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Electronic Supplementary Information

Biophysical and chemical handles to control the size of DNA nanoparticles produced by rolling circle amplification

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Table S1. DNA sequences for the primer and templates. Primer binding sequences are underlined.

		Sequence (5′ to 3′)
Primer		CTCTGGTGAGGACAGGACTT
Template	T1	CTCACCAGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGTCCTGTC
	T2	CTCACCAGAGCCGCATGCCATCCGCCCAGTTGACAAAAAAAC CGCATGCCATCCGCCCAGTTGACAAGTCCTGTC
	T3	CTCACCAGAGCCACCACCACCAACACCACCACCACCAAAAA AACCACCACCACCAACACCACCACCACCAAGTCCTGTC

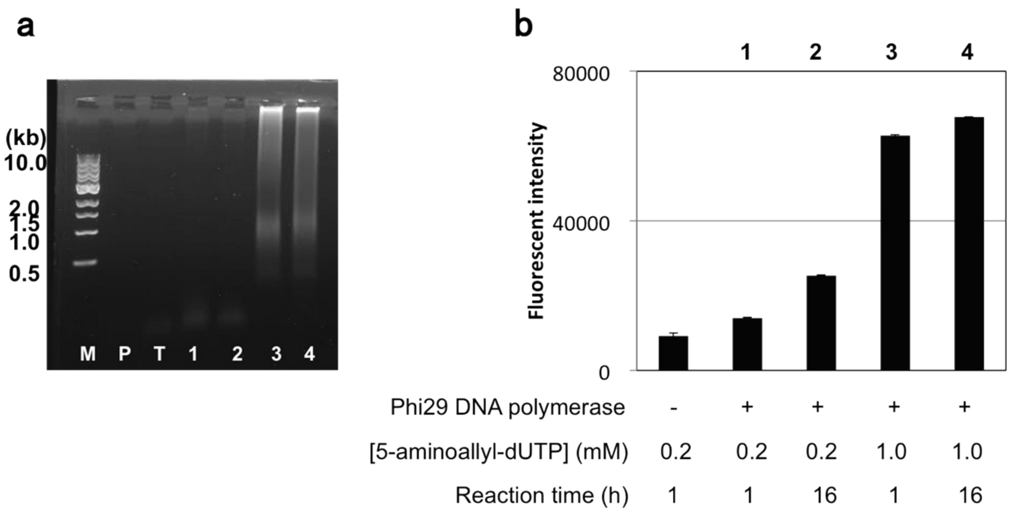

Figure S1. (a) Agarose gel (0.5%) analysis of the products obtained after RCA reactions for 1 (lane 1 and 3) and 16 h (lane 2 and 4) at 0.2 (lane 1 and 2) and 1.0 mM (lane 3 and 4) of 5-aminoallyl-dUTPDNA in the absence of dTTP. (b) Fluorescence intensity of SYBR gold-stained RCA products. M, P, and T denote size marker, primer, and template, respectively.

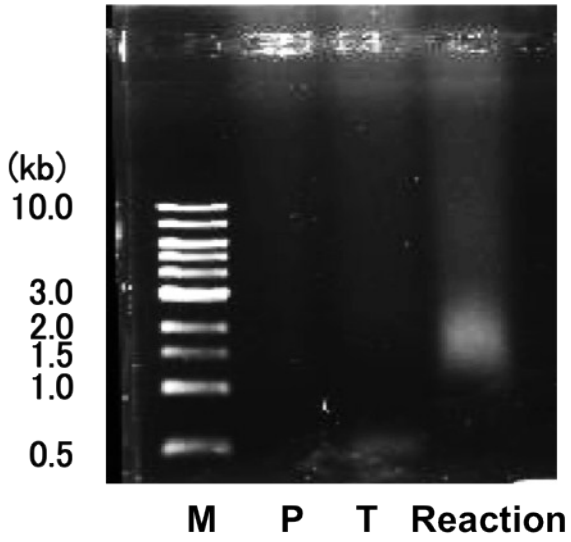

Figure S2. The product of the RCA reaction using Therminator DNA polymerase (from New England Biolabs Inc.) was analyzed on agarose gel (0.5%). The reaction was performed at 75°C by using the same manner employed for phi29-mediated RCA reactions. M, P, and T denote size marker, primer, and template, respectively.

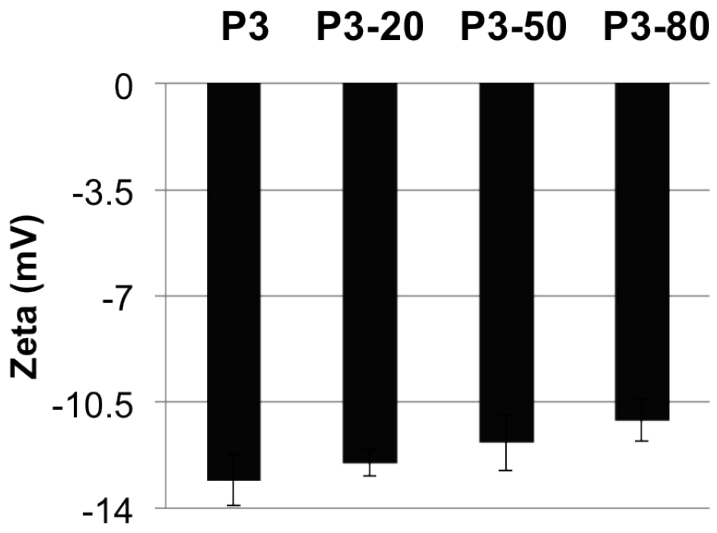

Figure S3. Zeta-potentials of DNA nanoparticles produced by RCA reactions.

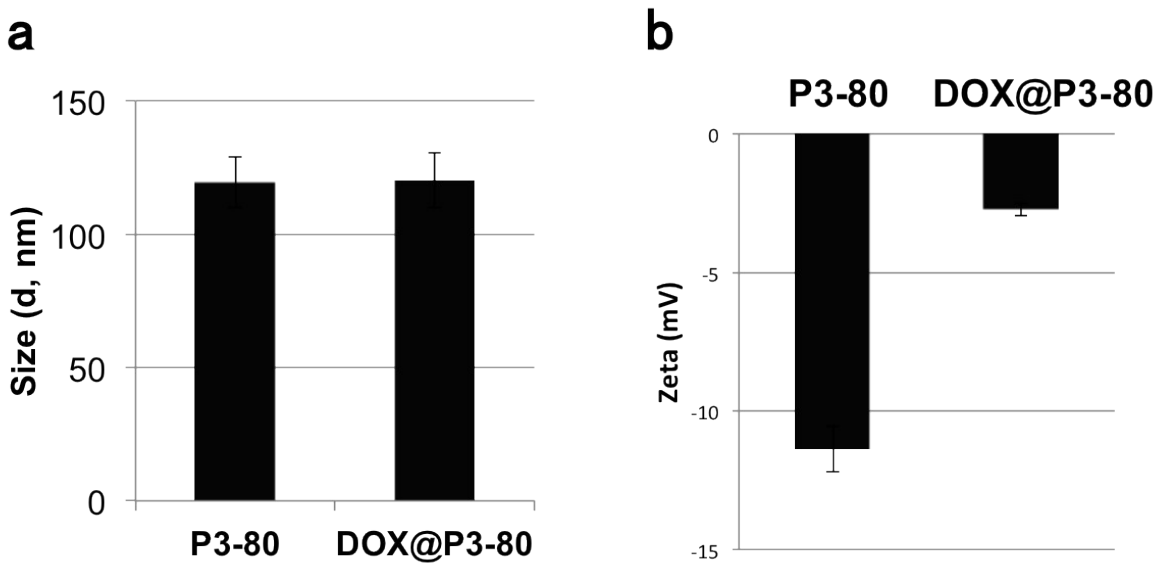

Figure S4. (a) Sizes of P3-80 and DOX@P3-80 measured by dynamic light scattering (DLS) (b) Zeta-potentials of P3-80 and DOX@P3-80.

Preparation of circular template for RCA reaction

5'-Phosphorylated T1, T2 and T3 (Bioneer, Korea) (Table S1) (10 µM each) were incubated with primer (15 µM), 10X ligation buffer (1X) and T4 DNA ligase (400,000 units/ml) (NEB, UK) for overnight at 16°C. After ligation, each circular template was purified by 10% non-denaturing gel electrophoresis followed by ethanol precipitation as described in the literature[1] Concentrations of the circular templates were determined by UV absorption at 260 nm. The extinction coefficients of the T1, T2 and T3 were calculated using the Oligo Analyzer 3.1 (www.idtdna.com).

RCA reaction

The solutions containing circular templates (150 nM), dNTP (0.2 mM) (Promega, USA), 5'allylamino dUTP (TriLink Biotechnologies, USA) BSA (1X), primer (150 nM), phi29 polymerase (10,000 units/ml) (Lucigen, USA) and its reaction buffer (1X) were incubated for 1 h at 37°C. Then, reaction mixtures were inactivated for 10 min at 65°C.

Agarose gel electrophoresis

Agarose gels (0.5%) (Lonza, USA) were run in 0.5X TBE buffer (Biosesang, Korea) with 100V for 45 min at 25°C. After electrophoresis, the images were visualized using fluorescence scanner (GelDoc, DNR, Israel). RCA products were stained by SYBR gold (1X) (Molecular Probes, USA).

Dynamic light scattering (DLS)

The hydrodynamic sizes of the RCA products were measured in Zetasizer (Malvern, UK) by following a literature procedure.[2] The concentration of samples used for the DLS analysis was 150 nM.

Measurement of fluorescence intensity of SYBR gold-stained RCA products

RCA products were stained with SYBR gold for 15 min at 25°C. Fluorescence intensity of the solutions was measured by using a fluorescence microplate reader (Appliskan, Thermo Fisher Scientific, USA). Excitation at 485 nm and emission at 513 nm were employed for the measurement.

Atomic force microscopy (AFM)

AFM samples (10 µM) were deposited onto freshly cleaved mica (Pelco Mica sheets, Ted Pella, USA). After 1 h, the mica surface was rinsed with distilled water and immediately dried using nitrogen gas. The samples were scanned in non-contact mode on a Park XE-100 (park system Corp., Korea) with PPP-NCHR tips (Park systems Corp., Korea).[3] AFM data were analyzed using XEI 4.1.0 software.

Preparation of modified RCA products for drug delivery

The RCA products (P1-P3, P3-20, P3-50 and P3-80) were dialyzed in 0.1X PBS for overnight at 25°C by using Amicon filters (MWCO=100K, Millipore, USA). The samples were lyophilized and then resuspended in distilled water and quantified. The concentrations of the samples were determined by UV absorption at 260 nm.

Transfection of RCA products into drug resistant breast cancer cells

MCF-7/ADR cells were kindly donated from Dr. Dae-Duk Kim (College of Pharmacy, Seoul National University, Seoul, Korea).[4] MCF-7/ADR cells were plated in glass-bottomed 35 mm dishes (SPL, Korea) with RPMI 1640 media (Gibco, USA) containing 10% heat inactivated fetal bovine serum (Gibco, USA), 1% penicillin and streptomycin (Gibco, USA). After cells (2.5×10^4) were seeded in each dish, the dishes were incubated overnight at 37°C in humidified atmosphere containing 5% CO_2. Growth medium was removed from each cell sample, and the cells were washed twice with DPBS (Welgene, Korea). RCA products (10 nM) in fresh media (250 µL) lacking serum and the antibiotics was then added to a sample of cells and incubated for 6 h at 37°C in humidified atmosphere containing 5% CO_2. Cells then were washed twice with PBS and used for microscopy experiments.

Microscopic imaging of RCA products in MCF-7/ADR cells

For microscopic analysis, the nuclei were stained using 3 µg/mL Hoechst 34580 (Invitrogen, USA), and the cells were washed with DPBS (200 µL) twice. DPBS (200 µL) was then added. Live cells were imaged using a fluorescence microscopy (LSM 700, Carl Zeiss Microscopy, Germany). Excitation/emission filters used for Cy5 and Hoechst 34580 were 630-650/665-705 nm, and 340-380/432-482 nm, respectively.

Flow cytometry analysis

Cells were seeded on 12-well culture plates (SPL, Korea) and cultured for 24 h at 37°C with

5% CO_2 and then washed twice with DPBS. They were incubated with the Cy5-labeled RCA products prepared by using Cy5-labeled primer (10 nM) for 6 h at 37°C with 5% CO_2, harvested, and washed three times with DPBS. Then, 0.2 mL of trypsin replacement (TrypLE™, Gibco, USA) was added to each sample, and the samples were incubated for 5 min at 37°C. Then, 1 mL of media 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 1 mL of DPBS. Fluorescence intensity of the cells was determined by a flow cytometry (Guava, Millipore, USA). Samples of at least 10000 cells were analyzed in triplicate.

Preparation of DOX@P3-80

For DOX intercalation into the RCA products, DOX (1 μM) (Sigma-Aldrich, USA) was mixed with the P3-80 (1, 5 and 10 nM) for 1 h at RT.

Cellular uptake of free DOX and DOX@P3-80

MCF-7/ADR cells were plated in glass-bottomed 35 mm dishes with RPMI1640 media containing 10% heat inactivated fetal bovine serum, 1% penicillin and streptomycin, and incubated at 37°C overnight in humidified atmosphere containing 5% CO_2. The culture media were replaced with the fresh media containing free DOX (1 μM) or DOX (1 μM)-loaded P3-80 (DOX@P3-80), and then incubated at 37°C for 6 h in the CO_2 chamber. The cells were examined under the fluorescence microscopy using the same procedure described above. Excitation/emission filters used for DOX were 480-500/509-547 nm. For flow cytometry analysis, MCF-7/ADR cells were seeded with complete RPMI media (1 mL) onto 12-well plates and incubated at 37°C in the CO_2 chamber for 24 h. The media were replaced with the fresh media containing free DOX (1 μM) or DOX (1 μM)-loaded P3-80 (DOX@P3-80), and then incubated at 37°C for 6 h in the CO_2 chamber. The cells were washed with DPBS twice, harvested and suspended in DPBS (1 mL) for the flow cytometry

analysis with a green fluorescence filter.

Cell viability analysis

Cytotoxicity of free DOX and DOX loaded P3-80 were estimated via CCK-8 assay kit (Dojindo laboratories, Japan). MCF-7/ADR cells were seeded in 96-well culture plate (SPL, Korea) and incubated at 37°C in humidified atmosphere containing 5% CO_2. When the confluency of cells was 80%, the cells were washed with DPBS. Free DOX and DOX@P3-80 at various concentrations were added into the plate and incubated for 24 h at 37°C. The plates were washed twice with DPBS and treated with 10 µL CCK-8 solution in fresh medium (100 µL) lacking serum and the antibiotics. After 4 h incubation, the absorbance was measured at 450 nm using a microplate reader (SpectraMax™ Plus, Molecular Devices, USA).

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