

Supporting Information

A Tumor mRNA-mediated Bi-Photosensitizer Molecular Beacon as an Efficient Imaging and Photosensitizing Agent

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Experimental Section.

Synthesis of labeled oligonucleotides. Ce6–MB–Ce6 was assembled by attaching two Ce6 molecules to the opposite ends of a single MB. For Ce6–MB, Ce6 was conjugated to one end of MB, leaving the other end of MB free. For Ce6–MB–BHQ3, Ce6 and BHQ3 were respectively conjugated to the two ends of MB. Ce6–MB–Ce6, Ce6–MB and Ce6–MB–BHQ3 were synthesized and HPLC purified by TaKaRa Biotechnology Co., Ltd (Dalian, China). Sequences of MB and various targets were given in Table S1. The HPLC chromatogram, UV-vis spectrum and gel electrophoresis results of labeled DNA oligonucleotides were provided in Fig. S1 and Fig. S2. The UV-vis spectrum for Ce6–MB–Ce6 exhibited a higher degree of PS aggregation (for Q band at 654 nm was split and shifted to the blue) compared with that of Ce6–MB and Ce6–MB–BHQ3.

Table S1. Sequences of MBs and DNA targets

Oligonucleotides	sequences
MB	5'- <u>AGCAGA</u> TTG AGA AAG GGC TG <u>TCTGCT</u> - 3'
DNA target 1 (perfectly matched)	5'-CAG CCC TTT CTC AA-3'
DNA target 2 (single-base mismatched)	5'-CAG CCT TTT CTC AA-3'
DNA target 3(cyclin D1 gene segment)	5'-TAC ACC GAC AAC TC-3'
DNA target 4(K-ras gene segment)	5'-AGC TGG TGG CGT AG -3'
DNA target 5(pHER-2/neu gene segment)	5'-AGA CAT GAA GCT GC-3'

^aUnderlined letters represent the stem sequence; ^bLetters in red represent the mismatched site.

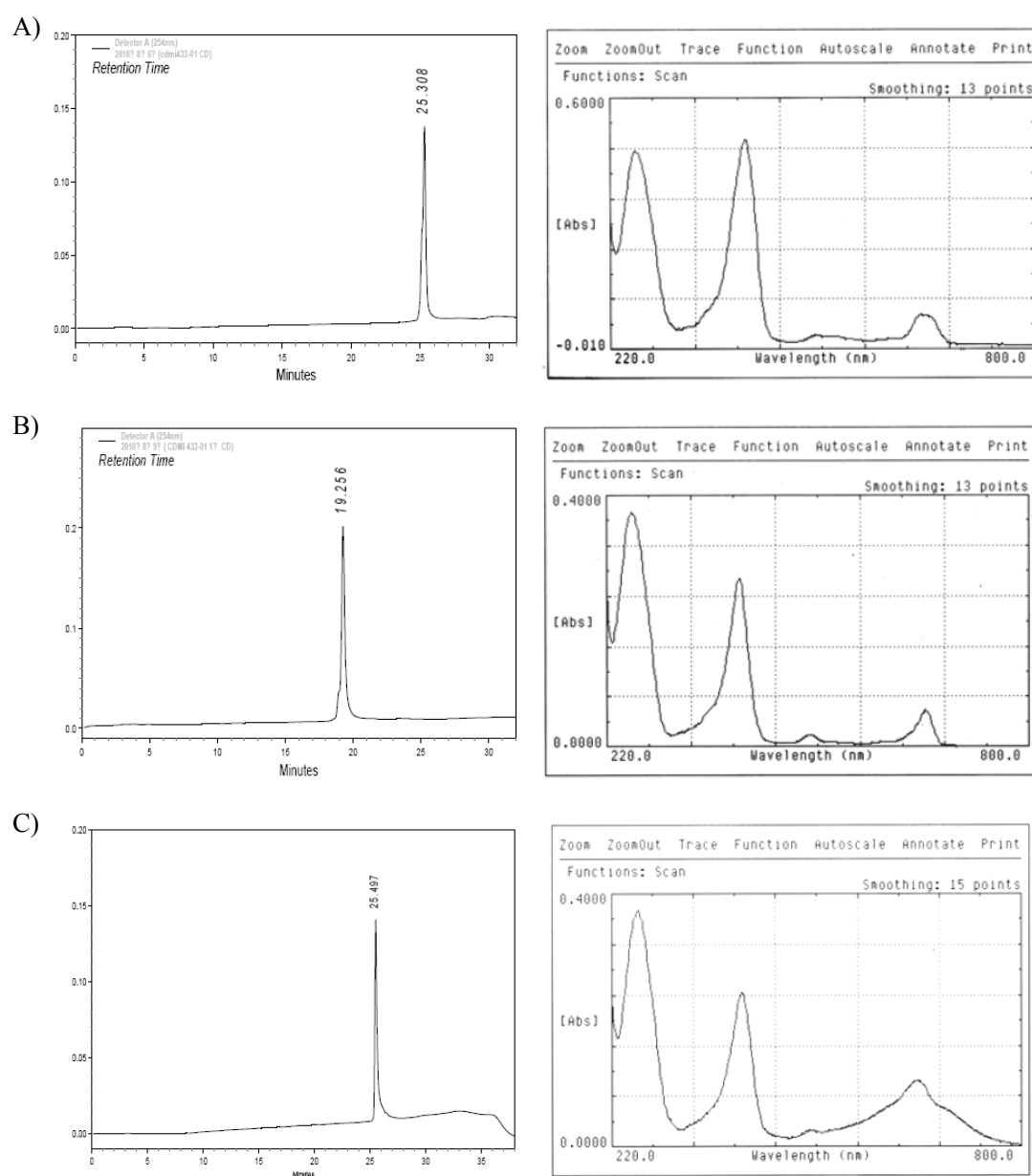


Fig. S1 The HPLC chromatogram and corresponding absorption spectra for (A) Ce6-MB-Ce6 (B) Ce6-MB and (C) Ce6-MB-BHQ3.

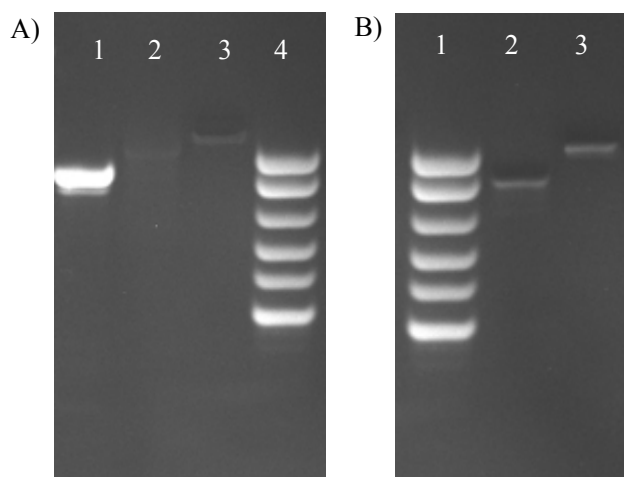


Fig. S2 Gel electrophoresis results of (A) Ce6–MB–Ce6 and Ce6–MB. Lane 1, unlabeled oligonucleotide; lane 2, Ce6–MB; lane 3, Ce6–MB–Ce6; lane 4, 20/30 ladder marker. (B) Ce6–MB–BHQ3. Lane 1, 20/30 ladder marker; lane 2, BHQ3–MB; lane 3, Ce6–MB–BHQ3.

The structure of MBs. The potential secondary structures of MBs were predicted in Fig. S3 by using mfold (www.idtdna.com). It indicated that the stem-loop structures would be the favorable conformations.

Parameter	Structure 1	Structure 2	Structure 3	Structure 4
Conformation				
ΔG , kcal mole ⁻¹	-3.31	-3.31	-2.68	-1.66
Description	Stem-and-loop structure			

Fig. S3 The two-dimensional structure of survivin MB.

The specificity of MB. Ce6–MB–Ce6 with a fixed concentration 200 nM was mixed with various synthesized DNA targets (2 μ M). The fluorescence intensity was measured with 404 nm excitation wavelength. The MB generated a much stronger fluorescent signal when mixed with the perfectly matched DNA target compared with other targets (cyclin D1 gene, K-ras gene, and pHER-2/neu gene segment).

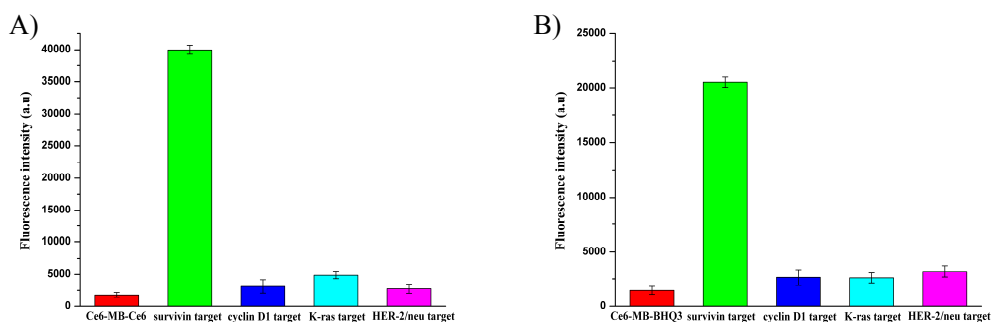


Fig. S4 Ce6–MB–Ce6 (A) or Ce6–MB–BHQ3 (B) was respectively mixed with various synthesized DNA targets. The fluorescence intensity was measured with a 404 nm excitation wavelength.

The fluorescence activation experiment.

Ce6–MB–Ce6 with a fixed concentration 200 nM in phosphate buffered solution (PBS at pH 7.4) was incubated with the perfectly matched DNA target or the single-base-mismatched DNA target as a function of DNA target concentrations (1 nM to 2 μ M) for 10 min. After incubation, the fluorescence was recorded on a Edinburgh FLS920 exciting at 404 nm and measuring emission from 620 to 720 nm.

The comparison of sensitivity (SB ratio) of Ce6–MB–Ce6 and Ce6–MB–BHQ3 was carried out. Ce6–MB was employed as a positive contrast. The concentration of MB was fixed at 200 nM. The fluorescence before and after incubation with the perfectly matched DNA target was measured with 404 nm excitation wavelength.

In kinetic studies, Ce6–MB–Ce6 (200 nM) was incubated with the perfectly matched DNA target (2 μ M) at 37 $^{\circ}$ C with different incubation time. The fluorescence intensity was measured with 404 nm excitation wavelength. Kinetic study of Ce6–MB–Ce6 in response to the perfectly matched DNA target was shown in Fig. S5.

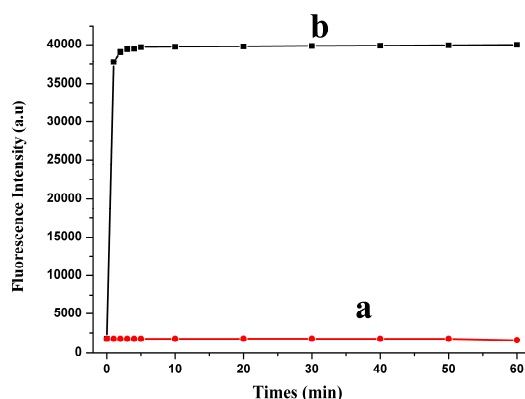


Fig. S5 Fluorescence intensity of Ce6–MB–Ce6 (200 nM) as a function of incubation time in the absence (trace a) and presence (trace b) of the perfectly matched DNA target (concentration of 2 μ M). The fluorescence intensity was measured at 37 $^{\circ}$ C with 404 nm excitation wavelength.

Nuclease Assay. Two groups of Ce6–MB–Ce6 were diluted to a concentration of 200 nM in 1mL of assay buffer (10 mM PBS, pH 7.4, 2.5 mM MgCl₂, and 0.5 mM CaCl₂) and placed in a 96-well fluorescence microplate at 37 $^{\circ}$ C. After equilibrating for 10 min, 1.3 μ L of DNase I in assay buffer (2 U/L) was added to one group. The fluorescence of these samples was monitored for 60 min. Then survivin DNA target of 2 μ M was delivered to the two groups and incubated for 10 min. When cooled slowly to room temperature, the fluorescence intensity was measured with 404 nm excitation and 665 nm emission wavelength.

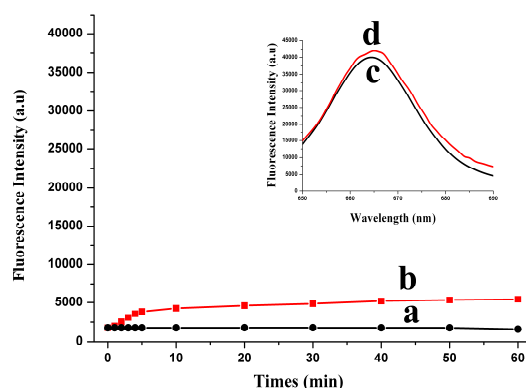


Fig. S6 The nuclease stability of Ce6–MB–Ce6 in the presence of DNase I. Fluorescence curve of 200 nM Ce6–MB–Ce6 in buffer in the absence (trace a) and presence (trace b) of DNase I. Fluorescence spectra after hybridization Ce6–MB–Ce6 with DNA target in the absence (trace c, inset) and presence (trace d, inset) of DNase I. The fluorescence intensity was measured with 404 nm excitation wavelength.

The 1O_2 activation experiment.

Disodium of 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABMD) was employed as a probe molecule to assess 1O_2 generation. ABMD molecules can react with 1O_2 to yield an endoperoxide, which caused a decrease in the intensity of ABMD absorption. The corresponding absorption spectrum of pure ABMD was given in Fig. S7 and each labeled-MB was shown in Fig. S1).

The Ce6–MB–Ce6 (2 μ M) was incubated with the perfectly matched DNA target in PBS (pH at 7.4) for 10 min. Then ABMD (0.1 mM) was added into the above solution. The photo-oxidation of ABMD was monitored for 30 min under the irradiation with a diode laser at 655 nm and the UV-vis spectra were recorded every 5 min.

The ability of 1O_2 generation of Ce6–MB–Ce6 and Ce6–MB–BHQ3 was then evaluated. Ce6–MB was used as a positive control to quantify the ability of PS to produce 1O_2 . The comparison of them is carried out under the above condition. The decreased intensity of ABMD absorption correlated well with 1O_2 quantities. The decreased intensity of ABMD absorption was measured after 30 min under the irradiation with a diode laser at 655 nm.

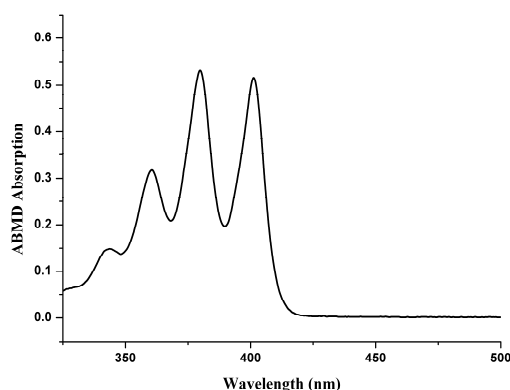


Fig. S7 UV-vis absorption spectrum of ABMD (0.1 mM).

Cell Culture. The breast cancer cell line SK-BR-3 and normal immortalized human mammary epithelial cell line MCF-10A were cultured in Dulbecco's modified Eagles medium (DMEM, Gibco, 1% antibiotics penicillin/streptomycin, 100 U/ml) plus 10% fetal bovine serum (FBS, Gibco) and maintained at 37 °C in 5 % CO₂.

Confocal laser scanning microscopy. SK-BR-3 and MCF-10A cells were plated on chamber slides for 24 hours. Cells are incubated with 500 nM Ce6-MB-Ce6, Ce6-MB-BHQ3 for 1h at 37 °C. Then cells were washed 3 times with PBS at room temperature and examined using a confocal laser scanning microscopy with a 633 nm laser (Leica TCS SPE, Germany).

Flow cytometry. Cells were treated with Ce6-MB-Ce6 and Ce6-MB-BHQ3 as described above and washed 3 times with PBS at room temperature. Flow cytometry was performed using a BECKMEN-KULTER FC500, exciting at 633 nm (see Fig. S8).

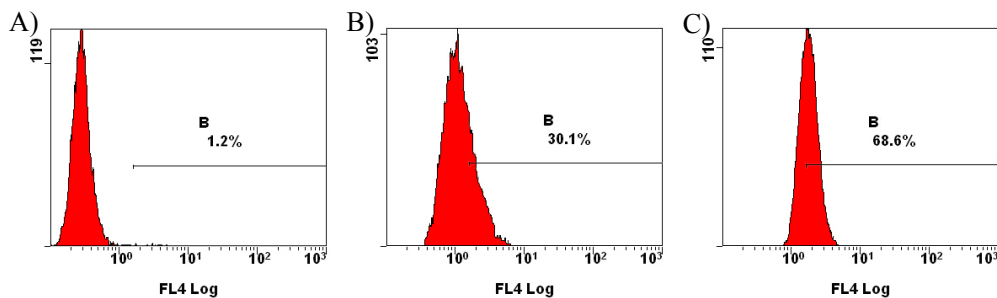


Fig. S8 Flow cytometry data were collected from SK-BR-3 cells alone (A), SK-BR-3 cells incubated with Ce6-MB-BHQ3 (B) and Ce6-MB-Ce6 (C).

Real-time Reverse Transcription-PCR. Total RNA from sorted cells was extracted with the RNeasy Mini Kit (Qiagen, Valencia, CA). cDNA synthesis was performed using an iScript kit (Bio-Rad). Real-time PCR was carried out with SYBR GreenI (Qiagen) on an ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA). The primer pairs for detecting the expression of survivin gene were survivin forward 5'-TCCACTGCCCCACTGAGAAC-3' and the reverse 5'-TGGCTCCCAGCCTTCCA-3'. GAPDH was used as an internal control. The relative expression of survivin gene was analyzed using the $2^{-\Delta\Delta CT}$ method.

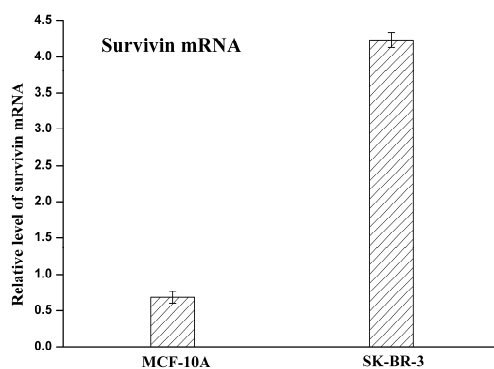


Fig. S9 Detection of the level of survivin mRNA by real-time RT-PCR. Relative level of survivin mRNA was calculated from the quantity of survivin PCR products and the quantity of GAPDH PCR products.

MTT cell viability assay. Cells were grown in 96-well plates for 24 h. Cells were washed with prewarmed PBS buffer and incubated with prewarmed fresh media for 30 min before the addition of Ce6–MB–Ce6, Ce6–MB–BHQ3 and Ce6–MB (2 μ M). Cells were incubated with them for 24 h at 37 $^{\circ}$ C, respectively, and then washed 3 times with PBS buffer. PDT treatment was performed using a 655 nm diode laser with one of the different light fluences (0, 1, or 5 J/cm²). The cells were allowed to continue growth for 24 h. The MTT reagent was added to the medium at 0.5 mg/ml. Four hours later, the medium was removed and replaced with DMSO. The absorbance was measured with a microplate reader at 490 nm.

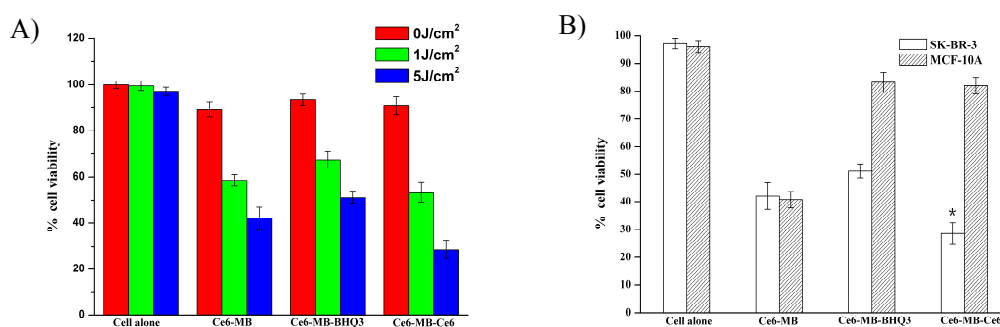


Fig. S10 (A) The photodynamic cytotoxicity of Ce6-MB-Ce6 to SK-BR-3 as a function of light dose. (B) The SK-BR-3 and MCF-10A cells were respectively incubated with 2 μ M Ce6–MB–Ce6, Ce6–MB–BHQ3 or Ce6–MB under irradiation with 5 J/cm². Asterisk indicates significant difference between SK-BR-3 and MCF-10A cells.

Determination of apoptotic cell death. The apoptotic cell death induced by Ce6–MB–Ce6 and Ce6–MB–BHQ3 was examined with DAPI stained SK-BR-3 cells cultured in 12-well plates. After incubated with 2 μ M Ce6–MB–Ce6 or Ce6–MB–BHQ3 for 24 h at 37 $^{\circ}$ C, respectively, cells were then PDT treated by using a 655 nm diode laser with 5 J/cm². The cells were incubated with a fixative solution (Tissue Fixative, OH, US) for 15 min. Then the fixative solution was replaced with 1 ml DAPI solution (final concentration 10 μ g/ml) for each well. The DAPI staining solution was removed and the cells were washed with PBS twice before coverslips

were mounted on the slides with Permount. Then cells were imaged using a confocal laser scanning microscopy with a laser (405 nm) (Leica TCS SPE, Germany).

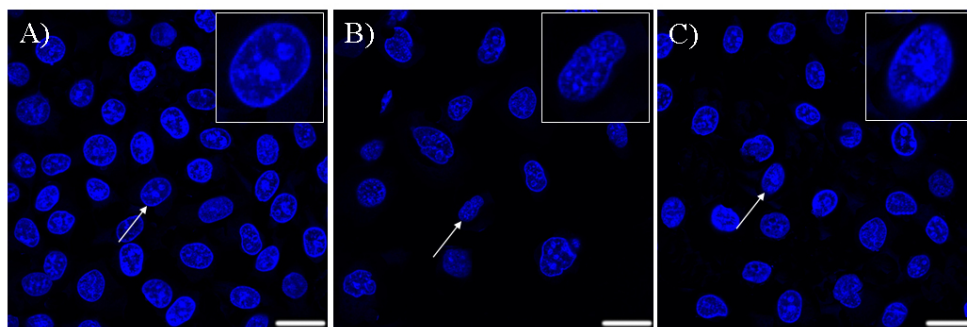


Fig. S11 Ce6-MB-Ce6-induced apoptosis in SK-BR-3 cells detected by DAPI (blue) staining. SK-BR-3 cells were incubated with 2 μM Ce6-MB-Ce6 (B), 2 μM Ce6-MB-BHQ3 (C) under irradiation with 5 J/cm^2 before adding DAPI for observation. SK-BR-3 cells alone as a contrast (A).