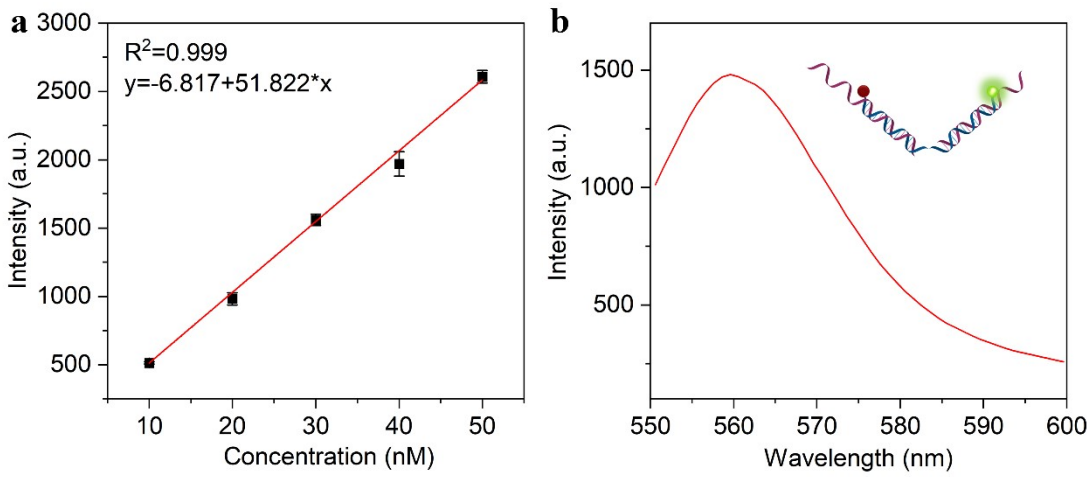

Fig. S6. Fluorescence based method was used to calculate the amount of T123L loaded on AuNR. a) Calibration curve of fluorescence intensity of Cy3 with T123L concentration. b) Fluorescence emission spectra of T123L released by DTT treated AuT123L. (AuNRs concentration was 0.31 nM).

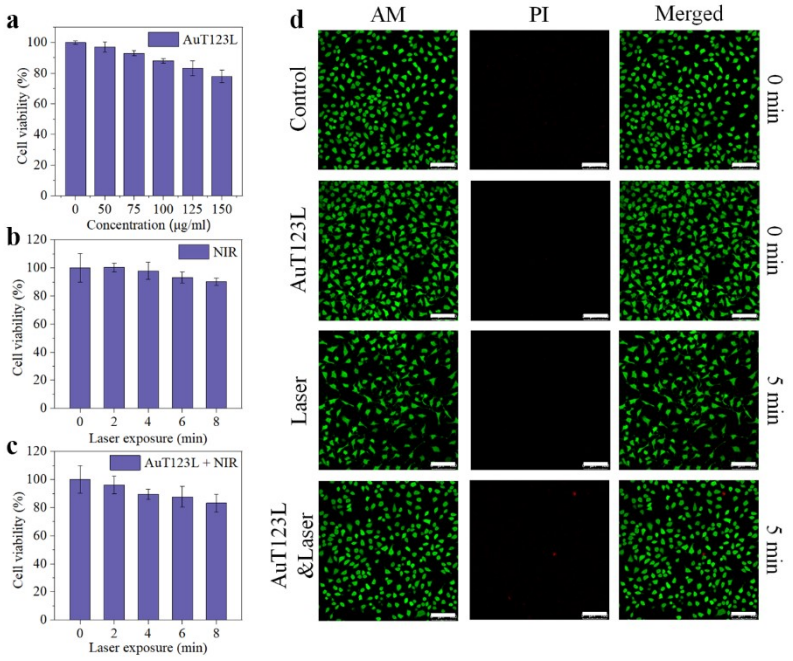

Fig. S7. Cytotoxicity and phototoxicity of the AuT123L nanoprobe. a) Cell viabilities after incubation of Hela cells with the AuT123L nanoprobe at various concentrations for 24 h. b) Cell viabilities of Hela cells irradiated for different periods of time by NIR laser. c) Cell viabilities of Hela cells after incubation with the AuT123L nanoprobe induced PTT for different lengths of time. d) Fluorescence images of calcein AM/PI-costained Hela cells incubated with the AuT123L nanoprobe and treated with and without the NIR laser. Scale bar: 100 μm.

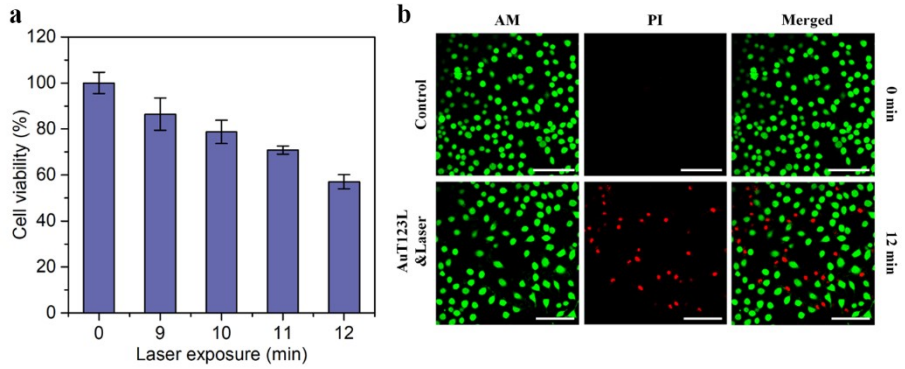

Fig. S8. Cytotoxicity and phototoxicity of the AuT123L nanoprobe. a) Cell viabilities of Hela cells after incubation with the AuT123L nanoprobe induced PTT for different lengths of time. b) Fluorescence images of calcein AM/PI-costained Hela cells incubated with the AuT123L nanoprobe with and without the NIR laser.

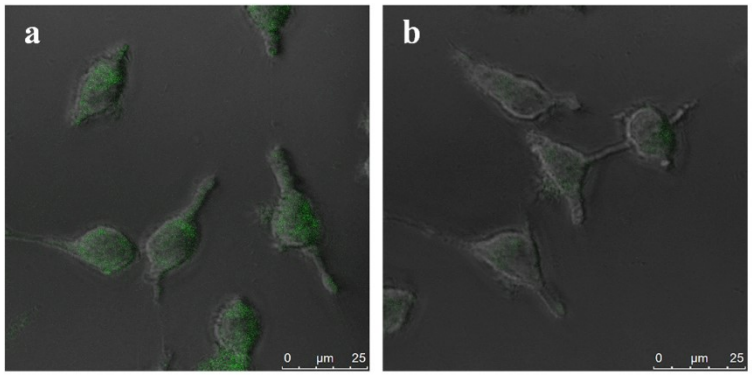

Fig. S9. Confocal microscopy images of Hela cells after incubation with AuT123L at a) 37 °C and b) 4 °C

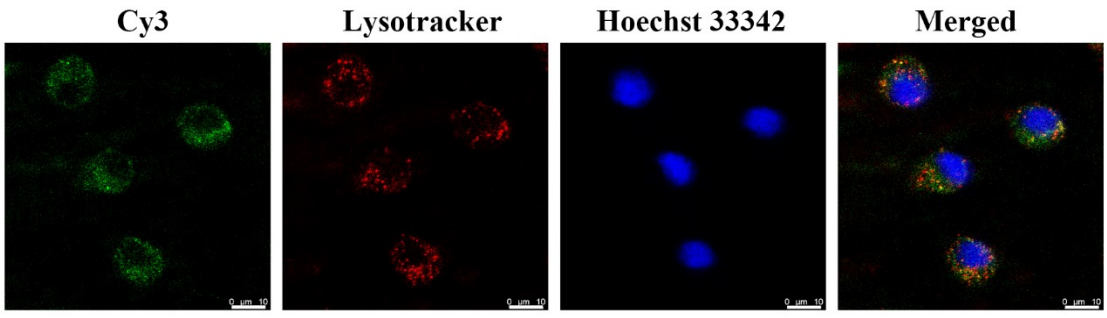

Fig. S10. Confocal microscopy images of HeLa cells pretreated with AuT123L (only Cy3 was modified) and further stained with Lyso-Tracker Red. The green fluorescence of Cy3 showed the location of AuT123L, and the red fluorescence of Lysotracker showed the location of acidic organelles such as lysosome and endosome. Scale bar: 10 μm.

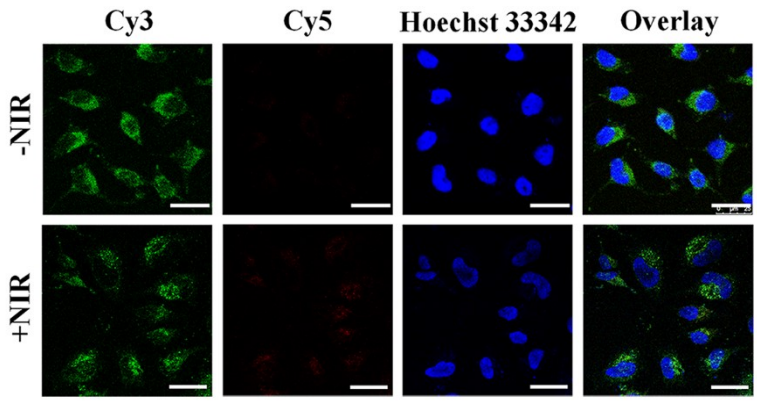

Fig. S11. Confocal microscopy images of Hela cells treated with AuT123L (top) and AuT123L + NIR (bottom); the excitation wavelength was 488 nm, and the emissions were collected in the range of 525 − 600 nm (first channel, Cy3) and 640 − 720 nm (second channel, Cy5), respectively. Scale bar: 25 μm.

3. Reference

1 You, Q.; Zhang, K.; Liu, J.; Liu, C.; Wang, H.; Wang, M.; Ye, S.; Gao, H.; Lv, L.; Wang, C.; Zhu, L.; Yang, Y. *Adv. Sci.*, 2020, **7**, 1903341.