Supplementary Information for the Manuscript

Block ionomer complexes consisting of siRNA and aqueous RAFT-synthesized hydrophilic-*block*-cationic copolymers: The influence of cationic block length on gene suppression

Andrew C. Holley,[a] Keith H. Parsons,[a] Wenming Wan,[a] Daniel F. Lyons,[b] G. Reid Bishop,[c] John J. Correia,[b] Faqing Huang,[d] and Charles L. McCormick[a,d]*

[a]The Department of Polymer Science and Engineering, The University of Southern Mississippi, Hattiesburg MS 39406, USA

[b]The Department of Biochemistry, The University of Mississippi Medical Center, Jackson MS 39211, USA

[c]The Department of Chemistry and Biochemistry, Belhaven University, Jackson MS 39202, USA

[d]The Department of Chemistry and Biochemistry, The University of Southern Mississippi, Hattiesburg MS 39406, USA

*Corresponding author e-mail: charles.mccormick@usm.edu

Contents of Supporting Information:

Materials and Methods:

Cell viability assays were performed using a Vybrant MTT Cell Proliferation Assay Kit (Invitrogen). KB-GLuc (KB cells over expressing *Gaussia* Luciferase) cells were seeded in a 96-well microplate (Nucleon) with a cell density of 12000 cells/well. Cells were incubated with a fixed concentration of hydrophilic-*block*-cationic copolymer (**P2**-**P4**, ~0.2 mg/mL) to mimic siRNA delivery conditions. Cells were incubated for 24 h and 48 h before adding 10 μL of a 12mM MTT reagent to each well. The cells were further incubated for an additional 4 h, followed by adding 100 μL of a SDS (10 %)/HCl (0.01 M) solution to each well. The absorbance was read at 570 nm with a Synergy2 MultiMode Microplate reader (BioTek).

All calorimetric experiments were carried out using a Calorimetric Sciences Corporation Nano DSC-II solution differential scanning calorimeter (DSC). Sodium cacadylate buffer (10 mM, pH 7.2) was used for the running buffer. The GLuc DNA (analogue of *Gaussia* Luciferase siRNA) concentration was maintained at 75 μM while the concentrations of poly[(HPMA-*stat*-APMA)-*block*-DMAPMA] (**P2**-**P4**) copolymers were adjusted to maintain a nitrogen-to-phosphate (N:P) ratio equal to 1 (i.e. neutral complexes). CpCalc (Version 2.1, Calorimetric Sciences Corp.) was used to subtract buffer-buffer scans from buffer-sample scans. Linear-polynomial baselines were applied to each scan for the determination of molar heat capacity values. Since $\Delta H = \int(\Delta C_p/dT)$, the area of each peak yields the calorimetric enthalpy (ΔH_{Cal}), and the peak maximum yields the melting temperature (T_m). Furthermore, $\Delta S = \int [(\Delta C_p/T)/dT]$, so the calorimetric Gaussian fits were re-plotted as $\Delta C_p/T$ versus T to yield a new curve; the area of which is the entropy. The van't Hoff enthalpy[1] (ΔH_{VH}) was determined by methods reported by Crothers[2] and Breslauer[3] (equation 1)

$$\Delta H_{VH} = \frac{b}{\frac{1}{T_1} - \frac{1}{T_2}}$$

in which b represents the molecularity in cal/(mol*K), T_1 is the temperature at half-width and half-max below the T_m, and T_2 is the temperature at half-width and half-max above the T_m (in Kelvin). After determining the enthalpy and entropy, the Gibbs free energy may be ascertained by the following relationship $\Delta G = \Delta H_{Cal} - T\Delta S$ at 37 °C (310.15 K). Since solution DSC has no ASTM standard, the GLuc

DNA was utilized as a standard, because most importantly, the DNA sample has a well-defined structure (e.g. no triplex or quadruplex formation), and furthermore, the complexes are derived from GLuc DNA. The ΔH_{Cal} of GLuc DNA was normalized to its ΔH_{VH}, and the corresponding ΔH_{Cal} of the GLuc DNA/hydrophilic-*block*-cationic copolymer complexes were scaled appropriately.

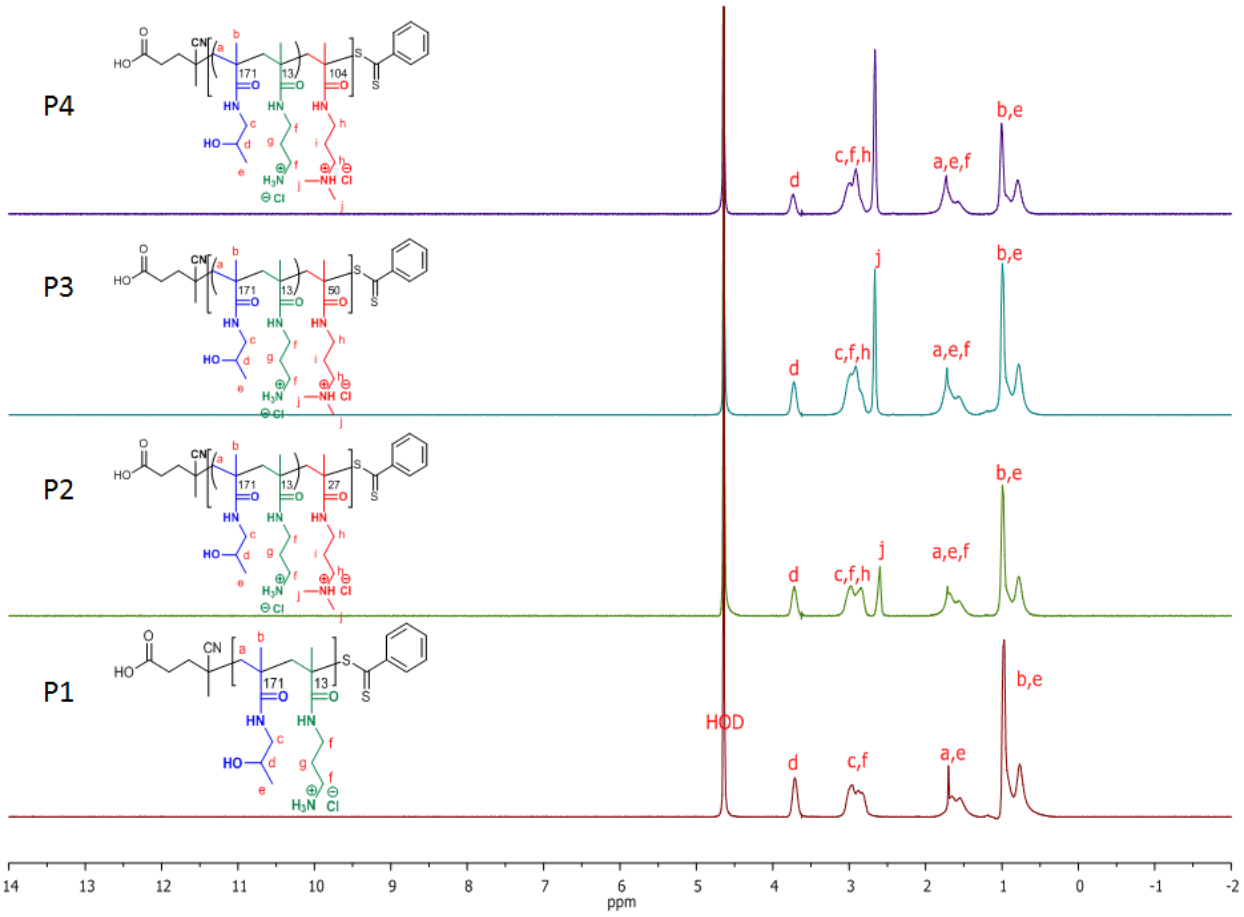

Figure S1. ^{1}H NMR of **P1**, **P2**, **P3**, and **P4** (the macroCTA and subsequent chain extensions with DMAPMA).

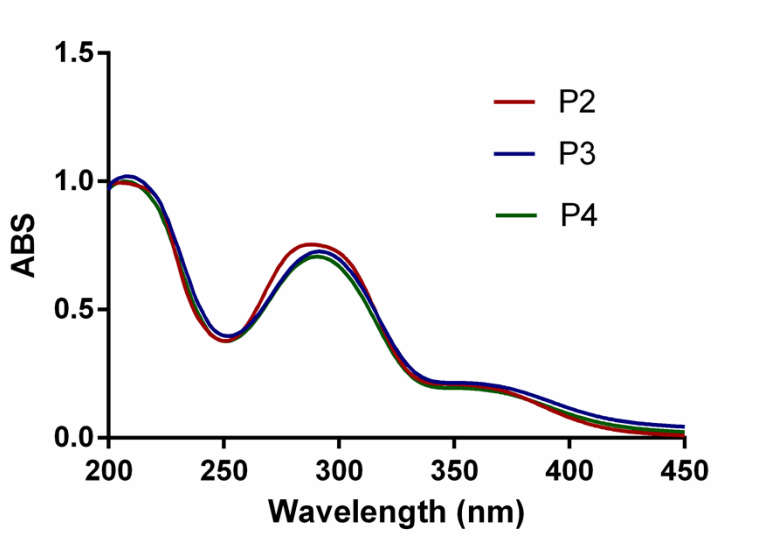

Figure S2. Uv-Vis spectroscopy of conjugated folic acid **P2** (red), **P3** (Blue), and **P4** (Green) hydrophilic-*block*-cationic copolymers.

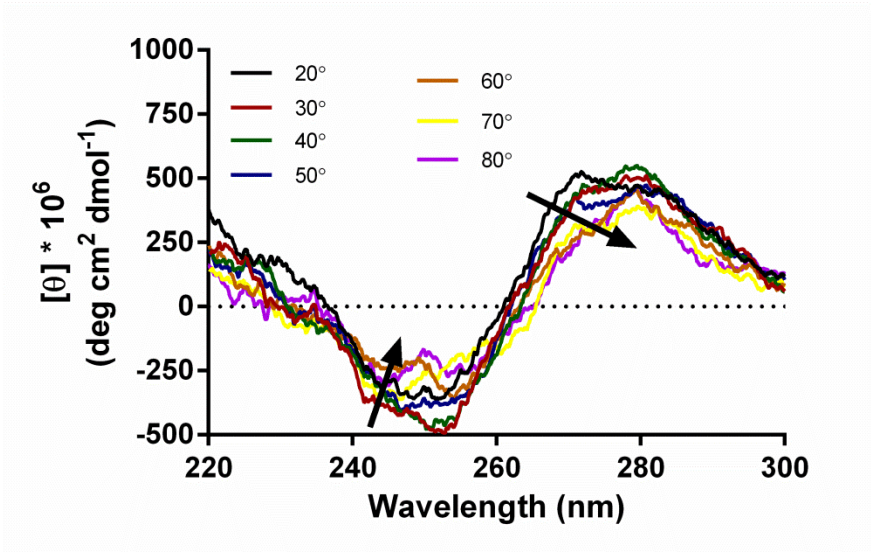

Figure S3. Circular dichroism melting spectra of GLuc DNA.

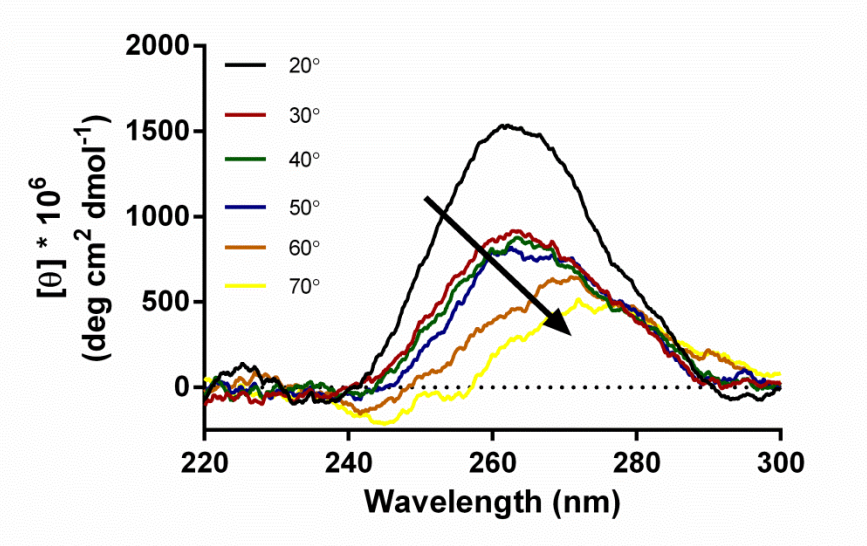

Figure S4. Circular dichroism melting spectra for siRNA.

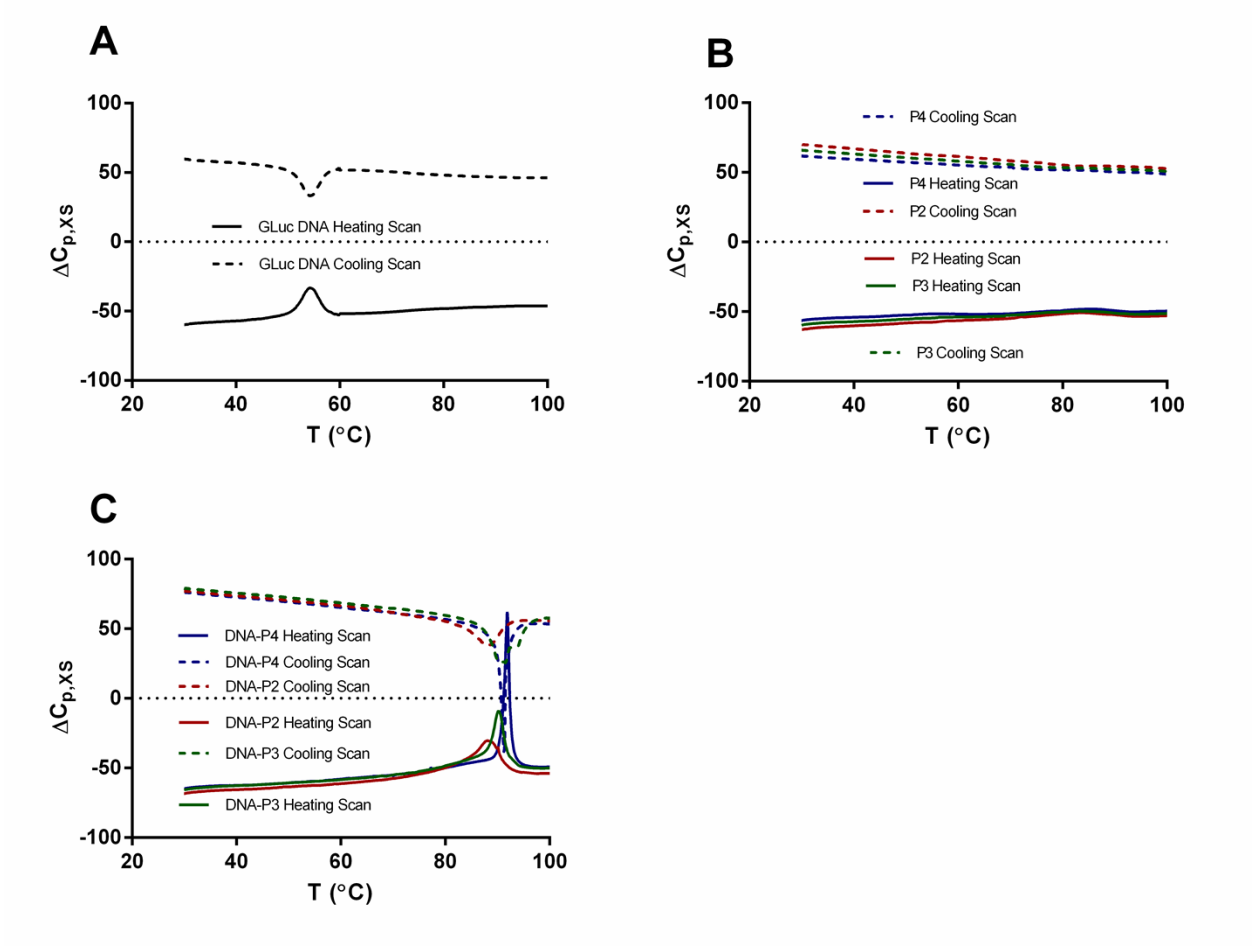

Figure S5. Heating and cooling solution DSC thermograms for (A) GLuc DNA, (B) hydrophilic-*block*-cationic copolymers (**P2**-**P4**), and (C) DNA-hydrophilic-*block*-cationic copolymer complexes. No hysteresis is evident between heating and cooling (i.e. these systems are reversible).

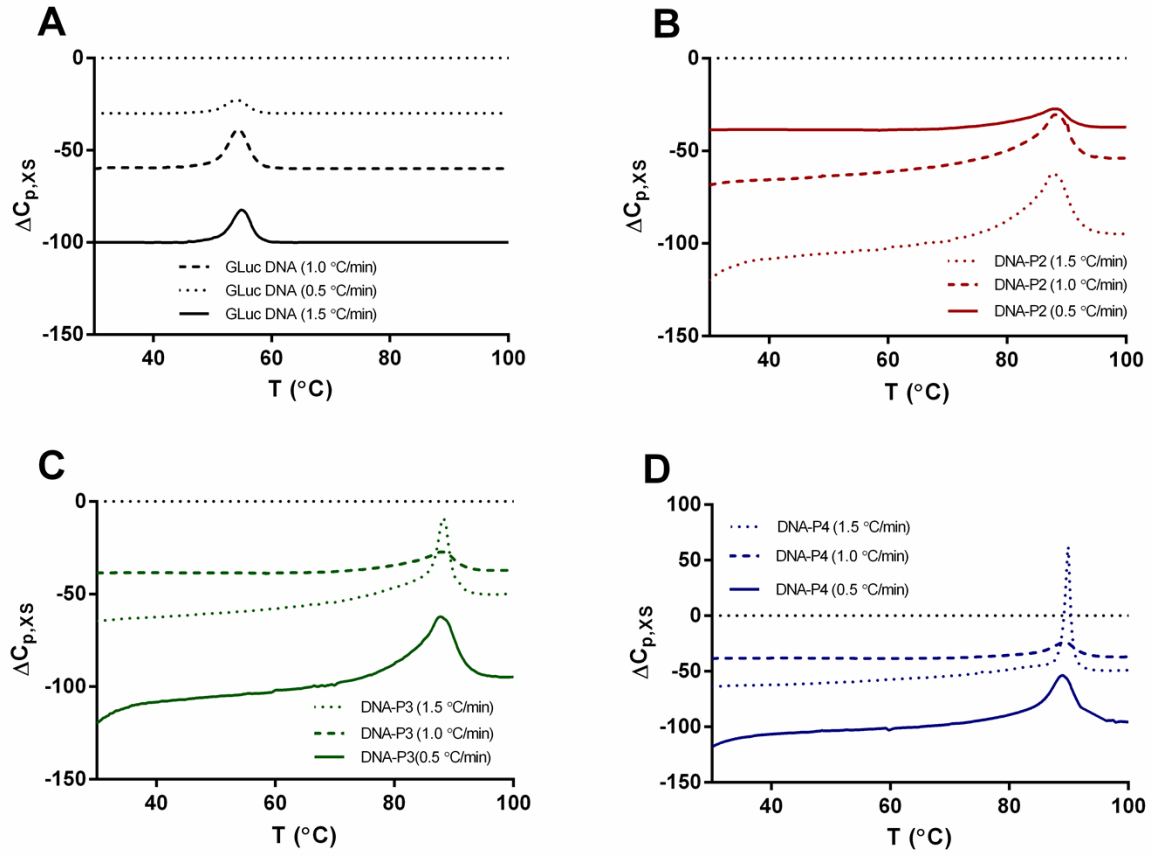

Figure S6. Solution DSC thermograms illustrating the scan rate dependence for (A) GLuc DNA, (B) DNA-**P2** complexes, (C) DNA-**P3** complexes, and (D) DNA-**P4** complexes. The melting temperature (T_m) remains constant with respect to scan rate (i.e. these systems are in equilibrium).

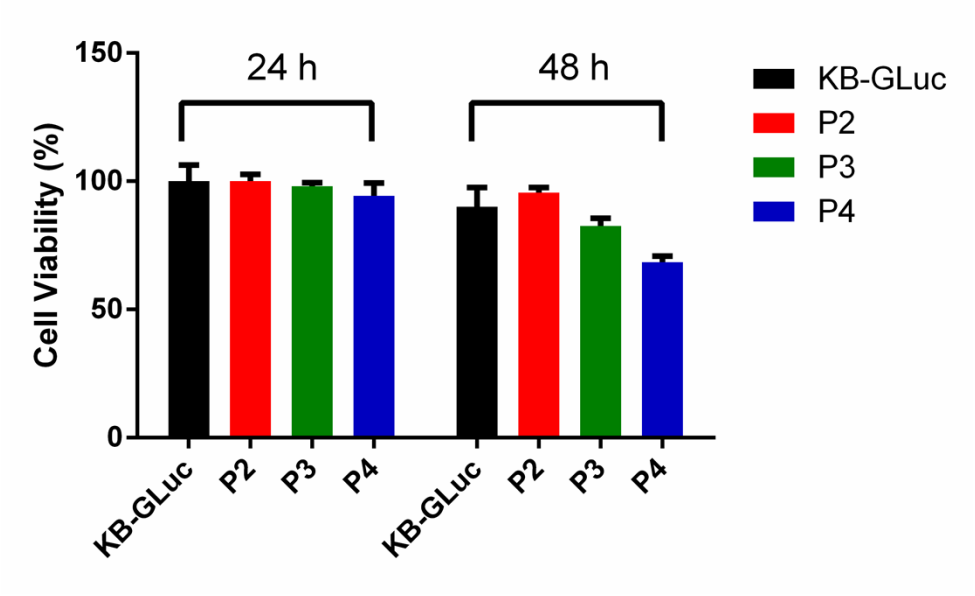

Figure S7. Cell viability assays of **P2**, **P3**, and **P4** after 24 and 48 hours. The cell viability was determined relative to KB-GLuc cells. Error bars represent the standard deviation from triplicate experiments. The concentration of hydrophilic-*block*-cationic copolymer was maintained ~0.2 mg/mL to represent delivery conditions.

Table S1. The melting temperature (T_m) calorimetric enthalpy (ΔH_{Cal}), van't Hoff enthalpy (ΔH_{VH}), binding enthalpy ($\Delta H_{Binding}$), entropy (ΔS) Gibb's free energy (ΔG) for GLuc DNA and GLuc DNA-hydrophilic-*block*-cationic copolymer complexes.

Sample	ΔH_{Cal} (kcal/mol)	ΔH_{VH} (kcal/mol)	ΔS (kcal/K*mol)	ΔG (kcal/mol)	T_m (°C)	$\Delta H_{Binding}$ (kcal/mol)
Gluc DNA	240	240	106	204	54.4	N/A
DNA-P2	420	367	120	340	88.4	160
DNA-P3	483	686	140	413	90.2	223
DNA-P4	461	1900	143	393	91.8	201

References:

1. D. S. Pilch, in *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, Inc., 2001.

2. J. Gralla and D. M. Crothers, *J. Mol. Biol.*, 1973, **78**, 301–319.

3. K. J. Breslauer, in *Energetics of Biological Macromolecules*, ed. G. K. A. B. T.-M. in E. Michael L. Johnson, Academic Press, 1995, vol. Volume 259, pp. 221–242.