Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information for the Manuscript

Block ionomer complexes consisting of siRNA and aRAFT-synthesized hydrophilic-block-cationic copolymers II: The influence of cationic block charge density on gene suppression

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Contents of Supporting Information:

Discussion of experiments pertaining to control copolymer P0

Mixing siRNA with non-ionic control polymer **P0**, which has no DMAPMA content, resulted in solutions exhibiting insufficient excess scattering intensity during DLS measurement to determine R_h (Table 2), indicating BICs were not formed. This phenomenon, combined with the highly negative ζ-potential near that of free siRNA, demonstrates the requirement of DMAPMA for BIC formation with these polymers. Furthermore, these results confirm that the remaining folic acid-free APMA units, protonated under physiological conditions, are unable to form stable electrostatic complexes with siRNA. These conclusions are corroborated by the DSC (Figure 2) and potentiometric titration experiments (Figure 3): **P0**-dsDNA exhibited a T_m (57.8 °C) near that of free dsDNA (T_m = 54.4 °C), and **P0**-PSS near-zero ΔG_{total} (-0.36 kJ/mol) as expected from a non-complexing copolymer. Interestingly, Bioanalyzer quantification revealed only 65.3 % free siRNA relative to the control. However, this apparent decrease in uncomplexed siRNA is likely the result of hydrogen bonding or other non-ionic copolymer-siRNA interaction.

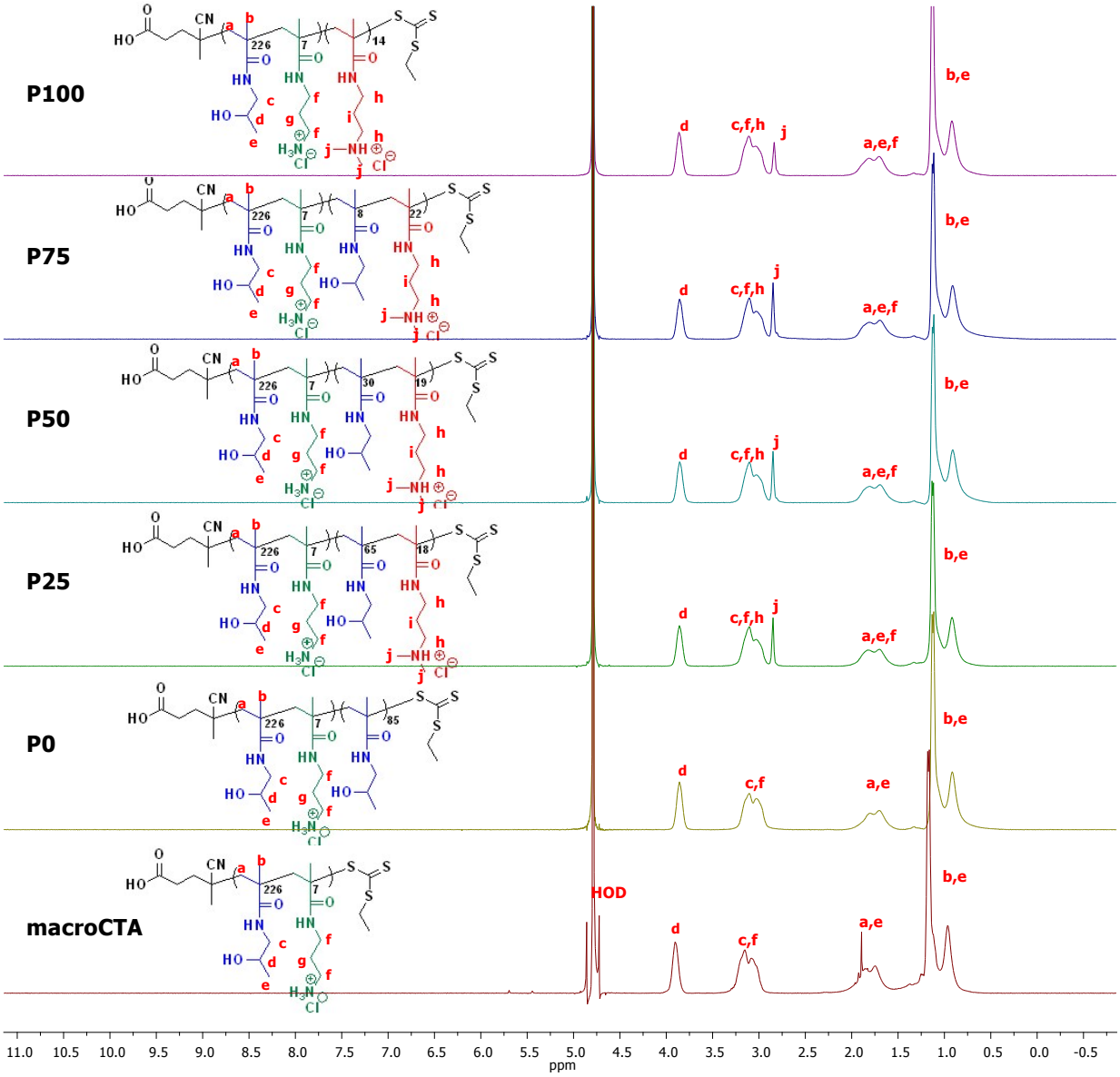

Figure S1. ^{1}H NMR of **P100**, **P75**, **P50**, **P25**, **P0**, and **macroCTA**

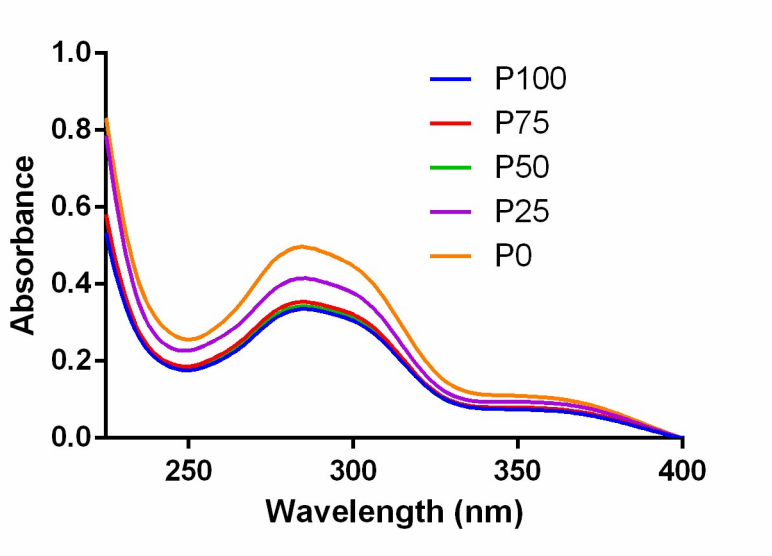

Figure S2. UV-Vis spectroscopy of conjugated folic acid **P100**, **P75**, **P50**, **P25**, and **P0** copolymers.

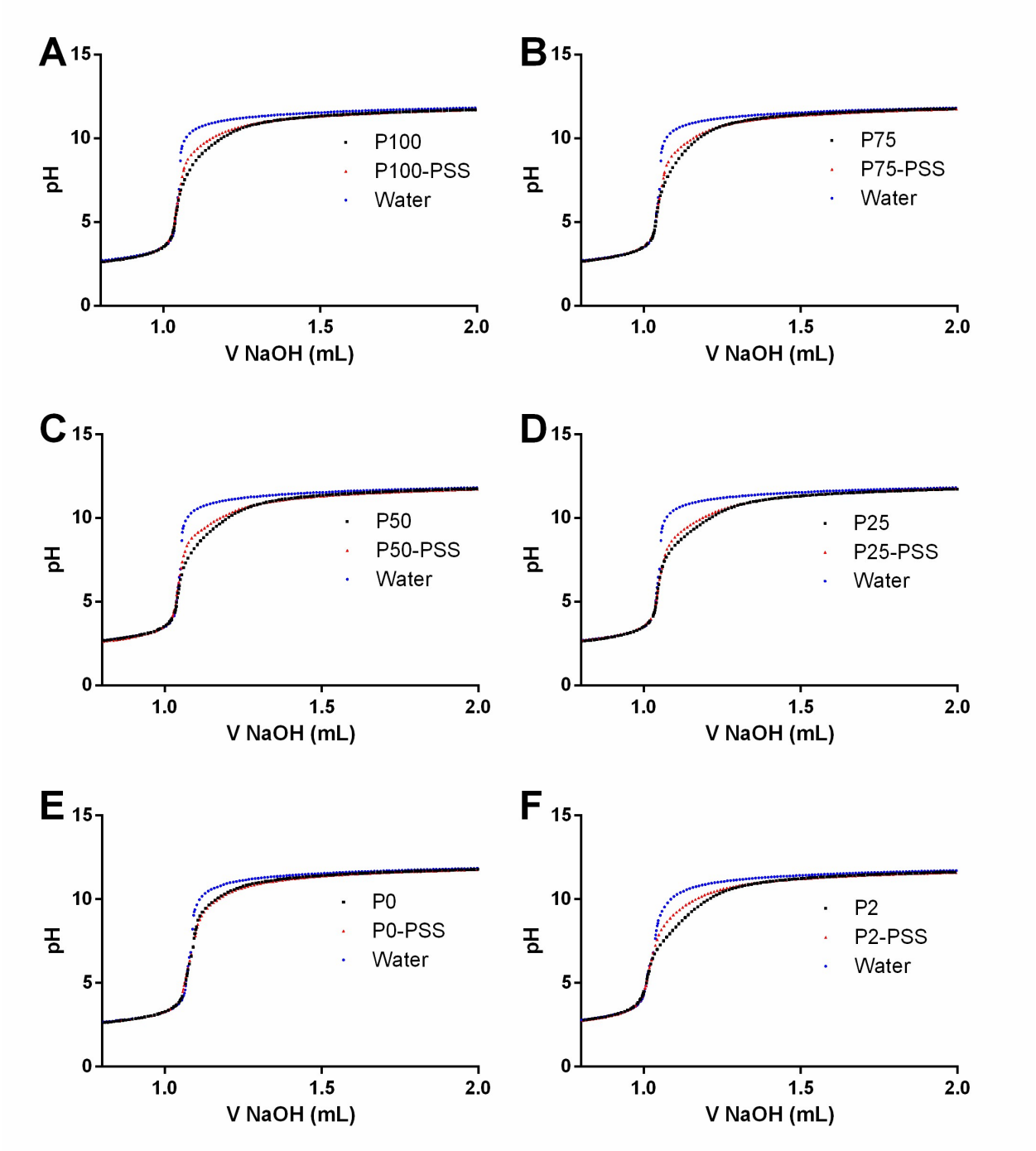

Figure S3. Potentiometric titration curves for (A) **P100**, (B) **P75**, (C) **P50**, (D) **P25**, (E) **P0**, and (F) **P2** and their respective block ionomer complexes with polystyrene sulfonate (PSS).

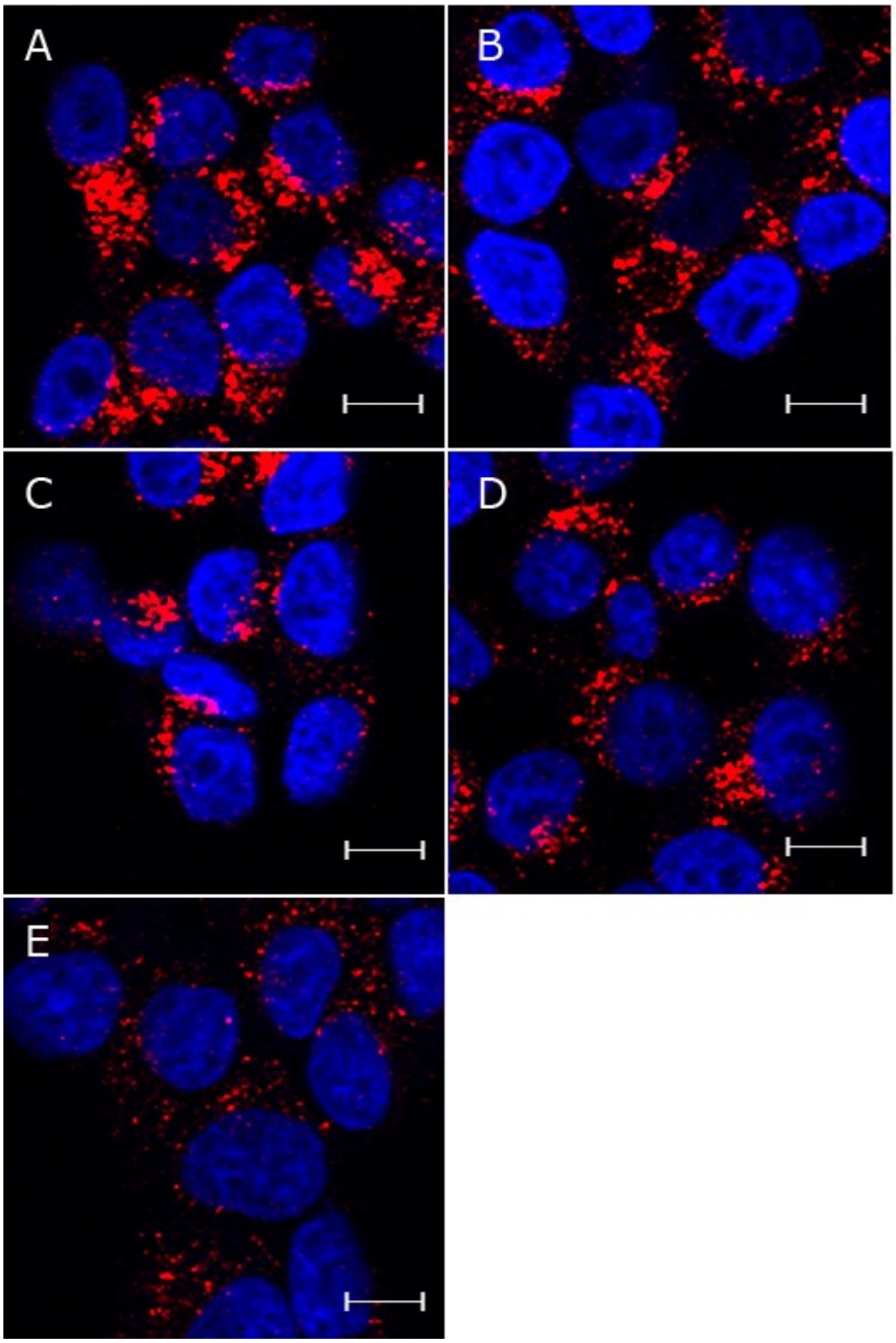

Figure S4. Confocal fluorescence microscopy images of KB cells treated with AlexaFluor594-tagged siRNA (red) delivered via (A) **P100**, (B) **P75**, (C) **P50**, (D) **P25**, and (E) **P0**. Nuclei were stained with DAPI (blue). Scale bars = 10 μm.

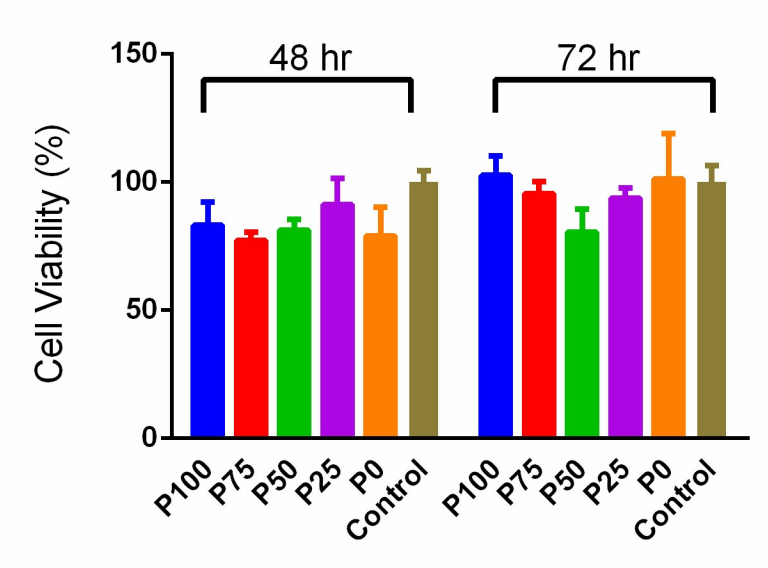

Figure S5. Cell viability assays of **P100**, **P75**, **P50**, **P25**, and **P0** after 48 and 72 hours. The cell viability was determined relative to KB cells. Error bars represent the standard deviation from triplicate experiments.

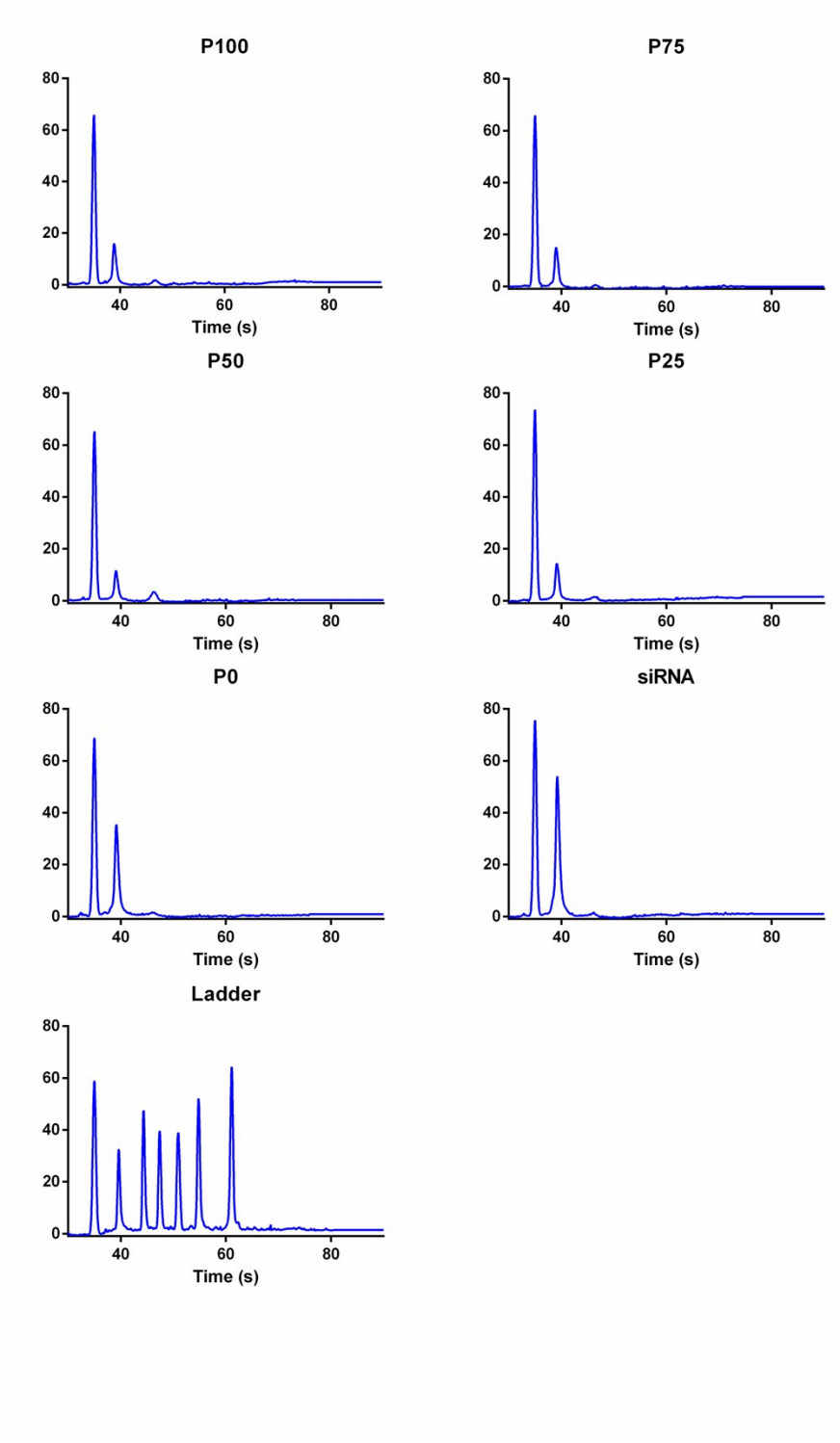

Figure S6. Agilent 2100 Bioanalyzer electropherograms for copolymer-siRNA complexes, free siRNA, and ladder. Uncomplexed siRNA concentration determined from area of peak at ~39 s. Peak at ~35 s corresponds to 4-nucleotide marker.