Supporting Information

pH-Sensitive Ternary Nanoparticles for Nonviral Gene Delivery

Ming-Hua Zhang,[a] Zhi-Peng Gu,[b] Xi Zhang[a]* and Min-Min Fan[a]*

1. Synthesis procedure and analysis data of tumor acidity-responsive PEGylated anionic polymer

1.1 Protection of the Amine group of β-aminoethanol with $(BOC)_2O$

Sodium hydroxide (5.00 g) and β-aminoethanol (3.82 g) were dissolved in 25 mL mixture solution of water and methanol (V/V=2/1). While stirring in an ice bath, 12 mL of a methanol solution of *tert*-butyl dicarbonate ($(Boc)_2O$, 18.9 g) was added dropwise, producing white precipitation. The reaction mixture was filtrated and the filtrate was extracted with ethyl acetate three times after stirring at room temperature for 24 h. The extraction was then washed with water until neutral, and followed with the wash of saturation salt solution. The mixture was then dried by anhydrous sodium sulfate, and the solvent was removed under reduced pressure to obtain the yellowish viscous Boc-aminoethanol.

1.2 Synthesis of Boc-PEOn/PAGEm

Boc-aminoethanol (2.64 g) was dried for 1 h at 100 °C under reduced pressure by an oil pump, and dissolved in 15 mL anhydrous 1,4-dioxane. Sodium hydride (60%, w/w) (1.03 g) was added after the above solution was heated for a few minutes at the desired temperature (60 °C), and the distilled ethylene oxide (EO, 50mL) was then added dropwise by a constant pressure infundibulum connected with a condensing tube (4−8 °C circulation water) and a drying tube to maintain the reaction system anhydrous. After 36 h, AGE (22 mL) was added to the above reaction solution, and the mixture was stirred at 60 °C for 24 h. Afterward, a little water was added to the reaction mixture to terminate the reaction, and the mixture was adjusted to neutral pH using hydrochloric acid (HCl, 5 M). The solvent was removed under reduced pressure. The residue was redissolved in dichloromethane, and dried with anhydrous sodium sulfate. Sodium sulfate was removed by filtration, and the calrified filtrate was concentrated in vacuo. After repeated precipitation with cold diethyl ether for three times, the Boc-PEOn/PAGEm was obtained as the product, and then verified by ^{1}H NMR (Fig. S1).

1.3 Synthesis of Boc-PEOn/PAGEm-Cys (PPC)

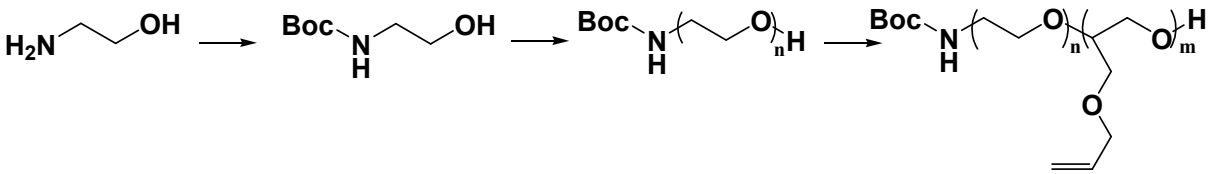
Boc-PEOn/PAGEm-Cys (PPC) was synthesized according to the procedure of Koyama et al.[1] Briefly, 3 mL methanol with Boc-PEOn/PAGEm (0.773 g) was added dropwise to the solution of cysteamine hydrochloride (Cys, 2.26 g) in 6 mL methanol. After stirring at room temperature for 48 h, the methanol was removed by evaporation. The resulting mixture was dialyzed against pure water for 3 days though cellulose membrane (MWCO 1000 Da). Lyophilization of the solution led to a yellowish product, namely Boc-PEOn/PAGEm-Cys. The structure of resultant was verified by ^{1}H NMR (Fig. S2).

1.4 Synthesis of Boc-PEOn/PAGEm-Cys-DMMA(PPC-DA)

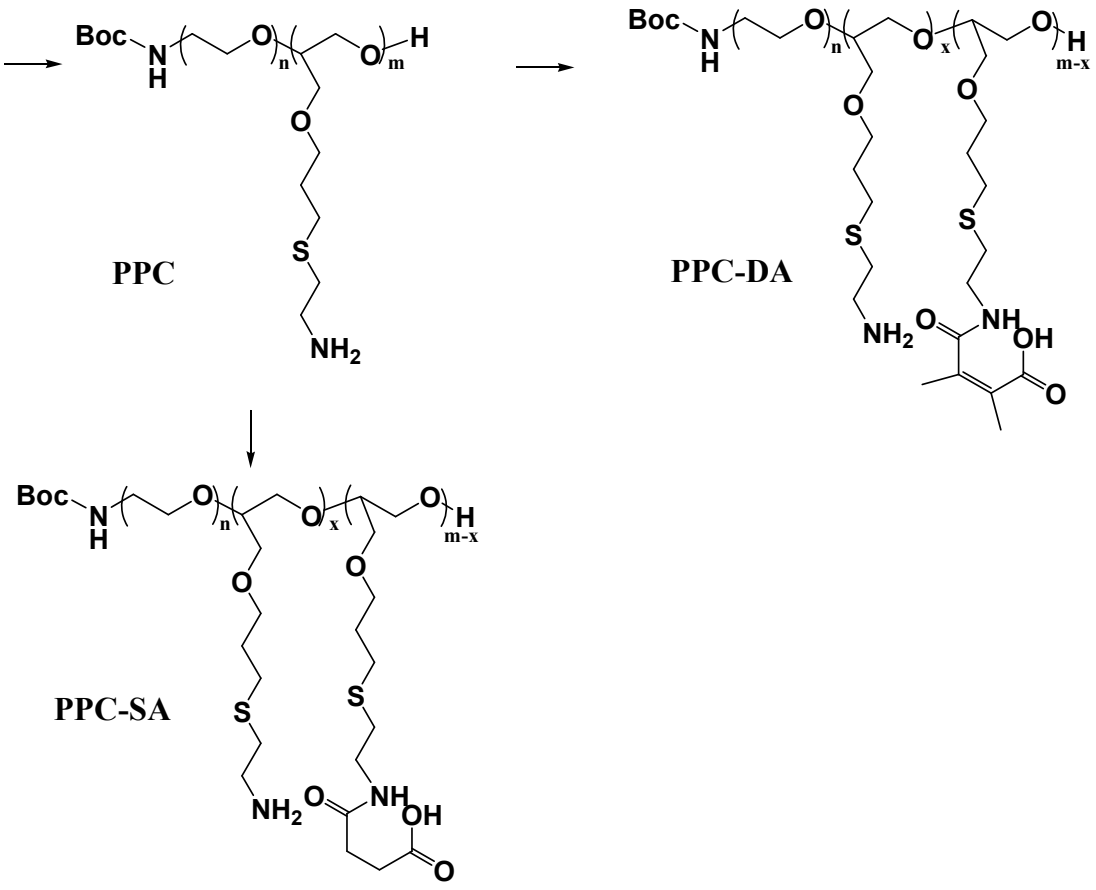
PPC (5.3 mg) was dissolved in 20 mL of distilled water, and the pH was adjusted to pH 8.5 with 0.2 M NaOH solution. After stirring vigorously at room temperature for 30 min, 100 equivalents (to amino groups) of 2,3-dimethylmaleic anhydride (DMMA, 148.36 mg) were added in several portions. The pH of solution was maintained in the range of 8-9 using 1 M NaOH solution. The reaction was continued at room temperature for 6 h. Afterward, the excess DMMA was removed by ultrafiltration (Millipore, MWCO 3000 Da), and PPC-DA was obtained by lyophilization. The final product was characterized by ^{1}H NMR analysis (Fig. S3).

1.5 Synthesis of Boc-PEOn/PAGEm-Cys-SA(PPC-SA)

The synthesis of PPC-SA was similar to that of PPC-DA by replacing DMMA with succinic anhydride (SA). The final product was verified by ^{1}H NMR analysis (Fig. S4).



Aminoethanol **Boc-aminoethanol** **Boc-amino-PEOn** **Boc-PEOn/PAGEm**



PPC **PPC-DA**

PPC-SA

Scheme S1 Synthesis procedures and the structures of of PPC-SA and PPC-DA.

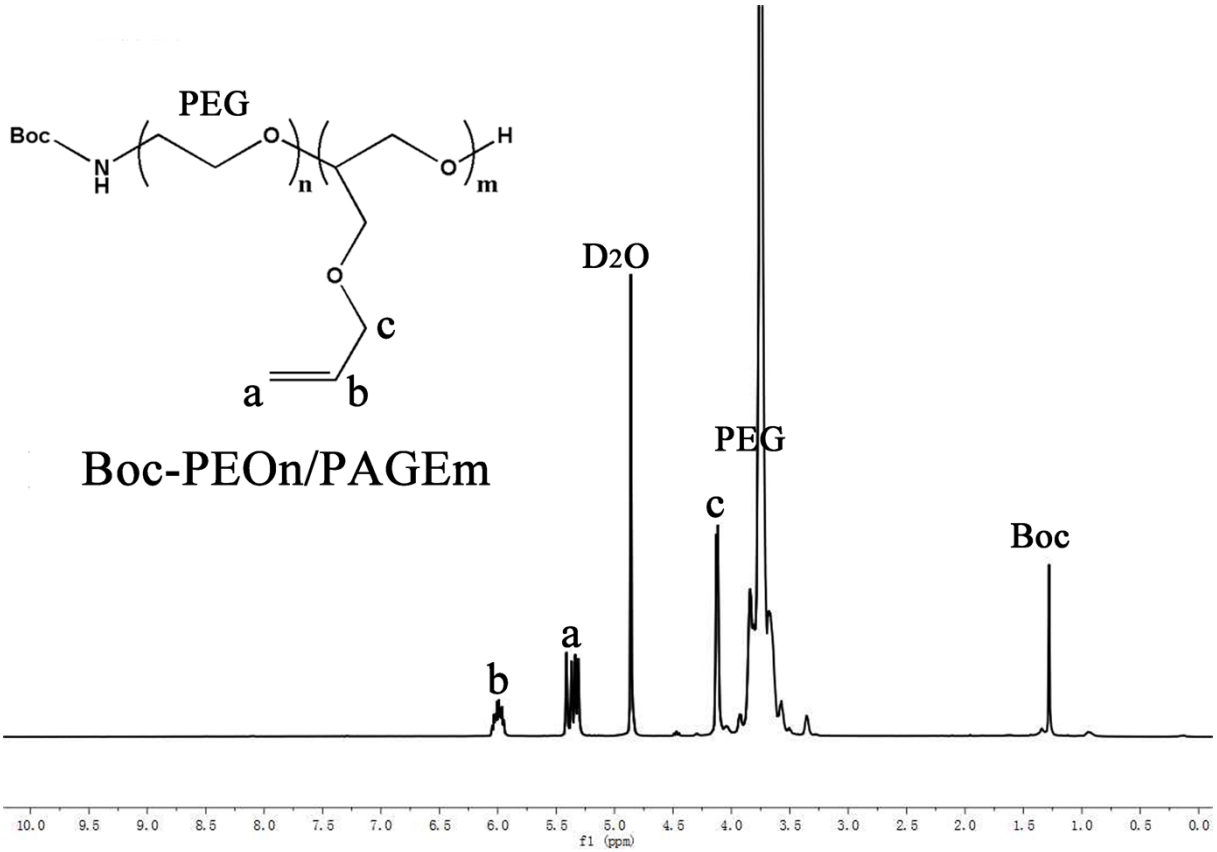


Fig. S1 ^{1}H NMR (600 MHz) of Boc-PEOn/PAGEm in D_2O at 25 °C.

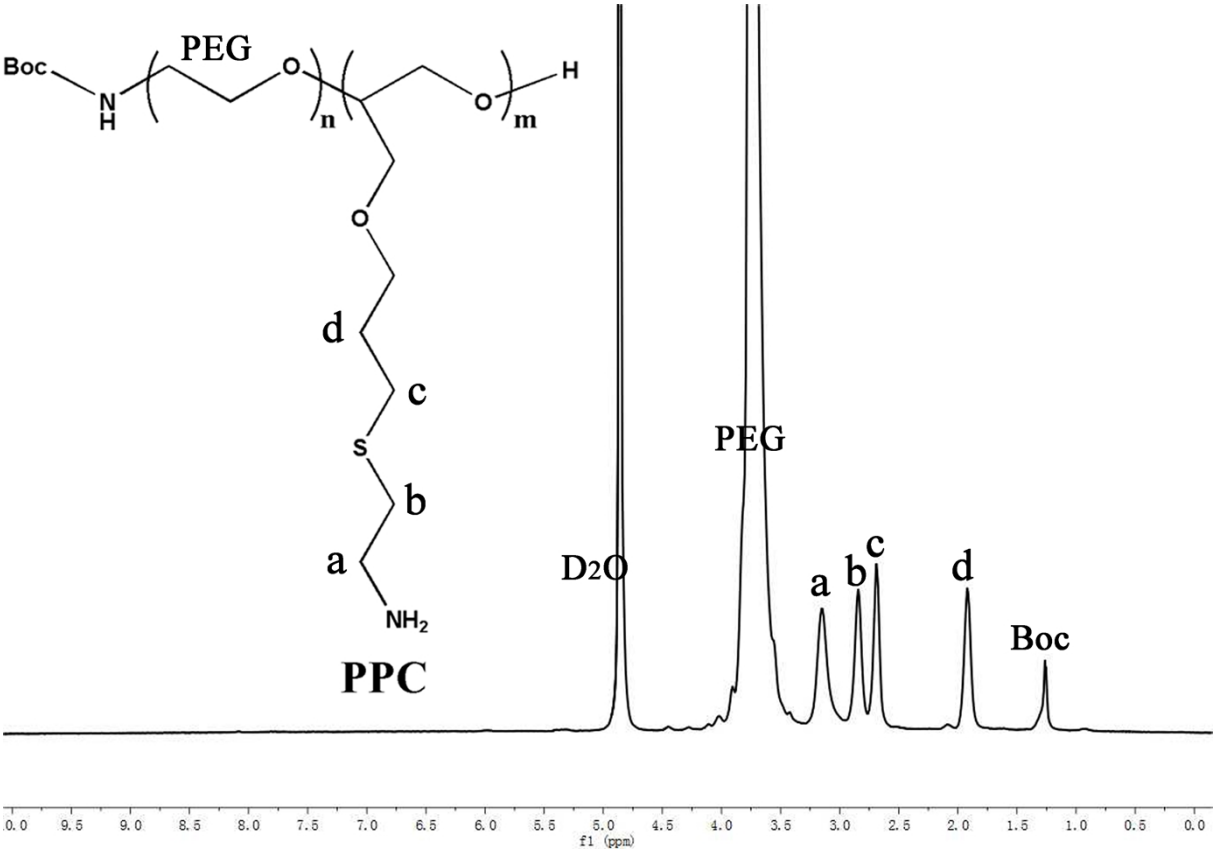


Fig. S2 ^{1}H NMR (600 MHz) of Boc-PEOn/PAGEm-Cys (PPC) in D_2O at 25 °C.

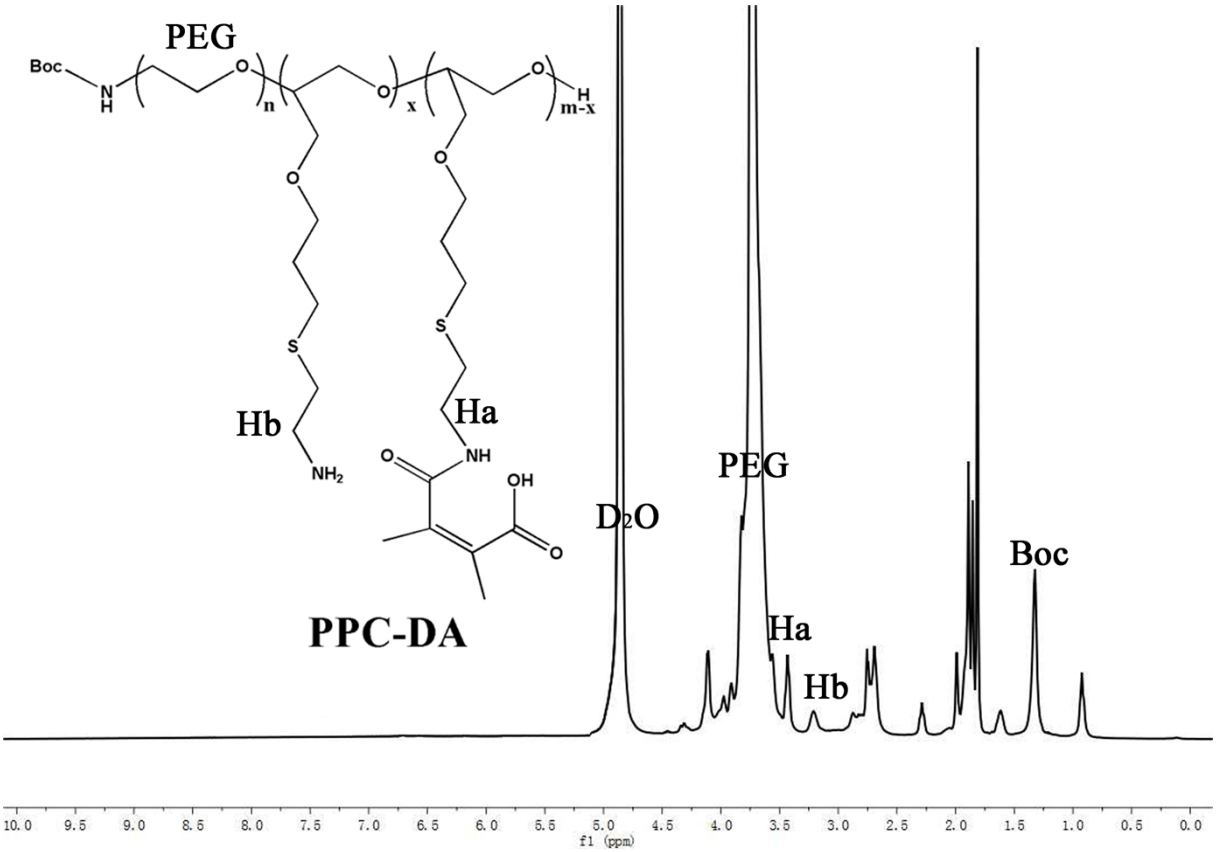


Fig. S3 ^{1}H NMR (600 MHz) of Boc-PEOn/PAGEm-Cys-DMMA (PPC-DA) in D_2O at 25 °C.

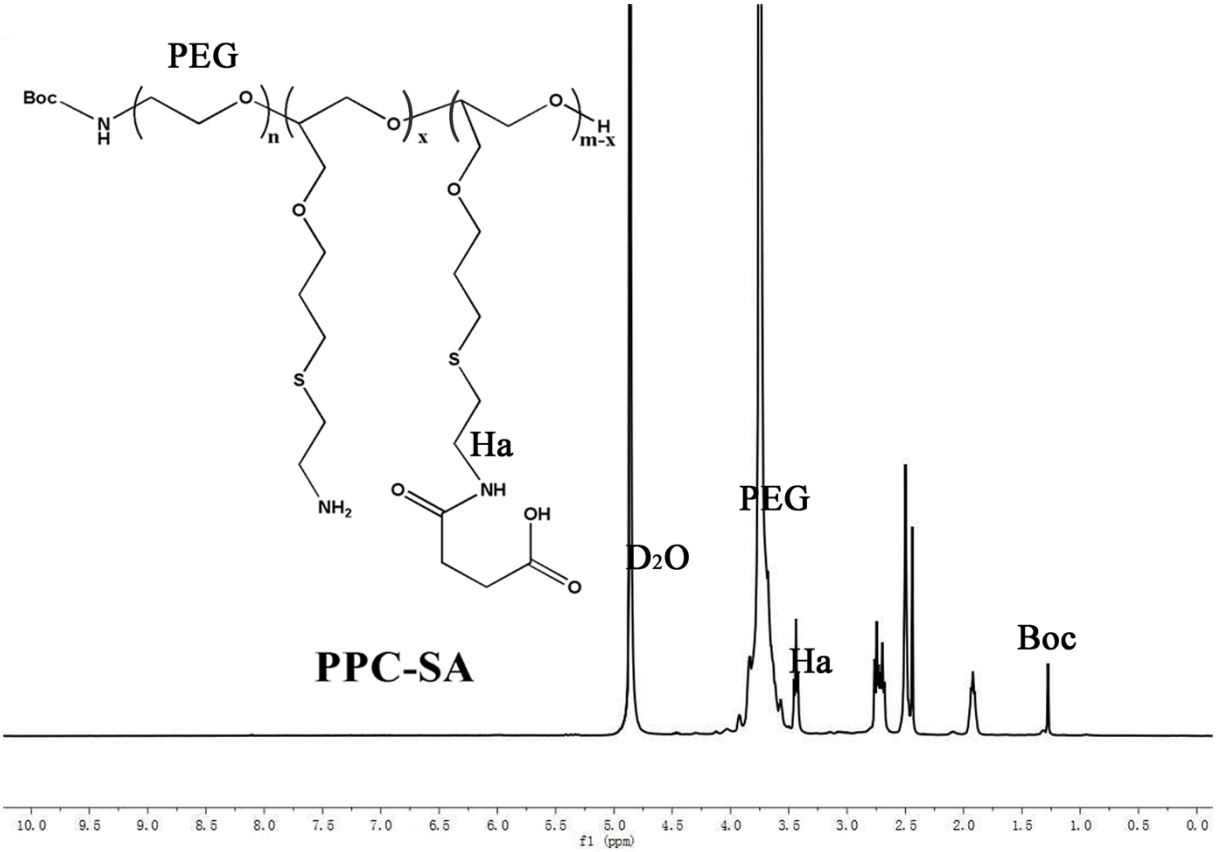


Fig. S4 ^{1}H NMR (600 MHz) of Boc-PEOn/PAGEm-Cys-SA (PPC-SA) in D_2O at 25 °C.

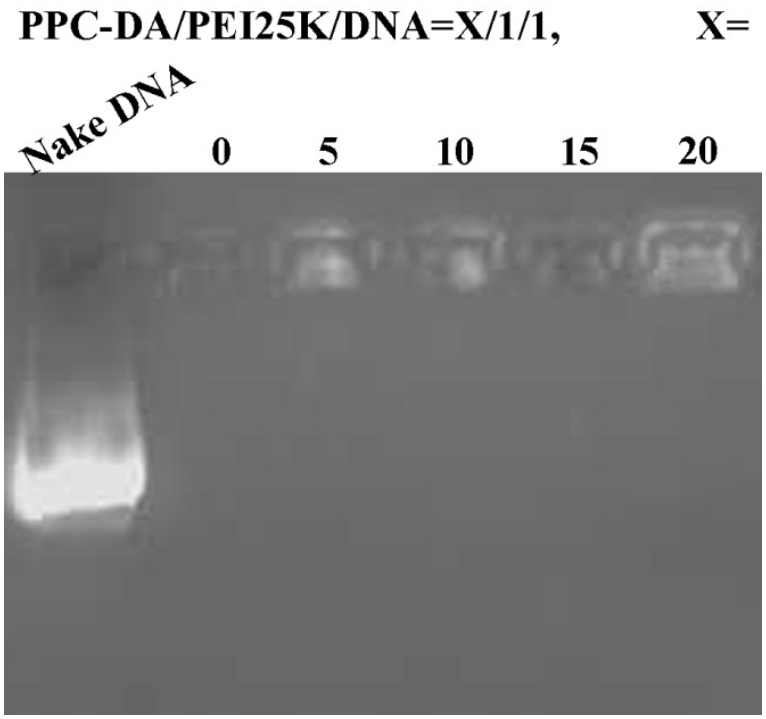


Figure S5. The DNA retardation assay of PPC-DA/PEI25K/DNA.

1 Y. Koyama, T. Ito, H. Matsumoto, A. Tanioka, T. Okuda, N. Yamaura, H. Aoyagi, T. Niidome, *J. Biomat. Sci., Polym. E.* 2003, **14**, 515.