Supporting Information

Efficiency of Cytosolic Delivery with Poly(beta-amino ester) Nanoparticles is Dependent on the Effective pKa of the Polymer

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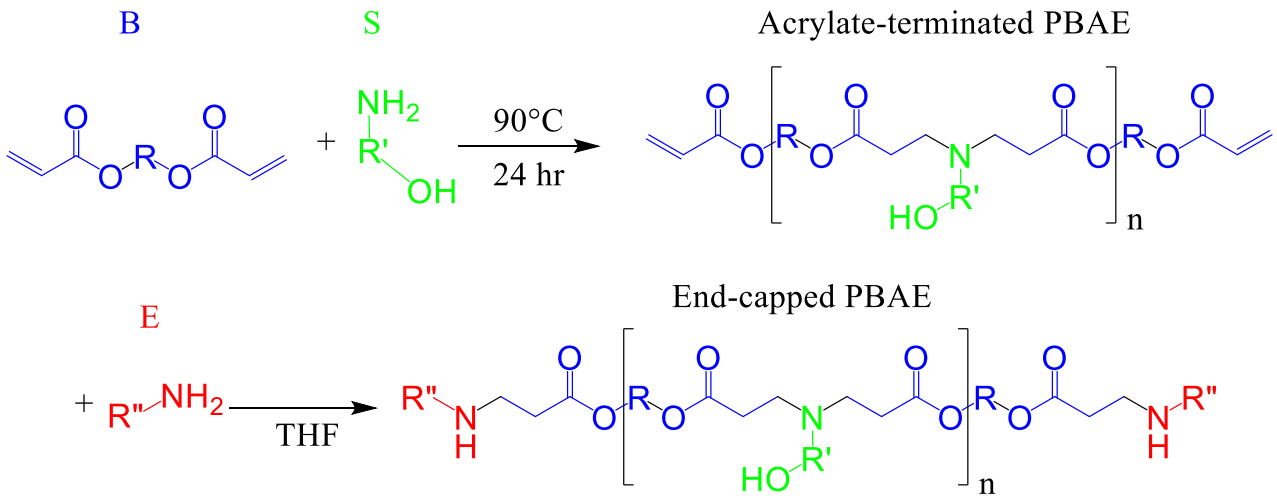

Figure S1. PBAE Synthesis. Diacrylate backbone monomer (B) is polymerized with one primary amine-containing sidechain monomer (S) in a neat solution by stirring for 24 hours at 90°C, forming the base polymer via Michael addition. This base polymer is then dissolved in anhydrous tetrahydrofuran (THF) and mixed with one end-cap small molecule (E) and stirred at room temperature for 1 hr to form the end-modified PBAE. The end-modified PBAE is then precipitated into diethyl ether, washed twice, and left under vacuum for 48 hours for complete removal of ether. The dry PBAE is dissolved in anhydrous DMSO at 100 mg/mL and stored at −20°C in small aliquots.

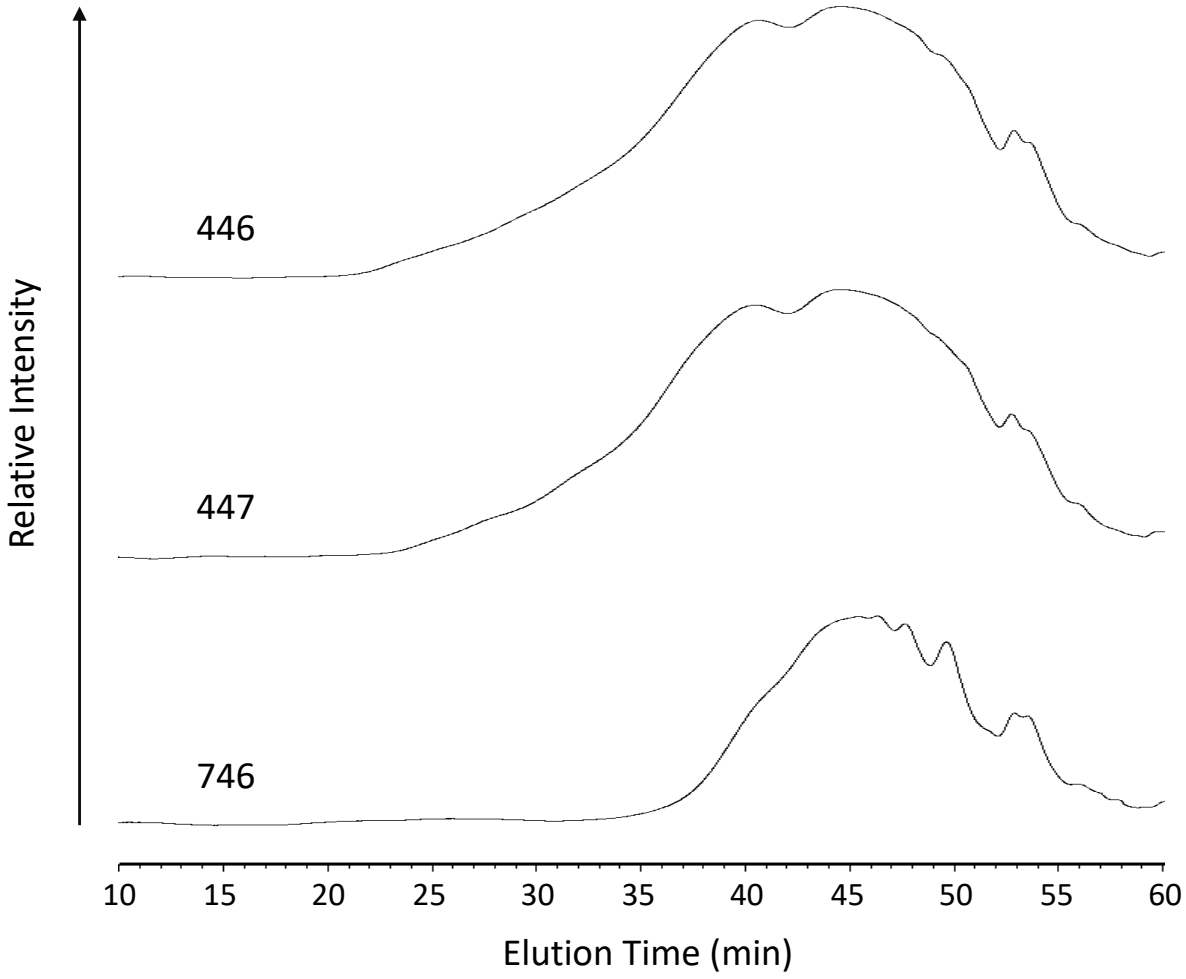

Figure S2. GPC characterization of PBAEs. The GPC traces for PBAEs 446 and 447 are similar as they are synthesized with the same base polymer and differ only in the end-capping group. PBAE 746 is synthesized with a different base polymer and is smaller than PBAEs 446 and 447.

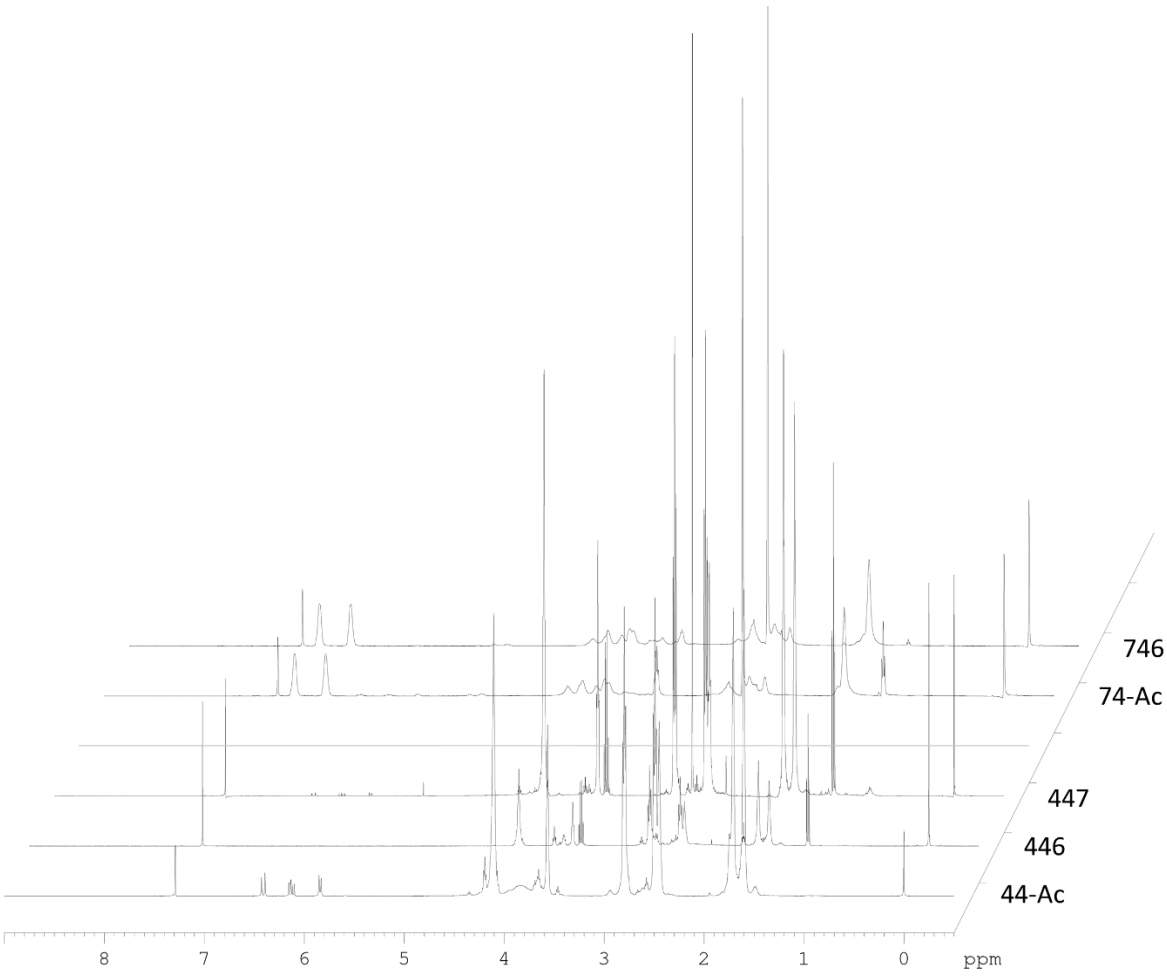

Figure S3. ^{1}H NMR spectra of PBAEs. Spectra of polymers 44-Ac and 74-Ac refer to polymers before the end-capping reaction was performed. Spectra of polymers 446, 447, and 746 are shown as well, after end-capping is complete.

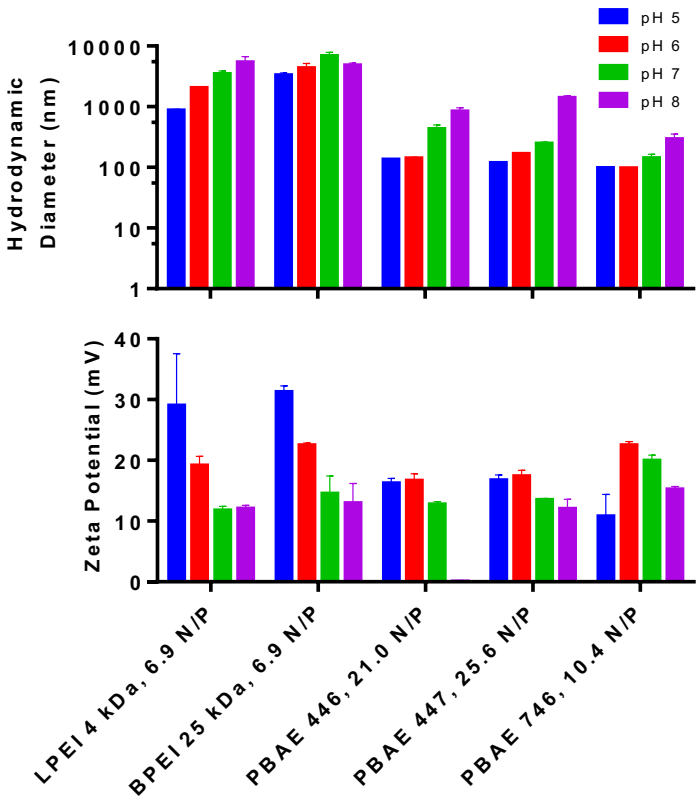

Figure S4. Supporting Figure 4. Hydrodynamic diameter and zeta potential of nanoparticles used for transfection as a function of pH.

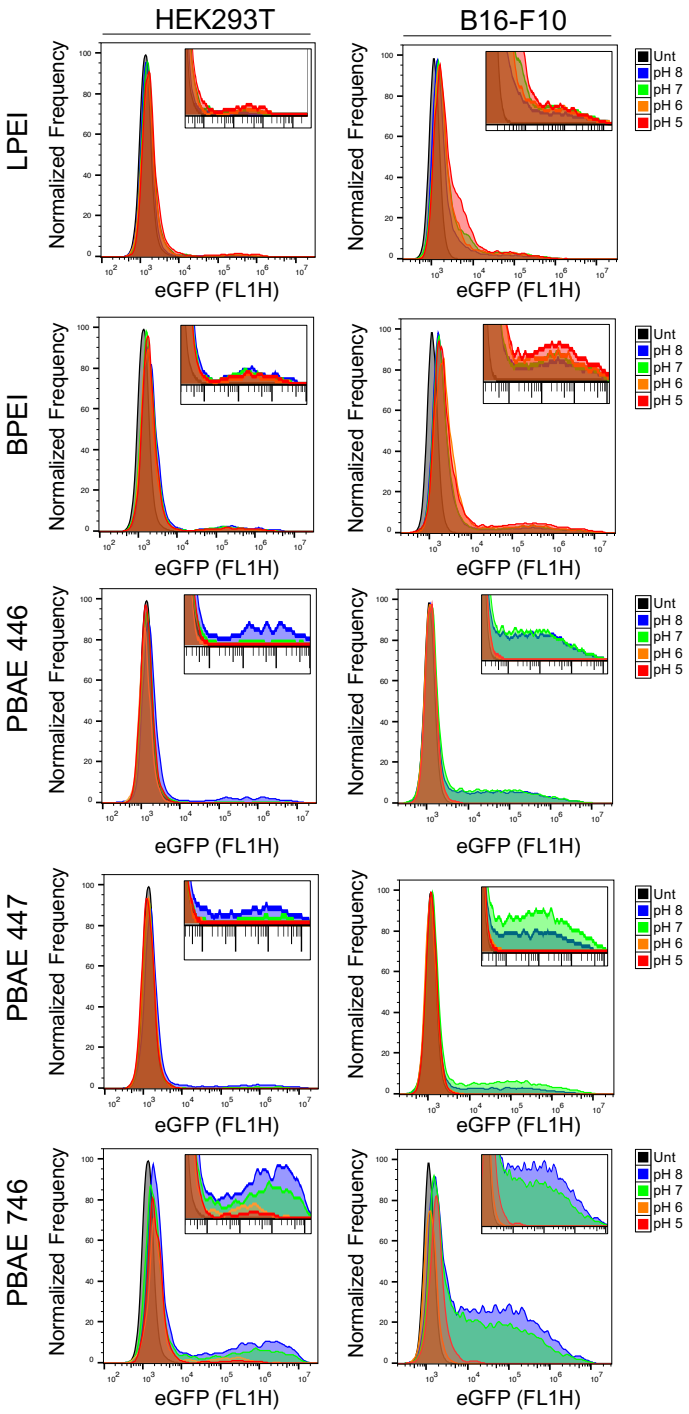

Figure S5. One-dimensional flow cytometry plots of eGFP expression. Each flow-plot shows mode-normalized frequency plots of the eGFP expression of the entire cell population when transfected with specified extracellular media pH. Inset panels show a zoom of the transfected cell population for better comparison between pH values. In both HEK293T cells and B16-F10 cells, no effective differences in transfected cell histograms are apparent for BPEI and LPEI, whereas for all PBAEs tested robust transfection is only achieved at pH 7 and pH 8.

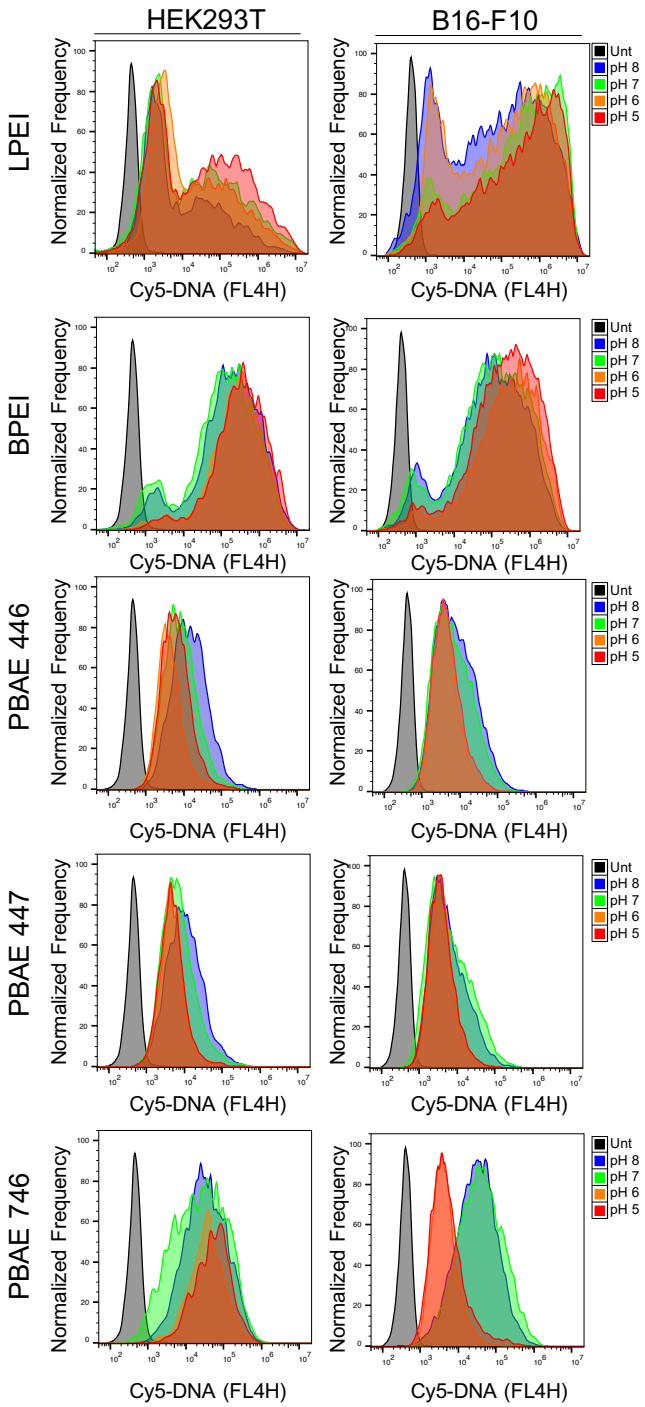

Figure S6. One-dimensional flow cytometry plots of Cy5-DNA nanoparticle uptake. Each flow-plot shows mode-normalized frequency plots of the Cy5-DNA uptake of the entire cell population when transfected with specified extracellular media pH. For all materials in both HEK293T cells and B16-F10 cells, only minor differences in cell uptake were apparent despite changing extracellular media pH. PBAE 746 with B16-F10 cells did demonstrate a different trend with greatly reduced nanoparticle uptake at pH 5 and 6 versus pH 7 and 8.