-

Supporting Information for Aicelles from the Self ass

Phototheranostic DNA Micelles from the Self-assembly of DNA-BODIPY Amphiphiles for the Thermal Ablation of Cancer Cells

Siriki Atchimnaidu, Devanathan Perumal, Kaloor S. Harikrishanan, Hari Veera Prasad Thelu, Reji Varghese*

Indian Institute of Science Education and Research-Thiruvananthapuram Trivandrum-695551, India

Email: reji@iisertvm.ac.in

TABLE OF CONTENTS

SI No.	Content	Pages
1	Experimental methods	02-03
2	Fluorescence quantum yield calculation	03-03
3	Calculation of photothermal activity	03-04
4	Details of FACS and CLSM analyses	04-04
5	Details of MTT analyses	05-05
6	Synthesis and characterisation of 2	06-09
7	Synthesis and characterisation of DNA1 and DNA2	09-11
8	Sequence of DNA3 and DNA4	11-11
9	Additional AFM images of DNA1	11-11
10	AFM images of DNA1@DNA3	11-11
11	AFM images of DNA2 and DNA2@DNA4	12-12
12	Additional TEM images of DNA1	12-12
13	TEM images of DNA1@DNA3, DNA2 and DNA2@DNA4	12-13
<mark>14</mark>	DLS analyses of DNA1 micelles in serum	<mark>13-13</mark>
15	Zeta potential analyses of DNA1 and DNA2	14-14
<mark>16</mark>	Time dependent DLS analyses of DNA1 micelles	<mark>14-14</mark>
17	Absorption and emission spectra of 1	14-14
18	Absorption and emission spectra of DNA2	15-15
19	Photothermal properties of DNA1	15-15
20	Photothermal properties of DNA2	16-16
21	Additional CLSM images of DNA1 micelle-treated HeLa cells	17-17
22	CLSM images of DNA2 micelle-treated HeLa cells	17-17
23	MTT analysis of DNA2 micelle-treated HeLa cells	18-18
24	CLSM images of DNA1@DNA3 micelles-treated CCRF-CEM cells	18-18
25	CLSM images of DNA1@DNA3 micelles-treated Ramos cells	19-19
26	CLSM images of DNA2@DNA4 micelle-treated CCRF-CEM cells	19-19
27	CLSM images of DNA2@DNA4 micelle-treated Ramos cells	20-20
28	MTT analyses of DNA2@DNA4 micelle-treated CCRF-CEM & Ramos	20-20
	cells under dark and laser illumination	
29	Spectral data for all the intermediates	21-29
30	References	30-30

Experimental methods

All chemicals used for the organic syntheses were purchased from Sigma Aldrich, TCI and were used as received. Phosphoramidites for automated solid-phase DNA syntheses were purchased from Glen Research. TLC analyses were performed on aluminium-plates coated with silica gel 60 F254, and column chromatography was performed on 200-400 mesh silica gel. Melting points were measured on the Stuart SMP30 melting point apparatus and are uncorrected. ¹H, ¹³C, ¹¹B, ¹⁹F and ³¹P NMR spectra were recorded on a Bruker Avance 500 MHz and 700 MHz spectrometers using 1, 1, 1, 1-tetramethylsilane (TMS) for ¹H, and ¹³C, 15 % BF₃.OEt₂ in CDCl₃, CFCl₃, and 85 % H₃PO₄ in H₂O for ¹¹B, ¹⁹F, and ³¹P as the internal standards respectively. Water used for all studies was Milli Q deionized water (18.2 M Ω cm). Mass measurements were performed on a Shimadzu GCMSQP-2010 in EI mode. ESI-HRMS measurements were performed on a Thermo scientific orbitrap mass spectrometer. Measurements were performed in multi-mode i.e. ESI and APCI simultaneously in negative ion mode. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analyses were performed on Bruker UltrafleXtreme MALDI-TOF mass spectrometer. Oligonucleotides were synthesized on an H-8 K&A DNA synthesizer. Oligonucleotides were purified on reverse phase Agilent 1260 Infinity Binary LC HPLC (Phenomenex Luna C-18 column). AFM analyses were carried out on Multimode SPM (Veeco Nanoscope V). Samples were prepared by drop-casting a 2 µL solution of the sample on a freshly cleaved mica surface and dried under air. The probe used for imaging was an antimony doped silicon cantilever with a resonant frequency of 300 kHz and a spring constant of 40 N m⁻¹. TEM analyses were carried out on a FEI Tecnai G2 Spirit Bio Twin F20 (120 kV) TEM. Samples were prepared by depositing 2 µL of the sample on a 400-mesh carbon-coated copper grid (Ted Pella, Inc.) which was negatively glow discharged (PELCO easiGlow, glow discharge cleaning system) for 1 min before use. Samples were allowed to adsorb on the grid for 2 min and then an excess sample was wicked with a piece of filter paper. Absorption spectra were recorded using a quartz cuvette of 10 mm path length on a Shimadzu UV-3600 Vis-NIR spectrophotometer having a Peltier controlled cell holder. Steady-state fluorescence spectra were recorded on a Horiba Jobin Yvon Fluorimeter equipped with a thermostat Peltier cell holder, in a quartz cuvette of 10 mm path length. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano ZS equipped with a 655 nm laser. Experiments were performed at both 20 °C at a back-scattering angle of 173°. Photothermal experiments were done with 635 nm continuous laser from CNI optoelectronics Technology Co., Ltd. China. The CCRF-CEM and Ramos cells (suspension

culture) were obtained from American Type Culture Collection (ATCC), cultured in RPMI-1640 media. HeLa cells were obtained from NCCS Pune, cultured in DMEM media supplied with 10% Fetal bovine serum with 1% penicillin-streptomycin and cultured at 37 °C in 5 % CO₂ incubator. Both the mediums, FBS, and antibody were purchased from Gibco-Thermo Fisher Scientific and the media. flow cytometry analysis carried out using the FACS LSR Fortessa Flow Cytometer (BD, USA). Confocal images have been carried out using Nikon Eclipse Ti with a 100 X oil immersion objective. MTT assay has been carried on Tecan Infinite 200 PRO microplate reader.

Fluorescence quantum yield (ϕ_f) calculation

Fluorescence quantum yields of **1**, **DNA1** and **DNA2** were calculated by comparison with Nile Blue, which has a quantum yield of 0.27 in methanol as a reference using the following equation.^{S1}

$$\phi_{sample} = \phi_{Reference} \times \frac{A_{Sample}}{A_{Reference}} \times \frac{Abs_{Reference}}{Abs_{Sample}} \times \frac{n_{Sample}^2}{n_{Reference}^2}$$

 Φ = quantum yield, A = area under the fluorescence curve, Abs = Absorbance, n = refractive index of the solvent.

Photothermal activity

DNA micelles in the aqueous solution (2.0 mL) was taken in a cuvette and irradiated with a 635 nm laser for 10 min. Pure water was used as a control. A digital thermometer was used to measure the temperature every one minute. The photothermal conversion efficiency (η) was calculated according to a reported method.^{S2}

$$\eta = \frac{hA(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A\lambda})}$$

Where 'h' and 'A' are the heat transfer coefficient and surface area of the cuvette cell, respectively. ' T_{surr} ' and ' T_{max} ' are initial and final temperature of the solution. ' Q_{dis} ' represents the heat dissipation of solvent (water) which was measured by a power meter. 'I' is the incident laser power, and 'A λ ' is the absorbance at 635 nm. A temperature driving force 'hA' was calculated by the following equation:

$$hA = \frac{\Sigma_{mi}C_i}{\tau_s}$$

where 'm' and 'C' are the mass (1 g) and heat capacity (4.2 J g⁻¹) of water, respectively. ' τ_s ' is the sample system time constant calculated by the following equation.

$$\tau_{s} = \frac{t}{\ln \theta}$$

where ' θ ' is the dimensionless driving force and 't' is time.

The Photothermal conversion efficiencies of **DNA1** and **DNA2** are 52 % and 50 %, respectively.

Fluorescence-activated cell sorting (FACS) analyses

CCRF-CEM and Ramos cells were seeded at the rate of 0.4×10^6 cells in a 6 well plate (Corning) and grown in 5 % CO₂ incubator at 37 °C for 24 h. **DNA1@DNA3** at an effective concentration of 500 nM were incubated with cells at different time intervals (0.5, 1, 2, and 6 h), and were washed 3 times with 1X PBS. Cells were then collected in 1mL PBS for flow cytometry analysis. $\lambda_{ex} = 650$ nm and collection Filter-PE- 660 nm was used.

Confocal laser scanning microscopic (CLSM) analyses

HeLa cells were plated on 12 mm coverslips (Hi-media) at a seeding density of 0.5 x 10⁵ in 24 well culture plate and allow to grow for 24 h in 5 % CO₂ incubator at 37 °C. After that cells were incubated with 1µM of **DNA1** and **DNA2** for 6 h, after washing with 1 x PBS for 3 times, then cells were fixed using 4 % paraformaldehyde for 10 minutes followed by 1X PBS wash for 3 times. The cells were further fixed using 0.01 % Triton X-100 for 10 minutes and repeat the washing with 1X PBS for 3 times, cells were stained with nuclear stain i.e. DAPI (10 µg/mL) for 30 sec and then wash with 1X PBS. The coverslips were then mounted on a glass slide with prolong gold anti-fade agent for confocal imaging with a 100 X oil immersion objective. Images have been collected by exciting at different wavelengths for DAPI $\lambda_{ex} = 407$ nm, $\lambda_{emi} = 425-475$ and for BODIPY $\lambda_{ex} = 560$ nm, $\lambda_{emi} = 570-620$ nm. For imaging CCRF-CEM and Ramos cells, the cells were plated at the seeding density of 0.2 x 10⁶ in 6 well plate and cultured for 24 h followed by the addition of **DNA1@DNA3** and **DNA2@DNA4** and cells were incubated for 0.5 h. Subsequently, the same procedures were used to fix the cells. The cells were coated on a coverslip by a spin coating method using Eppendorf centrifuge at 2000

rpm for 5 min followed by mounting the coverslip on a slide like previous procedures. Then slides were imaged in the presence of immersion oil with 100X objective.

Cell viability assay (MTT)

For in vitro PTT, HeLa cells were seeded to 96-well plates as a triplicate with a density of 1×10^4 cells per well and were grown for 24 h. Then, they were incubated with different concentrations of DNA micelles for 6 h. The cells were then washed with PBS followed by the addition of fresh medium and irradiated by a 635 nm laser at 0.75 W/cm² for 10 min. After irradiation, cells were incubated in dark for another 24 h. Cytotoxicity was analyzed by MTT assay. 10 µL of 12 mM MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5- diphenyltetrazolium bromide) was added and kept for 4 h incubation at 37 °C in 5 % CO₂ incubator. 150 µL DMSO was then added to solubilize the formazan crystals and absorbance was measured at 565 nm using a microplate reader. CCRF-CEM and Ramos cells were seeded at the density of 3x 10⁴ cells/well in a 6 well culture plate (corning) and grown in 5 % CO₂ incubator at 37 °C for 24 h. The cells were incubated with different concentrations of DNA1@DNA3 and DNA2@DNA4. After 0.5 h, the cells were washed with 1 X PBS and again they were cultured with fresh media in 96 well plate as a triplicate and kept undisturbed for 4 h, The cells were then irradiated by a 635 nm laser at 0.75 W/cm² for 10 min. After irradiation, cells were incubated in dark for another 24 h before the addition of MTT reagent. The same protocols as above mentioned are followed to find the photocytotoxicity.

Synthesis and characterization of 2



Scheme S1: Scheme for the synthesis of 2.

Synthesis of 1a: To a solution of tetraethyleneglycol monomethyl ether (10 g, 48.0192 mmol)



in dichloromethane, Et_3N (10mL) and p-toluene sulfonyl chloride (13.73 g, 72.0288 mmol) were added at 0 °C, the reaction mixture was slowly brought to

room temperature and stirred for 4 h. Reaction mixture was then poured into water and dichloromethane was added. The organic layer was washed with 3M HCl, followed by sodium bicarbonate and water. The organic layer was separated and dried over anhydrous Na₂SO₄, the solvent was evaporated under reduced pressure. The crude product obtained was then purified by column chromatography using dichloromethane:methanol (98:2) as eluent to get the desired

product as a colorless liquid (95 %). $R_f = 0.35$; ¹H NMR (500 MHz, CDCl₃), δ (ppm)= 7.72 (d, J = 8.15 Hz, 2H), 7.26 (d, J = 7.90 Hz, 2H), 4.08 (t, J = 4.6 Hz, 2H), 3.61 (t, J = 3.75 Hz, 2H), 3.51 (m, 6H), 3.48 (m, 4H), 3.47 (t, J = 4.7 Hz, 2H), 3.30 (s, 3H), 2.37 (s, 3H); ¹³C NMR (125 MHz, CDCl₃), δ (ppm) = 144.79, 133.05, 129.82, 127.99, 71.94, 70.75, 70.61, 70.53, 69.24, 68.69, 59.03, 21.64; GC-MS (EI)-m/z for C₁₆H₂₆O₇S: 362.13 (cal) 362.10 (expt).

Synthesis of 1b: To a solution of **1a** (1 g, 8.1886 mmol) in THF, 4-hydroxybenzaldehyde (4.45 g, 12.2830 mmol) and K₂CO₃ (3.39 g, 24.5658 mmol) were added and stirred for 8 h at 55 °C.



The reaction mixture was cooled to room temperature and diluted with water followed by the addition of

ethyl acetate. The organic layer was then separated, dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography using petroleum ether:ethyl acetate (60:40) as eluent to get the desired product as a colorless liquid (90 %). $R_f = 0.28$; ¹H NMR (500 MHz, CDCl₃), δ (ppm) = 9.81(s, 1H), 7.75 (d, J = 8.55 Hz, 2H), 6.95 (d, J = 8.55 Hz, 2H), 4.14 (t, J = 4.7 Hz, 2H), 3.81 (t, J = 4.90 Hz, 2H), 3.65 (q, 2H), 3.62 – 3.56 (m, 8H), 3.47 (t, J = 5.00 Hz, 2H), 3.30 (s, 3H); ¹³C NMR (125 MHz, CDCl₃), δ (ppm) = 189.78, 162.85, 130.93, 129.04, 113.87, 70.92, 69.89, 69.61, 69.60, 69.51, 68.45, 66.76, 58.01; GC-MS (EI)-m/z for C₁₆H₂₄O₆: 312.15 (cal), 312.05 (expt).

Synthesis of 1c: To a solution of 4-hydroxybenzaldehyde (2 g, 16.3666 mmol) in THF, K₂CO₃ (9 g, 65.4664 mmol) and 3-bromo-1-propanol (4.54 g, 32.7332 mmol) were added and stirred

for 8 h at 55 °C. The reaction mixture was then cooled to room temperature and diluted with ethyl acetate followed by the addition

of water. The organic layer was separated, dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure. The crude product obtained thus was purified by column chromatography using petroleum ether: ethyl acetate (70: 30) as eluent to get the desired product as a colorless liquid (92 %). $R_f = 0.34$; ¹H NMR (500 MHz, CDCl₃), δ (ppm) = 9.81 (s, 1H), 7.76 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.65 Hz, 2H), 4.14 (t, *J* = 6.05 Hz, 2H), 3.80 (t, *J* = 5.85 Hz, 2H), 2.03-1.99 (m, 2H); ¹³C NMR (175 MHz, CDCl₃), δ (ppm) = 190.85, 163.93, 132.03, 130.02, 114.78, 65.63, 59.78, 31.85; GC-MS (EI)-m/z for C₁₀H₁₂O₃: 180.078 (cal), 180.0 (expt).

Synthesis of 1d: To a solution of 1c (0.3 g, 1.6655 mmol) and 2, 4-dimethylpyrrole (0.4 g, 4.

1637 mmol) in dry dichloromethane, catalytic amount of trifluoroacetic acid was added and stirred at room temperature for 3 h. Reaction mixture was then brought to 0 °C and subsequently



added 2,3-dichloro-5, 6-dicyano-1, 4-benzoquinone (0.5 g). Reaction mixture was then stirred at room temperature for 2 h. To this reaction mixture, Et_3N (5mL) followed by $BF_3.OEt_2$ (5mL) were added and stirred at room temperature for another 3 h, diluted with water followed by the addition of dichloromethane. The organic layer was separated and dried over

anhydrous Na₂SO₄, the solvent was evaporated under reduced pressure, the crude product obtained was then purified by column chromatography using dichloromethane: methanol (99: 1) as eluent to get the desired product as a greenish-brown powder (30 %). R_f = 0.25; ¹H NMR (500 MHz, CDCl₃), δ (ppm) = 7.15 (d, *J* = 8.50 Hz, 2H), 7.00 (d, *J* = 8.45 Hz, 2H), 5.97 (s, 2H), 4.18 (t, *J* = 5.95 Hz, 2H), 3.91 (t, *J* = 5.8 Hz, 2H), 2.54 (s, 6H), 2.12-2.07 (m, 2H), 1.43 (s, 6H); ¹³C NMR (125 MHz, CDCl₃), δ (ppm) = 159.38, 155.29, 143.15, 141.79, 131.84, 129.25, 127.23, 121.11, 115.05, 65.65, 60.27, 32.02, 14.60, 14.57, ¹¹B NMR (160 MHz, CDCl₃), δ (ppm) = 0.96-0.54 (t, *J* = 33.08, 1B); ¹⁹F NMR (470 MHz, CDCl₃), δ (ppm) = (-146.21 to -146.42) (q, *J* = 32.85, 2H); M.P.: 171 °C; HR-MS (ESI positive)-m/z [M+H]⁺)-m/z for C₂₂H₂₆N₂BF₂: 399.1977 (cal), 399.2042 (expt).

Synthesis of 1: To the solution of **1d** (0.070 g, 0.1757 mmol) and **1b** (0.137 g, 0.4394 mmol) in dry toluene, glacial acetic acid (1 mL) and piperidine (2 mL) were added and refluxed for



4h by the continuous purging of N₂ gas. The solution was then cooled to room temperature and the crude product obtained was purified by column chromatography using dichloromethane: methanol (95:5) as eluent to get the desired product **1** as the blue color semi-solid (80 %). R_f = 0.4; ¹H NMR (700 MHz, CDCl₃), δ (ppm) = 7.63 – 7.58 (m, 6H), 7.24 – 7.20 (m, 4H), 7.02 (d, *J* = 6.10 Hz, 2H), 6.96 (d, *J* = 6.15 Hz, 4H), 6.63 (S, 2H), 4.21 – 4.19 (m,

6H), 3.94 (t, J = 4.15 Hz, 2H), 3.91 (t, J = 3.40 Hz, 4H), 3.78 – 3.76 (m, 4H), 3.73 – 3.66 (m, 16H), 3.57(t, J = 3.32 Hz, 4H), 3.40 (s, 6H), 2.13 (t, J = 4.22 Hz, 2H), 1.64 (s, 6H); ¹³C NMR (125 MHz, CDCl₃), δ (ppm) = 159.59, 159.35, 152.58, 141.82, 138.20, 135.62, 133.57, 129.77, 129.73, 129.04, 128.99, 128.23, 127.50, 125.30, 117.43, 117.31, 114.94, 71.94, 70.86, 70.63, 70.61, 70.51, 69.68, 67.98, 67.52, 65.65, 60.23, 59.03, 32.05, 30.33, 25.61, 14.86; ¹¹B NMR (160 MHz, CDCl₃), δ (ppm) = 1.19 (t, J = 33.16, 1B); ¹⁹F NMR (470 MHz, CDCl₃), δ (ppm)

= -138.19 to -138.40 (q, J = 28.48, 2H); HR-MS (ESI positive) -m/z [M+Na]⁺ for $C_{54}H_{69}O_{12}N_2BF_2Na$: 1009.4804 (cal), 1009.4794 (expt).

Synthesis of 2: To a solution of **1** (0.080 g, 0.0801 mmol) in dry dichloromethane (3 mL) and diisopropylamine (0.0349 g, 0.18 mmol), 2-cyanoethyl-N, N-diisopropylchloro



phosphoramidite (0.0379 g, 0.16 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, it was extracted with dichloromethane, the organic layer was washed with saturated NaHCO₃ and the solvent was removed under reduced pressure. The crude compound was purified by column chromatography (neutral alumina) by using dichloromethane as eluent to give the desired product **2** as the blue color semi-solid (95 %). TLC (dichloromethane: methanol 98:2): R_{f}

= 0.30; ³¹P NMR (500 MHz, CDCl₃) δ (ppm) = 148.65. After confirming the phosphoramidite using ³¹P NMR, compound **2** was immediately used for DNA synthesis on a DNA synthesizer using standard protocols with appropriated modifications.

Synthesis of DNA1 and DNA2



Scheme S2. Scheme showing the synthesis of DNA1 and DNA2.

BODIPY derivative **2** was incorporated at the 5'-end of the DNA during automated solid-phase DNA synthesis using a modified program with an extended coupling time of 30 minutes. **DNA1** and **DNA2** were synthesized on 1 μ mol scale with 500 Å CPG as the support. After completion of DNA synthesis, excess phosphoramidite was removed by repeated washing with dichloromethane and acetonitrile for several times and dried under vacuum. Bead was then transferred into the 1 mL vial, 28 % ammonia solution was added and vortexed for 24 h at room temperature. Beads were then removed by filtering using centrifugal filters (0.45 μ m filter size, Millipore Ultra free MC). After removing the ammonia using nitrogen gas purging, oligonucleotides were purified by using reverse-phase HPLC with the ammonium acetate buffer (20 mM, pH = 7.2) and acetonitrile as a solvent system. The Purity of **DNA1** and **DNA2** were confirmed by HPLC and characterized using MALDI-TOF analyses.



Scheme S3. Chemical structures of DNA1 and DNA2.



Figure S1. Analytical HPLC traces of (a) DNA1 and (b) DNA2.



Figure S2. MALDI-TOF spectra of (a) DNA1 and (b) DNA2.

Table S1. Sequence of DNA3 and DNA4.

DNA Oligonucleotide sequence $(5' \rightarrow 3')$

DNA3ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGATTTTT
TGGGTGCGADNA4ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGATTTTT
TATATTGATAAGGAT



Figure S3. Additional AFM images of **DNA1**. $([DNA1] = 1 \mu M)$



Figure S4. AFM images of DNA1@DNA3. ([DNA1@DNA3] = 1 μ M)



Figure S5. AFM images of **DNA2**. [**DNA2**] = 1μ M



Figure S6. AFM images of DNA2@DNA4. [DNA2@DNA4] = $1 \mu M$



Figure S7. Additional TEM images of **DNA1**. $[DNA1] = 1 \mu M$



Figure S8. TEM images of DNA1@DNA3. [DNA1@DNA3] = 1 μ M



Figure S9. TEM images of **DNA2**. [**DNA2**] = $1 \mu M$



Figure S10. TEM images of DNA2@DNA4. [DNA1@DNA4] = $1 \mu M$



Figure S11: Comparison of DLS size distributions of **DNA1** micelles prepared in serum and water. [**DNA1**] = $1 \mu M$



Figure S12. Zeta potential analyses of (a) DNA1 and (b) DNA2. $[DNA1/2] = 1\mu M$



Figure S13: Comparison of DLS size distributions of freshly prepared DNA1 micelle and the same sample after an year.



Figure S14. (a) Absorption and (b) emission spectra of 1 in THF. $[1] = 1 \mu M$



Figure S15. (a) Absorption and (b) emission spectra of DNA2 at 20 °C and 90 °C. $[DNA2] = 1 \mu M$



Figure S16. Photothermal properties of **DNA1** micelles. (a) Graph showing the concentrationdependent temperature elevation for **DNA1** micelle in water upon laser irradiation (635 nm, 0.75 W/cm^2). (b) Graph showing the laser intensity-dependent temperature elevation for **DNA1** micelles in water (2 μ M). (c) Photothermal effect of **DNA1** micelles in water when irradiated with a laser (635 nm, 1 W/cm²). The laser was switched off after irradiation for 10 min. (d) Graph of the cooling period of the time versus negative natural logarithm of the temperature for **DNA1** micelle.



Figure S17. Photothermal properties of **DNA2** micelles. (a) Graph showing the concentrationdependent temperature elevation for **DNA2** micelle in water upon laser irradiation (635 nm, 0.75 W/cm^2). (b) Graph showing the laser intensity-dependent temperature elevation for **DNA2** micelles in water (2 μ M). (c) Photothermal effect of **DNA2** micelles in water when irradiated with a laser (635 nm, 1 W/cm²). The laser was switched off after irradiation for 10 min. (d) Graph of the cooling period of the time versus negative natural logarithm of the temperature for **DNA2** micelle.



Figure S18. Additional CLSM images of **DNA1** micelles-treated HeLa cells. [**DNA1**] = 1μ M



Figure S19. CLSM images of DNA2 micelles-treated HeLa cells. $[DNA2] = 1 \mu M$



Figure S20. MTT analyses of DNA2 micelle-treated HeLa cells under dark (blue) and under laser irradiation (green).



Figure S21. Additional CLSM images of DNA1@DNA3 micelles-treated CCRF-CEM cells. $[DNA1@DNA3 = 0.5 \mu M]$



Figure S22. Additional CLSM images of DNA1@DNA3 micelles-treated Ramos cells. [DNA1@DNA3 = 0.5μ M]



Figure S23. CLSM images of DNA2@DNA4 micelle-treated CCRF-CEM cells. [DNA2@DNA4 = 0.5μ M]



Figure S24. CLSM images of DNA2@DNA4 micelles-treated Ramos cells. [DNA2@DNA4 = 0.5μ M]



Figure S25. MTT analyses of **DNA2@DNA4** micelles-treated CCRF-CEM and Ramos cells under (a) dark (b) laser irradiation.



Figure S26. ¹H (above; 500 MHz, CDCl₃) and ¹³C (below; 125 MHz, CDCl₃) - NMR spectra of 1a.



Figure S27. ¹H (above; 500 MHz, CDCl₃) and ¹³C (below; 125 MHz, CDCl₃) - NMR spectra of 1b.



Figure S28. ¹H (above; 500 MHz, CDCl₃) and ¹³C (below; 175 MHz, CDCl₃) - NMR spectra of 1c.



Figure S29. ¹H (above; 500 MHz, CDCl₃) and ¹³C (below; 125 MHz, CDCl₃) - NMR spectra of 1d.



Figure S30. ¹¹B (above; 160 MHz, CDCl₃) and ¹⁹F (below; 470 MHz, CDCl₃) - NMR spectra of 1d.



Figure S31. ¹H (above; 700 MHz, CDCl₃) and ¹³C (below; 125 MHz, CDCl₃) - NMR spectra of 1.



Figure S32. ¹¹B (above; 160 MHz, CDCl₃) and ¹⁹F (below; 470 MHz, CDCl₃) - NMR spectra of 1.



Figure S33. ³¹P NMR spectum of 2 (500 MHz, CDCl₃).



Figure S34. GC-MS spectrum of 1a.



Figure S35. GC-MS spectrum of 1b.



Figure S36. GC-MS spectrum of 1c.



Figure S37. HR-MS spectra of (a) 1d and (b) 1.

References:

- (S1) (a) G. Jones, W. R. Jackson, C.-Y. Choi and W. R. Bergmark, *J. Phys. Chem.*, 1985, 89, 294; (b) S. Ghosh, D. Banik, A. Roy, N. Kundu, J. Kuchlyan and N. Sarkar, *Phys. Chem. Chem. Phys.*, 2014, 16, 25024.
- (S2) D. K. Roper, W. Ahn and M. Hoepfner, J. Phys. Chem. C., 2007, 111, 3636.