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**Supporting Information**

# Zn-promoted gene transfection efficiency for non-viral vectors: a mechanism study.

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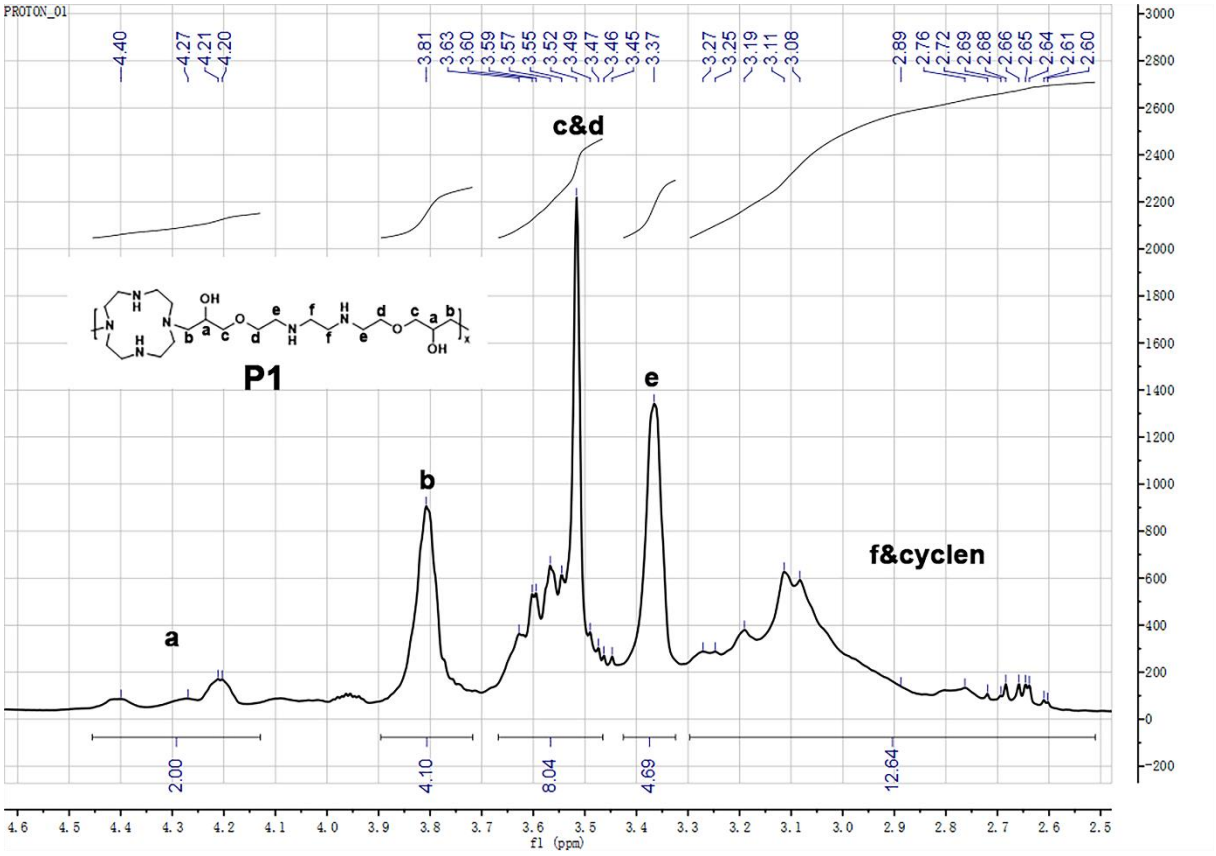
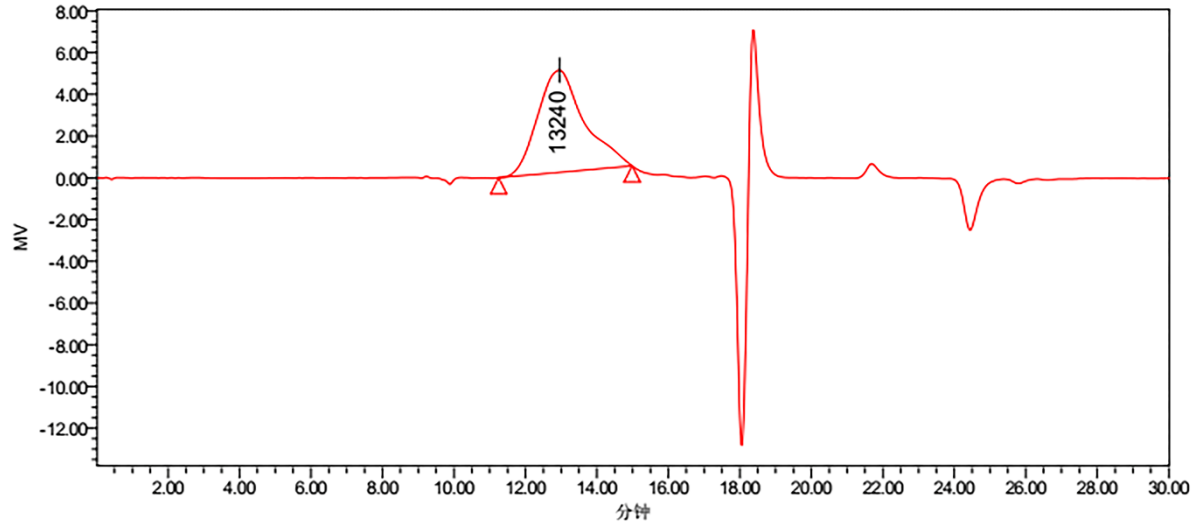



**Fig. S1.** $^{1}$H NMR spectrum of **P1**.




**GPC 结果**

| | 分布名 | Mn (道尔顿) | Mw (道尔顿) | MP | Mz (道尔顿) | Mz+1 (道尔顿) | 多分散性 | MW标记 1 (道尔顿) | MW标记 2 (道尔顿) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | 10560 | 14381 | 13240 | 19305 | 25217 | 1.36 | | |

**Fig. S2.** GPC analysis result of **P1**.

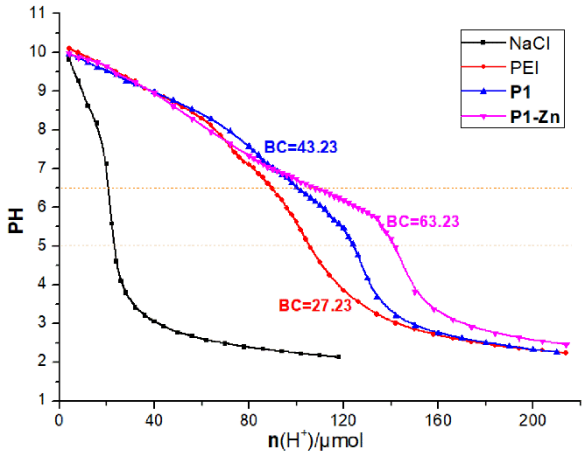



**Fig. S3.** Acid-base titration profiles and pH buffering capacity (BC) values (in the pH range of 5.0 ~ 6.5) of 25 kDa PEI, 150 mM NaCl and polymer solutions. Polymers or PEI (0.05 mmol of amino groups) were first treated with 1 N NaOH to adjust pH to 10.

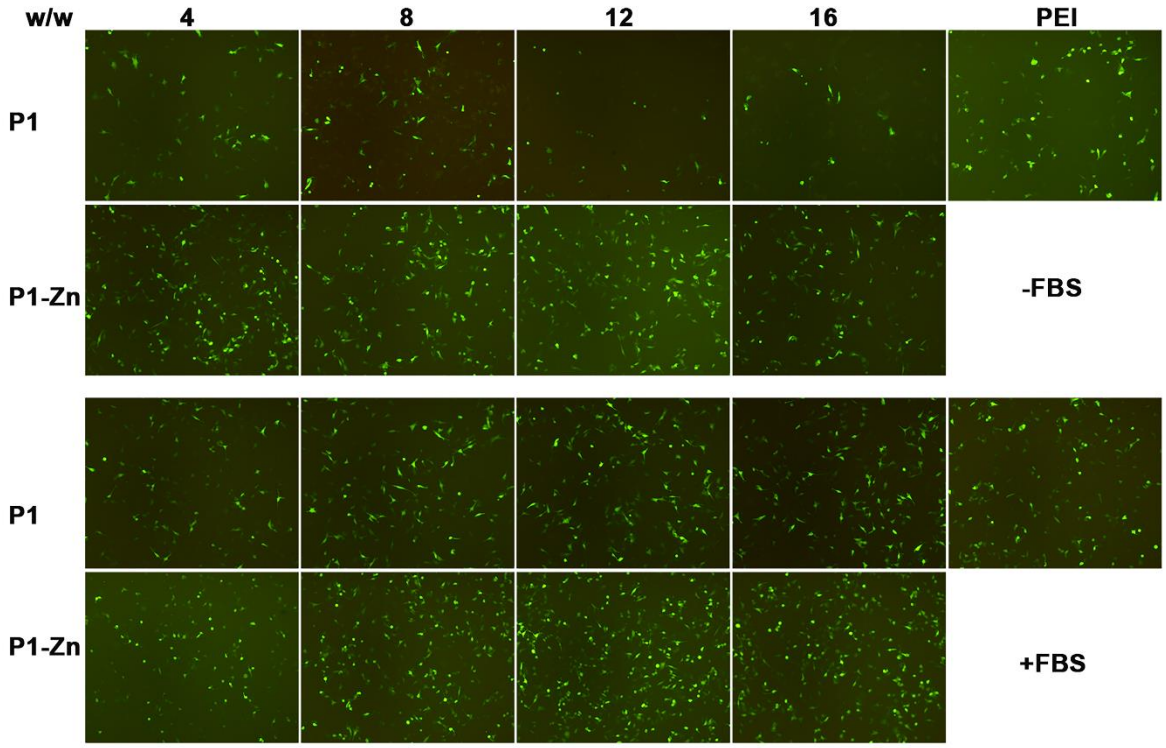



**Fig. S4.** Fluorescence microscopy images of B16 cells transfected by EGFP plasmid at various w/w ratios for 24 h. Scale bar: 200 μm.

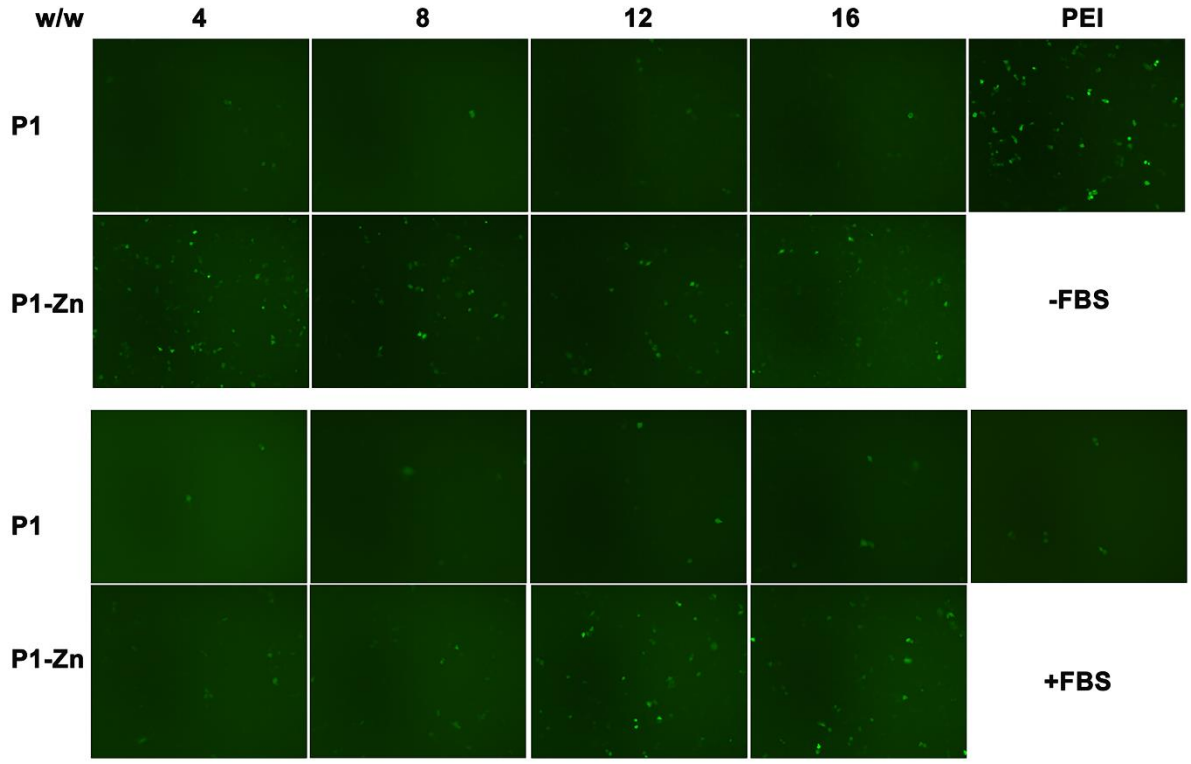



**Fig. S5.** Fluorescence microscopy images of 7402 cells transfected by EGFP-N1 plasmid at various w/w ratios for 24 h. Scale bar: 200 μm.

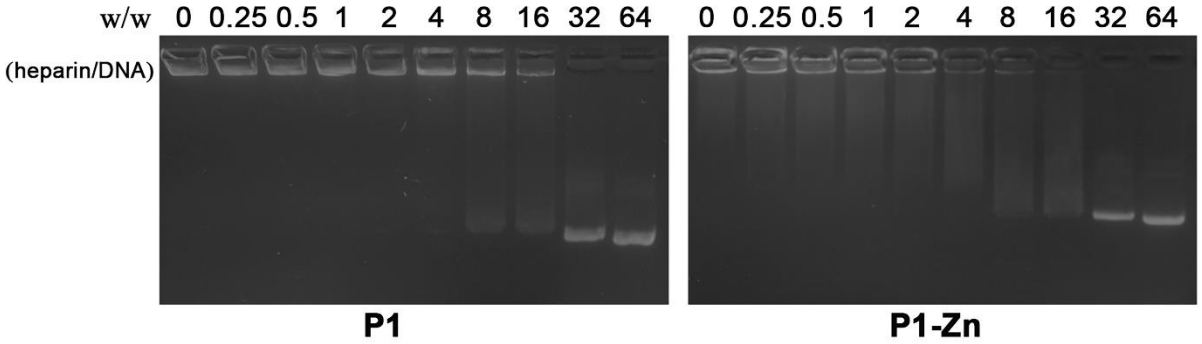



**Fig. S6.** Release of DNA from polyplxes with the addition of heparin at various heparin/DNA weight ratios. w/w = 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32;64; polymers/DNA: w/w = 2

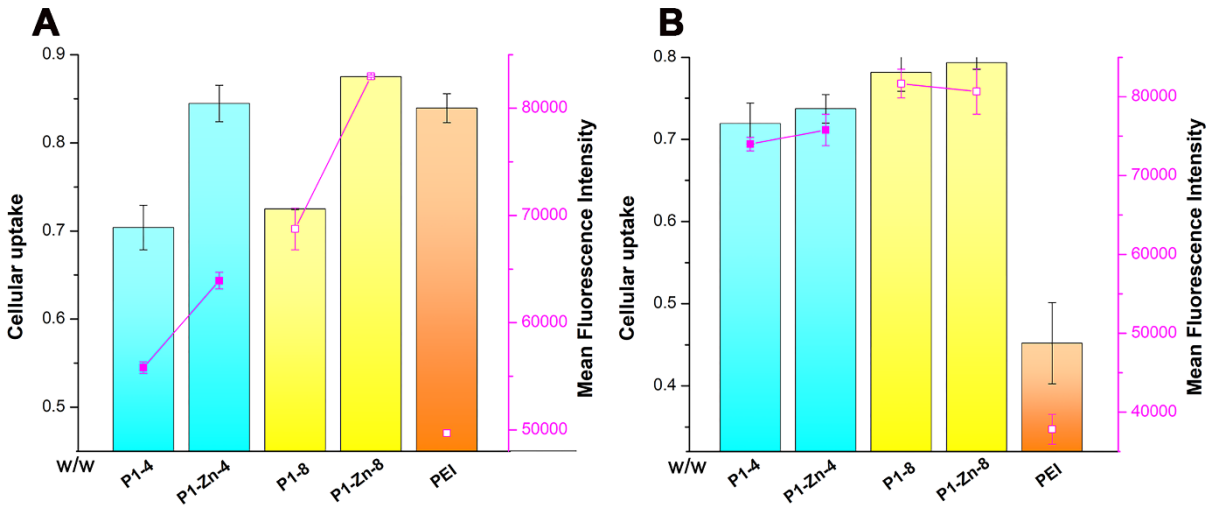



**Fig. S7.** Cellular uptake (columns, percentage of Cy5-positive cells) and mean fluorescence intensity (dots and lines) of the polyplexes derived from **P1** and **P1-Zn** in HEK293 cells quantified by flow cytometry analysis. (A) Without serum; (B) With 10% serum. The weight ratios were indicated below the horizontal axis. Data represent mean ± SD (n = 3).

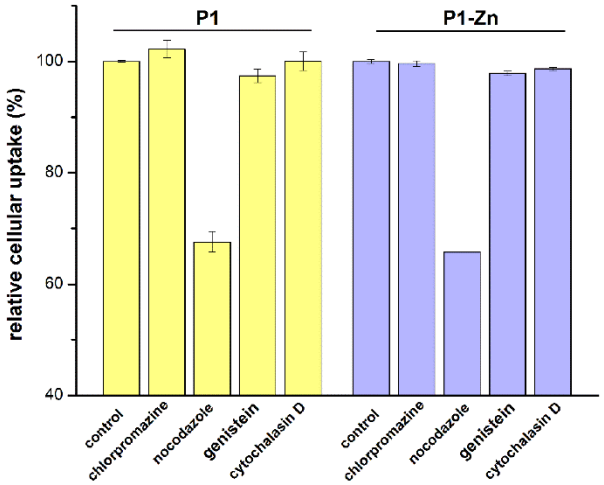



**Fig. S8.** Relative Cellular uptake of polyplexes/DNA at optimal transfection w/w ratio in HeLa cells in the presence of various endocytic inhibitors quantified by flow cytomety analysis.

**Supplementary experimental details**

*Gel permeation chromatography (GPC)*

The molecular weight (Mw) of polymers and polydispersity index [PDI, molecular weight/number average molecular weight (Mw/Mn)] of the prepared polycations were determined by using a gel permeation chromatography (GPC) system, which consisted of a Waters 515 pump, a linear 7.8 × 300 mm column (Waters Corp, Milford, MA, USA), an 18-angle laser scattering instrument (Wyatt Technology Corporation, USA), and an OPTILAB DSP interferometric refractometer (Wyatt Technology Corporation, USA). Sodium acetate-acetic acid (0.5 M, pH 4.6) was used as eluent through a membrane filter of 0.02 mm at a flow rate of 1 mL min-1.

*Agarose gel electrophoresis*

Gel retardation experiments can be used to evaluate the encapsulation of DNA by polymers. The pUC-19 DNA (5 μL,0.125 ug) was added to polymers with different mass ratios (the mass of polymer compared to DNA), then the multi-component solution was diluted to 10 μL. After incubating at 37°C for 30 minutes, 2.5 μl of loading buffer was added. Then, the mixture was electrophoresed on a 1% (w/v) agarose gel containing Gel Red™ and placed in triacetate (TAE) running buffer at 135 v 30 min. After the electrophoresis was completed, the gel was taken out and exposed to the gel imaging system to collect images.

*Ethidium bromide* (EB) *displacement assay*

The ability of polymers to condense DNA was studied using EB exclusion assays. Fluorescence spectra were measured at room temperature in air by a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer and corrected for the system response. EB (2.5 mL, 1 mg/mL) was put into quartz cuvette containing 2.5 mL of 10 mM Hepes solution. After shaking, the fluorescence intensity of EB was measured. Then CT DNA (10 mL, 1 mg/mL) was added to the solution and mixed symmetrically, and the measured fluorescence intensity was the result of the interaction between DNA and EB. Subsequently, the solution of polymer (1 mg/mL, 2 mL for each addition) was added to the above solution for further measurement. All the samples were excited at 520 nm and the emission was measured at 600 nm. The pure EB solution and DNA/EB

solution without cationic polymer were used as negative and positive controls, respectively. The percent relative fluorescence (%F) was determined using the equation %F = (F-FEB)/(F0-FEB), wherein FEB and F0 denote the fluorescence intensities of pure EB solution and DNA/EB solution, respectively.

*Determination of particle size and potential*

Polymers were incubated with pUC-19 DNA at different mass raitos for 30 min. The sizes and zeta potentials of complexes measured by dynamic light scattering using a Zetasizer Nano ZS instrument (Malvern Instruments).