Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Peptide modified polycations with pH triggered lytic activity for efficient gene delivery

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Materials and Methods.

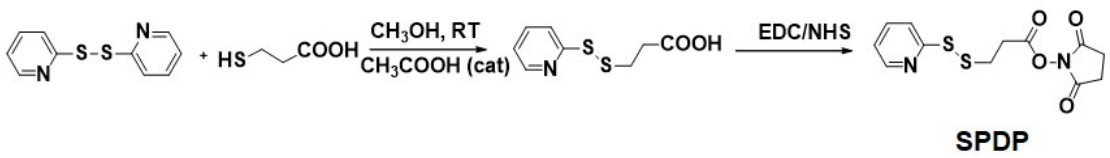
1. Materials. 2-(Dimethylamino)ethyl methacrylate (DMAEMA), triphosgene, N_{ε}-carbobenzoxy-L-lysine, 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB), 2,2'-azobis(2-methylpropionitrile) (AIBN), 2,2'-dithiodipyridine, 3-mercaptopropionic, hexylamine and methacryloyl chloride were purchased from Aladdin. DMAEMA was purified by passing through basic alumina filled column to remove the inhibitor before polymerization. Branched polyethylenimine (PEI, 25 kDa, Sigma) was dissolved in PBS with a concentration of 10 mg/mL followed by the pH adjustment using 1M HCl to ~ 6, and it was diluted with water to get the final concentration for experiments. Pyridyl disulfide ethyl methacrylate (PDSEMA) was synthesized as described previously.[1] Cysteine-C6M3 (NH_2-RLWHLLWRLWRRLHRLLRCCONH$_2$) was purchased from GL Biochem Ltd. (Shanghai, China). The luciferin substrate, plasmid encoding firefly luciferase (pGL3) was obtained from Promega (Madison, WI, USA). HeLa cells (ATCC CCL-2™) were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics/antimycotics (AbAm) (100 IU of penicillin, 100 ug/mL of streptomycin, and 0.25 ug/mL of amphotericin B).

2. Synthesis of SPDP. PDSPA (0.043 g, 0.2 mmol) was first dissolved in the mixture of DMSO and water (1 mL) followed the addition of by EDC (0.038 g, 0.20 mmol) and NHS (0.023 g, 0.20 mmol). The reaction was stirred at room temperature for 0.5 h to obtain 3-(2-pyridyldithio)propionic acid N-hydroxysuccinimide ester (SPDP), which was directly used for the next step.

3. Hemolysis of polycations. Hemolysis assay was used to evaluate the acid-triggered membrane-lytic activity of the synthetic materials at pH 7.4 (extracellular pH) and 5.7 (endosomal pH). Kunming mice were sacrificed to acquire pericardial blood. The blood was collected and gently blend in 10 mL EDTA containing vacutainers. Then the blood was centrifuged at 1500 rpm for 5 min at 4 ^{o}C. The total volume of blood was marked before removing the serum. The blood cells were then centrifuged and resuspended for two further times to remove remnant serum. After removing the saline solution at the last washing step, the red blood cells were resuspended into phosphate buffer (PB) at pH 7.4 or 5.7. The polymers at various concentrations (10 - 100 μg/mL) and 1% Triton X-100 as control, were added to the RBC suspensions in a 96-well conical plate and was allowed to incubate at 37 ^{o}C for 60 minutes. The suspension was collected and centrifuged for 5 minutes at 1500 rpm. 100 μL of supernatant was transferred from each group into a 96 well plate. Finally, the absorbance of the solution was measured with Multiskan FC Microplate Reader (Thermo, US). The hemolysis was calculated as follows:

$$H\ (\%) = \frac{(Ae - Ac)}{At} * 100\%$$

Where H represents the hemolysis, Ae, Ac, and At represents the absorbance of at 541nm for the experiment group, saline solution group, and Triton X-100 group, respectively.



Scheme S1. Synthetic route of SPDP.

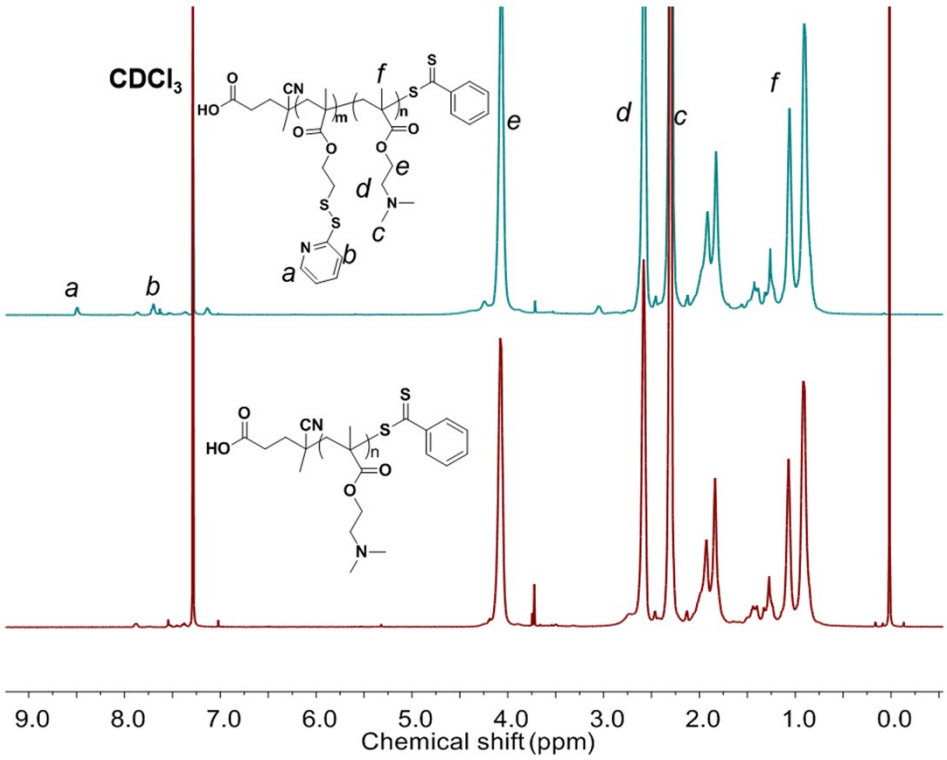


Fig. S1 ^{1}H NMR spectra of PDMAEMA and P(DMAEMA-co-PDSEMA).

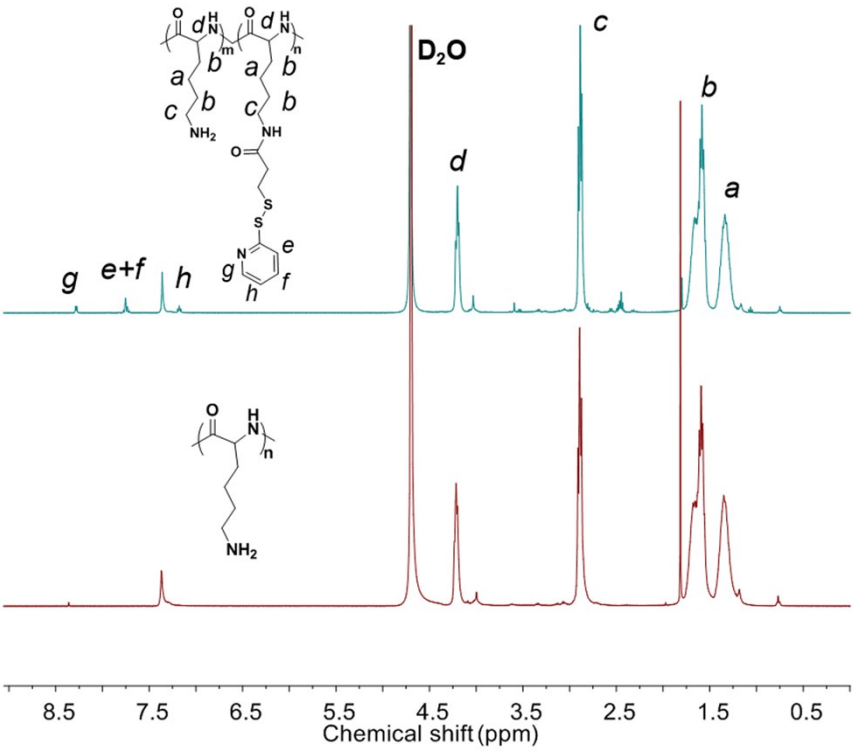


Fig. S2 ^{1}H NMR spectra of PLL and PLL-SPDP.

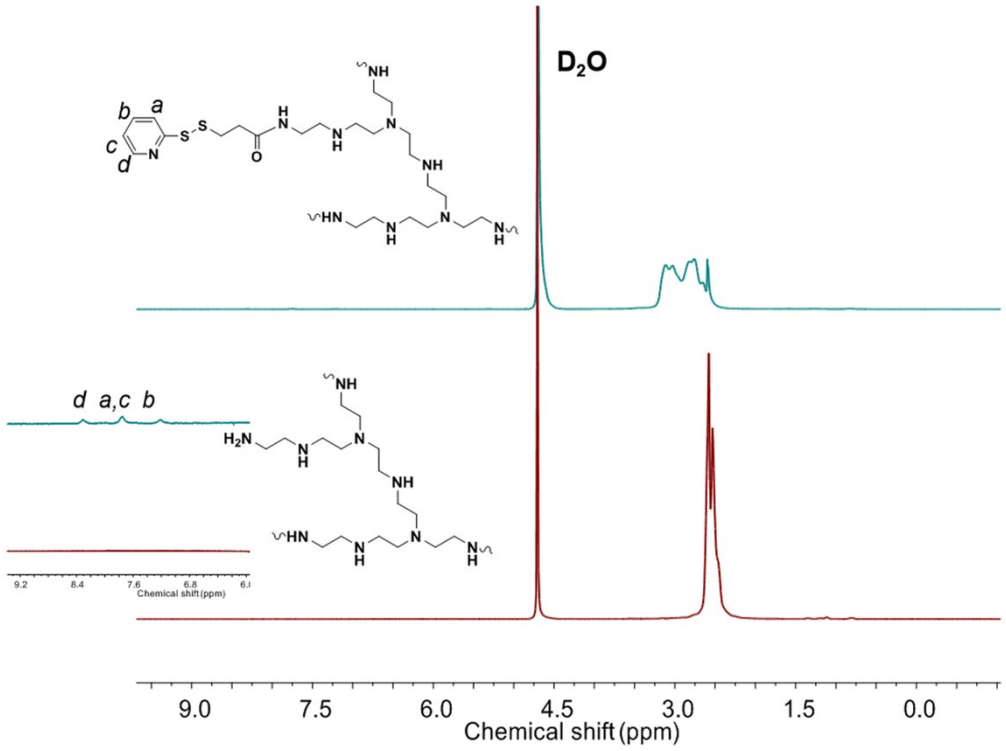


Fig. S3 ^{1}H NMR spectra of PEI and PEI-SPDP.

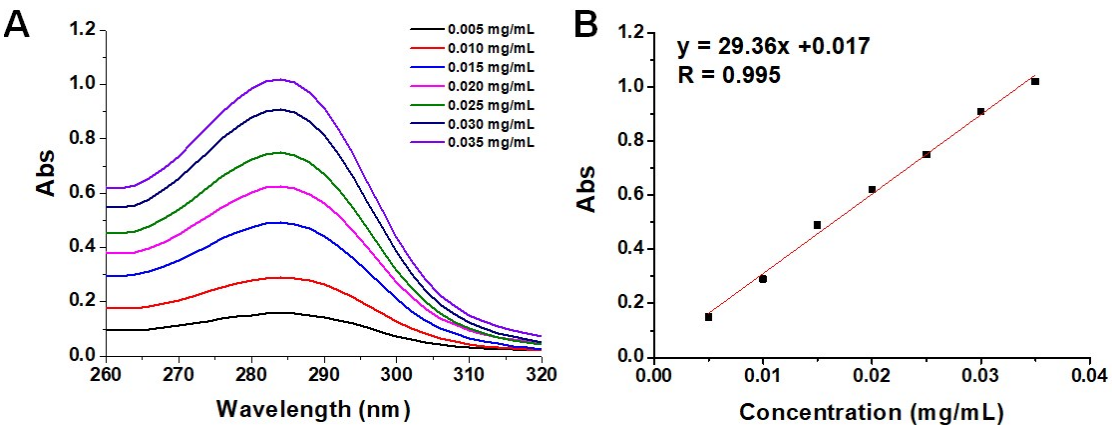


Fig. S4 A). UV-spectra of 3-(2-pyridyldithio)propionic acid with different concentrations. B). The relationship of 3-(2-pyridyldithio)propionic acid concentration and the corresponding absorption intensity.

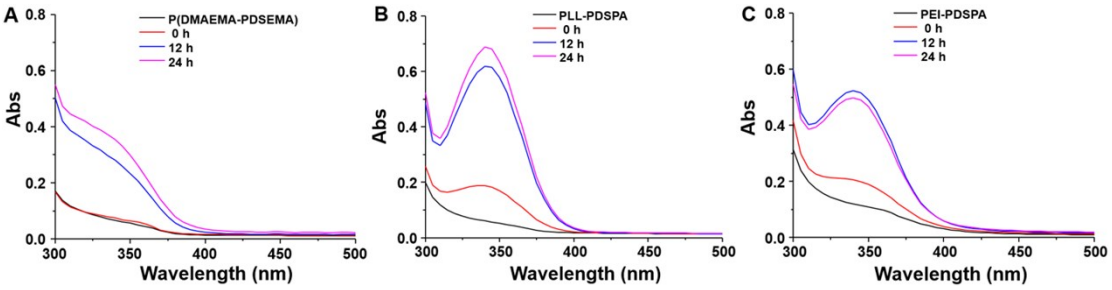


Fig. S5 UV spectra before and after the disulfide exchange reaction.

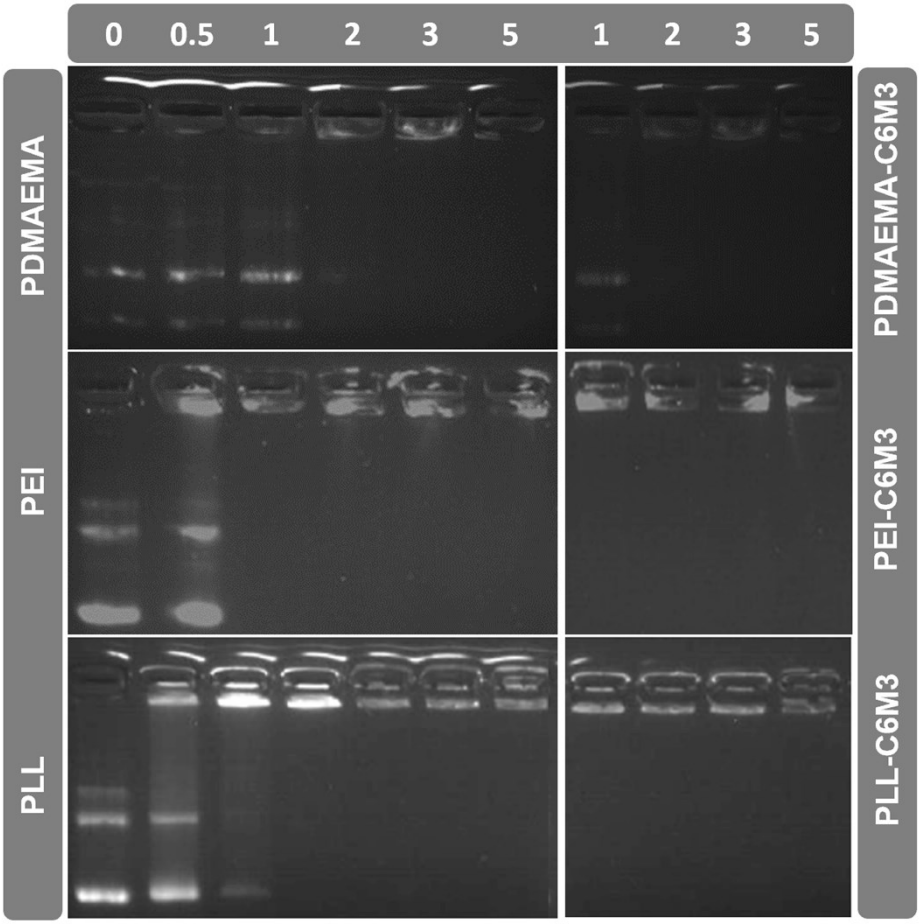


Fig. S6 Agarose gel electrophoresis of polymer/DNA complexes prepared at different N/P ratios.

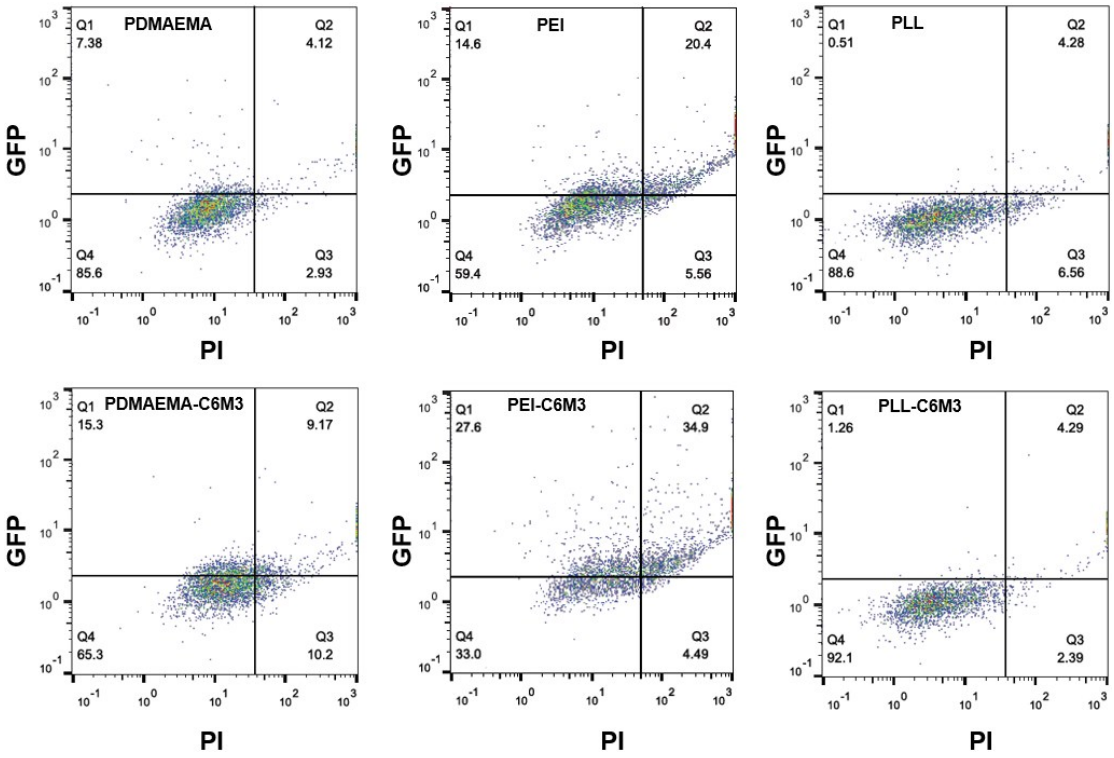


Fig. S7 Representative quadrant analysis of GFP transfection in HeLa cells with different polymers. Q1: GFP+/PI-; Q2: GFP+/PI+;Q3: GFP-/PI+; Q4: GFP-/PI-.

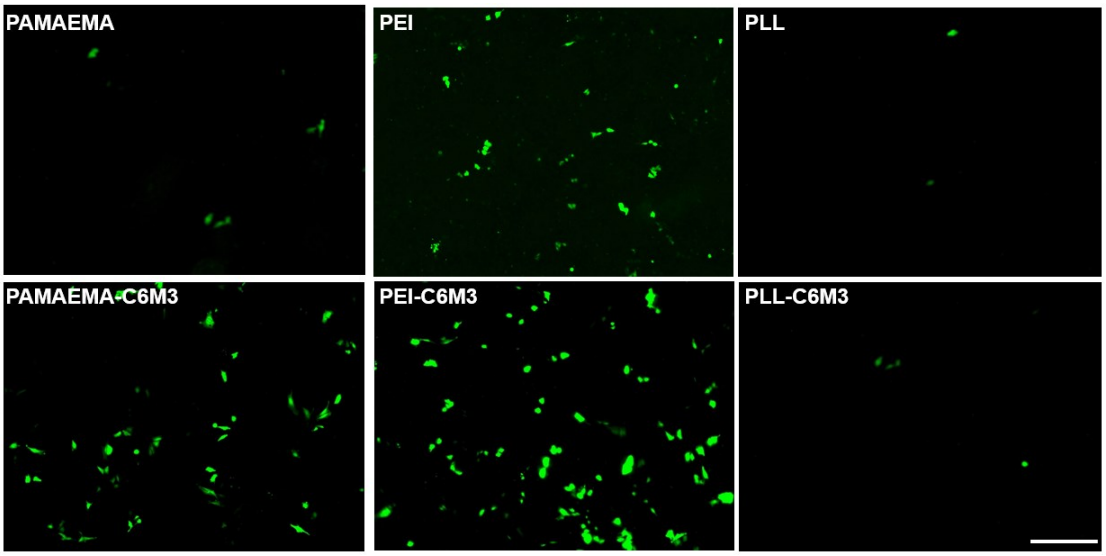


Fig. S8 Representative images of GFP expression mediated by various polyplexes in HeLa cells. Scale bar: 100 um.

REFERENCE

1 Y. L. Cheng, R. C. Yumul and S. H. Pun, *Angew Chem Int Edit*, 2016, **55**, 12013-12017.