Supplementary Information (ESI)

Macrocycle-Wrapped Polyethylenimine for Gene Delivery with Reduced Cytotoxicity

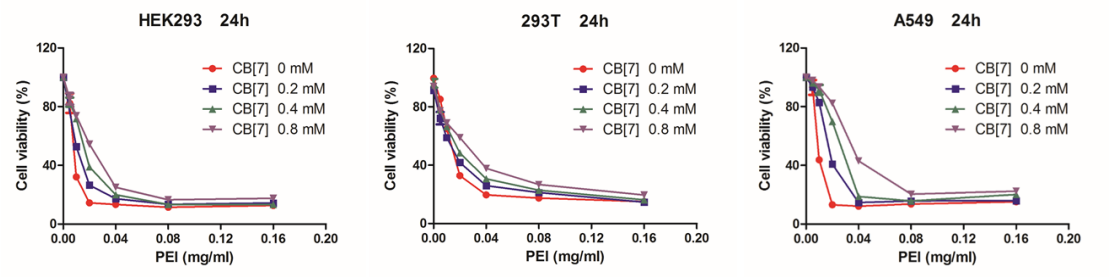
*Qiaoxian Huang,[a, ‡] Shengke Li,[a, ‡] Yuan-Fu Ding,[a,b] Hang Yin,[a] Lian-Hui Wang[b] and Ruibing Wang[a, *]*

[a] State Key Laboratory of Quality Research in Chinese Medicine, and Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau, China.

[b] Key Laboratory for Organic Electronics and Information Displays, Institute of Advanced Materials, Nanjing University of Posts & Telecommunications, 9 Wenyuan Road, Nanjing 210046, China.

Corresponding author Email: rwang@umac.mo.

(a)



(b)

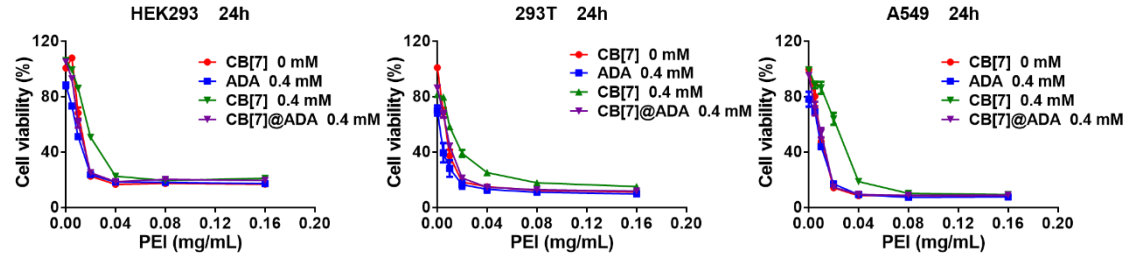


Fig. S1. Cytotoxicity of PEI (0.005, 0.010, 0.020, 0.040, 0.080, 0.160 mg/mL) (a) in the absence and presence of 0.2 mM, 0.4 mM and 0.8 mM CB[7]; (b) in the absence and presence of 0.4 mM ADA, 0.4 mM CB[7] and 0.4 mM CB[7]@ADA, respectively, on HEK293 (left), 293T (middle) and A549 (right) cell lines upon incubation for 24 h. Each data point represents the mean ± S.E.M. from a set of experiments (n = 4).

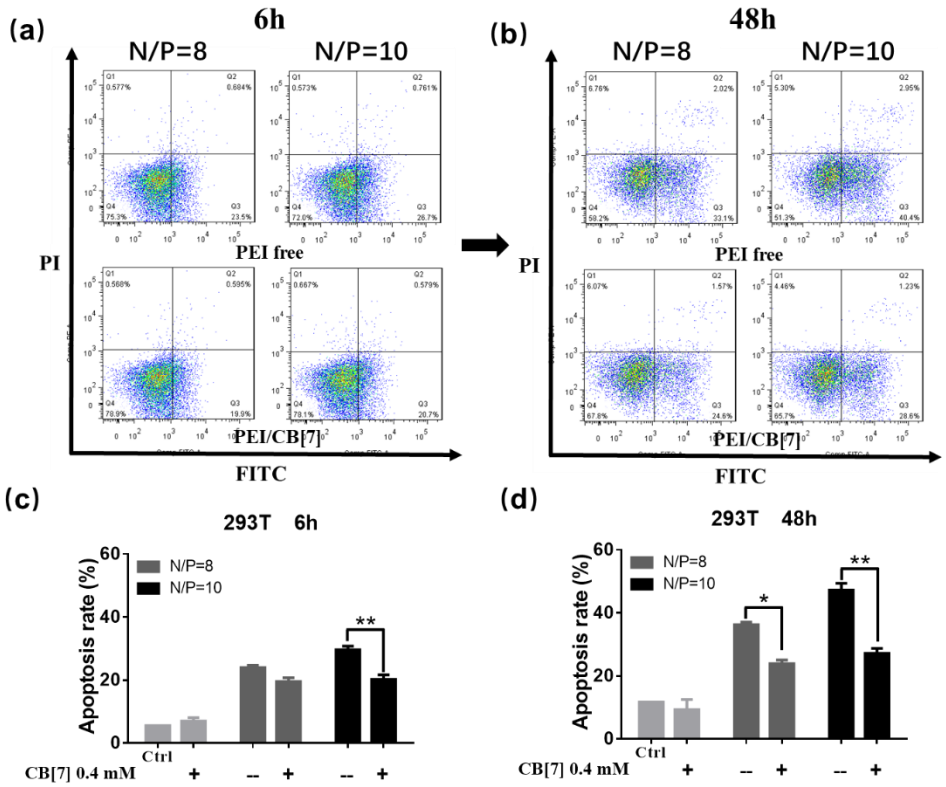


Fig. S2. Representative flow cytometry plots showing annexin V-FITC/PI staining in the process of transfection with N/P = 8 and 10, at 6 h (a) and 48 h (b) on 293T cell line. The apoptosis rates analyzed by flow cytometry and demonstrated with histograms at 6 h (c) and 48 h (d). Q1 (Annexin V-/PI+), dead cells; Q2 (Annexin V+/PI+), late-apoptotic and necrotic cells; Q3 (Annexin V+/PI-), early-apoptotic cells; Q4 (Annexin V-/PI-), viable cells (n = 3, mean ± S.E.M). *P < 0.05, **P < 0.01 vs. PEI at the same N/P ratio.

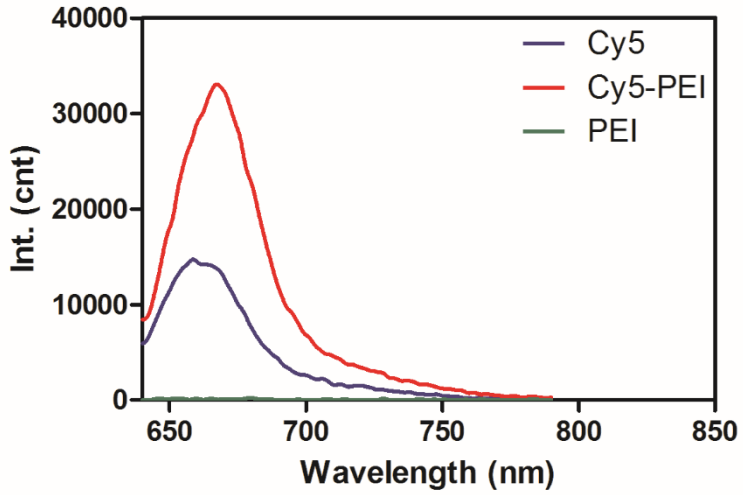


Fig. S3. Fluorescence spectroscopy of PEI, Cy5 and Cy5-PEI. (Ex: 630 nm).

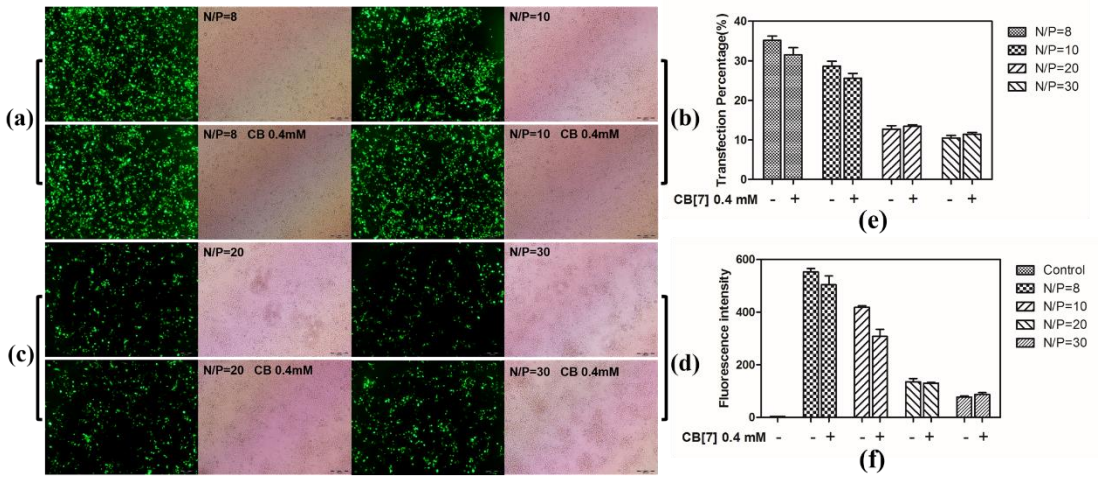


Fig. S4. Fluorescent microscopic images of pEGFP- transfected cell at different N/P ratios (8, 10, 20, 30) by PEI and PEI/CB[7] (a)-(d); Transfection efficiency of the pEGFP polyplexes with PEI or PEI/CB[7] to HEK293 cells in terms of the percentage of cells expressing GFP after 48 h transfection, in the form of histograms of the efficiency results (n=3, Mean ± S.E.M) (e), and mean Fluorescence Intensity (MFI) histograms (n=3, mean ± S.E.M) (f).

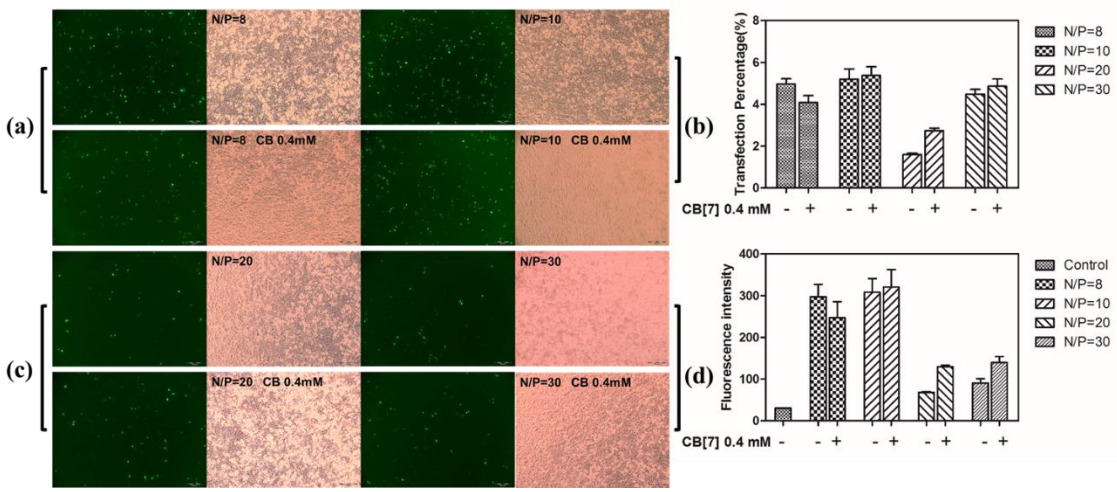


Fig. S5. Fluorescent microscopic images of pEGFP- transfected cell at different N/P ratios (8, 10, 20, 30) by PEI and PEI/CB[7] (a)-(d); Transfection efficiency of the pEGFP polyplexes with PEI or PEI/CB[7] to A549 cells in terms of the percentage of cells expressing GFP after 48 h transfection, in the form of histograms of the efficiency results (n=3, Mean ± S.E.M) (e), and mean Fluorescence Intensity (MFI) histograms (n=3, mean ± S.E.M) (f).

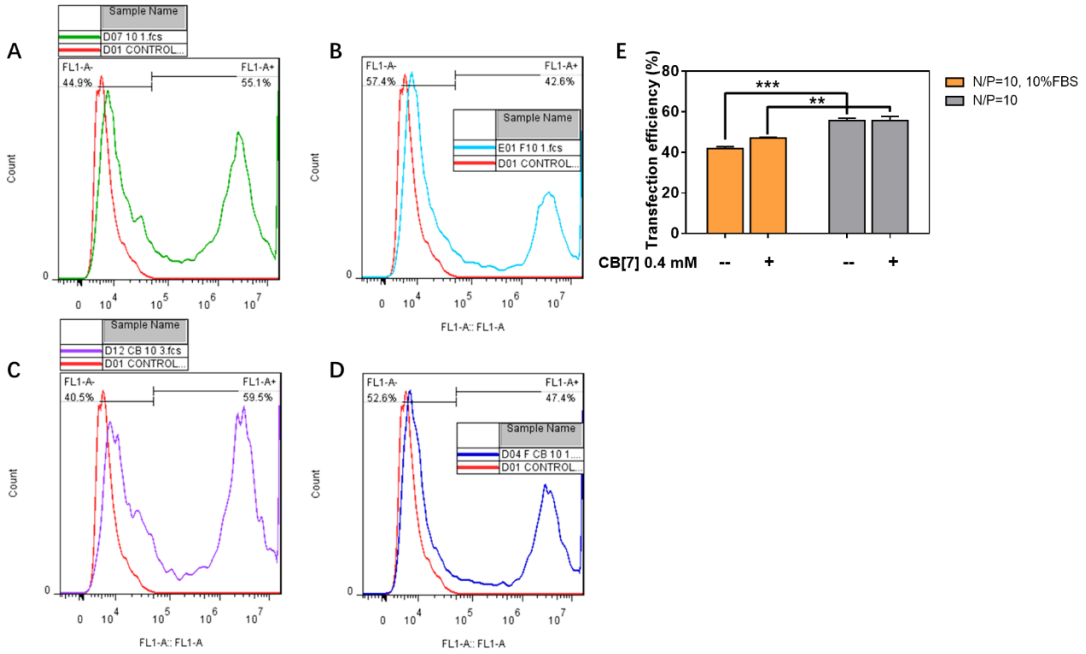


Fig. S6. A-D: Flow cytometry images of pEGFP- transfected cells (293T) at N/P ratio 10 by PEI and PEI/CB[7]. During the first 6 h, the cells were cultured in 10% serum (10% FBS) and serum-free culture medium, respectively; E: Transfection efficiency of the pEGFP polyplexes with PEI or PEI/CB[7] to 293T cells in terms of the percentage of cells expressing GFP after 48 h transfection, in the forms of histograms of the efficiency results (n = 3, Mean ± S.E.M. **P < 0.01, ***P < 0.001 denote a significant difference, 10% FBS vs. serum-free).