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Supporting Information for

A DNA-functionalized biomass nanoprobe for targeted photodynamic therapy of tumor and ratiometric fluorescence imaging-based visual cancer cell identification/antitumor drug screening

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Reagents and materials

1,3-Diphenylisobenzofurane was purchased from Shanghai Yuanye Biological Co., Ltd. Polyoxyethylenediamine (NH_2-PEG-NH_2, MW=2,000), oleic acid, 1-ethyl-(3-dimethyl aminopropyl) carbodiimide hydrochloric acid (EDC) and N-hydroxysuccinimide (NHS) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Sepantronium bromide (YM-155) was purchased from Med Chemexpress (MCE) China Ltd. Nucleic acid molecules used in this study were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China), which are shown in Table S1. Fetal Bovine Serum Qualified，DMEM basic (1×), RPMI Medium 1640 basic (1×), and 0.25% Tryosin-EDTA (1×) were purchased from Thermo Fisher (Suzhou) Instruments Co., Ltd. Mammary Epithelium Basal Medium Mammary Epithelial Cell Tested Contains NO Antimicrobial Agents purchased from Lonza Ltd. (USA). The mixture of penicillin and streptomycin (100×) and 1×PBS, 5×TBE were purchased from Beijing Solarbio Science & Technology Co., Ltd. PAGE gel coagulant, acrylamide/methylbispacrylamide solution (30%), and ammonium persulfate were purchased from Bioengineering (Shanghai) Co., Ltd. The cell lines: Hela cells (human cervical cancer cells), MCF-7 cells (human breast cancer cells), and MCF-10A cells (non-tumorigenic human breast epithelial cells) were purchased from the Cell Bank of the Typical Culture Storage Committee of the Chinese Academy of Sciences / Cell Resource Center of the Shanghai Institute of Life Sciences of the Chinese Academy of Sciences (Shanghai, China). All other chemical reagents in the experiment are purely for analysis and made in China, and the water used in the experiment was 18.2 MΩ·cm.

Primary instruments

The Cary Eclipse fluorescence spectrometer (Agilent Technologies, Santa Clara, CA, USA) was utilized to record the fluorescence spectrum. The Cary-60 UV-vis spectrophotometer (Agilent Technologies) was utilized to record the UV-vis absorption spectrum. A Perkin-Elmer Fourier transform infrared (FT-IR) spectrometer (Perkin-Elmer Inc./Thermo Fishser Scientific, Waltham, MA, USA) was utilized to characterize

the M-BQDs surface groups. A Rigaku X-ray powder diffractometer (Rigaku Corp., Tokyo, Japan) was used for X-ray diffraction (XRD) analysis. A Philips transmission electron microscope (Philips, Eindhoven, Netherlands) was utilized to characterize the particle size range of M-BQDs. An ESCALAB™ X-ray photoelectron spectrometer (Thermo Fisher, Waltham, MA, USA) was used for M-BQDs elemental analysis via X-ray photoelectron spectroscopy (XPS). The EL×800 enzyme labeling instrument (Bio-Tek Instruments, Winooski, VT, USA) was utilized for cytotoxicity analysis. The Zeiss LSM710 laser scanning confocal microscope system (Carl Zeiss Microscopy, GmbH, Jena, Germany) was used for living cell imaging *in vitro*.

Preparation of M-BQDs and the nanoprobe

After crushing 10 g of clean dried mint leaves in a crusher, 20 mL of absolute ethanol was added, and the crushed leaves were broken for 3 min. Then, the mint-leaf solution was poured into a beaker, mixed with 10 mL of acetone, stirred uniformly, left to stand for 30 min, and filtered to obtain the chlorophyll extract. Twenty mL of oleic acid and 0.03 g NH_2-PEG-NH_2 were added in a 250 mL flask, and the flask was connected via an air reflux tube and filled with argon. The solution was stirred and heated to 250 °C until its color turned to orange-red. After the solution was cooled to room temperature, 7 mL of the chlorophyll extract was added to this flask. Subsequently, the mixed solution was heated to 180 °C and reacted via stirring for 3 h. After cooling to room temperature, 5 mL of 12 M HCl was added to adjust the solution acidity to a strong acidity, and stirring was continued for 12 h at 30 °C. The mixture solution was transferred to a funnel, and 1 mL of ultra-pure water was added and shaken well. After static delamination, the lower-layer solution was transferred to a beaker, the pH of the solution was adjusted to neutral pH with saturated NaOH solution, then filtered with a 0.22 μm water system filter membrane to remove large particles. The filtrate was transferred to a dialysis bag (MWCO: 1000 Da) and dialyzed in ultra-pure water for 24 h to obtain the NICQDs solution.

Five mL of M-BQDs solution was mixed with 0.01 g NHS and 0.1 g EDC and stirred for 30 min at room temperature. One and a half mL of the resulting solution was mixed with 100 μL DNA-NH_2 solution (100 μM), and a covalent coupling reaction was

completed in a 37 °C thermostatic shaker. Then, 100 μL DNA-dylight680 solution (100 μM) was added into this reactant solution, and the hybridization reaction was carried out on a 37 °C constant temperature shaking table for 1 h. The complex solution was transferred to a 10 kD ultrafiltration tube, centrifuged at 10000 rpm for 15 min, and 1×PBS solution was added. The centrifugation steps were repeated three times to recover the unfiltered solution from the ultrafiltration tube and to obtain the nanoprobe solution.

Characterization of M-BQDs and the nanoprobe

The optical properties of M-BQDs and the nanoprobe were investigated via fluorescence spectroscopy and UV-vis spectroscopy. The surface functional groups and morphology of M-BQDs and the nanoprobe were characterized via TEM, XPS, FTIR, and XRD analyses.

Cytotoxicity test of the nanoprobe

To assess the cytotoxicity of the synthesized nanoprobe, the Hela cell line was selected for testing. The Hela cells were transferred into a 96-well cell culture plate and cultured for 24 h at 37 °C and 5% CO_2 in a thermostatic incubator until cell density reached 90%. The nanoprobe solutions (0, 50, 100, 150, 200 and 250 μg/mL) were added to the 96-well plates and incubated under identical conditions for 20 h. Then, 20 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) solution (5 mg/mL in PBS) was added and incubation was continued for 4 h. DMSO (100 μL) was added to every well, which was then placed on a microplate shaker for shaking for 5 min. A microplate reader measured the absorbance at 490 nm.

Photodynamic therapy of tumor cells

Hela cells were planted in 96-well plates, when the cell density cultured is close to 90%, the cells were divided into two groups. The nanoprobes with final concentrations of 0, 50, 80, 100, 120, 150 and 200 μg/mL were added respectively to the first group. The nanoprobe with a final concentration of 90 μg/mL was added into the second group. After the cells were cultured for 8 h, the medium was replaced. The cells in the first group was

irradiated with 660 nm laser (200 mW/cm^2) for 1.5 min, the cells in the second group was irradiated respectively with 660 nm laser (200 mW/cm^2) for 0, 1, 2, 3, 4 and 5 min. The cells were continue incubated for 24 h, then, incubated for 4 h with 20 μL MTT (5 mg/mL in PBS). DMSO (100 μL) was added to every well, which was then placed on a microplate shaker for shaking for 5 min. A microplate reader measured the absorbance at 490 nm.

Ratiometric fluorescence measurement of survivin mRNA

Different concentrations of survivin mRNA solutions were added into 100 μL of the nanoprobe solution, and the PBS solution is added to make the final volume 200 μL. After the solution was mixed evenly and left to sit for 5 min, the fluorescence intensity at the wavelengths of 678 and 720 nm was measured at the excitation wavelength of 405 nm.

Ratiometric fluorescence imaging of endogenous survivin mRNA in living cells

Hela, MCF-7, and MCF-10A cells were seeded at an appropriate density in three 35 mm confocal imaging plates, after each one incubated for 20, 48, and 48 h in an incubator (at 37 °C, 5% CO_2), and the nanoprobe solution (final concentration of 120 μg/mL) was added to each plate. After incubating for 8 h, the cell imaging at the 670 nm channel (650-690 nm) and the 720 nm channel (700-740 nm) was collected at 405 nm laser excitation via CLSM using a Zeiss LSM710 confocal laser scanning microscope system.

Table S1 The sequence of DNA/RNA fragments used in experiments

Name	Sequences (5′ to 3’-) Description
DNA-Dylight680	CCCAGCCTTCCAGCTCCTTGTT-Dylight680
DNA-NH_2	NH_2-TTTCAAGGAGCTGGAAG
Survivin mRNA	CAAGGAGCTGGAAGGCTGGG

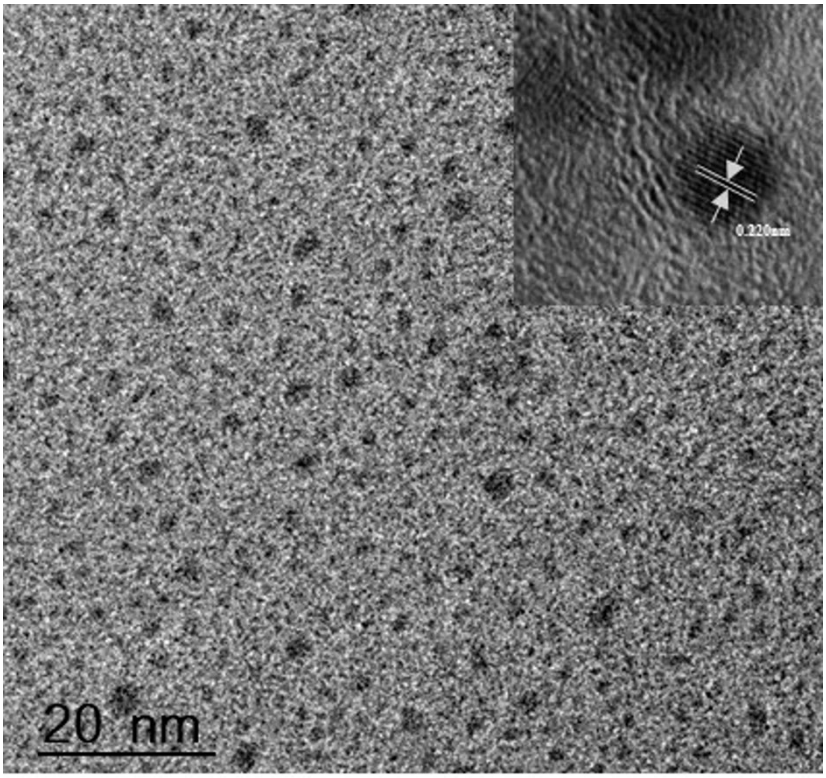

Fig. S1 The TEM and HRTEM images of the M-BQDs-dylight680.

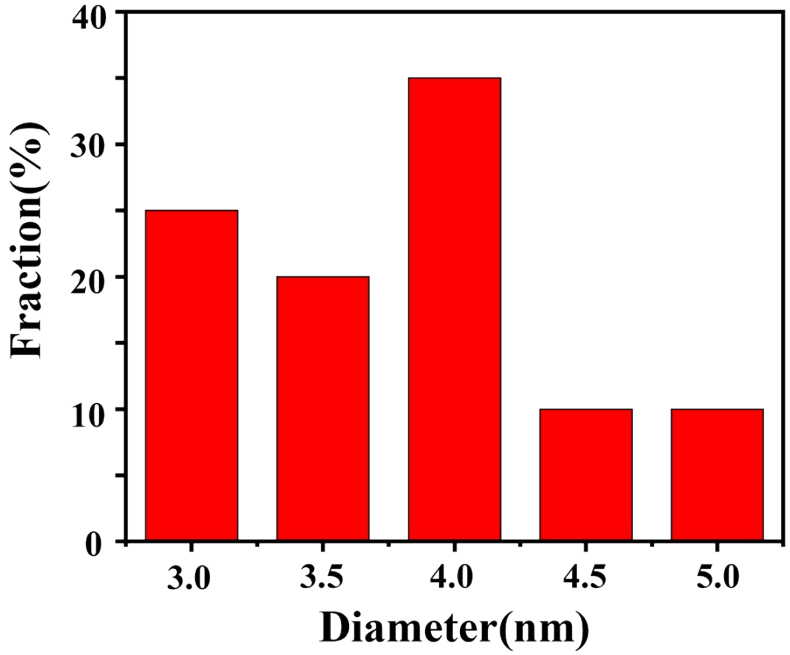

Fig. S2 The size distribution of the the M-BQDs-dylight680

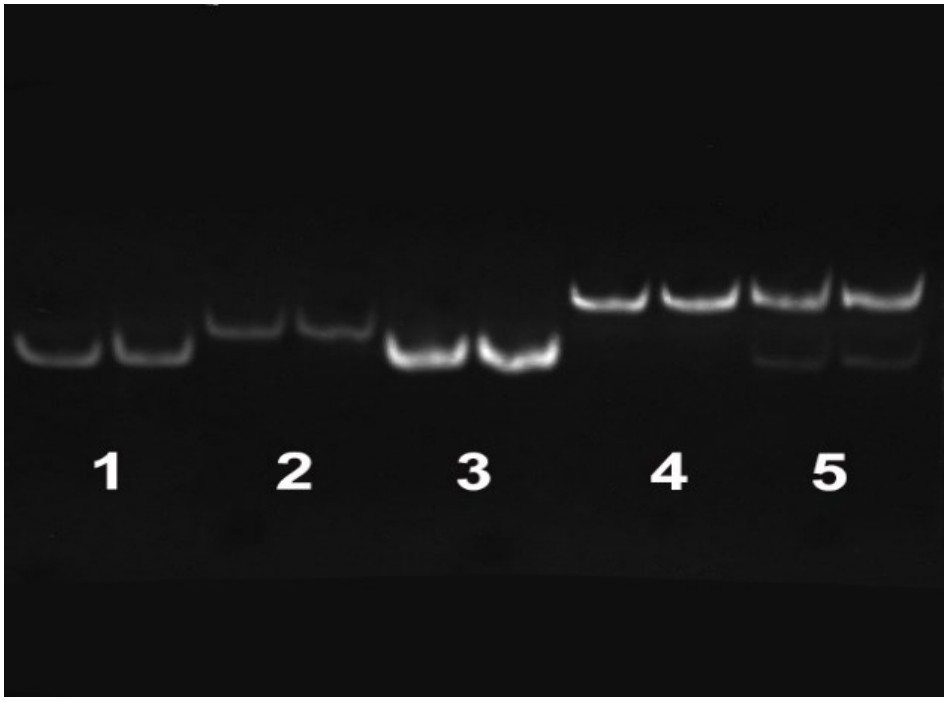

Fig. S3 Agarose gel electrophoresis image experiments for the special recognition of survivin mRNA. Samples for gel electrophoresis assays were prepared as follows: (1) amine-DNA; (2) survivin mRNA; (3) dylight680-aptamer; (4) amine-DNA+dylight680-aptamer; (5) amine-DNA+dylight680-aptamer+survivin mRNA.