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UV light-triggered unpacking of DNA to enhance gene transfection of azobenzene-containing polycations

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Materials: 2-Bromoisobutyryl bromide (98%), N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA, 99%), diethyl meso-2,5-dibromoadipate (DEDBA, 98%), branched polyethylenimine (PEI, 25 kDa, PEI25k) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, 99%) were obtained from Sigma-Aldrich Chemical Co. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 97%), 4,4'-diaminoazobenzene (95%), 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT, 98%) and CuCl (99.999%) were supplied by Alfa Aesar Co. YOYO-1 (1 mM solution in DMSO) was obtained from Invitrogen Co. All other chemicals were analytical grade and used without further purification. Plasmid pGL3-control with SV40 promoter and enhancer sequences encoding luciferase (5262 bp) was obtained from Promega. pEGFP-C1 encoding a red-shifted variant of wild-type green fluorescent protein (GFP) was purchased from Clontech. These plasmids were amplified in *Escherichia coli* and purified by the differential precipitation method.

Instrumentation and characterization: ^{1}H NMR spectra of azobenzene-based initiator and azobenzene-containing PDMAEMA were measured with a UNITY plus-500 NMR spectrometer (Varian, USA) using DMSO-d_6 and D_2O as solvent, respectively. The molecular weights and the molecular weight distributions of the

polymers were determined by gel permeation chromatography (GPC, Waters 510/M32). The mobile phase was THF and monodisperse polystyrene was used as calibration standard. UV-Vis absorption spectra of initiator in THF and polymer in water solutions were recorded on a Shimadzu UV-3600 spectrophotometer at room temperature.

Gel retardation assay: The DNA condensation ability of polymers was investigated by gel retardation. Polymer/DNA complexes were formed as described in Section 2.3. Then the complex solutions with loading buffer were loaded in a well for electrophoresis assay on a 1% agarose gel with ethidium bromide staining at 100 V for 40 min. The results were visualized by irradiation under UV light. To determine DNA dissociation from the complex under UV irradiation, the complexes were incubated for 20 min as described in Section 2.3, then for 10 min under UV irradiation, and analyzed by agarose gel electrophoresis in dark place.

Transmission electron microscopy: The morphology and size of the polymer/pDNA complexes at the selected weight ratios corresponding to the maximum transfection efficiency were observed by JEM-100CXII TEM (Jeol, Japan). Briefly, a 5 μl of each sample was carefully dropped on a copper grid for 5 min, and negatively stained by 1.5 wt% phosphotungstic acid (pH 6.7). Then the samples were imaged at an accelerating voltage of 100 kV.

Measurement of particle size and zeta potential: The particle size and zeta potentials of complexes at different weight ratios were measured using Zetasizer Nano ZS instrument (MALVERN Instrument). The complex solutions prepared as

described in Section 2.3 were diluted with PBS (pH 7.4) to the final pDNA concentration of 5 μg/ml. The measurements were carried out at 25 °C.

DNase I protection assay: 5 μl of complex solutions (0.5 μg pDNA) at desired weight ratios were prepared as described in Section 2.3. Then the complexes or naked pDNA were incubated with 4 μl 10× reaction buffer (20 mM Tris-HCl, 20 mM $MgCl_2$, pH 8.3) containing 1 unit of DNase I at 37 °C for 5, 30, 60 and 120 min. The reaction was terminated by addition of 3 μl 200 mM EDTA solution, and DNase I was denatured by further incubating for 10 min at 70 °C. Finally the samples were incubated with 20 μl 10 mg/ml heparin solution for another 3 hours to allow complete DNA dissociation from complexes and analyzed by agarose gel electrophoresis.

Cell culture: COS-7 cells (African green monkey kidney cells), HepG-2 cells (Human hepatoblastoma cells) and CHO-K1 cells (Chinese hamster ovary cells) were obtained from Peking Union Medical College (Beijing, China). COS-7 cells and HepG-2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone) with high glucose, containing 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin-streptomycin (Invitrogen) at 37 °C in 5% CO_2 humidified atmosphere. CHO-K1 cells were maintained in Ham's F-12 Medium (F-12, HyClone) with 10% FBS and 1% antibiotics.

Differential scanning calorimetry: 1 mg DPPC was dissolved in chloroform and then transferred to an aluminum pan. After evaporation of organic solvent, the dry lipid film was formed at the bottom of aluminum pan. The film was then hydrated in 10 μl aqueous solution of polymer (0.1 mg/μl) or in pure water, immediately followed

by hermetically sealing the pans. In order to disperse the lipid or lipid/polymer mixture in aqueous solution, the pan was equilibrated at 60 °C for 2 h with constant shaking. Thermal scans were carried out using a differential scanning calorimeter (DSC-204F, Netzsch, Germany) at a scan rate of 5 °C/min in a temperature range of 25-50 °C. An empty hermetically sealed aluminum pan was used as reference.

Time-resolved fluorescence measurement: The pDNA solution with a final concentration of 60 μM per nucleotide was prepared in PBS (pH=7.4). The EB-DNA complexes (ED) were prepared by drop-wise addition of EB solutions to the DNA solutions with continuous stirring and the final solutions were allowed to equilibrate for 5 min. The molar ratio of EB to nucleotide was 1: 15. Then for group I, an equal volume of polymer solution was drop-wise added into the solutions of ED with vigorous stirring and incubated at room temperature for 30 min. For group II, polymer/ED complexes were incubated for 20 min, and then illuminated under UV irradiation for 10 min. Finally time-resolved fluorescence spectra were recorded with FLS920 fluorometer (Edinburgh Instruments, Britain) equipped with a 450 W Xe lamp and a 1.5 ns pulse-width H_2 flash lamp. The excitation wavelength was 340 nm and the fluorescence was monitored at 610 nm. All fluorescence decays were taken by using the time-correlated single-photon counting (TCSPC) technique. The data were analyzed by reconvolution fit analysis with the instrument response function using the software package provided by the instruments. The decay process is expressed in mathematical terms as follows:

$$R(t) = A + \sum B_i e^{-t/\tau i}$$

Where B_i is pre-exponential factors, τ_i characteristic lifetimes; A is additional background constant. *R(t)* is called the sample decay model. For a 2-component system, the relative population of the first component is obtained from the following equation:

$$P_1 = \frac{B_1}{B_1 + B_2} \times 100\%$$

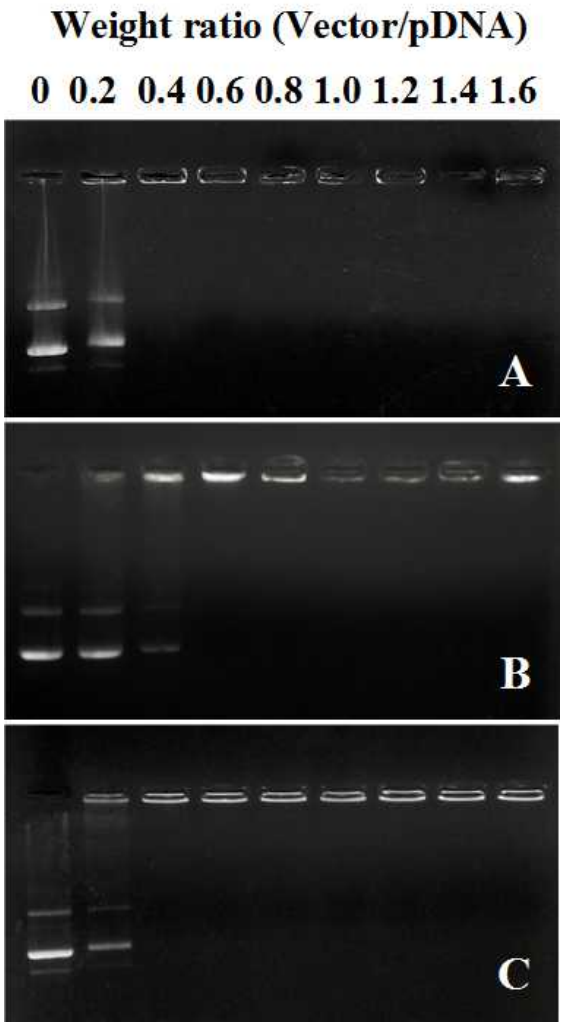

Figure S1. Agarose gel electrophoresis patterns of PDMAEMA$_{150}$/pDNA complexes (A), Azo-PDMAEMA$_{100}$/pDNA complexes (B) and Azo-PDMAEMA$_{150}$/pDNA complexes (C) at varied weight ratios.

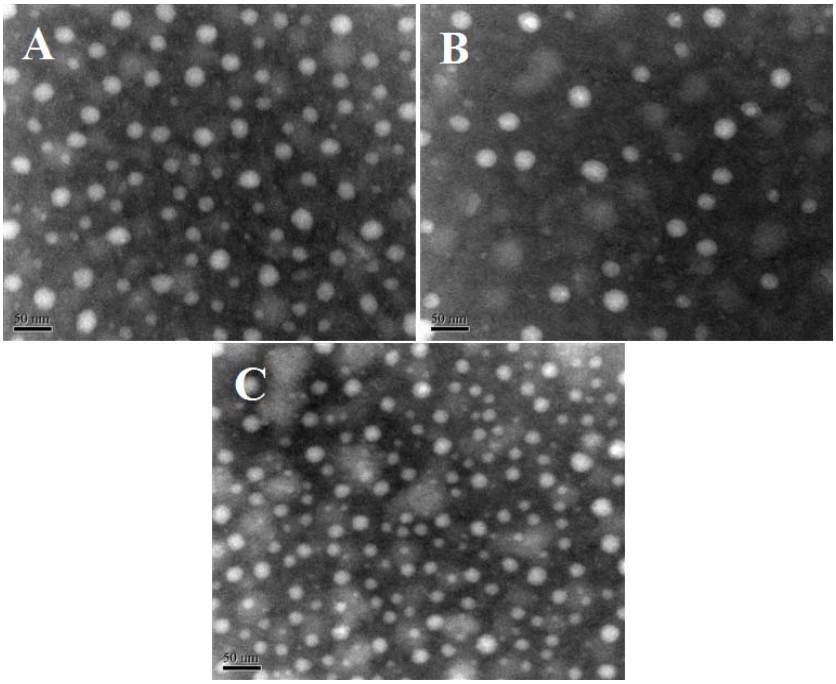

Figure S2. Morphologies of the complexes (weight ratio of 8:1) observed by TEM. (A): Azo-PDMAEMA$_{100}$/pDNA complexes; (B): Azo-PDMAEMA$_{150}$/pDNA complexes; (C): PDMAEMA$_{150}$/pDNA complexes.

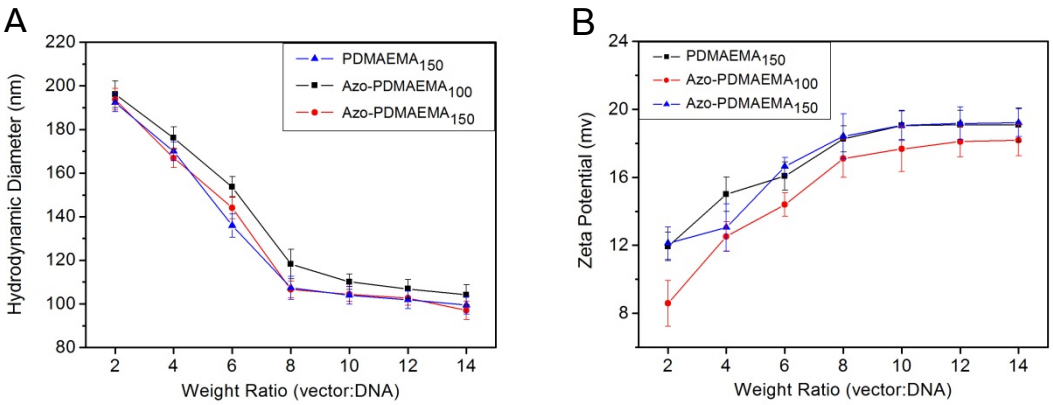

Figure S3. Hydrodynamic diameters (A) and Zeta potentials (B) of $PDMAEMA_{150}$/pDNA complexes, Azo-$PDMAEMA_{100}$/pDNA complexes and Azo-$PDMAEMA_{150}$/pDNA complexes at different weight ratios.

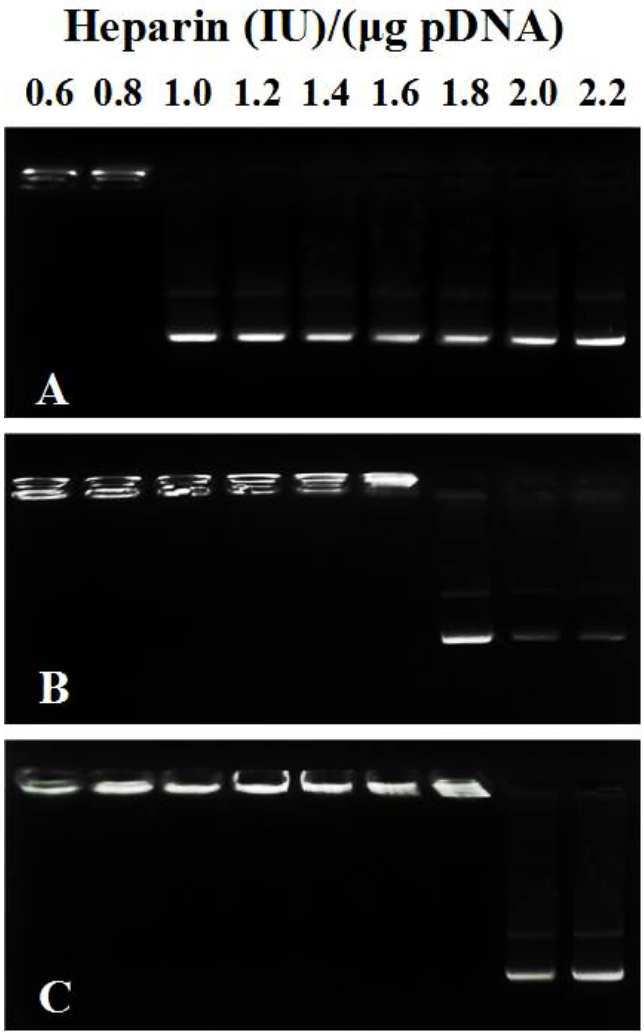

Figure S4. Electrophoresis patterns of the vector/pDNA complexes after treated with different amount of heparin: PDMAEMA$_{150}$/pDNA complexes at a weight ratio of 8:1 (A), Azo-PDMAEMA$_{100}$/pDNA complexes at a weight ratio of 8:1 (B), Azo-PDMAEMA$_{150}$/pDNA complexes at a weight ratio of 8:1 (C).

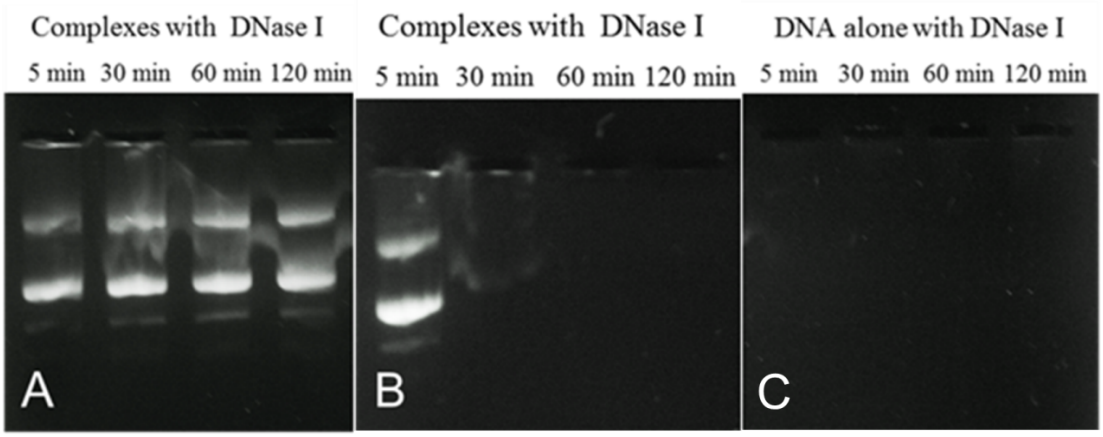

Figure S5. DNase I protection assay of Azo-PDMAEMA$_{150}$/pDNA complex at a weight ratio of 8:1 (A), PDMAEMA$_{150}$/pDNA complex at a weight ratio of 8:1 (B) and pDNA (C) when treated with DNase I for different time intervals.

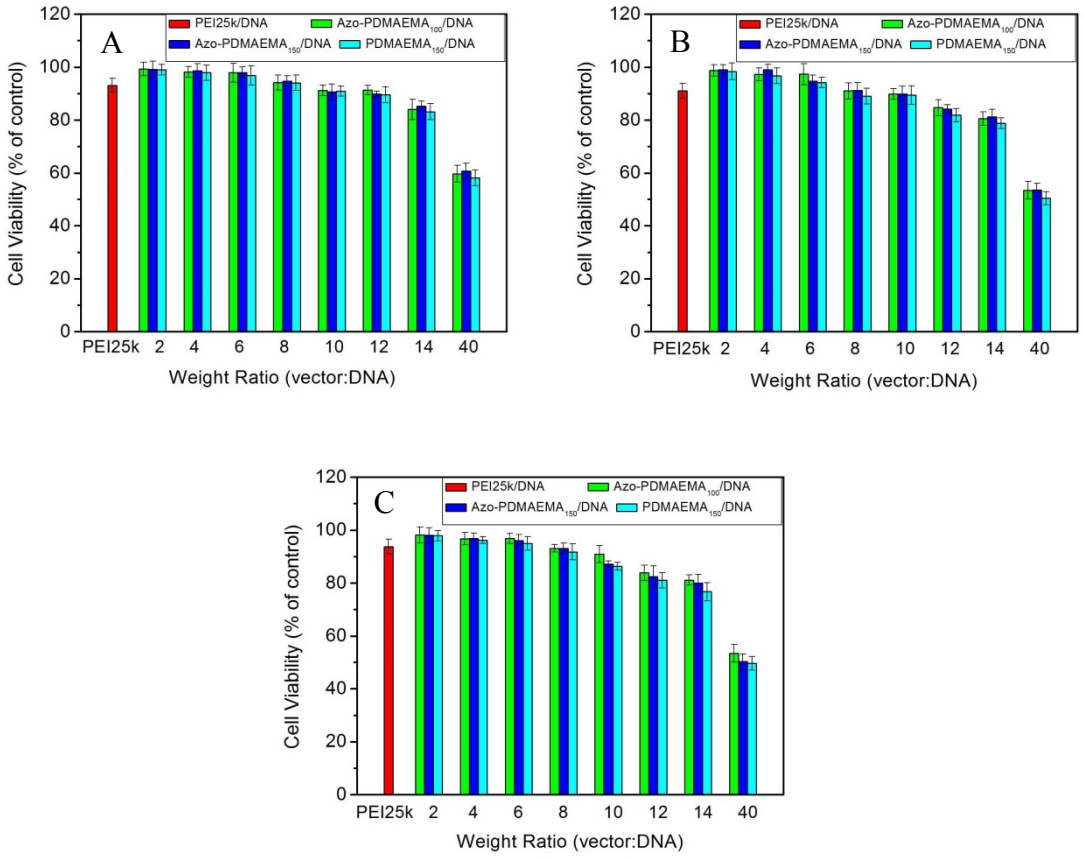

Figure S6. Cytotoxicity of three kinds of cationic polymers/pDNA complexes at different weight ratios against COS-7 cells (A), HepG-2 cells (B) and CHO-K1 cells (C).

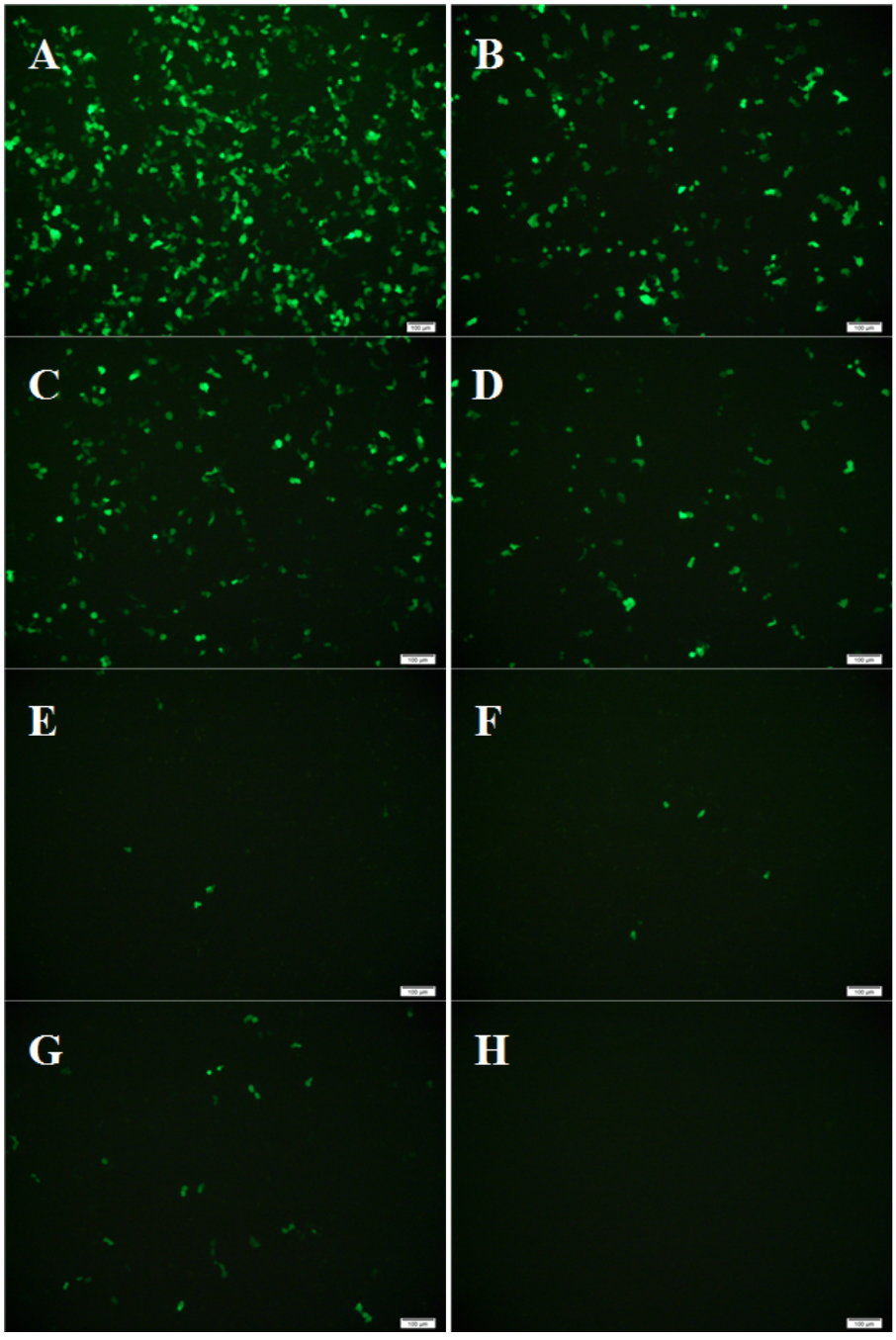

Figure S7. GFP expression of complexes in HepG-2 cells detected by fluorescence microscope. (A), (B): Azo-PDMAEMA$_{150}$/pDNA complexes at the weight ratio of 8:1; (C), (D): Azo-PDMAEMA$_{100}$/pDNA complexes at the weight ratio of 8:1; (E), (F): PDMAEMA$_{150}$/pDNA complexes at the weight ratio of 8:1; (G): PEI25k/pDNA complexes at the weight ratio of 2:1; (H): control. With UV irradiation: (A), (C), (E);

Without UV irradiation: (B), (D), (F). The scale bars in the photos are 100 μm.

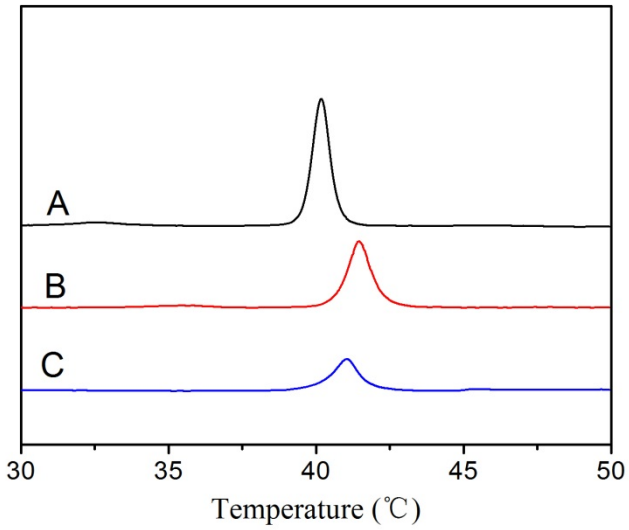

Figure S8. The DSC thermograms of the DPPC/polymer mixture in aqueous solution.

A: DPPC; B: $PDMAEMA_{150}$/DPPC; C: Azo-$PDMAEMA_{150}$/DPPC.

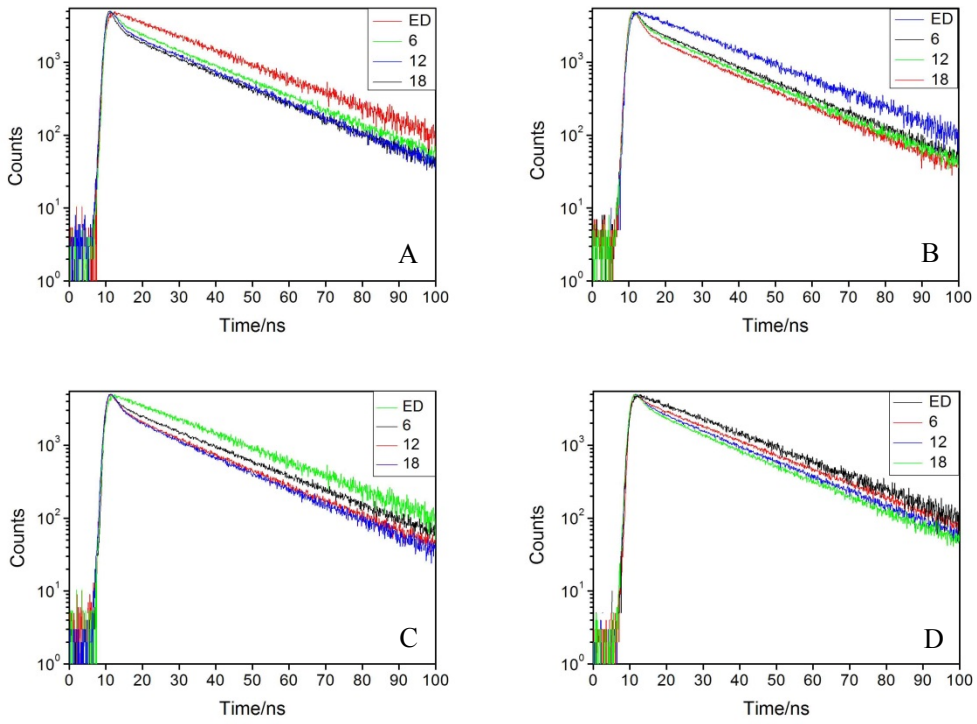

Figure S9. Fluorescence decay curves of EB/DNA complexes in the presence of the increased amount of cationic polymers: $PDMAEMA_{150}$/EB/pDNA complexes without UV irradiation (A); $PDMAEMA_{150}$/EB/pDNA complexes with UV irradiation (B); Azo-$PDMAEMA_{150}$/EB/pDNA complexes without UV irradiation (C); Azo-$PDMAEMA_{150}$/EB/pDNA complexes with UV irradiation (D).

Table S1. Molecular weight and polydispersity of the polymers prepared by ATRP.

Sample	Theoretical value of $\overline{Mn}$	Conversion of DMAEMA[a] (%)	$\overline{Mn}$ [b]	$\overline{Mw}$ [b]	PDI[b]
$PDMAEMA_{150}$	23750	79	11813	13957	1.18
$Azo\text{-}PDMAEMA_{100}$	16050	77	7589	9426	1.24
$Azo\text{-}PDMAEMA_{150}$	23900	74	10585	12320	1.16

[a]Conversion was determined gravimetrically.

[b]Measured by GPC using THF as eluent on the basis of a polystyrene calibration curve.

Table S2. Florescence lifetime obtained with two-exponential fit of the fluorescence decay curves of $PDMAEMA_{150}$/EB/pDNA complexes with and without UV irradiation (unit: ns).

Sample and weight ratio	After UV		Before UV		ΔP_1
	$\tau_1(P_1)$	$\tau_2(P_2)$	$\tau_1(P_1)$	$\tau_2(P_2)$	
EB	1.66(100%)	–	1.64(100%)	–	–
ED (EB/DNA)	–	22.76(100%)	–	22.77(100%)	–
Vector:ED = 6:1	1.79(58%)	20.34(42%)	2.07(54%)	20.64(46%)	– 4%
Vector:ED = 12:1	1.97(62%)	19.57(38%)	1.71(65%)	20.02(35%)	3%
Vector:ED = 18:1	1.81(67%)	19.91(33%)	1.71(66%)	20.35(34%)	– 1%

$\Delta P_1 = P_1$(Before UV) – P_1(After UV)

Table S3. Florescence lifetime obtained with two-exponential fit of the fluorescence decay curves of Azo-PDMAEMA$_{150}$/EB/pDNA complexes with and without UV irradiation (unit: ns).

Sample and weight ratio	After UV		Before UV		ΔP_1
	$\tau_1(P_1)$	$\tau_2(P_2)$	$\tau_1(P_1)$	$\tau_2(P_2)$	
EB	1.66(100%)	–	1.64(100%)	–	–
ED (EB/DNA)	–	22.76(100%)	–	22.77(100%)	–
Vector:ED = 6:1	2.08(34%)	21.78(66%)	1.87(48%)	21.15(52%)	14%
Vector:ED = 12:1	2.09(50%)	20.65(50%)	2.02(61%)	19.72(39%)	11%
Vector:ED = 18:1	2.15(54%)	19.90(46%)	1.96(65%)	19.13(35%)	11%

$\Delta P_1 = P_1$(Before UV) – P_1(After UV)