Supporting Information

A Multifunctional Polymeric Gene Delivery System for Circumventing Biological Barriers

Huajie Zhu,[a] Jinxia An,[a] Chengcai Pang,[a] Shuai Chen,[a] Wei Li,[a] Jinbiao Liu,[a] Qixian Chen,*[b] and Hui Gao*[a]

[a.] School of Chemistry and Chemical Engineering, Tianjin Key Laboratory of Organic Solar Cells and Photochemical Conversion, Tianjin University of Technology, Tianjin 300384 (P. R. China)

[b.] School of Life Science and Biotechnology, Dalian University of Technology, No. 2 Linggong Road, Dalian 116024 (P. R. China)

E-mail: qixian@dlut.edu.cn, hgao@tjut.edu.cn

Materials and methods

Materials

2-(*N,N*-Diethylamino)ethyl acrylate (DEAEA) and 2',7'-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma-Aldrich (Shanghai, China). Glycidyl methacrylate (GMA), 2-bromoisobutyryl bromide, bipyridyl, CuBr and 4-bromobenzophenone were purchased from Shanghai Adamas Reagent Co., Ltd (Shanghai, China). Trimethyl orthoformate, pyridinium-*p*-toluenesulfonate, neopentyl glycol (NPG), 2-hydroxyethyl acrylate, 4-methoxyphenol and 4-bromomethylphenylboronic acid pinacol ester were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). Prior to use, toluene and *N,N*-dimethylformamide (DMF) was purified according to a reduced pressure distillation approach. Dichloromethane (DCM) was purified by refluxing with treatment of CaH_2. *β*-cyclodextrin (*β*-CD) was subjected to recrystallization prior to use. Cell Counting Kit-8 (CCK-8) was employed in evaluation of cell viability (Dojindo, Japan). The Micro BCA Protein Assay Reagent Kit was purchased from Pierce Co., Inc. (Rockford, IL) and the luciferase assay kit was a product of Promega (Madison, USA). The Label IT Cy5 Kit (^{Cy5}DNA) was purchased from Mirus Bio

LLC (Madison, USA). All other reagents were obtained from Tianjin Chemical Reagent Co. (Tianjin, China).

Synthesis of 2-(5,5-Dimethyl-1,3-dioxan-2-yl) ethyl acrylate (DMDEA)

Monomer of DMDEA was prepared according to our previously reported procedures[1]. In brief, trimethyl orthoformate (21.2 g, 200 mmol) was dissolved in a DCM (50 mL) solution containing pyridinium-*p*-toluenesulfonate (0.506 g, 2 mmol) and NPG (15.2 g, 200 mmol). The reaction solution was under stirring for 12 h at 30 °C. The crude product was obtained by extraction in 10% NaOH aqueous solution (three times), and the organic layer was collected in anhydrous K_2CO_3. The dichloromethane solution was evaporated to yield colorless liquid under reduced pressure. 2-Methoxy-5,5-dimethyl-1,3-dioxane (3.0 g, 25 mmol), 2-hydroxyethyl acrylate (3.8 g, 32 mmol), pyridunium-*p*-toluenesulfonate (0.125 g, 0.5 mmol), and 4-methoxyphenol (0.1 g, 0.8 mmol) were added to toluene (25 mL), and stirred at 120 °C overnight. The solution was cooled, extracted three times with 10% NaOH aqueous solution, and the combined organic layer was dried over anhydrous K_2CO_3. After removing toluene by evaporation under vacuum, the residue was purified through a basic Al_2O_3 column eluting with petroleum ether/ethyl acetate (v/v = 10:1) to produce colorless oil (3.6 g, yield: 52%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.42-6.49 (q, 1H, CH_2CH), 6.13-6.22 (q, 1H, CH_2CH), 5.84-5.88 (q, 1H, CH_2CH), 5.37 (s, 1H, HCO_3), 4.34-4.38 (t, 2H, $CO_2CH_2CH_2O$), 3.88-3.91 (t, 2H, $CO_2CH_2CH_2O$), 3.75-3.79 (d, 2H, $CO_2CH_2CH_2O$), 3.39-3.43 (d, 2H, $CO_2CH_2CH_2O$), 0.99 (s, 6H, $C(CH_3)_2$).

Synthesis of poly[(2-acryloyl)ethyl(*p*-boronic acid pinacol ester)benzyldiethylammonium bromide] (BP)

Poly[(2-*N*,*N*-diethyl)aminoethyl acrylate) (PD) was synthesized based on atom transfer radical polymerization (ATRP) reaction. In brief, DEAEA (5.13 g, 30 mmol), 2,2'-bipyridyl (0.117 g, 0.75 mmol), 2-bromoisobutyryl bromide (0.0975 g, 0.5 mmol) as ATRP initiator was dissolved in anhydrous DMF, followed by addition of CuBr (0.0715 g, 0.5 mmol). The reaction solution was subjected to stirring at 80 °C under N_2 atmosphere. At 24 h post reaction, the above solution was cooled down at room temperature, followed by filtration through Al_2O_3 column to eliminate copper in THF. The filtrate was concentrated by evaporation and dissolved in CH_2Cl_2, followed by precipitation in chill hexane. The ultimate PD was purified by re-precipitation (three times) and dried under vacuum.

The synthesized PD (0.3 g) was reacted with 4-bromomethylphenylboronic acid pinacol ester (0.56 g) in 20 mL DMF at room temperature for 24 h. The resulting solutions were dialyzed against deionized water (three times) using a dialysis bag with MWCO of 3,500 Da and transferred to lyophilization to yield BP (0.23 g, yield: 90%).

Synthesis of BP-poly[2-(5,5-dimethyl-1,3-dioxan-2-yloxy)ethyl acrylate] copolymer (BP-PDM)

PD-PDM was synthesized by ATRP reaction. In brief, DEAEA (2.05 g, 12 mmol) and DMDEA (1.38 g, 6 mmol) were dissolved in 10 mL distilled DMF in a three-necked flask. Then 2-bromoisobutyryl bromide (0.039 g, 0.2 mmol) as ATRP initiator, bipyridyl (0.0468 g, 0.3 mmol) and CuBr (0.0286 g, 0.2 mmol) were sequentially added into the reaction system. The flask was deoxygenated at room temperature with nitrogen three times and then polymerized at 80 °C for 24 h. The reaction was subsequently quenched with an ice bath and diluted with proper amount of tetrahydrofuran (THF). The reaction suspension was passed through a neutral Al_2O_3 column with THF as an eluent to exclude copper. Following

evaporation of the solvent, the product was dissolved in a minimal volume of CH_2Cl_2 and precipitated twice in cold n-hexane.

PD-PDM (0.5 g) was reacted with 4-bromomethylphenylboronic acid pinacol ester (0.8 g) in 20 mL DMF at room temperature for 24 h. The resulting solutions were dialyzed 48 h against deionized water using a dialysis bag with a molecular weight cut-off of 3,500 Da and then lyophilized to obtained the product BP-PDM (0.36 g, yield: 88%).

Synthesis of l,2-Bis(4-bromophenyl)-l,2-diphenylethene (TPEBr)

As depicted in Scheme S1, the bromized TPE was prepared through McMurry Olefination reaction from 4-bromobenzophenone. Zinc (3.92 g, 60 mmol) was added into a 250 mL three-necked round-bottomed flask. The flask was degassed and flushed with dry nitrogen for three times, followed by addition of anhydrous tetrahydrofuran (THF) (60 mL). The mixture was cooled down in an ice-salt bath and titanium tetrachloride (3.32 mL, 30 mmol) was added in dropwise. The reaction suspension was under stirring at 25 °C for 30 min, and refluxed at 74 °C for 2 h. Then, the mixture was cooled down again. Pyridine (0.5 mL) was added under stirring for 10 min. Then, a THF solution (20 mL) of 4-bromophenone (5.22 g, 20 mmol) was added slowly. Following refluxing overnight, the reaction mixture was cooled to room temperature. The reaction was quenched with a 10% potassium carbonate aqueous solution and extracted by DCM three times. The organic layer was washed with saturated brine and dried with anhydrous sodium sulfate for 4 h. Following the sequential treatment of filtration and solvent evaporation, the residue was purified by silica gel column eluting with petroleum ether/DCM (v/v = 10:1) to obtain TPEBr as a white solid (2.94 g, yield: 60 %). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.25-7.19 (m, 4H, Ar H), 7.13-7.07 (m, 6H, Ar H), 7.03-6.96 (m, 4H, Ar H), 6.89-6.85 (m, 4H, Ar H).

Synthesis of 4,4'-(l,2-diphenylethene-l,2-diyl)bis(l,4-phenylene)diboronic acid (TPE)

In a 100 mL four-necked round-bottomed flask, 1,2-Bis(4-bromophenyl)-l,2-diphenylethene (0.40 g, 0.82 mmol) was dissolved in 20 mL of newly evaporated THF. The flask was cooled to -78 °C with an acetone-dry ice bath. Then, 1.0 mL (2.6 mmol) of n-butyllithium (2.5 M in hexane) was injected under nitrogen. After stirring for 1 h, 0.46 mL (4.0 mmol) of tri-methylborate was added and the mixture was allowed to react for 45 min. Then, the mixture was warmed to room temperature for overnight reaction. The reaction was quenched with 1 mL of dilute HCl solution. Following filtration and solvent evaporation, the product was purified by silica gel column chromatography using ethyl acetate/DCM (v/v = 1:10) as eluent. The product was obtained as yellow solid (0.16 g, yield: 42 %). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.96 [d, $B(OH)_2$], 7.54 (d, 4H, Ar H), 7.08-7.16 (m, 6H, Ar H), 6.92-6.99 (m, 8H, Ar H).

^{1}H NMR Characterization of copolymer

The ^{1}H NMR spectra of DMDEA, TPE and copolymers were recorded on a 400 MHz Bruker Avance-400 spectrometer (400 MHz, Bruker, Freemont, CA) using $CDCl_3$ and D_2O as solvents, respectively.

Fabrication and characterizaion of polyplexes

BP, BP-PDM and BP-PDM-PG were dissolved and mixed with pDNA under gentle vortex for 15 seconds to create the complex formation at varying N/P ratios (defined by the molar ratio of the total amino groups from the polymers to the phosphate groups from DNA). The size and zeta potential of the polyplexes were measured in three independent experiments at 25

°C using a Zetasizer Nano-ZS90 (Malvern Instruments, UK). Data are presented as the means ± SD (n = 3).

ROS- and pH-responsiveness measurements of polyplexes

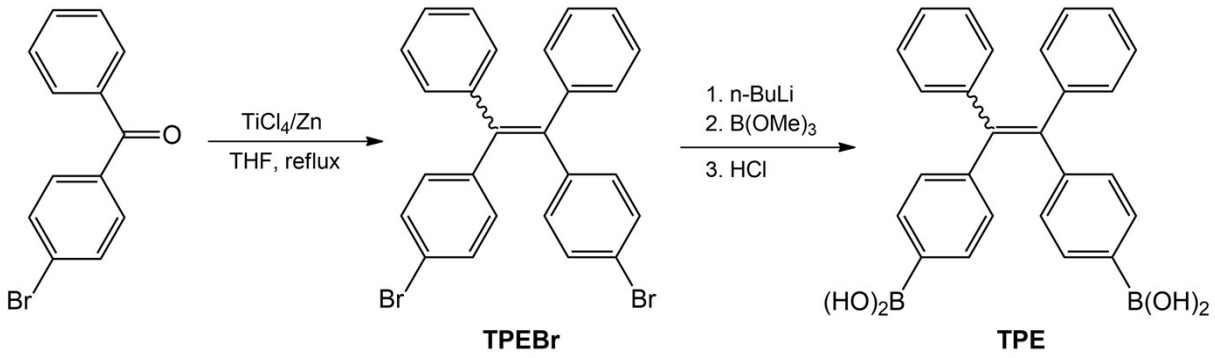
The charge reversal of BP-PDM-PG(TPE) were measured by the Zetasizer Nano-ZS90. The solution of BP-PDM-PG(TPE) was prepared as described above and exposed to white light irradiation at a power density of 10 mW cm^{-2}. The solution was incubated with shaking at 37 °C. Samples were taken at timed intervals and their zeta potentials were measured.

The changes of BP-PDM-PG(TPE) in particle size at pH 5.0 were similarly investigated by the Zetasizer Nano-ZS90. The solution of BP-PDM-PG(TPE) was prepared at the same concentration as zeta potential described above and adjust pH to 5.0. Samples were taken at timed intervals and their particle size and particle dispersion index (PDI) were measured. Data are presented as the means ± SEM (n = 3).

^{1}H NMR measurement was performed for BP-PDM-PG at pH 5.0 to gain the evidence of the hydrolysis of the pendent ortho ester of PDM segment. BP-PDM-PG (3 mg mL^{-1}) was dissolved in D_2O, adjusted by DCl to pH 5.0. The solution was incubated with different time intervals at 37 °C prior to ^{1}H NMR measurement.

Cell culture

Human cervical carcinoma HeLa cell lines were maintained in RPMI 1640 (GIBCO, Grand Island, USA) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, USA), penicillin (100 units/mL) and streptomycin (100 μg/mL) in a humidified environment containing 5% CO_2 at 37 °C.



Scheme S1. Synthetic route in preparation of aggregation-induced emission molecule TPE.

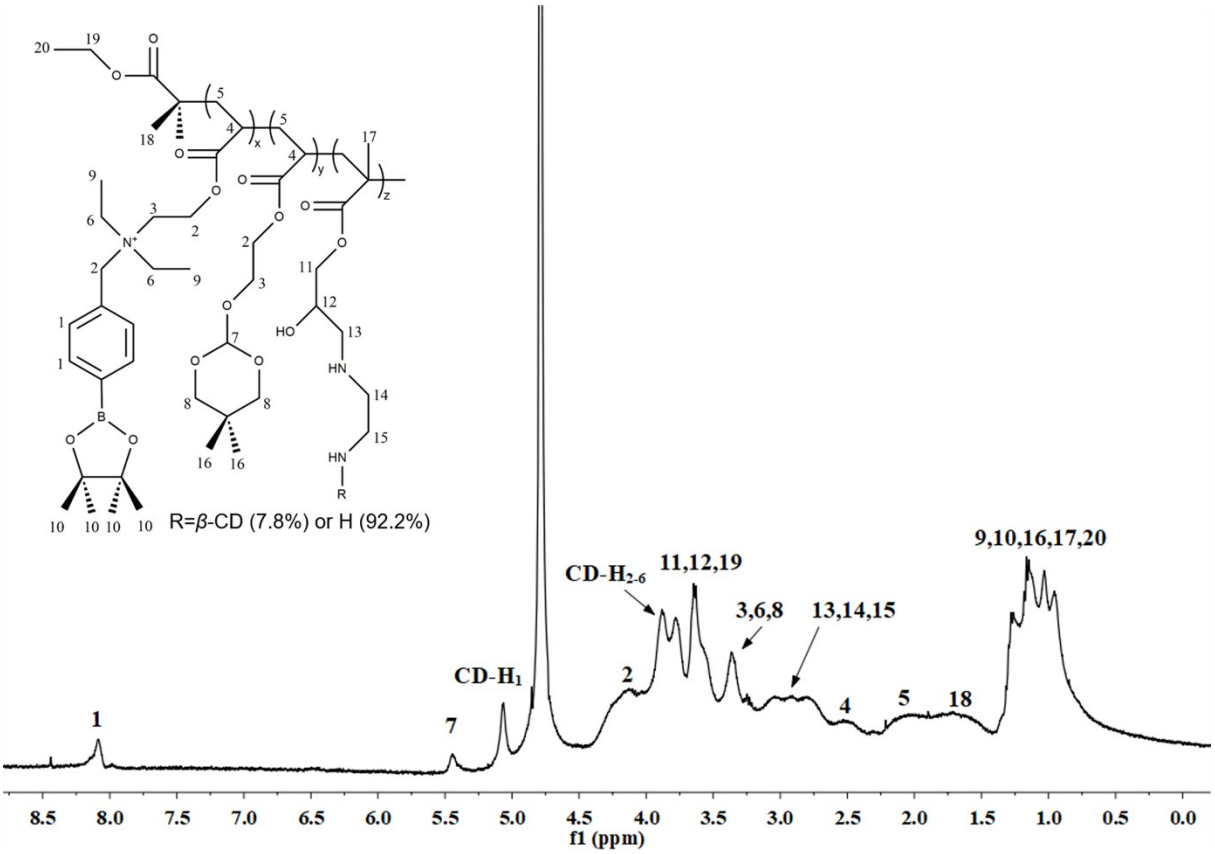


Fig. S1. ^{1}H NMR spectra of BP-PDM-PG in D_2O.

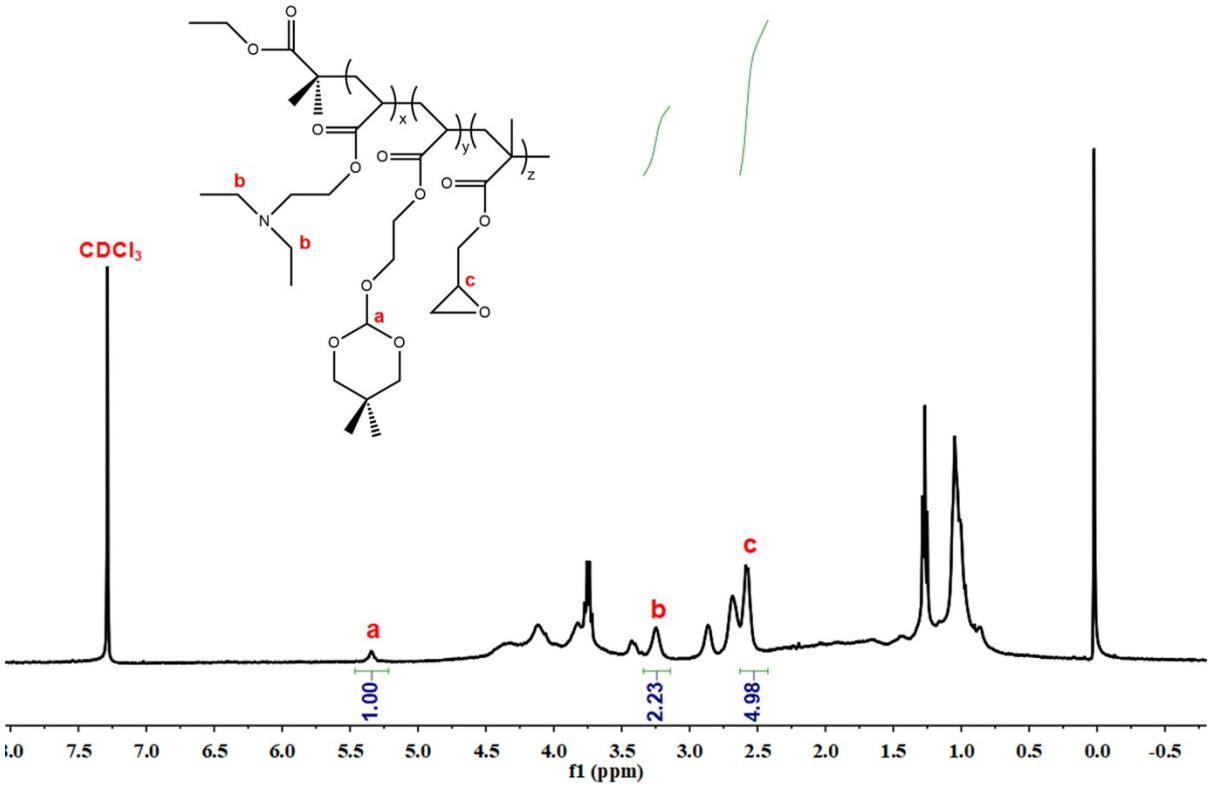


Fig. S2. ^{1}H NMR spectra of PD-PDM-PG in $CDCl_3$.

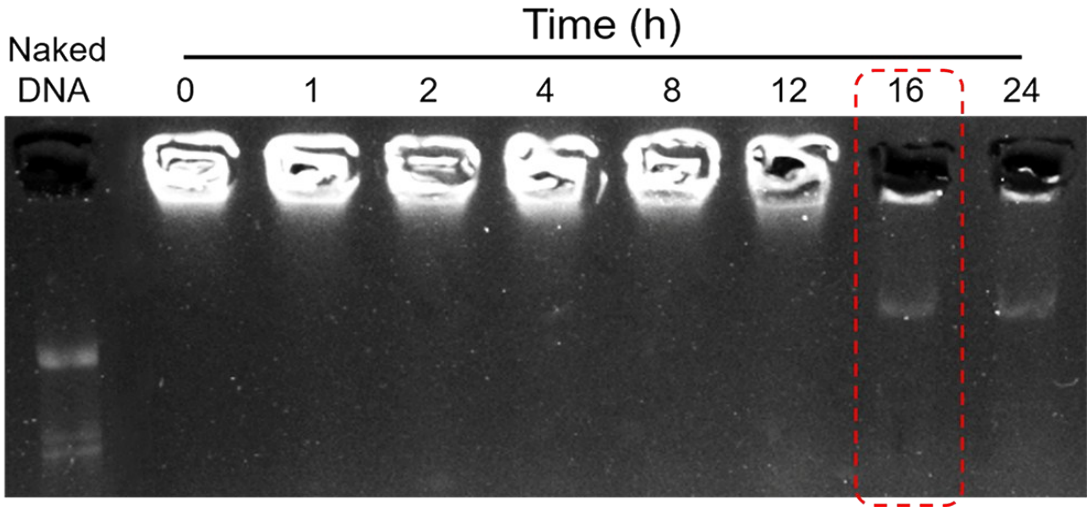


Fig. S3. Gel retardation assay of DNA released from polyplexes exposed to white light irradiation at 37 °C.

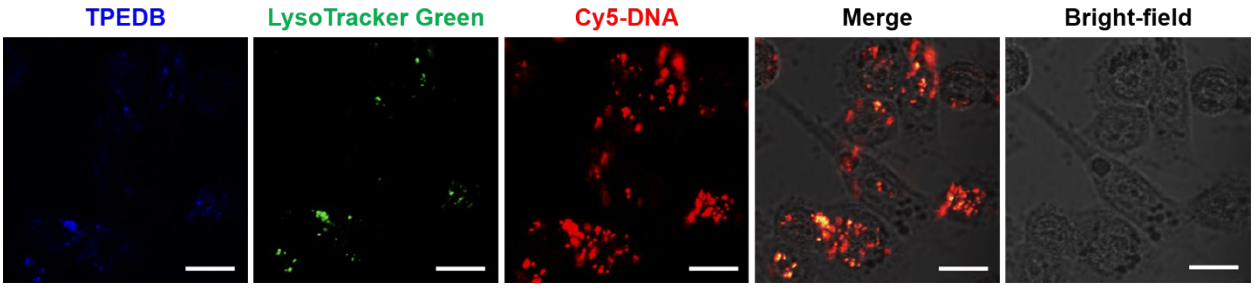


Fig. S4. Intracellular distribution of BP-PDM-PG(TPE)/^{Cy5}DNA polyplex of HeLa cells at the incubation time of 18 h by CLSM. The scale bar is 10 μm.

Reference

1. J. An, X. Dai, Z. Wu, Y. Zhao, Z. Lu, Q. Guo, X. Zhang and C. Li, *Biomacromolecules*, 2015, **16**, 2444-2454.