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Efficient *in vitro* and *in vivo* transfection of self-amplifying mRNA with linear poly(propylenimine) and poly(ethylenimine-propylenimine) random copolymers as non-viral carriers

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Experimental

MRNA Synthesis and silica purification.

Modified non-amplifying mRNA encoding luciferase was produced in a similar way as saRNA, using the pTK305 plasmid. This pTK305 plasmid is the result of the deletion of the viral non-structural proteins from the pTK160 plasmid. During IVT the uridine triphosphate was replaced by the N_1-methylpseudouridine (m1Ψ) analogue. Following IVT, the pDNA template was degraded, the mRNA was purified, the concentration and quality were determined and the mRNA was evaluated during gel electrophoresis according to previously described protocols (see **saRNA synthesis and silica purification**).

Polymer-based nanoparticle (polyplex) Formulation.

A polymer solution was established by dissolving the polymers in 20 mM NaOAc (pH 5.2) at a concentration of 2 mg/mL. this solution was aliquoted and stored at -20 °C. Freeze-thaw cycles were limited to five. SaRNA-polyplexes were formulated in one step by adding an equal volume of the saRNA solution to the polymer solution and pipetting 8 times up and down (both solutions diluted in either 20 mM NaOAc, pH 5.2 or in 20 mM HEPES, pH 7.4), followed by a 30 minute incubation at room temperature. The saRNA concentration was kept constant (25 ng saRNA/µL polyplex) and a polymer dilution series was constructed according to the desired N/P ratio's (N: mole of cationic nitrogen atoms in the polymer structure; and P: mole of anionic phosphates in the saRNA molecule). For *in vivo* administration, at the day of injection the saRNA solution and the polymer solutions were formulated in a 20 mM HEPES buffered glucose (5% w/v) (HBG) solution according to the desired N/P ratio.

Cell Culture, *In Vitro* Transfection of saRNA-Polyplexes and Bioluminescent imaging.

HeLa cells (a kind gift from prof. dr. Daisy Vanrompay), C2C12 cells and HepG2 cells (ATCC, Virginia, US) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, Waltham, Massachusetts, USA), containing 10% heat inactivated fetal bovine serum (FBS) (Biowest, Nuaillé, France) and 1% penicillin/streptomycin (P/S) (Thermo Fisher Scientific, Waltham, Massachusetts, USA). One day before transfection $5*10^4$/500 µL HeLa cells were seeded in each well of 24-well plates to obtain 70-80% confluency. Cells were washed with 1x DPBS and transfected with saRNA-polyplexes (500 ng saRNA) in Opti-MEM or full DMEM (i.e., with 10%FBS and 1% P/S). After 4 hours the medium containing the complexes was either replaced or not by full DMEM medium. Twenty-four hours after transfection, the medium was replaced, cells were washed with 1x DPBS and trypsinized. During the 10 minute trypsinization, cells were incubated at 37 °C and afterwards neutralized with full DMEM medium. Part of the neutralized cell suspension (36%) was transferred to a black 96-well plate. A D-luciferin solution (50 mg/ml; 1/10 dilution) was added to each well with transfected cells, and left to incubate at 37 °C for 10 minutes. Subsequently, the bioluminescent signal was measured using the IVIS lumina III (Xenogen Corporation, Alameda, California, US).

Particle Size and Zeta-Potential Analysis.

The particle size (hydrodynamic diameter (DH), Z-average) and zeta potential (ZP) of the saRNA-polylexes were measured using the Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). For the size measurements, 400 µL samples were made in either NaOAc buffer (pH 5.2, 20 mM) or HEPES buffer (pH 7.4, 20 nM) (25 ng/µL saRNA) and loaded into a disposable semi-micro polystyrene cuvette (BRAND, Wertheim, Germany). These measurements are based on the principle of Dynamic Light Scattering (DLS) using a laser beam of 632.8 nm and a detector at a scattering angle of 93°.

ZP measurements were performed with samples at a concentration of 10 ng/µL saRNA loaded in a folded capillary cell (polystyrene) cuvette (Malvern Panalytical, Malvern, UK). Both size and ZP measurements were conducted at 25 °C, with the following settings: dispersant 'water', with viscosity 0.8872 cP, refractive index of 1.33, and dielectric constant of 78.5.

The size of the saRNA-polyplexes was also determined with a NanoSight NS300 (Malvern Panalytical, Malvern, UK). To that end the saRNA-polyplex samples were diluted with NaOAc (20 mM, pH 5.2) or HEPES (20 mM, pH 7.4) to concentrations ranging from ~ 10 – 40 particles/frame or ~ 2 – 7 * e11 particles/mL, corresponding to concentrations of 0.25 – 0.5 µg/mL saRNA. The samples were loaded with a syringe pump (speed 50), irradiated by a 488-nm laser and visualised by a high-sensitivity sCMOS camera. The resulting experiment recordings were analysed using Nanoparticle Tracking Analysis (NTA')[1] 3.4 Build 3.4.003 software (Malvern Instruments) after capture in script control mode (3 recordings of 60 s per measurement). In total ~ 1,500 frames were created per sample to determine the size of the saRNA-polyplexes.

Electrophoretic Mobility Shift Assay (EMSA), Heparin Competition Assay and RNase A Protection Assay.

An *Electrophoretic Mobility Shift Assay (EMSA)* or *Gel Retardation Assay* was performed to assess the complexation status of the saRNA-polyplexes. To that end, the saRNA-polyplexes were formulated in NaOAc buffer as described earlier (see Polyplex Formulation). Next, 1% (w/v) agarose gels containing 1% (v/v) bleach (NaClO) were made in Tris-Borate-EDTA (TBE) buffer (0.045 M Tris-borate and 0.001 M EDTA, pH 8.3). After homogenization, the mixtures were heated to melt the agarose and then cooled before adding ethidium bromide (EtBr) (10 mg/mL) to a final 0,02% concentration. Next, the gels were poured into the mold and allowed to cool and set for at least 15 minutes at room temperature. Before loading the saRNA-polyplexes (500 ng saRNA), (10X) loading buffer (50% (w/v) sucrose in 20 mM HEPES buffer pH 7.4) was added to allow for RNA sedimentation in the wells. RNase-free water was added to all samples and to obtain identical total sample volumes. A 1kb+ DNA-ladder (Thermo Fisher Scientific,

Massachusetts, USA) and/or free saRNA, made with the same stock solution, were also loaded on the gels to identify the location of the free saRNA band. Absence of this band represents retention of the saRNA by the polymer. After loading the gel was run at 100 V for 30-35 minutes. Afterwards, the gel was exposed to UV light and images were taken and analysed with Fiji software.
To assess the strength of the saRNA-polyplexes, a *Heparin Competition Assay* was performed. To that end, 20 µl saRNA-polyplexes containing 500 ng saRNA were incubated during 1 hour at 37 °C with 1 µL of a 80 mg/mL heparin sodium (HS) dissolved in RNase-free H2O. After incubation the samples were analysed by agarose gel electrophoresis as described above.
The level of protection of the saRNA against nuclease degradation was investigated using in-house *RNase A Protection Assay*. During this assay saRNA and saRNA-polyplexes were exposed to RNase A (1 µL, 1 ng/µL) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 30 minutes at 37 °C, before or after the addition of 80 µg HS. In this experiment proteinase K (275 ng – 550 ng – 1.1 µg and 2.2 µg) (20 mg/mL; Thermo Fisher, Waltham, Massachusetts, USA), 1% (w/v) SDS (sodium dodecyl sulfate) dissolved in RNase-free water and 0.25 mM EDTA (ethylenediaminetetraacetic acid) were tested (alone or in combination) as RNase A inhibitor. Subsequently, the samples were loaded on an agarose gel, run and analysed as described above.

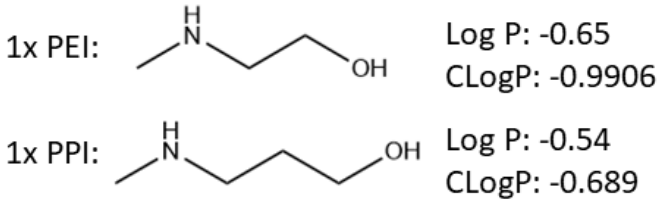


Fig. S1.: Chemical Properties of hypothetical L-PEI and L-PPI homopolymers (DP 1). The focus here is on the Log P value, which indicates the hydrophobicity of a chemical structure. Compared to L-PEI, L-PPI has a log P value which is less negative or higher. Therefore, L-PPI has a higher affinity for n-octanol which indicates that it's more hydrophobic/lipophilic compared to L-PEI.

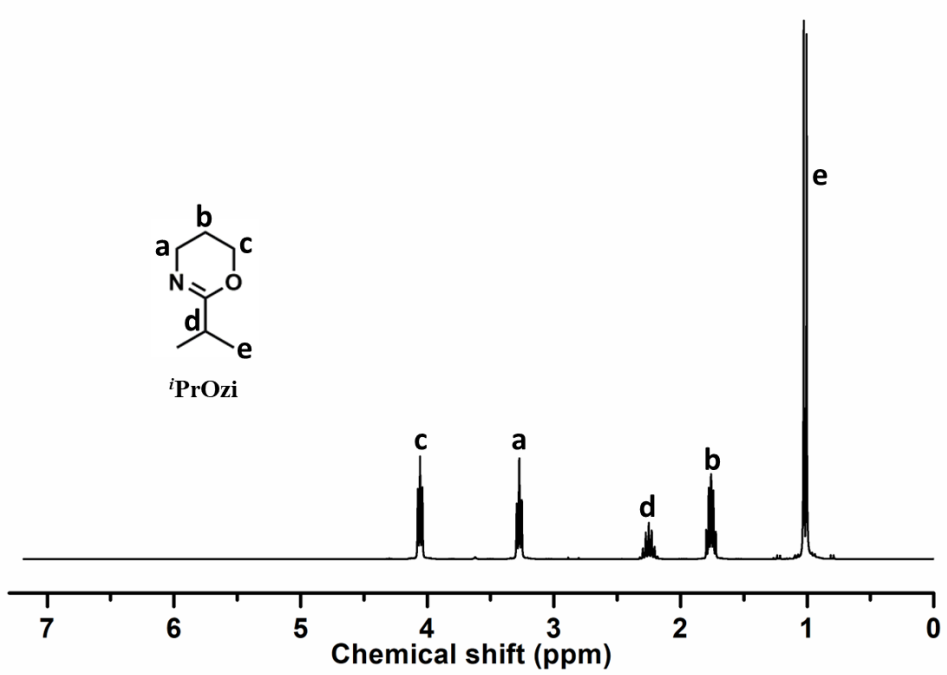


Fig. S2.: ^{1}H NMR spectrum of ^{i}PrOzi monomer in CDCl3.

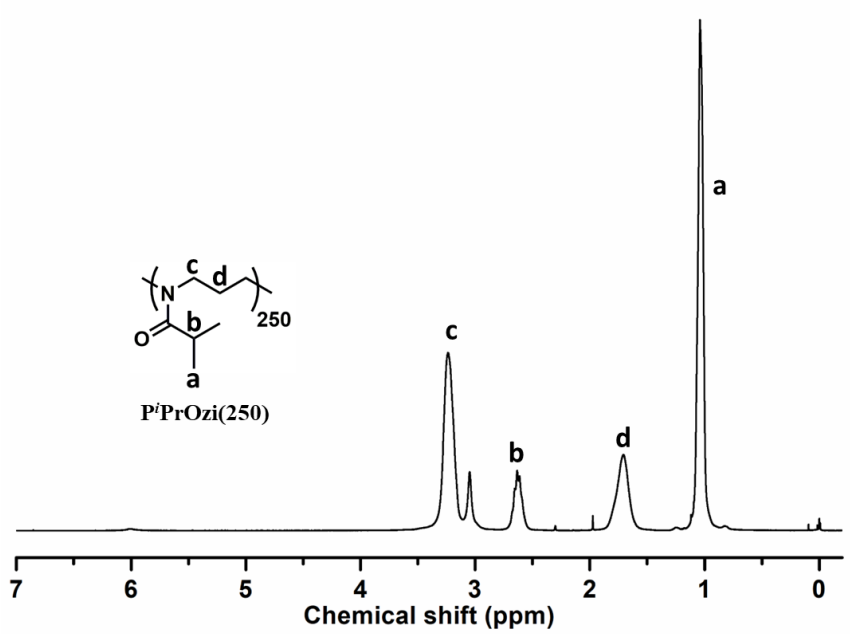


Fig. S3.: ^{1}H NMR spectrum of PiPrOzi(DP250) homopolymer in $CDCl_3$.

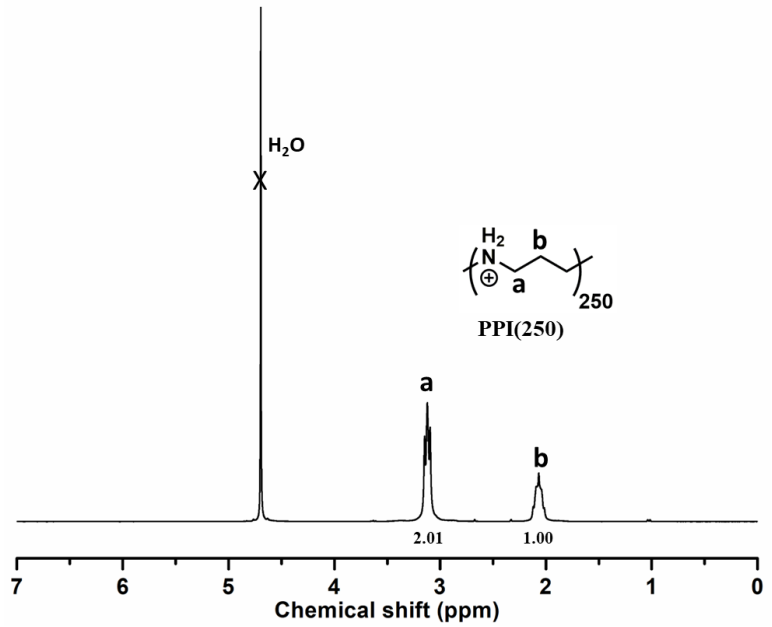


Fig. S4.: ^{1}H NMR spectrum of PPI(DP250) in D_2O.

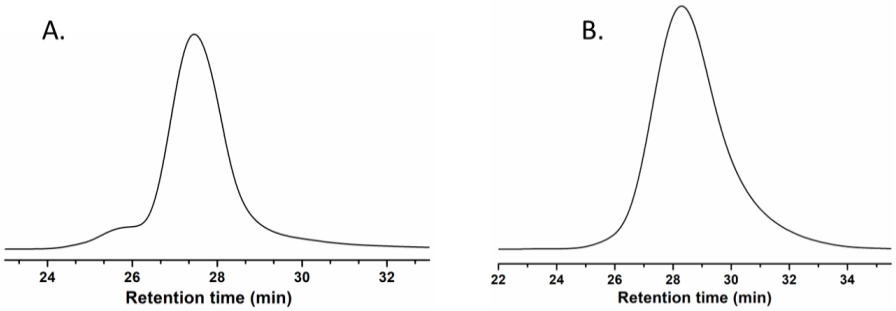


Fig. S5.: SEC traces of P^{i}PrOzi(DP250) in DMA (A) and PPI(DP250) (B) in aqueous medium.

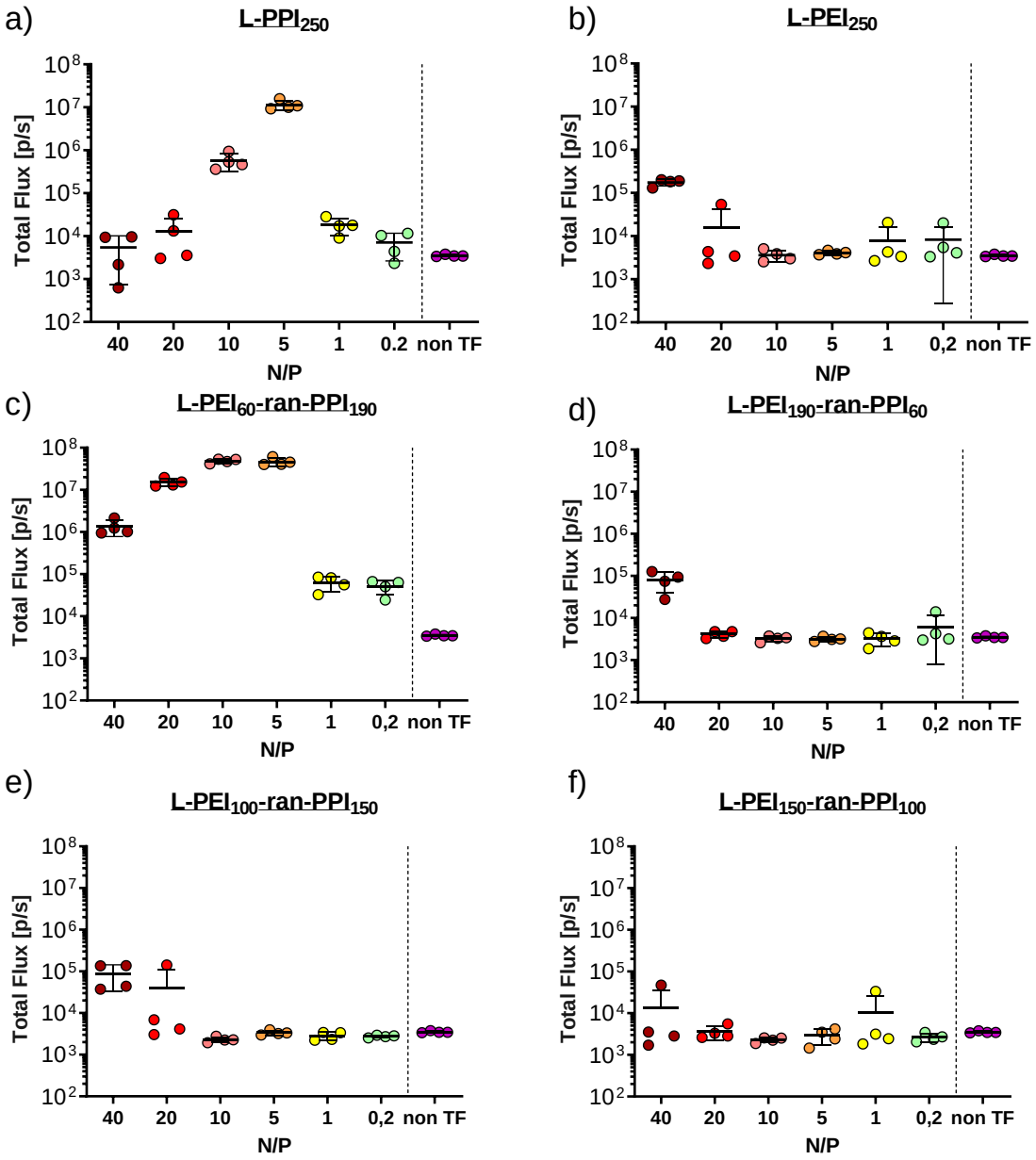


Fig. S6.: Bioluminescent signals of *in vitro* saRNA-polyplex transfections with L-PEIx-ran-PPIy (DP polymers) in HeLa cells. Cells (in OptiMEM) were transfected with L-PEI$_{60}$-*ran*-PPI$_{190}$ -based saRNA-polyplexes (N/P 5) containing 500 ng saRNA per well (24 well-plate). Polyplexes were formulated in NaOAc (20 mM, pH 5.2). The graph shows data 24 hours after transfection, medium was not changed. Data was obtained with the IVIS Lumina III (Total Flux [p/s]). Each circle represents one well of the 24 well-plate (N = 4).

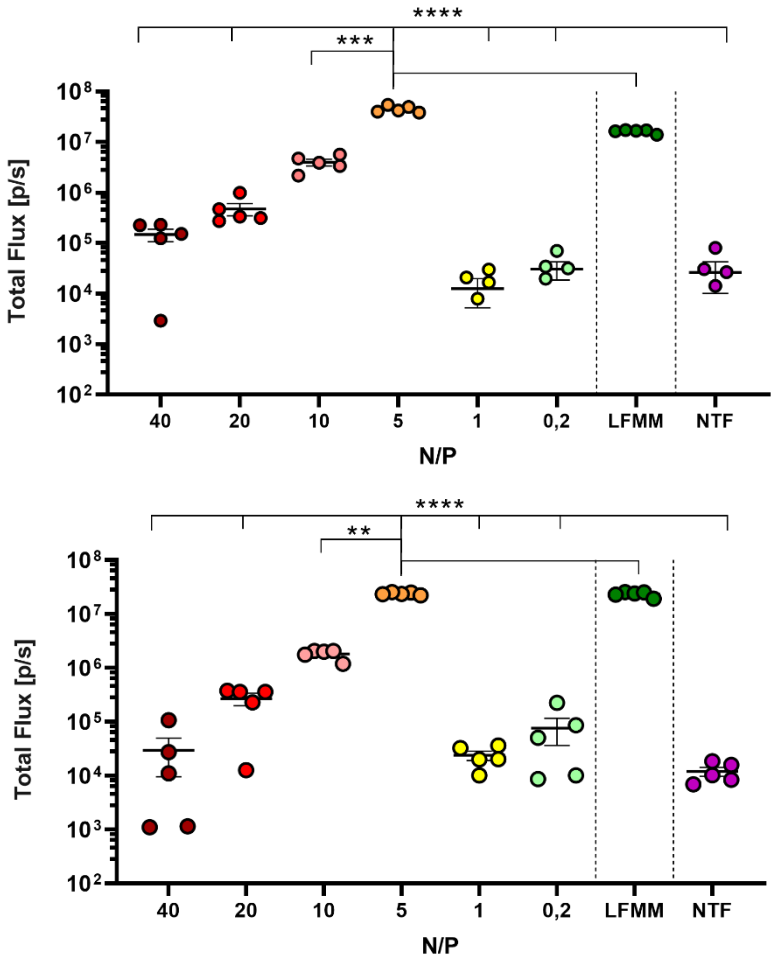


Fig. S7.: **Bioluminescent signals of *in vitro* saRNA-polyplex transfections with L-PEI$_{60}$-*ran*-PPI$_{190}$ in HeLa cells (repeat 1 and 2).** Cells (in OptiMEM) were transfected with L-PEI$_{60}$-*ran*-PPI$_{190}$ -based saRNA-polyplexes (N/P 5) containing 500 ng saRNA per well (24 well-plate). Polyplexes were formulated in NaOAc (20 mM, pH 5.2). The graph shows data 24 hours after transfection, medium was not changed. Data was obtained with the IVIS Lumina III (Total Flux [p/s]). Each circle represents one well of the 24 well-plate (N = 5), the line represents mean ± SEM. * indicates significance of $p \leq 0.05$, ** indicates significance of $p \leq 0.01$, *** indicates significance of $p \leq 0.001$, **** indicates significance of $p \leq 0.0001$. All conditions were compared to each other with one-way ANOVA, after log-transformation of the obtained data and testing for normality. Adjustment for multiple comparisons was performed using Tukey's multiple comparisons test. Abbreviations: NTF = non-transfected cells.

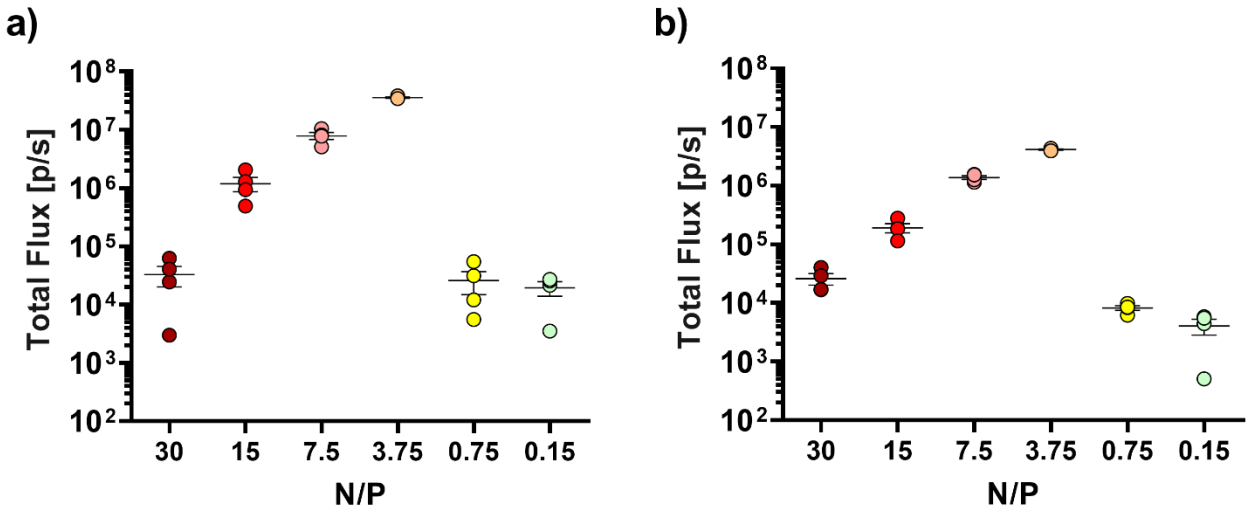


Fig. S8: Bioluminescent signals of in vitro RNA-polyplex transfections with L-PPI250 in HeLa cells. Cells were transfected with 500 ng RNA in a 24 well-plate. The RNA-polyplexes were composed of L-PPI250 in combination with luciferase-coding RNA, which was either self-amplifying mRNA (left, graph a) or non-replicating, N1mΨ-modified mRNA (right, graph b). Six N/P ratios were considered. The graphs show data 24 hours after transfection, without changing of the medium. The data were obtained with the IVIS Lumina II (Total Flux [p/s]).

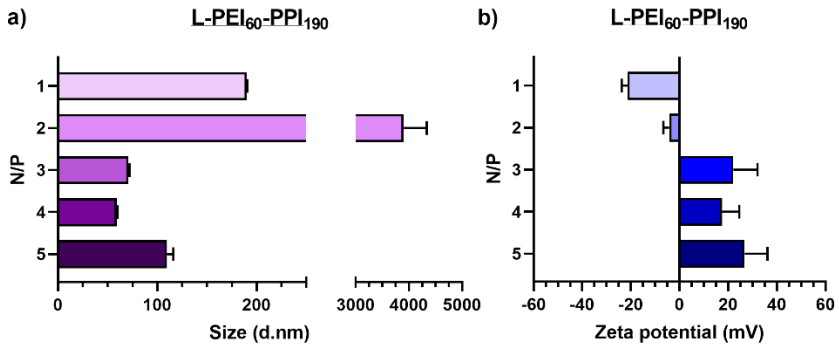


Fig. S9.: Size and zeta potential of the saRNA-based polyplexes, with L-PEI$_{60}$-*ran*-PPI$_{190}$ in NaOAC buffer. SaRNA-polyplexes are composed of L-PEI$_{60}$-*ran*-PPI$_{190}$ and saRNA with N/P ratios of 1, 2, 3, 4 and 5. The Z-average sizes data ((graphs a), mean and SD) are based on intensity distributions of the dynamic light scattering measurements. The zeta potential data (graphs b)) are based on laser doppler velocimetry measurements, both obtained with the ZetaSizer Nano-ZS.

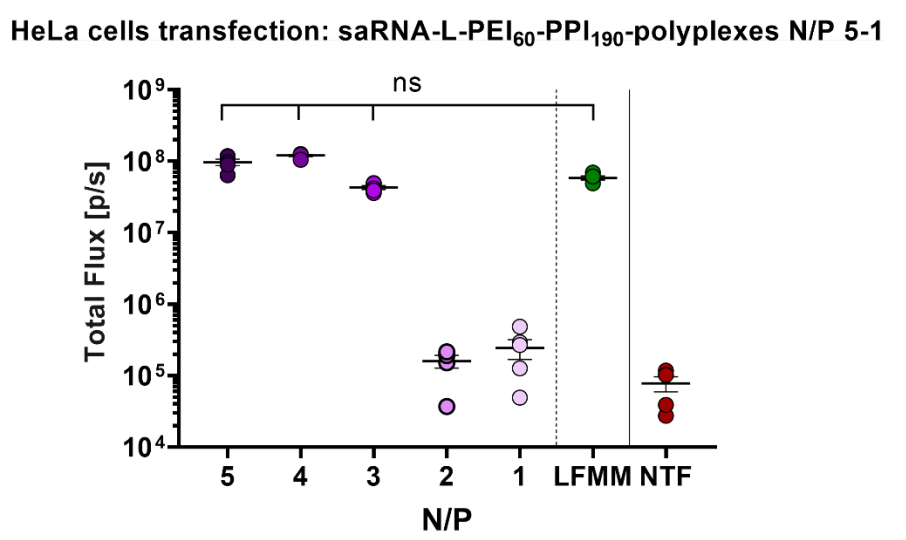
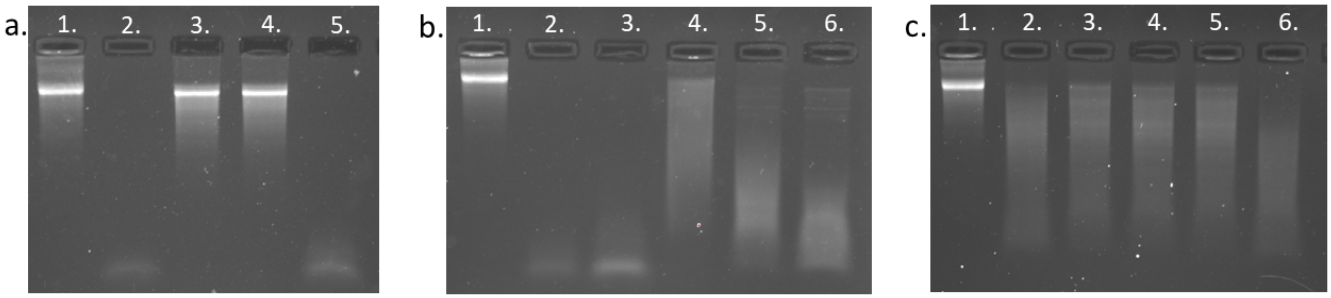


Fig. S10.: Bioluminescent signals of *in vitro* saRNA-polyplex transfections with L-PEI$_{60}$-*ran*-PPI$_{190}$ in HeLa cells. Cells (in OptiMEM) were transfected with L-PEI$_{60}$-*ran*-PPI$_{190}$-based saRNA-polyplexes containing 500 ng saRNA per well (24 well-plate). From left to right, the N/P ratios are: 5, 4, 3, 2 and 1. SaRNA-polyplexes were formulated in NaOAc (20 mM, pH 5.2). The graph shows data 24 hours after transfection, medium was not changed. Data was obtained with the IVIS Lumina III (Total Flux [p/s]). Each circle represents one well of the 24 well-plate (N = 4), the line represents mean ± SEM. * indicates significance of $p \leq 0.05$, ** indicates significance of $p \leq 0.01$, *** indicates significance of $p \leq 0.001$, **** indicates significance of $p \leq 0.0001$. All conditions were compared to each other with one-way ANOVA, after log-transformation of the obtained data and testing for normality. Adjustment for multiple comparisons was performed using Tukey's multiple comparisons test. Abbreviations: LFMM = Lipofectamine MessengerMax™, NTF = non-transfected cells.



Panel a.:

1. RNA only (40 min at room temperature (RT))
2. RNA + 1 ng RNase A (30 min at 37 °C) + 275 ng proteinase K (PK) (10 min at 55 °C)
3. RNA + RNase-free H_2O 30 min at 37 °C + 10 min at 55 °C,
4. RNA + 30 min at RT + 10 min @ 55 °C
5. RNA + 275 ng PK (10 min at 55 °C) + 1 ng/µL RNase A (30 min at 37 °C)

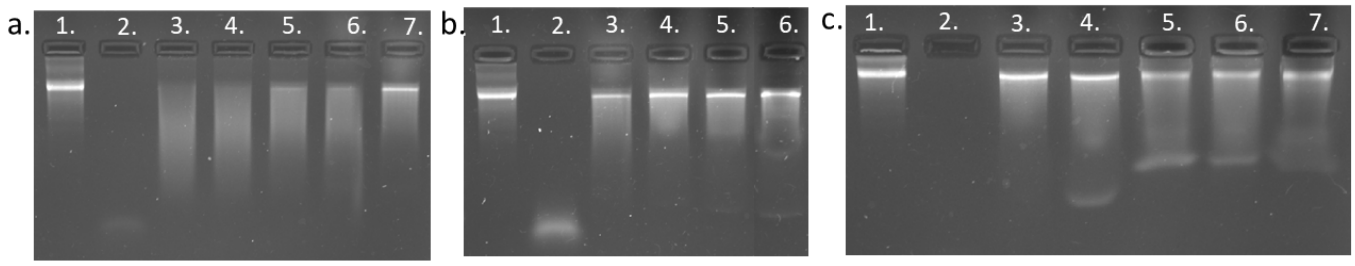
Panel b.:

1. RNA only (at RT)
2. RNA + 1 ng RNase A, 30 min at 37 °C
3. RNA + 275 ng PK (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
4. RNA + 1% SDS in RNase-free H_2O, 10 min at RT + 1 ng RNase A (30 min at 37 °C)
5. RNA + PK in 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
6. RNA + PK in 1% SDS and 0,25 mM EDTA (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)

Panel c.:

1. RNA only (30 min at 37 °C)
2. RNA + 1 ng RNase A (10 min at 55 °C)
3. RNA + 275 ng PK (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
4. RNA + 550 ng PK (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
5. RNA + 1,1 µg PK (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
6. RNA + 2,2 µg PK (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)

Fig. S11.: RNase A inhibition tests, part 1. The UV-pictures of the EtBr bleach agarose gels are shown. For each condition 500 ng saRNA was used. The detection limit of the gels is 10 ng saRNA. These tests failed to prevent saRNA degradation mediated by RNase A. PK: proteinase K (20 mg/mL), Panel a. and b.: 275 ng PK used alone, or in combination with 0.25 mM EDTA and/or SDS to incubate RNase A. Panel c.: 275 ng – 550 ng – 1.1 µg – 2.2 µg PK used.



Percentage: 100% 0% 82% 120% 100% 86% 100% 0% 129% 121% 103% 112% 75%

Panel a):

1. RNA only (30 min at 37 °C)
2. RNA + 1 ng RNase A (30 min at 37 °C) + 1 µL RNase-free H_2O (10 min at 55 °C)
3. RNA + 1 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
4. RNA + 2 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
5. RNA + 3 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
6. RNA + 4 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
7. RNA + 5 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)

Panel b):

1. RNA only (30 min at 37 °C)
2. RNA + 1 ng RNase A (30 min at 37 °C) + 1 µL RNase-free H_2O (10 min at 55 °C)
3. RNA + 5 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
4. RNA + 7,5 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
5. RNA + 10 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
6. RNA + 15 µL 1% SDS (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)

Panel c):

1. RNA only (30 min at 37 °C)
2. RNA + 1 ng RNase A (30 min at 37 °C)) + 1 µL RNase-free H_2O
3. RNA + 7,5 µL 1% SDS (in RNase-free H_2O) (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
4. RNA + 550 ng PK + 7,5 µL 1% SDS (in RNase-free H_2O) (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
5. RNA + 550 ng PK + 7,5 µL 1% SDS (in 0,25 M EDTA) (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
6. RNA + 550 ng PK + 5 µL 1% SDS (in RNase-free H_2O) (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)
7. RNA + 550 ng PK + 10 µL 1% SDS (in RNase-free H_2O) (10 min at 55 °C) + 1 ng RNase A (30 min at 37 °C)

Fig. S12.: RNase A inhibition tests, part 2. The UV-pictures of the EtBr bleach agarose gels are shown. For each condition 500 ng saRNA was used. The detection limit of the gels is 10 ng saRNA. These tests were successful. The densitometric analysis (Fiji) of the electrophoretic retardation of the polyplexes is shown below the pictures as percentages relative to the "saRNA only" signal from lane 1.

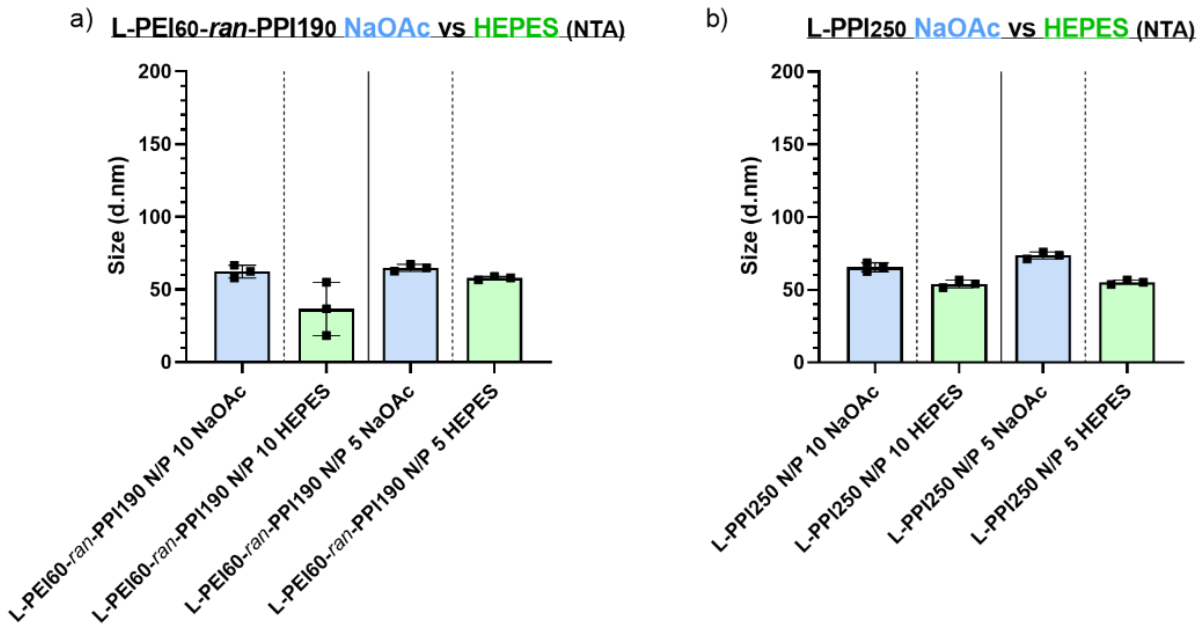


Fig. S13.: Size of the saRNA-based polyplexes, with L-PEI$_{60}$-*ran*-PPI$_{190}$ and L-PPI$_{250}$ (N/P 10 and 5) in NaOAC buffer (blue) versus in HEPES (green). Size data ((graphs a) and b), mode and SE) are based on intensity distributions of the nanoparticle tracking analysis measurements obtained with the NanoSight NS300.

	Size			
	DLS			
	L-PEI60-ran-PPI190 N/P 10 NaOAc	*L-PEI60-ran-PPI190 N/P 10 HEPES*	*L-PEI60-ran-PPI190 N/P 5 NaOAc*	*L-PEI60-ran-PPI190 N/P 5 HEPES*
	71,69	75,53	105,7	81,06
	71,85	73,95	108,4	77,87
	68,99	55,76	105,8	75,32
Average:	**70,84**	**68,41**	**106,63**	**78,08**
	L-PPI250 N/P 10 NaOAc	*L-PPI250 N/P 10 HEPES*	*L-PPI250 N/P 5 NaOAc*	*L-PPI250 N/P 5 HEPES*
	98,18	147,9	72,02	89,99
	70,74	52,71	68,67	56,58
	70,5	96,75	70,29	56,81
Average:	**79,81**	**99,12**	**70,33**	**67,79**
	NTA			
	L-PEI60-ran-PPI190 N/P 10 NaOAc	*L-PEI60-ran-PPI190 N/P 10 HEPES*	*L-PEI60-ran-PPI190 N/P 5 NaOAc*	*L-PEI60-ran-PPI190 N/P 5 HEPES*
	62,4	36,7	65	57,9
	58	18,4	62,6	56,7
	66,8	55	67,4	59,1
Average:	**62,40**	**36,70**	**65,00**	**57,90**
	L-PPI250 N/P 10 NaOAc	*L-PPI250 N/P 10 HEPES*	*L-PPI250 N/P 5 NaOAc*	*L-PPI250 N/P 5 HEPES*
	65,5	54,1	73,6	55,2
	62,4	51,4	71,2	53,6
	68,6	56,8	76	56,8
Average:	**65,50**	**54,10**	**73,60**	**55,20**
	Zeta potential			
	L-PEI60-ran-PPI190 N/P 10 NaOAc	*L-PEI60-ran-PPI190 N/P 10 HEPES*	*L-PEI60-ran-PPI190 N/P 5 NaOAc*	*L-PEI60-ran-PPI190 N/P 5 HEPES*
	27,5	40,5	32,7	34,3
	25,5	39,8	37,9	36,1
	27,4	39,9	38,9	39,6
Average:	**26,80**	**40,07**	**36,50**	**36,67**
	L-PPI250 N/P 10 NaOAc	*L-PPI250 N/P 10 HEPES*	*L-PPI250 N/P 5 NaOAc*	*L-PPI250 N/P 5 HEPES*
	33,8	19,2	45,1	30,2
	32,8	29,6	43,3	32,7
	33,9	28,1	41,9	35,2
Average:	**33,50**	**25,63**	**43,43**	**32,70**

Fig. S14.: Overview of the absolute values (and their average/mean) of the size (DLS and NTA) and zeta potential of the saRNA-polyplexes formulated in NaOAc buffer (20 mM, pH 5.2) (black) and HEPES buffer (20 mM, pH 7.4) (blue). The saRNA-polyplexes are either composed of saRNA complexed with L-PEI$_{60}$-*ran*-PPI$_{190}$ or L-PPI$_{250}$, with N/P ratio of 10 or 5. These data allow an exact determination of the buffer effect on the particle size and zeta potential.

PDI of polyplexes based on L-PEI$_{60}$-*ran*-PPI$_{190}$ complexed in NaOAc				PDI of polyplexes based on L-PPI$_{250}$ complexed in NaOAc			
N/P	PDI	**N/P**	PDI	**N/P**	PDI	**N/P**	PDI
40	0.757	**5**	0.287	**40**	0.427	**5**	0.346
	0.719		0.324		0.445		0.276
	0.743		0.293		0.458		0.363
20	0.333	**1**	0.483	**20**	0.474	**1**	0.525
	0.359		0.353		0.462		0.245
	0.333		0.356		0.507		0.319
10	0.375	**0.2**	0.791	**10**	0.349	**0.2**	0.983
	0.396		0.773		0.341		0.751
	0.358		0.735		0.337		0.691

Table S1: Overview of the polydispersity index (PDI) values of the saRNA-polyplexes composed of L-PEI$_{60}$-*ran*-PPI$_{190}$ (left) and L-PPI$_{250}$ (right) with N/P ratio: 40, 20, 10, 5, 1 and 0.2, formulated in NaOAc buffer (20 mM, pH 5.2). Measurements were performed in triplicate with the Malvern ZetaSizer Nano-ZS.

PDI of polyplexes based on L-PEI$_{60}$-*ran*-PPI$_{190}$ complexed in HEPES

N/P	PDI
10	0.320
	0.314
	0.286
5	0.281
	0.235
	0.240

PDI of polyplexes based on L-PPI$_{250}$ complexed in HEPES

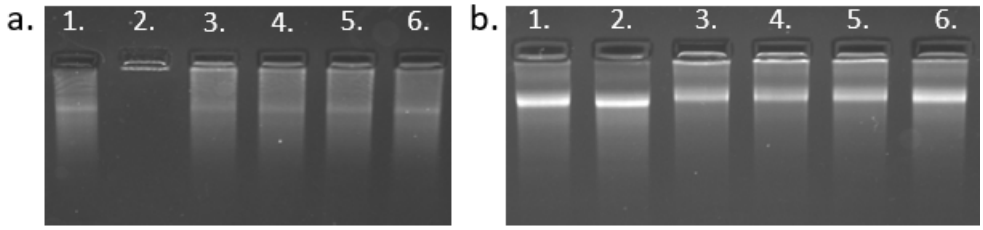
N/P	PDI
10	0.430
	0.336
	0.411
5	0.327
	0.337
	0.324

Table S2: Overview of the polydispersity index (PDI) values of the saRNA-polyplexes composed of L-PEI$_{60}$-*ran*-PPI$_{190}$ (left) and L-PPI$_{250}$ (right) with N/P ratio: 40, 20, 10, 5, 1 and 0.2, formulated in HEPES buffer (20 mM, pH 7.4). Measurements were performed in triplicate with the Malvern ZetaSizer Nano-ZS.

PDI of polyplexes based on L-PEI$_{60}$-*ran*-PPI$_{190}$ complexed in NaOAc

N/P	PDI	N/P	PDI
5	0.521	**2**	0.478
	0.448		0.378
	0.408		0.341
4	0.218	**1**	0.175
	0.213		0.161
	0.206		0.155
3	0.181		
	0.196		
	0.175		

Table S3: Overview of the polydispersity index (PDI) values of the saRNA-polyplexes composed of L-PEI$_{60}$-*ran*-PPI$_{190}$, with N/P ratio's 5, 4, 3, 2 and 1, formulated in NaOAc buffer (20 mM, pH 5.2). Measurements were performed in triplicate with the Malvern ZetaSizer Nano-ZS.



Percentage: 100% 0% 113% 84% 82% 80% 100% 96% 83% 77% 83% 99%

Panel a):

1. RNA only (in ice box)
2. Polyplex N/P 5 (L-PEI_{60}-*ran*-PPI_{190})
3. Polyplex N/P 5 + 80 µg HS (1 hour at 37 °C)
4. Polyplex N/P 5 + 100 µg HS (1 hour at 37 °C)
5. Polyplex N/P 5 + 120 µg HS (1 hour at 37 °C)
6. Polyplex N/P 5 + 160 µg HS (1 hour at 37 °C)

Panel b):

1. RNA only (in ice box)
2. RNA only (1 hour at 37 °C)
3. Polyplex N/P 20 + 80 µg HS (1 hour at 37 °C)
4. Polyplex N/P 10 + 80 µg HS (1 hour at 37 °C)
5. Polyplex N/P 5 + 80 µg HS (1 hour at 37 °C)
6. Polyplex N/P 1 + 80 µg HS (1 hour at 37 °C)

Fig. S15.: Heparin sodium (HS) competition assay with polyplexes based on L-PEI_{60}-*ran*-PPI_{190}. The UV-pictures of the EtBr bleach agarose gels are shown. For each condition 500 ng saRNA was used. The detection limit of the gels is 10 ng saRNA. These tests were successful. The densitometric analysis (Fiji) of the electrophoretic retardation of the polyplexes is shown below the pictures as percentages relative to the "saRNA only" signal from lane 1.