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Nano-formulation of a photosensitizer using DNA tetrahedron and its potential for *in vivo* photodynamic therapy

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Table S1. DNA oligonucleotides to construct Td.

S1	5'ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA-FAM
S2	5'TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
S3	5'TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
S4	5'TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT

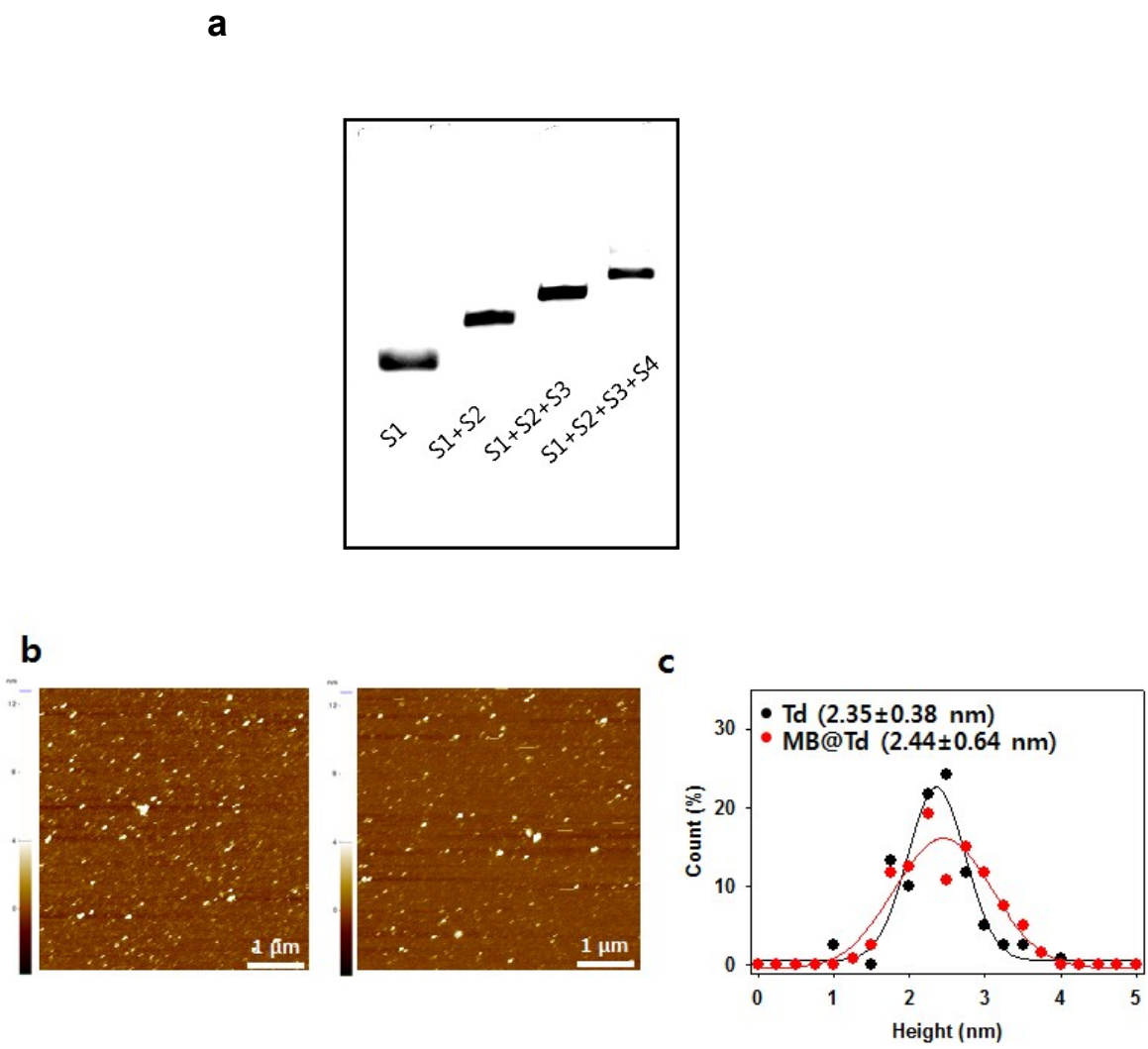

Fig. S1. (a) Native PAGE (6%) to verify assembly of Td. (b) AFM images of Td (left) and MB-loaded Td (right) showing the dried state of nanoparticles. (c) The averaged heights of Td (black) and MB@Td (red) were estimated from 120 representative particles in AFM images.

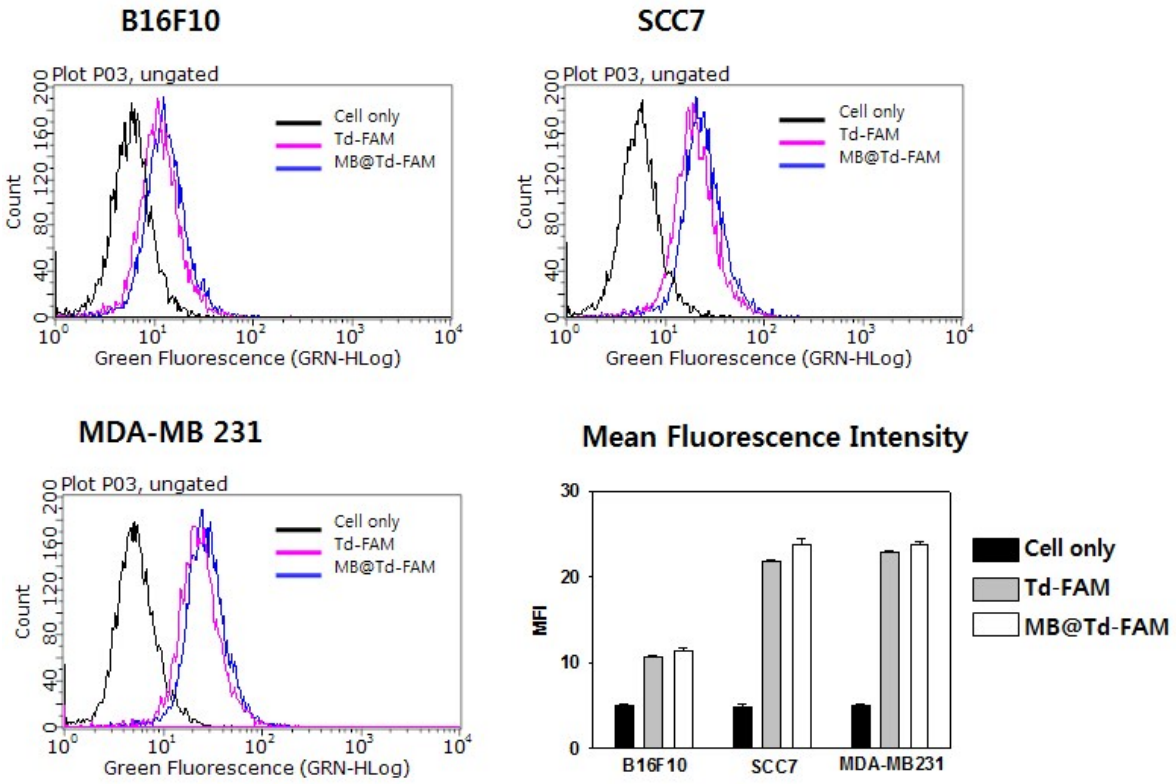

Fig. S2. Cellular uptake of Td before (magenta) and after (blue) intercalation of MB.

EXPERIMENTAL SECTION

DNA tetrahedron (Td): The DNA oligonucleotides required for the construction of Td were synthesized by using the standard protocols in a DNA synthesizer. The sequences of oligonucleotides to construct the DNA tetrahedron were as presented in Table S1. The base sequences of DNA strands were adopted from Tuberfield's Td. Td assembly was performed and characterized by following the previously reported procedures .[1]

Job plot, size and zeta potential: Job plot was established to determine binding ratio between Td and MB in the complex.[2,3] While keeping the total concentration of the MB and Td at 1 μM in TM buffer (10 mM Tris-HCl, 5 mM $MgCl_2$, pH=8.0), the difference in absorbance of the MB-Td complex (MB@Td) and the free MB at 660 nm was recorded after incubation for 1 h at room temperature. Linear regression analysis was carried out with curve fitting software. The size and zeta potential of Td was measured in the presence and absence of MB by Zetasizer™ (Malvern, UK).

AFM imaging: For the AFM imaging, the DNA samples (250 nM, 30 μL) were placed onto freshly cleaved mica for 1 h, washed with distilled water and then dried with compressed air. Then, samples were imaged in non-contact mode on an AFM instrument (XE-100, Park Systems, Korea) using non-contact cantilever (PPP-NCHR 10M, Park Systems, Korea). Finally, AFM data were processed with XEI 4.1.0.software.

Singlet oxygen generation: Optical detection of singlet-oxygen was determined by monitoring the bleaching of p-nitrosodimethylaniline (RNO, 120 μM) by the photosensitizer in the presence of histidine (30 mM).[4,5] PBS solutions (100 μL) containing RNO/L-histidine and either free MB (400 nM) or MB@Td (25 nM Td and 400 nM MB) were irradiated (excitation at 665 nm, 200 mW・cm^{-2}) through a pinhole mask for 3 min. Absorbance at 440 nm was measured at using a microwell plate reader (SpectraMax™ Plus, Molecular Devices, USA). The singlet oxygen generation level was estimated by the absorption decrease of the RNO/L-histidine solution.

Flow cytometry: Cells (SCC7, B16F10, and MDA-MB231) were seeded on 24-well culture plates with DMEM or RPMI media (Gibco, USA) containing 10% heat inactivated fetal bovine serum, 1% penicillin and streptomycin at a density of 10^5 cells/mL and cultured for 24 h 37 °C in humidified atmosphere containing 5% CO_2. Then growth medium was removed from each cell sample, and the cells were washed twice with PBS. The cells were treated with transfection mixtures containing MB@Td (10 nM) at varying concentration of MB in the absence of serum and the antibiotics, and incubated for 6 h at 37 °C in humidified atmosphere containing 5% CO_2. Then, trypsin replacement (200 μL, TrypLE™, Gibco, USA) was added to each sample, and the samples were incubated for 5 min at 37 °C. Then, the medium (1 mL) was added to each sample, and the resulting cell suspensions were transferred to conical tubes (Falcon™ tubes, BD Biosciences, USA) and centrifuged for 3 min at 2500 rpm. Supernatant was removed, and the cell pellets were resuspended in PBS (1 mL). Fluorescence intensity of the cells was estimated by flow cytometry (Guava easyCyte™, Millipore, USA) Samples of at least 5000 cells were analyzed in triplicate.

***In vitro* PDT assay**: Cells were cultured in 96-well plates and treated with MB@Td (10 nM Td and 160 nM MB) or free MB (160 nM) for 6 h under the same conditions used for the cytometric analysis. After washing with fresh media, the plate was incubated for 24 h with or without laser irradiation (665 nm and 200 mW・cm^{-2}) on each well. The cell viability was then evaluated via MTT assay.

Fluorescence microscopy: The MB-treated SCC7 cells were irradiated (665 nm and 200 mW・cm^{-2}) through a

pinhole mask for 3 min. After irradiation, the cells were washed with PBS and incubated in culture media. Apoptotic cells were stained with a commercially available live-dead cell staining kit (LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells, Invitrogen, USA) by following the manufacturer's instructions. After incubation for 5 min in the staining solution, the cells were washed with PBS. The cells were finally imaged by a fluorescence microscope (Deltavision, Applied Precision, USA) equipped with a filter set (excitation 480-500 nm; emission 509-547 nm for calcein stained live cell, excitation 630-650 nm; emission 665-705 nm for EthD-1 stained dead cell).

Preparation of tumor model for *in vivo* study: The animal study was approved by the animal care and use committee of Korea Institute of Science and Technology and all mice were handled in accordance with institutional regulations. For tumor model preparation, mice were anaesthetized with intraperitoneal injection of zoletil-rompun mixture. Animal disease models were prepared on BALB/c nude mice (male, 5 weeks old, Orient Bio Inc., Korea). Tumors were established by subcutaneous inoculation of SCC7 cells (1.0×10^6 cells suspended in the culture medium) into the thigh of mice.[6]

***In vivo* PDT**: After tumor growth to ~50 mm3 size, the mice were administered with peritumoral injection of free MB (16 μM MB, 100 μL), MB@Td (16 μM MB and 1 μM Td, 100 μL), or PBS (100 μL). After 30 min post injection, the tumors were exposed to a laser light for 15 min. The body weight of the mice was measured using an electronic balance every 2 or 3 days. The tumor volume was estimated by using the digital caliper every 2 or 3 days before laser therapy and calculated according to the formula $(a^2b)/2$, where a and b were the respective width and length of the tumor.

Histological analysis: After last therapy, the tumors and organs were removed from treated. Organs (lung, liver, and kidney) and tumors were fixed in 4% formaldehyde, embedded in paraffin, and cut in 5 μm sections. For fluorescent histological inspection, sections were stained with annexin V (Annexin V-FITC Apoptosis Detection Kit, Sigma-Aldrich, USA) for apoptotic cells and Hoechst 34580 (Invitrogen, USA) for nuclei, and then analyzed by fluorescence microscopy (Leica DMI3000 B and Nuance Multispectral Imaging System) equipped with a filter set (excitation 355-425 nm; emission 470 nm for nuclei staining, excitation 420-490 nm; emission 515 nm for annexin V-FITC stained apoptosis).

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