Supporting Info:

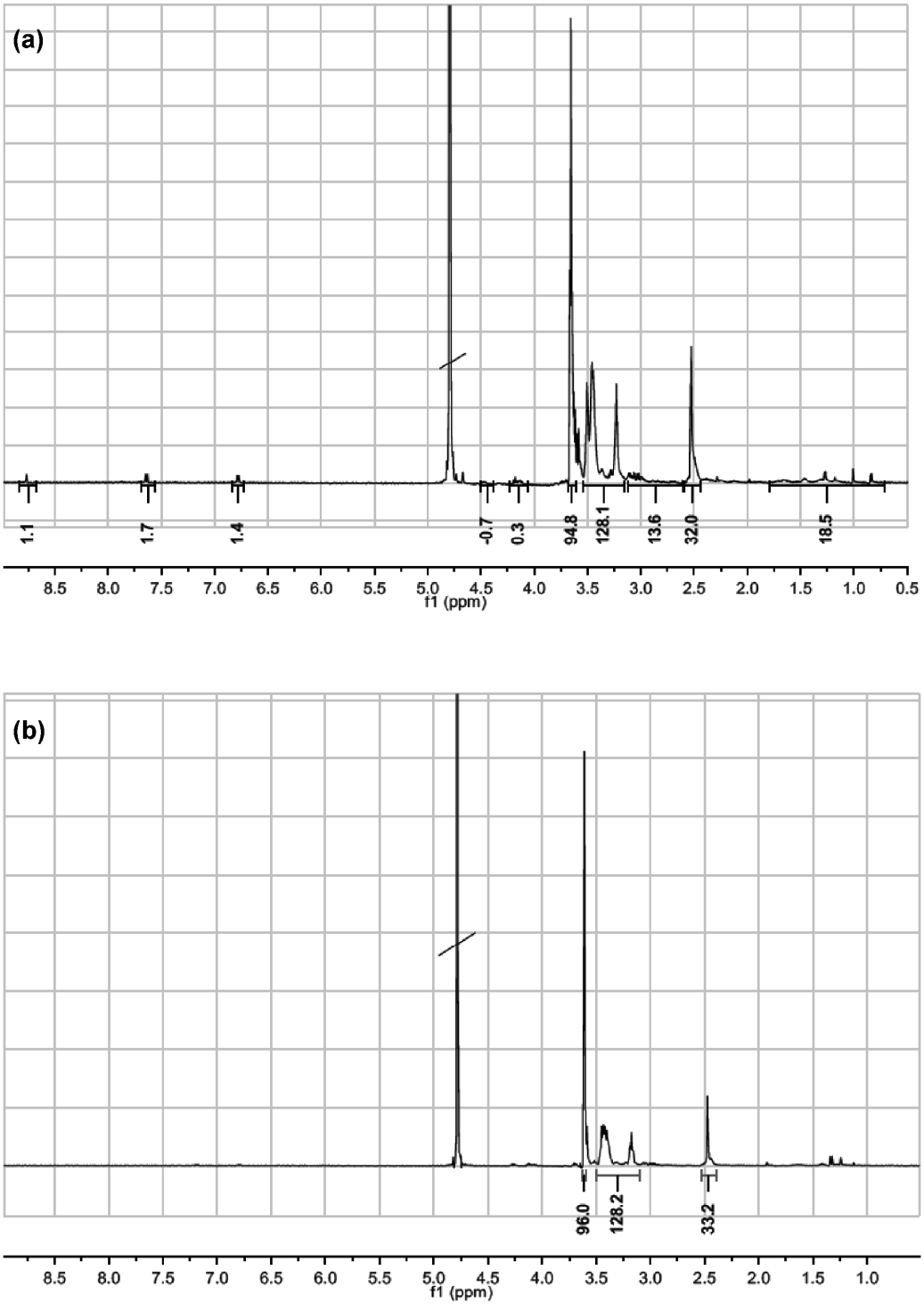


Figure S 1. ^{1}H-NMR spectra of FolA-PEG$_{24}$-K(Stp$_4$-C)$_2$ (**356**, a) and A-PEG$_{24}$-K(Stp$_4$-C)$_2$ (**188**, b) in D_2O.

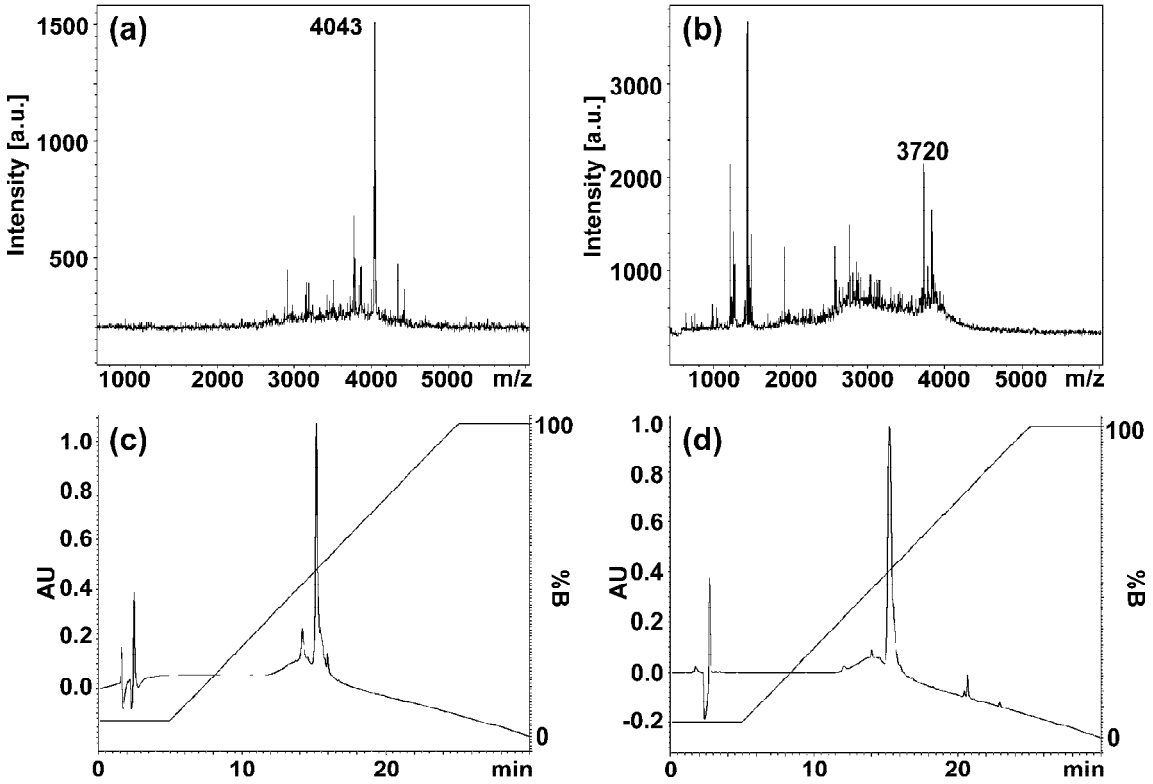


Figure S 2. Analysis of structural analogs of polymer **356**. MALDI-TOF-MS spectra and chromatogram of the analytical RP-HPLC of FolA-PEG_{24}-K(Stp_4-S)$_2$ (**420**) (a,c) and A-PEG_{24}-K(Stp_4-C)$_2$ (**188**) (b,d). Calculated mass $[M+H]^+$: FolA-PEG_{24}-K(Stp_4-S)$_2$: 4043, A-PEG_{24}-K(Stp_4-C)$_2$: 3720.

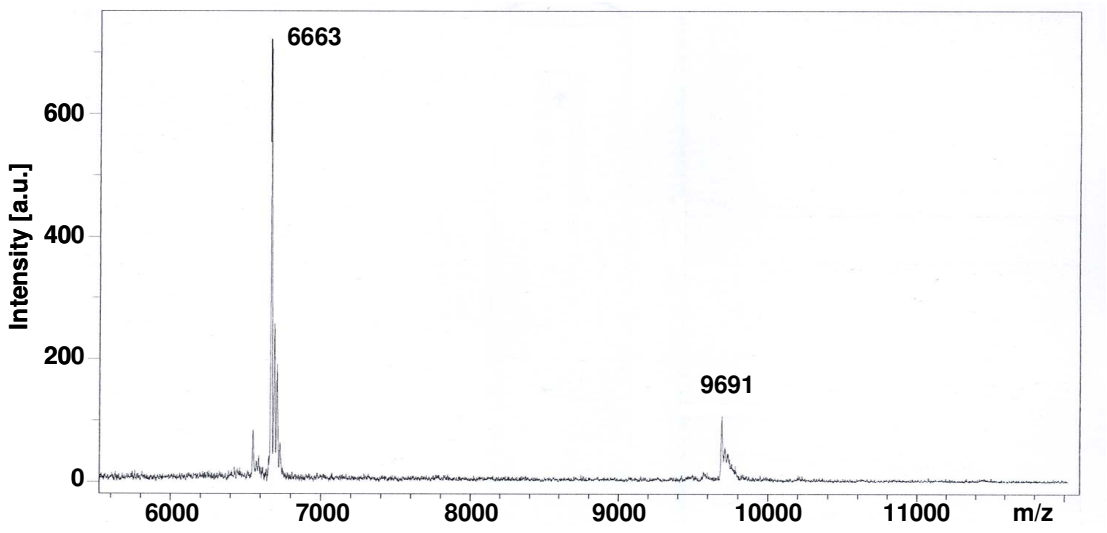
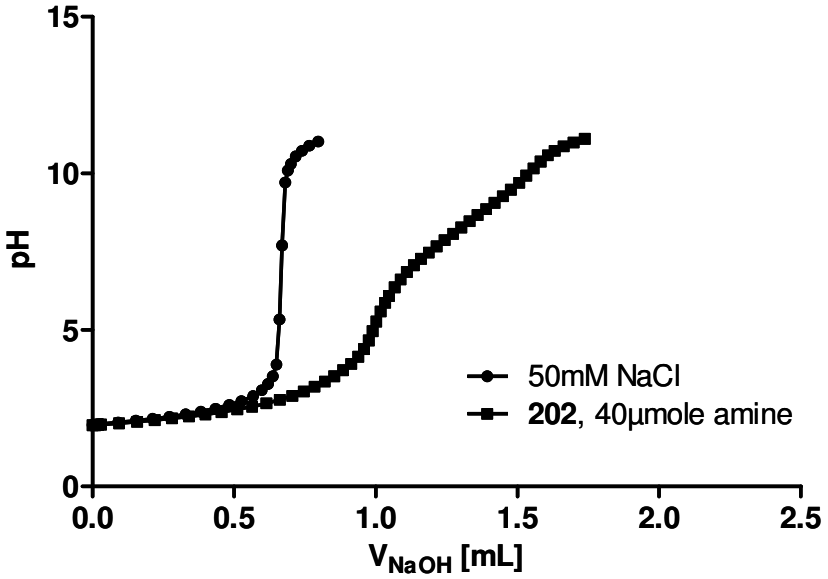


Figure S 3. MALDI-TOF-MS spectrum of siGFP-Inf7. After purification via ion exchange chromatography and analysis with agarose gel electrophoresis, the Inf7-siRNA conjugate was identified by MALDI-TOF-MS analysis. Calculated mass: unconjugated antisense strand: 6668 Da; conjugated sense strand: 9691 Da.

Figure S 4. Agarose gel shift assay of oligomer **356** /siRNA-Inf7 polyplexes formed at lower molecular ratios. Polyplexes were formed as described under polyplex formation using N/P ratios 2, 3, 4, 5 and 6. N/P 0: free siRNA.



Protonation at pH 7.4	
40 µmole amine	46.7%

Figure S 5. pH-titration of polycation **202**. The full titration of the polyamine structure using NaOH revealed a broad buffering range. Calculating the relative protonation state at pH 7.4 resulted in the value listed in the table.

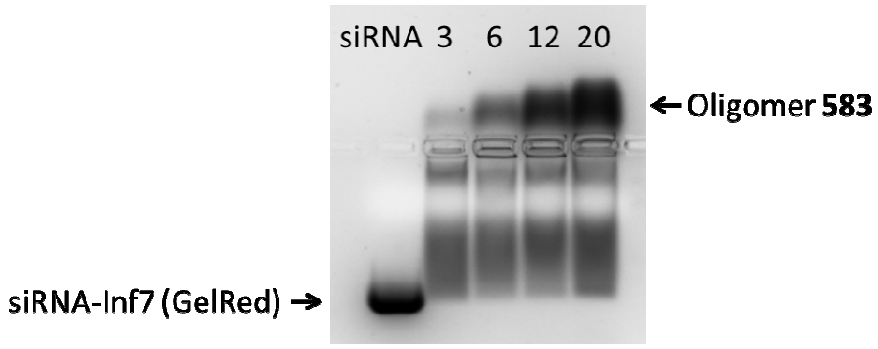


Figure S 6. Gel retardation assay using Alexa488 labeled polymer **583**. Polyplex formation was performed using siRNA-Inf7 in combination with Alexa488 labeled oligomer. Numbers (3-20) indicate N/P mixing ratios. siRNA is stained by GelRed stain, oligomer **583** is visible by Alexa fluorescence (not GelRed fluorescence). Loading of high amounts of polyplexes onto the agarose gel (5 µg siRNA) and long exposure time were required for detection of free oligomer. Free oligomer has the same migration behaviour as indicated by the arrow (determined in a separate gel, not shown here). Note that siRNA polyplexes which do not enter the gel are not visible. The smear of GelRed stained siRNA (lanes 3 -20) represents very minor partly complexed siRNA amounts visible only at this high 5 µg siRNA concentration.

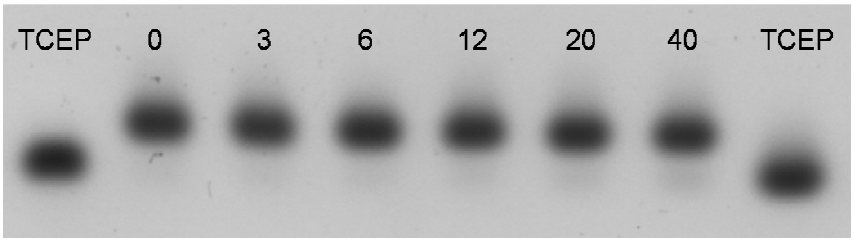


Figure S 7. Agarose gel shift assay of siRNA-Inf7 after incubation with free thiol groups. 200 ng siRNA-Inf7 was mixed as described under 'Polyplex formation' using free cysteine instead of polymer. Numbers (3-40) indicate the theoretical N/P ratio that would lead to the same amount of cysteines (75 pmol – 1 nmol) as used in the assay. TCEP: control, siRNA-Inf7 after reduction with TCEP, releasing unconjugated siRNA.

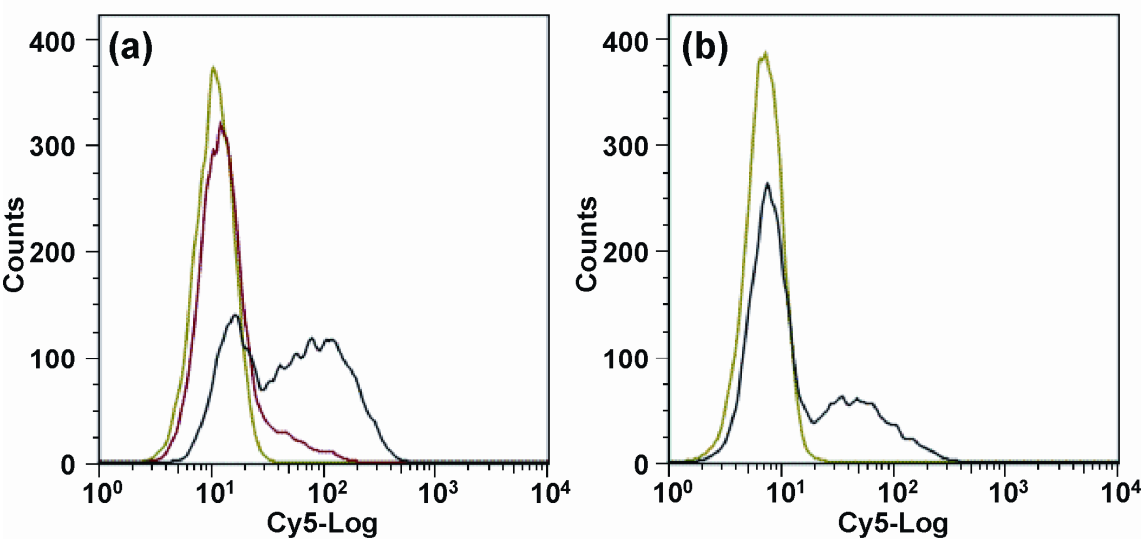


Figure S 8. FACS analysis of the receptor mediated cell uptake of FolA-PEG_{24}-K(Stp_4-C)$_2$ (**356**) polyplexes (N/P 16). A: cell association assay on ice. KB cells were incubated on ice with FolA-PEG_{24}-K(Stp_4-C)$_2$ without (black line) and with (red line) preincubation with free folic acid saturated medium. Untransfected control cells: green line. B: Neuro2A cells having a low folic acid receptor level, were transfected using functional FolA-PEG_{24}-K(Stp_4-C)$_2$ (dark line). Control cells: green line.

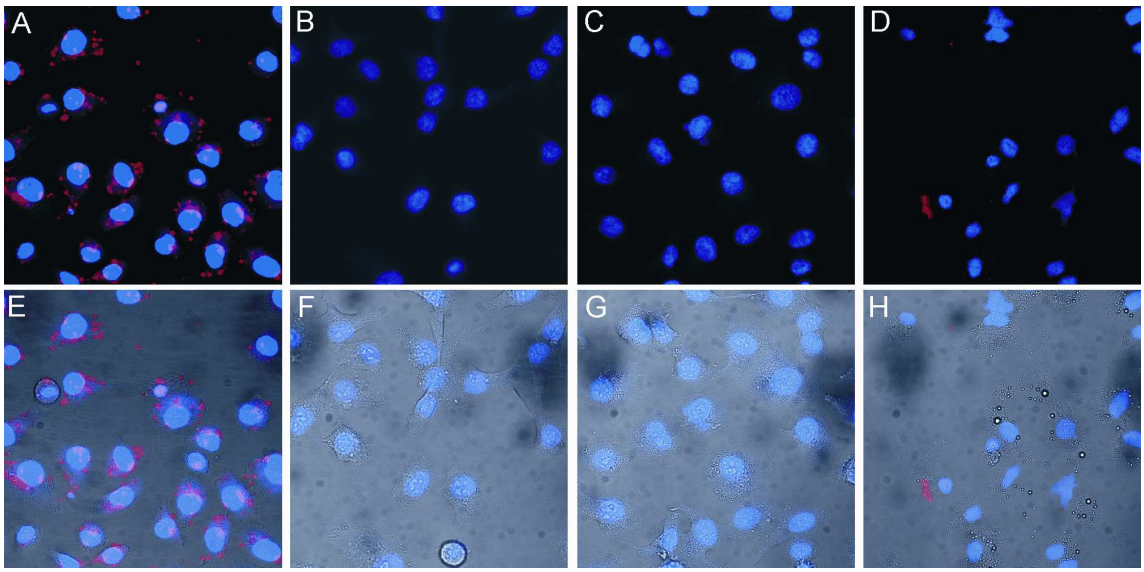


Figure S 9. Fluorescence microscopic pictures of the internalization of FolA-PEG_{24}-K(Stp_4-C)$_2$ polyplexes. A-D: overlay of DAPI and Cy5 channel; E-H: overlay of DAPI, Cy5 and transmission channel. A,E: KB cell transfected with FolA-PEG_{24}-K(Stp_4-C)$_2$ (**356**); B,F: KB cells transfected with FolA-PEG_{24}-K(Stp_4-S)$_2$ (**420**); C,G: KB cells transfected with A-PEG_{24}-K(Stp_4-C)$_2$ (**188**); D,H: Neuro2A cells transfected with FolA-PEG_{24}-K(Stp_4-C)$_2$ (**356**). For all transfection Cy5-siAHA1-Inf7 was used.

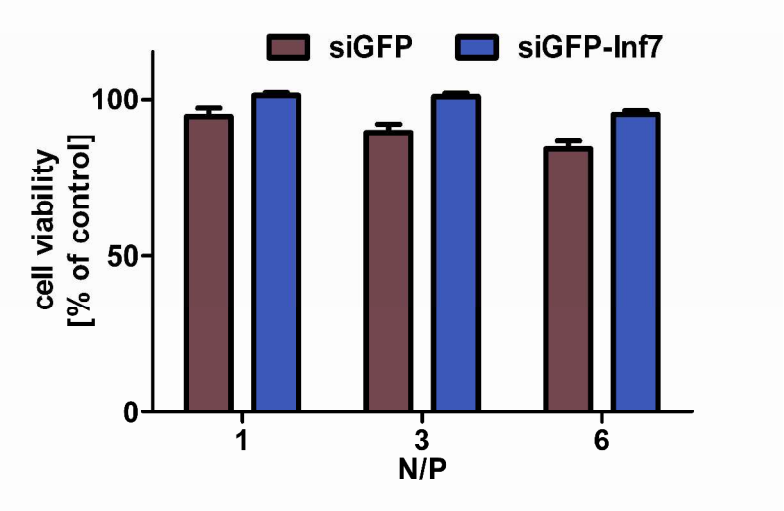


Figure S 10. Cell viability of cells after treatment with conjugated or unconjugated siRNA. Neuro2A/eGFPLuc cells were transfected with 400 nM siRNA-Inf7 (blue bars) or 400 nM unmodified siRNA (purple bars) using a functional polycationic carrier at indicated N/P ratios. Cell viability, determined via MTT assay is shown as percentage of untreated cells.

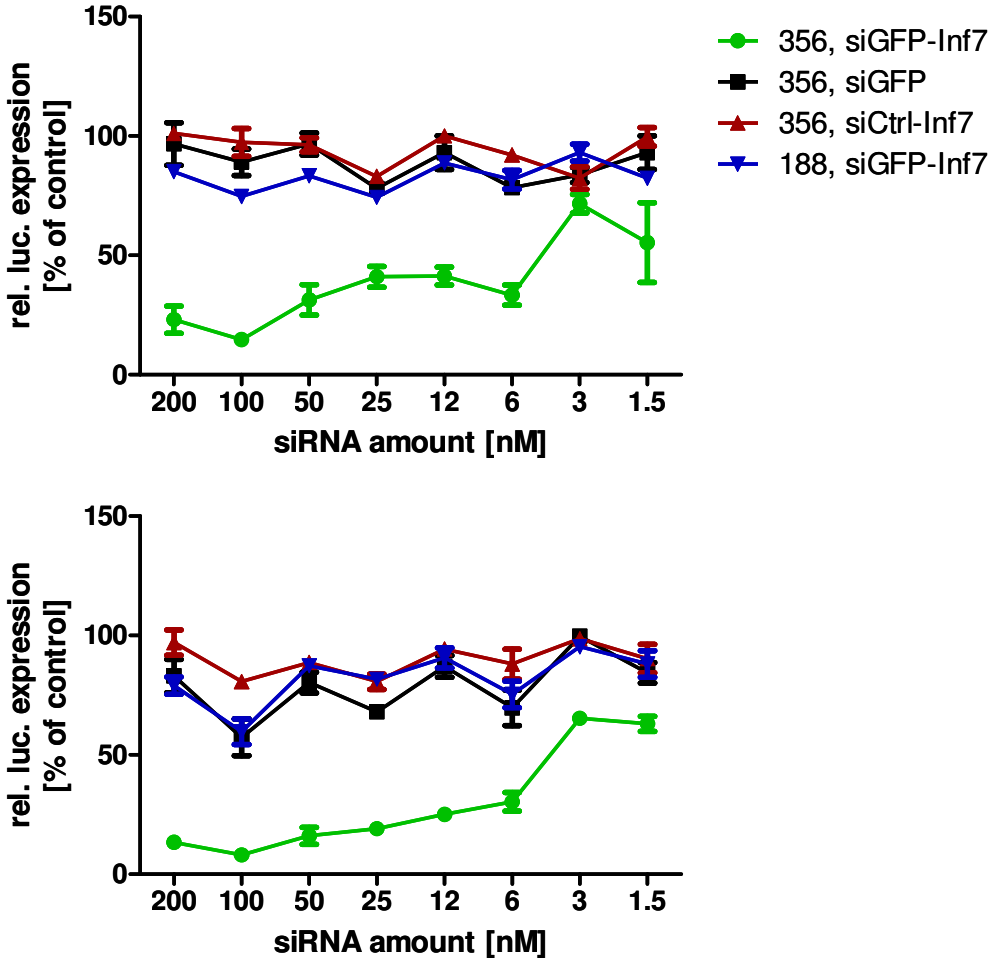


Figure S 11. Dose titration of polyplexes. Transfection were performed as described under materials and methods using oligomers **356** and **188** in combination with siGFP and siCtrl with or without conjugated Inf7. Starting with 200 nM siRNA and an N/P ratio of 12 (upper panel) or 20 (lower panel), the siRNA amount was stepwise reduced, keeping the oligomer amount constant.

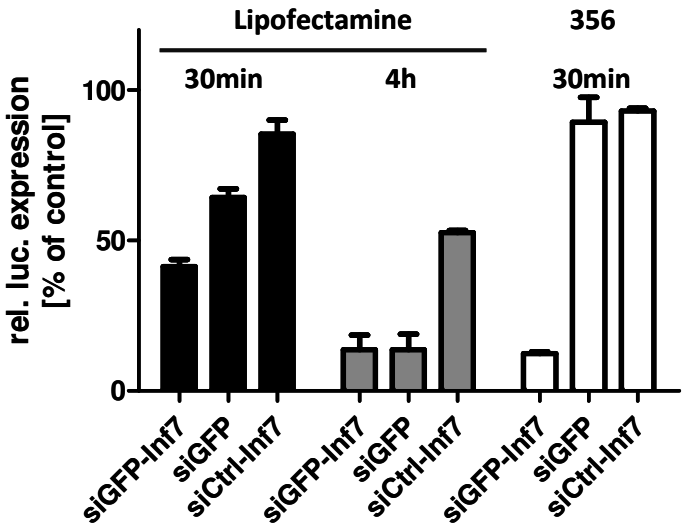


Figure S 12. Silencing efficiency of oligomer **356** (N/P 12) in comparison to lipofectamine. Lipofectamine was transfected with FCS free medium using 4h incubation time as described by the manufacturer, or 30min, as used for the targeted structures. Note that also gene silencing by lipofectamine at 30 min was improved by incorporation of Inf7-siRNA.

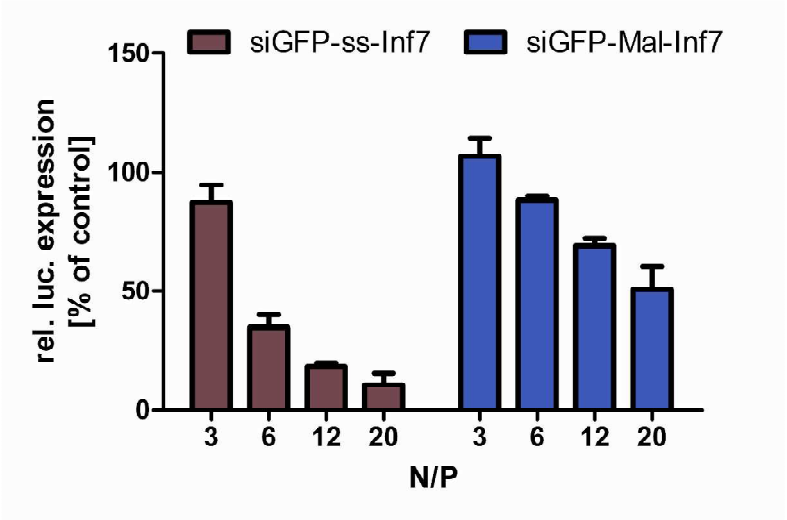
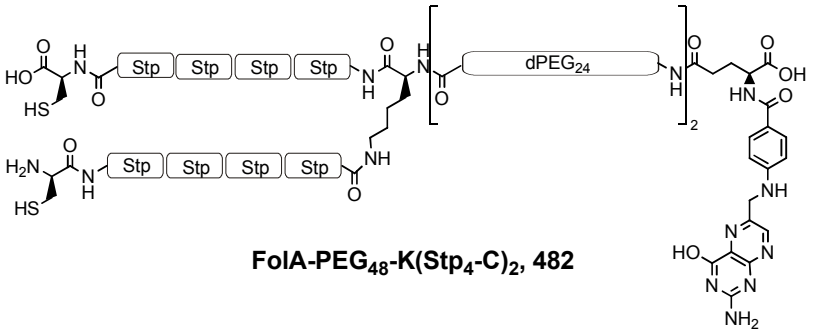


Figure S 13. Comparison of silencing efficiency of siRNA-Inf7 using a reducible or non reducible linker. siGFP-ss-Inf7, having a reducible disulfide bond or siGFP-Mal-Inf7, with a non reducible maleimide bond were used in combination with FolA-PEG_{24}-K(Stp_4-C)$_2$ (**356**) to transfect KB cells stably expressing eGFP-Luciferase. Reporter gene knockdown is shown as percentage of untreated cells.



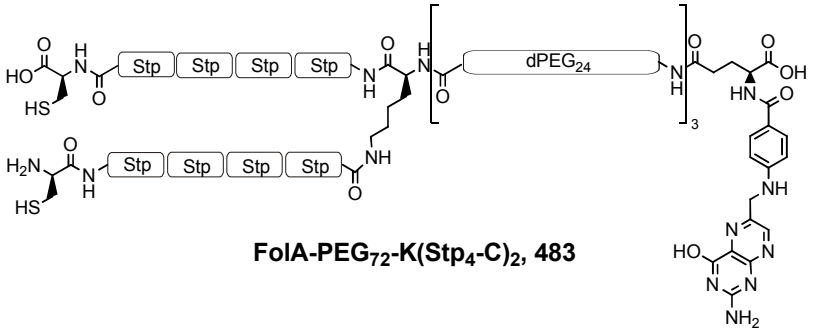


Figure S 14. Structure of polycationic oligomer with two (**482**) or three (**483**) repeats of $dPEG_{24}$.

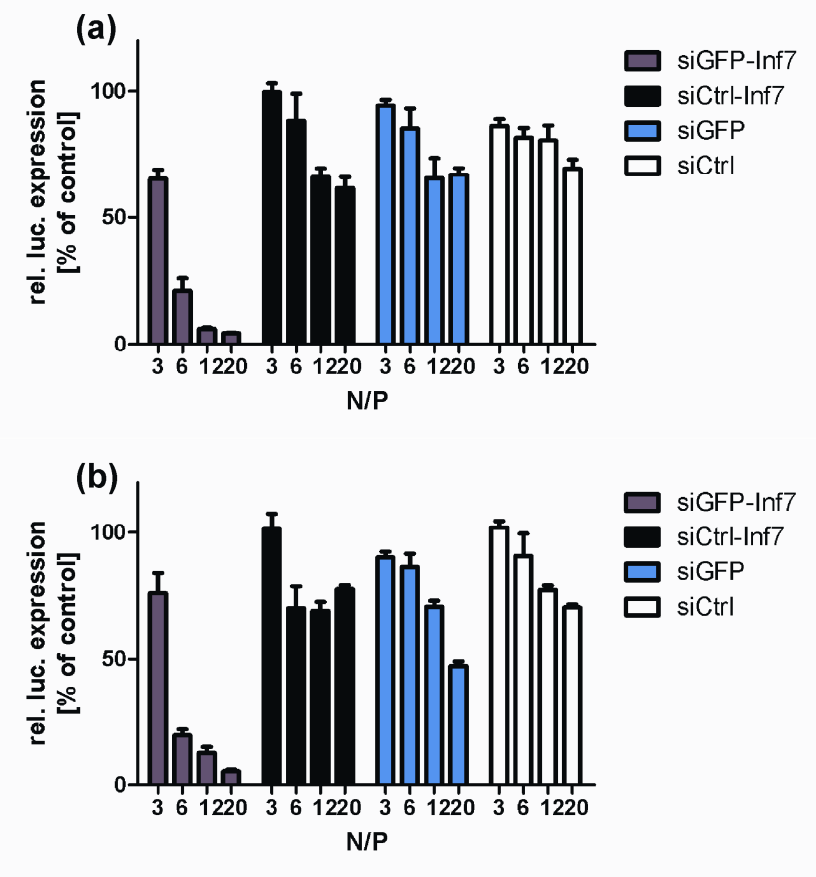


Figure S 15. Silencing efficiency using carriers with elongated PEG chains. KB cells stably expressing eGFP-Luciferase were transfected using (A) FolA-PEG_{48}-$K(Stp_4\text{-}C)_2$ (**482**) or (B): FolA-PEG_{72}-$K(Stp_4\text{-}C)_2$ (**483**).

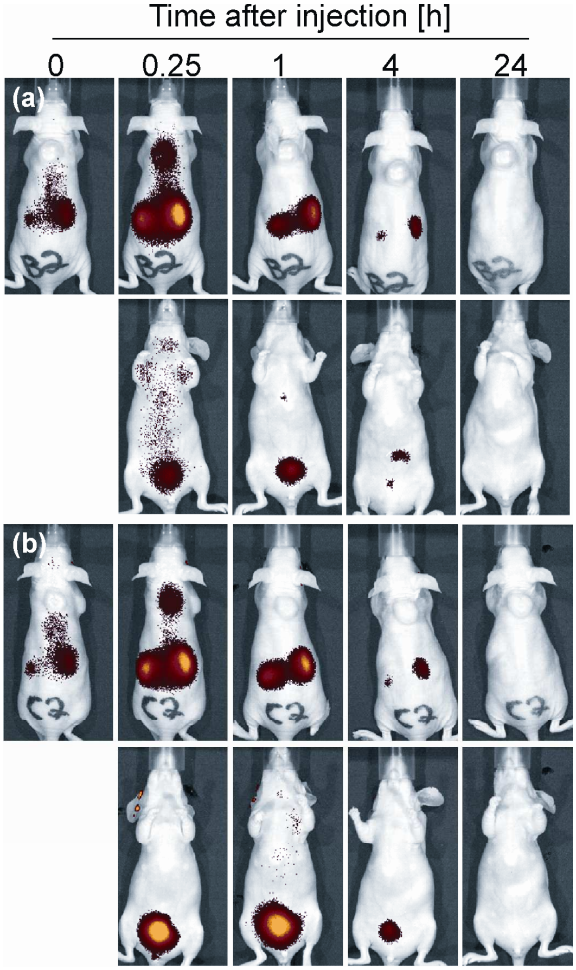


Figure S 16. Time dependent distribution of the polyplexes during the first 24 h after intravenous injection using (A) FolA-PEG_{48}-K(Stp_4-C)$_2$ (**482**) or (B) FolA-PEG_{72}-K(Stp_4-C)$_2$ (**483**). Upper panel: ventral position, lower panel: dorsal position.

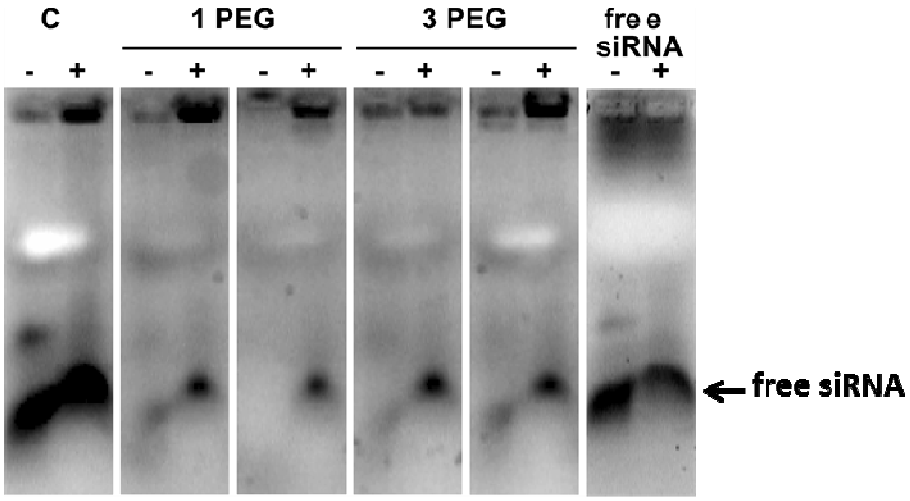


Figure S 17. Detection of free siRNA and polyplexes in urine samples of mice after intravenous injection of siRNA polyplexes or free siRNA. Urine of each two mice treated with siRNA polyplexes using FolA-PEG$_{24}$-K(Stp$_4$-C)$_2$ **356** ('1 PEG') , FolA-PEG$_{72}$-K(Stp$_4$-C)$_2$ **483** ('3 PEG') or treated with uncomplexed siRNA ('free siRNA') was collected 4 h after application and analyzed by gel electrophoresis. To distinguish between siRNA still present in polyplexes and free siRNA, urine samples were analyzed directly (-) or after dissociation of polyplexes by using heparine and TCEP (+). C: control (free siRNA standard). Note that free siRNA is visible in urine samples of mice treated with free siRNA, and in polyplex treated mice only after treatment with heparin and TCEP treatment.

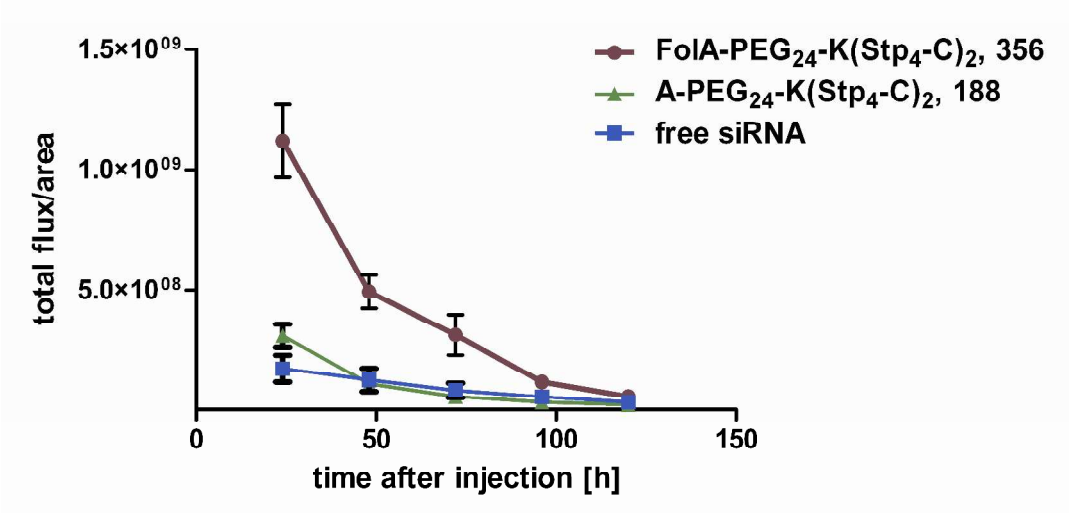


Figure S 18. Quantitative analysis of the tumor retention of siRNA after intratumoral injection of free siRNA or in combination with FolA-PEG$_{24}$-K(Stp$_4$-C)$_2$ (**356**) or A-PEG$_{24}$-K(Stp$_4$-C)$_2$ (**188**).

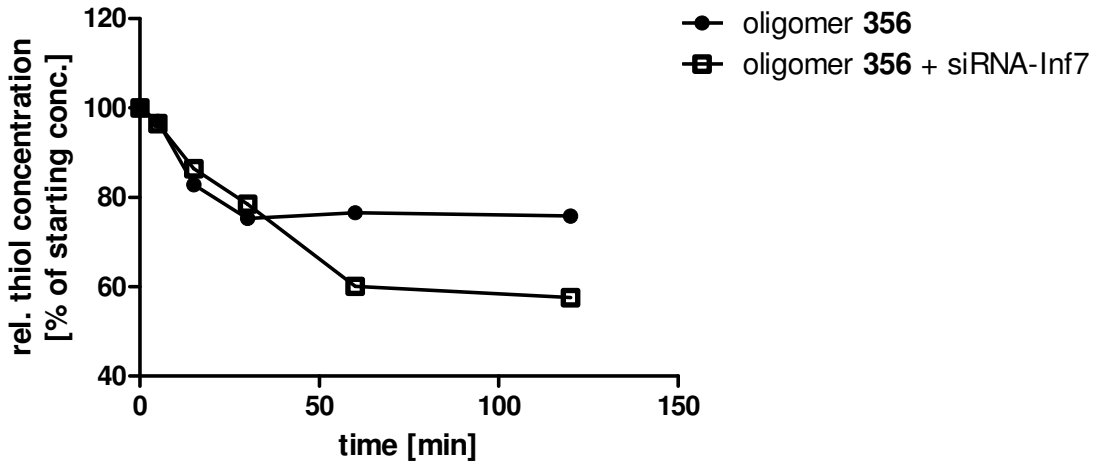


Figure S 19. Reduction of free thiol groups in presents or absence of siRNA-Inf7 during polyplex formation. Polyplexes at N/P 12 were formed as described in Methods, or the same amount of free oligomer **356** was incubated for 2 h. At indicated time points the amount of free thiol groups was determined using the Ellman's assay.* Reduction of free thiol groups was used as indicator of disulfide formation.

Consistent with previous observations (Schaffert et al 2011,[27] Fröhlich et al 2012, [29]), the presence of nucleic acid as template results in enhanced disulfide formation of polyplex bound oligomer. Free oligomer or unbound oligomer (about 80% at N/P 16) do not show the observed enhanced oxidation.

* Ellman's assay: 0.4 mg of DTNB dissolved in 1 mL of the corresponding Ellman's buffer (0.2 M Na2HPO4 with 1 mM EDTA at pH 8.0) was used as stock solution. For UV/VIS absorption measurement Ellman's stock diluted 1 : 10 in Ellman's buffer was taken as blank. Samples were diluted in Ellman's buffer and 10% (v/v) of the stock solution. After 15 min at 37 °C the solutions were measured at 412 nm. Concentrations of the free thiols at 0 min were set to 100%. For the determination of free thiol groups over time, the polymer or polyplex samples were processed as described above.

Table S 1. Hydrodynamic diameter (d_h) of polyplexes formed using Alexa488 labeled polycation (**583**) in combination with siRNA-Inf7 at N/P 6, determined by fluorescent correlation spectroscopy.

	Population 1		Population 2	
N/P	**d_h [nm]**	**% of total**	**d_h [nm]**	**% of total**
0 (free polymer)	2.3	100%	---	---
6	2.2	63%	7.4	37%

Table S 2. Zeta potential of PEGylated and non PEGylated polyplexes.

	Zeta potential [mV]	
N/P	**A-PEG_{24}-$K(Stp_4\text{-}C)_2$, 188**	**A-$K(Stp_4\text{-}C)_2$, 202**
3	0.0 (±1.6)	9.3 (±1.8)
6	0.0 (±1.7)	12.7 (±1.9)
12	0.0 (±2.4)	14.2 (±1.7)
20	0.0 (±2.4)	12.9 (±1.9)
40	0.1 (±2.7)	---

Table S 3. Hydrodynamic diameter (d_h) of polyplexes formed using Cy 5 labeled siRNA-Inf7 in combination with FolA-PEG_{48}-K(Stp_4-C)$_2$ (**482**) or FolA-PEG_{72}-K(Stp_4-C)$_2$ (**483**) at N/P 16, determined by fluorescent correlation spectroscopy.

Carrier	d_h [nm]
FolA-PEG_{48}-K(Stp_4-C)$_2$, **482**	6.4
FolA-PEG_{72}-K(Stp_4-C)$_2$, **483**	8.8

Table S 4. Diameter of particles formed with siRNA and non targeted non pegylated polycation **202**.

N/P	A-K(Stp4-C)2, 202
3	59 (±4)
6	186 (±13)
12	90 (±6)
20	433 (±45)
40	919 (±290)

Table S 5. Structure of used polycationic oligomers.

ID	Structure
46	C-Stp_3-C-K$(OleA)_2$
188	A-PEG_{24}-K$(Stp_4$-C$)_2$
202	A-K$(Stp_4$-C$)_2$
356	C-Stp_4-K$(Stp_4$-C)-PEG_{24}-FolA
420	S-Stp_4-K$(Stp_4$-S)-PEG_{24}-FolA
482	C-Stp_4-K$(Stp_4$-C)-$(PEG_{24})_2$-FolA
483	C-Stp_4-K$(Stp_4$-C)-$(PEG_{24})_3$-FolA
583	C-Stp_4-K$(Stp_4$-C)-PEG_{24}-Alexa488