Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Dynamic DNA Nanostructure with Switchable and Size-Selective Molecular Recognition Properties

Chan-Jin Kim[a], Eun Hye Jeong[b], Hyukjin Lee[b], and So-Jung Park*[a]

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

[b]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

Materials and instrumentations

Oligonucleotides were purchased from Bioneer (Daejeon, Korea). All other reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO) or Fisher Scientific (Hampton, New Hampshire). Ultrapure water (18.2 MΩ) was used for all experiments. Dynamic light scattering (DLS) and zeta potential measurements were performed using Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) with a 632.8 nm laser at a scattering angle of 173°. All DLS measurements were conducted in triplicate. Transmission electron microscopy (TEM) images were obtained using JEM-2100F electron microscope (JEOL Ltd., Tokyo, Japan) operating at 200 kV accelerating voltage. Extinction spectra were obtained using UV-vis spectrophotometer equipped with a temperature controller system (Agilent Technologies, Santa Clara, US). Molecular weights (M_n) and polydispersity index (PDI) of synthesized polymers were estimated 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).

Synthesis and characterization of PS and PS-*b*-PNIPAM

PS_{68} was synthesized by the RAFT polymerization method. Briefly, styrene (15.9 mL, 134 mmol) was 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. The solution was degassed by three freeze-pump-thaw cycles and then heated at 70 °C for 5 h. The polymerization was quenched by opening the cap of the flask and introducing air (i.e., oxygen). The synthesized PS was precipitated by adding MeOH (~200 mL), and the

precipitated polymers were recovered by filtration. The same procedure was repeated three times and the final precipitates were dried in vacuum.

The synthesized PS_{68} was used as a macro-CTA for the synthesis of three PS_{68}-*b*-PNIPAM. PS_{68} (0.1 g, 13.3 μmol), *N*-Isopropylacrylamide (NIPAM) (0.92 g, 8.13 mmol for PS_{68}-*b*-$PNIPAM_{372}$, 0.61 g, 5.39 mmol for PS_{68}-*b*-$PNIPAM_{239}$, or 0.30 g, 2.65 mmol for PS_{68}-*b*-$PNIPAM_{52}$), and V-501 (0.00075 g, 2.68 μmol) were dissolved in 5 mL acetone in a Schleck flask. The reagents were degassed with three freeze-pump-thaw cycles. The polymerization was conducted at 70 °C for 24 h. The reaction was quenched by opening the cap of the flask. The synthesized PS_{68}-*b*-PNIPAM was precipitated using cold ethyl ether (~200 mL) three times and recovered by filtration.

Synthesis of PS-*b*-DNA1(FAM)

PS-*b*-DNA1(FAM) was synthesized by coupling 5'-amine-modified DNA1(FAM) and carboxylic acid-terminated PNIPAM by solid phase synthesis method.[1] For the synthesis of PS_{68}-*b*-DNA1(FAM), PS_{68} (225.0 mg, 30.0 μmol), *N,N*-diisopropylethylamine (DIEA) (52.3 μL, 300 μmol), and HATU (11.4 mg, 29.9 μmol) were first dissolved in DMF and then the solution was vortexed for 10 min to activate carboxylic acid terminus. Subsequently, DNA1(FAM) attached on controlled pore glass (CPG) beads (1 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 DMF (~10 × 5 mL), chloroform (~10 × 5 mL), and acetone (~10 × 5 mL) to remove unreacted polymers and HATU. DNA1(FAM)-*b*- PS_{68} and uncoupled DNA strands were deprotected and cleaved from the CPG beads by the incubation in concentrated ammonium hydroxide at 55 °C for 5 h. After the cleavage reaction, the CPG beads were filtered and washed with water (1 × 7 mL) and then DNA block copolymers were extracted from the beads by DMF (1 × 5 mL).[2] Extracted PS_{68}-*b*-DNA1(FAM) was characterized by 15% polyacrylamide gel electrophoresis in 1 X Tris/boric

acid/EDTA (TBE) buffer at 200 V for 40 min. The DNA bands were visualized by the UV irradiation at 254 nm.

Fluorescence resonance energy transfer (FRET) Efficiency

The FRET efficiency was determined based on the changes in the fluorescence intensity of FAM (donor) with the addition of Cy3-labeled complimentary oligonucleotide (acceptor), using eq S1, where F_D is the donor fluorescence intensity in the absence of the acceptor and F_{DA} is the donor fluorescence intensity in the presence of the acceptor.[3]

$$\text{FRET efficiency (\%)} = \left(1 - \frac{F_{DA}}{F_D}\right) \times 100 \qquad \text{(S1)}$$

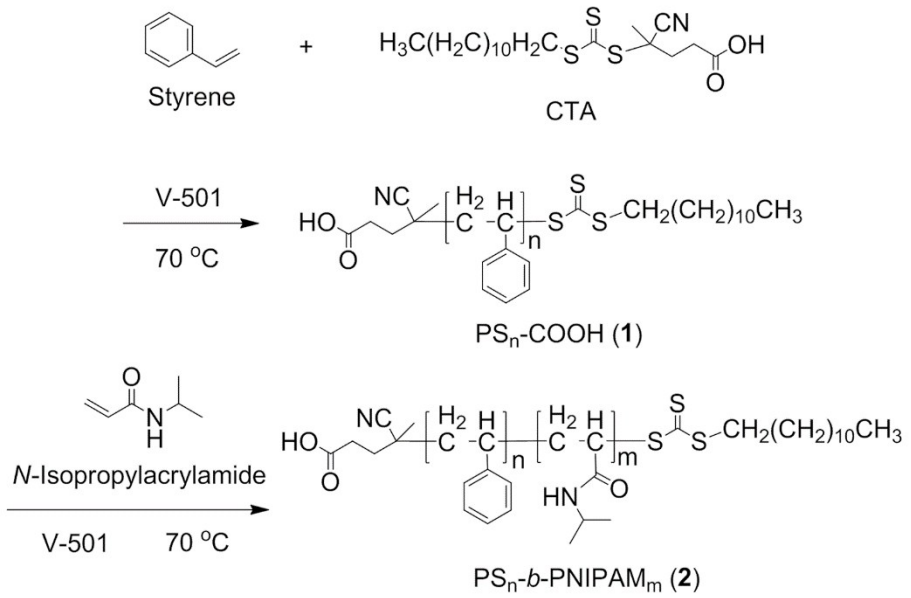
Thermal denaturation curves based on FRET measurements

Temperature-dependent FRET measurements were performed for hybrid micelles and DNA1(FAM) hybridized with DNA1'(Cy3) to obtain DNA thermal denaturation curves. For these experiments, hybrid micelles and DNA1(FAM) were each mixed with DNA1'(Cy3) in 0.3 M PBS, where the concentration of DNA block copolymers, DNA1(FAM), and DNA1'(Cy3) were set to 100 nM, respectively. 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 0.3 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.

Preparation of DNA functionalized gold nanoparticles (AuNP-DNA1')

Gold nanoparticles (AuNPs) were synthesized by well-established citrate reduction method.[4] Briefly, $HAuCl_4$ (0.06 g) was first dissolved in 196 mL water (0.78 mM). This solution was refluxed until boiling, and then 4 mL of 184 mM sodium citrate solution was quickly added to the solution under vigorous stirring. The mixture was refluxed for additional 30 min. The color of the solution changed from yellow to deep red. The average size of the gold nanoparticles was determined to be 10.5 ± 1.0 nm by TEM. Nanoparticle concentration was determined by measuring the optical density (OD) at 520 nm using the extinction coefficient of 1.21×10^8 $M^{-1}cm^{-1}$.[5]

The citrate-stabilized gold nanoparticles were functionalized with thiol-modified DNA according to the procedure reported by Storhoff et al.[5] The concentration of synthesized gold nanoparticles were adjusted to 17 nM. Thiol-modified DNA were added to the solution at a final concentration of 3.61 μM and placed on an orbital shaker at 150 rpm for 16 h prior to salt aging process. In order to avoid particle aggregation, 100 mM phosphate buffer (PB, pH 7) and 1 M NaCl were added slowly over 3 h with 20 min intervals to the final concentration of 10 mM PB (pH 7) and 100 mM NaCl. The solution was kept at this condition for 40 h followed by centrifugation at 12,000 rpm for 30 min to remove excess reagents. The supernatant was discarded and DNA-functionalized AuNPs were redispersed in 0.3 M PBS at a final concentration of 15 nM.



Scheme S1. Synthesis of PS and PS-*b*-PNIPAM by the RAFT polymerization method.

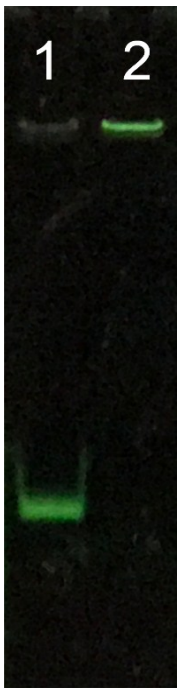


Figure S1. 15 % precast gel electrophoresis data. Lane 1: Plain DNA1(FAM), Lane 2: PS_{68}-*b*-DNA1(FAM).

Table S1. DNA sequences.

Name[a]	Sequence
DNA1(FAM)	5'-A_3-ATCCTTATCAATATTCAA-FAM-3'
DNA1'(Cy3)	5'-Cy3-TTGAATATTGATAAGGAT-3'
Thiol-modified DNA1'	5'-SH-A_{10}-TTGAATATTGATAAGGAT-3'

[a]DNA1 and DNA1' are complementary.

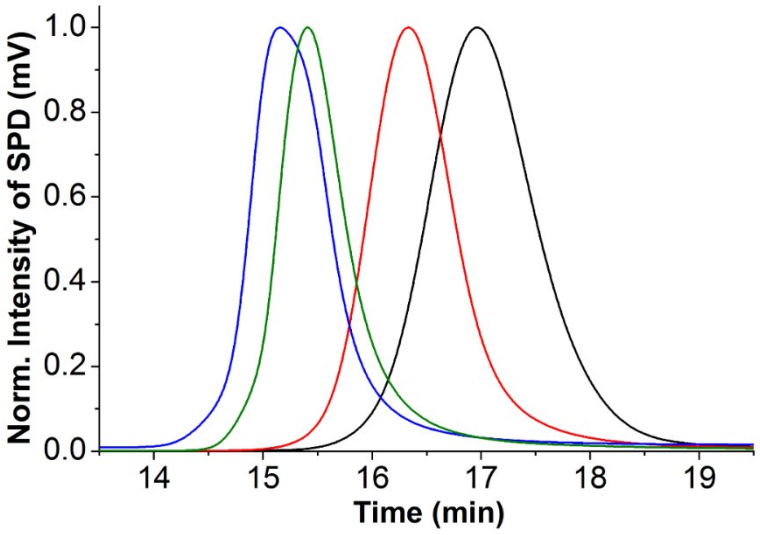


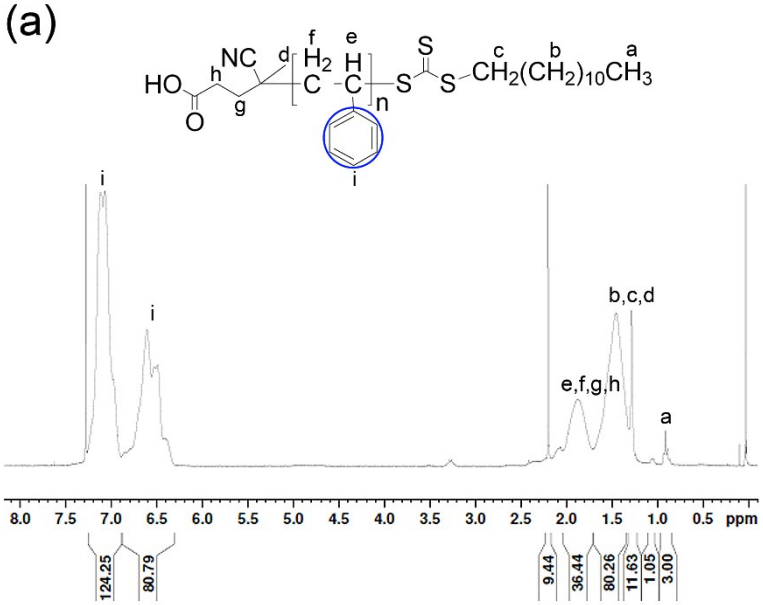
Figure S2. GPC chromatogram (SPD at 254 nm trace) of PS_{68} (black curve), PS_{68}-*b*-$PNIPAM_{372}$ (blue curve), PS_{68}-*b*-$PNIPAM_{239}$ (green curve), and PS_{68}-*b*-$PNIPAM_{52}$ (red curve).

Table S2. Molecular weights of synthesized polymers.

Polymer	M_n (g/mol)[a]	M_w/M_n[a]
PS_{68}	7500	1.19
PS_{68}-*b*-$PNIPAM_{372}$	49600	1.09
PS_{68}-*b*-$PNIPAM_{239}$	34500	1.10
PS_{68}-*b*-$PNIPAM_{52}$	13400	1.11

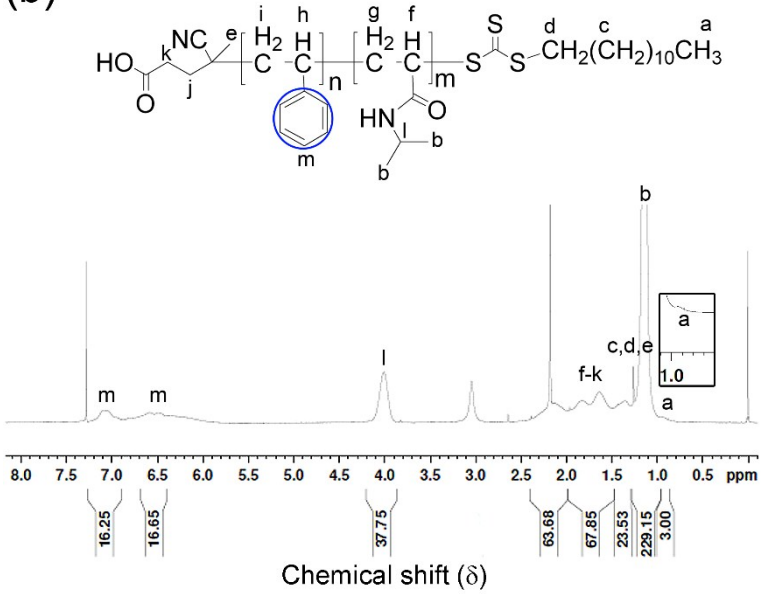
[a]M_n and PDI were determined by GPC with polystyrene standards.

(a)



Chemical shift (δ)

(b)



Chemical shift (δ)

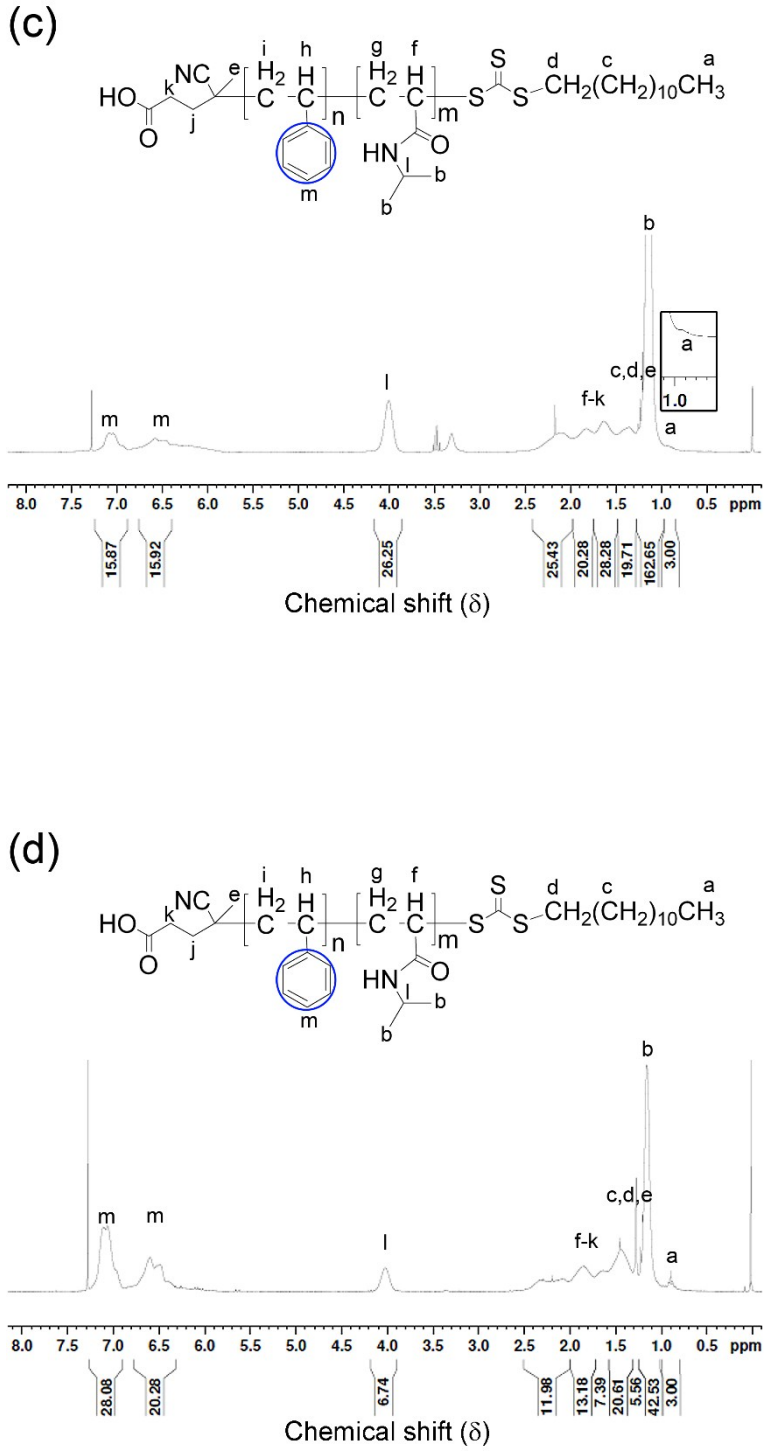


Figure S3. ^{1}H NMR spectra and chemical structures of (a) PS_{68}, (b) PS_{68}-*b*-$PNIPAM_{372}$, (c) PS_{68}-*b*-$PNIPAM_{239}$, and (d) PS_{68}-*b*-$PNIPAM_{52}$.

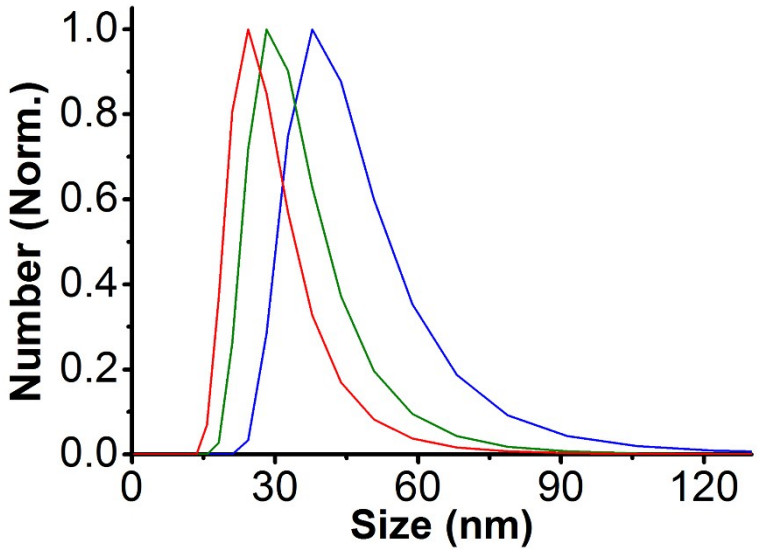


Figure S4. DLS data of switchable micelles prepared by the binary self-assembly of SDM_{372} (blue), SDM_{239} (green), SDM_{52} (red) at 25 ^{o}C. These hydrodynamic diameters, detected by DLS, are the average of three measurements.

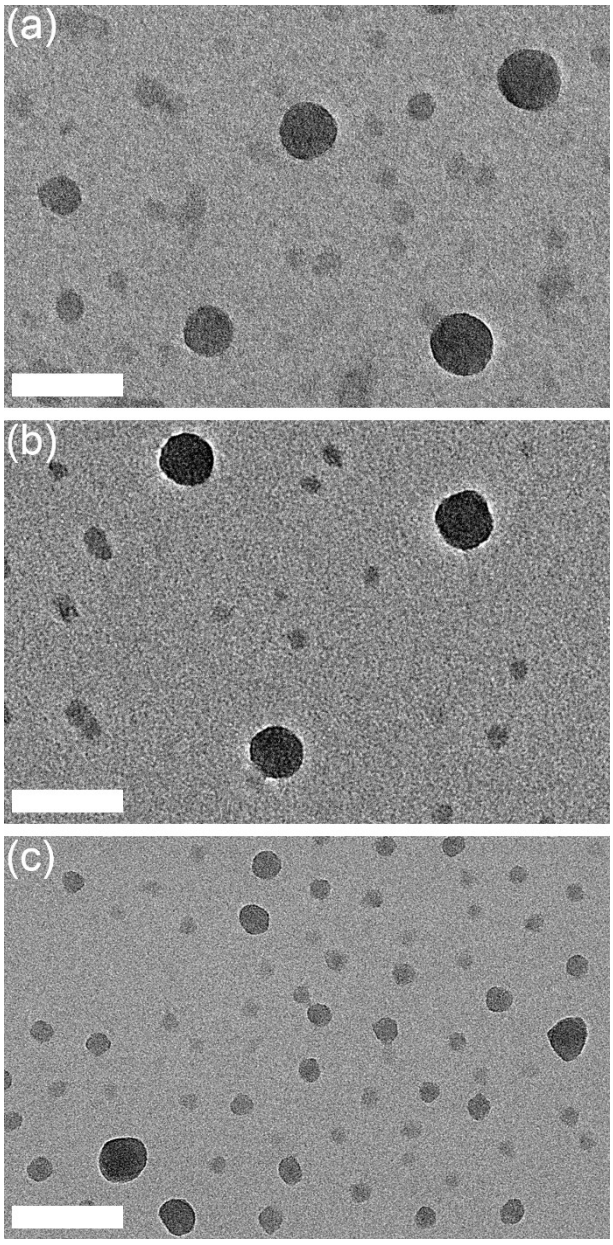


Figure S5. TEM images of (a) SDM_{372}, (b) SDM_{239}, and (c) SDM_{52} (scale bars: 100 nm).

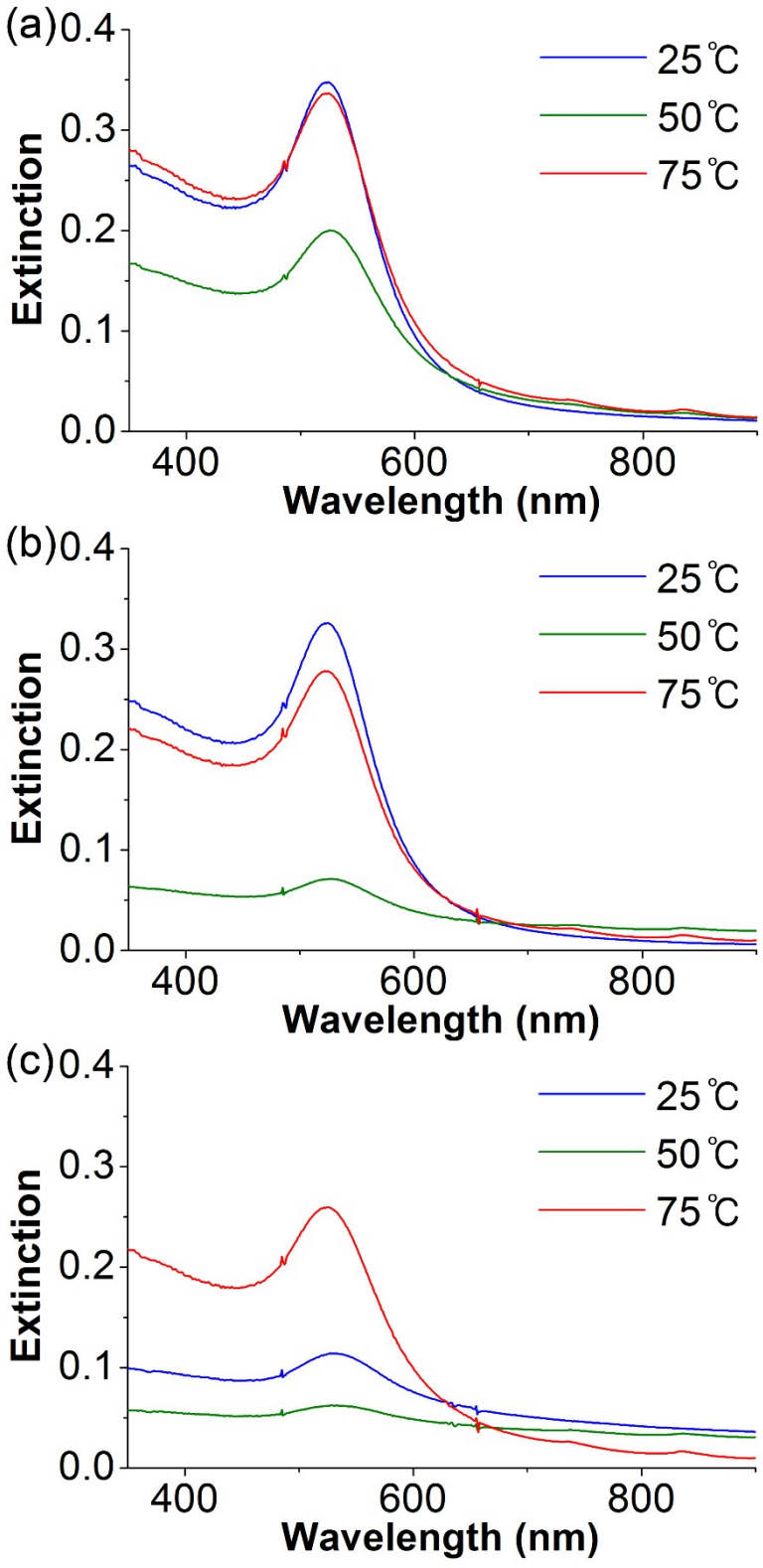


Figure S6. Extinction spectra of AuNP-DNA1' mixed with (a) SDM_{372} (blue), (b) SDM_{239} (green), and (c) SDM_{52} (red) in 0.3 M PBS at each temperature.

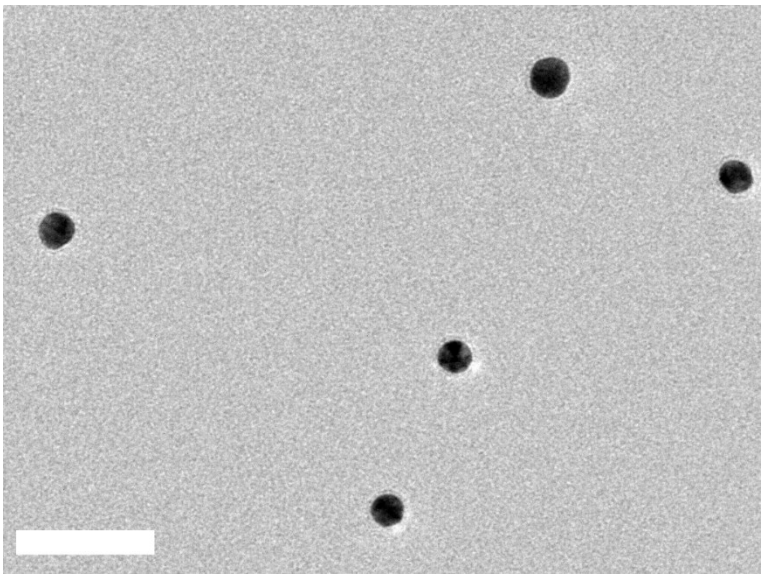


Figure S7. TEM images of AuNP-DNA1' mixed with SDM_{239} in 0.3 M PBS at 75 ^{o}C (scale bars: 50 nm).

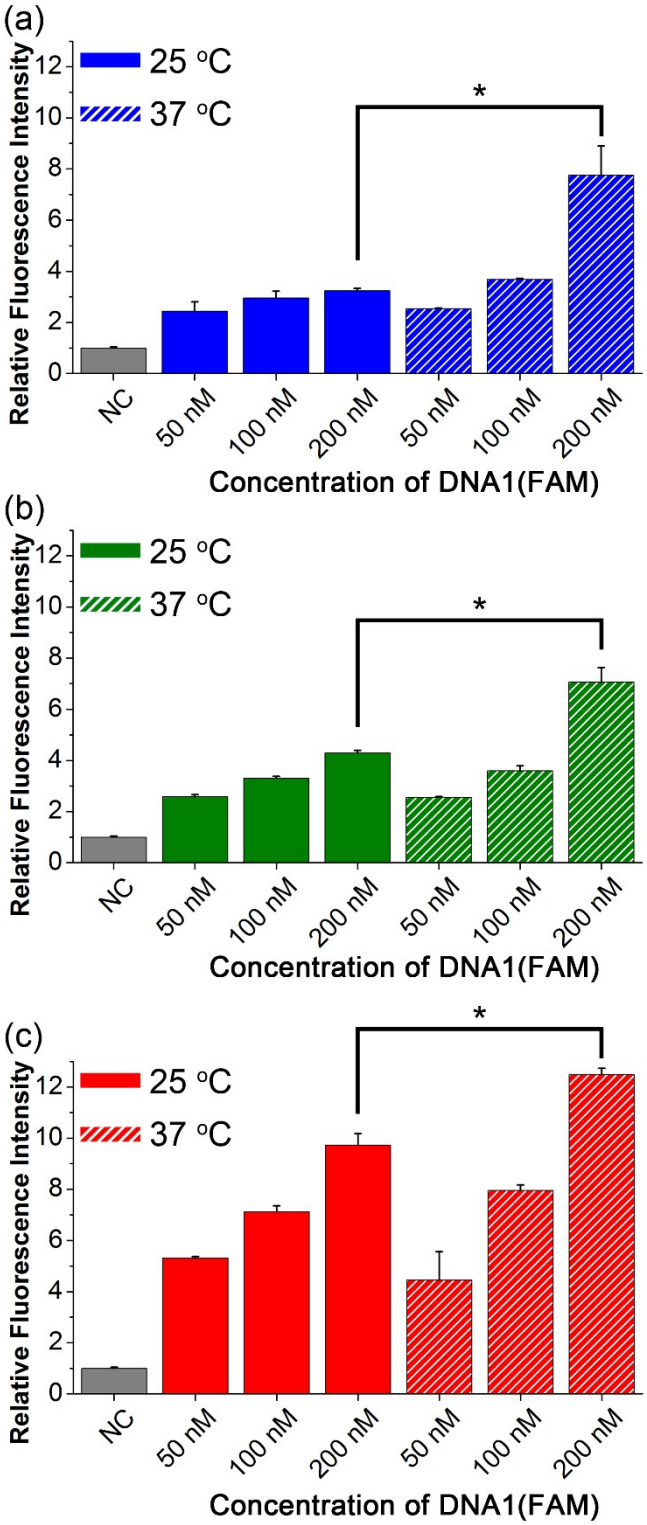


Figure S8. Flow cytometry analysis on the intracellular uptake of (a) SDM_{372} (blue), (b) SDM_{239} (green), and (c) SDM_{52} (red) in HeLa cells at 25 °C and 37 °C (*$p < 0.05$). NC represents negative control.

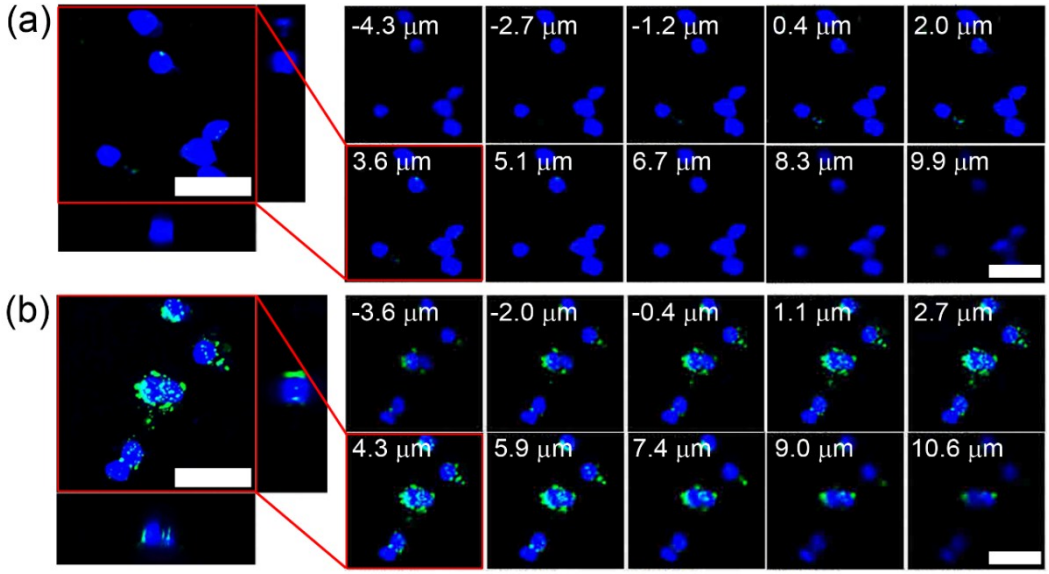


Figure S9. Confocal microscope analysis including Z-stacks on the intracellular uptake of SDM_{372} (200 nM PS_{68}-*b*-DNA1(FAM) and 800 nM PS_{68}-*b*-$PNIPAM_{372}$) in HeLa cells at (a) 25 ^{o}C and (b) 37 ^{o}C (scale bars: 50 μm).

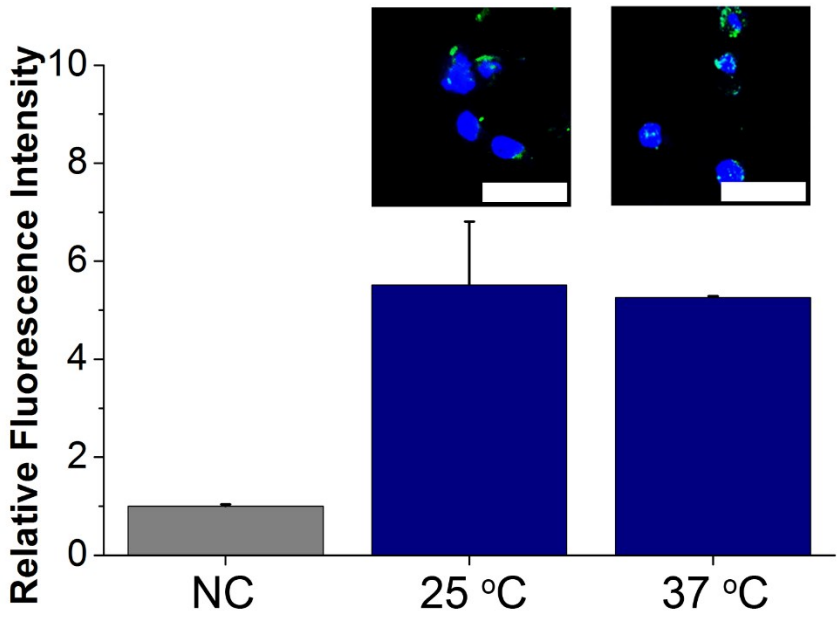


Figure S10. Relative intracellular uptake of PS-*b*-DNA micelles at 25 °C and 37 °C for 200 nM DNA block copolymer concentration estimated by flow cytometry and confocal microscope analysis (scale bars: 50 μm).

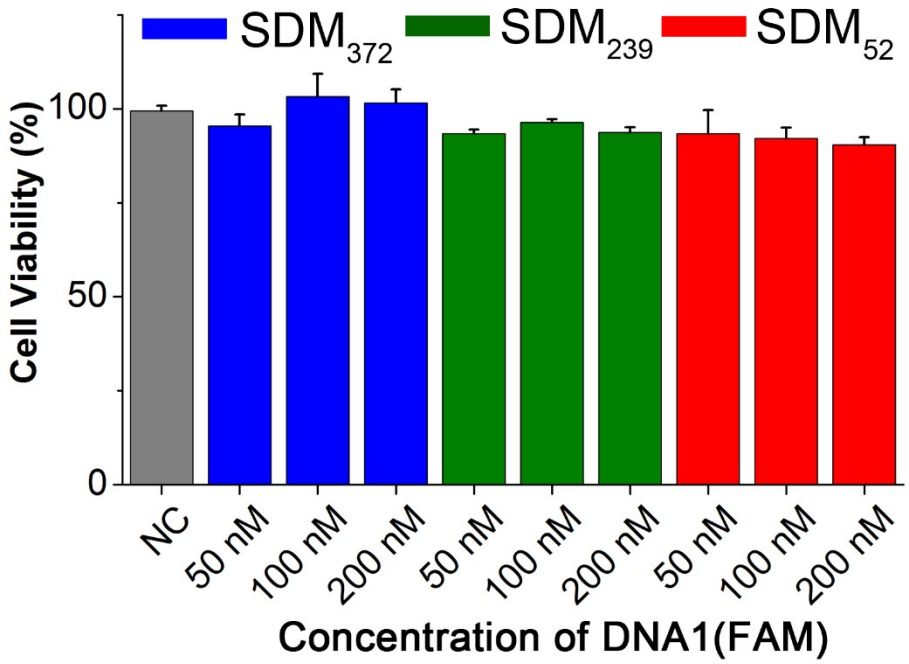


Figure S11. Evaluation of cytotoxicity test in HeLa cells of SDM_{372} and SDM_{239}, and SDM_{52}.

References

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4. J. Turkevich, P. C. Stevenson and J. Hillier, *Discuss. Faraday Soc.*, 1951, **11**, 55-75.
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