Electronic Supplementary Information (ESI)

Thermal-activated nanocarriers for the manipulation of cellular uptake and photothermal therapy on command

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Experimental Section

Chemicals and Materials: Hydrogen tetrachloroaurate trihydrate ($HAuCl_4$), cobalt chloride ($CoCl_2$), trisodium citrate, L-ascorbic acid, sodium borohydride ($NaBH_4$), sodium azide (NaN_3), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 95%), sodium iodide (NaI), copper(II) sulfate ($CuSO_4$), N-isopropylacrylamide (NIPAAm, 99%), acrylamide (AAm, 99%), copper(I) bromide (CuBr), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA), tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The disulfide-containing initiator, bis (2-hydroxyethyl) disulfide bis (2-bromo propionate) ($BHEDS(BP)_2$) was synthesized according to the literature (see Ref. 35 of the main text). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sunshine Biotech. Co. Ltd. (Nanjing, China). mPEG -Alkyne (MW=2000) was purchased from Shanghai Yare Biotech, Inc. (Shanghai, China). Phosphate buffer saline (PBS, 10 mM, pH=7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 1.4 mM KH_2PO_4 and 8.7 mM Na_2HPO_4. Tris-HCl (20 mM, pH=7.6) containing 146.0 mM NaCl, 5.0 mM KCl, 5.0 mM $MgCl_2$ was used in the AS1411 dissolution. All aqueous solutions were prepared using DEPC treated ultrapure water from a Milli-Q system (Millipore, USA). AS1411 strands were obtained from Sangon Biological (Shanghai) Co. Ltd. (Shanghai, China).

The sequences of AS1411 (from 5' to 3'): SH-C6TTT TTT TTG GTG GTG GTG GTT GTG GTG GTG GTG G-FAM.

Apparatus and Characterization: UV-vis spectra were determined using a UV-3600 spectrophotometer (Shimadzu, Japan). Fluorescence spectra for quantification of the aptamer loading were recorded on a RF-5301PC fluorescence spectrometer. MTT assay was recorded at 570 nm using a Varioskan Flash (Thermo Scientific, USA). Fourier transform infrared (FT-IR) spectra were collected on a Nicolet 6700 FT-IR spectrometer. Dynamic light scattering (DLS) measurements were performed on a 90 Plus Nanoparticle Size Analyzer (Brookhaven, USA). Zeta potential was tested on a nano-zeta potential analyzer (Malvern Instruments, USA). Gel permeation chromatography (GPC) analysis was recorded on a Waters 244 (Milford, MA, USA) with THF as eluent. NMR spectra were determined on a Bruker DPX 300 MHz spectrometer with internal standard tetramethylsilane (TMS) and solvent signals as internal references; the chemical shifts (δ) were expressed in ppm and J values were given in Hz. Confocal laser scanning microscopy (CLSM) images were obtained using a Leica TCS SP5 confocal microscope (Germany). Transmission electron microscopy (TEM) images were performed using a JEM 200CX transmission electron microscope (JEOL, Japan) with an accelerating voltage of 200 kV. HRTEM images were carried out on a JEOL-2100 transmission electron microscope (JEOL, Japan). A diode laser (LE-LS-795-TFC) with wavelength of 795 nm was used in all the laser irradiation experiments.

Synthesis of hollow gold nanoshells (HGNSs): Hollow gold nanoshells (HGNSs) were synthesized according to the method published by Schwartzberg et al.[1] Briefly, cobalt nanoparticles were first synthesized by adding 100 μL of 0.4 mol L^{-1} $CoCl_2$ and 400 μL of 0.1 mol L^{-1} sodium citrate to 100 mL of pre-deoxygenated deionized water, and the solution was degassed by bubbling with argon for 20 min. Upon the addition of 400 μL of 0.25 mol L^{-1} $NaBH_4$ to that deaerated solution, the pale pink solution turned brown, indicating the formation of cobalt nanoparticles. The solution was allowed to react for ~ 40 min under constant nitrogen flow at room temperature until the complete hydrolysis of the $NaBH_4$. Thereafter, 30 mL of the cobalt nanoparticles solution was added into 10 mL deionized water containing 15 μL of 0.1 mol L^{-1} $HAuCl_4$, resulting in reduction of Au ions onto the surface of cobalt nanoparticles and the oxidation of cobalt. Then the remaining cobalt core was further oxidized by air. The solution was centrifuged at 8500 rpm for 10 min, obtaining the final product HGNSs. The

resonance wavelength of the HGNSs was measured using UV-vis spectroscopy. Particle size was determined using both transmission electron microscopy (TEM) and dynamic light scattering (DLS).

Synthesis of pNIPAAm-co-pAAm copolymer conjugated with PEG ($P39_{PEG}$): pNIPAAm-co-pAAm copolymer was synthesized via atom transfer radical polymerization according to the published protocol.[2] The following reagents were mixed in a Schlenk flask: NIPAAm (3.56 g), AAM (0.32 g), PMDETA (70 μL), BHEDS $(BP)_2$ (0.03 g), deionized water (36 mL) and methanol (24 mL). After the solution was degassed by freeze–pump–thaw cycles for three times, the flask was filled with argon and then CuBr (0.020 g) was quickly added to the frozen mixture. Before the mixture was melt at room temperature, the flask was evacuated and back-filled with argon three times again. The reaction solution was magnetically stirred overnight at room temperature. After evaporation of the solvent, the crude product was dissolved in water and purified by dialysis, obtaining the copolymer with LCST at 39 °C (P39).

Then, P39 (0.2 g), NaN_3 (0.10 g) and NaI (0.10 g) were dissolved in 10 mL of DMF. After the mixture was stirred at room temperature for 24 h, ether was added into the solution to precipitate the copolymer. Copolymer was separated by centrifugation and washed by ether three times. The crude products was dissolved in water and purified by dialysis to obtain the P39-N_3.

mPEG-Alkyne was conjugated with P39-N_3 *via* copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC).[3] First, P39-N_3 (40 mg) was dissolved in H_2O (2 mL) and then mixed with a solution of mPEG-Alkyne (100 μL, 1 mg mL^{-1}). After the solution was degassed for 10 min and filled with argon, $CuSO_4$ (200 μL, 7 mg mL^{-1}), sodium ascorbate (500 μL, 7 mg mL^{-1}), THPTA (500μL, 7 mg mL^{-1}) were injected into the mixture. Subsequently, the reaction solution was magnetically stirred at room temperature for 48 h. The copolymer was dialyzed to remove the unreacted molecules and PEG terminated copolymer $P39_{PEG}$ was obtained.

Conjugation of AS1411 and $P39_{PEG}$ on HGNSs (denoted as PEG/apt-HGNSs): For PEG/apt-HGNSs preparation, a thiol-ended and FAM-labeled AS1411 (5'SH C6-TTTTTTTTGGTGGTGGTGGTTGTGGTGGTGGTGG-FAM 3') was deprotected by TCEP at first. Briefly, 20 μL of 20 mmol L^{-1} TCEP was added to 200 μL of 10 μmol L^{-1} AS1411 solution and shaken

for 3 h at room temperature. Then, 45 μL of deprotected AS1411, 68 μL of 20 μmol L^{-1} $P39_{PEG}$ solutions and 100 μL 5×TBE were added to 500 μL of HGNSs (3.75×10^{11} particles mL^{-1}). Then the mixture was gently shaken overnight at a speed of 150 rpm. In order to increase the surface concentration of aptamers on HGNSs, we salted the mixture solution with 10 μL of 5 mol L^{-1} solution of NaCl for ten times.[4] The HGNSs were aged overnight to achieve maximum aptamer loading. To remove the unattached aptamer and polymers, the solution was centrifuged and washed with deionized water for three times to obtain PEG/apt-HGNSs. In order to estimate the number of aptamers attached to the HGNSs, fluorescence intensity of the FAM-labeled aptamers in the solution before and after the conjugation was measured. The fluorescence of the supernatant was measured to determine the amount of aptamers unreacted with the HGNSs. On the basis of above results, it was calculated that approximate 475 AS1411 strands were functionalized on a single nanoparticle.

Measurement of photothermal performance: Different concentrations of PEG/apt-HGNSs nanoparticles were added into a 96-well plate. 795 nm stabilized CV laser with a beam diameter approximately 6 mm (LEO Photonics) was used to irradiate the solution for 8 min. The temperature change of the solution was measured with a TM902C thermodetector once half a minute.

Cell culture: Human cervical carcinoma cell (HeLa), human breast cancer cell (MCF-7), human hepatocellular liver carcinoma cell (HepG2), normal human liver cell (L-O2) and the mouse fibrosis cell (NIH 3T3) were obtained from the Institute of Cell Biology at the Chinese Academy of Sciences (Shanghai, P. R. China) and cultured in complete DMEM (Nanjing KeyGen Biotech Co. Ltd.) containing L-glutamine (2 mM), penicillin (80 units mL^{-1}), streptomycin (0.08 mg mL^{-1}) and 10 % fetal bovine serum (FBS) at 37 ºC under 5% CO_2 atmosphere. At the logarithmic growth phase, the cells were incubated with different nanoparticles in cultured medium.

Cytotoxicity of PEG-HGNSs and PEG/apt-HGNSs: A total of 5000 cells were plated into 96-well plate at 37 °C with 5% CO_2 in complete medium and incubated for 24 h. Then the cells were cultured with new medium including free HGNSs, PEG-HGNSs, apt-HGNSs or PEG/apt-HGNSs with various concentrations for another 24 h. Cytotoxicity of nanoparticles was measured by a standard MTT assay. The medium was removed and fresh medium (100 μL) containing MTT (10 μL, 5 mg mL^{-1}) was added

into each well. After 4 h of incubation, the medium was replaced with DMSO (100 μL). To assess the relative viability of the cells, absorbance intensity of the solution at 570 nm was determined with Varioskan Flash (Thermo Scientific). Relative cell viability was expressed as: ([OD] test/ [OD] control) × 100% and each group was repeated for four times.

Confocal images of HeLa cells after incubated with FAM-labeled nanocarriers: HeLa cells and NIH 3T3 cells were seeded onto 20 mm confocal dishes (25000 cells per well) and incubated for 24 h. After removal of the medium, 0.5 mL of medium containing 50 μg/mL PEG/apt-HGNSs were added in which the aptamers were labeled with FAM dye. Then cells were irradiated with NIR laser (2 W cm^{-2}, 15 min) and incubated for a further 2 h. For the control, cells were not irradiated with NIR laser. The cells were washed three times with PBS buffer and the confocal images were captured using a Leica TCS SP5 microscope.

***In vitro* photothermal-controlled cellular uptake experiments:** HeLa cells were seeded into 48-well plate (20000 cells per well) in DMEM medium and grown for 24 h. After removal of the medium, PEG-HGNSs (with or without AS1411) suspended in DMEM (200 μL, 50 μg mL^{-1}) was added to each well and incubated for 2 h. Some cell groups were irradiated (CW laser, 795 nm at 2.0 W cm^{-2}) for 15 min (5 min, three times), while the others were kept in the dark as a control. After incubation at 37 °C for 2 h, the medium was removed and the cells were gently washed with PBS buffer three times to remove unbound HGNSs. Then, cells were trypsinized, collected and treated with 200 μL of new aqua regia overnight to dissolve the cells and HGNSs for ICP-MS analysis.

***In vitro* photothermal therapy of tumor cells:** Cells were seeded into 96-well plate (5000 cells per well) in DMEM medium and grown for 24 h. After removal of the medium, PEG/apt-HGNSs was added to each well and incubated for 2 h. Some cell groups were irradiated (2.0 W cm^{-2}, 15 min) for photothermal-controlled cellular uptake and the others without laser irradiation. After incubation for another 2 h, all wells were gently washed with PBS buffer and replenished with fresh medium. Thereafter, NIR laser light with an output power of 3.1 W cm^{-2} was used to irradiate all wells for 5 min and further incubated for 24 h. Cell viability was measured using the MTT assay as the above process.

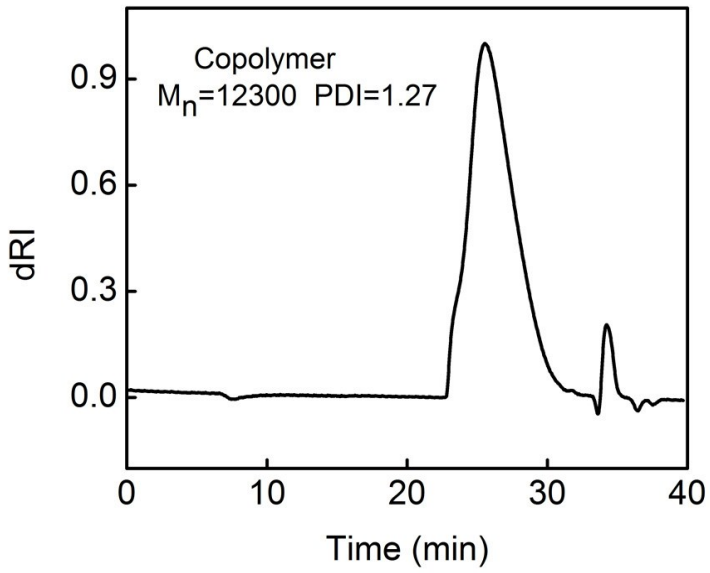


Fig. S1 Molecular weight of pNIPAAm-co-AAm measured by gel permeation chromatography (GPC) with THF as eluent.

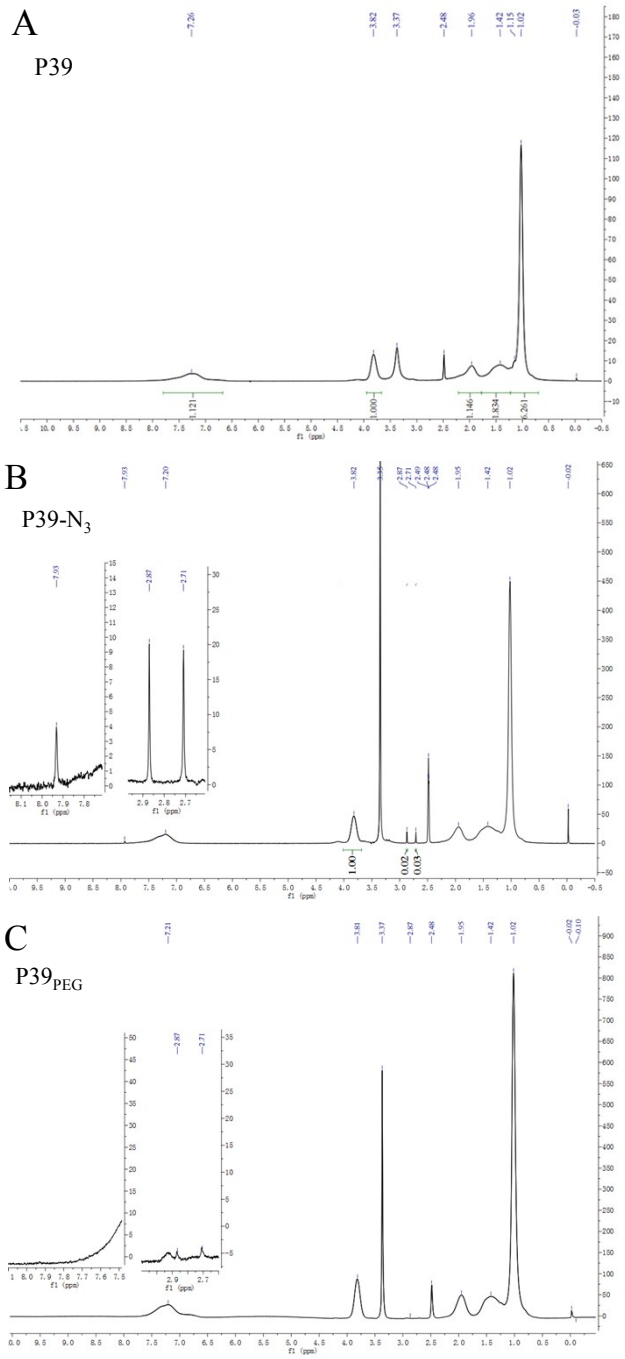


Fig. S2 ^{1}H NMR spectra of P39, P39-N_3, $P39_{PEG}$.

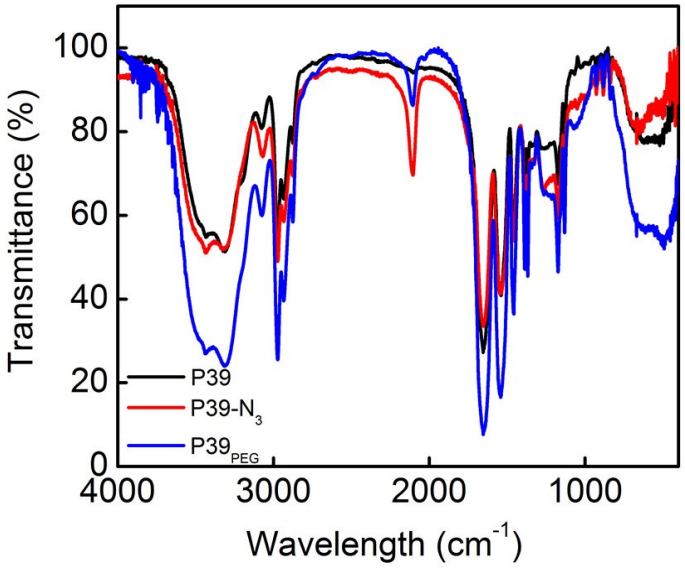


Fig. S3 FTIR spectra of P39, P39-N_3, $P39_{PEG}$.

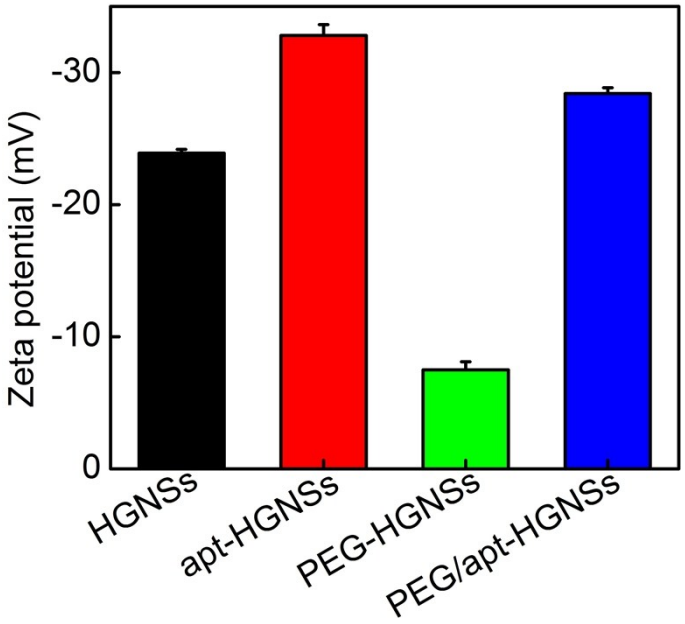


Fig. S4 The variations of zeta potential values during the preparation of the nanocarriers.

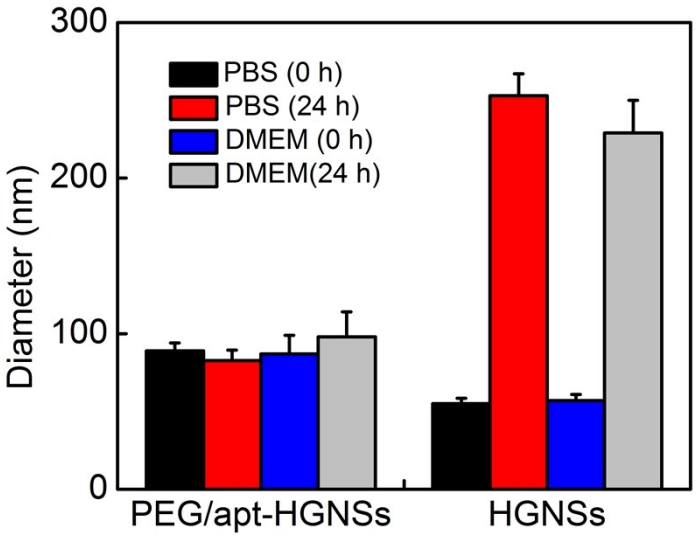
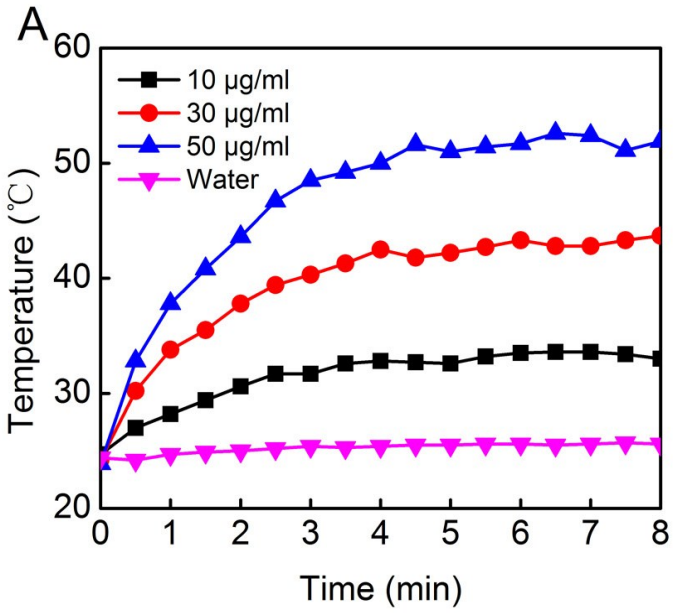
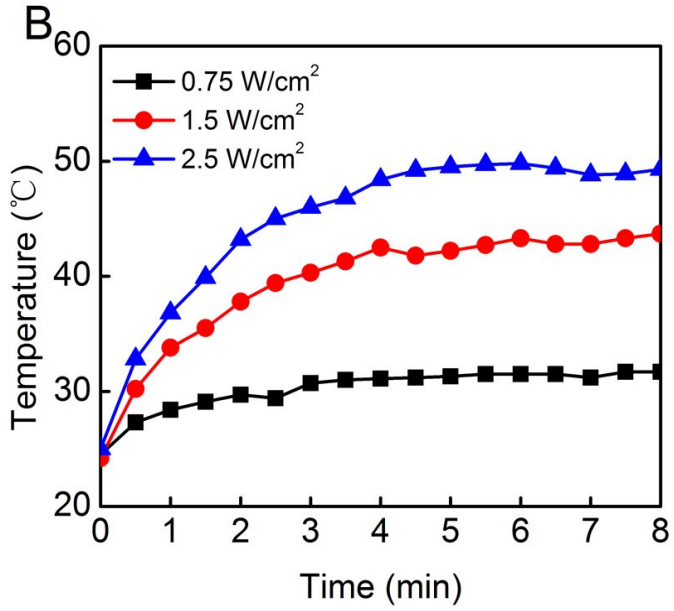


Fig. S5 The long-term stability of PEG/apt-HGNSs and HGNSs in PBS and DMEM cultured medium. The DLS measurements were performed after the solutions were shaken at a speed of 100 rpm for 0 h and 24 h.


A
10 μg/ml
30 μg/ml
50 μg/ml
Water
Temperature (℃)
Time (min)
20
30
40
50
60
0
1
2
3
4
5
6
7
8


B
0.75 W/cm2
1.5 W/cm2
2.5 W/cm2
Temperature (℃)
Time (min)
20
30
40
50
60
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1
2
3
4
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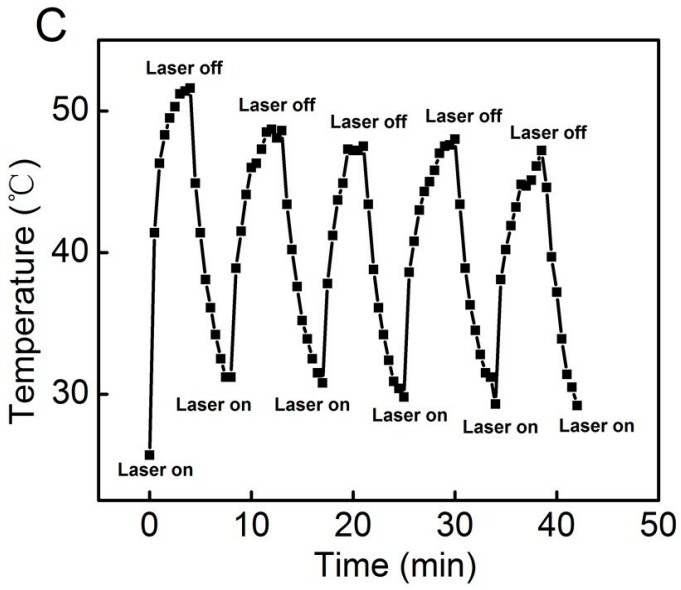

Fig. S6 (A) Phtotothermal behaviors of distilled water and PEG/apt-HGNSs solutions with different concentrations under laser irradiation at 1.5 W cm^{-2}. (B) Photothermal behaviors of PEG/apt-HGNSs solutions (30 μg mL^{-1}) exposed to 795 nm laser at different power densities. (C) The temperature changes of PEG/apt-HGNSs solution (50 μg mL^{-1}) after repeated laser irradiation (1.5 W cm^{-2}, 4 min), and natural cooling.

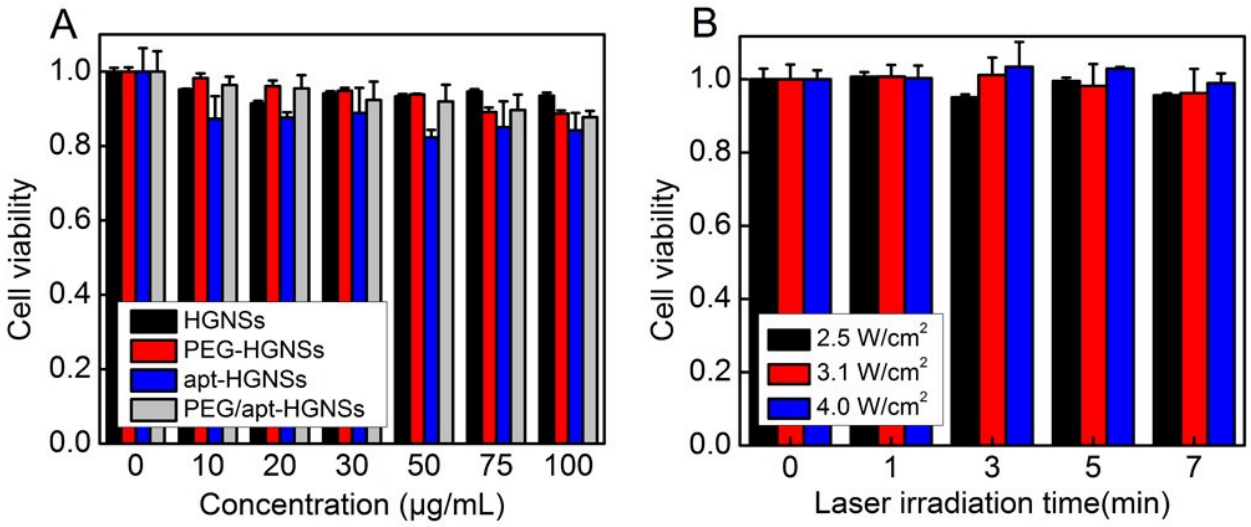

Fig. S7 (A) Cell viability of HeLa cells after incubated with different concentration of HGNSs, PEG-HGNSs, apt-HGNSs and PEG/apt-HGNSs for 24 h. (B) Cell viability for HeLa cells after irradiated at different laser power densities.

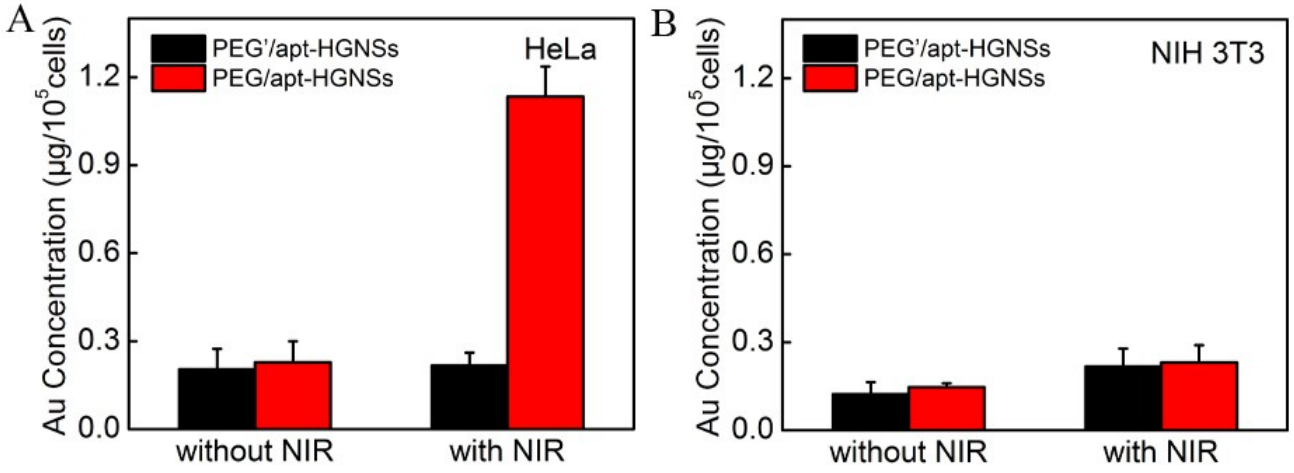

Fig. S8 Cellular uptake of PEG'/apt-HGNSs and PEG/apt-HGNSs by HeLa cells (A) and NIH 3T3 cells (B) with and without laser irradiation. In the group of PEG'/apt-HGNSs, the $P39_{PEG}$ was replaced by thiol-tailed PEG with a molecule weight of 10 kDa whose length had no response to temperature variation, which completely blocked the interaction of aptamer with its target receptor.

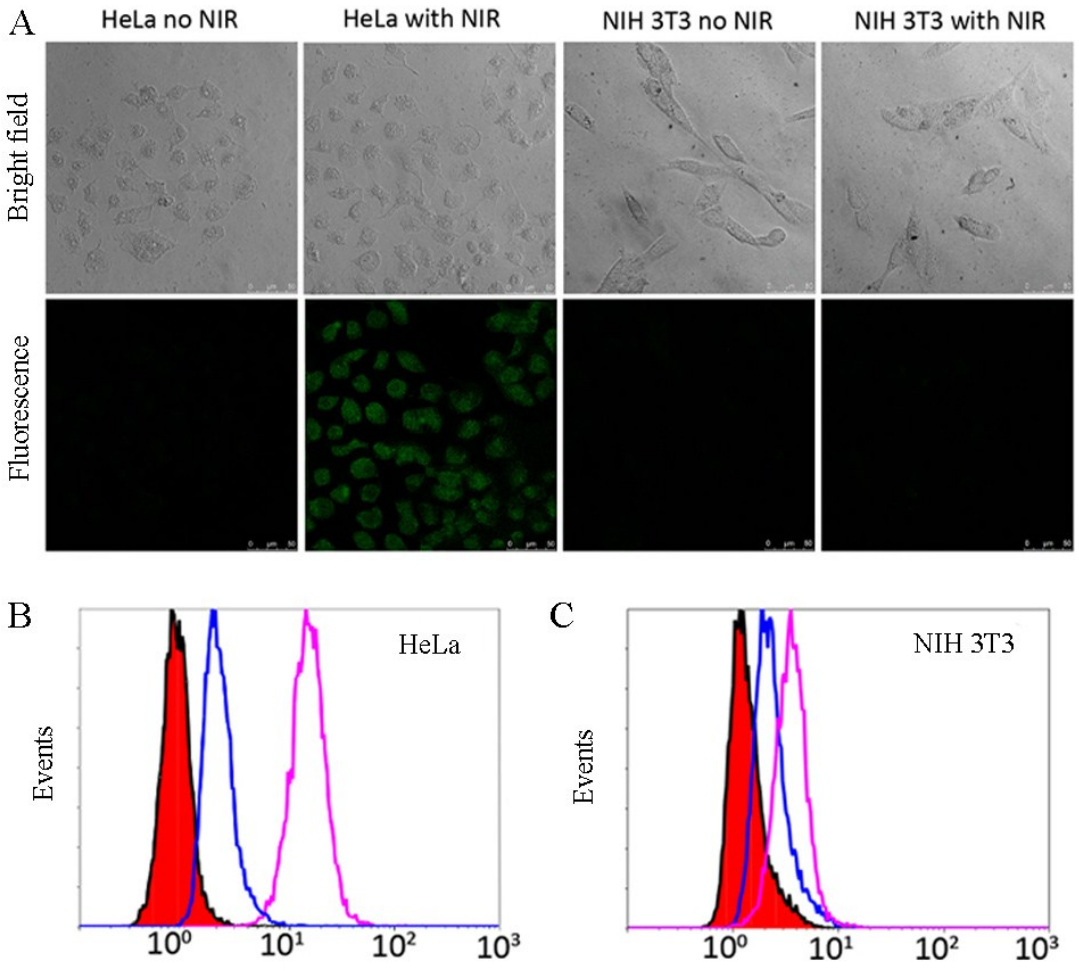

Fig. S9 (A) CLSM images of HeLa cells and NIH 3T3 cells incubated with FAM-labeled PEG/apt-HGNSs with and without treatment by 795 nm laser. Flow cytometry results of HeLa cells (B) and NIH 3T3 cells (C) after incubation with FAM-labeled PEG/apt-HGNSs with (pink line) and without (blue line) treatment by 795 nm laser. The black line indicated the control group. From the confocal and cytometric results, the fluorescence signal of FAM-labeled nanoparticles (green) in HeLa cells was higher with laser irradiation than that without laser irradiation, but there was no change in NIH cells. The increase in FAM signal intensity suggested that more PEG/apt-HGNSs were internalized to HeLa cells under NIR irradiation.

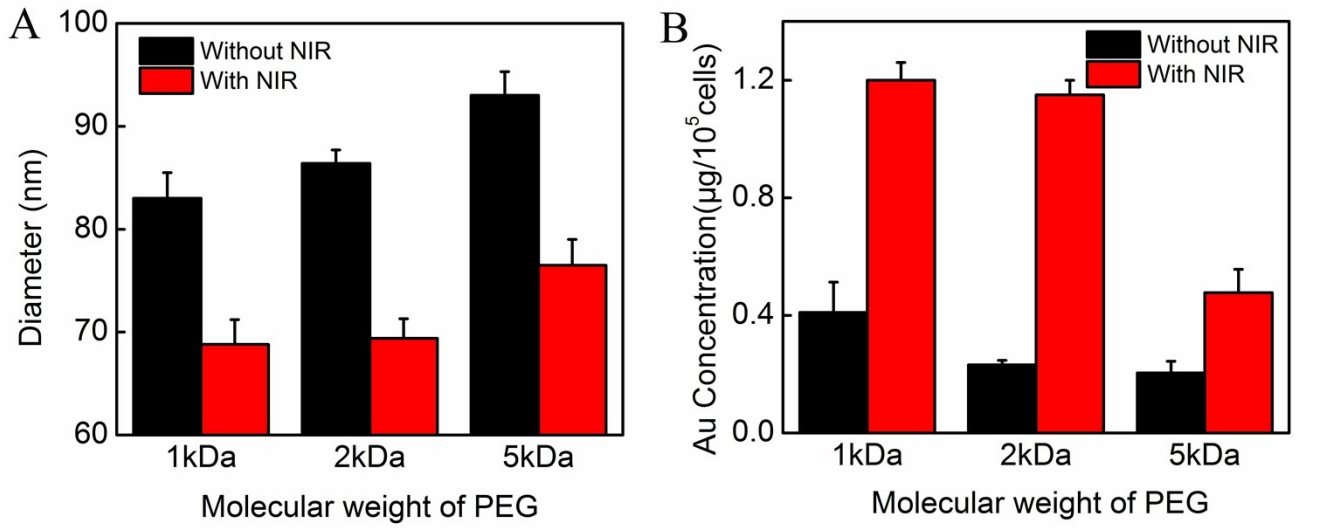

Fig. S10 (A) Hydrodynamic diameter and (B) cellular uptake of PEG/apt-HGNSs with different molecular weight of PEG tail. When the length of PEG tail increased to 5 kDa, the diameter of the nanoparticles under NIR irradiation reduced to 74.5 nm, which was larger than that of apt-HGNSs (68.4 nm), indicating that the aptamers were encapsulated in the PEG corona, thus leading to the drastic decrease in cellular uptake in (B).

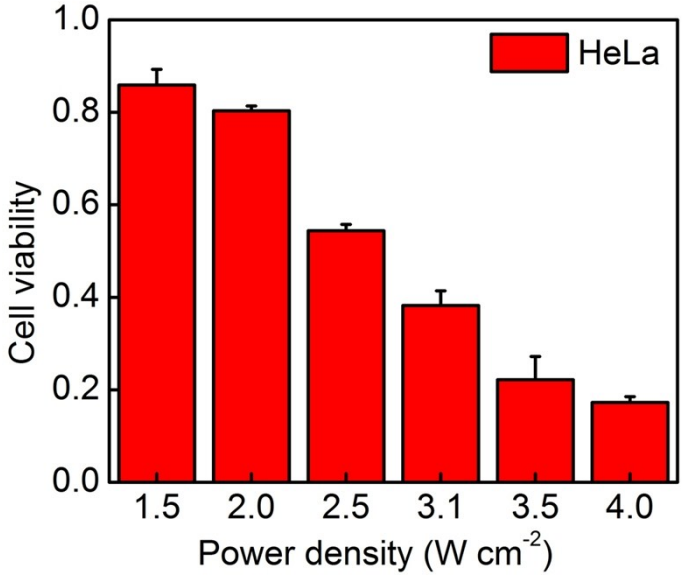

Fig. S11 Cell viability of HeLa cells under the NIR irradiation with different power densities. The cells were first incubated with nanocarriers under NIR irradiation (795 nm, 2.0 W cm^{-2}) for 15 min to enhance the cellular uptake; after incubation for 2 h, the cells were cultured in fresh medium for

another 2 h and then exposed under the NIR laser at different power densities for 5 min. After 24 h, MTT assay were performed to assess the cell viability.

Reference:

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