Supporting Information

Investigating the Effects of Block versus Statistical Glycopolycations Containing Primary and Tertiary Amines for Plasmid DNA Delivery

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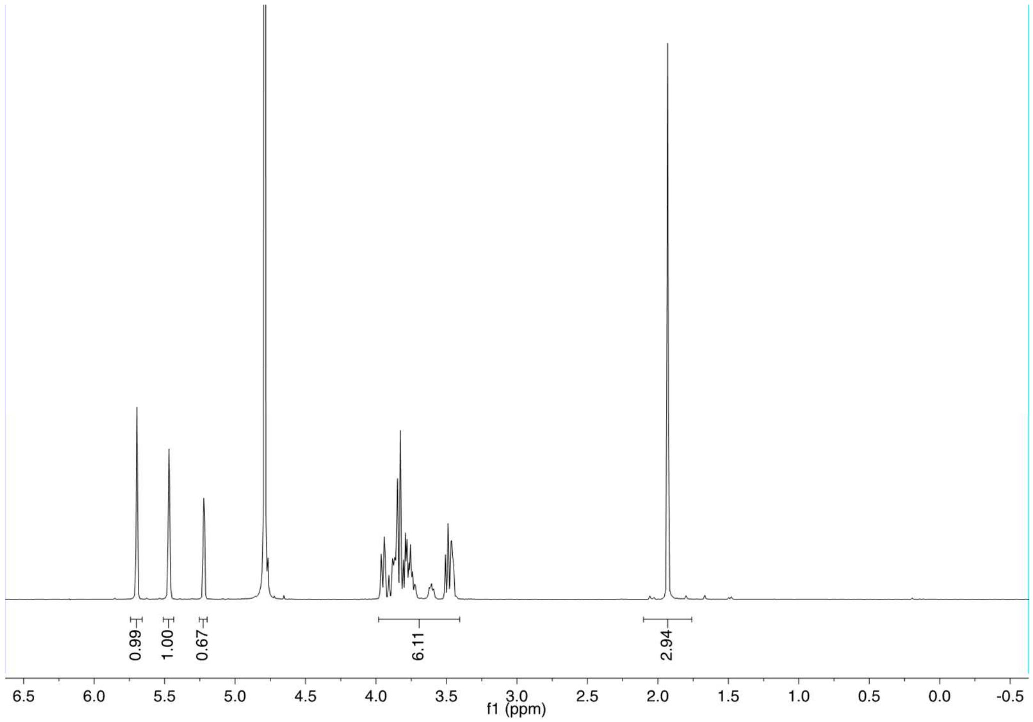

Figure S1. [1]H NMR spectra of MAG.

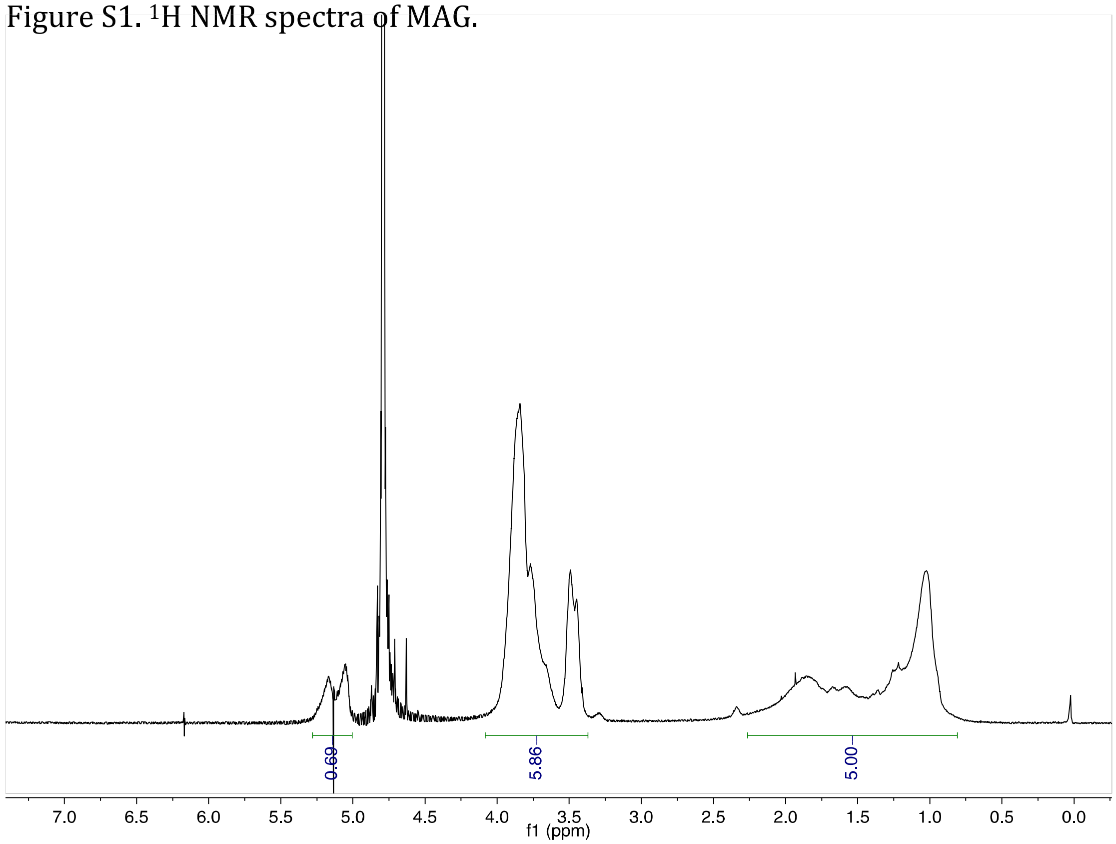

Figure S2. [1]H NMR spectra of poly($\mathbf{G}_{46}$)

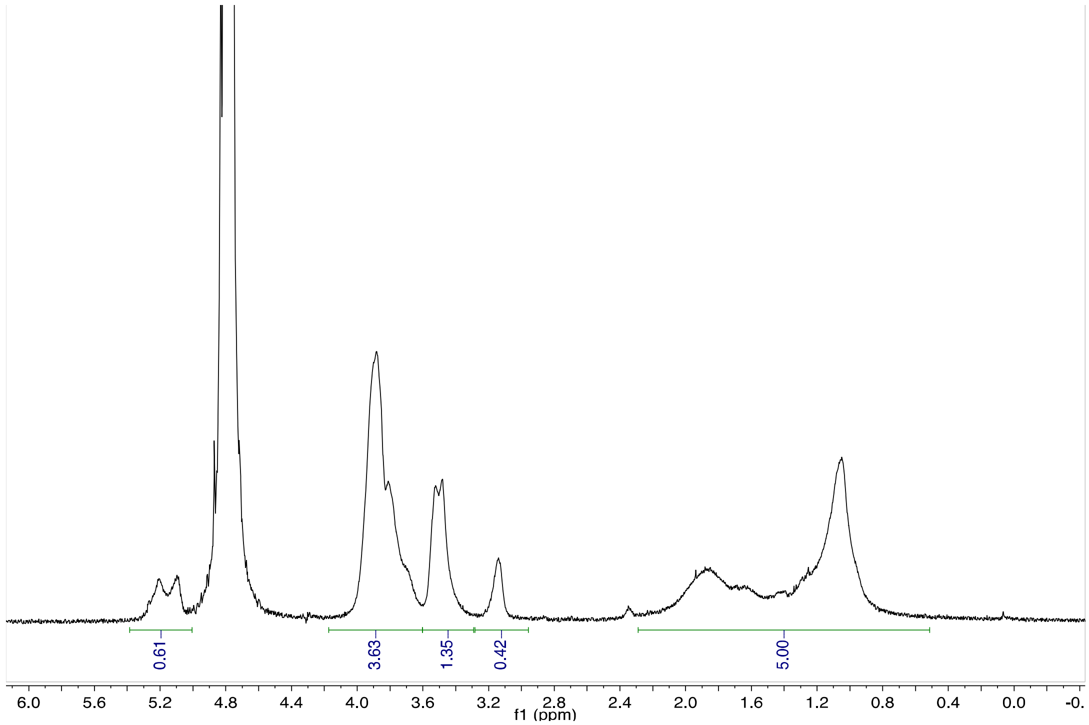

Figure S3. ^{1}H NMR spectra of poly($\mathbf{G}_{46}$-b-$\mathbf{P}_{13}$)

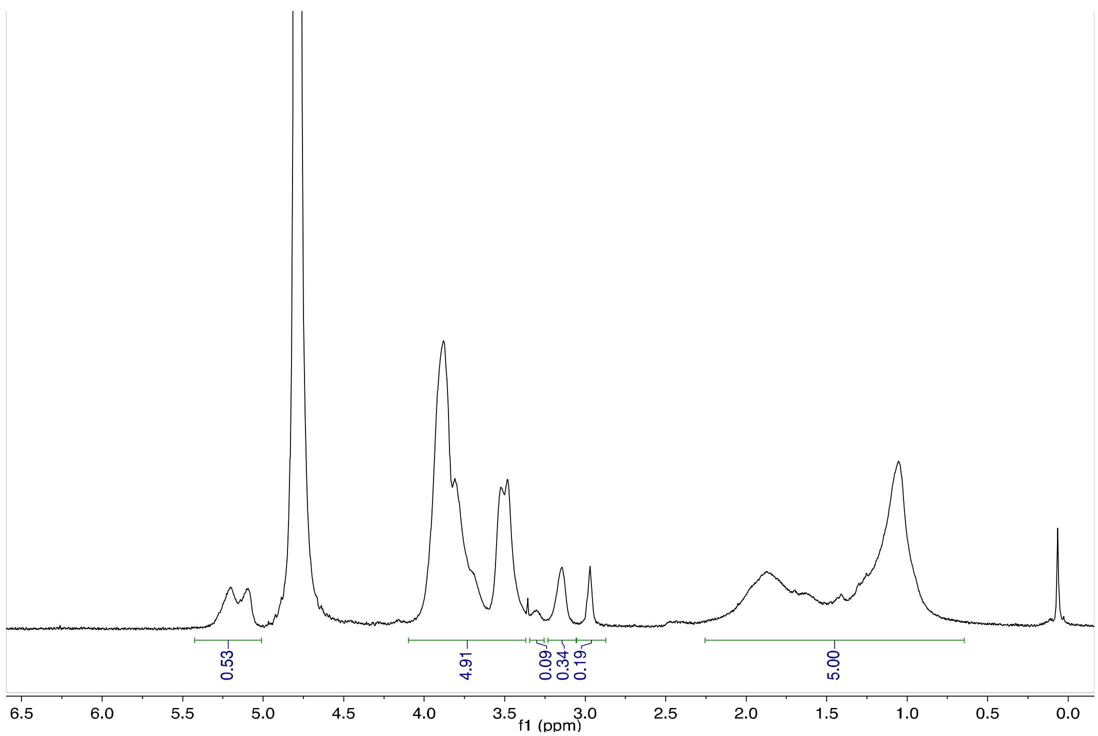

Figure S4. ^{1}H NMR spectra of poly($\mathbf{G}_{46}$-b-$\mathbf{P}_{10}$-b-$\mathbf{T}_{2}$)

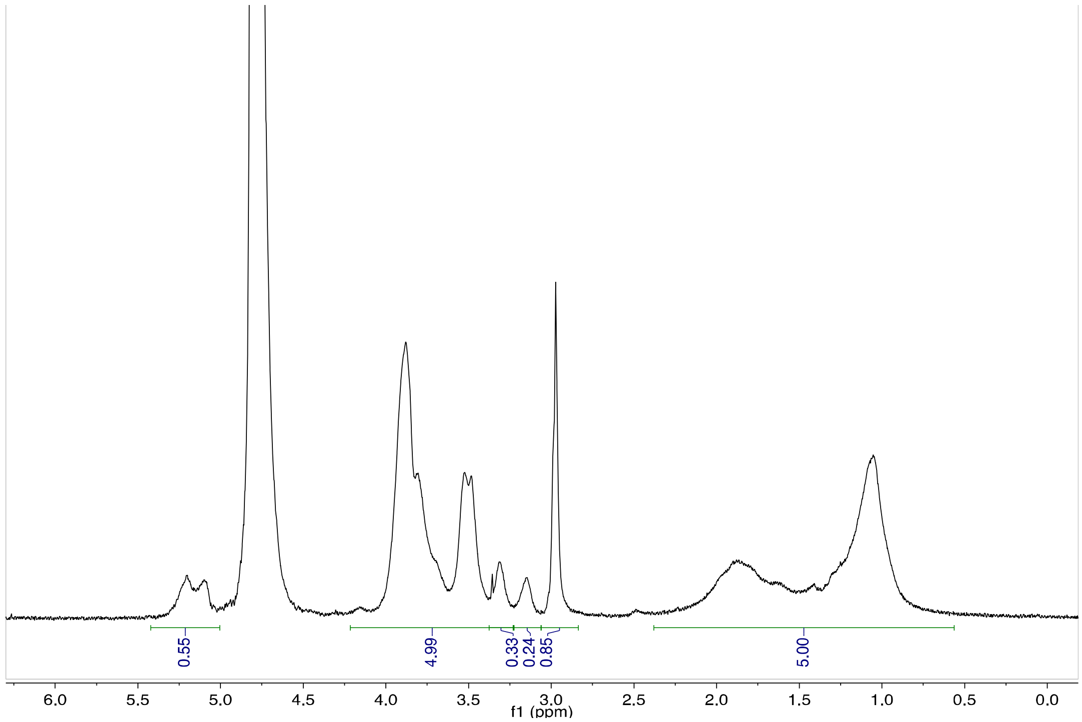

Figure S5. ^{1}H NMR spectra of poly($\mathbf{G}_{46}$-*b*-$\mathbf{P}_{8}$-*b*-$\mathbf{T}_{9}$)

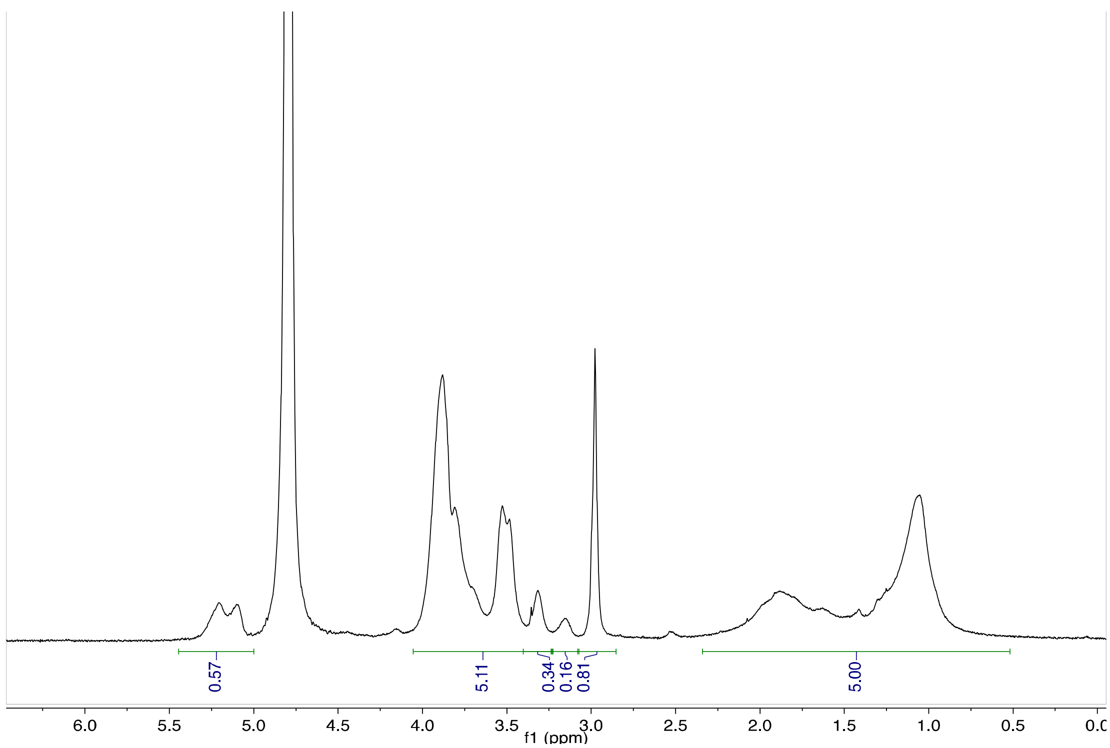

Figure S6. ^{1}H NMR spectra of poly($\mathbf{G}_{46}$-*b*-$\mathbf{P}_{6}$-*b*-$\mathbf{T}_{17}$)

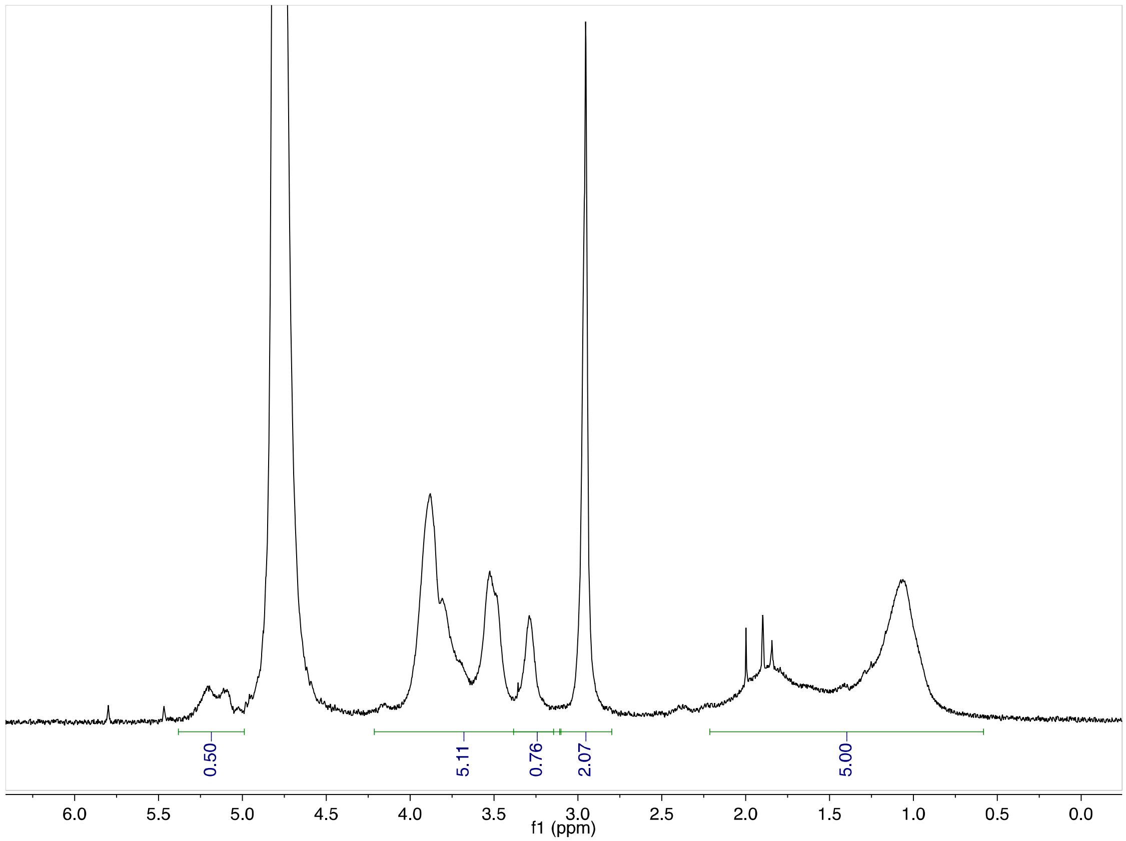

Figure S7. [1]H NMR spectra of poly(**G**$_{46}$-*b*-**T**$_{26}$)

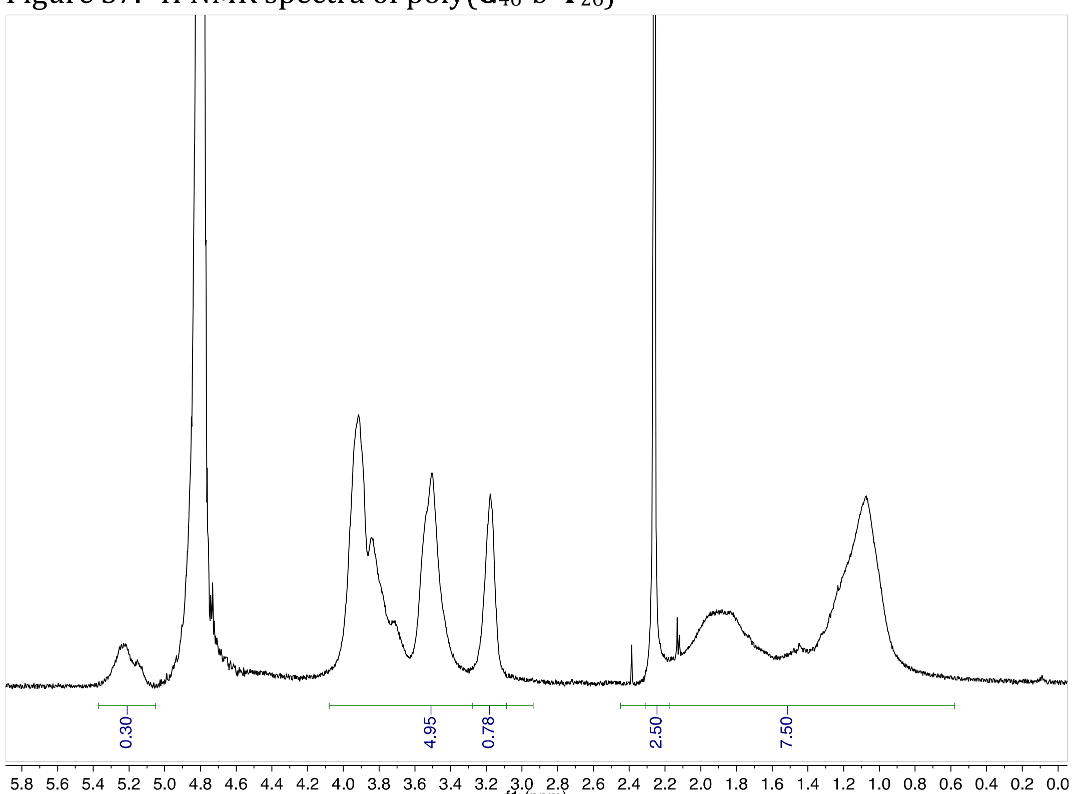

Figure S8. [1]H NMR spectra of poly(**G**$_{45}$-*s*-**P**$_{35}$)

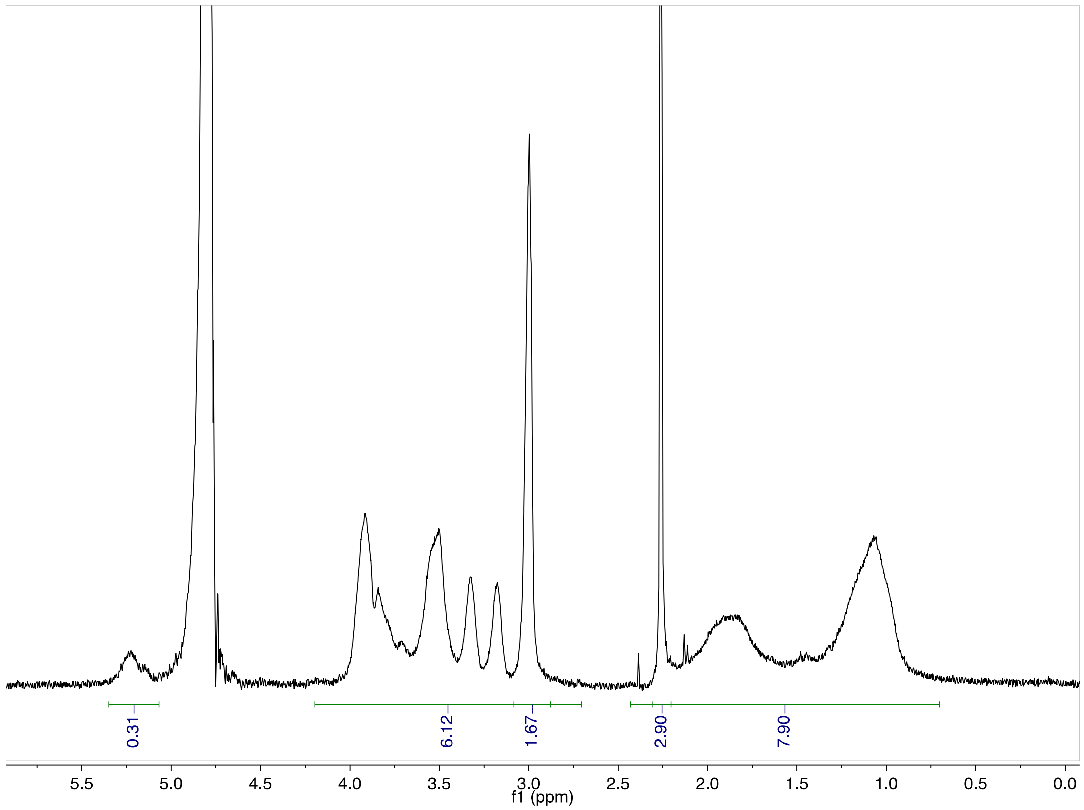

Figure S9. [1]H NMR spectra of poly(**G**$_{62}$-*s*-**T**$_{23}$)

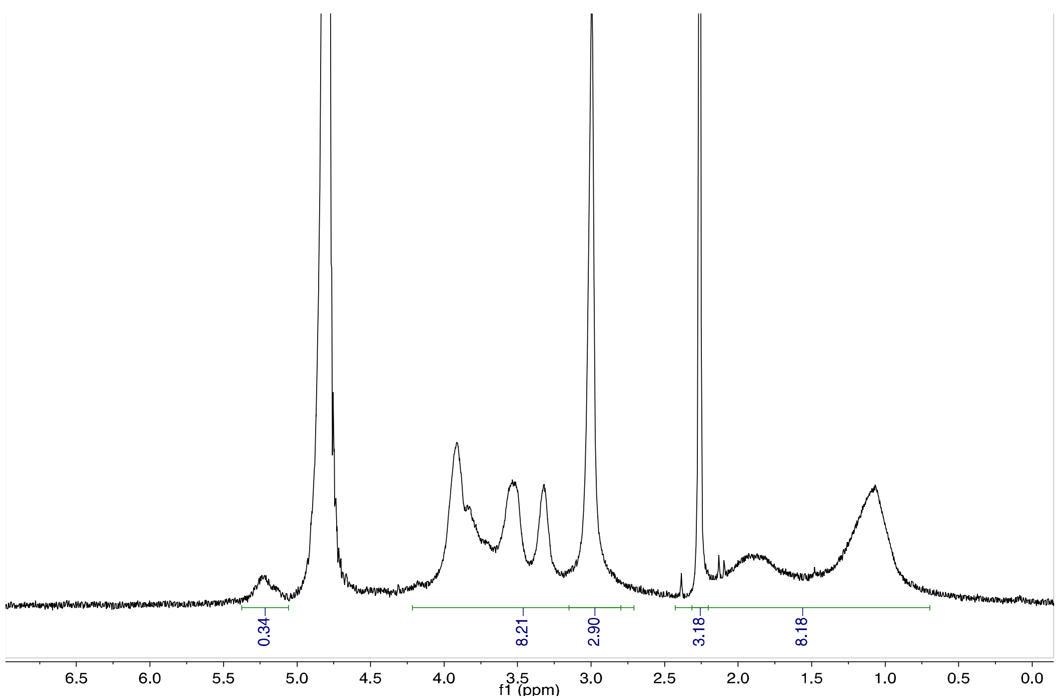

Figure S10. [1]H NMR spectra of poly(**G**$_{32}$-*s*-**P**$_{40}$-*s*-**T**$_{21}$)

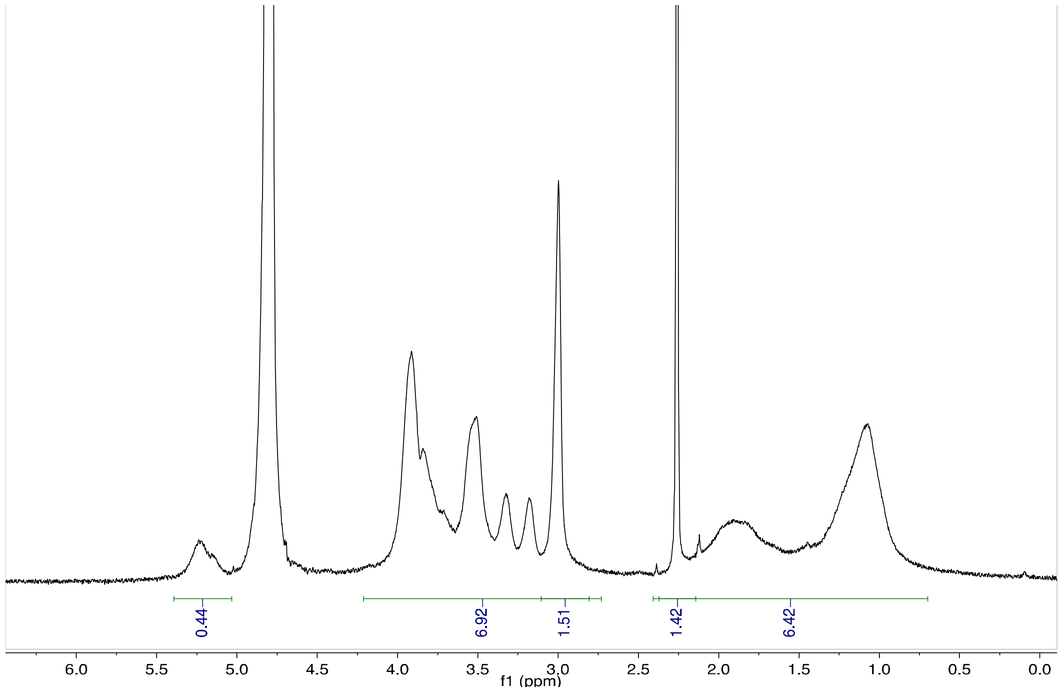

Figure S11. ^{1}H NMR spectra of poly($\mathbf{G}_{47}$-*s*-$\mathbf{P}_{28}$-*s*-$\mathbf{T}_{18}$)

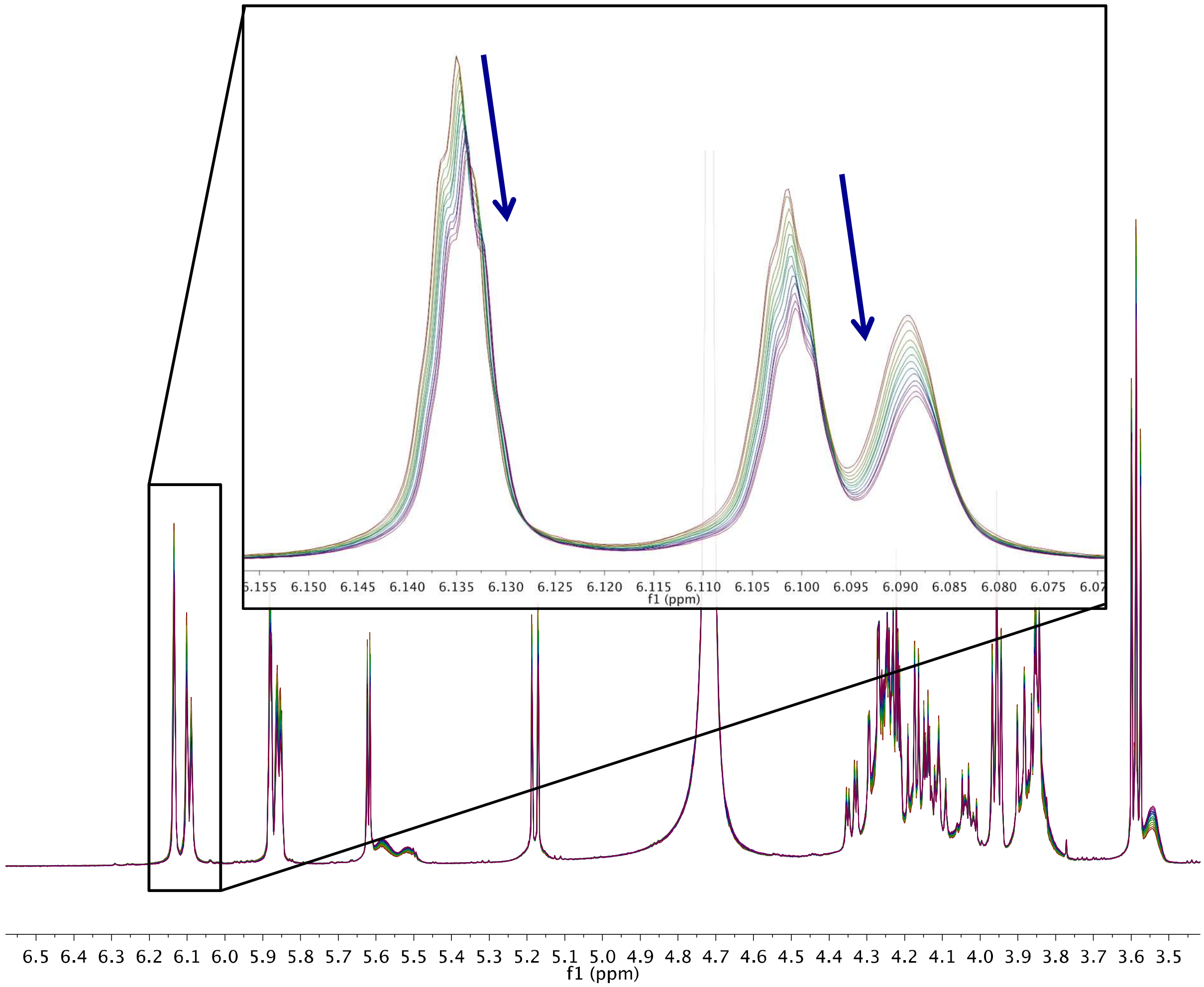

Figure S12. Overlays of ^{1}H NMR at 70 °C to monitor the consumption of two different monomers to find r_1 and r_2 in the reactivity ratio study. The monomers in this case were MAG and AEMA

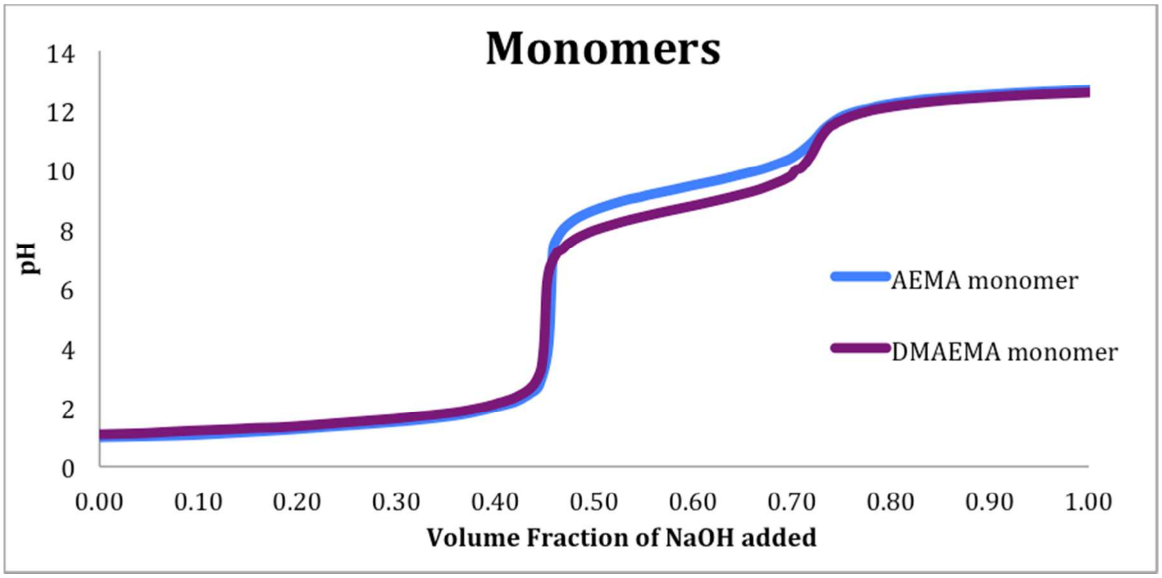

Figure S13. Comparison of the titration curves between AEMA and DMAEMA monomers.

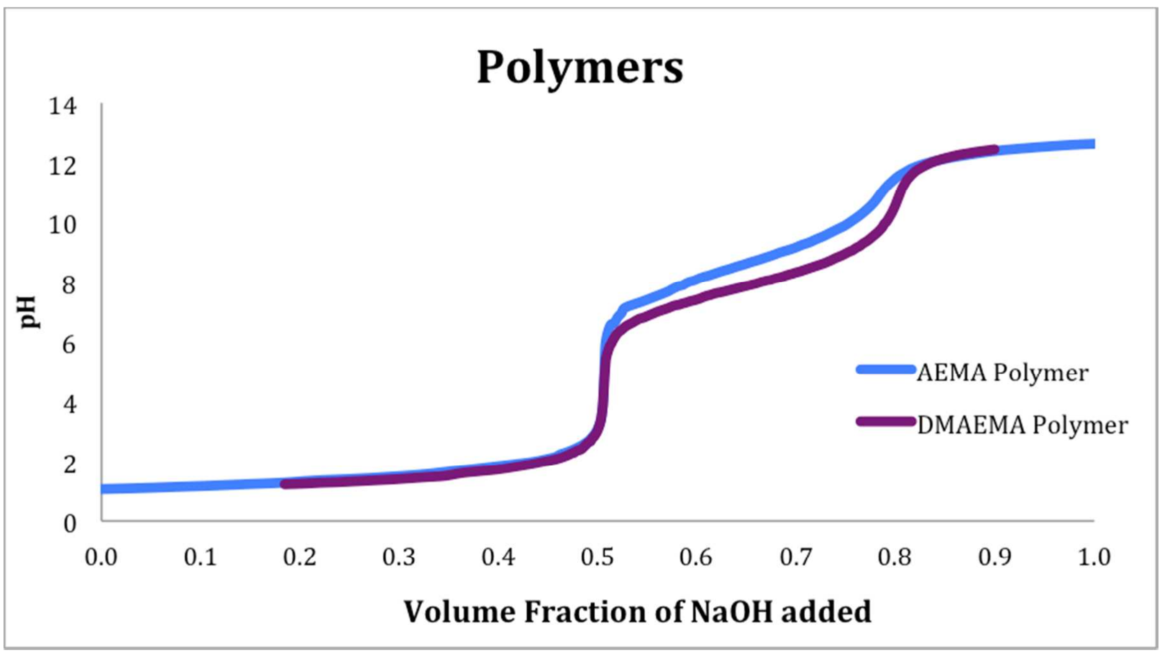

Figure S14. Comparison of the titration curves between AEMA and DMAEMA polymers.

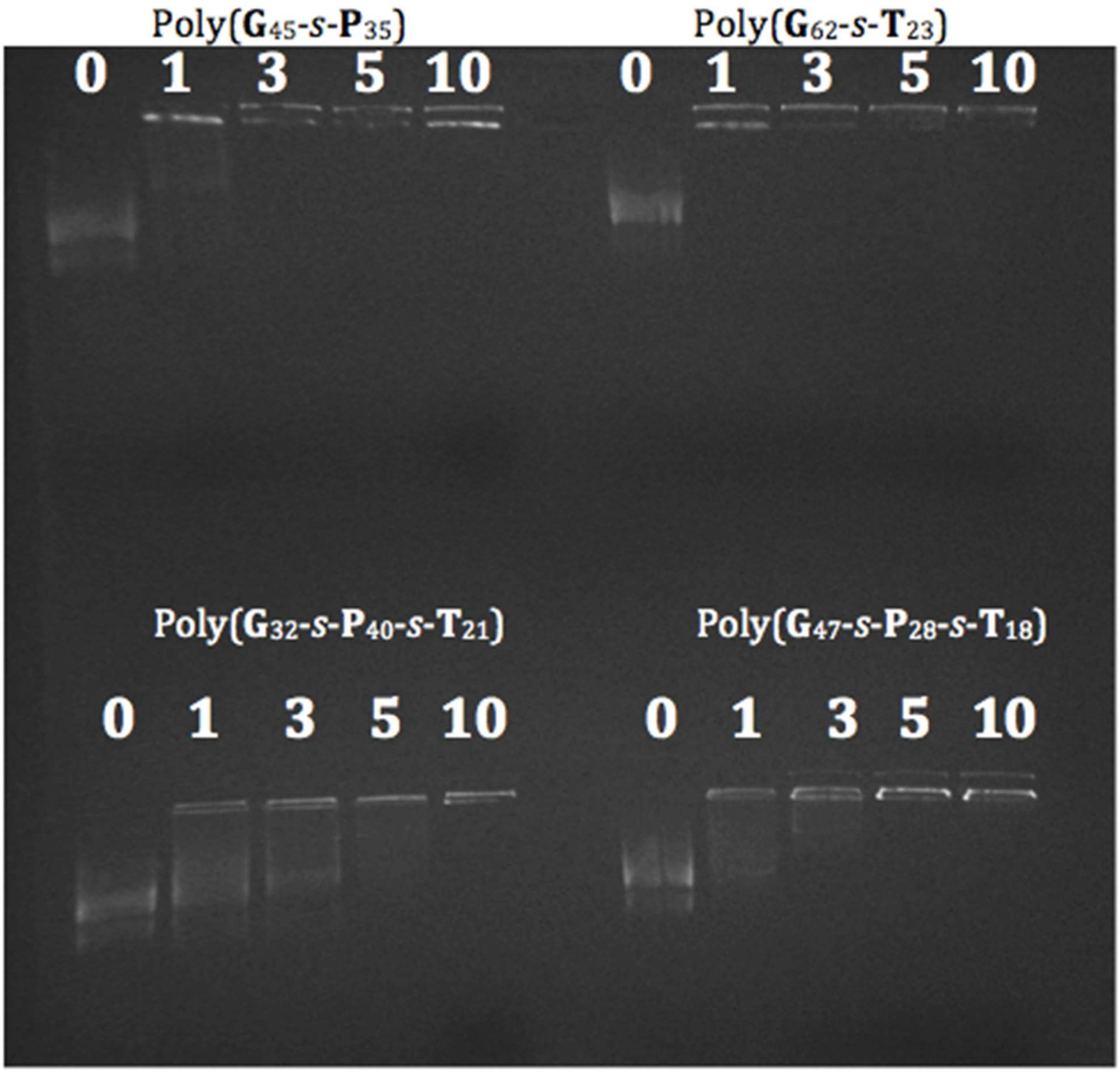

Figure S15. Gel Electrophoretic shift assay of the four statistical copolymers at N/P ratios 0, 1, 3, 5 and 10.

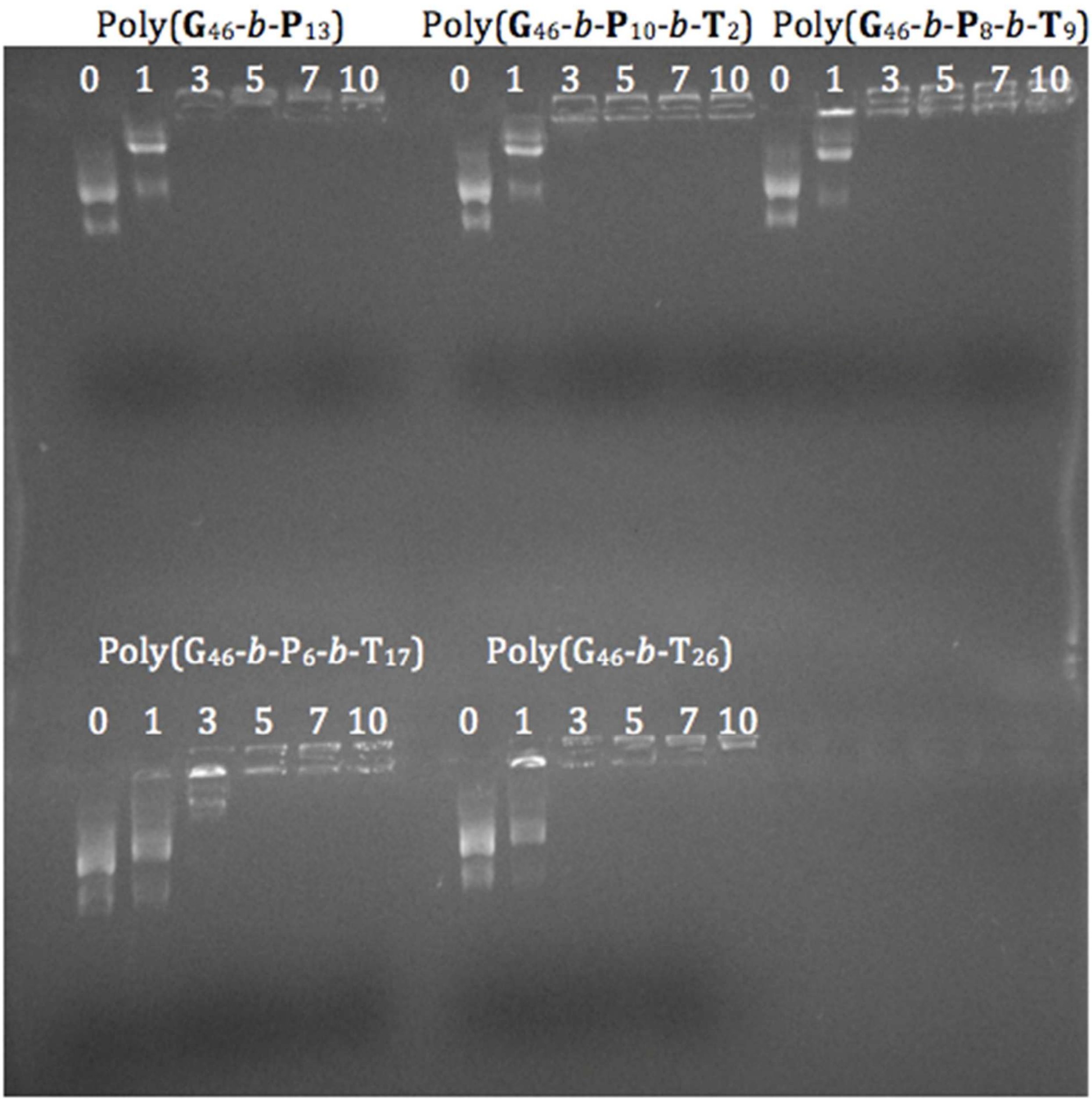

Figure S16. Gel Electrophoretic shift assay of the five block copolymers at N/P ratios 0, 1, 3, 5, 7 and 10.

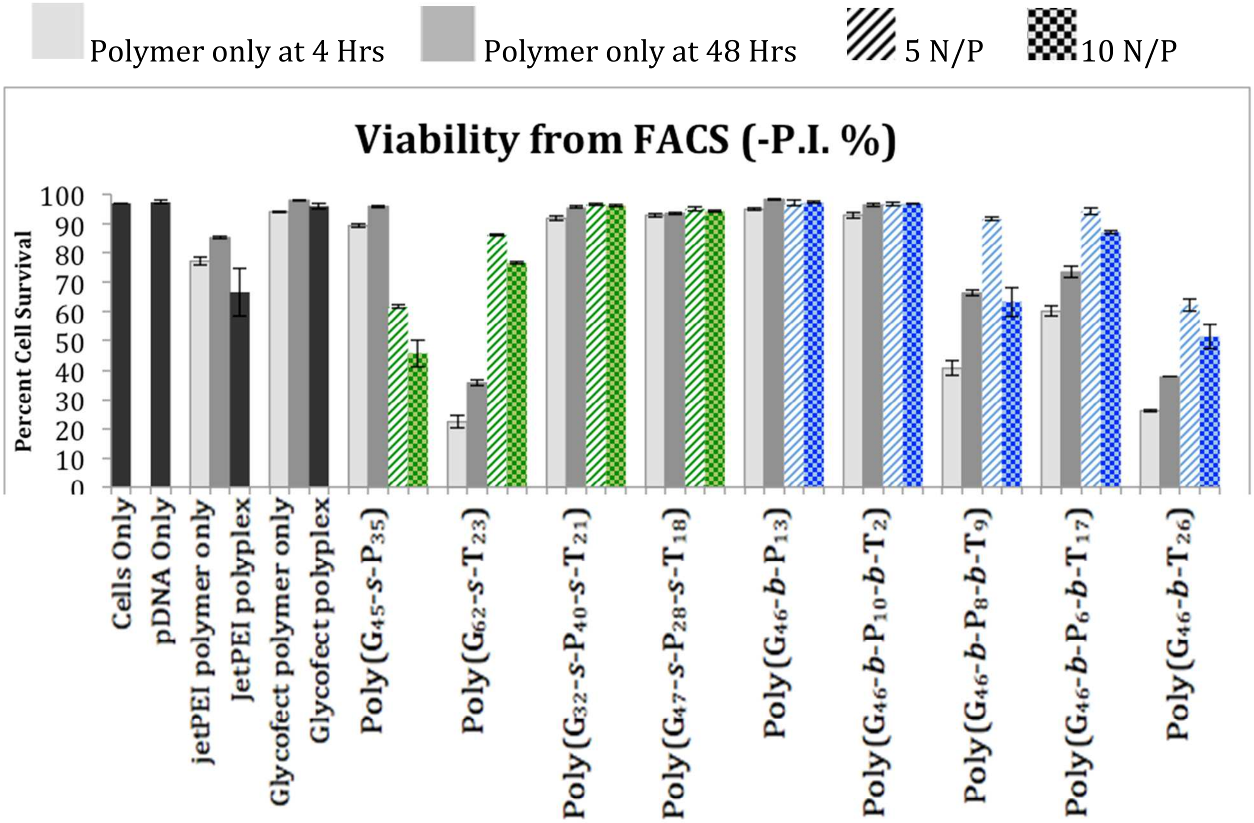

Figure S17. From Flow Cytometry – the percent cell survival according to negative propidium iodide staining of cells. Cells were treated with polymer only, and polyplexes at 5 N/P and 10 N/P. Polymer only samples were analyzed at 4 and 48 hours post transfection.

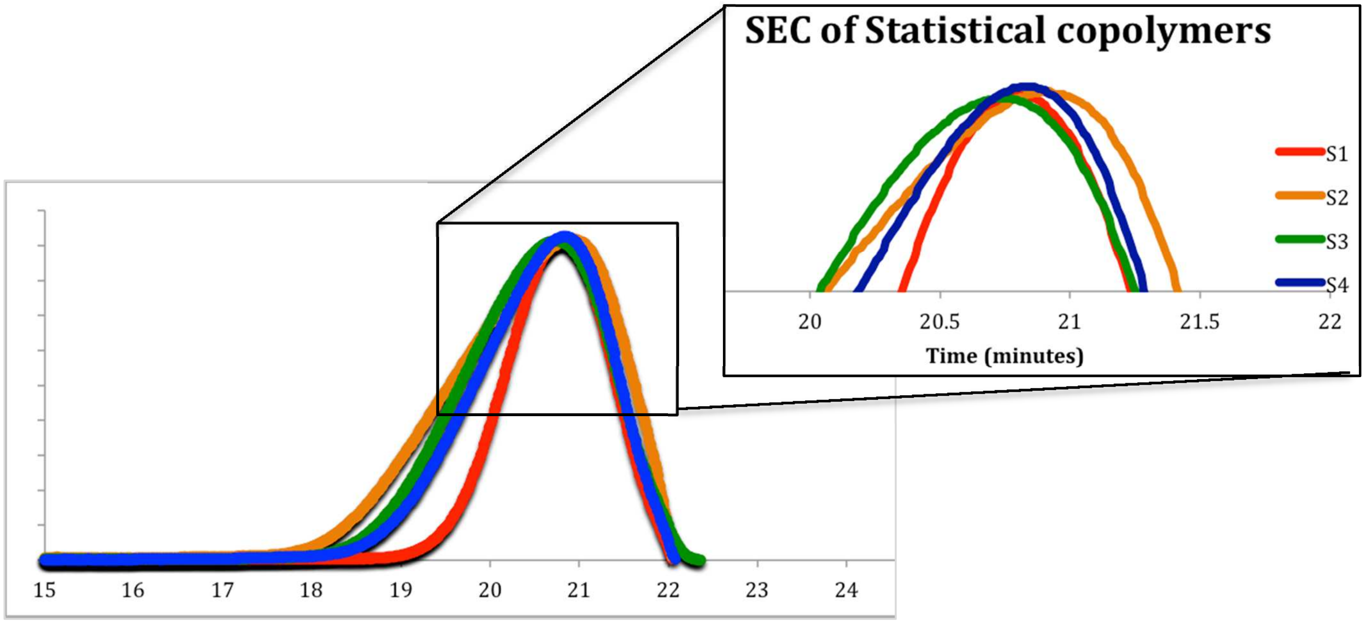

Figure S18.

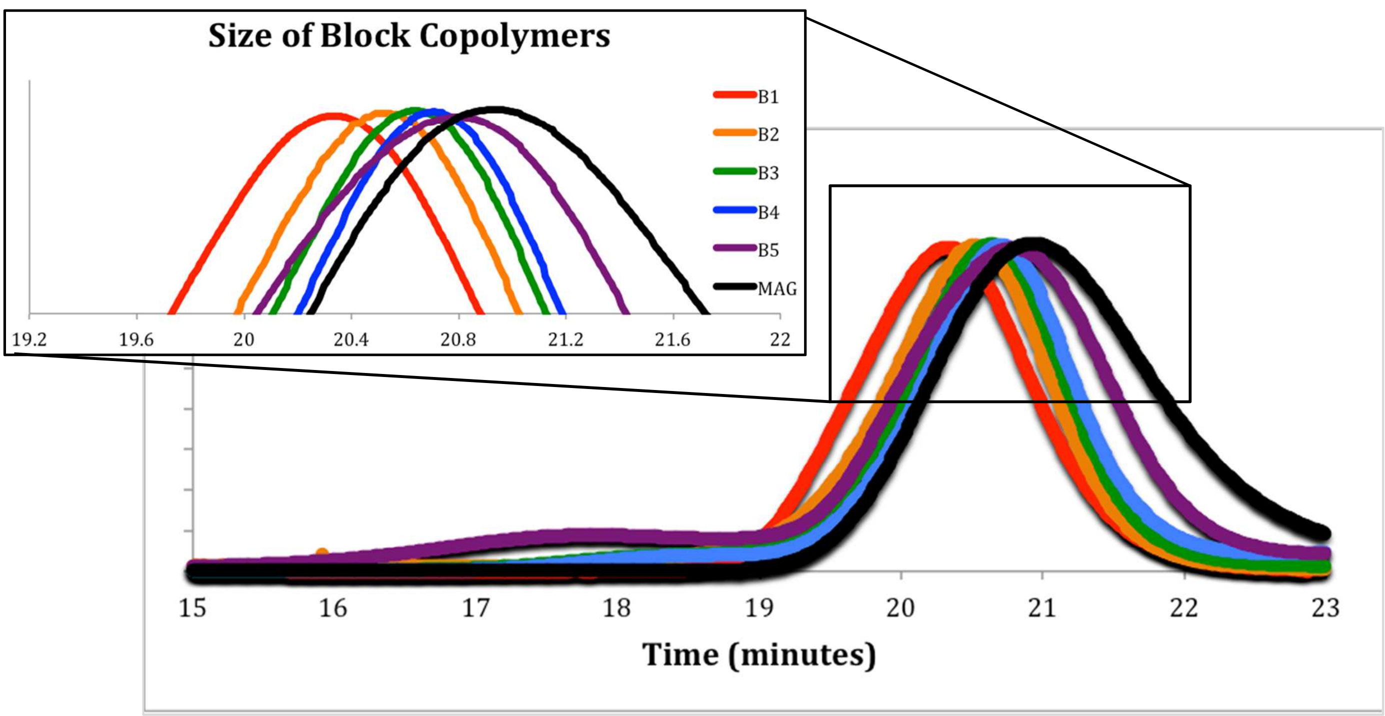

Figure S19.

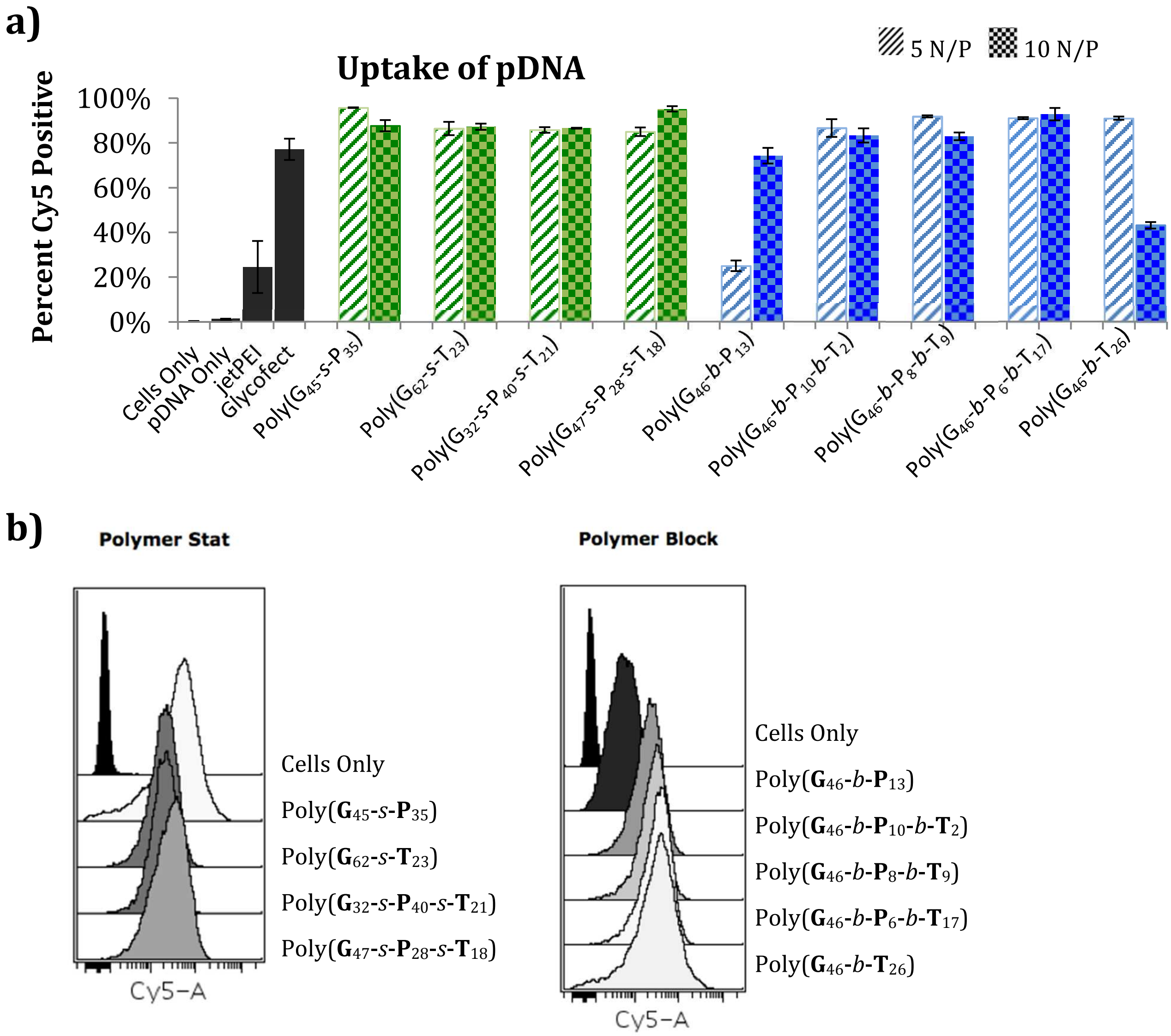

Figure S20. a) The amount of live cells that contain Cy-5 labeled pDNA and b) the intensity of the Cy-5 signal in cells transfected with polyplexes at 5 N/P. Cy5 represents the intensity of the Cy5 signal only in live cells – as determined by the negative population of propidium iodide.

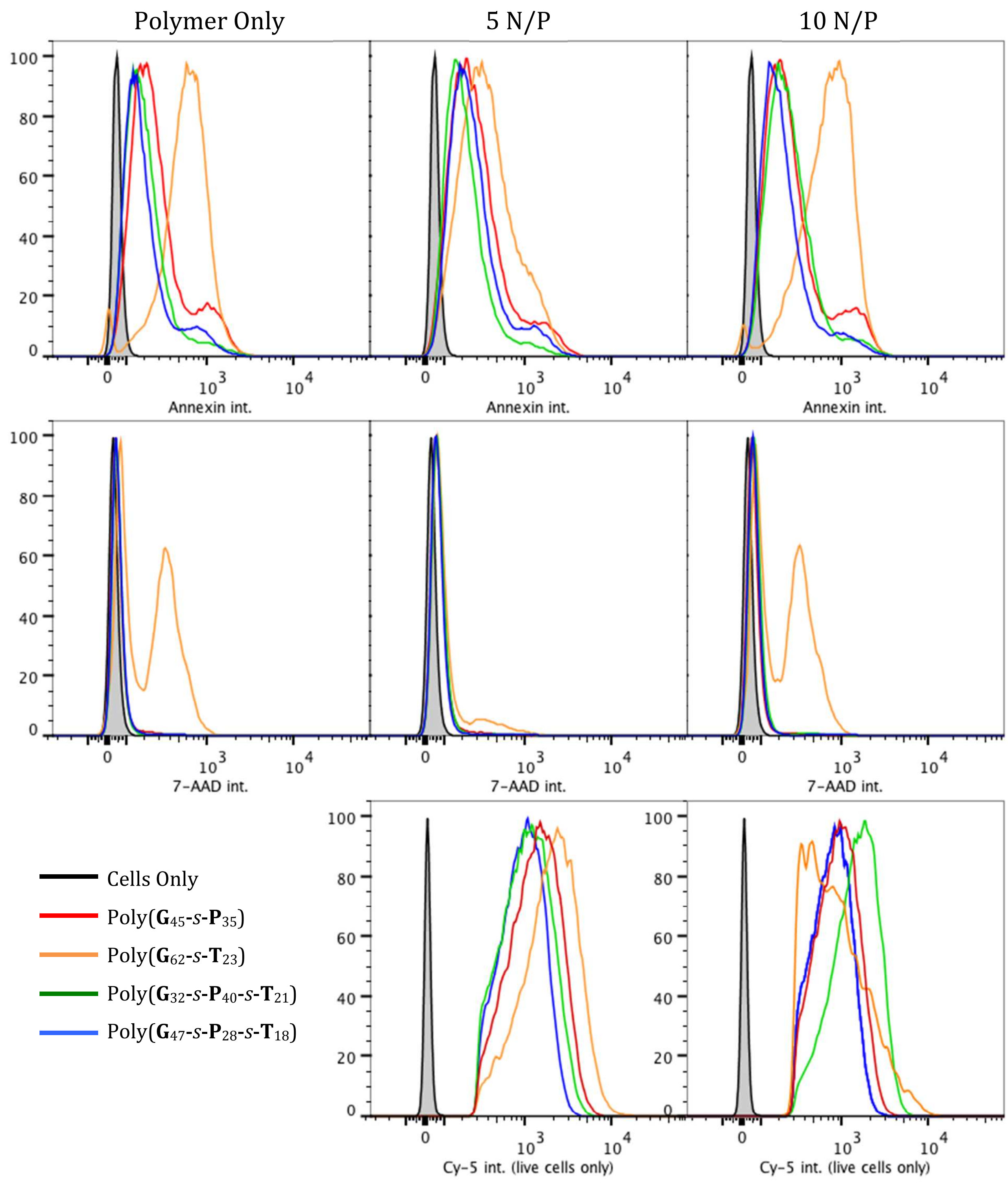

Figure S21. Normalized concatenated histogram overlays of the four statistical copolymers. The x-axis is Annexin V, 7-AAD, and Cy5 intensity for the rows and the columns are Polymer Only, 5 N/P, and 10 N/P, respectively. Cy5 represents the intensity of the Cy5 signal only in live cells – as determined by Annexin V negative population.

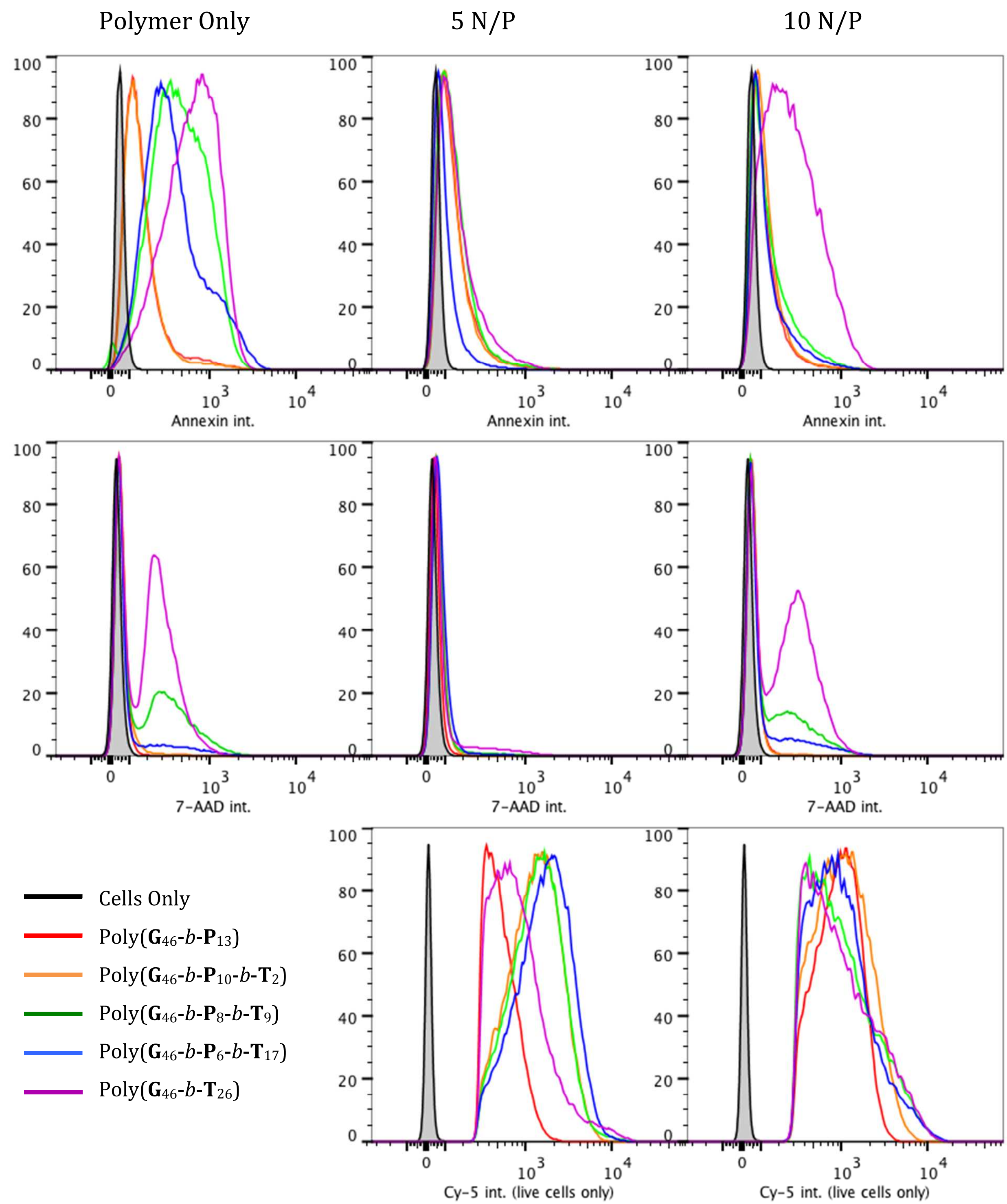

Figure S22. Normalized concatenated histogram overlays of the five block copolymers. The x-axis is Annexin V, 7-AAD, and Cy5 intensity for the rows and the columns are Polymer Only, 5 N/P, and 10 N/P, respectively. Cy5 represents the intensity of the Cy5 signal only in live cells – as determined by Annexin V negative population.

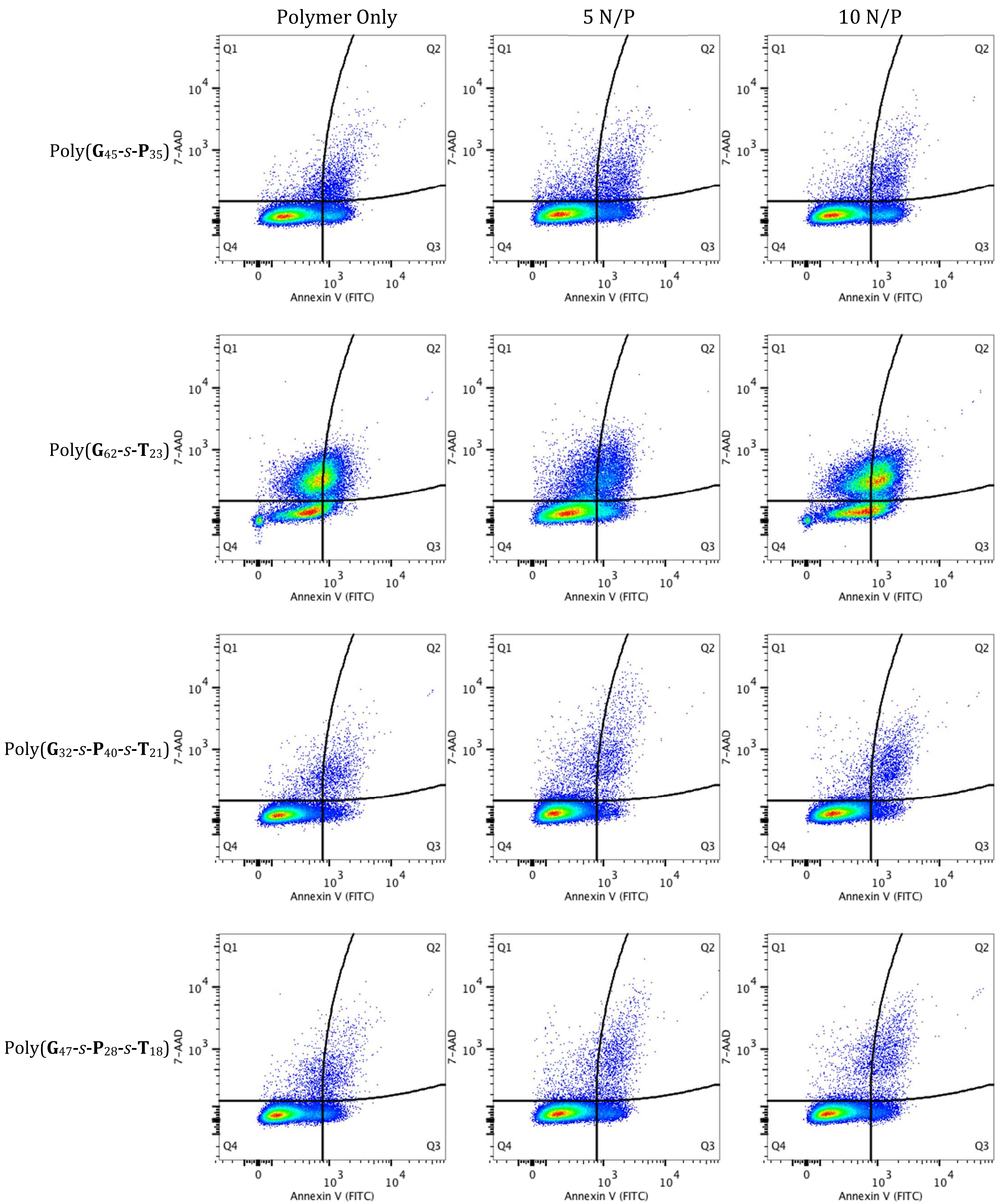

Figure S23. Flow cytomertry data for the four statistical copolymers. Triplicate sample was concatenated into one file. The x-axis is the Annexin V (FITC) stain, the y-axis is the 7-AAD stain, and the columns represent the Polymer only, 5 N/P, and the 10 N/P samples.

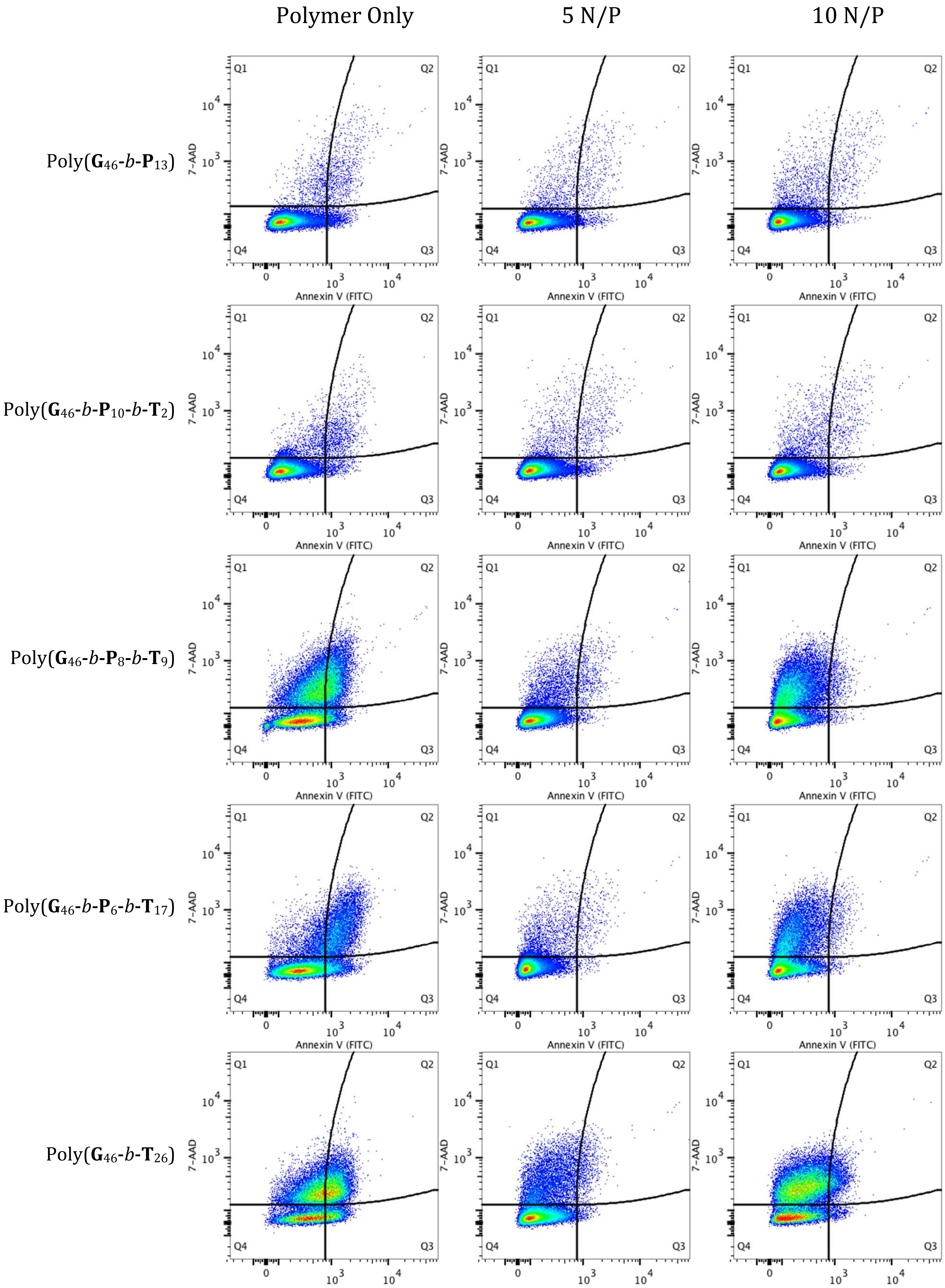

Figure S24. Flow cytomertry data for the five block copolymers. Triplicate sample was concatenated into one file. The x-axis is the Annexin V (FITC) stain, the y-axis is the 7-AAD stain, and the columns represent the Polymer only, 5 N/P, and the 10 N/P samples.

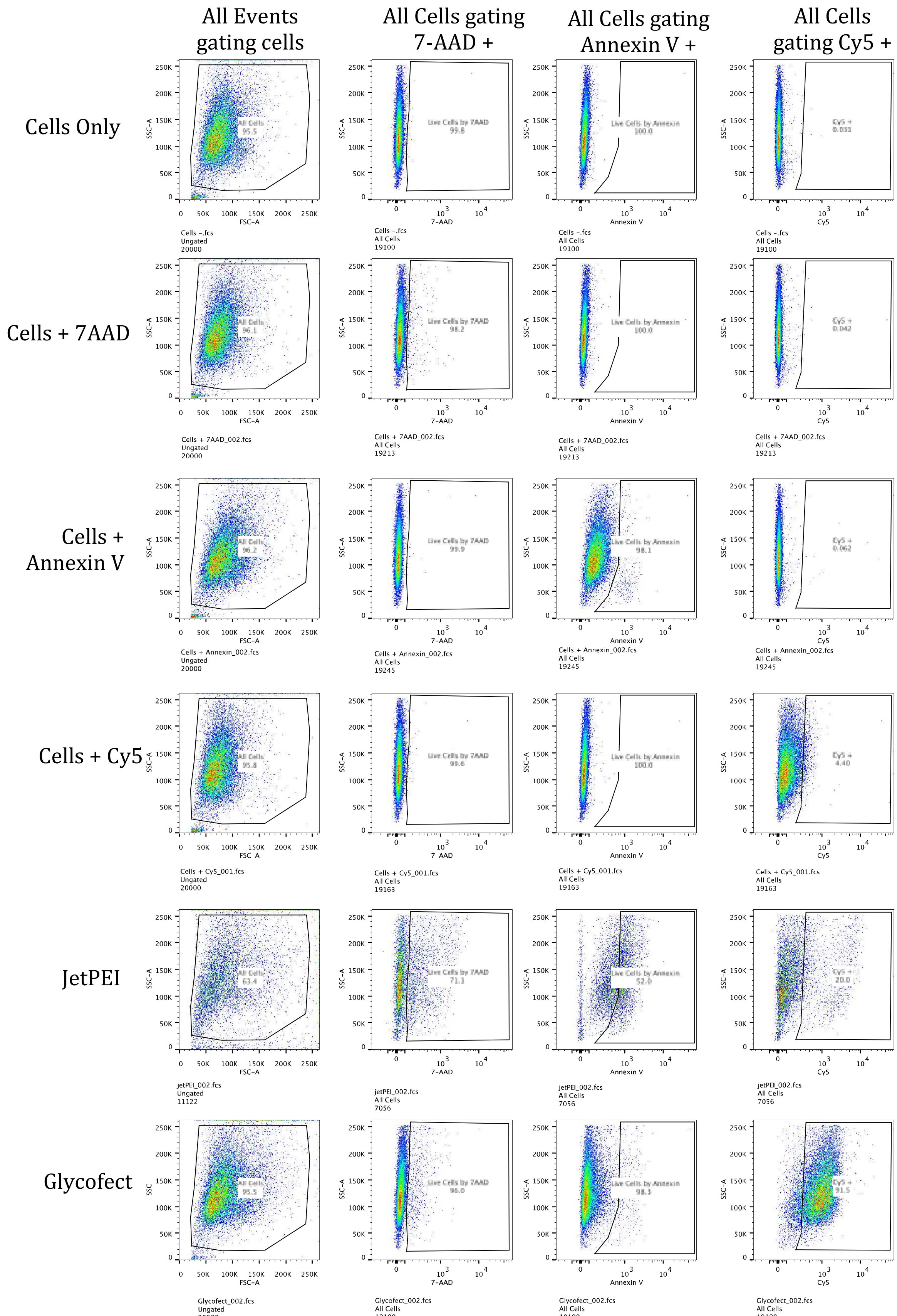

Figure S25a. Gating hierarchy and controls for flow cytometry data.

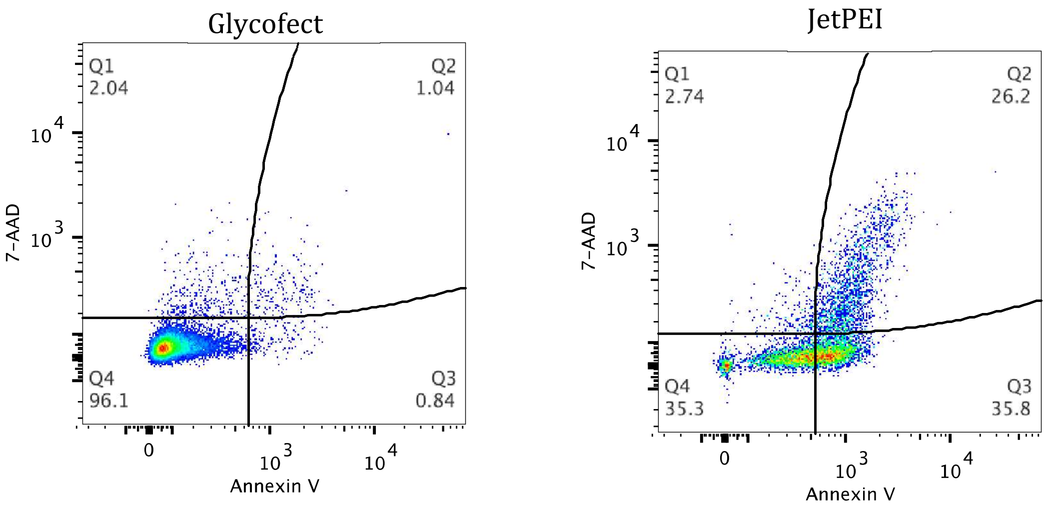

Figure S25b. Controls Glycofect and JetPEI from flow cytometry data. The x-axis is the Annexin V (FITC) stain, the y-axis is the 7-AAD stain

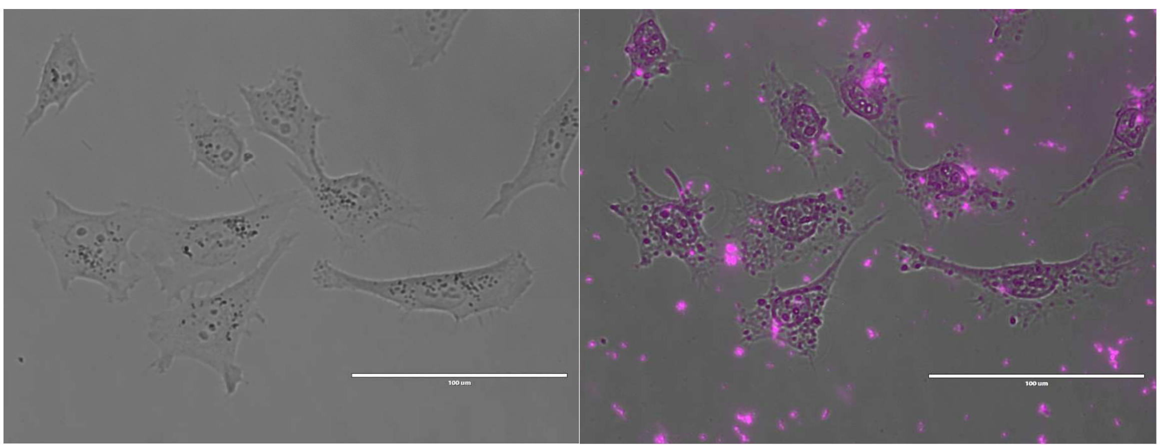

Figure S26. Microscopy images taken of the cells before Cy-5 labeled JetPEI polyplexes were added and four hours post transfection. Purple overlay is fluorescence microscopy taken at 679 nm. Scale bar represents 100 μm.

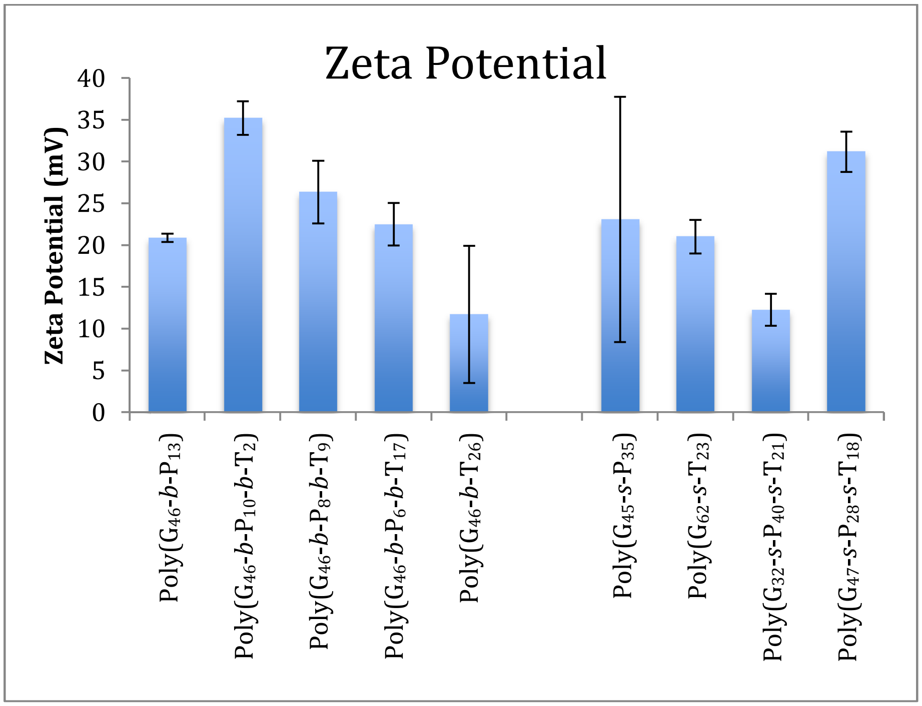

Figure S27: The zeta potential of the formulated polyplexes at 10 N/P diluted in water. Standard deviation was taken from three separate ζ measurements. All formulations had a positive zeta value.

Table S28. The size of the polyplexes measured by DLS, polyplexes were formed in water and then added to opti-MEM and size was measured at 0, 2, 4, and 6 hours.

Polymer	N/P	0 Hour	2 Hours	4 Hours	6 Hours	6 Hr in H_2O
Poly(G_{45}-*s*-P_{35})	5	221	806	1200	1485	78
	10	176	491	871	1121	59
Poly(G_{62}-*s*-T_{23})	5	170	410	563	689	53
	10	158	385	544	722	64
Poly(G_{32}-*s*-P_{40}-*s*-T_{21})	5	281	374	432	371	58
	10	192	613	645	697	65
Poly(G_{47}-*s*-P_{28}-*s*-T_{18})	5	143	491	700	715	68
	10	118	159	249	483	55
Poly(G_{46}-*b*-P_{13})	5	79	108	85	96	84
	10	78	89	92	73	109
Poly(G_{46}-*b*-P_{10}-*b*-T_2)	5	78	113	151	202	66
	10	130	214	224	252	159
Poly(G_{46}-*b*-P_8-*b*-T_9)	5	180	308	607	776	66
	10	163	298	577	712	72
Poly(G_{46}-*b*-P_6-*b*-T_{17})	5	181	289	549	720	69
	10	173	322	432	567	72
Poly(G_{46}-*b*-T_{26})	5	130	169	208	266	87
	10	145	301	476	564	97