

Enhanced Gene Transfection Capability of Polyethylenimine by Incorporating Boronic Acid Groups

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Chemicals

4-(Bromomethyl)phenylboronic acid and 25 kDa PEI were purchased from Aldrich. Fluorescein isothiocyanate (FITC) were purchased from Molecular Probes, Inc. Dubelcco's Modified Eagle's Medium (DMEM), penicillin-streptomycin, trypsin, Dubelcco's phosphate buffered saline and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma. Dubelcco's Modified Eagle's Medium (DMEM)-liquid (without glucose) was purchased from Gibco. 1800 Da PEI was purchased from Alfa Aesar. Plasmid pGL3 under the control of SV40 promoter and with enhancer sequences encoding luciferase was obtained from Promega, Madison, WI, USA. Plasmids were propagated in *Escherichia. colia.* in Luria Bertani (LB) medium containing 60 µg/ml ampicillin respectively at 37 °C and purified using E. Z. N. A. Fastfilter Endo-free Plasmid Midi kits (Omega) according to the manufacturer's instruction. The purity of DNA was assessed spectrophotometrically by measuring absorbance at wavelengths of 260 and 280 nm (OD_{260}/OD_{280} 1.8 or greater) and confirmed using 0.7% agarose gel electrophoresis containing GelRedTM. The DNA concentration was determined by measuring the UV absorbance at 260 nm. DNA aliquots of pGL3 were stored at -20 °C prior to use.

Synthesis of PEI₁₈₀₀-PB_x

PEI₁₈₀₀-PB_{1.9}: A solution of 1800 Da PEI (0.481 g, 0.267 mmol) and 4-(bromomethyl)-phenylboronic acid (0.238 g, 0.534 mmol) in 5 mL of methanol was stirred in a 50°C oil bath for 24 h. The reaction solution was precipitated in diethyl ether three times, and dried under high vacuum to give 0.411 g of light yellow viscous glue in 61% yield. ¹H NMR (300 MHz, D₂O): δ 7.39 (br s, 2H), 7.11 (br s, 2H), 3.65 and 3.45 (br s, 2H), 2.66–2.47 (m, 88H).

PEI₁₈₀₀-PB_{3.2} (0.437 g) and PEI₁₈₀₀-PB_{5.4} (0.407 g) were prepared similarly with increased 4-(bromomethyl)phenylboronic acid/PEI feed ratio in 79% and 95% yield, respectively. The ¹H NMR spectra are shown in Figure S1.

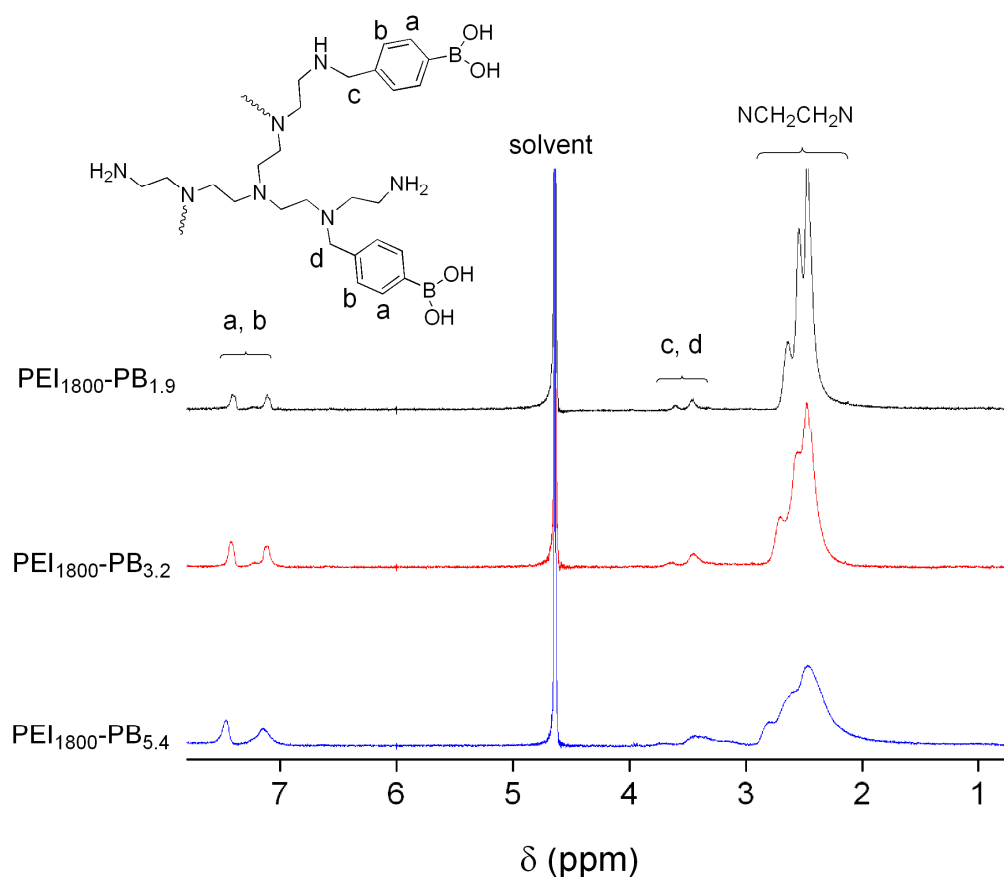


Figure S1. ^1H NMR spectra of $\text{PEI}_{1800}\text{-PB}_x$ (300 MHz, D_2O).

Cell culture

HepG2, COS-7 and HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (containing 1×10 mU/mL of penicillin, 0.1 mg/mL of streptomycin) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were grown in corning S1 incubated at 37°C under 5% CO_2 and 95% air atmosphere to $\sim 80\%$ confluence. A subculture was performed every 2–3 days.

Cytotoxicity Assay of $\text{PEI}_{1800}\text{-PB}_x$

The cytotoxicity assay was carried out on the basis of an MTT assay on HepG2 and COS-7 cells. The cells were seeded in 96-well plates at an initial density of 5000 cells/well in 200 μL of DMEM complete medium. The cells were allowed to grow for 24 h. The original media were replaced with 100 μL of fresh media. The $\text{PEI}_{1800}\text{-PB}_{1.9}$, $\text{PEI}_{1800}\text{-PB}_{3.2}$, $\text{PEI}_{1800}\text{-PB}_{5.4}$, 1800 Da and 25 kDa PEI solutions were added to the media at concentrations ranging from 0.001 to 1.0 mg/mL.

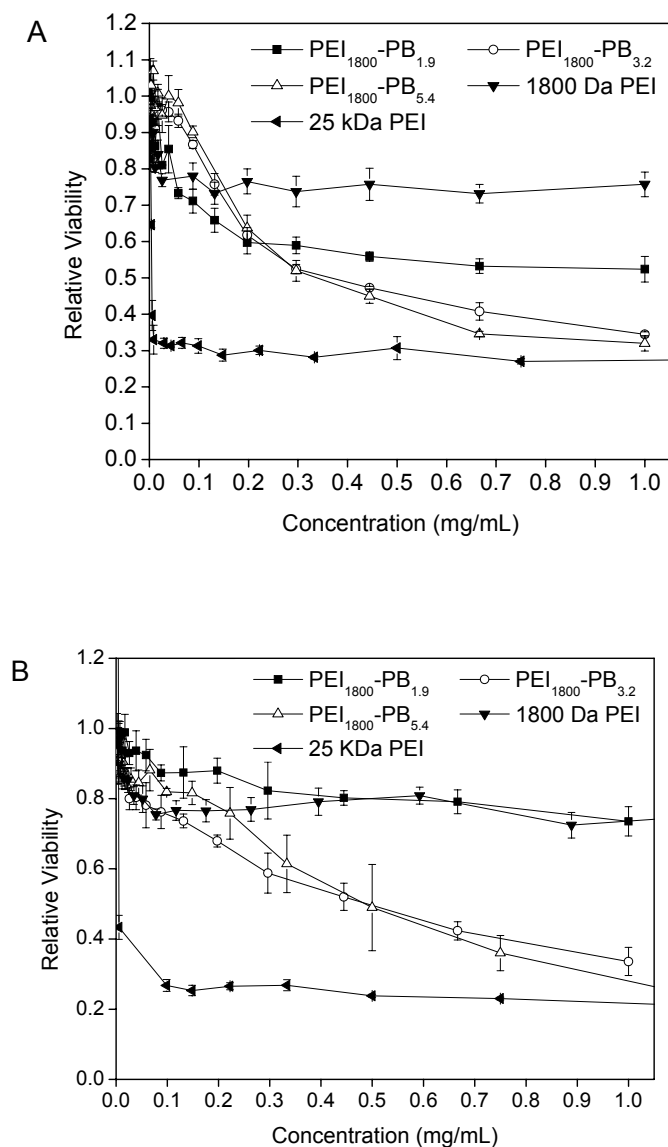


Figure S2. Relative cell viability of (A) HepG2 and (B) COS-7 cells at 24 h post-addition of PEI₁₈₀₀-PB_x, 1800 Da PEI, and 25 kDa PEI as demonstrated by MTT assay.

Each dosage was replicated in 4 wells. Treated cells were incubated at 37°C under a humidified atmosphere of 95% air and 5% CO₂ for 24 h. MTT reagent (20 µL in pH 7.4 PBS, 5 mg/mL) was added to each well, and the cells were incubated for another 4 h at 37°C. 100 µL of DMSO was added to each well until all crystals dissolved. The absorbance at 570 nm of the solution in each well was recorded using a Microplate Reader (Bio-Rad model 550). Cell viability was calculated according to the following equation:

$$\text{Cell viability (\%)} = (\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}) \times 100$$

where OD_{sample} is the absorbance of the solution of the cells cultured with the polymer and PEI; OD_{blank} is the absorbance of the medium; and OD_{control} is the absorbance of the solution of the

cells cultured with the medium only.

Formation of PEI₁₈₀₀-PB_X/pGL3 Polyplexes

PEI₁₈₀₀-PB_X/pGL3 polyplexes were formed at a defined ratio of amino nitrogen groups in the vector to the phosphate groups of the plasmid (N/P ratio) by mixing in 150 mM NaCl. After incubation at room temperature for 30 min, the polyplexes were electrophoresed on a 0.7% agarose gel containing GelRed with Tris-acetate (TAE) running buffer (pH 8) at 80 V for 80 min. DNA bands were visualized by a 254 nm UV illuminator and photographed with a Vilber Lourmat imaging system.

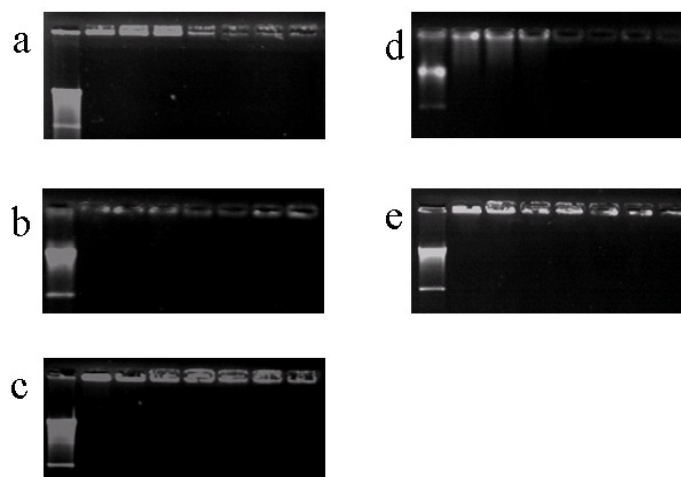


Figure S3. Agarose gel electrophoreses of pGL3 released from the polyplexes with (a) PEI₁₈₀₀-PB_{1.9}, (b) PEI₁₈₀₀-PB_{3.2}, (c) PEI₁₈₀₀-PB_{5.4}, (d) 1800 Da PEI, and (e) 25 kDa PEI at N/P ratios of 0, 0.5, 1, 2, 4, 6, 8, 10 (stripes from left to right).

Intrinsic Viscosity Measurements

Intrinsic viscosity of PEI₁₈₀₀-PB_X series was measured using a capillary viscometer. In 150 mM NaCl aqueous solution (pH 7.4, 25°C), the intrinsic viscosities of PEI₁₈₀₀-PB_{1.9}, PEI₁₈₀₀-PB_{3.2}, and PEI₁₈₀₀-PB_{5.4} were measured to be 7.24, 7.08, and 7.07 L/g, respectively, while those of 600 Da PEI and 1800 Da PEI purchased from Alfa Aesar were measured to be 6.05 and 7.34 L/g, respectively.

Particle Sizes and Zeta Potential Measurements

The particle size and zeta potential were measured with a Malvern Nano-ZS ZEN3600 Zeta-sizer at room temperature. The polyplexes at various N/P ratios were prepared by adding appropriate volume of pGL3 solution (in 40 mM Tris - HCl buffer solution, pH 7.4) to appropriate volume of PEI₁₈₀₀-PB_X in 150 mM NaCl solution. Then the polyplexes were incubated at room temperature for 30 min. After that the polyplexes were diluted by 150 mM NaCl solution and pure water to 1 mL volume prior to measure respectively.

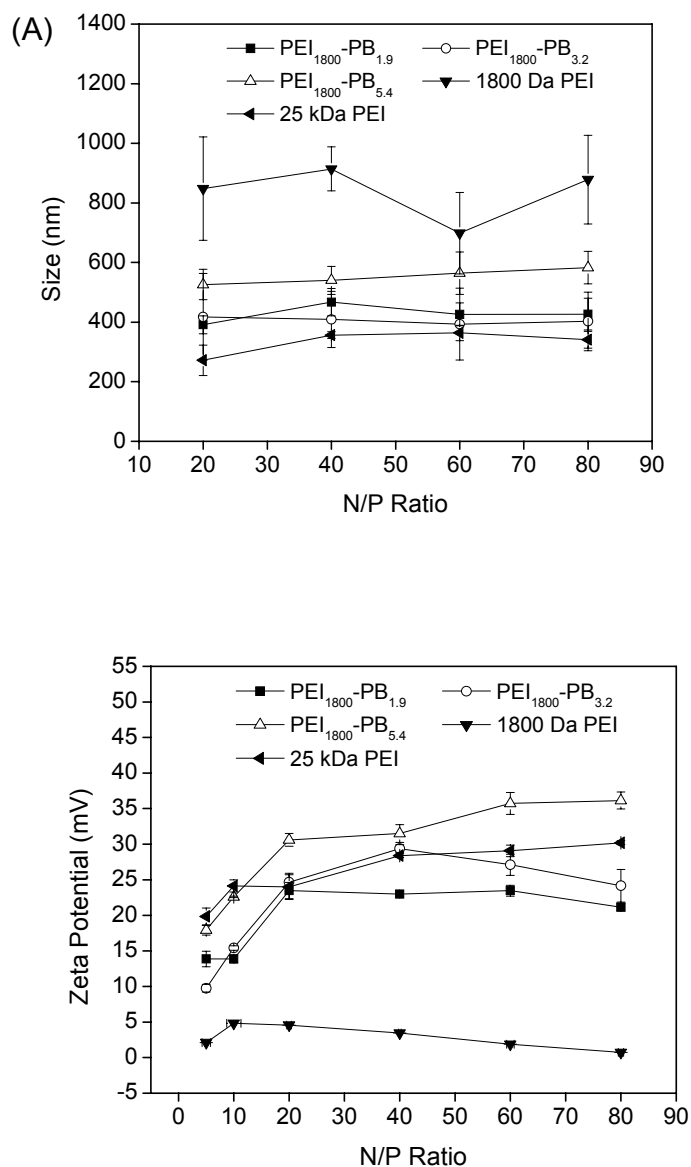


Figure S4. (A) DLS size of PEI₁₈₀₀-PB_x/pGL3 polyplexes in 150 mM NaCl solution, (B) surface charge of PEI₁₈₀₀-PB_x/pGL3 polyplexes in 10 mM NaCl solution at various N/P ratios.

Preparation of FITC-Labeled Polymers and Live Cell Laser Scanning Confocal Microscopy

To study the cellular uptake of polymers, PEI₁₈₀₀-PB_{3.2} and 1800 Da PEI was labeled with FITC similarly to a literature procedure (M. M. Caulum, B. M. Murphy, L. M. Ramsay, C. S. Henry, *Anal. Chem.* **2007**, 79, 5249-5256). Briefly, FITC (3.3 mg) was reacted with PEI₁₈₀₀-PB_{3.2} (10.8 mg) or 1800 Da PEI (7.7 mg) in CH₃OH (2 mL) overnight at room temperature (25°C) to produce FITC-PEI-PB and FITC-PEI. Both FITC-PB and FITC-PEI are dark orange products insoluble in the methanol solvent and therefore precipitate upon formation.

Both FITC-PB and FITC-PEI were washed three times with methanol and allowed to dry under vacuum.

Live cell confocal microscopy was used to monitor FITC-PB and FITC-PEI uptake into HepG2 and COS-7 cells. Cells were seeded into 6-well plates at a density of 1.0×10^5 cells/well with 1 mL of DMEM containing 10% FBS and incubated at 37°C for 24 h. Then, 2.0 mL of fresh DMEM containing polymers (50 $\mu\text{g/mL}$) was added into each well and incubated with cells for 4 h at 37°C. Before observation, medium was removed, cells in each well were washed by 1.0 mL PBS solution (pH 7.4) five times, and then 2 mL of fresh DMEM with FBS was added. The fluorescent images of cells were observed under excitation at 488 nm using confocal laser scanning microscopy (Nikon C1-si TE2000, Japan). All confocal images were slice images to distinguish internalized PEI₁₈₀₀-PB_{3.2} or PEI from that adhered to the outside cellular membrane.

Transfection Experiments at Different Glucose Concentration

The in vitro transfection efficiency of PEI₁₈₀₀-PB_{3.2}/pGL3 polyplexes (N/P = 20) was evaluated in HepG2 cells by the procedure as described in the previous paper (Q. Peng, Z. L. Zhong and R. X. Zhuo. *Bioconjugate Chem.*, 2008, **19**, 499–506). The media used for the transfection experiments were prepared by adding appropriate amount sugar to glucose-free Dubelcco's Modified Eagle's Medium (DMEM)-liquid from Gibco. The results are shown in Figure S5.

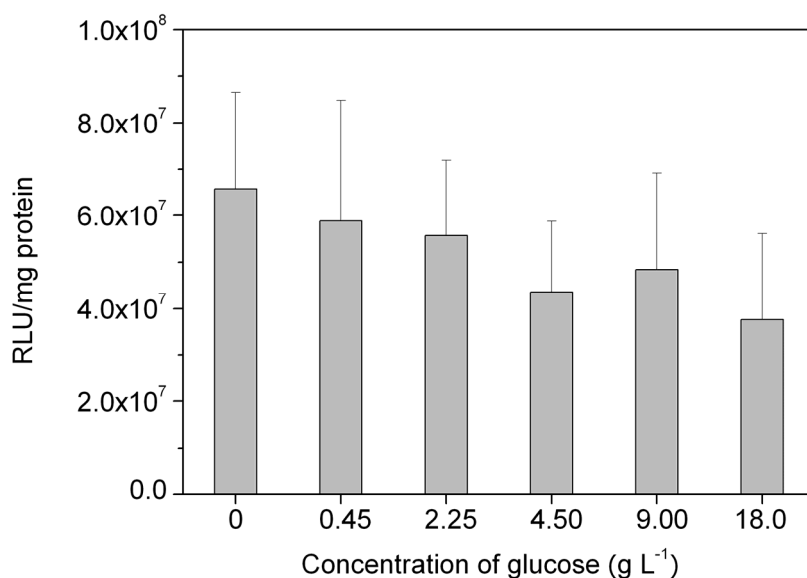


Figure S5. In vitro transfection efficiency of PEI₁₈₀₀-PB_{3.2}/pGL3 polyplex (N/P = 20) in HepG2 cells in the presence of different concentration of glucose. Data are averages of four parallel experiments.