Supplementary information

Phosphonium Polymethacrylates for Short Interfering RNA Delivery: Effect of Polymer and RNA Structural Parameters on Polyplex Assembly and Gene Knockdown

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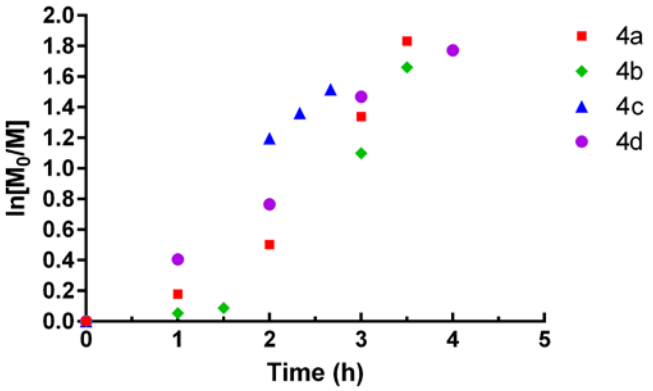
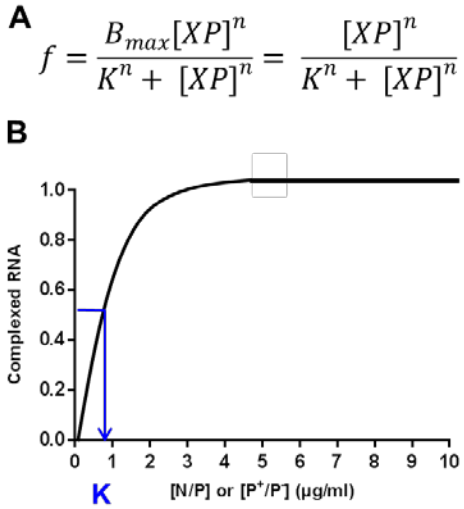


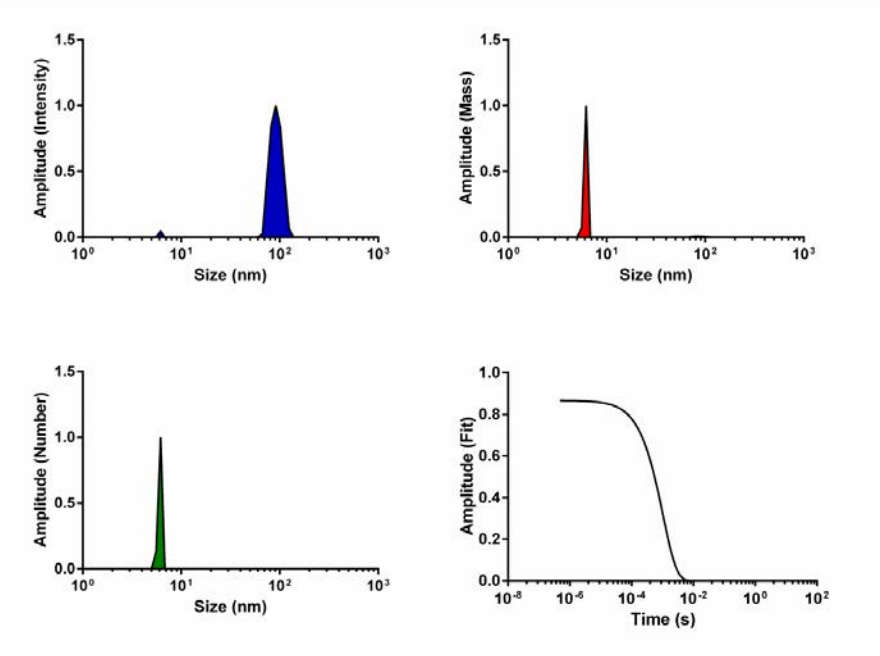
S1: Kinetic plots for the RAFT polymerization of methacrylate monomers yielding polymers **4a** (■), **4b** (♦), **4c** (▲) and **4d** (•). Reactions were performed with CTP, V-501 and monomers (**3a-d**) using a molar ratio of $[M]_0$:$[CTA]_0$:$[I]_0$=100:1:0.5 in D_2O: EtOH (3:1 vol/vol).



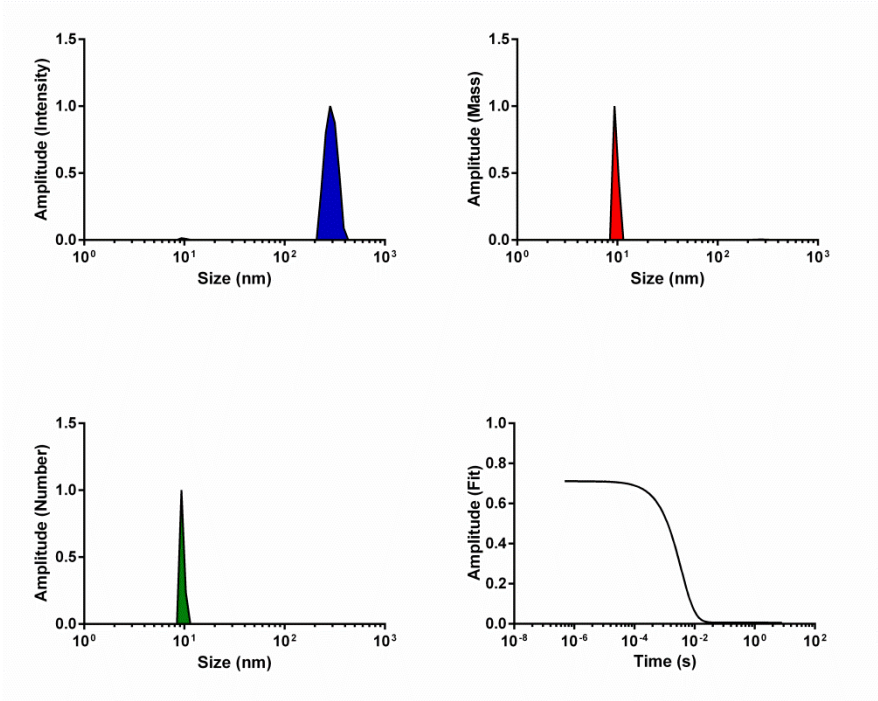
S2: A) Modified Hill's equation. B_{max} = Maximum specific binding (B_{max}=1), XP= polymer concentration represented as N^+/P ratio (ammonium polymer) or $P^{+/}P^-$ (phosphonium polymer) ratio**,** K= Binding constant to achieve a half-maximum binding at 30 minutes, n= Hill coefficient; B) Illustration of Hill's equation model and binding constant K.

S3: Dynamic light scattering and zeta potential measurements for RNA polyplexes at N^+/P^- or P^+/P^- ratio 20 (as analyzed by mass).

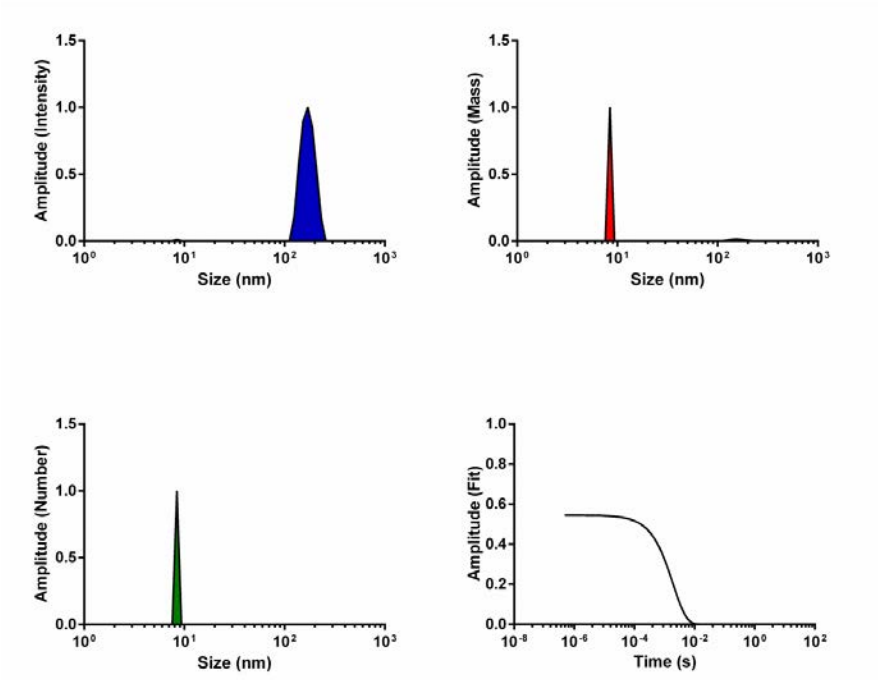
Polyplexes	R_h (nm)	Subpopulation, R_h (nm)	Subpopulation (%)	Zeta- potential (mV)
siRNA	1.02 ± 0.07	–	–	−21.9 ± 5.3
4a	8.8 ± 2.2	95.8 ± 53.5	2.9 ± 1.9	30.1 ± 6.9
4b	13.7 ± 5.6	223.2 ± 138.6	1.4 ± 1.5	24 ± 2.8
4c	14.8 ± 6.9	174.5 ± 85.3	6.3 ± 6.1	30.5 ± 3.7
4d	4 ± 1	–	–	31.3 ± 3.4



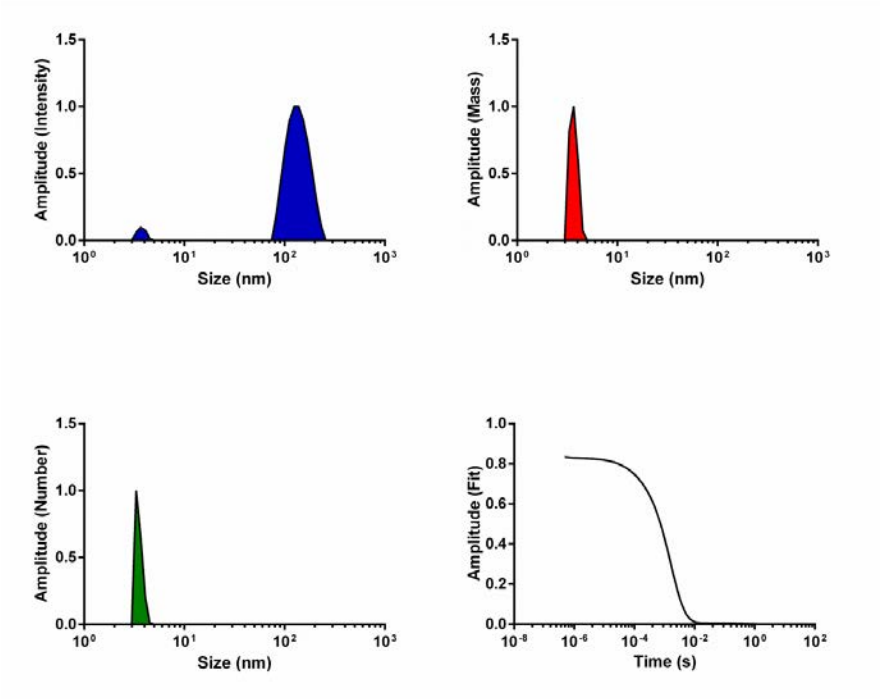
S4: Dynamic light scattering of RNA polyplexes formed with polymer **4a** at N^+/P^- ratio 20. Hydrodynamic radius (Rh, nm) was measured by intensity (A), mass (B) or number (C). (D) Illustration of correlation function. Experiment were performed in triplicate, with three independent measurements (n=3).



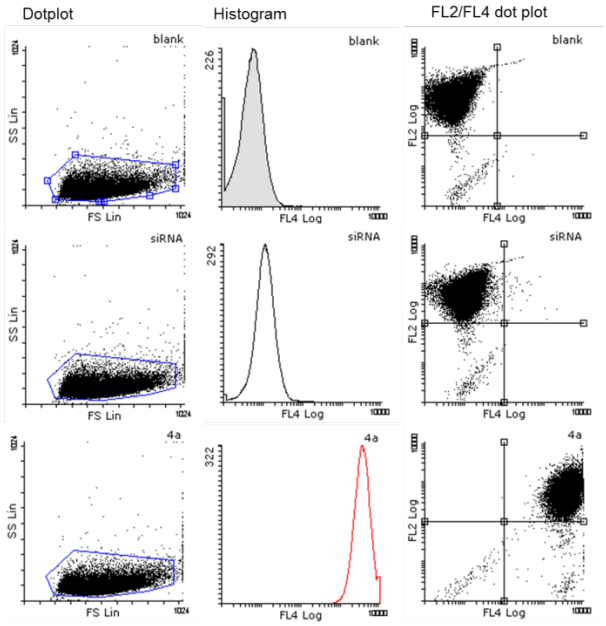
S5: Dynamic light scattering of RNA polyplexes formed with polymer **4b** at N^+/P^- ratio 20. Hydrodynamic radius (Rh, nm) was measured by intensity (A), mass (B) or number (C). (D) Illustration of correlation function. Experiment were performed in triplicate, with three independent measurements (n=3).

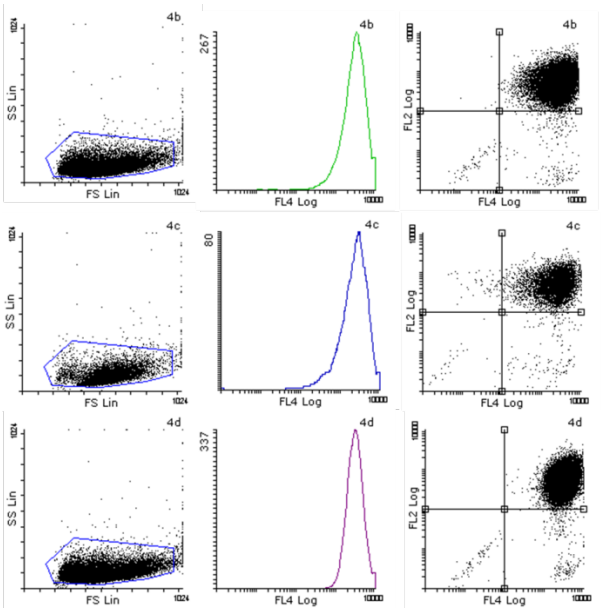


S6: Dynamic light scattering of RNA polyplexes formed with polymer **4c** at N^+/P^- ratio 20. Hydrodynamic radius (Rh, nm) was measured by intensity (A), mass (B) or number (C). (D) Illustration of correlation function. Experiment were performed in triplicate, with three independent measurements (n=3).

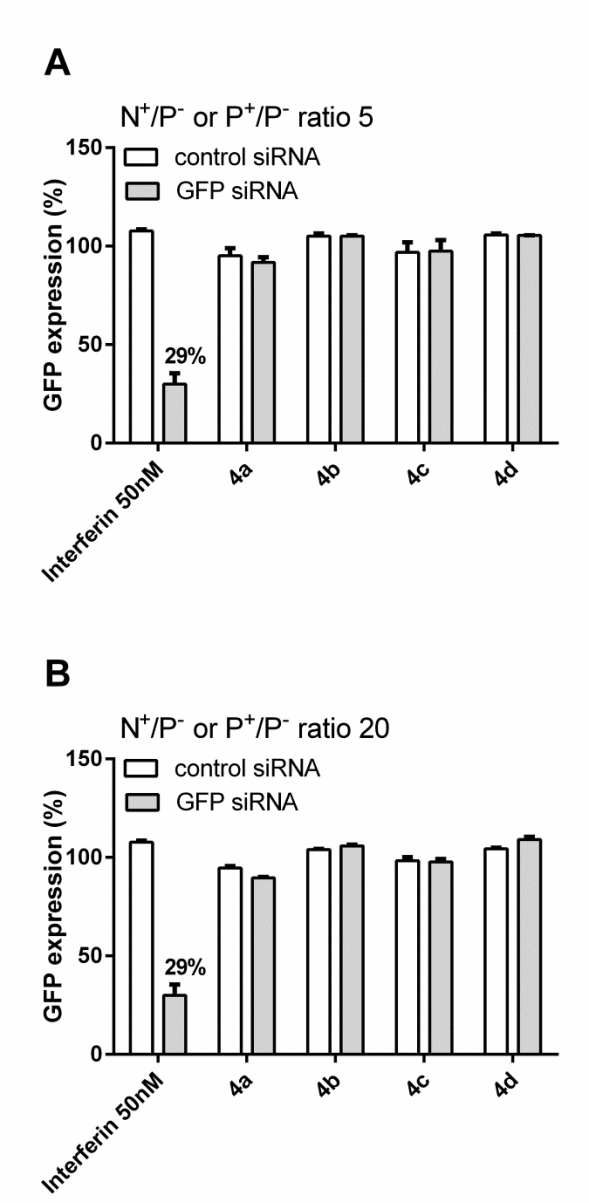


S7: Dynamic light scattering of RNA polyplexes formed with polymer **4d** at N^+/P^- ratio 20. Hydrodynamic radius (Rh, nm) was measured by intensity (A), mass (B) or number (C). (D) Illustration of correlation function. Experiment were performed in triplicate, with three independent measurements (n=3).

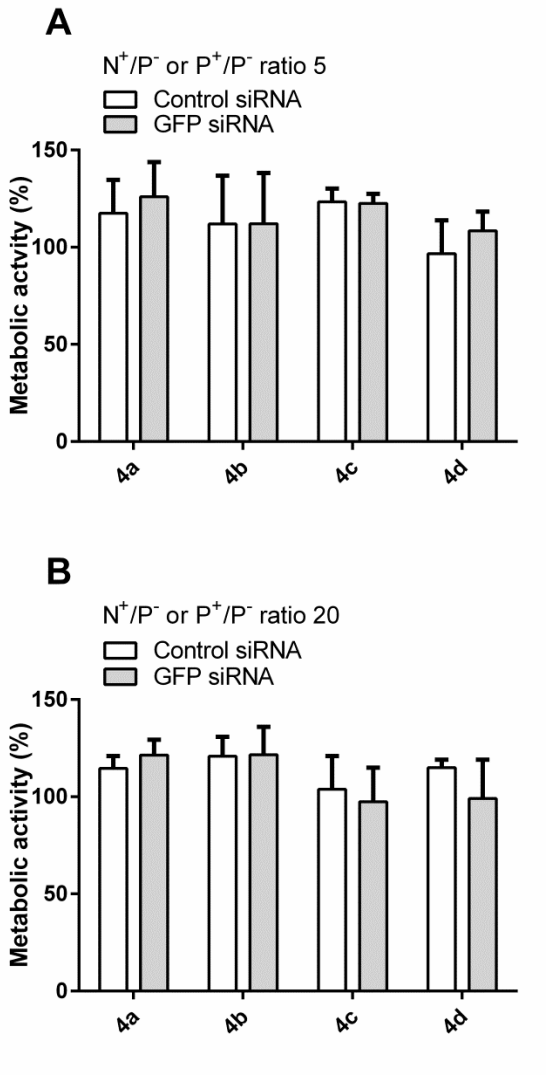




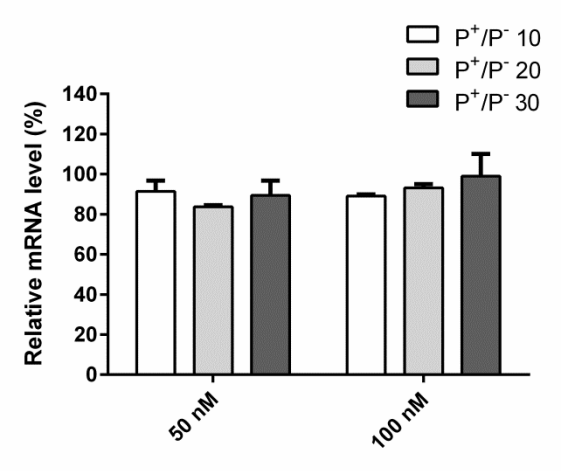
S8: 3T3 cellular uptake using flow cytometry. The main population is plotted in a side scatter/forward scatter dot plot and gated (R-1) to exclude cell debris. The gate population is plotted on a histogram (FL-4 = AF-647) and FL-2/FL-4 dot plot showing an increase of FL-4 fluorescence in samples containing siRNA-polyplexes.



S9: No GFP knockdown is achieved in 3T3 cells when employing polymers (**4a-d**) at different N^+/P^- or P^+/P^- ratio using 187 nM siRNA after 48 hs incubation as analyzed by flow cytometry. A) Polymers at N^+/P^- or P^+/P^- ratio = 5. B) Polymers at N^+/P^- or P^+/P^- ratio = 20. In contrast, commercially available Interferin successfully transfects siRNA at concentration of 50 nM. Data is represented as GFP expression (%), mean ± SEM (n=2).



S10: 3T3 cellular viability after polyplex exposure at different N^+/P^- or P^+/P^- ratio using 187 nM siRNA. No effect on cellular viability was observed when polyplexes were exposed to the cells and are incubated for 48 hs. (A) N^+/P^- or P^+/P^- ratio = 5 (B) N^+/P^- or P^+/P^- ratio = 20. Data is represented as metabolic activity (%), mean ± SEM (n=2).



S11: Knockdown studies with siRNA targeting Survivin. HeLa cells were transfected with polymer **4d** using a P^+/P^- ratio of 10, 20, 30, with 50 or 100 nM siRNA. Survivin and GAPDH mRNA levels were measured by qRT-PCR 24 hs after transfection. Survivin mRNA levels were normalized to GAPDH mRNA levels. Data is represented as relative mRNA level (%), mean ± SEM (n=2).