Zwitterionic Nanocarrier Surface Chemistry Improves siRNA Tumor Delivery and Silencing Activity Relative to Polyethylene Glycol.

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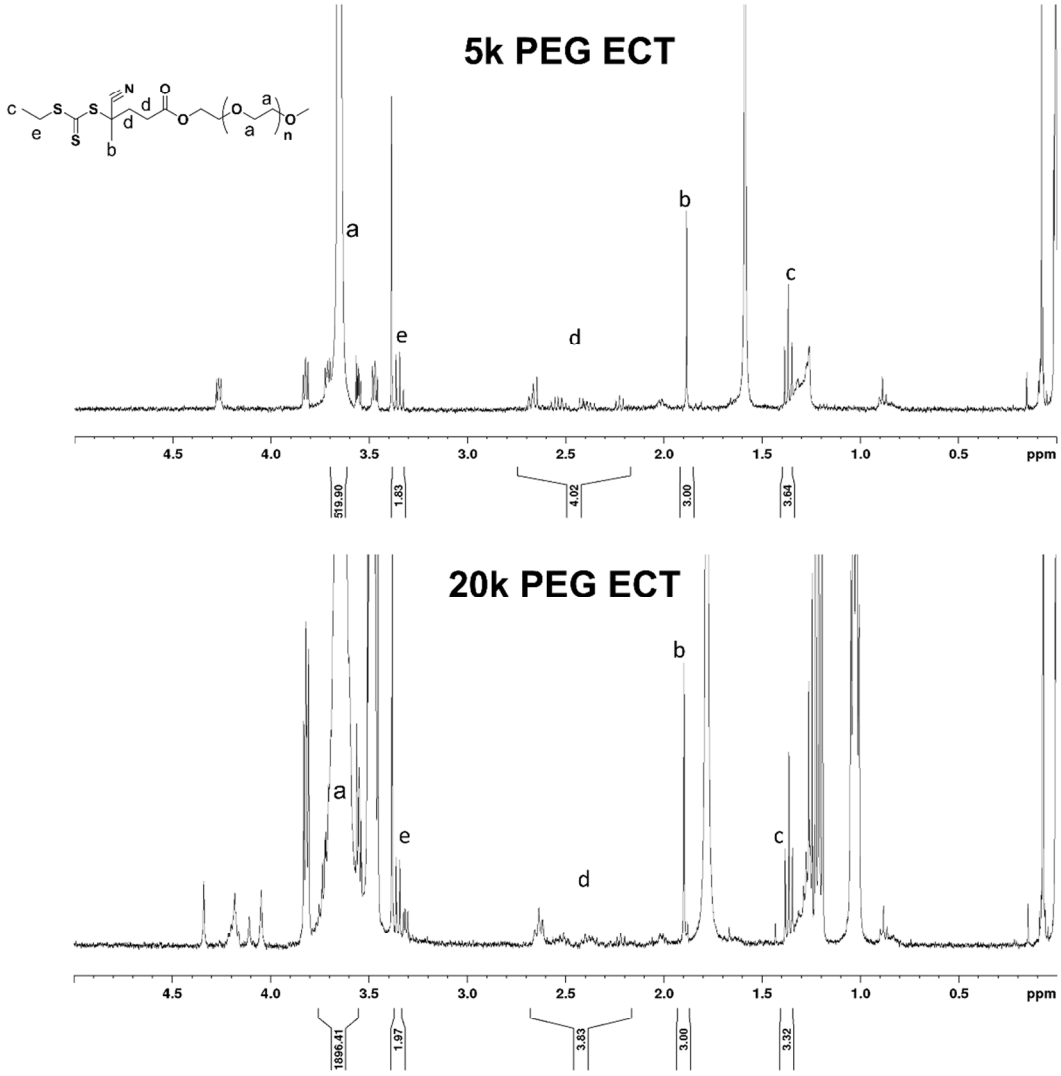
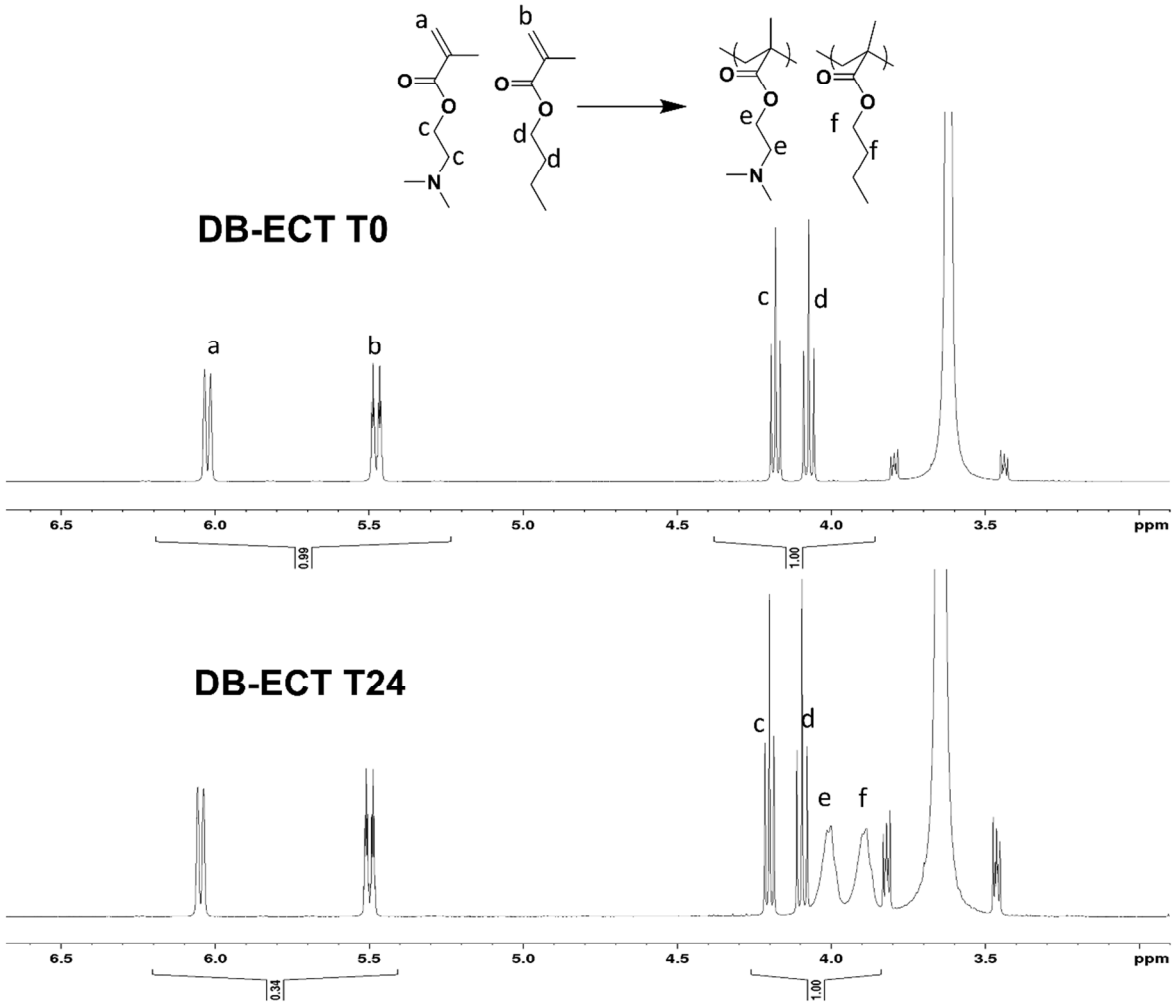
Contents of Supplemental Information

PEG-ECT Conjugation



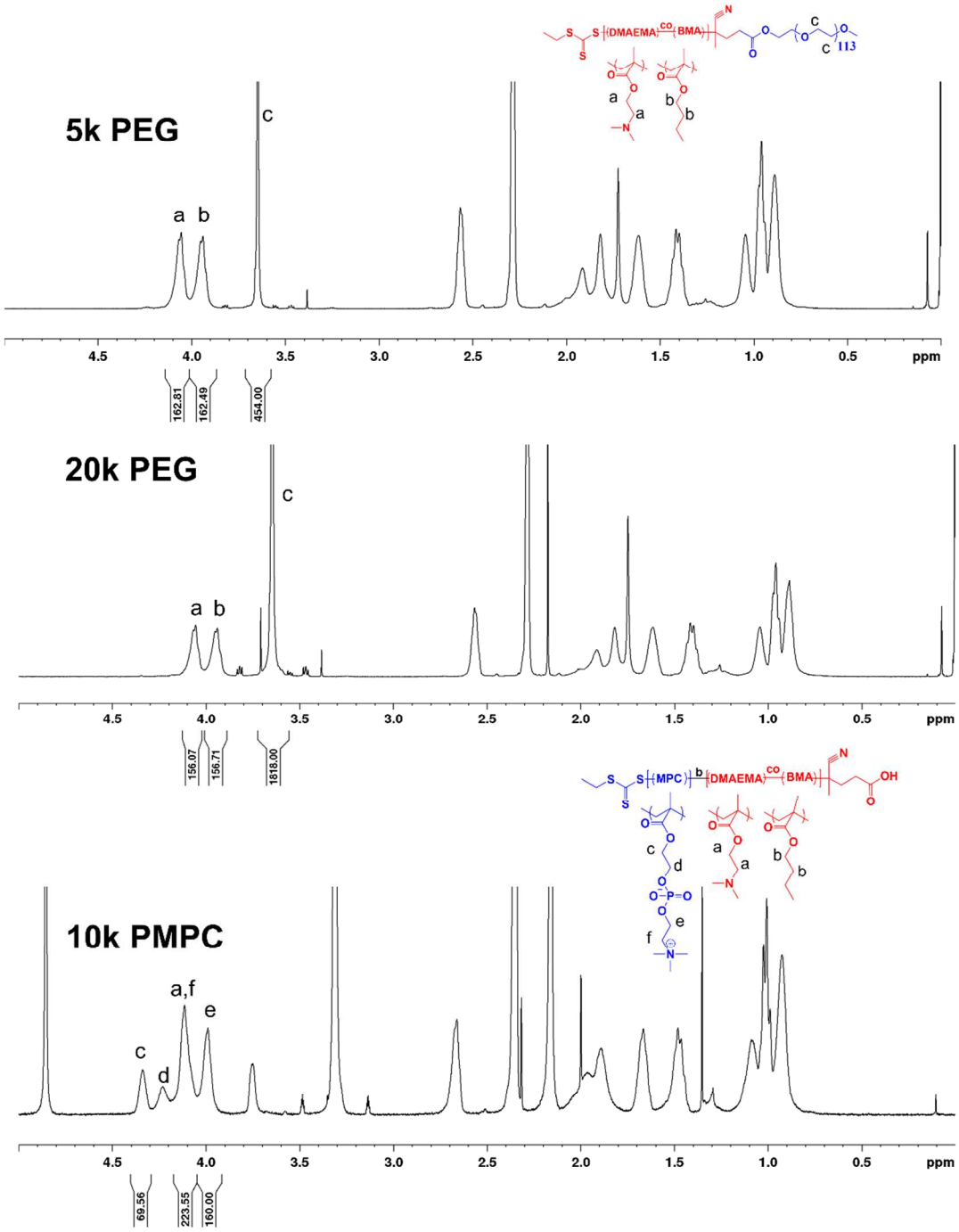
Supplemental Figure S1- ^{1}H-NMR data in CDCl3 for 5k PEG ECT and 20k PEG ECT. Conjugation efficiency of PEG to ECT was quantified by integrating PEG peak relative to the (CCNC**H3)** ECT peak (δ 1.88 s), and dividing the expected number of protons for that given PEG peak (454 for 5k PEG, 1818 for 20k PEG) by the integral of the PEG (δ3.65 s). Efficiencies were 87% for 5k PEG ECT and 96% for 20k PEG ECT.

DB ECT Synthesis

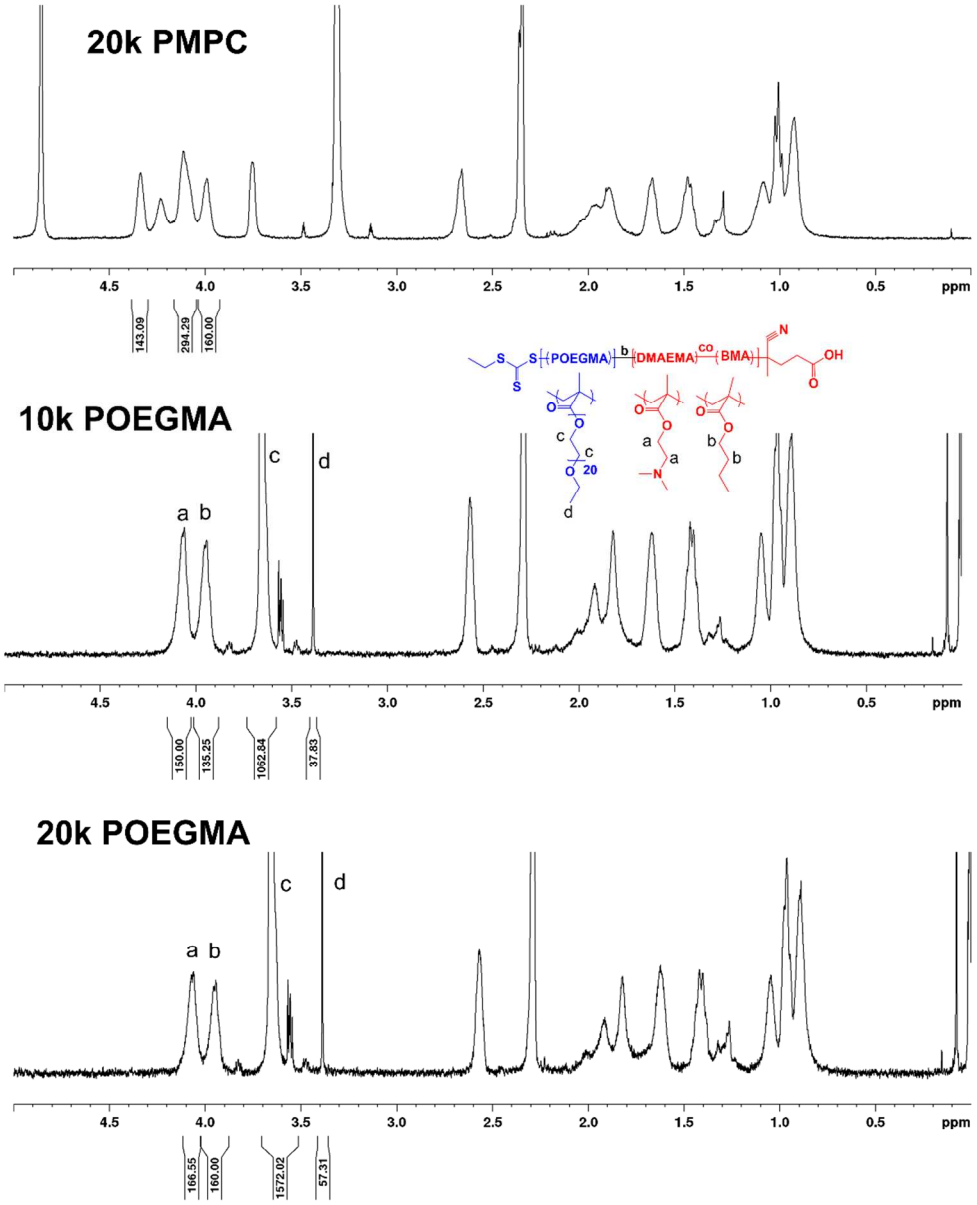


Supplemental Figure S2- Polymerization of core poly(DMAEMA-co-BMA) block. Polymerization conversion was calculated by integrating monomer peaks at δ6.05s and δ5.5s, while setting integrals of polymer/monomer peaks in the range of δ3.8-4.2s to 1 at 0 hrs and 24 hrs. Conversion was calculated by [1-(integral at T24/integral at T0)]. CDCl3 solvent.

[1]H-NMR of Polymer Panel

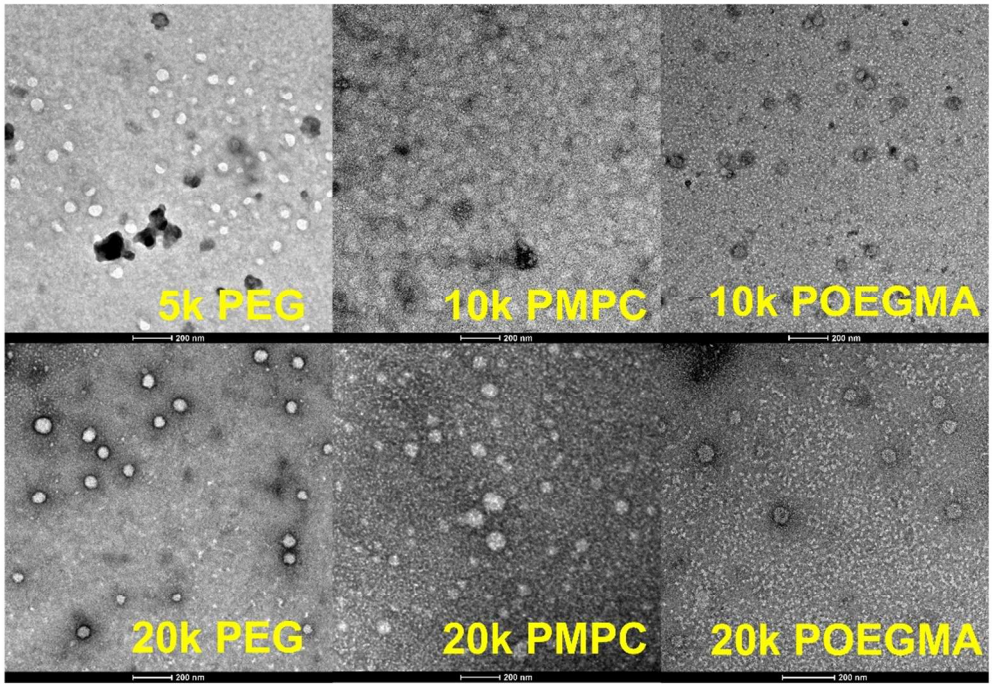


Supplemental Figure S3- [1]H-NMR of polymer panel-continued on next page



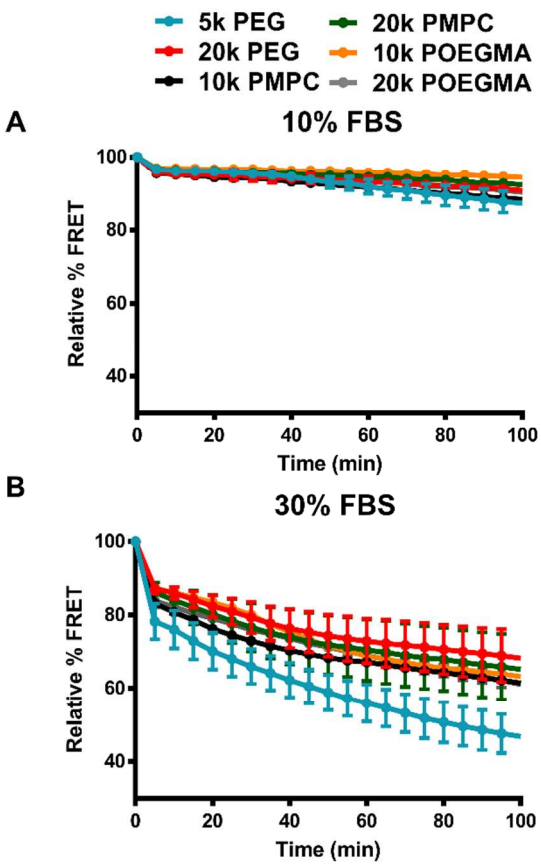
Supplemental Figure S3- ^{1}H-NMR of polymer panel. Polymer repeating units for 5k PEG and 20k PEG were calculated by comparing DMAEMA (δ4.05 s) and BMA (δ3.95 s) peak integrals to PEG integrals (δ3.65s) (in CDCl3). For PMPC (MeOD) and POEGMA polymers (CDCl3), PMPC and POEGMA peaks (labeled) were integrated relative to known DMAEMA and BMA lengths (from DB ECT NMR). DMAEMA:BMA ratios of ~50:50 were confirmed using this method.

TEM of polyplexes



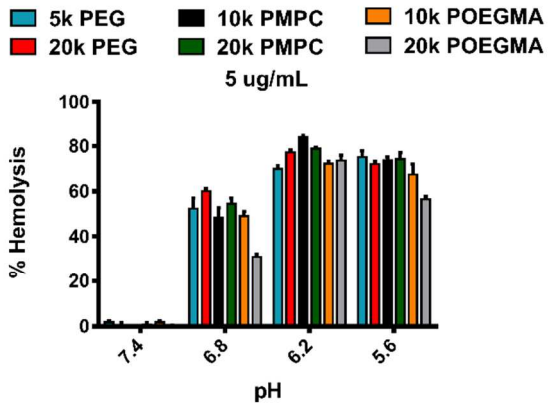
Supplemental Figure S4: TEM images of polyplex suspensions at N^+:P^- 20, 0.5 mg/mL polymer, counterstained with 3 % uranyl acetate. All scale bars represent 200 nm. TEM confirms DLS data that particle sizes are in 100-150nm range. Note that dried particles are typically smaller in appearance on TEM than hydrated particles measured by DLS.

FBS Stability

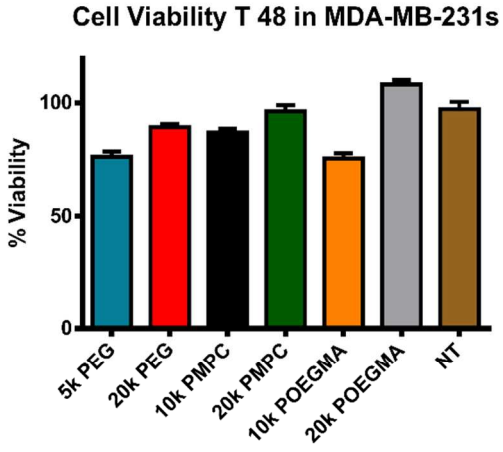


Supplemental Figure S5- Alternative polyplex coronas (N^+:P^- 20) improve stability in FBS compared to traditional 5k PEG coronas. (A) Over 100 minutes, polyplexes remain stable in 10% serum. In 30% serum (B), polyplexes are partially destabilized over longer time periods, with no significant differences between PMPC, POEGMA, and 20k PEG coronas.

***In vitro* polyplex characterization**

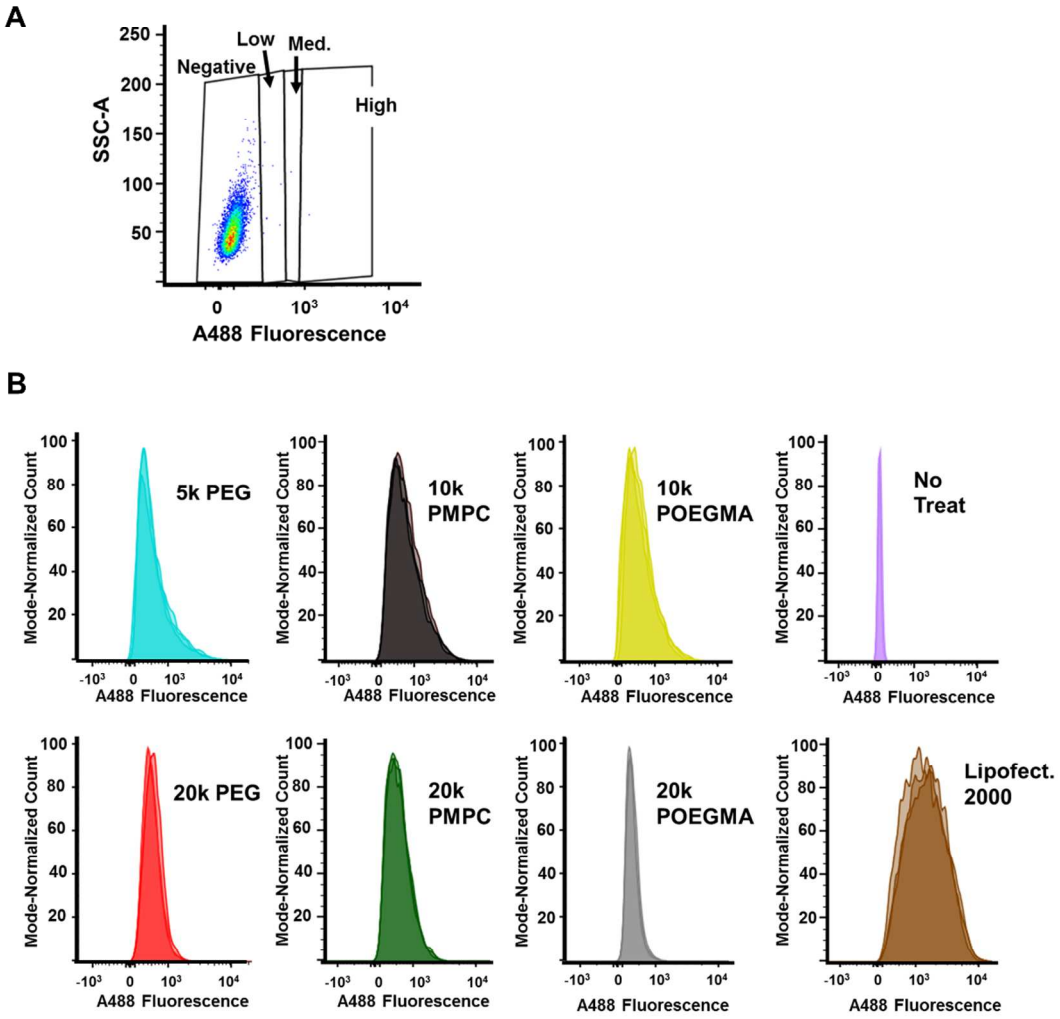


Supplemental Figure S6- Hemolysis panel of polymer library at lower concentration of polymer. Polyplexes were formulated at N^+:P^- ratios of 20:1. Polyplexes at 5 µg/mL polymer showed similar hemolytic properties to 40 µg/mL samples. Results represent average of n=3 experiments.



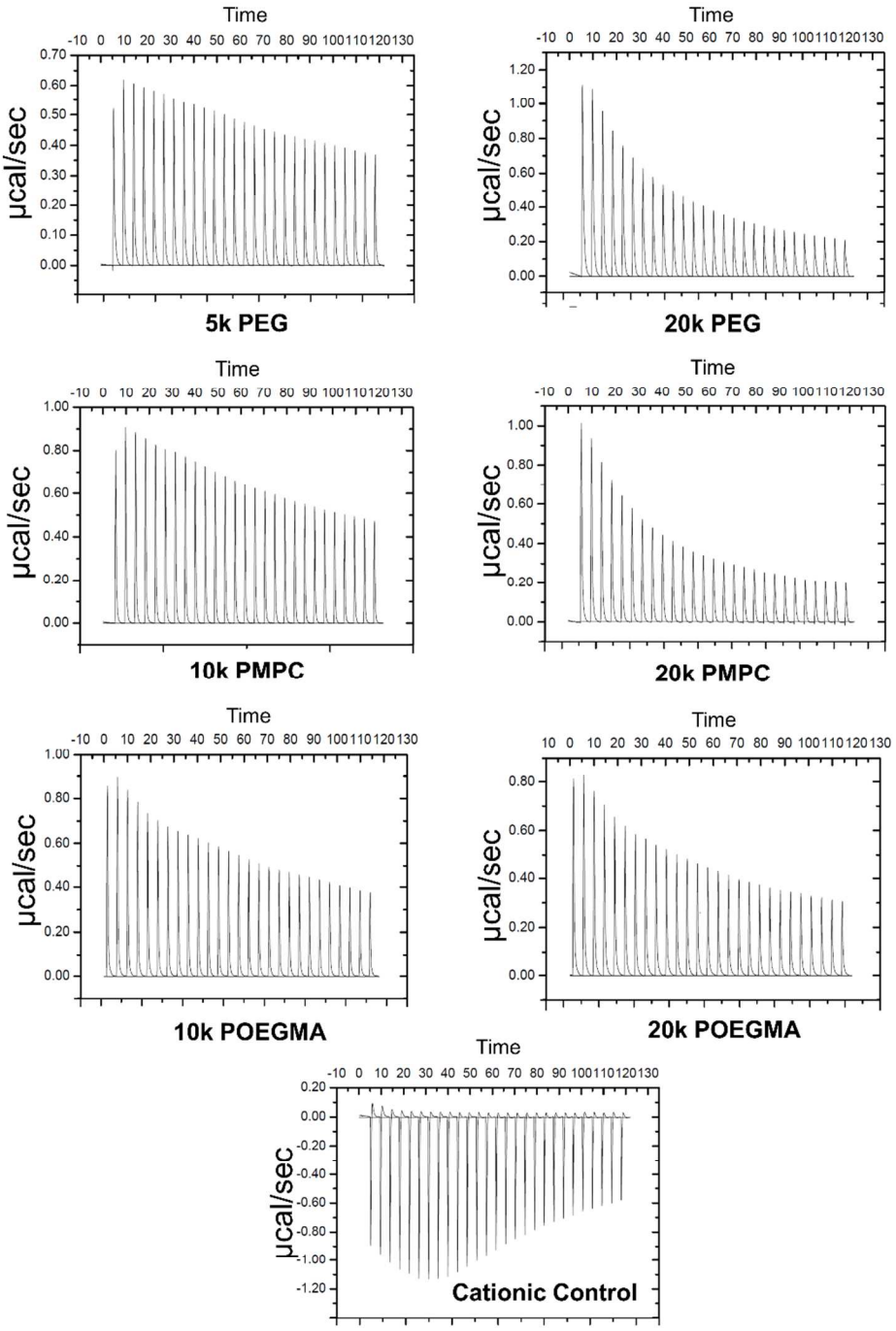
Supplemental Figure S7: Cell viability in MDA-MB-231s 48 hours post polyplex introduction. All polyplexes maintained greater than 75% cell viability. Polyplexes were delivered with 100 nM scrambled siRNA at N^+:P^- 20.

Flow Gating and Histograms



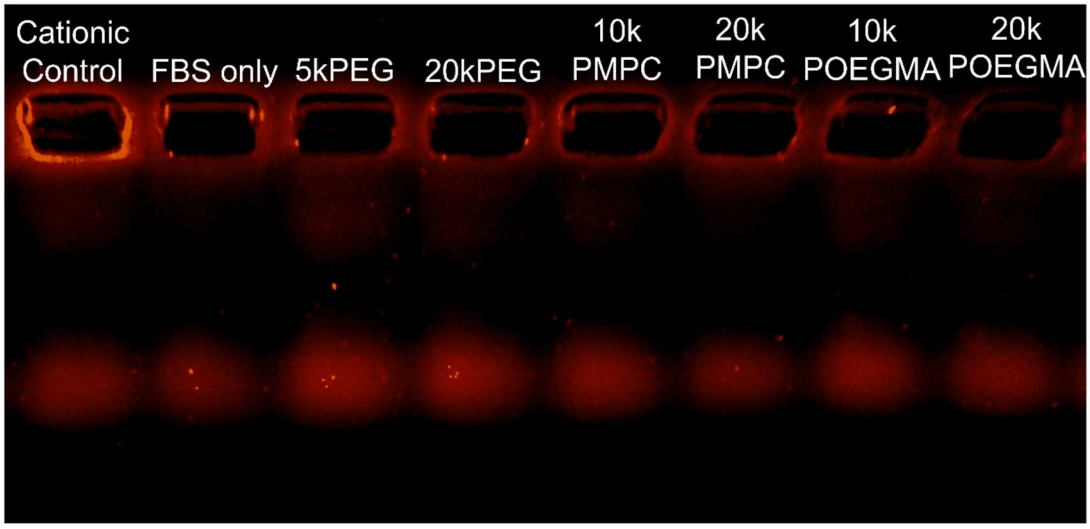
Supplemental Figure S8: (A) Example of low-medium-high gating on Alexa488 fluorescence. (B) Histograms for all flow samples. MDA-MB-231 cells were treated with polyplexes bearing 100 nM A488-siRNA for 24 hours (N^+:P^- 20).

Raw ITC Panel



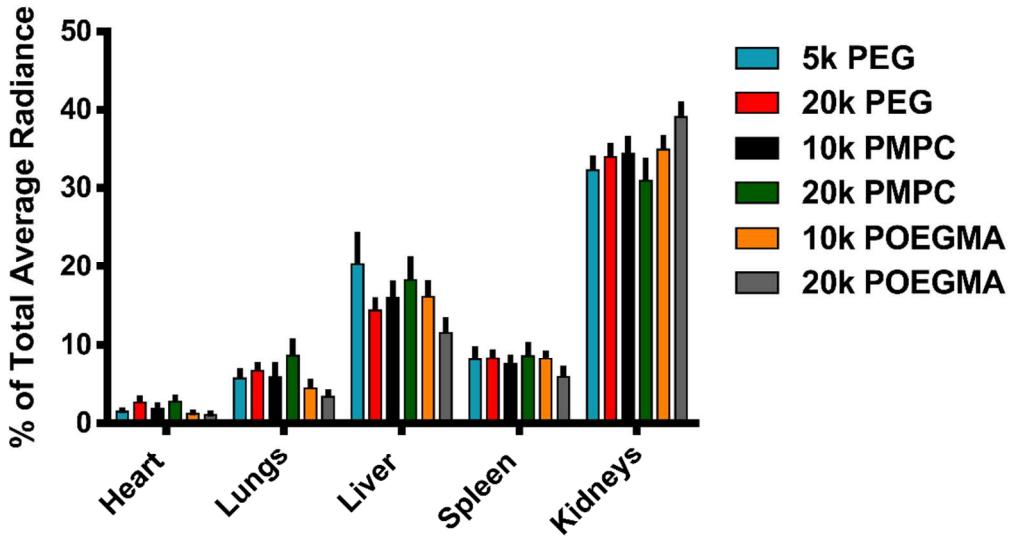
Supplemental Figure S9- Raw ITC panel. Panels display raw thermodynamic ITC data, with µcal/sec on the y axis and time on the x axis. Each peak represents heat change associated with a 10 µL injection of 15 mg/mL BSA (exothermic is negative).

Gel Retention Assay

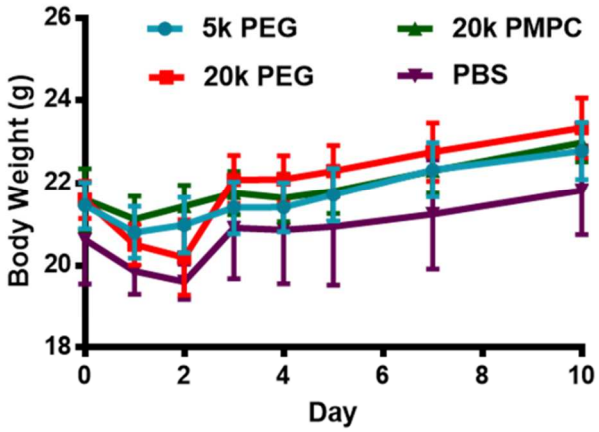


Supplemental Figure S10: Gel retention assay of FBS-incubated polyplexes. Polyplexes (100nM siRNA, N^+:P^- 20) were incubated with 5% FBS and run on an agarose gel, which was then stained for protein using SYPRO Ruby. The cationic control exhibits intense protein staining within the loading well, while the appearance of binding to all other polyplexes was negligible and appeared similar to the FBS only control.

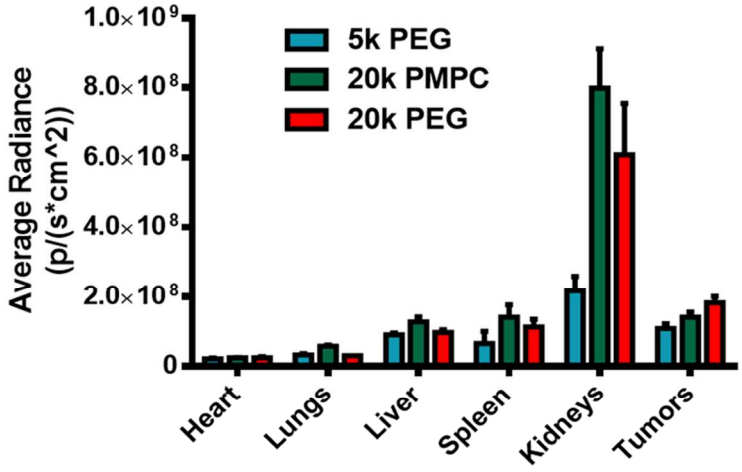
In Vivo Polyplex Biodistribution and Mouse Body Weights



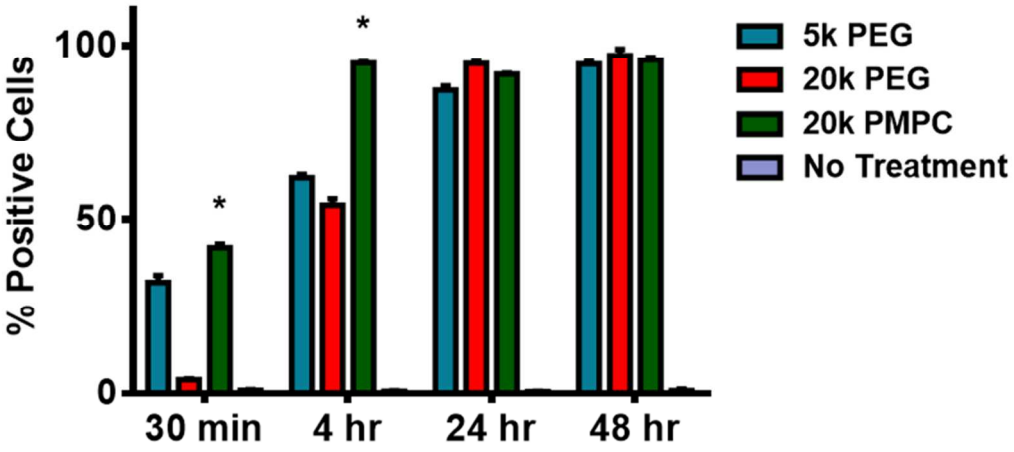
Supplemental Figure S11- Tissue biodistribution of Cy5-siRNA 20 min post-injection. Data represent average of n=5 animals. Polyplexes were administered at a dose of 1 mg/kg siRNA, N^{+}:P^{-} 20.



Supplemental Figure S12 – Body weight measurements of tumor bearing mice throughout 10-day study period show that there were no significant differences in body weight between mice receiving different polyplex treatments.



Supplemental Figure S13- Biodistribution of PEGylated vs zwitterated polyplexes at 24 hours in tumor-bearing mice (N^+:P^- 20). At 24 hours, although 20k PMPC DB and 20k PEG DB average fluorescence values in tumors were both higher than 5k PEG DB, there was no significant difference between the high molecular weight coronas.



Supplemental Figure S14- Percent of cells taking up polyplexes *in vitro* over time. 20k PMPC polyplexes penetrated nearly 100% of cells *in vitro* after only 4 hours, significantly more than 5k PEG or 20k PEG polyplexes.