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Supplemental Information

A Targeted and Stable Polymeric Nanoformulation Enhances Systemic Delivery of mRNA to Tumors

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Supplemental data items:

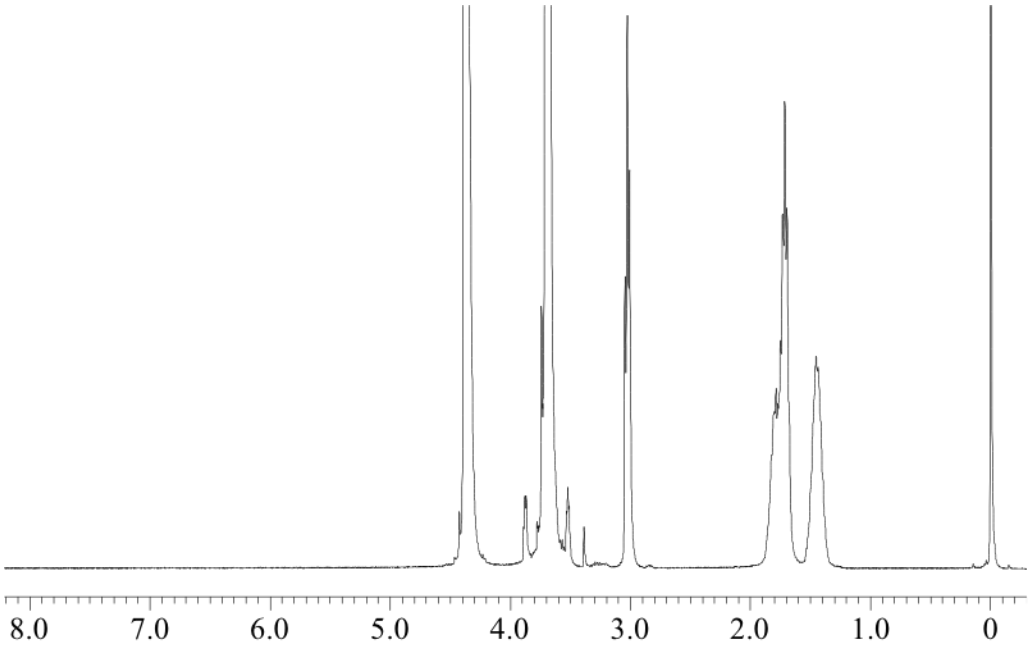


Figure S1 ^{1}H-NMR spectra of PEG-PLys in D_2O at 80 °C.

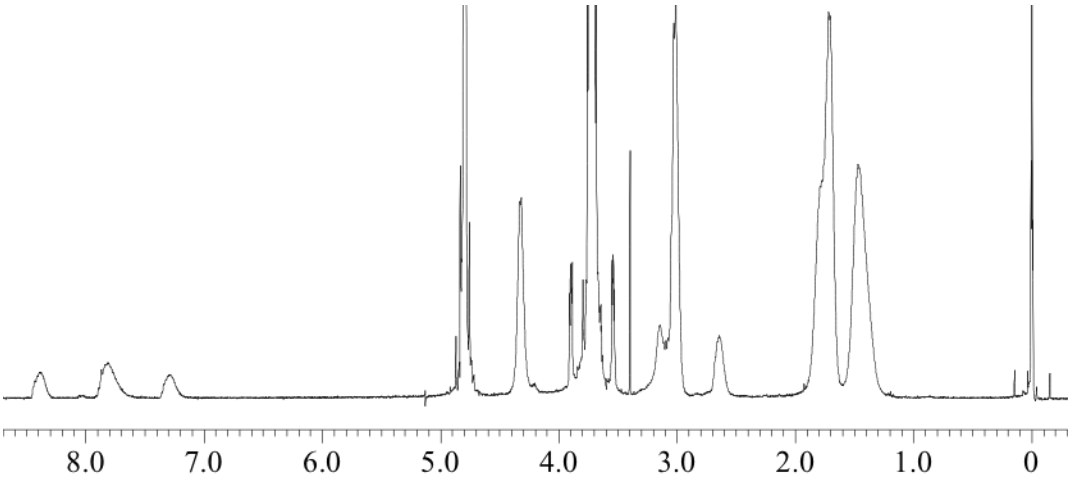


Figure S2 ^{1}H-NMR spectra of PEG-PLys(PDP) in D_2O at 80 °C.

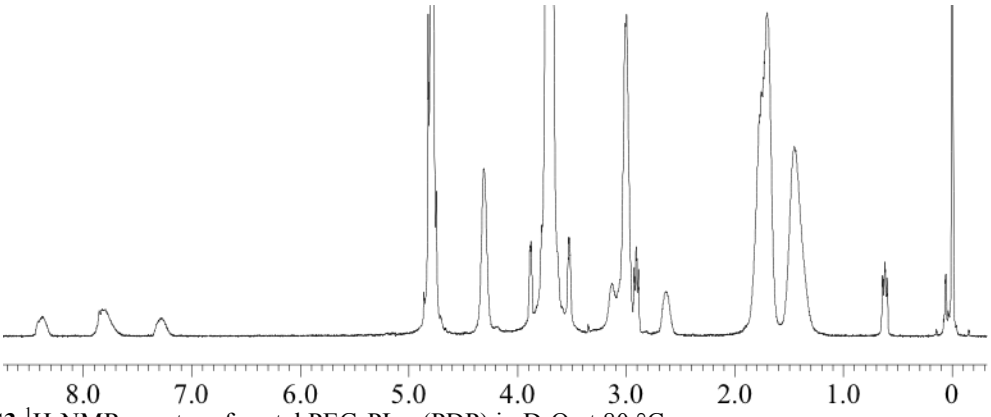


Figure S3 ^{1}H-NMR spectra of acetal PEG-PLys(PDP) in D_2O at 80 °C.

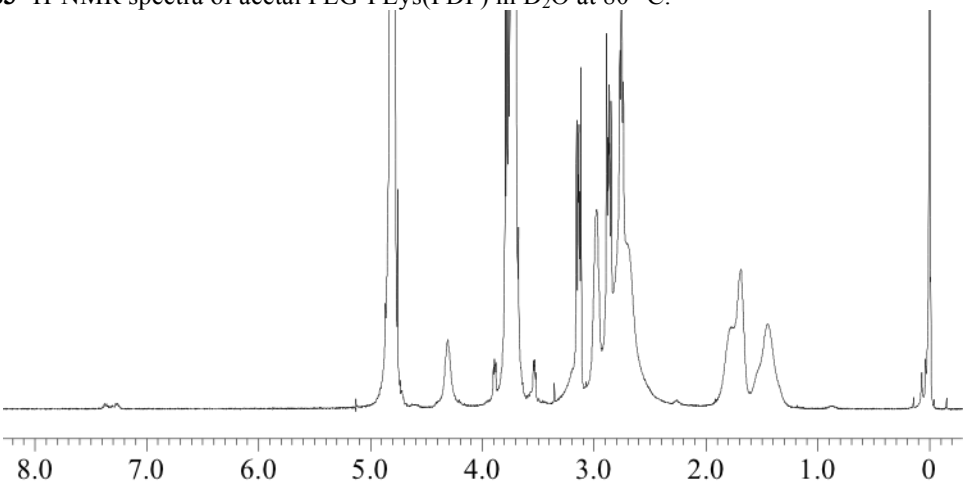


Figure S4 ^{1}H-NMR spectra of cRGD-PEG-PLys(PDP) in D_2O at 80 °C.

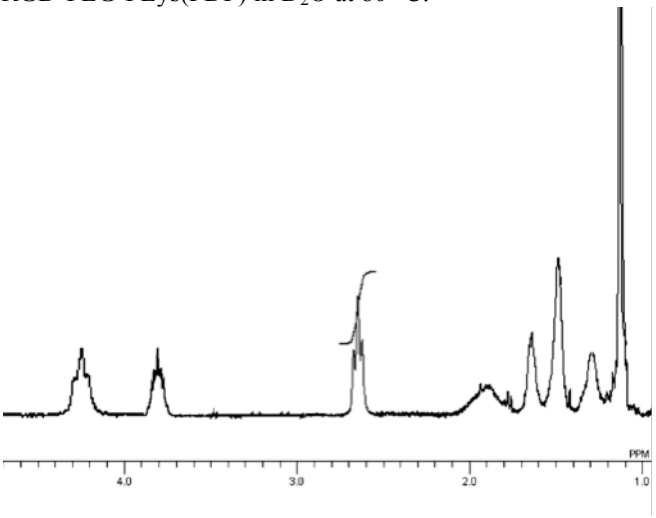


Figure S5 ^{1}H-NMR spectra of PNIPAM-PLys(PDP) in D_2O at 20 °C.

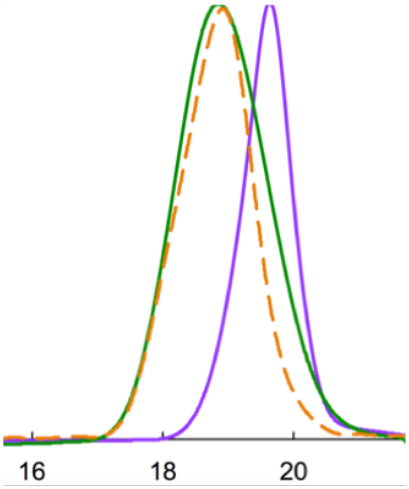


Figure S6 GPC trace of PEG-PLys (orange), cRGD-PEG-PLys(SH) (green) and PNIPAM-PLys(SH) (purple).

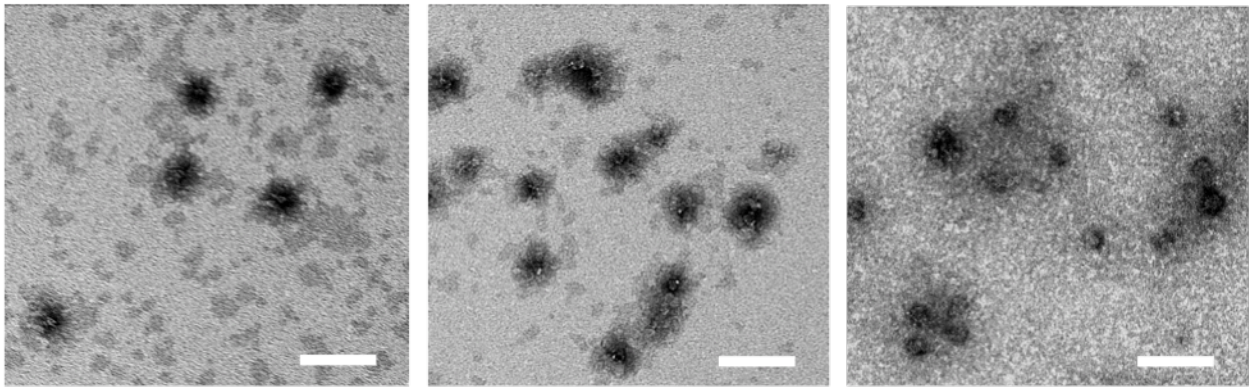


Figure S7 TEM morphology of diverse polymeric formulations. Polymeric formulation of PEG-PLys(SH) (left), PEG/PNIPAM-PLys(SH) at 25 °C and PEG/PNIPAM-PLys(SH) at 37 °C. Scale bar: 200 nm.

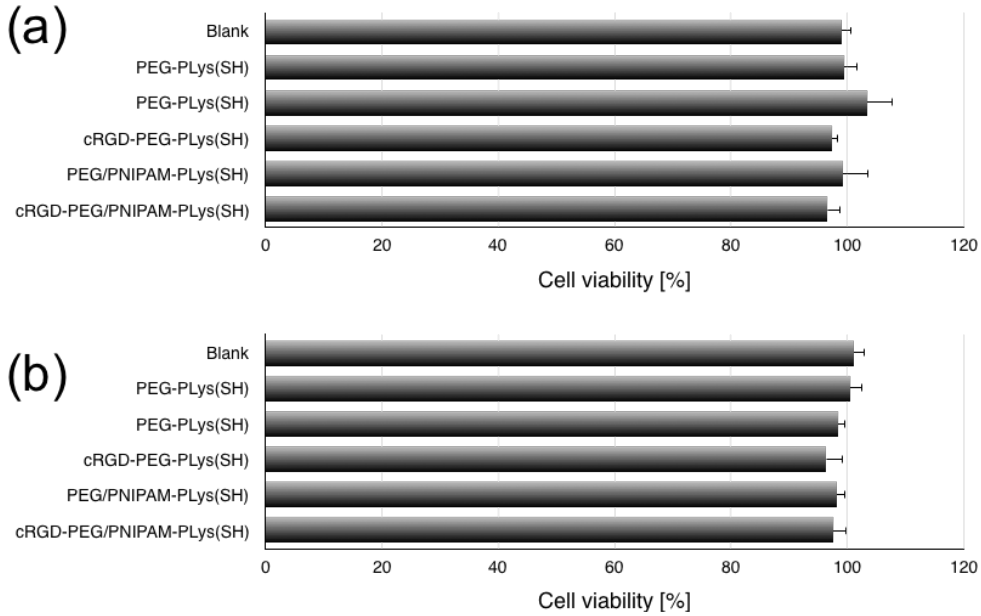
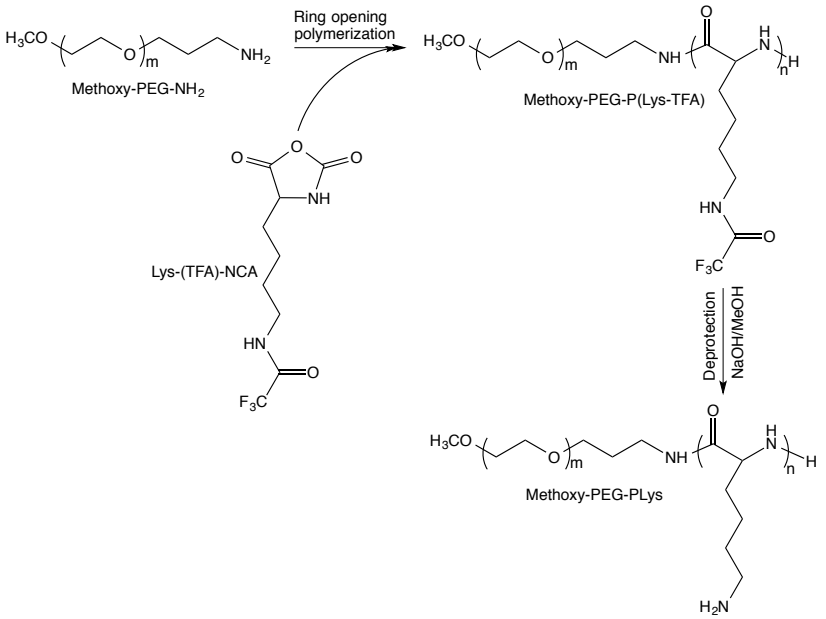
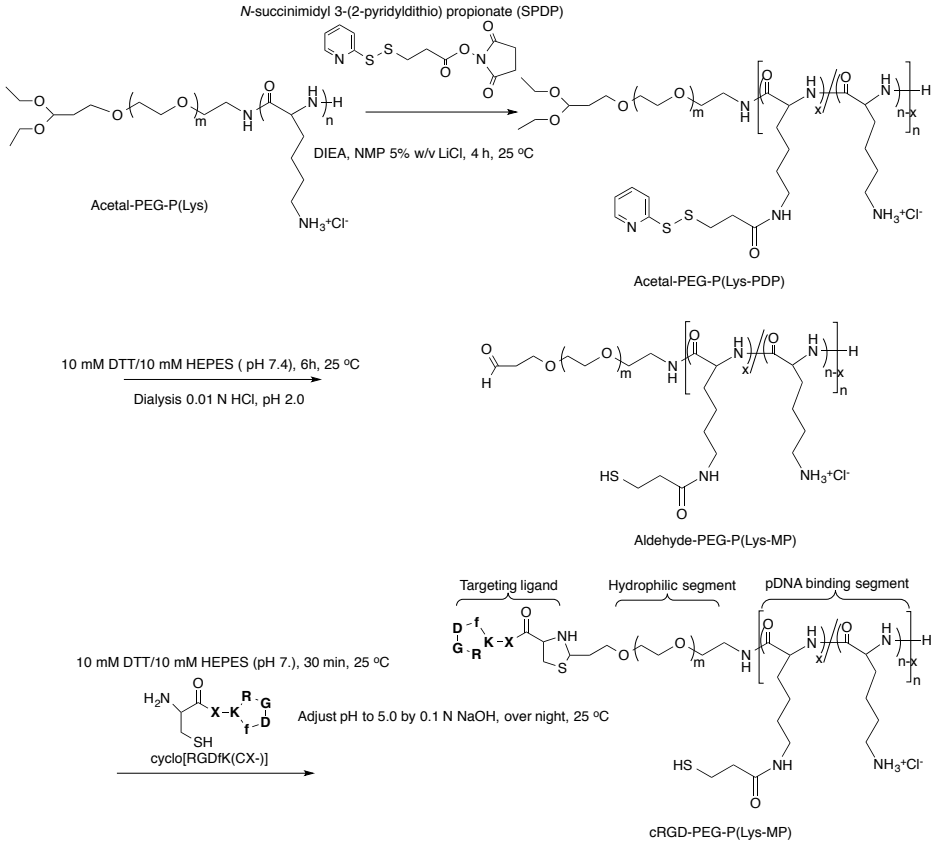


Figure S8 Cell viability of U87 cells and HUVECs in presence of diverse polymeric formulations.

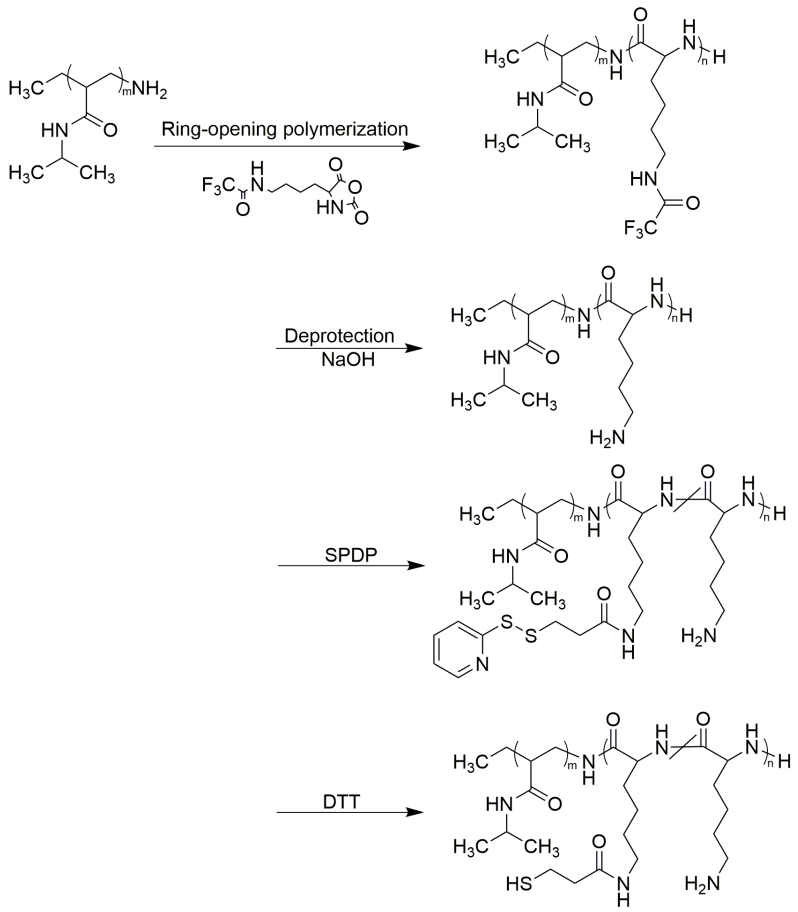
Supplemental Methods:



Scheme S1 Synthetic scheme to prepare block copolymer PEG-PLys



Scheme S2 Synthetic scheme to prepare block copolymer cRGD-PEG-PLys(SH).



Scheme S3 Synthetic scheme to prepare block copolymer PNIPAM-PLys(SH)

Transmission electron microscopy (TEM) characterization

The morphology of polymeric formulations was observed using the H-7000 TEM machine (Hitachi Ltd., Tokyo, Japan) operated at an acceleration voltage of 75 kV. In brief, copper TEM grids (Nisshin EM Corp., Japan) containing carbon-coated collodion membrane were glow-discharged for 30 s using an Eiko IB-3 ion coater (Eiko Engineering Co. Ltd., Japan) for hydrophilization. The hydrophilized grids were immersed into uranyl acetate (UA) (2% w/v)-treated PM solutions for 30 s to achieve effective staining. The sample-deposited grids were blotted onto the filter paper to remove excess solution, followed by air-drying for 30 min. The grids were transferred to the TEM observation.

Cytotoxicity

U87 cells [or Human Umbilical Vein Endothelial Cells (HUVECs)] were plated onto 24-well culture dishes (20,000 cells/well) (or collagen I coated 24-well culture dishes for HUVECs) in 400 µl DMEM containing 10% FBS and 1% antibiotics (penicillin and streptomycin) (or HUVEC-Umbilical Vein Endo, EGM-2, amp AMP for HUVECs, Lonza, Basel, Switzerland) and incubated in a humidified atmosphere with 5% CO_2 at 37 °C. After 24 h of incubation, the medium was replaced with 400 µl of fresh medium, followed by the addition of polymeric formulation solutions equivalent to 0.5 µg of pDNA/well. After 24 h incubation, the medium was replaced with fresh medium, followed by another 24 h of incubation. The cells were washed three times with ice-cold PBS, followed by the addition of 200 µl of fresh medium. Cell viability was assessed on the basis of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-*2H*-tetrazolium (WST-8) reduction to WST-8 formazan by the dehydrogenase

activity of viable cells using the Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan) according to manufacturer's instructions. In brief, 20 µl of the CCK-8 reagent was added to each well and allowed to develop orange-colored WST-8 formazan for 2 h. The UV absorbance of WST-8 formazan in each well was quantified at 450 nm using a microplate reader (Model 680, Bio-Rad, UK). The cell toxicity was expressed as the percentage of cell viability normalized against the control cells treated with 10 mM HEPES (pH 7.4) (n = 4).