Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

The Core Composition of DNA Block Copolymer Micelles Dictates DNA Hybridization Properties, Nuclease Stabilities, and Cellular Uptake Efficiencies

Chan-Jin Kim[1], Ga-Hyun Kim[1], Eun Hye Jeong[2], Hyukjin Lee[2,*], and So-Jung Park[1,*]

[1] Department of Chemistry and Nanoscience, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Republic of Korea

[2] College of Pharmacy, Graduate School of Pharmaceutical Science, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Republic of Korea

*Correspondence to: sojungpark@ewha.ac.kr, hyukjin@ewha.ac.kr

Materials and instrumentations. All oligonucleotides used this study were purchased from Bioneer (Daejeon, Korea). 10X Tris/Borate/EDTA (TBE) buffer, 30 % acrylamide/bis Sol. 29:1, ammonium persulfate, and TEMED (*N,N,N′,N′*-tetramethylethylenediamine) were purchased from Bio-Rad (Hercules, California, USA). 2-Vinylpyridine was purchased from Tokyo Chemical Industry (Tokyo, Japan). All other reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Ultrapure water (18.2 MΩ) was used for all experiments. Dynamic light scattering (DLS), zeta potential, and static light scattering (SLS) measurements were performed using Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) with a 632.8 nm laser at a scattering angle of 173°. Transmission electron microscopy (TEM) images were obtained using JEM-2100F electron microscope (JEOL Ltd., Tokyo, Japan) and JEM-2100BU (JEOL Ltd., Tokyo, Japan) operating at 200 kV accelerating voltage. Molecular weights (M_n) and polydispersity index (PDI) of synthesized polymers were determined by gel permeation chromatography (GPC) system (Shimadzu, Kyoto, Japan) with polystyrene standards setting at 40 °C with a flow rate of 1 mL/min using THF as eluent. Chemical structures of synthesized polymers were determined by proton nuclear magnetic resonance (^{1}H NMR) spectra, which were recorded on a Bruker Advance 300 MHz NMR spectrometer (Bruker Biospin Corp., Germany). All cells were cultured in DMEM medium containing 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (P/S) (100 U/mL) (Invitrogen Corp, Caelsbad, CA, USA) in 5% CO_2 at 37 °C. The growth medium for HeLa cells was Dulbecco's Modified Eagle Medium (DMEM) with high glucose (1 g/L) and sodium pyruvate, respectively.

Synthesis and characterization of PS, P2VP, and PMA. Carboxylic acid terminated PS_{62}, $P2VP_{64}$, and PMA_{73} were synthesized by the RAFT polymerization method. For PS_{62}, styrene

monomers (15.9 mL, 134 mmol) were dissolved in 5 mL acetone with an initiator, 4,4'-Azobis(4-cyanopentanoic acid) (V-501), (0.01 g, 35.7 μmol) and a chain transfer agent (CTA), 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid, (0.072 g, 178 μmol) in a Schleck flask. For $P2VP_{64}$, 2-vinylpyridine monomers (5.96 mL, 55.30 mmol) were dissolved in 6 mL DMF with V-501 (0.01 g, 35.7 μmol) and a CTA (0.07 g, 178 μmol) in a Schleck flask. For PMA_{73}, methyl acrylate monomers (1.79 mL, 19.8 mmol) were dissolved in 5 mL DMF with V-501 (0.0139 g, 49.5 μmol) and a CTA (0.1 g, 248 μmol) in a Schleck flask. These solutions were degassed by three freeze-pump-thaw cycles and then heated at 70 ^{o}C for 5 h for $PS_{62,}$ for 12 h for $P2VP_{64}$, and for 24 h for PMA_{73}, respectively. The polymerization reactions were stopped by letting the air (*i.e.*, oxygen) into the flask. The synthesized PS, P2VP, and PMA were each precipitated by adding methanol (~200 mL) or ethyl ether (~200 mL) respectively and recovered by filtration. The same purification procedure was repeated three times and the final precipitates were dried under vacuum overnight.

Synthesis of DNA block copolymers. DNA-*b*-PS, DNA-*b*-P2VP, and DNA-*b*-PMA were synthesized through the coupling of carboxylic acid terminated polymers to 5'-amine-modified DNA1(FAM) attached on CPG beads, following a modified literature procedure.[1] For the synthesis of DNA1(FAM)-*b*-PS_{62}, DNA1(FAM)-*b*-$P2VP_{64}$, and DNA1(FAM)-*b*-PMA_{73}, synthesized polymers (103.5 mg, 15.0 μmol for PS_{62}, 106.5 mg, 15.0 μmol for $P2VP_{64}$, or 100.5 mg, 15.0 μmol for PMA_{73}), *N*,*N*-diisopropylethylamine (DIEA) (26.1 μL, 150 μmol), and HATU (5.7 mg, 15.0 μmol) were first dissolved in DMF and then the solution was vortexed for 10 min to activate carboxylic acid terminus. Subsequently, DNA1(FAM) on CPG beads (0.5 μmol scale) was added to this solution and the mixture was kept on an orbital shaker at

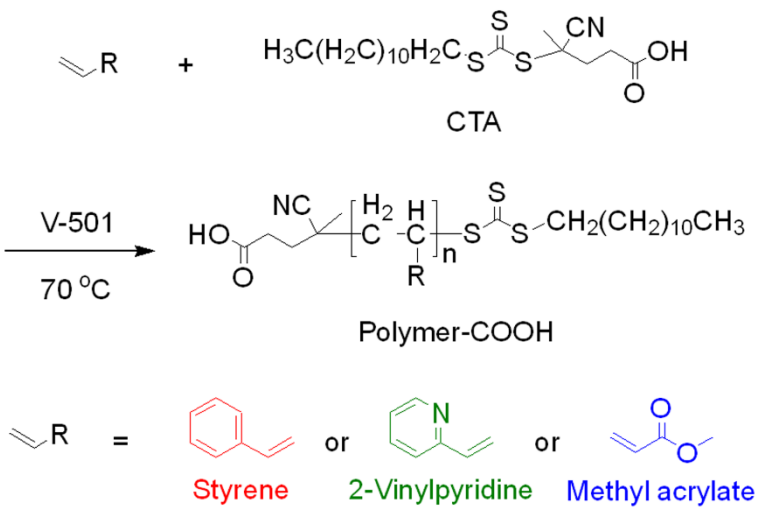
400 rpm for 24 h. The CPG beads were then filtered from the dispersion and washed with sufficient organic solvent (~50 mL of DMF, ~50 mL chloroform, and ~50 mL acetone) to remove uncoupled polymers. Synthesized DNA block copolymers were cleaved from CPG beads by treating the beads with 1 mL concentrated ammonium hydroxide at 55 ^{o}C for 5 h. For purification of DNA1(FAM)-*b*-PS_{62}, the CPG beads were filtered and washed with 7 mL water, and then DNA1(FAM)-*b*-PS_{62} strands were extracted from the beads with 5 mL DMF.[2] The purified DNA1(FAM)-*b*-PS_{62} was stored in DMF until use. For purification of DNA1(FAM)-*b*-$P2VP_{64}$ and DNA1(FAM)-*b*-PMA_{73}, the CPG beads were filtered and washed with 7 mL water, 5 mL DMF with a few drops of concentrated ammonium hydroxide, and 5 mL DMSO with a few drops of concentrated ammonium hydroxide. The resulting solution containing DNA1(FAM)-*b*-$P2VP_{64}$ or DNA1(FAM)-*b*-PMA_{73} was dried by lyophilization. DNA1(FAM)-*b*-$P2VP_{64}$ or DNA1(FAM)-*b*-PMA_{73} was then dispersed in 400 μL water/glycerol (8:2) mixture and purified by gel electrophoresis using 15 % polyacrylamide gel in 1 x Tris/boric acid/EDTA (TBE) buffer at 200 V for 35 min. Because DNA1(FAM)-*b*-$P2VP_{64}$ and DNA1(FAM)-*b*-PMA_{73} forms micelles in aqueous solution, it remained in the loading well. The gel containing DNA1(FAM)-*b*-$P2VP_{64}$ or DNA1(FAM)-*b*-PMA_{73} was cut and incubated in water overnight with vigorous stirring. The extracted DNA1(FAM)-*b*-$P2VP_{64}$ or DNA1(FAM)-*b*-PMA_{73} strands were separated from the gel by the filtration (0.2 μm cellulose acetate membrane filter). Finally, the extracted DNA1(FAM)-*b*-$P2VP_{64}$ or DNA1(FAM)-*b*-PMA_{73} was dialyzed against water for 2 days to remove salts followed by lyophilization to remove water. The purified DNA1(FAM)-*b*-$P2VP_{64}$ or DNA1(FAM)-*b*-PMA_{73} was stored in DMSO until use.

Estimation of surface DNA density. The radius of the hydrophobic polymer core can be estimated from the aggregation number using (eq S1),[3]

$$\frac{4}{3}\pi \times R_c^3 = \frac{N \times M \times N_{AV}}{\rho} \qquad \text{(S1)}$$

where R_c, N, M, N_{AV}, and ρ are the calculated core radius, the aggregation number for DNA block copolymer micelles, molecular weights of hydrophobic polymers, Avogadro's number, and the density of the PS block (1.05 g/mL)[4] and the PMA block (1.22 g/mL)[5], respectively. The core diameters of DNA block copolymer micelles are calculated to be 20.8 nm and 10.2 nm for DNA-*b*-PS micelles and DNA-*b*-PMA micelles, respectively from the aggregation number and the density of polymers. The DNA density on the surface of PS or PMA core is estimated to be 3.18×10^{13} strands/cm^2 and 1.86×10^{13} strands/cm^2 for DNA-*b*-PS micelles and DNA-*b*-PMA micelles, respectively, by dividing the number of oligonucleotides per micelle (*i.e.*, aggregation number) with the surface area of hydrophobic polymer core. Note that this calculation generates somewhat overestimated values of the DNA density, especially for PMA, as the polymer core is likely to be somewhat swollen in the solution phase. The surface DNA density is calculated to be 8.48×10^{12} strands/cm^2 for DNA-*b*-PS micelles and 2.47×10^{12} strands/cm^2 for DNA-*b*-PMA micelles when using the micelle size obtained from TEM data, which are lower than those obtained using the polymer density. Note that accurate size measurements of soft micelles in the solution phase are quite challenging. As the shape of soft micelles are likely to change upon solvent drying, TEM-based sizing can produce overestimated values, which are translated into underestimated DNA densities especially for less hydrophobic polymers. Nonetheless, both methods point to a larger DNA density for DNA-*b*-PS, providing a clear qualitative comparison of the surface DNA density.

Melting transition curves based on FRET measurements. Melting transition curves were obtained by performing temperature-dependent FRET measurements for DNA block copolymer micelles with DNA1(FAM) corona hybridized with DNA1'(Cy3). Typically, DNA block copolymer micelles (DNA concentration: 100 nM) were mixed with 100 nM DNA1'(Cy3) in 0.1 or 0.3 M PBS. The solutions were kept at room temperature for 2 h to ensure the hybridization between DNA1 and DNA1'. Fluorescence spectra were obtained while increasing temperature from 20 ^{o}C to 80 ^{o}C in 0.1 or 0.3 M PBS using a temperature gradient of 1 ^{o}C/min with 1 min equilibration time at each temperature. The melting temperature of each sample was determined by taking the peak position of the first derivative of the thermal denaturation curve.



Scheme S1. Synthetic scheme of PS, P2VP, and PMA by RAFT polymerization.

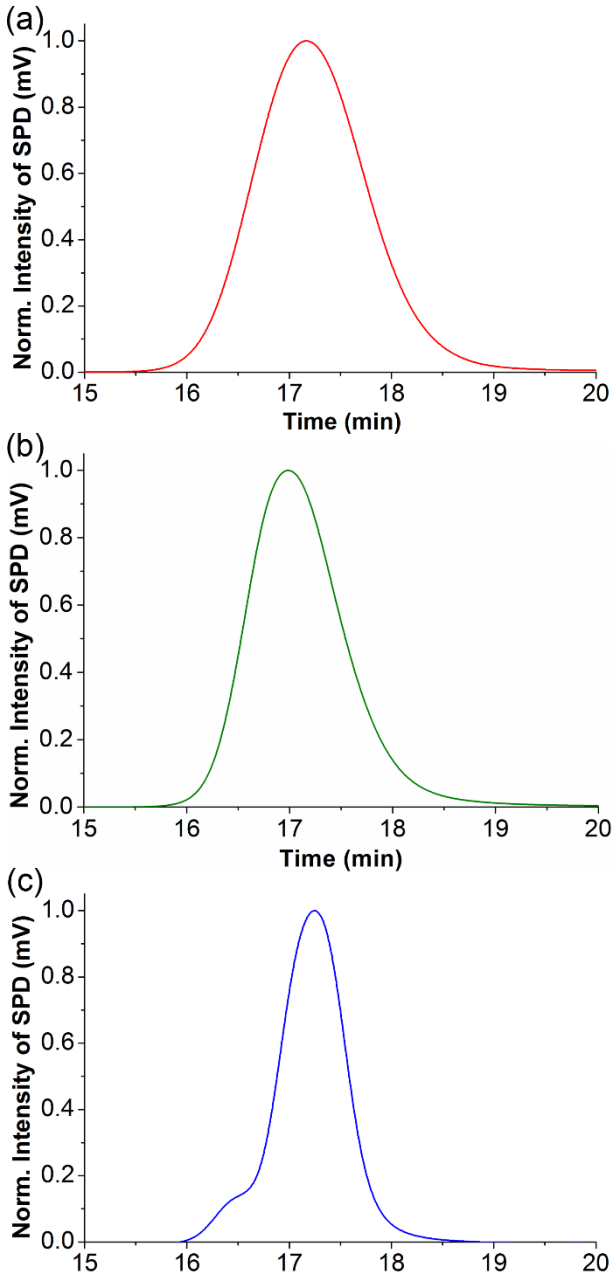


Figure S1. GPC chromatogram (SPD at 254 nm trace) of (a) PS_{62} (red curve), (b) $P2VP_{64}$ (green curve) and (c) PMA_{73} (blue curve).

Table S1. Molecular weights of synthesized polymers.

Polymer	M_n (g/mol)[a]	M_w/M_n[a]
PS_{62}	6900	1.22
$P2VP_{64}$	7100	1.14
PMA_{73}	6700	1.10

[a]M_n and PDI were determined by GPC with polystyrene standards.

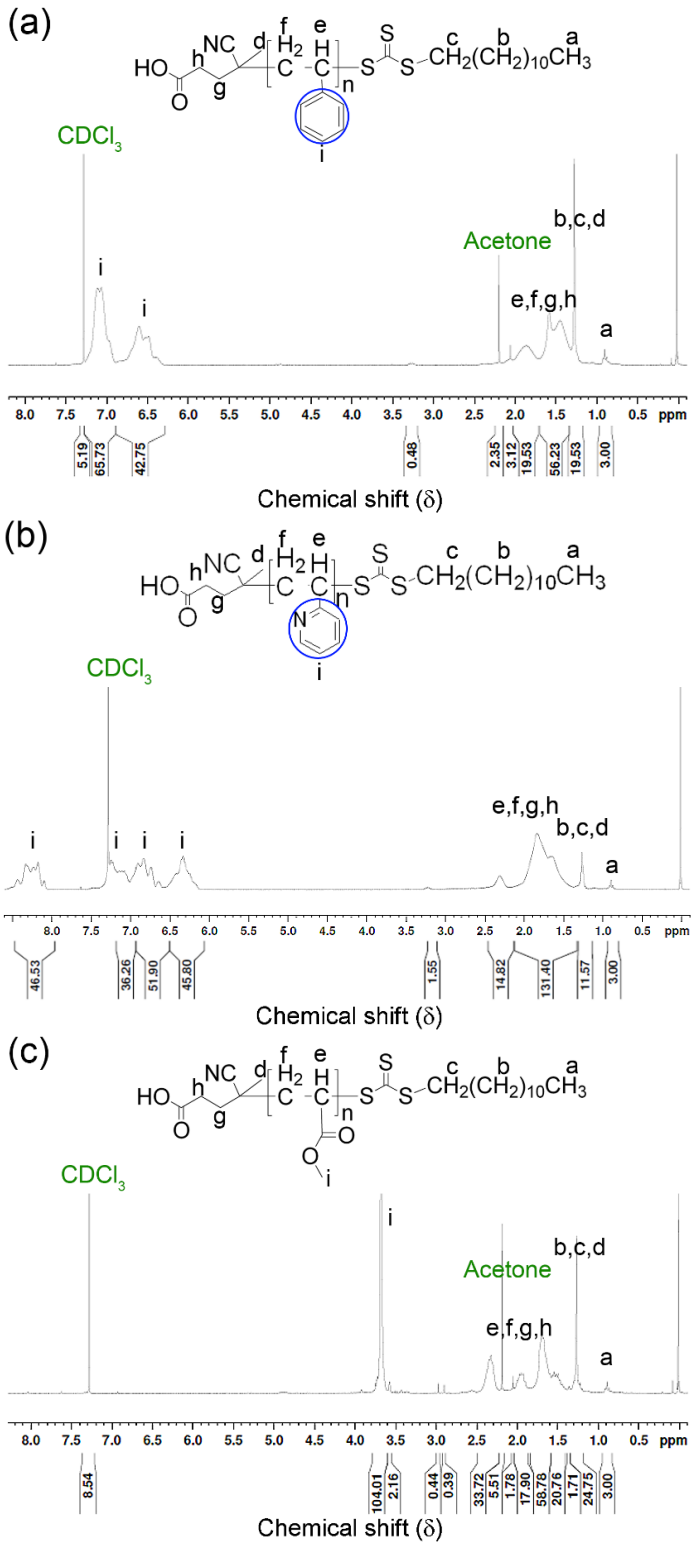


Figure S2. ^{1}H NMR spectra and chemical structures of (a) PS_{62}, (b) $P2VP_{64}$ and (c) PMA_{73}.

Table S2. DNA sequences

Name[a]	Sequence
DNA1(FAM)	5'-A_{10}-ATCCTTATCAATATT-FAM-3'
DNA1'(Cy3)	5'-Cy3-AATATTGATAAGGAT-T_{10}-3'

[a]DNA1 and DNA1' are complementary to each other.

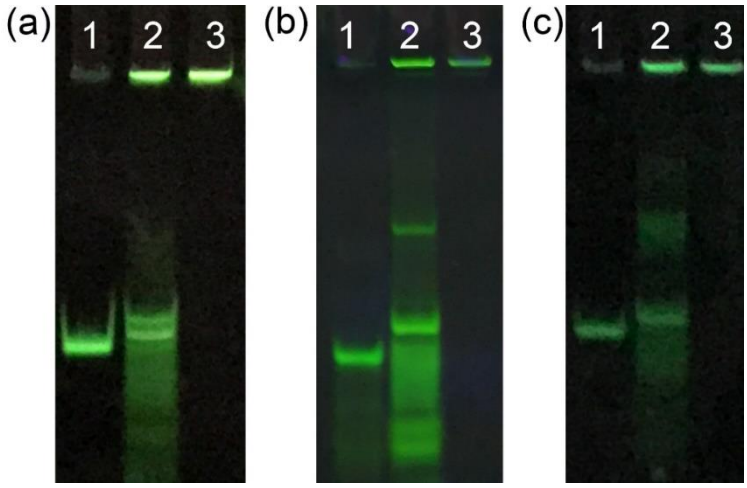


Figure S3. 15 % PAGE gel electrophoresis data. (a) Lane 1: Plain DNA1(FAM), Lane 2: crude sample (before purification), Lane 3: purified DNA1(FAM)-*b*-PS_{62}. (b) Lane 1: Plain DNA1(FAM), Lane 2: crude sample (before purification), Lane 3: purified DNA1(FAM)-*b*-$P2VP_{64}$. (c) Lane 1: Plain DNA1(FAM), Lane 2: crude sample (before purification), Lane 3: purified DNA1(FAM)-*b*-PMA_{73}.

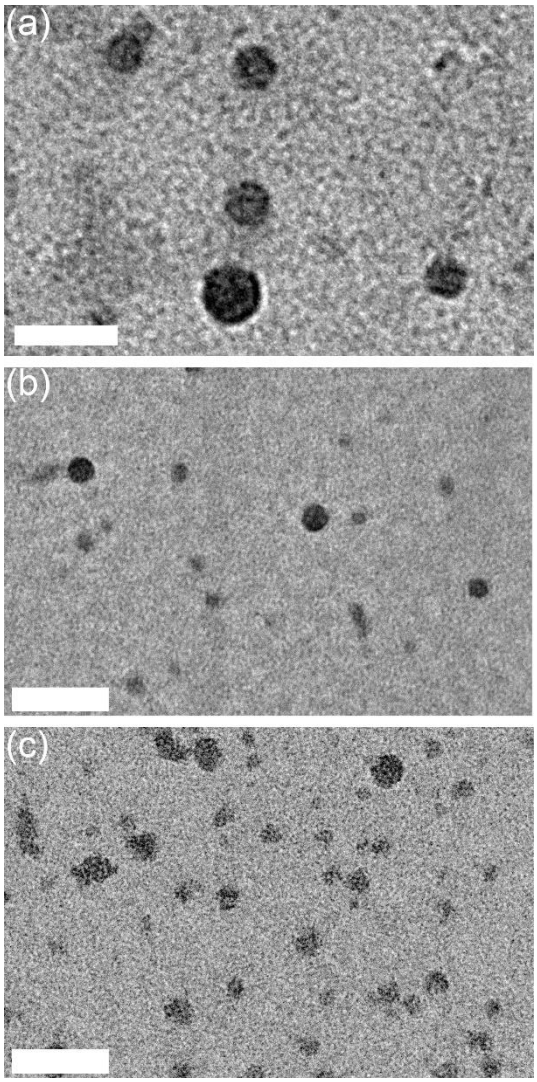


Figure S4. TEM data (scale bars: 100 nm) for (a) DNA1(FAM)-*b*-PS_{62} micelles, (b) DNA1(FAM)-*b*-$P2VP_{64}$ micelles, and (c) DNA1(FAM)-*b*-PMA_{73} micelles.

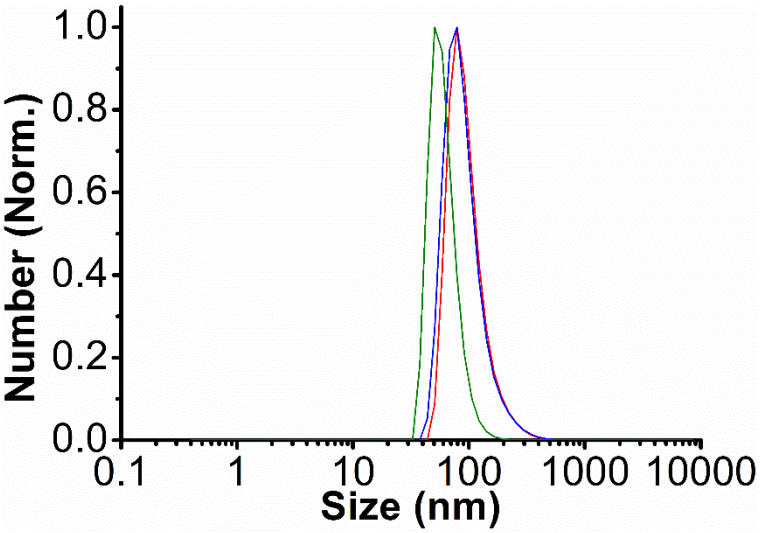


Figure S5. DLS measurements of DNA1(FAM)-*b*-PS_{62} micelles (red), DNA1(FAM)-*b*-$P2VP_{64}$ micelles (green), and DNA1(FAM)-*b*-PMA_{73} micelles (blue) at 25 ºC. The data are the averages of three measurements.

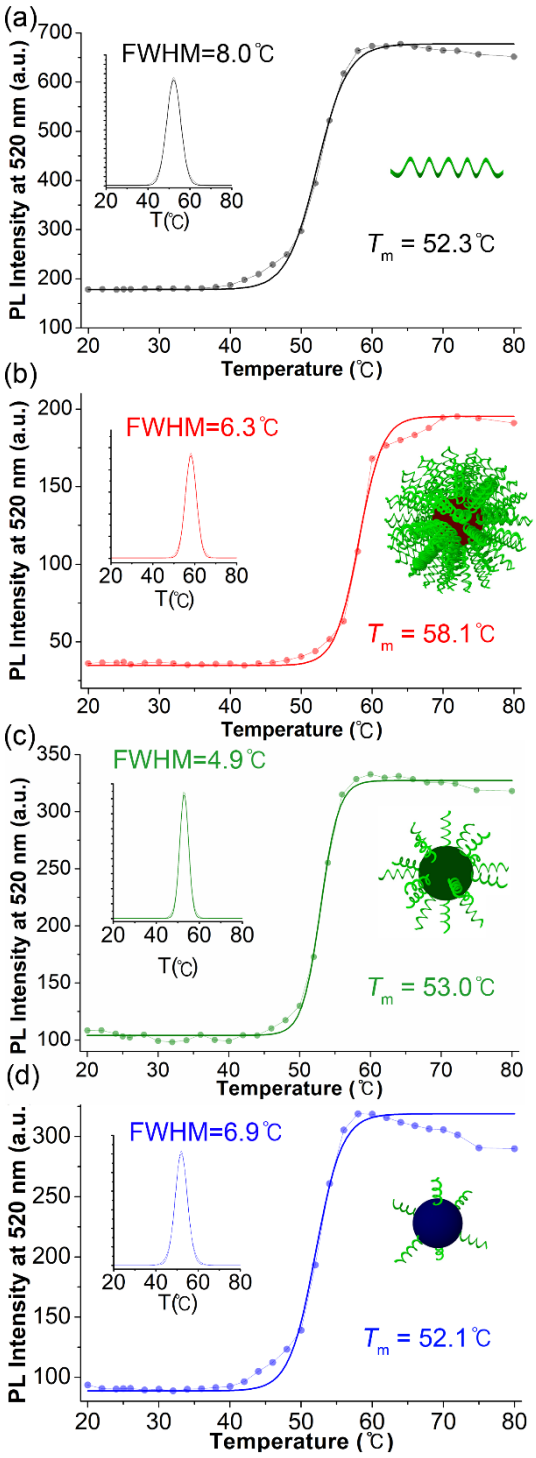


Figure S6. Melting transition curves of (a) plain DNA1(FAM) (black), (b) DNA1(FAM)-*b*-PS_{62} micelles (red), (c) DNA1(FAM)-*b*-$P2VP_{64}$ micelles (green), and (d) DNA1(FAM)-*b*-PMA_{73} micelles (blue) in 0.1 M PBS. The recovery of fluorescence intensity is indicative of dehybridization between DNA1(FAM) and DNA1'(Cy3).

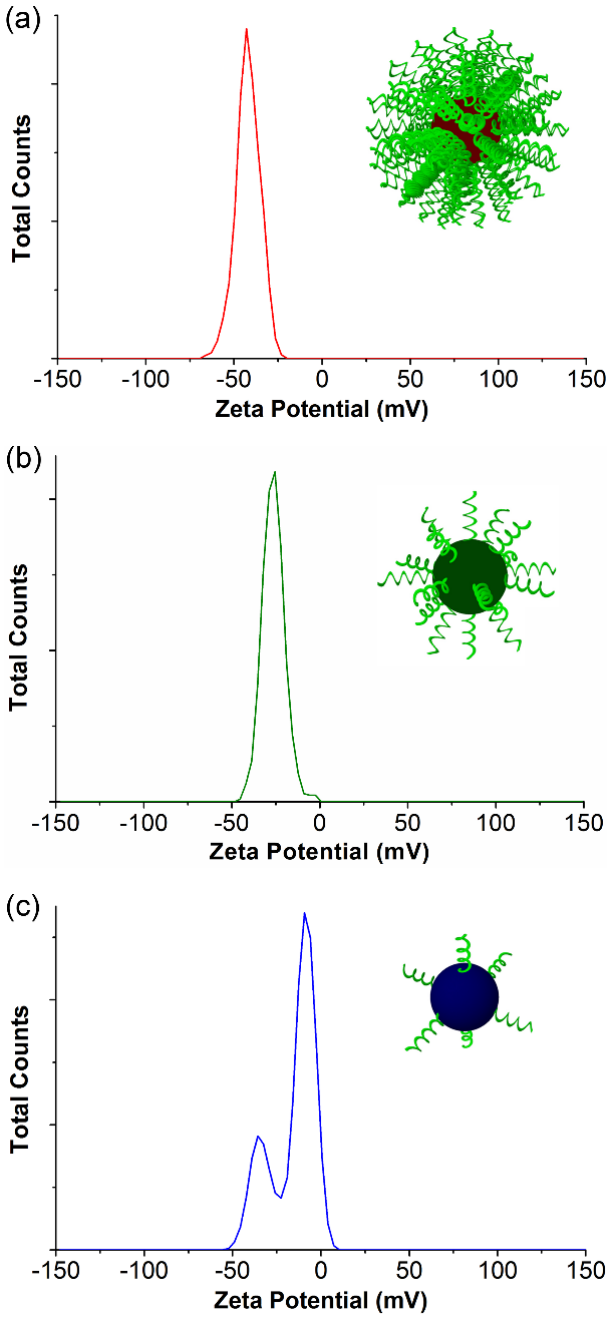


Figure S7. Zeta-potential of (a) DNA1(FAM)-*b*-PS_{62} micelles, (b) DNA1(FAM)-*b*-$P2VP_{64}$ micelles, and (c) DNA1(FAM)-*b*-PMA_{73} micelles.

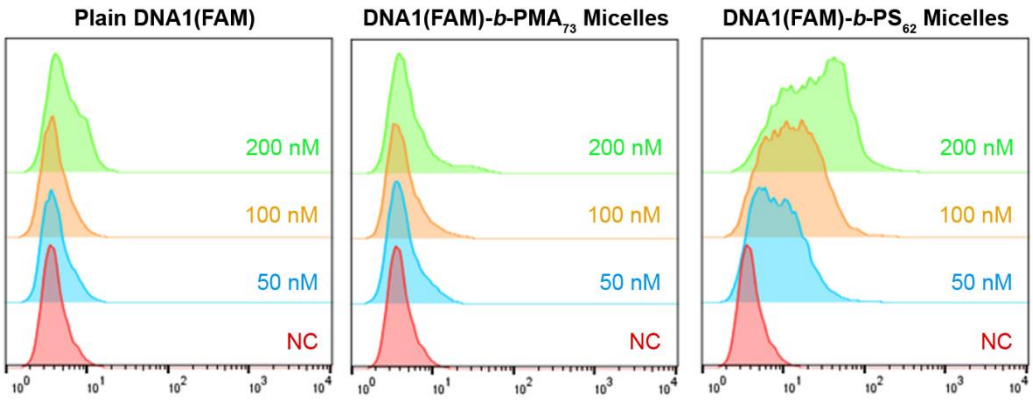


Figure S8. Flow cytometry analysis of intracellular uptake of plain DNA1(FAM), DNA1(FAM)-*b*-PMA_{73} micelles and DNA1(FAM)-*b*-PS_{62} micelles for HeLa cells. For each sample, 10,000 cells (gated events) were counted, and the FAM fluorescence at 488 nm was detected with logarithmic setting.

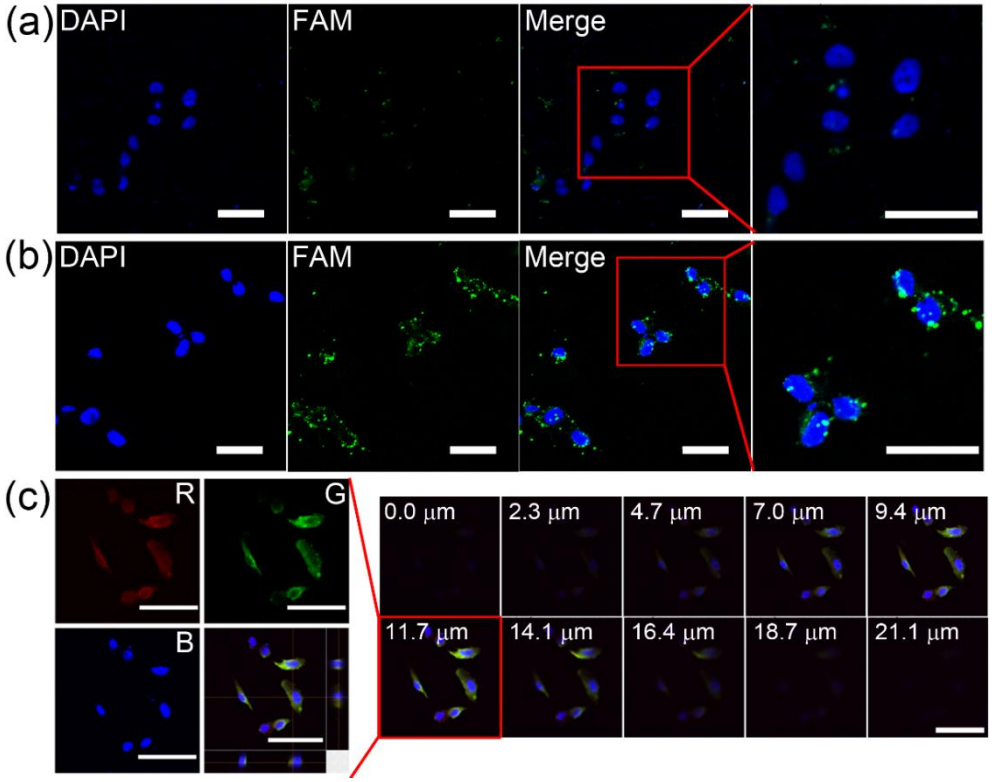


Figure S9. Confocal microscope analysis (scale bars: 50 μm) of (a) DNA1(FAM)-*b*-PMA_{73} micelles and (b) DNA1(FAM)-*b*-PS_{62} micelles after incubation in HeLa cells for 4 h. (c) Confocal microscope analysis including Z-stacks of DNA1(FAM)-*b*-PS_{62} micelles.

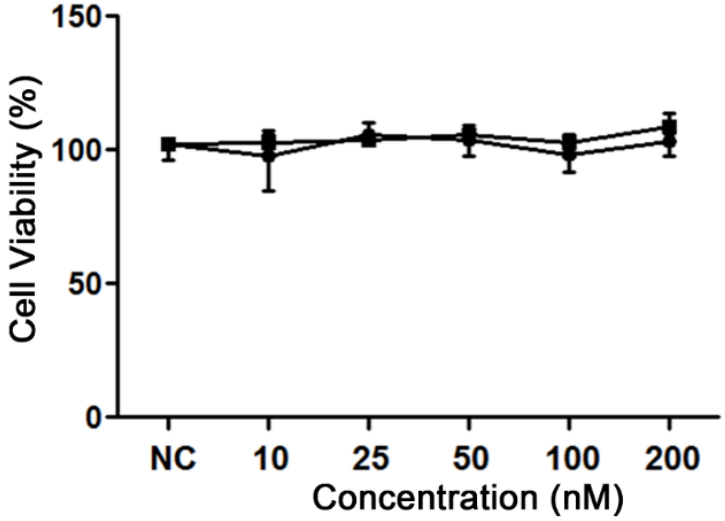


Figure S10. Evaluation of cytotoxicity of DNA1(FAM)-*b*-PMA_{73} micelles (closed cicles) and DNA1(FAM)-*b*-PS_{62} micelles (closed squeares) in HeLa cells.

References

1. A. M. Rush, M. P. Thompson, E. T. Tatro and N. C. Gianneschi, *ACS Nano*, 2013, **7**, 1379-1387.

2. Z. Li, Y. Zhang, P. Fullhart and C. A. Mirkin, *Nano Lett.*, 2004, **4**, 1055-1058.

3. K. Khougaz, I. Astafieva and A. Eisenberg, *Macromolecules*, 1995, **28**, 7135-7147.

4. S. George, R. Champagne-Hartley, G. Deeter, D. Campbell, B. Reck, D. Urban and M. Cunningham, *Macromolecules*, 2015, **48**, 8913-8920.

5. M. R. Whittaker, C. N. Urbani and M. J. Monteiro, *J. Polym. Sci., Part A: Polym. Chem.*, 2008, **46**, 6346-6357.