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Supporting Information

A Library of Aminoglycoside-derived Lipopolymer Nanoparticles for Delivery of Small Molecules and Nucleic Acids

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Polymer	Lipid	Molar ratio (polymer:lipid)	LPNs Polymer-lipid (molar ratio)	Yield (%)
NR	C2	1:2	NR-C2 (1:2)	37
	C2	1:5	NR-C2 (1:5)	34
	C4	1:2	NR-C4 (1:2)	41
	C4	1:5	NR-C4 (1:5)	32
	C6	1:2	NR-C6 (1:2)	38
	C6	1:5	NR-C6 (1:5)	42
	C6	1:10	NR-C6 (1:10)	40
	C14	1:2	NR-C14 (1:2)	33
	C14	1:5	NR-C14 (1:5)	38
	C14	1:10	NR-C14 (1:10)	50
	C18	1:2	NR-C18 (1:2)	51
	C18	1:5	NR-C18 (1:5)	43
	C18	1:10	NR-C18 (1:10)	46
PR	C2	1:2	PR-C2 (1:2)	33
	C2	1:5	PR-C2 (1:5)	46
	C4	1:2	PR-C4 (1:2)	34
	C4	1:5	PR-C4 (1:5)	48
	C6	1:2	PR-C6 (1:2)	52
	C6	1:5	PR-C6 (1:5)	50
	C6	1:10	PR-C6 (1:10)	45
	C14	1:2	PR-C14 (1:2)	44
	C14	1:5	PR-C14 (1:5)	38
	C14	1:10	PR-C14 (1:10)	31
	C18	1:2	PR-C18 (1:2)	36
	C18	1:5	PR-C18 (1:5)	41
	C18	1:10	PR-C18 (1:10)	41
AR	C2	1:2	AR-C2 (1:2)	34
	C2	1:5	AR-C2 (1:5)	37
	C4	1:2	AR-C4 (1:2)	31
	C4	1:5	AR-C4 (1:5)	39
	C6	1:2	AR-C6 (1:2)	54
	C6	1:5	AR-C6 (1:5)	51
	C6	1:10	AR-C6 (1:10)	41
	C14	1:2	AR-C14 (1:2)	45
	C14	1:5	AR-C14 (1:5)	42
	C14	1:10	AR-C14 (1:10)	41
	C18	1:2	AR-C18 (1:2)	41
	C18	1:5	AR-C18 (1:5)	36
	C18	1:10	AR-C18 (1:10)	39

Table S1. Nomenclature & average yields of aminoglycoside-derived lipopolymer nanoparticles (LPNs). Abbreviations used for parental aminoglycoside-derived polymers: NR for Neomycin-RDE polymer, PR for Paromomycin-RDE polymer and AR for Apramycin-RDE polymer, where RDE = Resorcinol diglycidylether. Yields were calculated as described in the Experimental section.

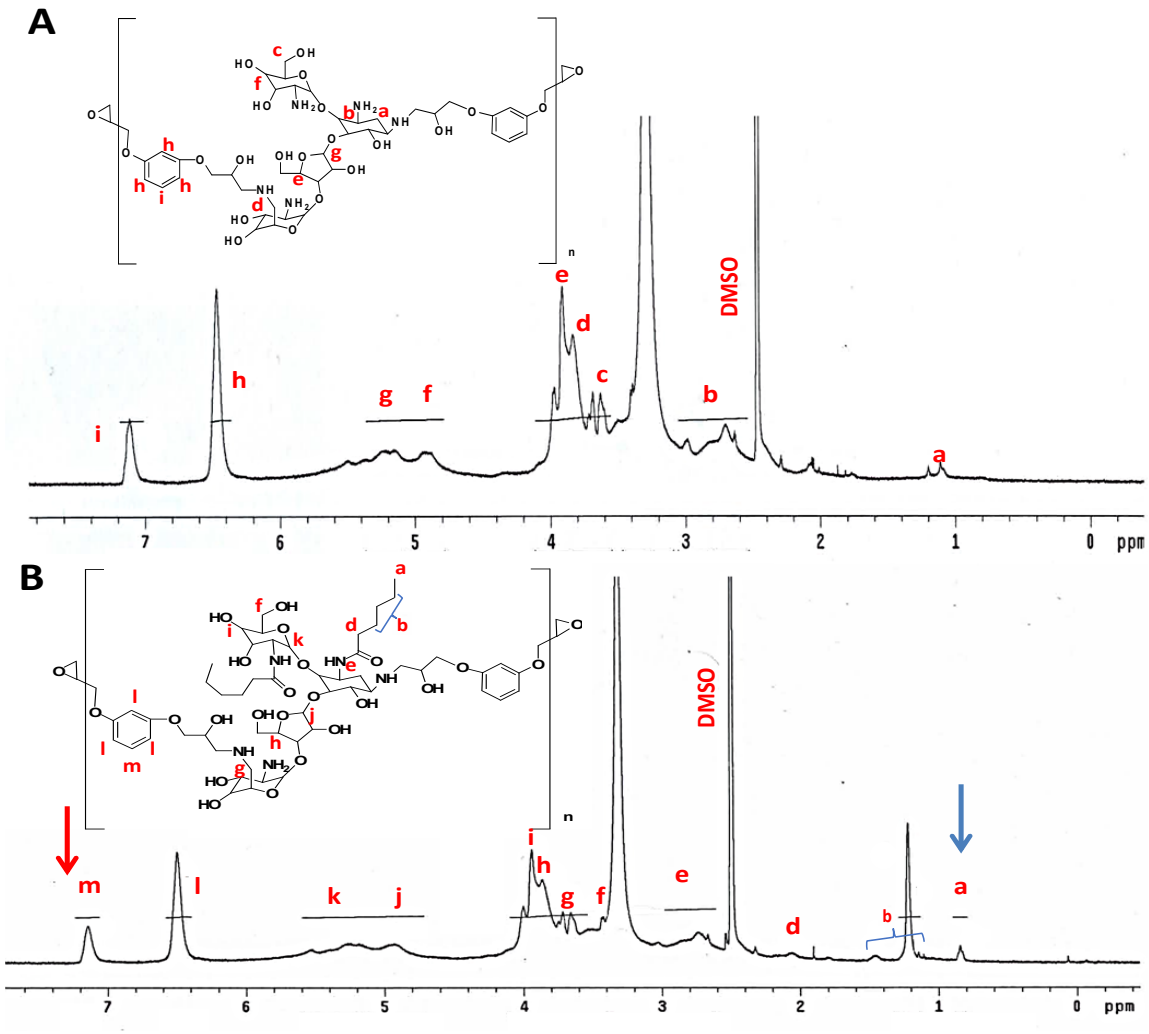


Figure S1. ^{1}H-NMR spectra of **(A)** PR polymer and **(B)** PR-C6 (1:2) its lipopolymer in DMSO-D_6 solvent. Red and blue arrows show the protons shifts of the lipid and polymer, respectively. These shifts were used to calculate the degree of substitution. In B, the peak designated as 'b' corresponds to a combined peak of 3 adjacent -CH_2 protons which result in higher intensity of the peak compared to protons in peaks designated as 'a' or 'd'.

Lipopolymer	Polymer (mmol)	Lipid (mmol)	Lipid/Polymer (mol ratio)[a]	Lipid/Polymer (mol ratio)[b]	Degree of Substitution (%)[c]
NR-C6 (1:2)	0.01	0.02	2.0	0.72	5.3
NR-C6 (1:5)	0.01	0.05	5.0	0.93	6.3
NR-C6 (1:10)	0.01	0.1	10.0	1.4	14
NR-C14 (1:2)	0.01	0.02	2.0	0.3	7.5
NR-C14 (1:5)	0.01	0.05	5.0	0.63	13.9
NR-C14 (1:10)	0.01	0.1	10.0	2.73	32
NR-C18 (1:2)	0.01	0.02	2.0	0.65	9.33
NR-C18 (1:5)	0.01	0.05	5.0	0.98	14
NR-C18 (1:10)	0.01	0.1	10.0	2.33	33.3
PR-C6 (1:2)	0.01	0.02	2.0	0.65	13
PR-C6 (1:5)	0.01	0.05	5.0	0.85	17
PR-C6 (1:10)	0.01	0.1	10.0	2.2	34
PR-C14 (1:2)	0.01	0.02	2.0	0.53	16.2
PR-C14 (1:5)	0.01	0.05	5.0	0.85	22.5
PR-C14 (1:10)	0.01	0.1	10.0	1.25	28.6
PR-C18 (1:2)	0.01	0.02	2.0	0.45	9
PR-C18 (1:5)	0.01	0.05	5.0	0.8	16
PR-C18 (1:10)	0.01	0.1	10.0	1.13	22.6
AR-C6 (1:2)	0.01	0.02	2.0	0.97	14.25
AR-C6 (1:5)	0.01	0.05	5.0	1.69	22.25
AR-C6 (1:10)	0.01	0.1	10.0	3.1	37.6
AR-C14 (1:2)	0.01	0.02	2.0	0.36	9.2
AR-C14 (1:5)	0.01	0.05	5.0	0.58	14.5
AR-C14 (1:10)	0.01	0.1	10.0	0.73	18.25
AR-C18 (1:2)	0.01	0.02	2.0	0.49	12.27
AR-C18 (1:5)	0.01	0.05	5.0	0.74	18.5
AR-C18 (1:10)	0.01	0.1	10.0	1.78	34.5

Table S2. Polymers and lipopolymers generated in this study and the extent of lipid substitution based on ^{1}H-NMR. [a]Feed ratio. [b]Ratio calculated from ^{1}H-NMR spectrum. [c] Percentage of amines modified (calculated from the ^{1}H-NMR spectra by comparing the methyl protons of alkyl group to proton on aminoglycoside as shown in Figure S1). Three independent experiments (n=3) were carried out.

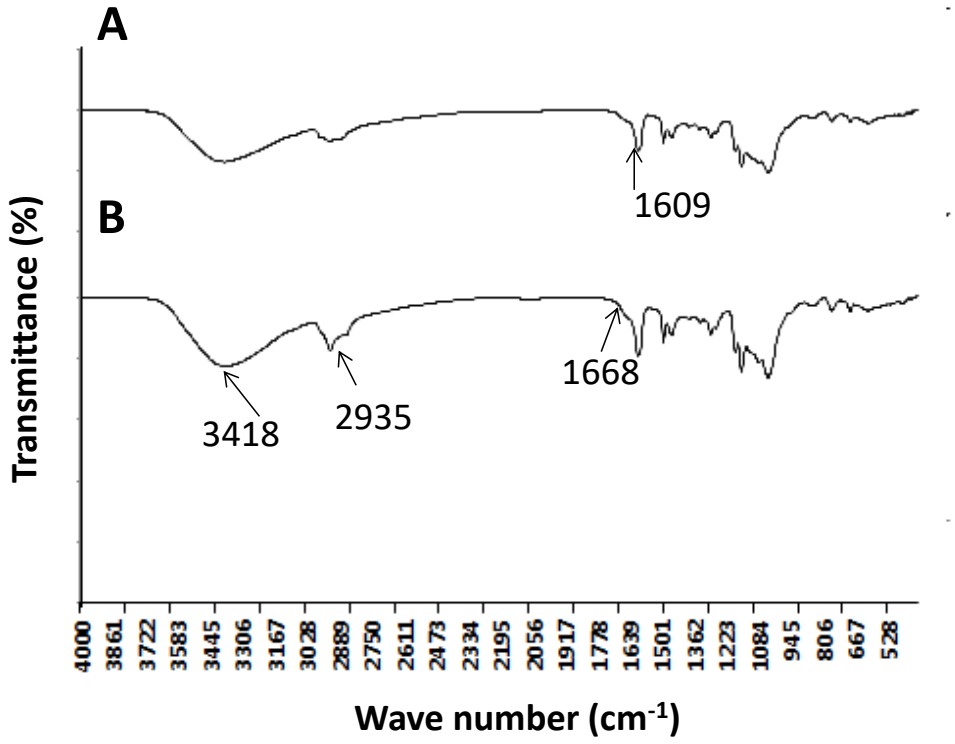


Figure S2. FTIR spectra of **(A)** PR polymer and **(B)** PR-C6 (1:2) lipopolymer with C_6 lipid conjugated to PR polymer. An increase in % transmittance for alkyl C-H (sp^3) stretching at 2935 cm^{-1} and amide bond formation at 1668 cm^{-1} are seen in case of PR-C6 (1:2) lipopolymer compared to the parental polymer, which is indicative of the lipid-conjugation. Three independent experiments (n=3) were carried out.

No.	SAMPLE	C (% weight)	H (% weight)	N (% weight)
1	NR-C6 (1:5)	44.08 ± 0.106	7.09 ± 0.233	5.09 ± 0.339
2	NR-C6 (1:2)	43.72 ± 0.509	7.07 ± 0.042	5.19 ± 0.113
3	NR	42.47 ± 0.240	6.94 ± 0.198	5.73 ± 0.212
4	PR-C14 (1:2)	45.73 ± 0.735	7.12 ± 0.325	5.14 ± 0.544
5	PR-C6 (1:2)	45.01 ± 0.120	7.29 ± 0.219	4.82 ± 0.106
6	PR	42.60 ± 0.297	6.96 ± 0.445	5.71 ± 0.417

Table S3. CHN analyses (% weight) of lead LPNs and parental polymers (NR, PR). Two independent experiments (n=2) were carried out.

No.	SAMPLE	M_n	M_w	PDI
1	NR-C6(1:2)	3086	3814	1.16
2	NR-C6(1:5)	3489	4063	1.24
3	PR-C6(1:2)	3843	4962	1.18
4	PR-C6(1:5)	4057	5282	1.31
5	AR-C6(1:2)	2886	3570	1.23
6	AR-C6(1:5)	2827	3463	1.22
7	NR	3312	3853	1.27
8	PR	3928	4638	1.32
9	AR	2783	3193	1.26

Table S4. Molecular weights* (MWs) with polydispersity index (PDI) of lead LPNs and parental polymers NR, PR, and AR, which also self-assemble into nanoparticles.
*Number-averaged (M_n) and weight-averaged (M_w) molecular weights (in Daltons; Da), and polydispersity index (PDI) of lipopolymers were measured using Gel Permeation Chromatography (GPC).

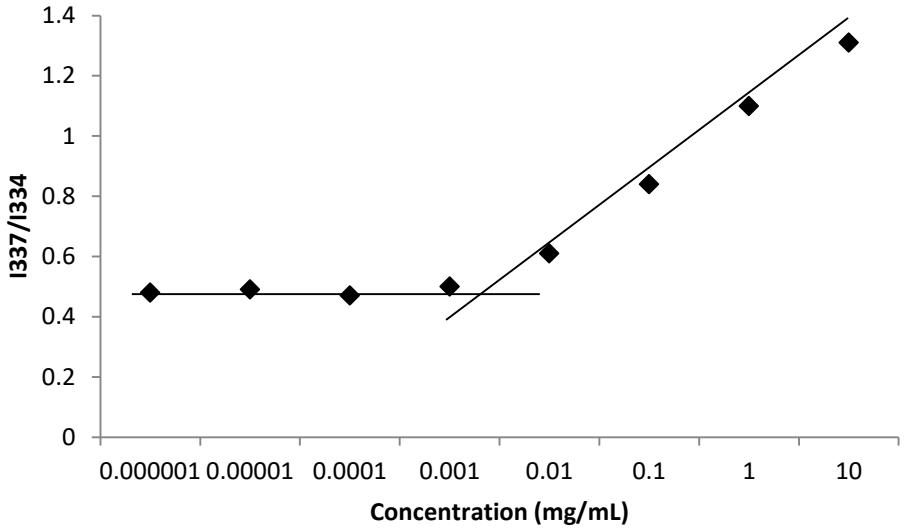


Figure S3. Repreentative data for the determination of critical micellar concentration (CMC) PR-C14 (1:2) micelles using the pyrene fluorescence assay. Please see experimental section for details.

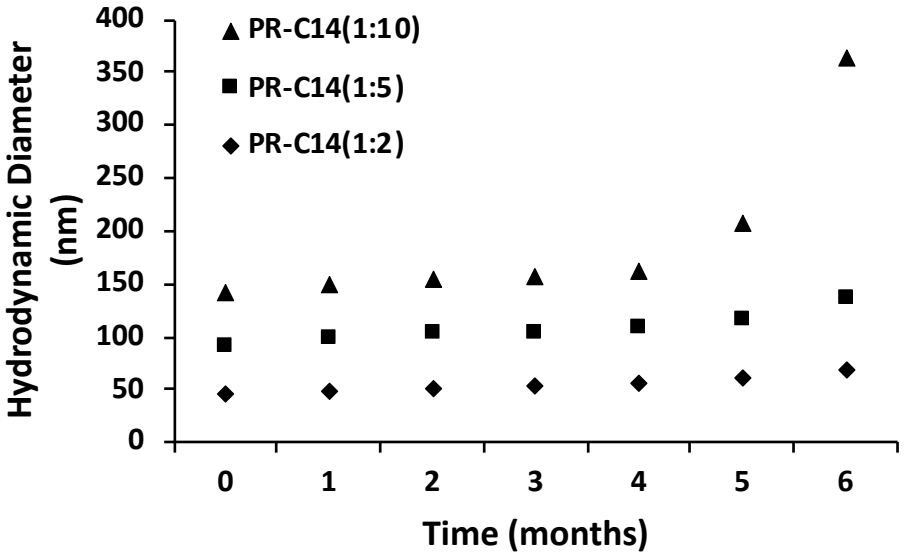


Figure S4. Stability studies of LPNs carried out over a period of six months in PBS (pH 7.4) by determining the corresponding hydrodynamic diameter using dynamic light scattering.

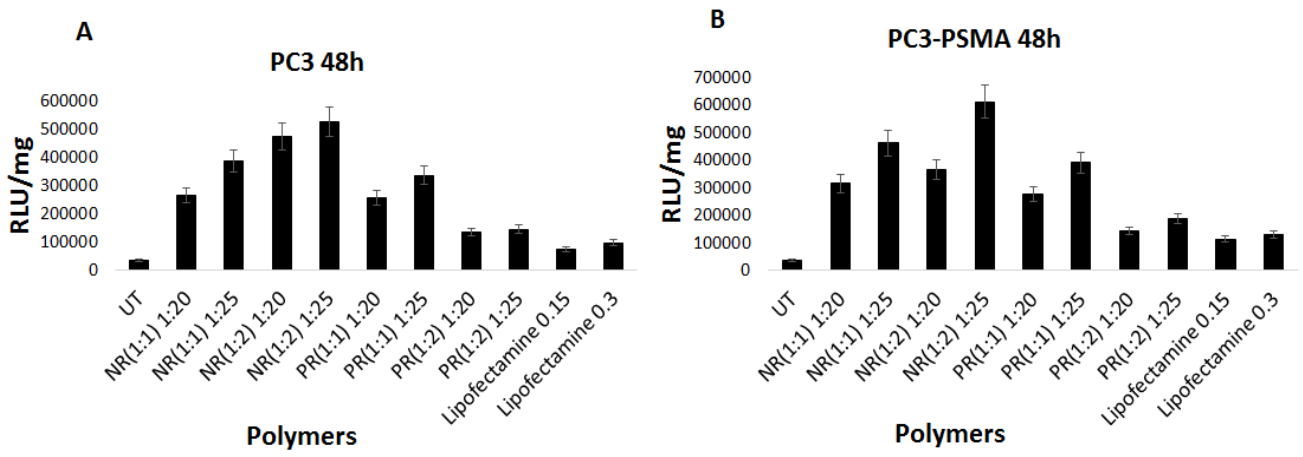
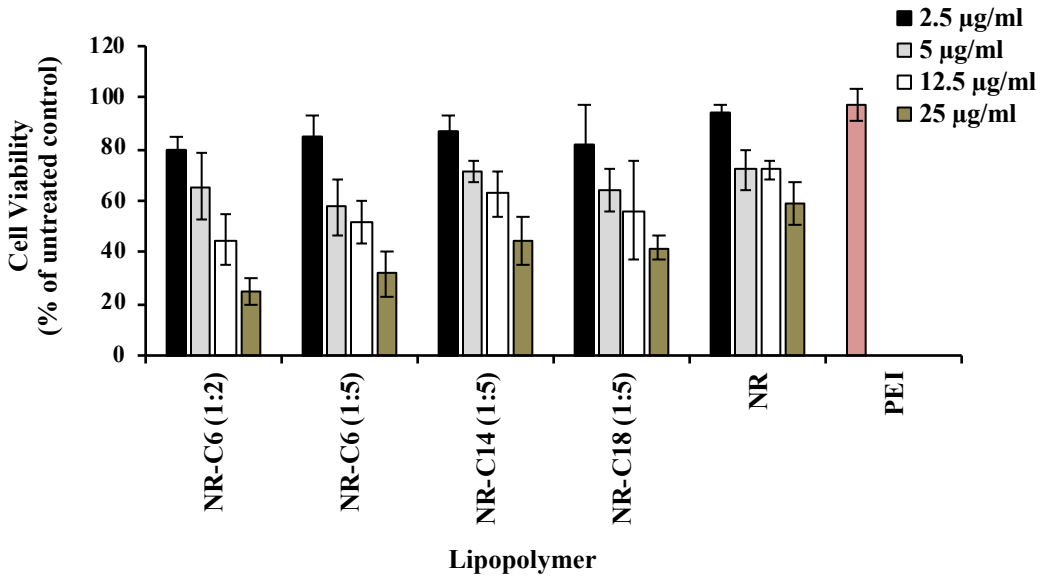
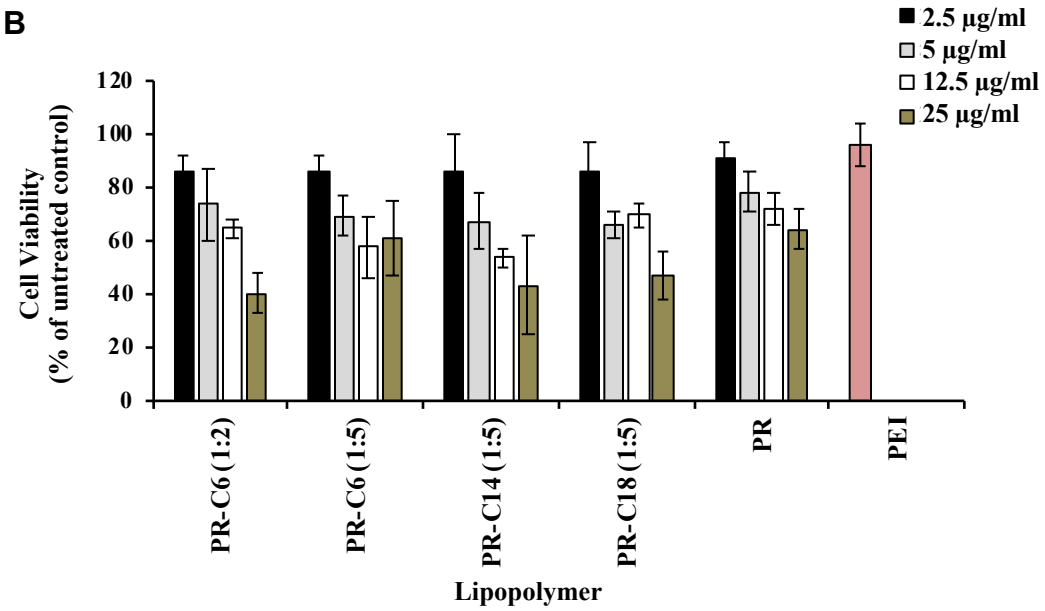


Figure S5. Transgene (luciferase) expression following delivery of pGL4.5 plasmid DNA (pDNA) to PC3 and PC3-PSMA human prostate cancer cells using parental polymers (NR and PR) at two different aminoglycoside:resorcinol diglycidylether molar ratios. Luciferase expression was evaluated for 20:1 to 25:1 polymer:pDNA (w/w). Transgene expression efficacies of polymers were quantified in terms of relative luciferase units (RLU) and normalized to total protein content (mg), resulting in RLU/mg values, which were used for comparison between different delivery vehicles. Transgene expression efficacies of lipopolymers were compared with lipofectamine. Mean values ± one standard deviation from three independent experiments (n=3) are shown.

A


2.5 µg/ml
5 µg/ml
12.5 µg/ml
25 µg/ml
Cell Viability
(% of untreated control)
120
100
80
60
40
20
0
NR-C6 (1:2)
NR-C6 (1:5)
NR-C14 (1:5)
NR-C18 (1:5)
NR
PEI
Lipopolymer

B


2.5 µg/ml
5 µg/ml
12.5 µg/ml
25 µg/ml
Cell Viability
(% of untreated control)
120
100
80
60
40
20
0
PR-C6 (1:2)
PR-C6 (1:5)
PR-C14 (1:5)
PR-C18 (1:5)
PR
PEI
Lipopolymer

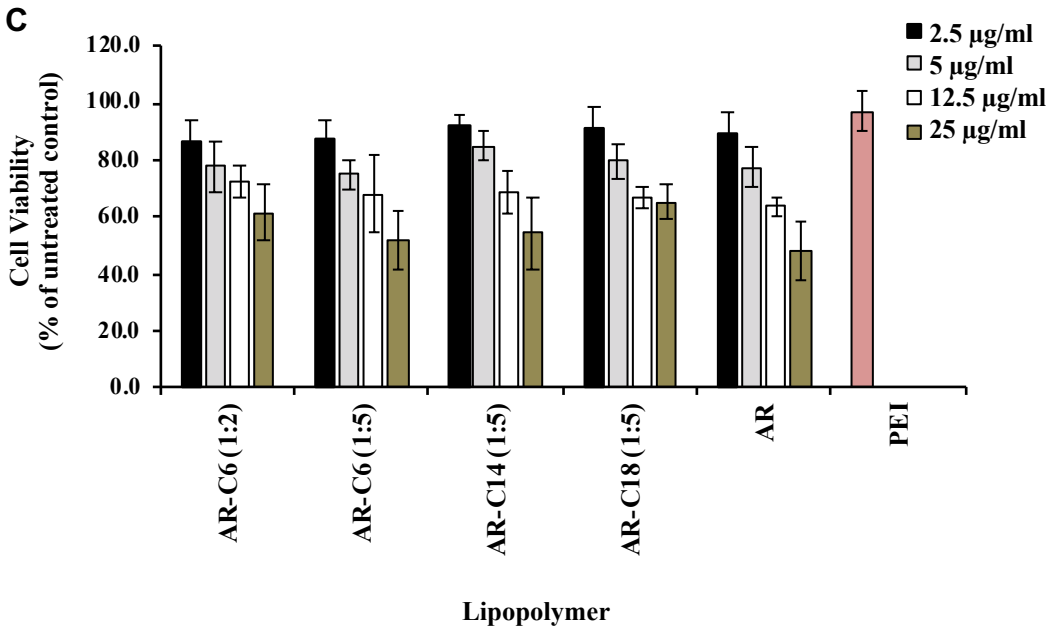


Figure S6. Cell viability of PC3 cells following treatment with different polymers (LPNs and parental polymers) at different doses i.e. 2.5, 5, 12.5, 25 µg/ml. Cell viability was determined using the MTT assay, and in all cases, compared to untreated cells as the control. Data represent mean ± one standard deviation of three independent experiments (n=3). Here polymer doses of 2.5, 5, 12.5 and 25 µg/ml are equivalent to the polymer:pDNA weight ratios of 5:1, 10:1, 25:1 and 50:1 respectively used in the transgene expression studies.The concentration of PEI was 0.5 µg/ml.

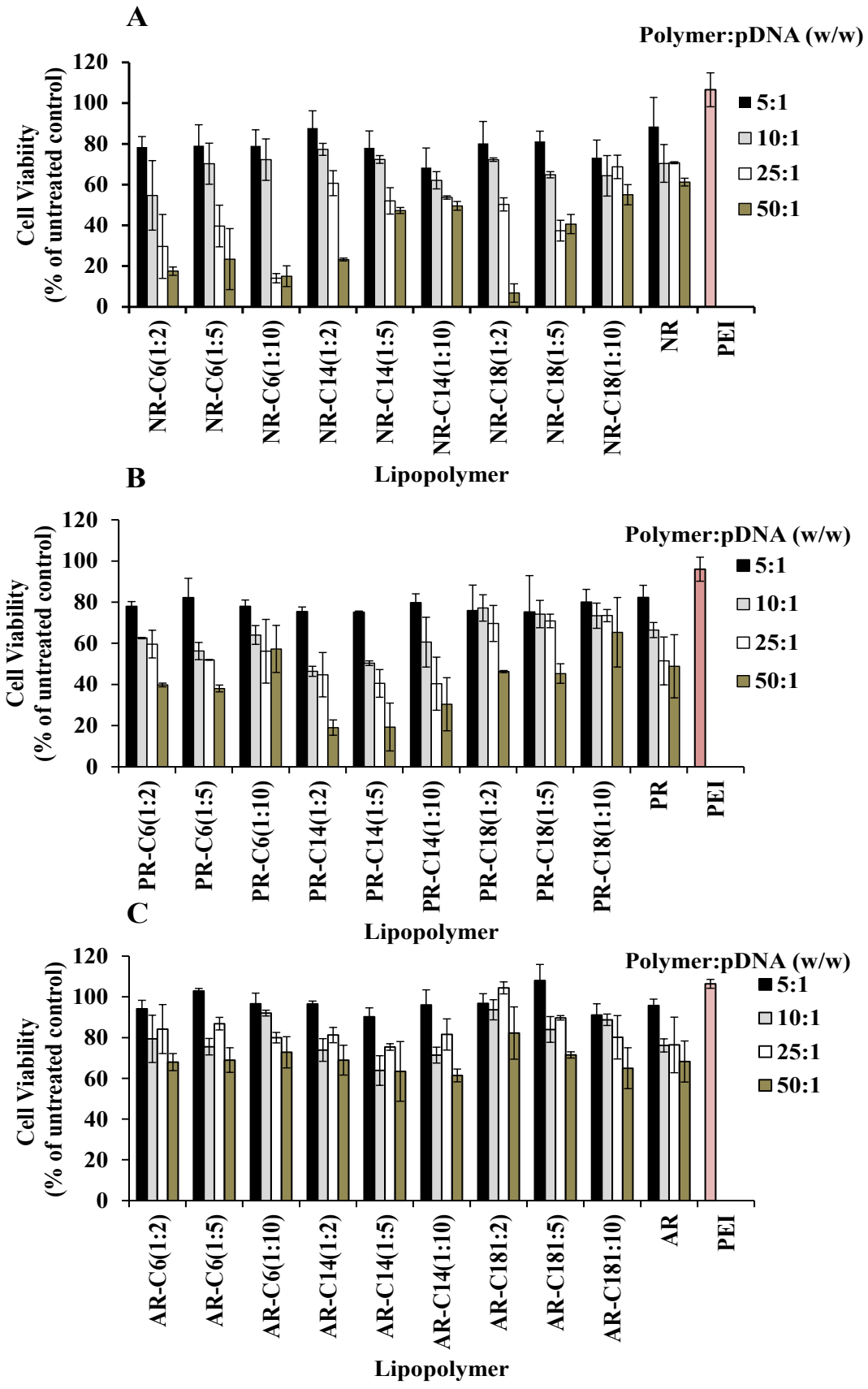

A
Polymer:pDNA (w/w)
5:1
10:1
25:1
50:1
Cell Viability
(% of untreated control)
120
100
80
60
40
20
0
NR-C6(1:2)
NR-C6(1:5)
NR-C6(1:10)
NR-C14(1:2)
NR-C14(1:5)
NR-C14(1:10)
NR-C18(1:2)
NR-C18(1:5)
NR-C18(1:10)
NR
PEI
Lipopolymer
B
Polymer:pDNA (w/w)
5:1
10:1
25:1
50:1
Cell Viability
(% of untreated control)
120
100
80
60
40
20
0
PR-C6(1:2)
PR-C6(1:5)
PR-C6(1:10)
PR-C14(1:2)
PR-C14(1:5)
PR-C14(1:10)
PR-C18(1:2)
PR-C18(1:5)
PR-C18(1:10)
PR
PEI
Lipopolymer
C
Polymer:pDNA (w/w)
5:1
10:1
25:1
50:1
Cell Viability
(% of untreated control)
120
100
80
60
40
20
0
AR-C6(1:2)
AR-C6(1:5)
AR-C6(1:10)
AR-C14(1:2)
AR-C14(1:5)
AR-C14(1:10)
AR-C181:2)
AR-C181:5)
AR-C181:10)
AR
PEI
Lipopolymer

Figure S7. Cell viability of PC3 cells following treatment with polyplexes generated using LPNs and parental polymers at different polymer:pDNA weight ratios i.e. 5:1, 10:1, 25:1 and 50:1. The highest efficacy, weight ratio (i.e. 1:1) was used in case of PEI (25 kDa). Cell viability was determined using the MTT assay, and in all cases, compared to untreated cells as the control. Data represent mean ± one standard deviation of three independent experiments (n=3).

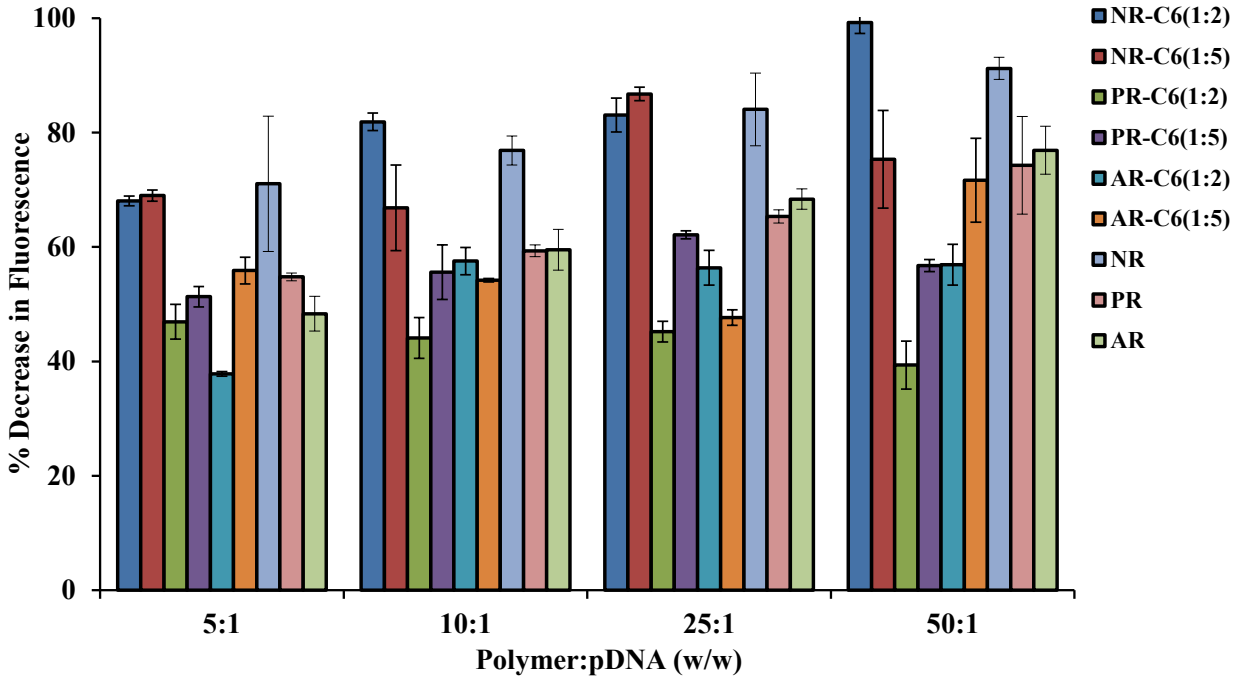


Figure S8. Plasmid DNA (pDNA) binding of lead LPNs and their respective parental polymers as assessed by the ethidium bromide (EtBr) displacement assay. The complexes were prepared at optimal weight ratios of lipopolymer to pGL4.5 pDNA in PBS (150 mM salt concentration, pH 7.4), and the extent of polymer binding was assessed in triplicate. Data represent mean ± one standard deviation of three independent experiments (n=3); higher % fluorescence decreased values indicate greater binding to DNA.

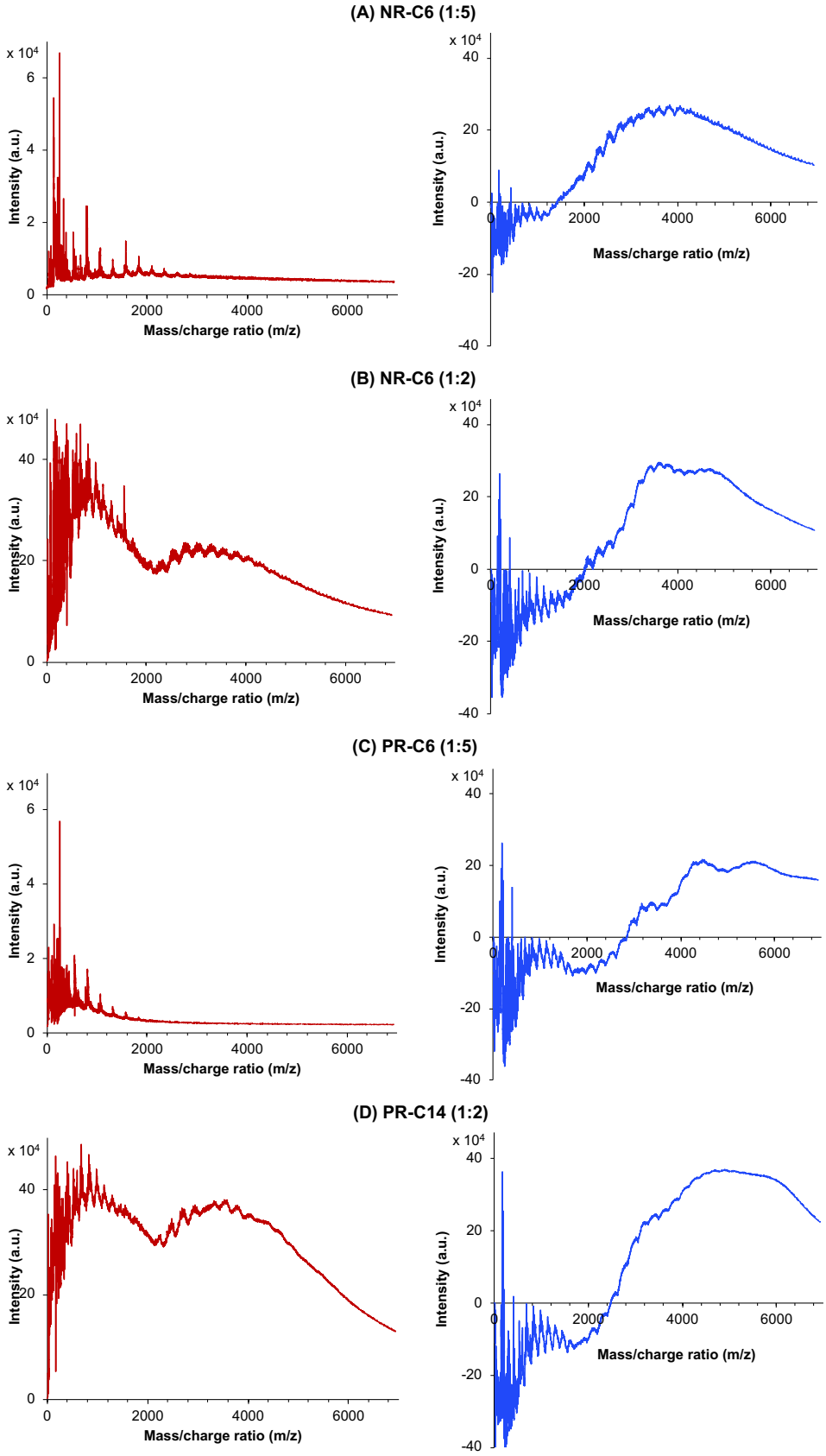
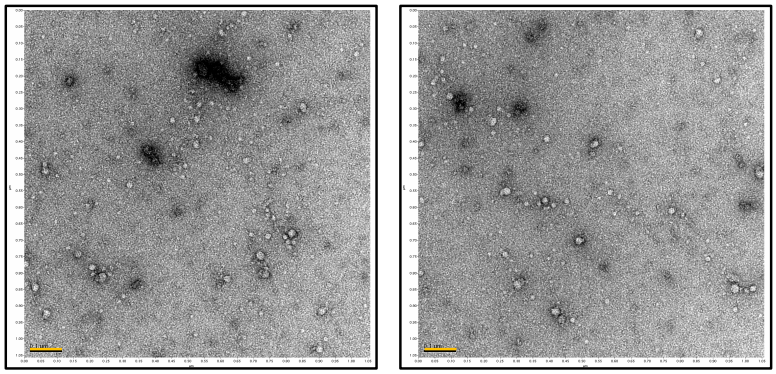

(A) NR-C6 (1:5)
x 10^4
Intensity (a.u.)
Mass/charge ratio (m/z)
(B) NR-C6 (1:2)
(C) PR-C6 (1:5)
(D) PR-C14 (1:2)

Figure S9. MALDI-TOF mass spectra of four lead lipopolymers: **(A)** NR-C6 (1:5), **(B)** NR-C6 (1:2), **(C)** PR-C6 (1:5), **(D)** PR-C14 (1:2) before dialysis (on the left & marked in red) and after dialysis (on the right & marked in blue). Results indicate that excess/unreacted lipid acid chlorides were removed with the dialysis process. Here y-axis of all the plots represent relative intensities (arbitrary units or a.u.) of the generated ions.

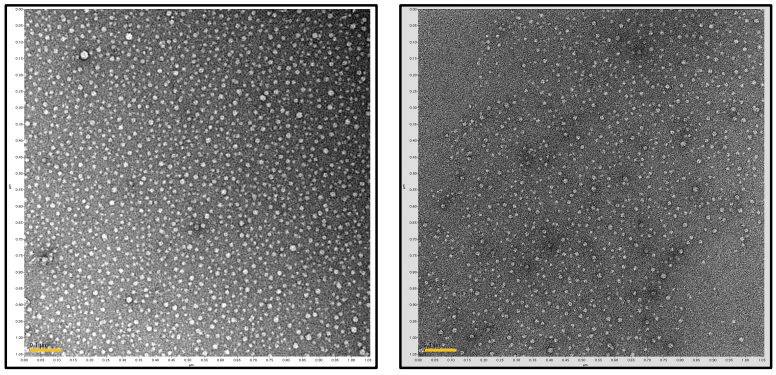
A.

PR-C6 (1:5) –Polymer alone



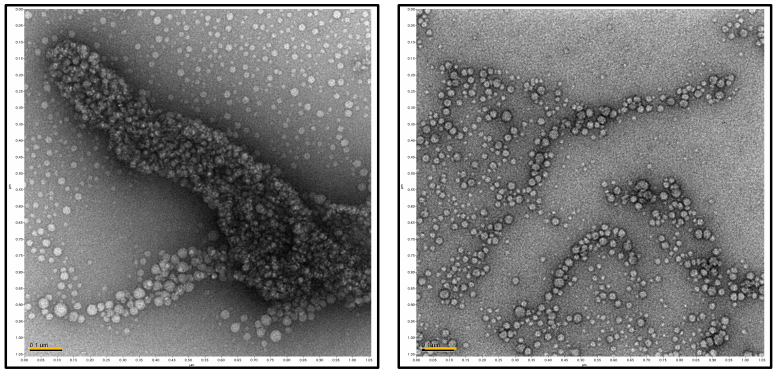
B.

PR-C6 (1:5) Polymer:pDNA – 5:1 ratio



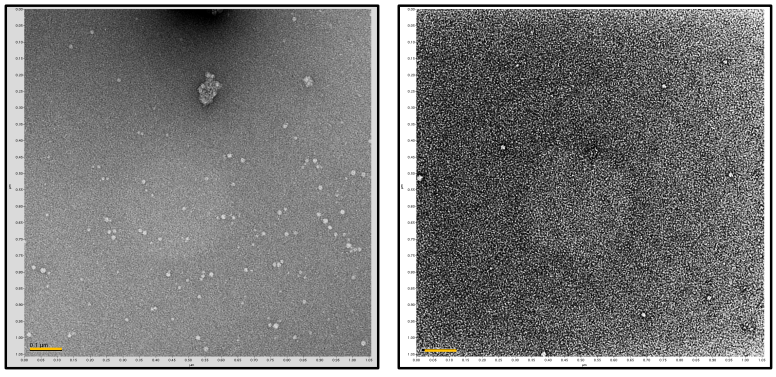
C.

PR-C6 (1:5) Polymer:pDNA – 25:1 ratio



D.

NR-C6 (1:2) –Polymer alone



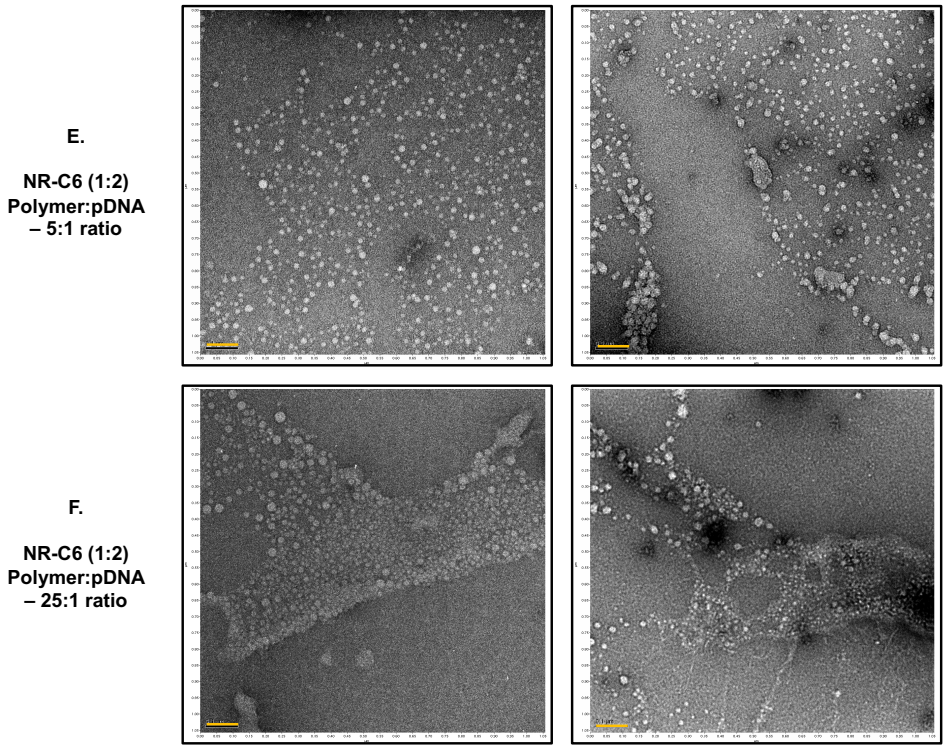


Figure S10. Additional representative electron micrographs of the negatively stained lead lipopolymers PR-C6 (1:5) and NR-C6 (1:2) and their respective lipopolyplexes at LPN:pDNA ratios of 5:1 and 25:1. White color indicates LPNs or LPN:pDNA complexes and black color indicates the background of the image. Scale bars (in yellow) represent 100 nm in all the images.