Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Material

Self-assembled PEGylated Amphiphilic Polypeptides for Gene Transfection

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Kinetic study observed by IR measurements

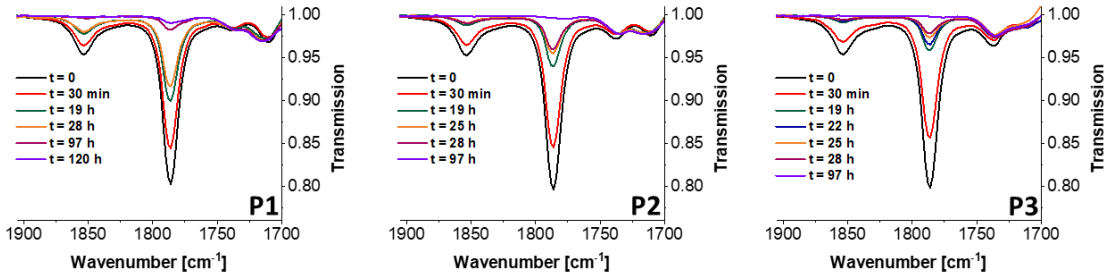


Figure S1: Reaction process observed via disappearance of NCA bands at 1854 and 1782 cm^{-1} along reaction time.

Detailed amounts of reactants used for polymer synthesis

Table S1: Summary for HO-PEG-*b*-P(Bzl-*L*-Glu-*stat*-Boc-*L*-Lys) block copolymers **P1** – **P3**.

	Bzl-L-Glu		**Boc-L-Lys**		**HO-PEG-NH_2**		**M/I**	**DMF**	**t**	**Yield**	
	m	n	m	n	m	n		V			
	mg	mmol	mg	mmol	mg	mmol		mL	h	mg	%
P1	214	0.81	527	1.93	275	0.055	50	10.06	120	663	74
P2	363	1.38	383	1.41	278	0.056	50	10.09	97	633	70
P3	501	1.90	226	0.83	274	0.055	50	10.05	97	667	76

Determination of k_P

Equation S1: Calculation of k_P.

$$\ln\left(\frac{T_{100\%}}{T_{\%t}}\right) = k \cdot t = k_P \cdot [I]_0 \cdot t \quad \rightarrow \quad k_P = \frac{k}{[I]_0} \tag{1}$$

$T_{100\%}$	Transmittance at 1853 nm (rel. to baseline value at 1900 nm) at t = 0
$T_{\%t}$	Transmittance at 1853 nm (rel. to baseline value at 1900 nm) at t
k	rate constant resp. rise of lin. regression fit in L mol^{-1} h^{-1}
t	Time in h^{-1}
k_P	Polymerization rate constant in h^{-1}
$[I]_0$	Concentration of the initiator in mol L^{-1}

*Assignments of signals in ^{1}H-NMR spectra to polymer structure of **P1** – **P3***

P1: ^{1}H-NMR (d-TFA, 300 MHz, 298 K): δ [ppm] = 7.25 (Ph-*H*, 50H), 5.10 (CH_2-Ph, 21H), 4.90 – 4.32(C*H*-backbone, 36H), 3.82 (O-CH_2-CH_2 of PEG, 452H), 3.16 – 3.09 (CH_2-C(O)O of Bzl-*L*-Glu & CH_2-NH-C(O)O of Boc-*L*-Lys, 46H), 2.53 – 1.99 (CH-CH_2, 34H), 1.98 – 1.15 (C(CH_3)$_3$ & CH$_2$-CH_2-CH_2 of Boc-*L*-Lys, 389H)).

P2: ^{1}H-NMR (d-TFA, 300 MHz, 298 K): δ [ppm] = 7.20 (Ph-*H*, 116H), 5.07 (CH_2-Ph, 46H), 4.90 – 4.27(C*H*-backbone, 47H), 3.82 (O-CH_2-CH_2 of PEG, 452H), 3.31 – 2.96 (CH_2-C(O)O of Bzl-*L*-Glu & CH_2-NH-C(O)O of Boc-*L*-Lys, 46H), 2.70 – 1.96 (CH-CH_2, 81H), 1.95 – 1.16 (C(CH_3)$_3$ & CH$_2$-CH_2-CH_2 of Boc-*L*-Lys, 355H)).

P3: ^{1}H-NMR (d-TFA, 300 MHz, 298 K): δ [ppm] = 7.16 (Ph-*H*, 180H), 5.04 (CH_2-Ph, 72H), 4.83 – 4.37(C*H*-backbone, 51H), 3.81 (O-CH_2-CH_2 of PEG, 452H), 3.27 – 2.92 (CH_2-C(O)O of Bzl-*L*-Glu & CH_2-NH-C(O)O of Boc-*L*-Lys, 30H), 2.74 – 2.02 (CH-CH_2, 112H), 2.01 – 1.25(C(CH_3)$_3$ & CH$_2$-CH_2-CH_2 of Boc-*L*-Lys, 259H)).

Determination of the monomer distribution via area calculation in ^{1}H NMR of the polymer backbone

Table S2: Content of Boc-*L*-Lys *via* calculation of ^{1}H-NMR signal of the polymer backbone using Peak Analyzer of Origin 9.1.0G (© by OriginLab Corporation)

	Area		**Boc-*L*-Lys**	
	Boc-*L*-Lys	Bzl-*L*-Glu	%	DP
P1	78.0155	51.9659	60	22
P2	85.6290	112.5786	43	19
P3	61.9589	202.3175	23	12

Determination of absolute molar mass via SEC MALS

For each block copolymer **P1** – **P3**, five solutions with different concentrations between 1 and 10 mg mL^{-1} were prepared. Each of the solutions was injected directly into the detector at a pump rate of 36 mL h^{-1} until the dRI intensity value reached a plateau. Between each calibration standard, the measuring system was rinsed with ultrapure DMF until the dRI intensity reached the initial values again. For each calibration solution, the maximum intensity section was manually selected. The dn/dc values were determined by plotting the intensity as a function of the concentration of the polymer in the DMF and calculating the slope of the straight line by linear regression using the software Astra (version 7.1.0.29). The results of the SEC MALS investigations are summarized in **Figure S1**. The determination of the absolute molar mass was done by a regular SEC measurement using the SEC MALS setup in DMF containing 0.1 wt% LiBr, see **Figure S2**.

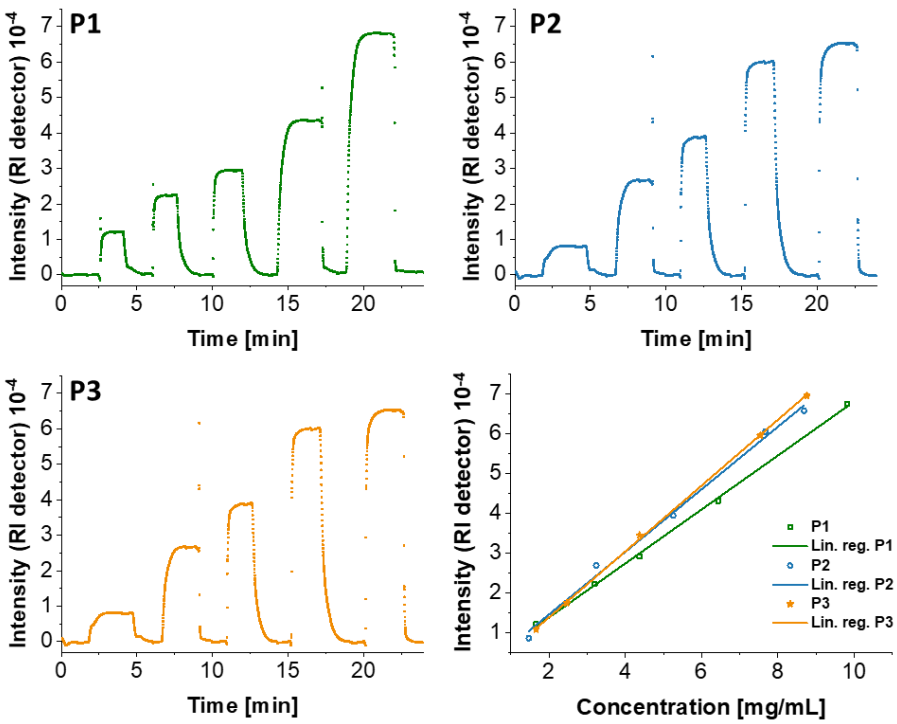


Figure S1: Injection profile of SEC MALS measurement of **P1** (top left), **P2** (top right) and **P3** (bottom left). Bottom right: Results of linear regression by plotting the dRI intensity as a function of the concentration of the polymer in the DMF.

*Elugram of SEC MALS measurements of PEG macro initiator and **P1** – **P3***

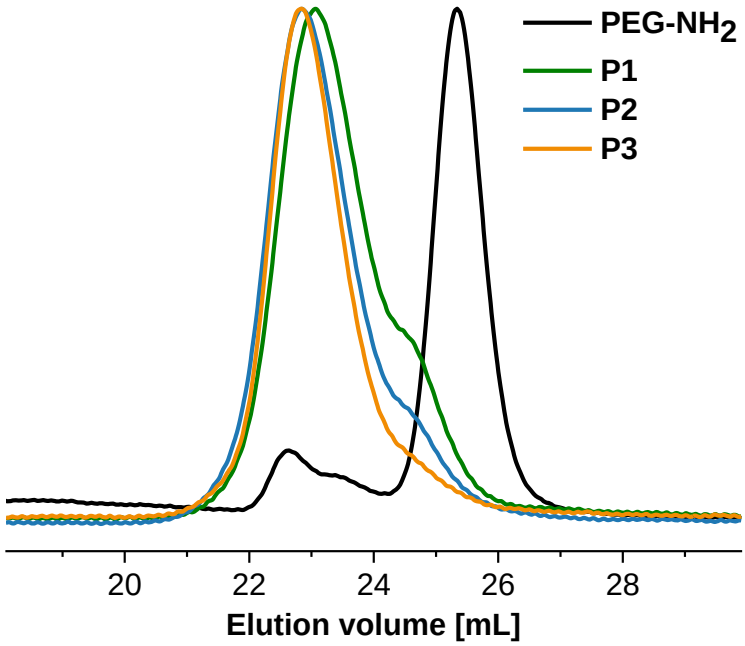


Figure S2: SEC plot of PEG-NH_2 initiator and block copolymers **P1** – **P3** measured in DMF containing 0.09 wt% LiBr as eluent using SEC MALS setup.

*IR and CD measurements of **P1 - P3***

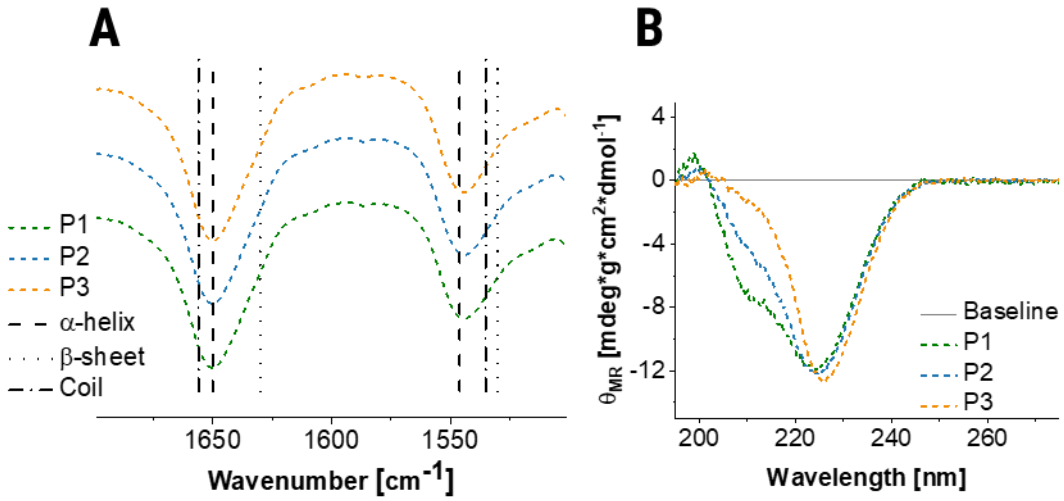


Figure S4: A Stacked IR spectra of **P1** – **P3** with characteristic wavenumber of amide CO stretching for α-helix (solid, 1650 & 1546 cm^{-1}), b-sheet (points, 1630 & 1530 cm^{-1}) and coil (dash-points, 1656 & 1535 cm^{-1}). **B** Overlay CD spectra of **P1** – **P3** measured in MeOH (c_{Pi*} = 0.50 mg mL^{-1}). Mean residual ellipticity θ_{MR} calculated with **Equation S3**.

*Detailed amounts of reactants used for deprotection of **P1*** – **P3***

Table S3: Summary for HO-PEG-*b*-P(Bzl-*L*-Glu-*stat*-*L*-Lys) block copolymers **P1*** – **P3***.

		Precursor		**CH_2Cl_2**	**TFA**	**Yield**	
		m	n_{Lys}	V	V		
		mg	mmol	mL	mL	mg	%
P1*	**P1**	154	0.43	1.16	0.40	83	66
P2*	**P2**	159	0.27	1.19	0.40	103	76
P3*	**P3**	157	0.15	1.18	0.40	157	67

*^{1}H-NMR investigations of **P1*** – **P3****

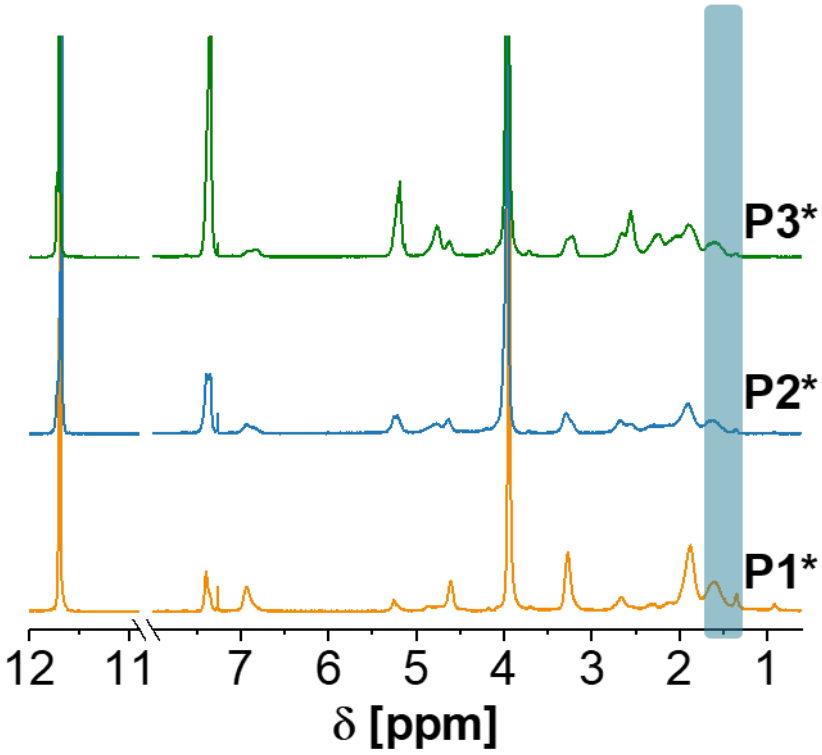


Figure S5: Stacked ^{1}H-NMR spectra of **P1*** – **P3*** measured in d-TFA containing traces of $CDCl_3$ for axis calibration at 298 K and 300 MHz. Expected area of the signals of the Boc protons marked in blue.

*Assignments of signals in ^{1}H-NMR spectra of polymer structures **P1*** – **P3***

P1*: ^{1}H-NMR (d-TFA + 1 drop $CDCl_3$, 300 MHz, 298 K): δ [ppm] = 7.41 (Ph-*H*, 50H), 6.94 (CH_2-N*H*$_2$, 61H), 5.25 (C*H*$_2$-Ph, 20H), 4.99 – 4.42 (C*H*-backbone, 79H), 3.96 (O-C*H*$_2$-C*H*$_2$ of PEG, 452H), 3.28 (C*H*$_2$-C(O)O of Bzl-*L*-Glu & C*H*$_2$-NH-C(O)O of *L*-Lys, 122H), 2.90 – 2.08 (CH-C*H*$_2$, 101H), 2.07 – 1.11 (C(C*H*$_3$)$_3$ & CH_2-C*H*$_2$-C*H*$_2$ of Boc-*L*-Lys, 369H)).

P2*: ^{1}H-NMR (d-TFA + 1 drop $CDCl_3$, 300 MHz, 298 K): δ [ppm] = 7.37 (Ph-*H*, 103H), 6.93 (CH_2-N*H*$_2$, 26H), 5.23 (C*H*$_2$-Ph, 43H), 5.02 – 4.43 (C*H*-backbone, 58H), 3.96 (O-C*H*$_2$-C*H*$_2$ of PEG, 452H), 3.44 – 3.11 (C*H*$_2$-C(O)O of Bzl-*L*-Glu & C*H*$_2$-NH-C(O)O of *L*-Lys, 50H), 2.68 – 2.08 (CH-C*H*$_2$, 104H), 2.07 – 1.44 (C(C*H*$_3$)$_3$ & CH_2-C*H*$_2$-C*H*$_2$ of Boc-*L*-Lys, 156H)).

P3*: ^{1}H-NMR (d-TFA + 1 drop $CDCl_3$, 300 MHz, 298 K): δ [ppm] = 7.34 (Ph-*H*, 153H), 6.84 (CH_2-N*H*$_2$, 13H), 5.19 (C*H*$_2$-Ph, 62H), 4.94 – 4.52 (C*H*-backbone, 50H), 3.95 (O-C*H*$_2$-C*H*$_2$ of PEG, 452H), 3.38 – 3.11 (C*H*$_2$-C(O)O of Bzl-*L*-Glu & C*H*$_2$-NH-C(O)O of *L*-Lys, 29H), 2.88 – 2.17 (CH-C*H*$_2$, 108H), 2.16 – 1.43 (C(C*H*$_3$)$_3$ & CH_2-C*H*$_2$-C*H*$_2$ of Boc-*L*-Lys, 117H)).

Determination of the molar mass of the monomer hybrids, the mean residual ellipticity θ_{MR} and the helicity

Equation S2: Calculation of the molar mass of the monomer hybrids.

$$M_{monomer\ hybrid} = \frac{DP_{L-Lys} \cdot M_{L-Lys} + DP_{Bzl-L-Glu} \cdot M_{Bzl-L-Glu}}{M_{L-Lys} + M_{Bzl-L-Glu}} \quad (2)$$

$M_{monomer\ hybrid}$	Molar mass of monomer hybrid consisting of Bzl-*L*-Glu and Boc-*L*-Lys in g mol^{-1}
DP_{L-Lys}/ $DP_{Bzl-L-Glu}$	Degree of polymerization of Boc-*L*-Lys, *L*-Lys resp. Bzl-*L*-Glu
M_{L-Lys}/$M_{Bzl-L-Glu}$	Molar mass of Boc-*L*-Lys-, *L*-Lys- resp. Bzl-*L*-Glu-unit

Equation S3: Calculation of the mean residual ellipticity.

$$\theta_{RM} = \frac{\theta \cdot M_{monomer\ hybrid}}{10 \cdot c \cdot l} \quad (3)$$

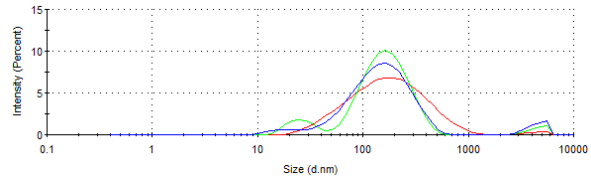
θ_{RM}	Mean residual ellipticity in deg cm^2 dmol^{-1}
θ	Ellipticity in deg
c	Polymer concentration in g/L
l	Layer thickness of the quartz cuvette in cm

Equation S4: Calculation of the helicity.

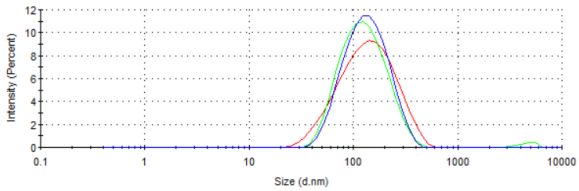
$$\%_{\alpha-helices} = \frac{-[\theta_{222}] + 3{,}000}{39{,}000} \quad (4)$$

$\%_{\alpha-helices}$	Helicity in %
$[\theta_{222}]$	Value of ellipticity at 222 nm

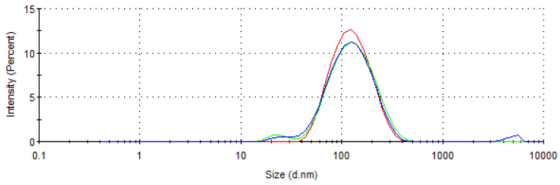
DLS measurements of aqueous complexes



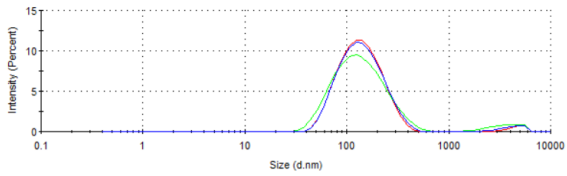
P1*$_{Aq}$



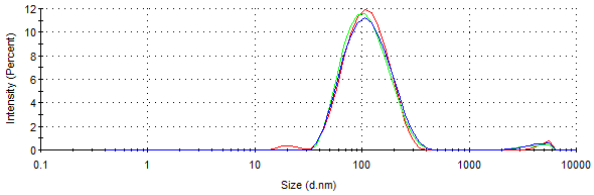
P1*$_{Aq}$(GFP) N/P 5



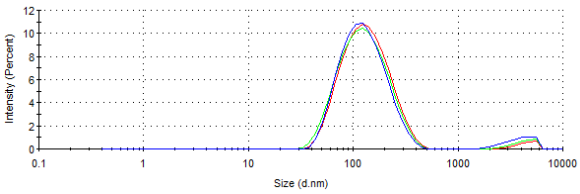
P1*$_{Aq}$(GFP) N/P 10



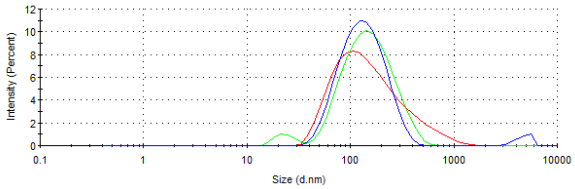
P1*$_{Aq}$(GFP) N/P 20



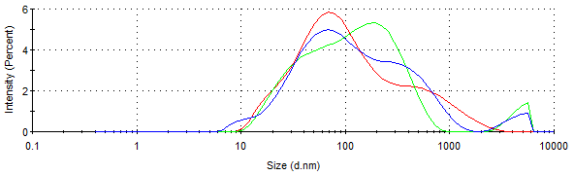
P1*$_{Aq}$(ctrl) N/P 5



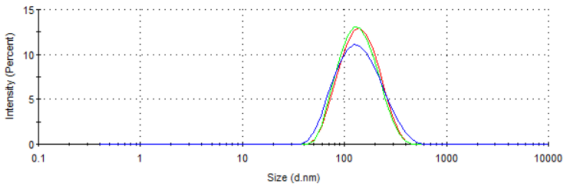
P1*$_{Aq}$(ctrl) N/P 10



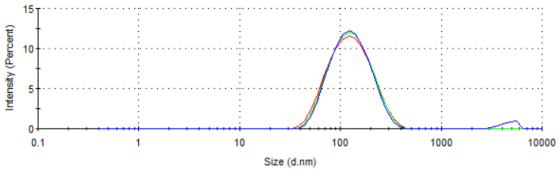
P1*$_{Aq}$(ctrl) N/P 20



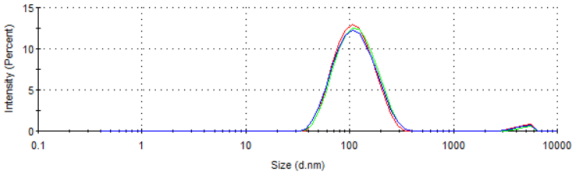
P2*$_{Aq}$



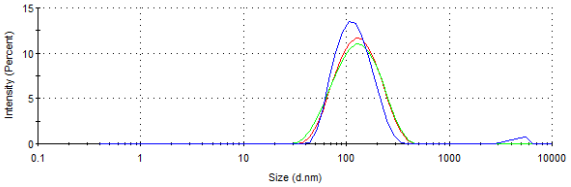
P2*$_{Aq}$(GFP) N/P 5



P2*$_{Aq}$(GFP) N/P 10



P2*$_{Aq}$(GFP) N/P 20



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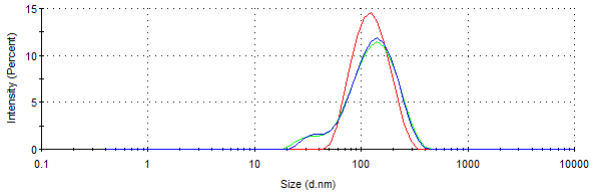
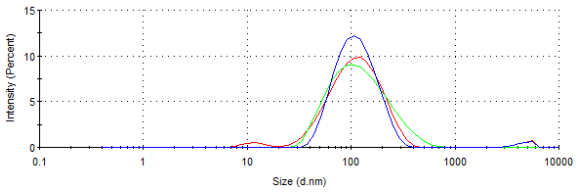


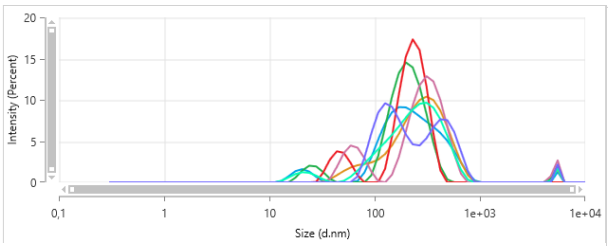
P2*$_{Aq}$(ctrl) N/P 10



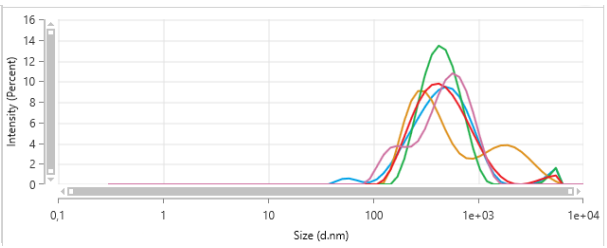
P2*$_{Aq}$(ctrl) N/P 20

Figure S6: Overview of DLS measurements of complexes. Measurement of three batches, each measured three times without purification, each 3 runs with 30 s run duration at 25 °C.

DLS measurements of NP formulations after centrifugation



P2*$_{C}$



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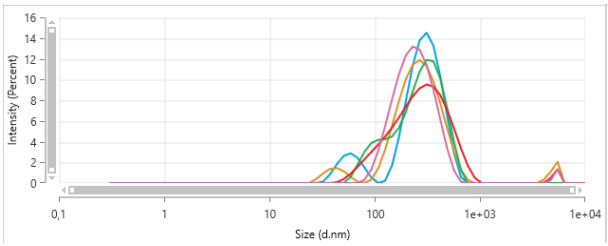
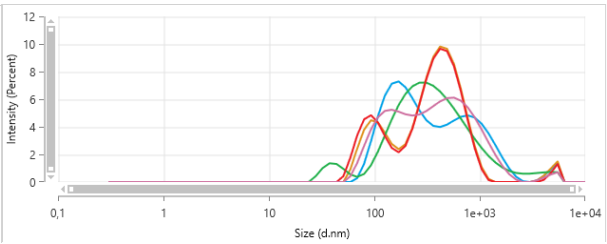
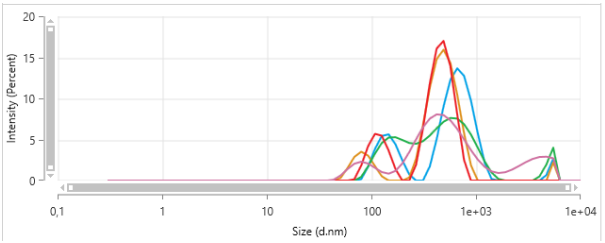


P2*$_{C}$(ctrl)



P2*$_{DA}$



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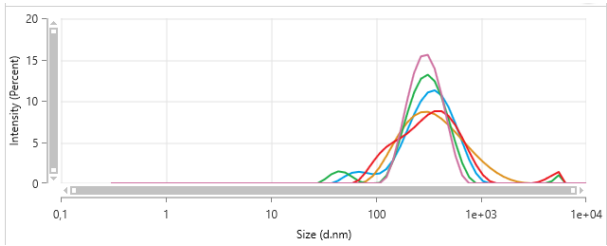
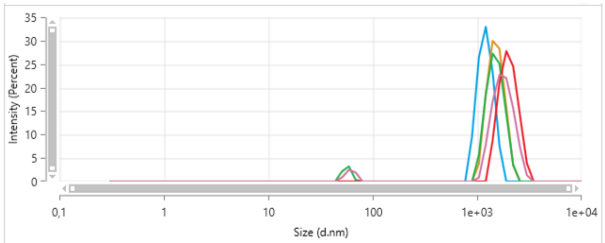
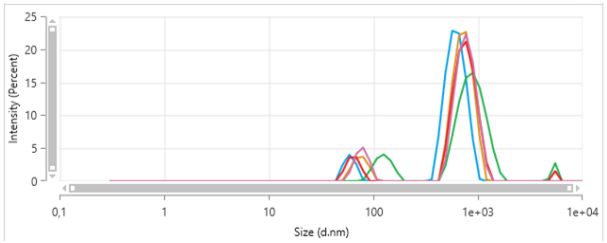


P2*$_{DA}$(ctrl)



P2*$_{D}$



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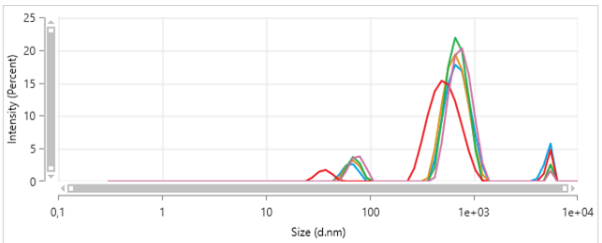

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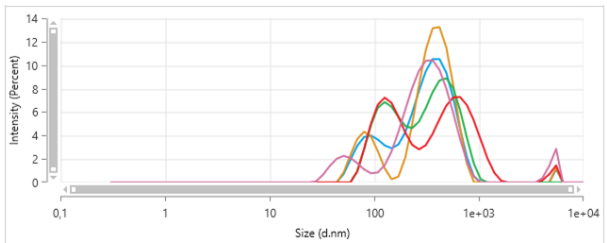
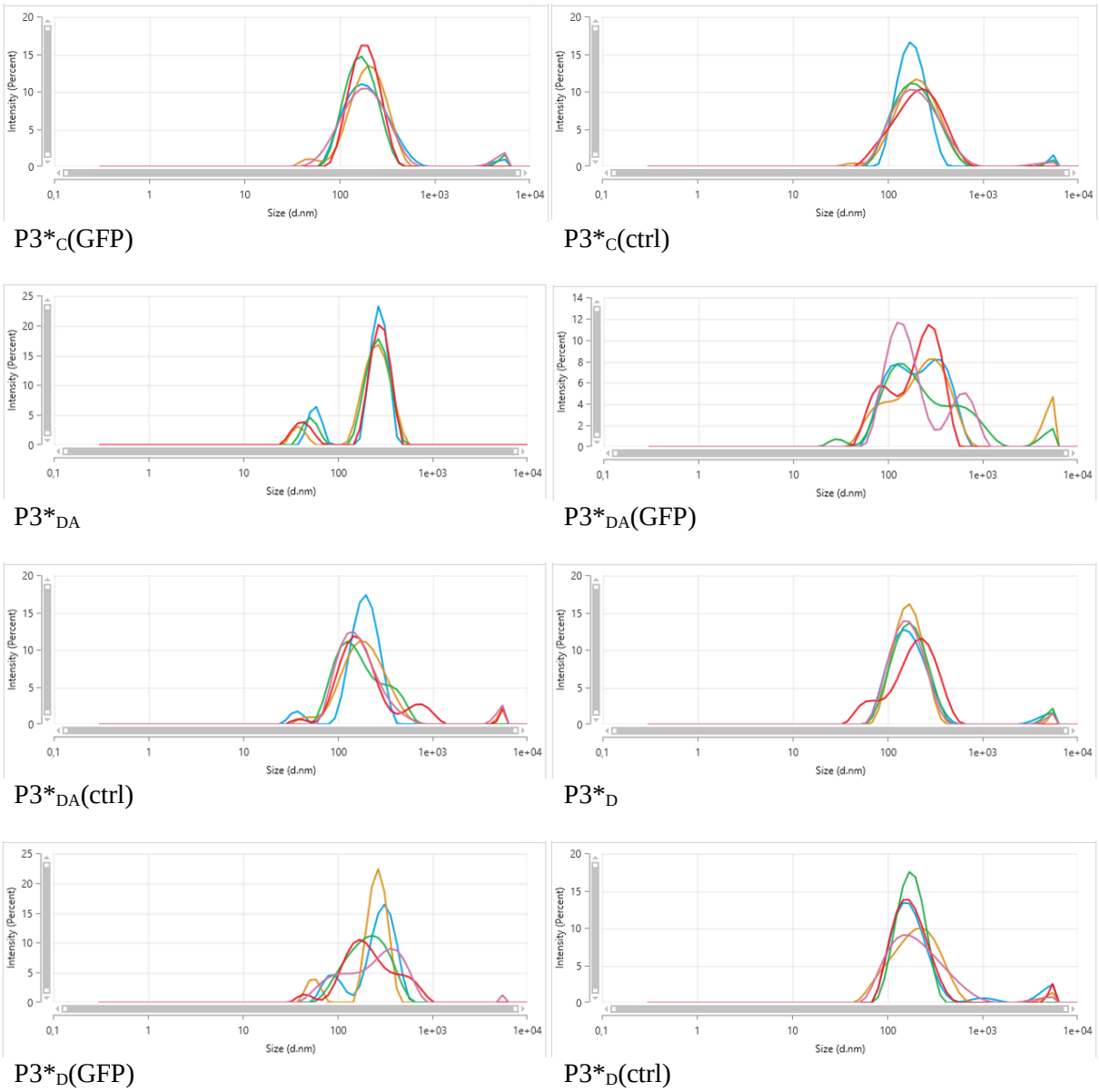


P3*$_{C}$



P3*$_{C}$(GFP) P3*$_{C}$(ctrl)

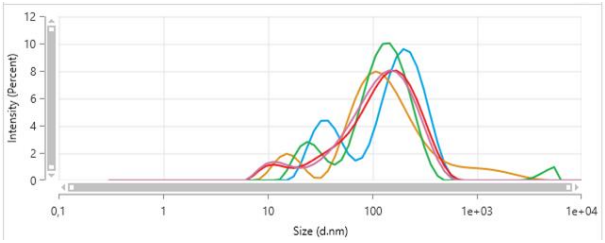
P3*$_{DA}$ P3*$_{DA}$(GFP)

P3*$_{DA}$(ctrl) P3*$_{D}$

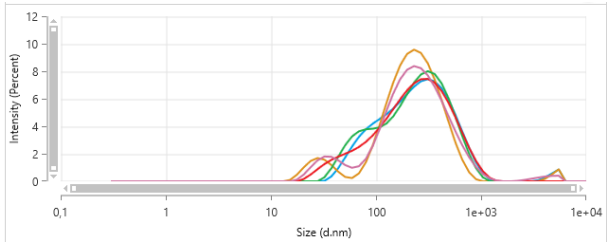
P3*$_{D}$(GFP) P3*$_{D}$(ctrl)

Figure S7: Overview of DLS measurements of centrifugated NP suspensions. Five measurements, each 15 runs with 1.68 s run duration at 25 °C.

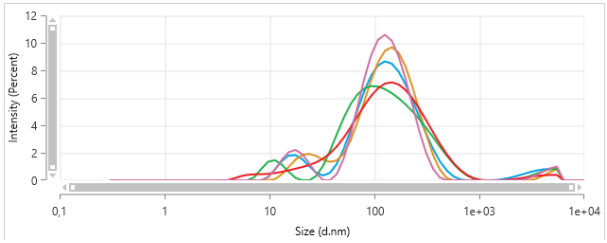
DLS measurements of NP formulations after dialysis



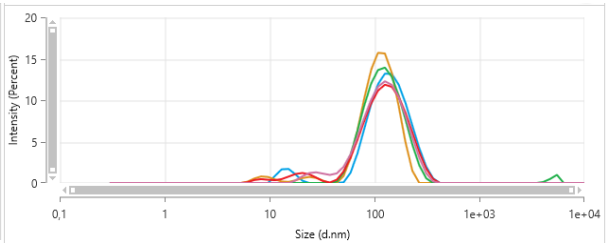
P2*$_{C}$



P2*$_{C}$(GFP)



P2*$_{C}$(ctrl)



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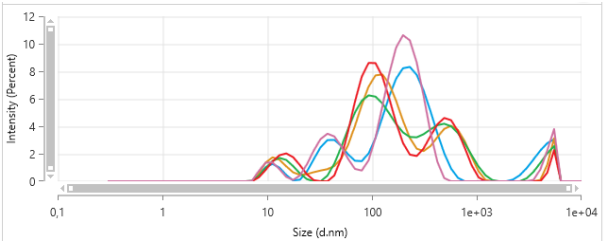

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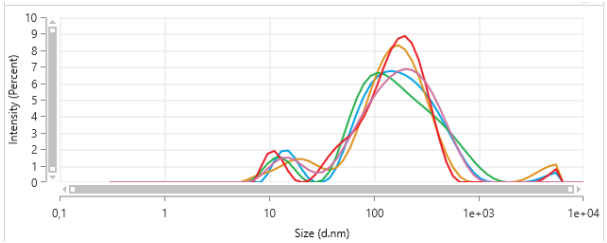

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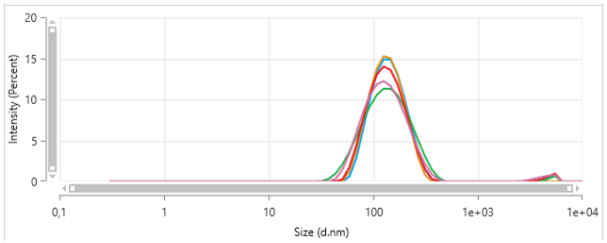
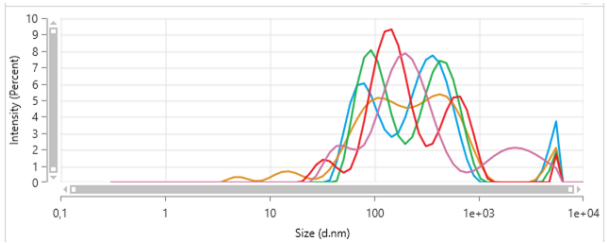
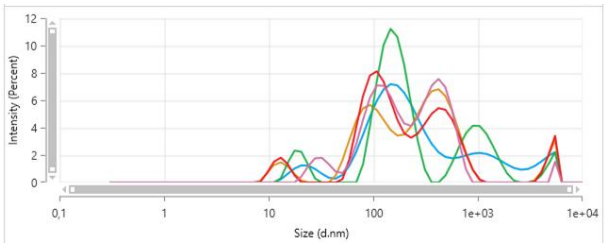


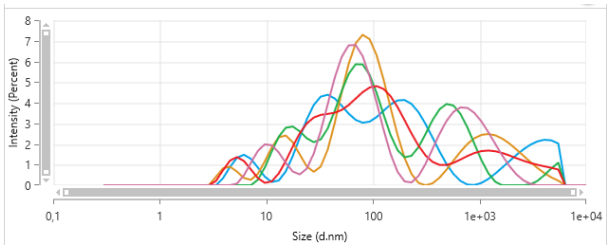
P2*$_{D}$



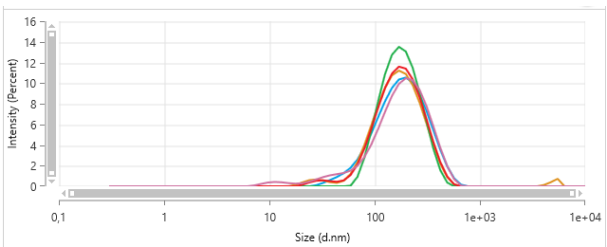
P2*$_{D}$(GFP)

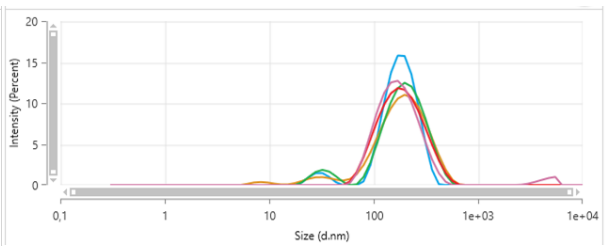


P2*$_{D}$(ctrl)



P3*$_{C}$





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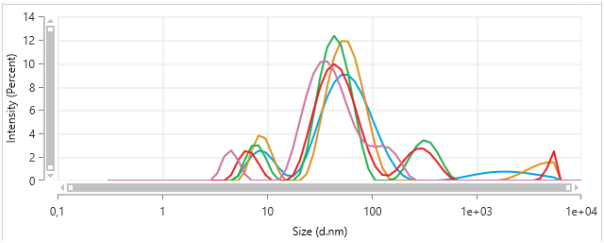
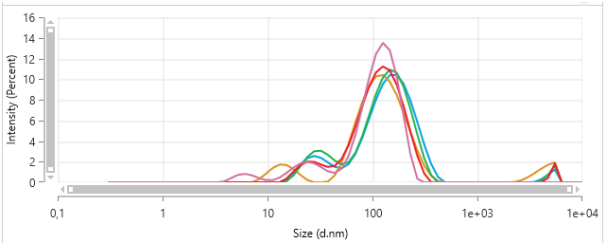


P3*$_{C}$(ctrl)



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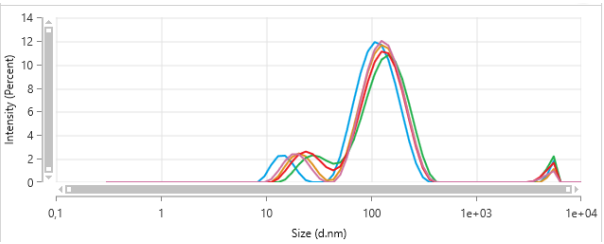

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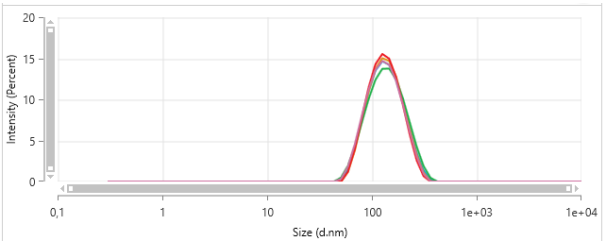
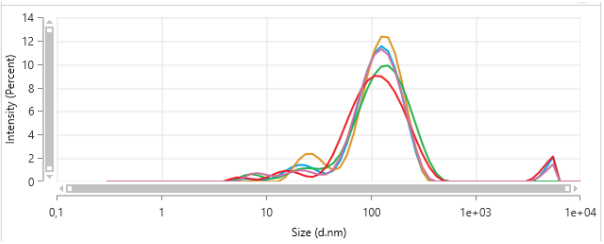


P3*$_{DA}$(ctrl)



P3*$_{D}$

P3*$_{D}$(GFP)

P3*$_{D}$(ctrl)

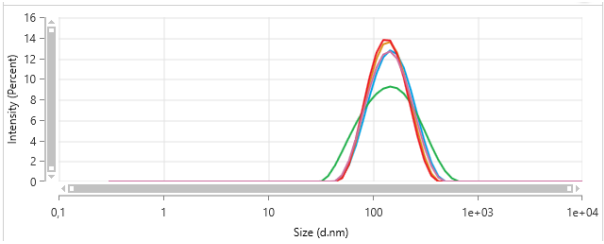


Figure S8: Overview of DLS measurements of dialyzed NP suspensions. Five measurements, each 15 runs with 1.68 s run duration at 25 °C.

DLS data of NP formulations after purification by centrifugation

Table S4: Overview of characterization data of NP formulations from polypeptides **P2*** and **P3*** after purification by centrifugation.

Sample#	N/P ratio	Zeta potential[a)]	DLS[b)]	
			Size	PDI
		mV	nm	
P2*$_C$	-	17.6	226	0.47
P2*$_C$(GFP)	58	30.5	390	0.34
P2*$_C$(ctrl)	58	13.4	231	0.45
P2*$_{DA}$	-	16.5	246	0.47
P2*$_{DA}$(GFP)	58	21.7	417	0.64
P2*$_{DA}$(ctrl)	58	19.4	267	0.33
P2*$_D$	-	13.2	>1000	0.46
P2*$_D$(GFP)	58	25.8	711	0.78
P2*$_D$(ctrl)	58	25.9	699	0.73
P3*$_C$	-	15.6	265	0.55
P3*$_C$(GFP)	33	18.9	177	0.25
P3*$_C$(ctrl)	33	19.4	180	0.23
P3*$_{DA}$	-	11.6	351	0.47
P3*$_{DA}$(GFP)	33	16.2	216	0.46
P3*$_{DA}$(ctrl)	33	13.6	207	0.37
P3*$_D$	-	16.8	160	0.27
P3*$_D$(GFP)	33	15.9	295	0.45
P3*$_D$(ctrl)	33	17.4	184	0.32

Pi*$_Y$(GFP): i = 2 or 3 (ID of polypeptide); GFP = mEGFP pDNA, (cargo); Y = solvents (subscripted: C = Cyrene, D = DMSO, DA = mixture of DMSO and acetone). Cyrene formulations were done by sonication. DMSO:acetone and DMSO formulations were done by nanoprecipitation. [a)] Measured three times before purification at 25 °C. [b)] Five measurements after purification by centrifugation, each 15 runs with 1.68 s run duration at 25 °C.

*Polymer concentrations in aqueous formulations of **P1*** and **P2***

Table S5: Overview of polymer concentrations used for the aqueous complex formulations of **P1*** and **P2*** using pDNA at a final concentration of 30 µg mL^{-1}.

Sample#	N/P ratio	Final polymer concentration in complex µg mL^{-1}
$P1^*_{Aq}$(GFP/ctrl)	5	203.4
	10	406.9
	20	813.7
$P2^*_{Aq}$(GFP/ctrl)	5	258.5
	10	517.1
	20	1034.2

PrestoBlue™ cell viability assay and degradation

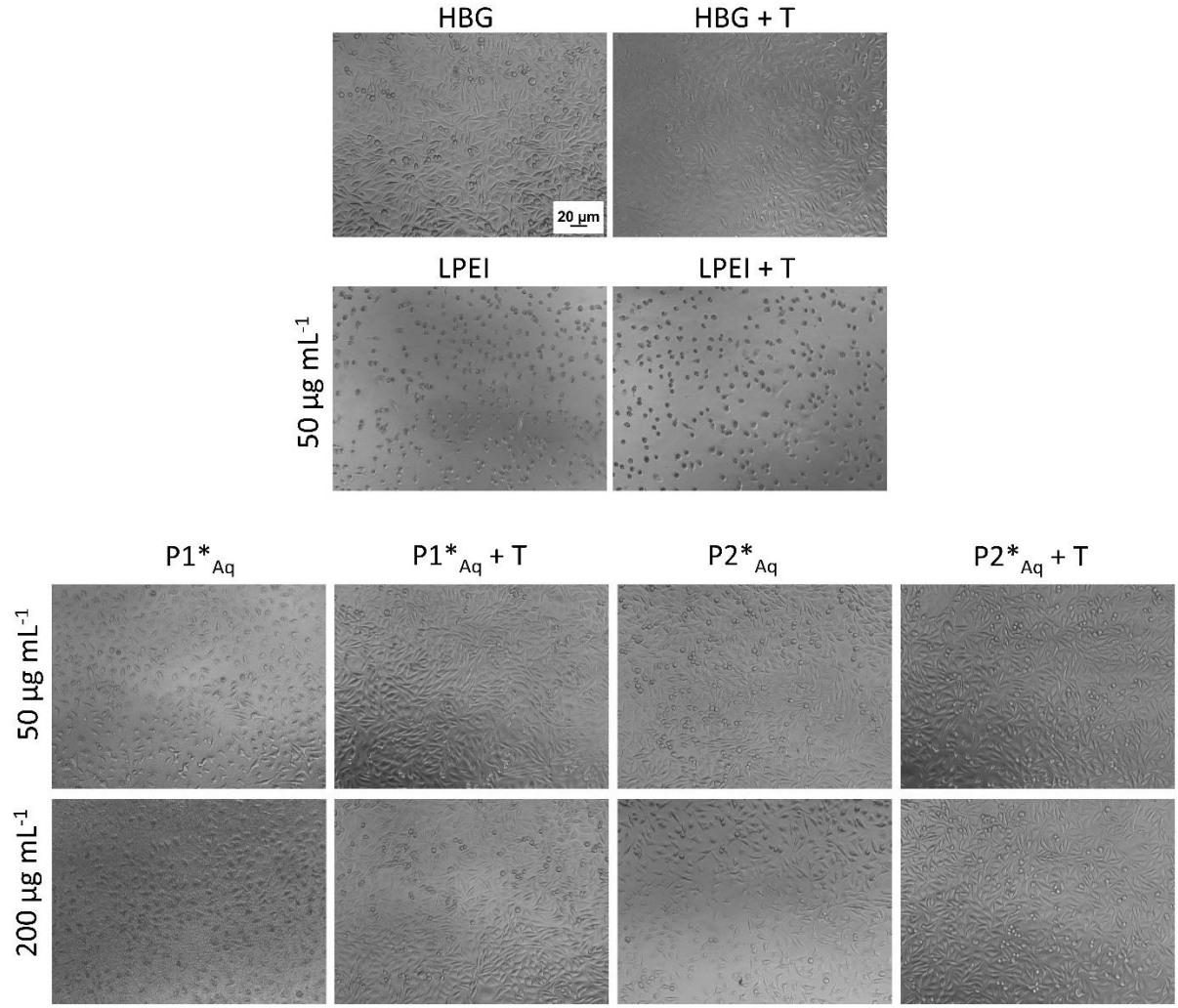


Figure S9: Microscopy images of L-929 cells in cytotoxicity and degradation assay. For evaluation of cytotoxicity and biodegradability L-929 cells were incubated with **P1*** and **P2*** polymer dissolved in acetate buffer (**P1*$_{Aq}$**, **P2*$_{Aq}$**), dialyzed **P2*$_{D,d}$** and **P3*$_{D,d}$** NPs without pDNA for 24 h and imaged *via* light microscopy. Degradability of **P1*$_{Aq}$** and **P2*$_{Aq}$** was evaluated by incubation with trypsin for 1 h at 37 °C and subsequent incubation with L-929 cells. **P1*$_{Aq}$** and **P2*$_{Aq}$** without trypsin treatment and LPEI as non-degradable polymer without and with trypsin treatment were used as controls.

Flow cytometry - Gating strategy in transfection experiments

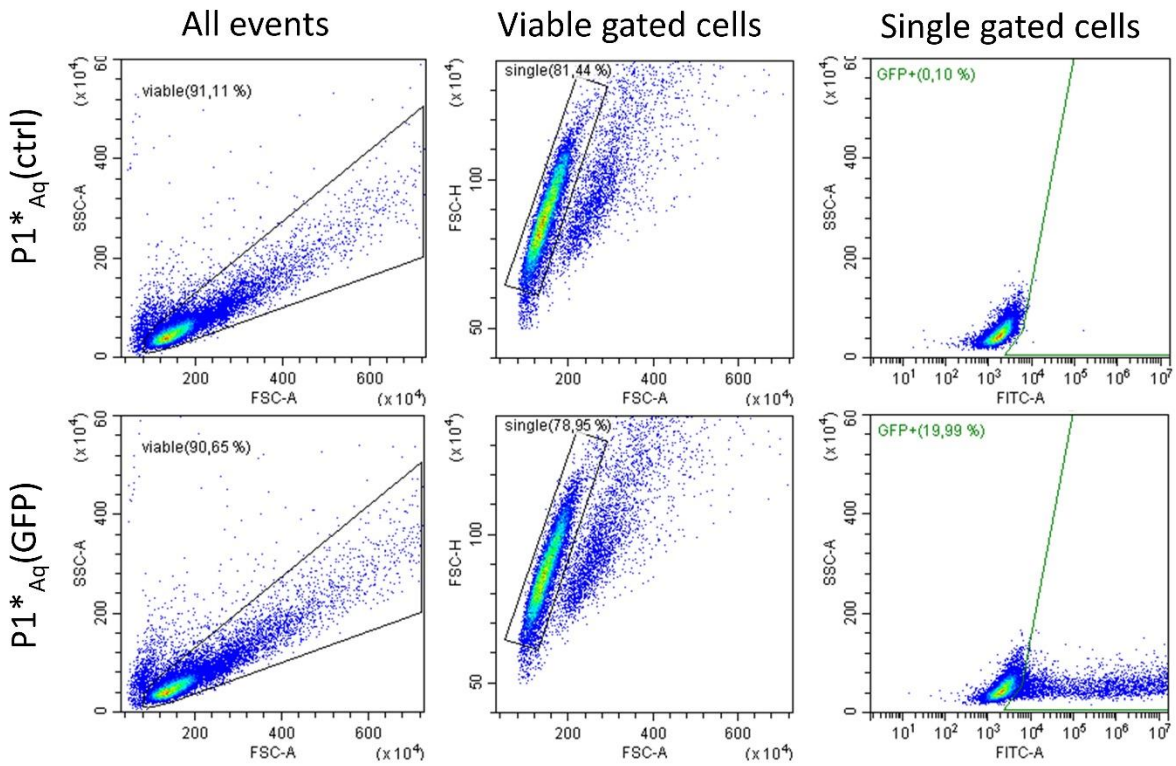


Figure S10: Gating strategy for transfection experiments in HEK293T cells. The gating strategy for transfection experiments is exemplary shown for HEK293T cells incubated with **P1*$_{Aq}$(GFP)** complexes (N/P 5, 6 mg mL^{-1} pDNA) for 48 h. Viable cells were gated according to the FSC/SSC pattern. Viable gated cells were further gated using the area of FSC signal plotted against the FSC height (FSC-H/FSC-A plot) to discriminate single cells from doublets in the sample. GFP positive cells were identified by gating the single cells to the unstained control (complex with pKMyc pDNA, **P1*$_{Aq}$(ctrl)**).

Transfection of HEK293T cells – Microscopy

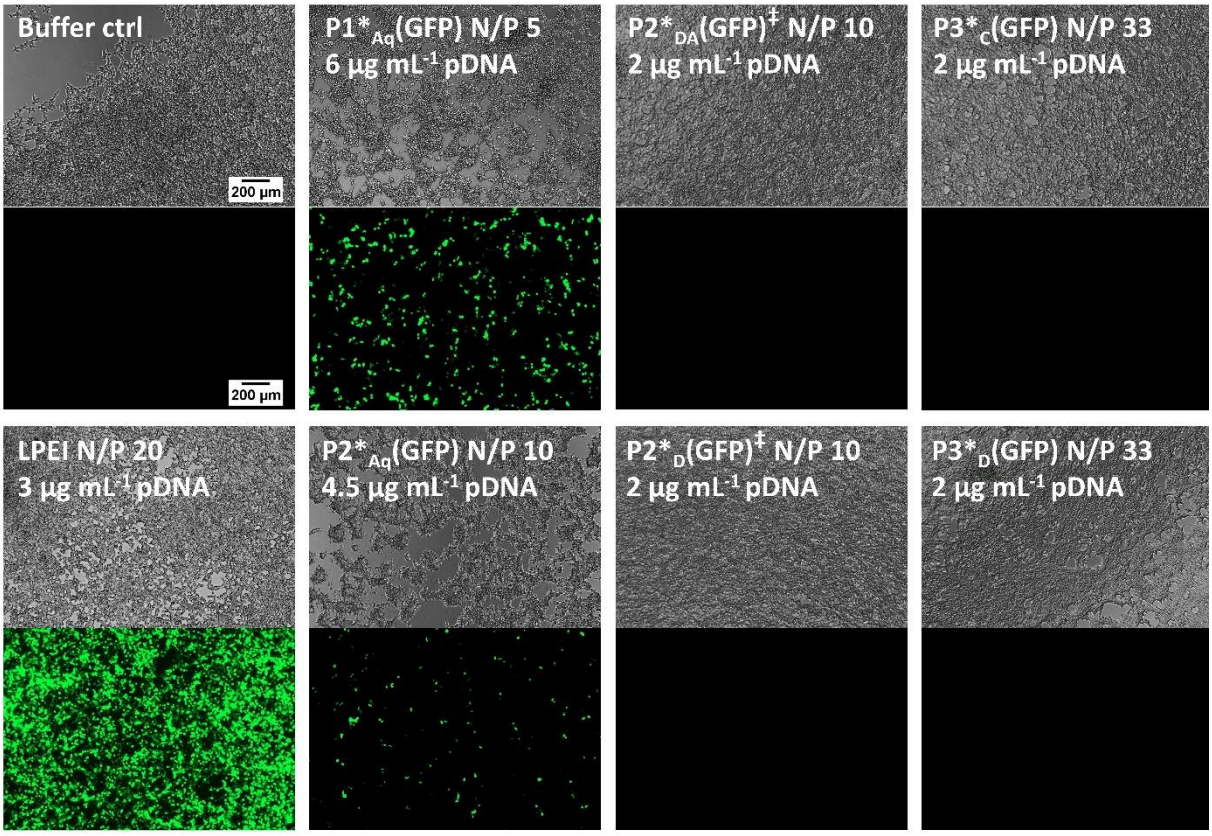


Figure S11: Images of transfection in HEK293T cells. HEK293T cells were incubated with particles at varying N/P ratios (5 - 33) and pDNA concentrations (2 - 6 µg mL^{-1}) for 48 h and subsequently imaged *via* fluorescence microscopy.

Linear polyethyleneimine (LPEI) transfection in HEK293T cells

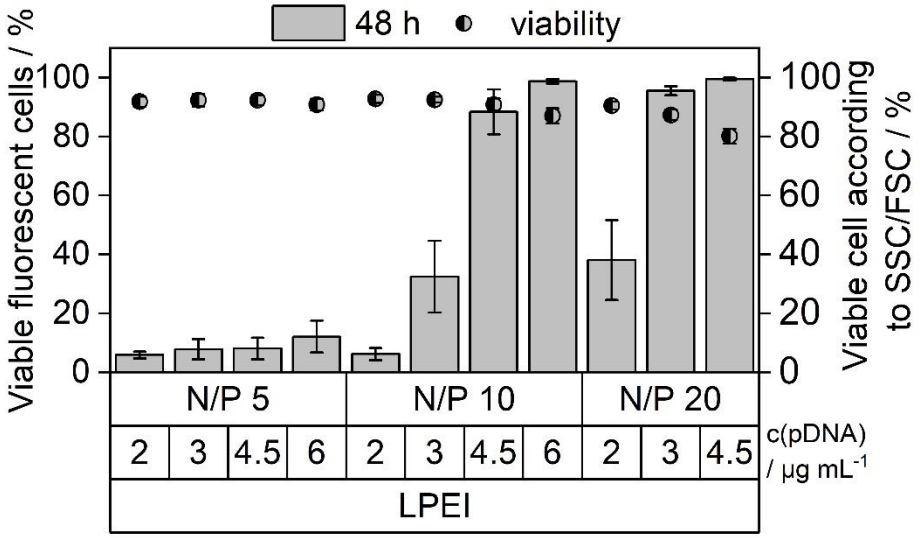


Figure S12: Transfection of HEK293T cells with LPEI. HEK293T cells were incubated with LPEI polyplexes particles at varying N/P ratios (5 - 20) and pDNA concentrations (2 - 6 µg mL^{-1}) for 48 h and subsequently measured *via* flow cytometry. Cell viability during transfection experiments was evaluated by gating the cells according to the SSC/FSC pattern. Transfection efficiency is displayed as percentage of viable fluorescent cells of all viable singe cells. The samples were gated to the respective control (polyplex with pKMyc pDNA, non-fluorescent control, ctrl) (n = 3 ± SD).