Supporting Information

Multifunctional DNA nanoprobe for tumor-targeted synergy therapy by integrating chemodynamic therapy with gene silencing

*Qiaorong Tang[1], Qianqian Li[1], Lu Shi, Wei Liu, Baoxin Li and Yan Jin**

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, Key Laboratory of Applied Surface and Colloid Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710119, China.

* Corresponding author: Prof. Yan Jin, Email: jinyan@snnu.edu.cn, Fax: 86-29-81530727.

[1] These authors contributed equally to this work.

Experimental section

Materials and reagents

All oligonucleotides listed in Table S1 were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). Silver nitrate, hydrogen peroxide and sodium borohydride were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Reactive oxygen species (ROS) assay kit was purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). DEPC-treated water, PI staining kit and DAPI stain solution were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). SYBR Green I was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). All metal salts were purchased from Xi'an chemical reagent Co., Ltd. (Xi'an, China). All other reagents were of analytical reagent grade. Deionized water obtained from a Millipore filtration system was used all throughout the experiment. Human cervical carcinoma (HeLa) cells and human normal liver (HL-7702) cells were purchased from KeyGen Biotech Co., Ltd. (Nanjing, China). Dulbecco′s modified Eagle′s medium (DMEM), RPMI-1640 and phosphate buffer saline (PBS, pH 7.4) were purchased from HyClone Thermofisher (Beijing, China).

Apparatus

Gel electrophoresis was performed on a vertical electrophoresis system (Bio-Rad Laboratories Inc., America). Transmission electron microscopy (TEM) images were recorded using a Tecnai G2 F20 Field Transmitance Electron Microscropy (FEI, America). Atomic force microscopy (AFM) was performed with Bruker-Dimension Icon (Bruker Inc., USA). X-ray photoelectron spectroscopy (XPS) was carried out on an XPS spectrometer (AXIS ULTRA, Kratos Analytical Ltd.). Fluorescence microscopy images were recorded using an IX73 (Olympus, Japan) with iXon X3885 EMCCD (Andor, England). The UV-Vis absorption spectra were measured by using a U-3900H UV-Vis Spectrophotometer (Hitachi, Japan). All fluorescence measurements were performed on FluoroMax-4 fluorescence spectrophotometer (HORIBA Scientific, America).

Assembly of DTNS

The preparation of DTNS was according to previous reported one-step thermal annealing method. Briefly, four strands: P1, P2, P5 and P6, were respectively dissolved in DEPC-treated water to 50 μM as mother liquor. Then, 4 μL of each strand was simultaneously added to 184 μL of Tris-HCl buffer and heated to 95 ℃ for 5 min, followed storing at 4 ℃ ice box for more than 1 h to form 1

μM DTNS. In the same way, P1, P2, P3, and P4 were mixed and thermally annealed to obtain a DNA tetrahedron with dual aptamers (named D-sgc8-DTNS). P1, P2, P3, and P6 were mixed in Tris-HCl buffer. After thermal annealing treatment, a DNA tetrahedron with a single aptamer (called S-sgc8-DTNS) was obtained. In addition, S1, S2, P3, and P4 were assembled in the same way to form a DNA tetrahedron with dual aptamers and Anta-21 (referred to as D-sgc8-DTNS-Anta-21). All assembled DNA nanostructures are stored at 4°C for later use.

Polyacrylamide gel electrophoresis (PAGE)

PAGE to verify the formation of D-sgc8-DTNS was performed following the methods in previous work[1, 2].

Preparation of D-sgc8-DTNS-AgNCs-Anta-21

Briefly, the assembled D-sgc8-DTNS-Anta-21 reacted with 10 mM $AgNO_3$ for 15 min on ice. Then, the 10 mM $NaBH_4$ solution freshly prepared was quickly added to the above solution, violently shaken for 1 min. D-sgc8-DTNS-AgNCs-Anta-21 were obtained after being placed overnight in the dark at room temperature. The ratio of the final concentration of DTNS to $AgNO_3$ and $NaBH_4$ was 1:12:12. The morphology and size of D-sgc8-DTNS-AgNCs-Anta-21 was characterized by TEM.

Cell culture

HeLa cells (human cervical cells) were seeded in DMEM, supplemented with 10% fetal calf serum (Gibco), 100 U/mL penicillin and 0.01 mg/mL streptomycin and incubated under 5% CO_2 at 37 °C. HL-7702 cells (human normal liver cells) were seeded in RPMI-1640, supplemented with 10% fetal calf serum (Gibco), 100 U/mL penicillin and 0.01 mg/mL streptomycin and incubated under 5% CO_2 at 37 °C.

Intracellular ROS imaging

The intracellular ROS level was measured with fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). First, HeLa cells and HL-7702 were cultured for 12 h. Then, the cells were co-cultured with D-sgc8-DTNS and D-sgc8-DTNS-AgNCs for 10 h. Subsequently, 10 μM DCFH-DA was incubated with the cells for 20 min. After washing with PBS, the intracellular ROS level of the cells was determined by a fluorescent microscopy.

Targeted cellular internalization of DTNS, S-sgc8-DTNS and D-sgc8-DTNS

Firstly, SYBR Green I (SG I, 0.5 ×) was incubated with 100 nM DTNS, S-sgc8-DTNS and D-

sgc8-DTNS at room temperature for 2 h respectively. Then, cells were incubated with SG I-stained DTNS, S-sgc8-DTNS and D-sgc8-DTNS at 37 °C for 3 h. After washing with PBS, DAPI was co-incubated with cells for 20 min. Finally, the fluorescent images of cells were observed with fluorescence microscopy.

The antitumor cells activity of D-sgc8-DTNS-AgNCs-Anta-21

PI was used to investigate D-sgc8-DTNS-AgNCs-Anta-21 induced cell death by fluorescent imaging. HeLa cells were cultured as mentioned above for 12 h. Then, cells were incubated with D-sgc8-DTNS-AgNCs-Anta-21 (0, 5, 10, 20, 50 and 100 nM respectively) for 0, 12, 18, 24 h. The treated cells were incubated with PI (1 $\mu g \cdot mL^{-1}$) for 30 min. Finally, after washing three times with PBS, the red fluorescence of cells was observed by fluorescent microscopy.

Cell viability

The cell viability was determined by MTT assay. Briefly, different concentration of D-sgc8-DTNS-AgNCs, D-sgc8-DTNS-Anta-21 and D-sgc8-DTNS-AgNCs-Anta-21 were incubated with cells respectively for 24 h. Then, the cells further were incubated with 20 μL of MTT solution (5 $mg \cdot mL^{-1}$) for 4 h after washing once with PBS. And 150 μL DMSO was incubated with cells to make the formazan salt soluble. Finally, the absorbance at 490 nm was measured.

Flow cytometric assay of cell apoptosis

Flow cytometry was used to analyze cell apoptosis. The cells were seeded into cell culture dishes for 12 h (37 °C in 5% CO_2). Then, D-sgc8-DTNS (50 nM), D-sgc8-DTNS-AgNCs (50 nM), D-sgc8-DTNS-Anta-21 (50 nM) and D-sgc8-DTNS-AgNCs-Anta-21 (50 nM) were incubated with cells for another 12 h. After staining the cells with Annexin V-FITC/PI kit according to the manufacturer's instructions, the cells were performed to flow cytometric assay on flow cytometer.

Table S1. Sequences of the oligonucleotides used in the experiments.

Name	Sequence
P1	5'-ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTACCCCCCCCCCCCTTTTTTTTTTTCAACATCAGTCTGATAAGCTA-3'
P2	5'-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACCCCCCCCCCCCCTTTTTTTTTTTCAACATCAGTCTGATAAGCTA-3'
P3	5'-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTTTTTTTTTTATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA-3'
P4	5'-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTTTTTTTTTTATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA-3'
P5	5'-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC-3'
P6	5'-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT-3'
P1-FAM	5'-ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTACCCCCCCCCCCCTTTTTTTTTTTCAACATCAGTCTGATAAGCTA-FAM-3'
P2-FAM	5'-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACCCCCCCCCCCCCTTTTTTTTTTTCAACATCAGTCTGATAAGCTA-FAM-3'
cDNA	5'-TAGCTTATCAG-3'
BHQ1-cDNA	5'-BHQ1-TAGCTTATCAG-3'
S1	5'-ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTACCCCCCCCCCCCTTTTTTTTTTmU*mC*mAmAmCmAmUmCmAmGmU

	mCmUmGmAmUmAmAmG*mC*mU*mA*-Chol-3'
S2	5'-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACCCCCCCCCCCCCTTTTTTTTTTmU*mC*mAmAmCmAmUmCmAmGmUmCmUmGmAmUmAmAmG*mC*mU*mA*-Chol-3'
antagomir-21	5'-mU*mC*mAmAmCmAmUmCmAmGmUmCmUmGmAmUmAmAmG*mC*mU*mA*-Chol-3'
miRNA-21	5'-UAGCUUAUCAGACUGAUGUUGA-3'
miRNA-222	5'-AGCUACAUCUGGCUACUGGGU-3'
miRNA-223	5'-UGUCAGUUUGUCAAAUACCCCA-3'
miRNA-20a	5'-UAAAGUGCUUAUAGUGCAGGUAG-3'
TM-miRNA-21	5'-UAGCUUAUAACCCUGAUGUUGA-3'

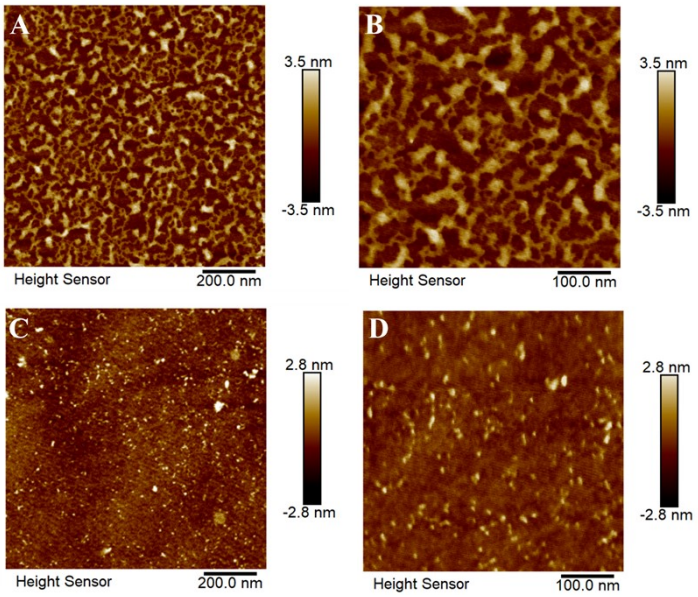

Figure S1. AFM characterization of D-sgc8-DTNS (A, B) and D-sgc8-DTNS-AgNCs-Anta-21 (C, D).

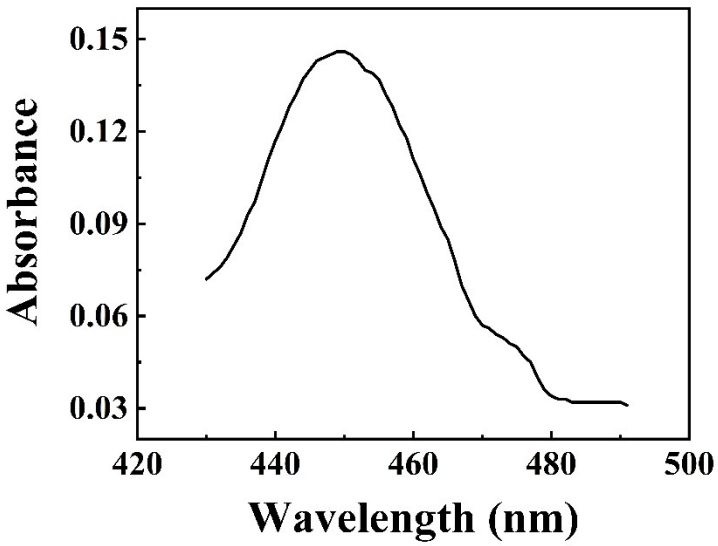

Figure S2. UV-vis absorbance spectra of D-sgc8-DTNS-AgNCs-antagomir-21.

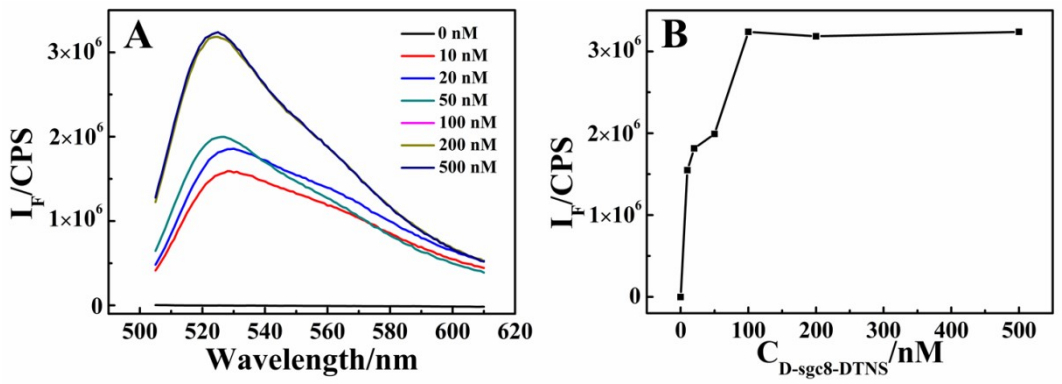

Figure S3. (A) Fluorescence spectra and (B) Fluorescence intensity of SG I under the different concentrations of D-sgc8-DTNS. The concentration of SG I is 0.5×.

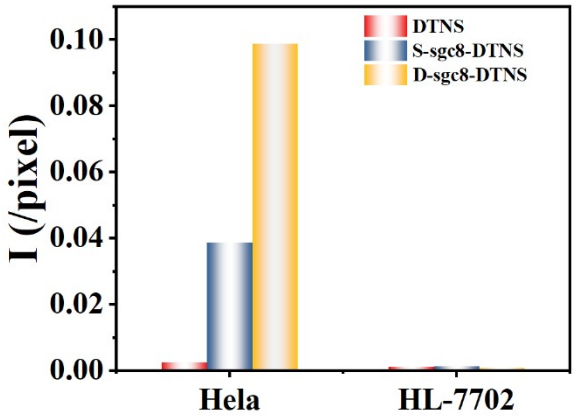

Figure S4. Mean fluorescent signal of HeLa cells and HL-7702 cells under different conditions to verify the targeted recognition of aptamer.

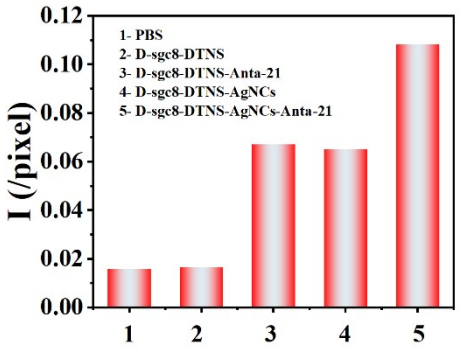

Figure S5. Mean fluorescent signal of HeLa cells treated in different groups. The concentration of D-sgc8-DTNS, D-sgc8-DTNS-Anta-21 and D-sgc8-DTNS-AgNCs-Anta-21 is 100 nM, respectively. Red fluorescence indicates the PI-stained dead cells.

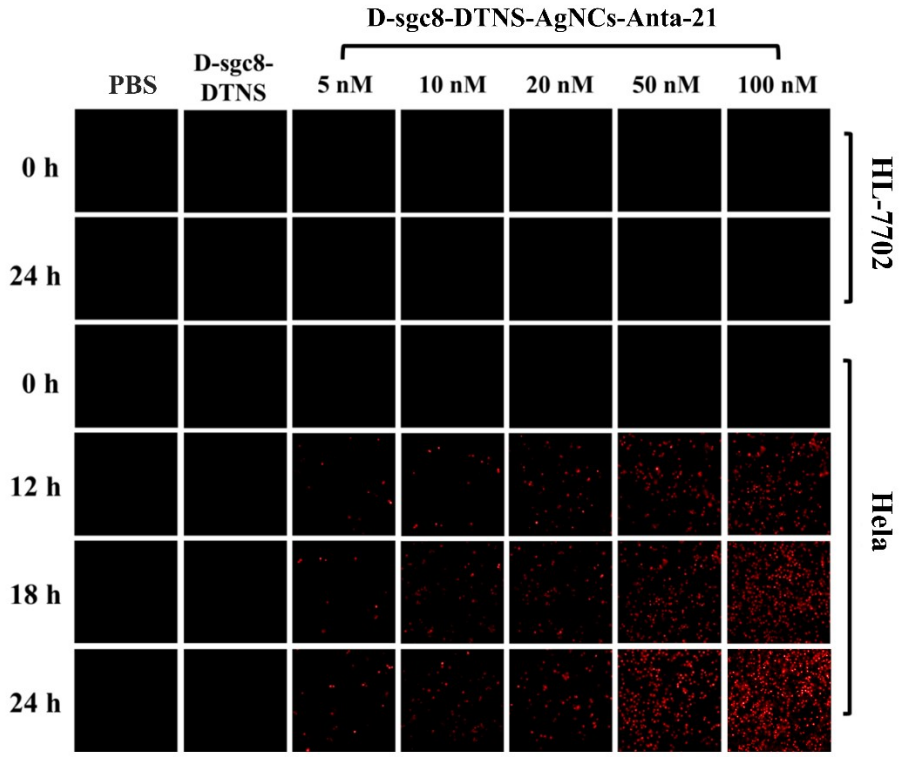

Figure S6. Fluorescence images of HeLa cells and HL-7702 cells under different concentrations of D-sgc8-DTNS-AgNCs-Anta-21 and different incubation time. Concentration of D-sgc8-DTNS-AgNCs-Anta-21 ranged from 5 nM to 100 nM. The concentration of D-sgc8-DTNS is 100 nM. Red fluorescence shows the dead cells stained with PI. Scale bar, 50 μm.

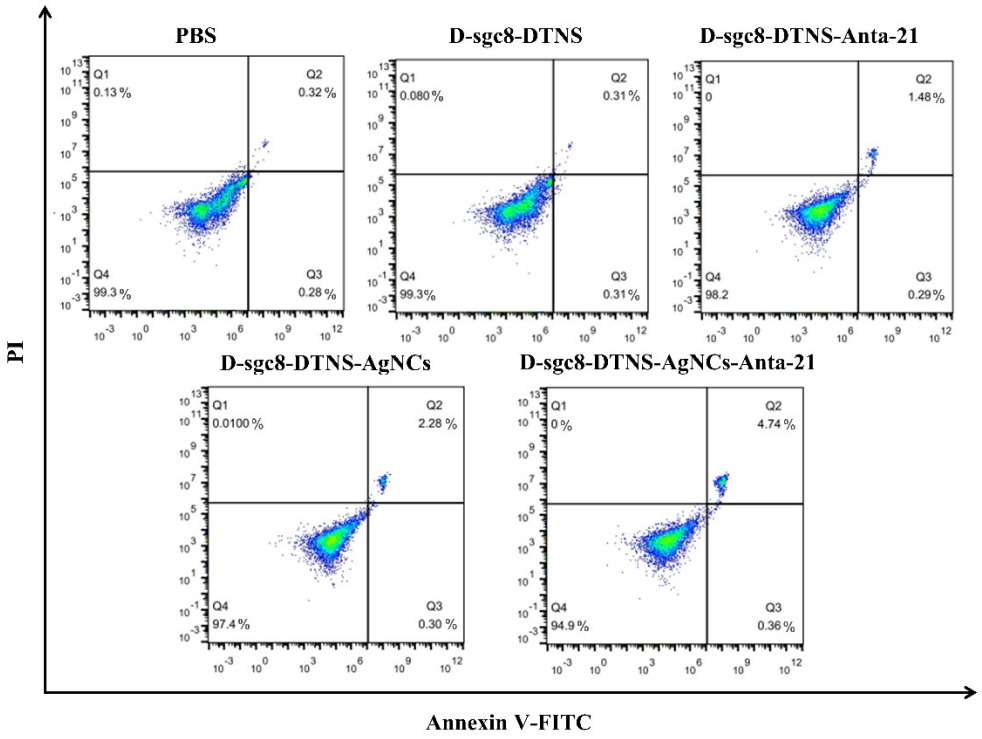

Figure S7. Flow cytometric apoptosis analysis of Annexin V-FITC/PI-stained HL-7702 cells after different treatments for 12 h.

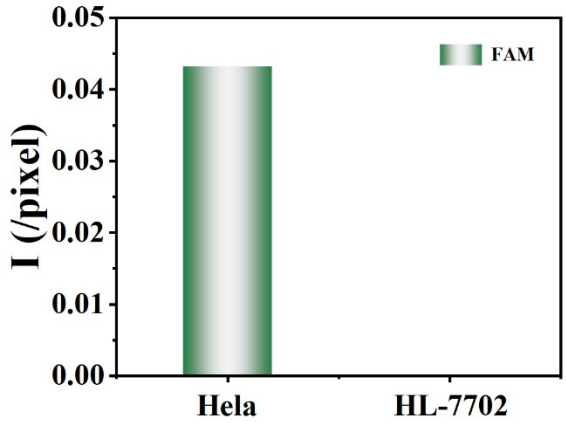

Figure S8. Mean fluorescent signal of D-sgc8-DTNS-AgNCs-FAM and BHQ1-cDNA complex after incubating with HeLa cells and HL-7702 cells.

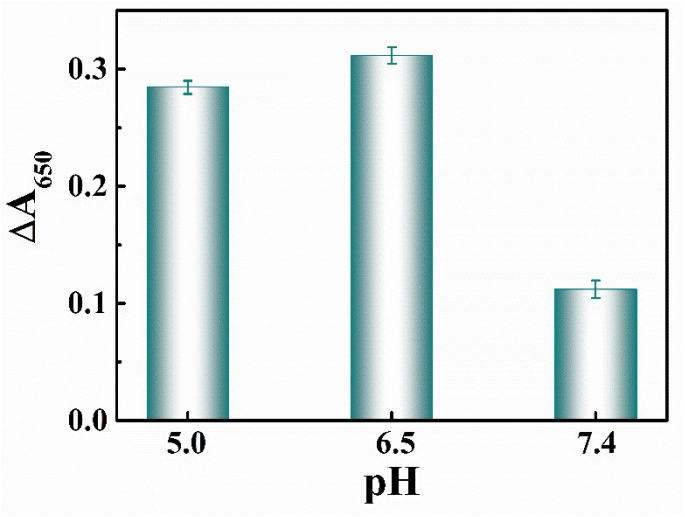

Figure S9. Effect of pH on the catalytic performance of DTNS-AgNCs.

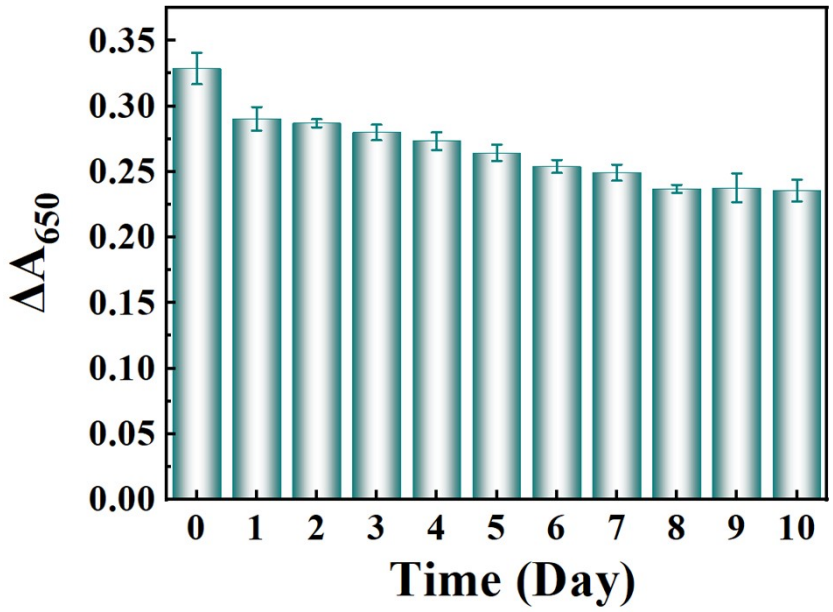

Figure S10. Influence of storage time in blood serum on the catalytic ability of DNA-AgNCs. The error bars represented the standard deviation of three repetitive measurements.

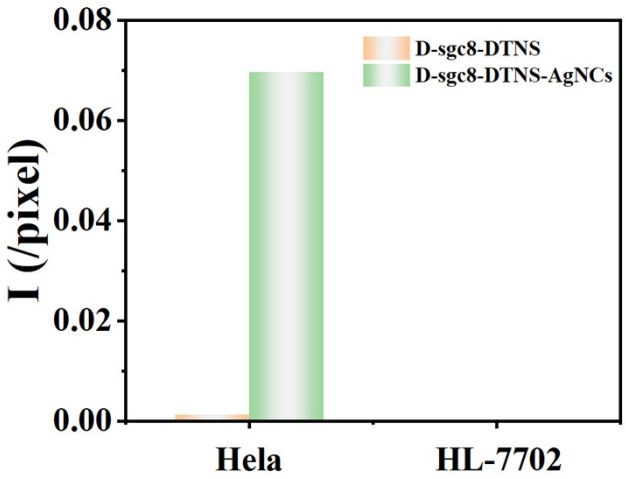

Figure S11. Mean fluorescent signal of HeLa cells and HL-7702 cells treated in different groups by ROS staining.

References

1. Q. Li, F. Wang, L. Shi, Q. Tang, B. Li, X. Wang and Y. Jin, *ACS Appl. Mater. Interfaces*, 2022, **14**, 37280-37290.
2. L. Shi, W. Liu, B. Li, C. J. Yang and Y. Jin, *ACS Appl. Mater. Interfaces*, 2021, **13**, 15008-15016.