Supporting Information

In-situ fluorescent monitoring of diagnosis and treatment: a versatile nanoprobe combining tumor targeting based on MUC1 and controllable DOX release by telomerase

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Experimental Section

Materials and Reagents. Oligonucleotides Mucin 1 aptamer （5'-HS-$(CH2)_6$-ACA CGG CAG TTG ATC CTT TGG ATA CCC TGG CGT GT-Alexa Fluor 405-3'）were synthesized and HPLC purified by TAKARA Biotechnology Co., Ltd. (Dalian, China). The longer sequence of molecular beacon （5'-FAM-CGT TGC $(AAA)_7$ GCA ACG AAC TCT GCT CGA CGG ATT-SH-3'） and telomerase primer（5'-AAT CCG TCG AGC AGA GTT-3'）were synthesized and HPLC purified by Sangon Biotech Co., Ltd. (Shanghai, China). (4, 5-dimethylthiazol-2-yl)-2-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Company. Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), Trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), Sodium dodecyl sulfate (SDS), NaCl were purchased from China National Pharmaceutical Group Corporation (Shanghai, China). Telomerase kit and Mucin 1 (MUC1) ELISA Kit was purchased from Elabscience Biotechnology Co., Ltd (Wuhan, China). The human cervical cancer cell lines Hela and human hepatocyte cell line HL-7702 was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cell culture products, unless mentioned otherwise, were purchased from GIBCO. All the chemicals were of analytical grade and used without further purification. Deionized water was obtained through a Sartorius Arium 611 VF system (Sartorius AG, Germany) to a resistivity of 18.2 MΩ·cm.

Instrumentation. The transmission electron microscopy (TEM) was carried out on a Hitachi HT7700 transmission electron microscope (JEOL Ltd., Japan). The UV-vis absorption spectra were obtained with a Hitachi U-3010 UV-vis spectrophotometer (JEOL Ltd., Japan). The fluorescent spectra were measured using Cary Eclipse fluorescence spectrophotometer (Varian, U.S.A.). Absorbance was measured in a RT-6000 microplate reader (Rayto, U.S.A.) in the MTT assay. Confocal fluorescence imaging studies were performed with a LEICATCS SP8 confocal laser scanning microscopy (Leica Co. Ltd., Germany) with an objective lens (60×).

Synthesis of Au NPs. The AuNPs were prepared by the classical citrate reduction route. All the glassware was first cleaned with a mixture of HCl and HNO_3 (ratio of HCl/HNO_3 = 3:1 in volume) and thoroughly rinsed with ultrapure water. Then 100 mL of 0.01% aqueous chlorauric acid solution was brought to a boil, and 3.5 mL of 1% sodium citrate solution was added quickly, which resulted in a change in solution color from pale yellow to deep red. The solution was further boiled for 15 min and then left to cool to room temperature while stirring. Transmission

electron microscopy (TEM) images indicated the particle sizes are ~13 nm. The prepared AuNPs were stored at 4 °C.

Preparation of the drug-loaded telomerase probes. The FAM-labeled molecular beacons were mixed with an equal amount of telomerase primers and annealed in 95°C water bath for 3 minutes. After cooling at room temperature for 2 hours, the single-stranded DNA was completely folded to form a hairpin shape, and the hairpin DNA was able to fully hybridize with the telomerase primer through base pairing. The obtained telomerase probes was mixed with a certain amount of DOX and shaken overnight at room temperature so that DOX could be fully inserted into the telomerase probe. The solution was repeatedly centrifuged using an ultrafiltration tube (5000 rpm for 10 minutes) to remove the un-embedded DOX, resulting in a pure drug-loaded telomerase probes.

Quantitation of Dox loaded on the drug-loaded telomerase probes. Different concentrations of telomerase probes (0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14 μM) were mixed with 0.6 μM DOX at room temperature overnight to ensure that DOX was fully embedded in the probes. When the concentration of the telomerase probes is greater than 0.12 μM, the fluorescence intensity no longer changes, indicating that 0.6 μM DOX is completely loaded into 0.12 μM telomerase probes. So we calculated the drug loading of each telomerase probe by completely loading 0.6 μM DOX with 1.2 μM telomerase probes and the drug loading amount of each telomerase probe is 5.

Fabrication of the nanoprobes or drug-loaded nanoprobes. AuNPs were modified with nucleic acid probes (telomerase probes/drug-loaded telomerase probes and MUC1 aptamers) according to published protocols with slight modifications. Briefly, the MUC1 aptamers and drug-loaded telomerase probes were uniformly mixed in a ratio of 1:2 and then added to the AuNPs (3nM) solution and stirred overnight. A mass fraction of (10%) sodium dodecyl sulfate (SDS) was added to the above solution to a final mass fraction of 0.1%. Phosphate buffer (NaH_2PO_4/Na_2HPO_4, 0.1 M, pH 7.4) was then added to a final concentration of 0.01 M. During the subsequent salt aging, 2 M NaCl solution was added dropwise to the mixed solution over 8 hours to give a final concentration of 0.1 M, and the resulting solution was further stirred at room temperature for 48 hours. The reaction solution was centrifuged at high speed (13000 rpm for 15 minutes) three times and the final product was resuspended in phosphate buffered PBS and stored in a refrigerator at 4°C. The concentration of the nanoprobes was determined by the quenching extinction coefficient of the gold nanoparticles of this size at 524 nm ($\varepsilon=2.7\times10^8$ L $mol^{-1}cm^{-1}$).

Quantitation of telomerase probes modified on the drug-loaded nanoprobes. The telomerase probes loaded on each AuNP was derived from the fluorescence intensity of the FAM fluorophore of telomerase probes loaded on AuNPs that were competed by mercaptoethanol (ME). The fluorescence standard curve of the FAM fluorophores was obtained by measuring the fluorescence intensity of a known concentration of FAM-labeled telomerase probes under the same buffer, ionic strength and the same concentration of ME conditions (Figure S4). We then converted the fluorescence intensity of the competing telomerase probes to the corresponding molarity via a standard curve (Figure S5). The calculation shows that each AuNP is loaded with 35 ± 2 telomerase probes.

Quantitation of the amount of drugs released by the drug-loaded nanoprobes. 6 nM drug-loaded nanoprobes were reacted with 5 ng/mL telomerase solution for different time (0 min-130 min). After the reaction, the solution was centrifuged (13000 rpm for 15 min) to measure the fluorescence intensity of DOX in the supernatant. The amount of drugs released by the drug-loaded nanoprobes was calculated from the intensity of the emitted light at λ_{max} = 590 nm in the supernatant and the release efficiency is the ratio of DOX release to DOX loading (DOX's standard fluorescence curve is shown in Figure S3).

Fluorescence Measurements. Fluorescence measurements were conducted on a Cary Eclipse fluorescence spectrophotometer. In a typical experiment, a certain concentration of probes (nanoprobes or drug-loaded nanoprobes) was added into the MUC1, telomerase or the mixed solution. After reaction at 37℃for different times, the solution was centrifuged (13000 rpm for 15 min) and resuspended in phosphate buffered saline (PBS) for fluorescence measurements. The fluorescence of Alexa Fluor 405 was collected between 415 and 500 nm by use of the maximal excitation wavelength at 405 nm, the fluorescence of FAM was collected between 500 and 650 nm by use of the maximal excitation wavelength at 488 nm, and the fluorescence of DOX was collected between 500 and 750 nm by use of the maximal excitation wavelength at 488 nm.

Cell Culture. Hela cells and HL-7702 cells were cultured at 37℃in a humidified atmosphere of 5% CO2 and 95% air using DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were passaged every 2−3 days.

Laser Scanning Confocal Microscopy Imaging. Cell lines (HeLa cells and HL-7702 cells) of 1×10^6 mL^{-1} were seeded in each confocal dish, and nanoprobes or drug-loaded nanoprobes was then added into each dish. After incubation at 37℃ for different times, the cells were sent for fluorescent confocal imaging. Fluorescence images were acquired on a CLSM with different laser transmitters. The fluorescence of Alexa Fluor 405 was collected between 415 and 480 nm by use of the maximal excitation wavelength at 405 nm, the fluorescence of FAM was collected between 500 and 540 nm by use of the maximal excitation wavelength at 488 nm, and the fluorescence of DOX was collected between 575 and 650 nm by use of the maximal excitation wavelength at 488 nm.

MTT Assays. Hela cells and HL-7702 cells (1×10^6 cells/well) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO2/95% air incubator for 24 h. After the original medium was removed, Hela cells and HL-7702 cells were incubated with nanoprobes (or drug-loaded nanoprobes) with different concentration. After 5 h, washed with PBS and fresh media (10% FBS) added for further cell growth (24 h). Then, 100 μL of MTT solution (0.5 mg/mL in PBS) was added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm with a RT 6000 microplate reader.

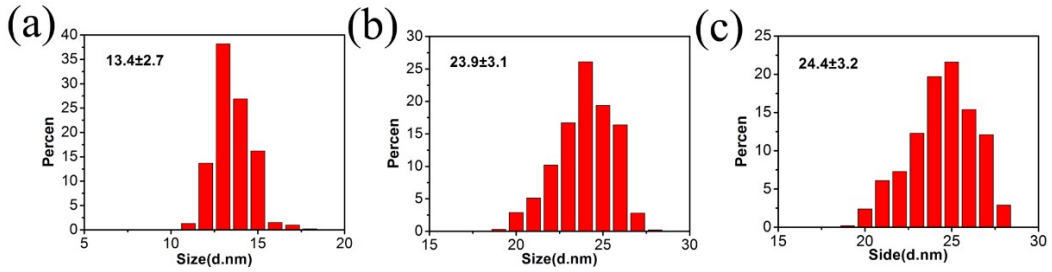

Figure S1. The characterization of versatile nanoprobes. DLS characterization of the prepared AuNPs(a), nanoprobes (b), drug-loaded nanoprobes (c).

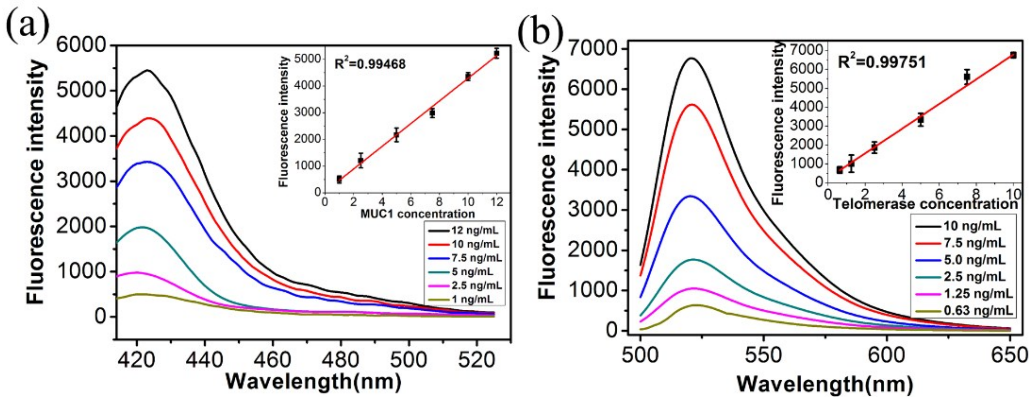

Figure S2. (a) Fluorescence spectra of the nanoprobes in the presence of various concentrations of MUC1 (1, 2.5, 5, 7.5, 10, 12 ng/mL). Inset: Standard curve for detection of MUC1 in solution with the MUC1 ELISA kit; (b) Fluorescence spectra of the nanoprobes in the presence of various concentrations of telomerase (0.63, 1.25, 2.5, 5, 7.5, 10 ng/mL). Inset: Standard curve for detection of telomerase activity in solution with the telomerase ELISA kit.

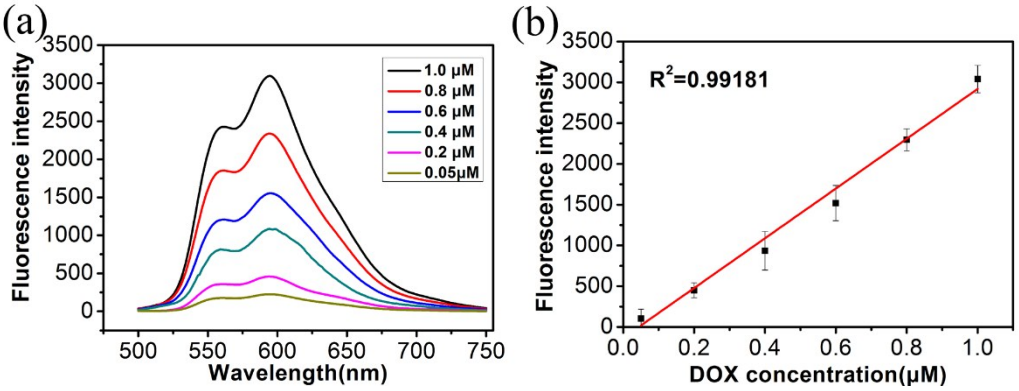

Figure S3. (a) Fluorescence spectra of different concentrations of DOX. (b) Standard linear calibration curve for the fluorescence intensity versus the corresponding DOX concentrations.

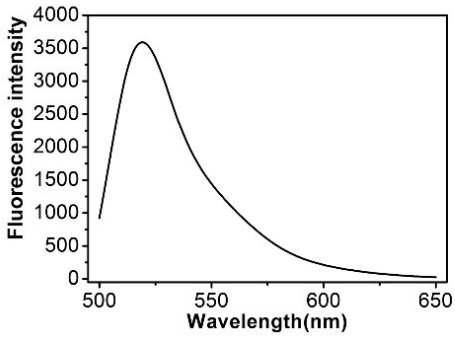

Figure S4. Fluorescence spectra of telomerase probes loaded on AuNPs that were competed by mercaptoethanol (ME).

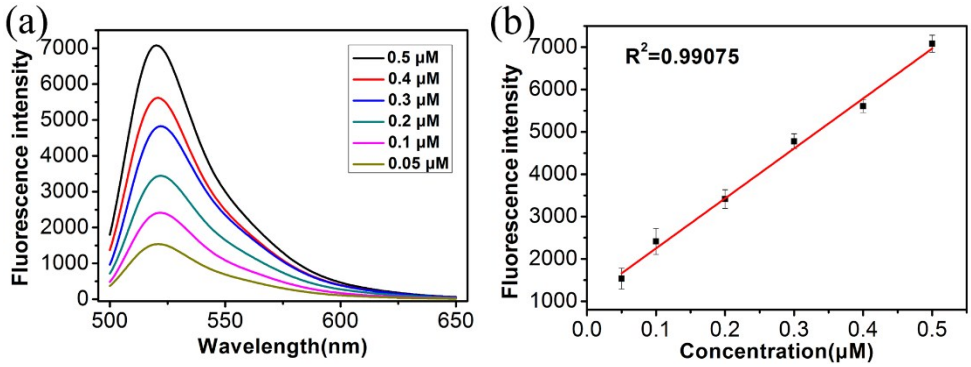

Figure S5. (a) Fluorescence spectra of different concentrations of FAM-labeled telomerase probes. (b) Standard linear calibration curve for the fluorescence intensity versus the corresponding target concentrations of FAM-labeled telomerase probes.

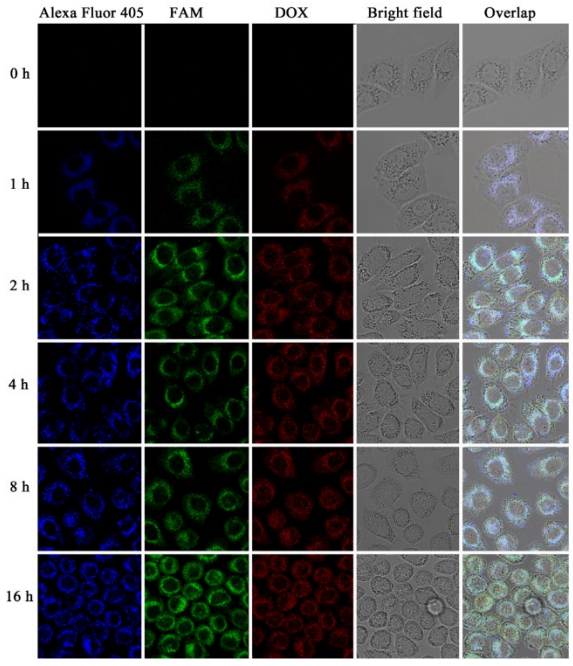

Figure S6. Fluorescent confocal images of HeLa cells after incubation with drug-loaded nanoprobes (7 nM) for the different time (0, 1, 2, 4, 8, 16 h).

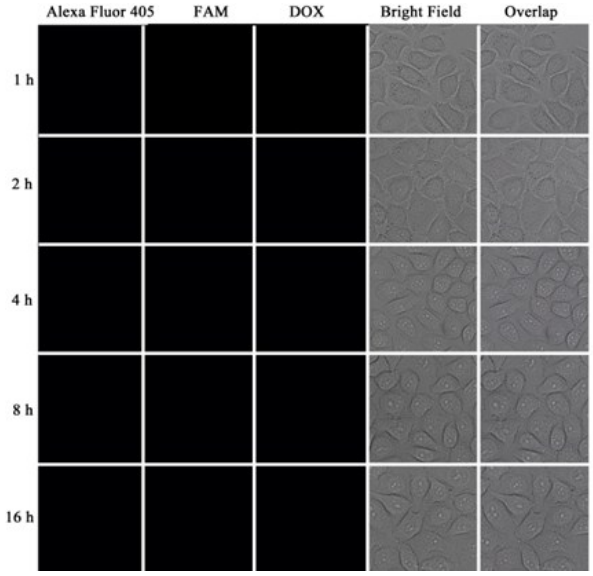

Figure S7. Fluorescent microscopy images of HL-7702 cells incubated with drug-loaded nanoprobes (7 nM) for the different time (1, 2, 4, 8, 16 h).

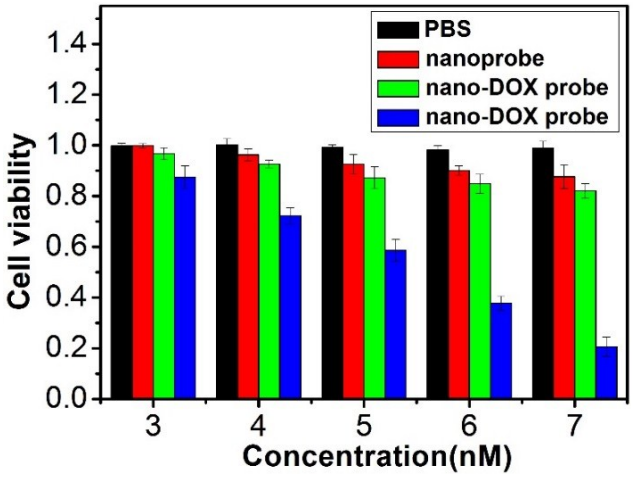

Figure S8. Cytotoxicity assays of Hela (target) cells (black，red，blue) and HL-7702 (control) cell lines(green) with PBS, nanoprobes and drug-loaded nanoprobes. Cells (1×10^6 cells/well) were incubated with nanoprobes or drug-loaded nanoprobes (3, 4, 5, 6, 7 nM) in culture medium with PBS at 37°C in an atmosphere of 5% CO_2 for 5 h. After probes treatment, cells were grown in fresh medium (10% PBS) for 24 h. Cytotoxicity was measured by the MTT assay.