Supporting information

Poly(*N*-methyl-*N*-vinylacetamide): A strong alternative to PEG for Lipid-Based Nanocarriers delivering siRNA

Materials	2
Characterization	3
Figure S1. ^{1}H NMR spectra of OD-XA and OD-PNMVA$_{31}$.	4
Protocol 1. Synthesis of OD-PNMVA.	5
Scheme S1. General synthesis of the DSPE-PNMVA derivatives	6
Figure S2. ^{1}H NMR spectra of DSPE-PNMVA$_{50}$ and precursors.	7
Protocol 2. Synthesis of SC-PNMVA-XA.	8
Table S1. Synthesis and characteristics of SC-PNMVA-XA	8
Protocol 3. Synthesis of DSPE-PNMVA-XA	9
Table S2. Synthesis and characteristics of DSPE-PNMVA-XA.	9
Protocol 4. Synthesis of DSPE-PNMVA-H by removal of the terminal xanthate	10
Table S3. Characteristics of DSPE-PNMVA after removal of the terminal xanthate.	10
Figure S3. Overlay of the SEC-RI and SEC-UV traces of DSPE-PNMVA$_{53}$-XA before treatment with LPO.	11
Protocol 5. Synthesis of Et-PNMVA.	12
Figure S4. Typical QCM traces for the deposition of a DOTAP/Chol/DOPE (44.44%/33.33%/22.22%) membrane.	13
Figure S5. Δ*f* (a) and Δ*D* (b) traces from QCM-D monitoring of interaction of 30 µM DSPE-PNMVA$_{24}$ with a DOTAP/Chol/DOPE (44.44%/33.33%/22.22%) membrane.	14
Figure S6. Mouse whole-body biodistribution imaging of DSPE-PEG and DSPE-PNMVA$_{24}$ grafted-lipoplexes and PBS injected mice after 5h and 24h of the first and the second injection.	15

Materials.

1,2 distearoylphosphatidylethanolamine (DSPE)(Aldrich), magnesium sulfate (Abcr), ammonium chloride (Aldrich), lauroyl peroxide (Fluka), 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V70, t½ = 10 h at 30 °C) (Wako), hydrochloric acid (Acros), sodium phosphate monobasic monohydrate (Aldrich), sodium phosphate dibasic heptahydrate (Aldrich), sodium chloride (VWR), 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP) (Avanti polar lipids), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) (Avanti polar lipids), cholesterol (Aldrich), ammonium peroxide (Aldrich), 3-mercaptopropionic acid (MPA) (Aldrich), hydrogen peroxide (30%, Aldrich), sulfuric acid (95-97%, Merck), silica gel for column chromatography (60 Å, ROCC S.A.), triethylamine (99%, Acros organic), *n*-hexane (> 99%, VWR), ethyl acetate (≥ 99.9%, VWR), acetonitrile (≥ 99.99 %, Fisher), acetone (Fisher) and isopropanol (Fisher) were used as received. *N*-methyl-*N*-vinylacetamide (Aldrich) was dried over calcium hydride, degassed and distilled under reduced pressure prior to use. Tetrahydrofuran (THF, ≥ 99.9 %, VWR), dichloromethane (CH_2Cl_2, VWR) were dried over molecular sieves (4 Å) and degassed prior to use.

Ethyl-2-((ethoxycarbonothioyl)thio)propanoate (Et-XA), O-ethyl S-(1-(octadecylamino)-1-oxopropan-2-yl) carbonodithioate (OD-XA) and 2-((((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)oxy)ethyl 2-((ethoxycarbonothioyl) thio)propanoate (SC-XA) were prepared according to previously described procedures (Journal of Controlled Release, 2023, 361, 87–101).

QCM-D sensors were gold coated, AT-cut quartz crystals with a fundamental frequency of about 5 MHz (Q-Sense, Sweden).

Characterization.

Size exclusion chromatography (SEC) of the polymers was carried out in DMF containing 0.025 M of LiBr at 55 °C with a Waters chromatograph equipped with two columns (PSS GRAM 100Å), a dual λ absorbance detector (Waters 2487) and a refractive index detector (Waters 2414). The system was operated at a flow rate of 1 mL/min. The molar mass of the SC-PNMVA-XA was determined by SEC equipped with a multiangle laser light scattering (MALLs) detector in DMF/LiBr (0.025 M). The Wyatt MALLs detector (120 mW solid-state laser, k 1/4 658 nm, DawnHeleos S/N342-H) measures the excess Rayleigh ratio Rh (related to the scattered intensity) at different angles for each slice of the chromatogram. The specific refractive index increment (d*n*/d*c*) of polymer was measured by using a Wyatt Optilab refractive index detector (k 1/4 658 nm). Data were processed with the Astra V software (Wyatt Technology). The system was operated at a flow rate of 1 mL/min. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) were recorded at 298 K with a Bruker AVANCE III HD spectrometer (B_0 = 9.04 T) (400 MHz) and treated with MestreNova software.

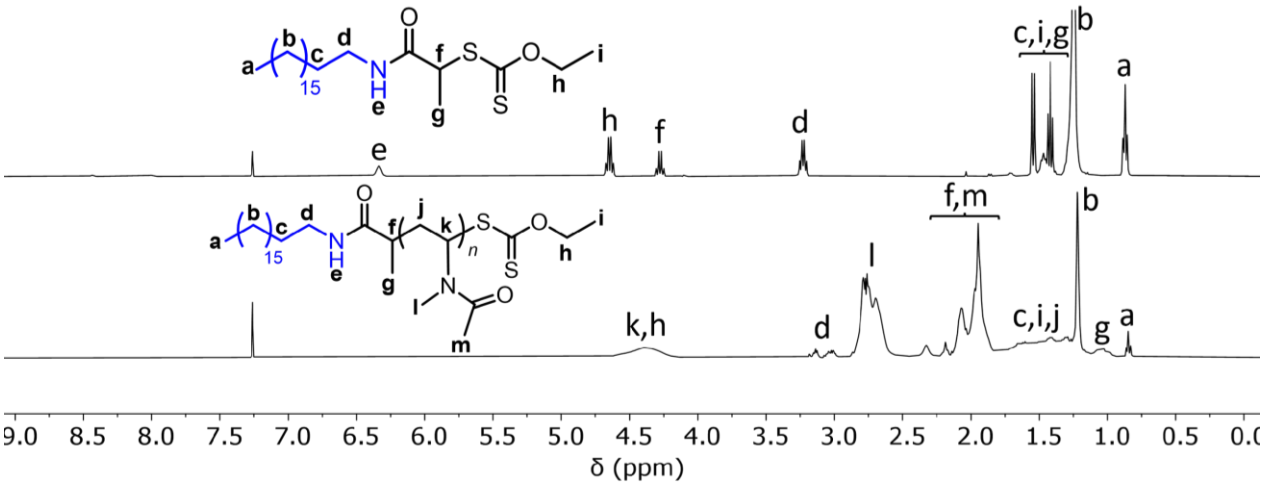
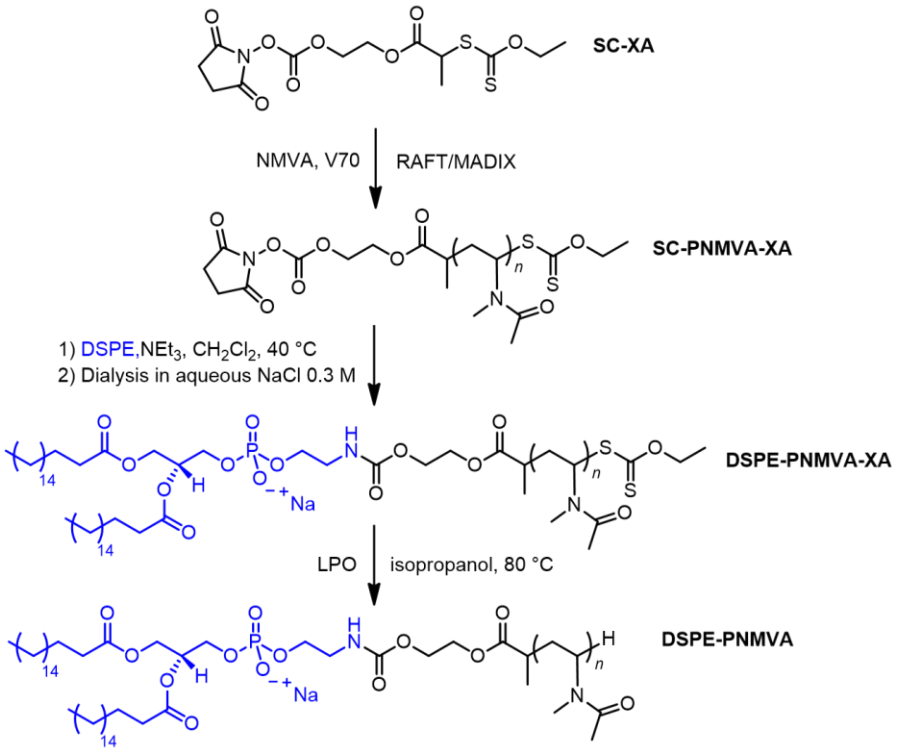


Figure S1. ^{1}H NMR spectra of OD-XA (upper spectrum) and OD-PNMVA$_{31}$ (lower spectrum).

Protocol 1. Synthesis of OD-PNMVA. OD-XA (703 mg, 1.58 mmol) and V70 (487 mg, 1.58 mmol) were placed under inert atmosphere in a Schenck tube and added with distilled, dried and degassed NMVA (7.8 g, 79 mmol). The reaction mixture was then stirred at 35 °C. After 6 h, the reaction was stopped and the monomer conversion measured by ^{1}H NMR in CD_2Cl_2 reached 50 %. The mixture was diluted in dichloromethane followed by precipitation in diethyl ether. The polymer was recovered by filtration, dried under vacuum at 40 °C for 12 h. The polymer was further purified by dialysis in acetone through a 1 kDa porous membrane for 24 h and in milliQ water through a 500 Da porous membrane for 12 h, followed by lyophilization. The desired OD-$PNMVA_{31}$ (0.98 g) was recovered as a pale yellow powder and characterized by SEC in DMF using a PS calibration ($M_{n\ SEC}$ = 1800 g/mol, $Đ$ =1.21) and ^{1}H NMR in CD_2Cl_2 ($M_{n\ NMR\ PNMVA}$ = 3100 g/mol, DP = 31).



Scheme S1. General synthesis of the DSPE-PNMVA derivatives.

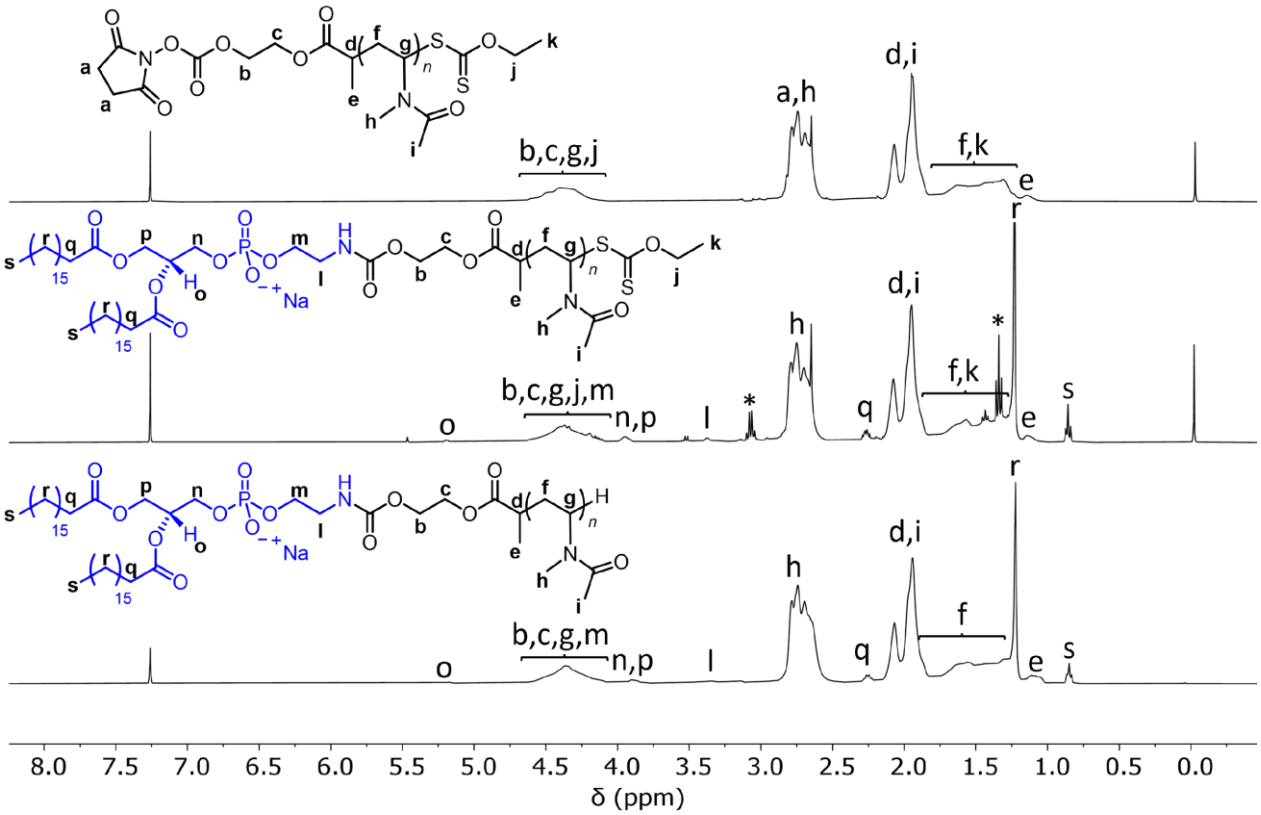


Figure S2. ^{1}H NMR spectra of DSPE-PNMVA$_{50}$ and precursors. (* = residual diethyl ether)

Protocol 2. Synthesis of SC-PNMVA-XA. SC-XA (519 mg, 1.36 mmol) and V70 (420 mg, 1.36 mmol) were placed a under inert atmosphere in a Schenck tube and added with distilled, dried and degassed NMVA (6.8 g, 68.1 mmol). The reaction mixture was then stirred at 35 °C. After 5 h, the reaction was stopped and the monomer conversion measured by ^{1}H NMR in $CDCl_3$ reached 70 %. The mixture was diluted in dichloromethane and the polymer was purified twice by precipitation in diethyl ether. After drying under vacuum at 40 °C for 24 h, SC-PNMVA$_{40}$-XA (2.71 g) was collected as a pale yellow powder and characterized by SEC in DMF using a PS calibration ($M_{n,\,SEC}$ = 3800 g/mol, $Đ$ = 1.20) and MALLS (dn/dc = 0.071) ($M_{n,\,MALLS}$ = 4300 g/mol, $Đ$ = 1.16). This procedure was adapted for the production of SC-PNMVA$_{30}$-XA.

Table S1. Synthesis and characteristics of SC-PNMVA-XA.

compounds	Time (h)	Conv.[a] (%)	$M_{n,th}$[b] (g/mol)	$M_{n,\ SEC}$[c] (g/mol)	$Đ$ [c]	$M_{n,\ MALLS}$[d] (g/mol)	$Đ$ [d]
SC-PNMVA$_{30}$-XA	4	65	3600	3000	1.12	3400	1.17
SC-PNMVA$_{40}$-XA	6.5	70	3800	3800	1.20	4300	1.16

Conditions: $[NMVA]_0/[SC\text{-}XA]_0/[V70]_0$ = 50/1/1, 35 °C, in bulk. [a] Determined by ^{1}H NMR in $CDCl_3$ on the crude mixture by integrating the signals of the methyl group of the monomer (at 3.08 ppm, 3H) and the signals of the methyl group of the polymer (at 2.73 ppm, 3H). [b] $M_{n,\ th} = [NMVA]_0/[CTA]_0 \times conv. \times MM_{NMVA} + MM_{SC\text{-}XA}$. [c] $M_{n,\ SEC}$ determined by SEC DMF/LiBr (PS calibration) on the crude mixture. [d] Determined by MALLS in DMF/LiBr (dn/dc = 0.071 mL.g^{-1}) after purification of the polymer by precipitation two times in diethyl ether and drying under vacuum at 40 °C.

Protocol 3. Synthesis of DSPE-PNMVA-XA. SC-PNMVA$_{40}$-XA (2.0 g, 5.88 mmol), DSPE (440 mg, 5.88mmol) and triethylamine (2.5 g, 24.57 mmol) were placed in a 25 mL round-bottom flask under nitrogen atmosphere and dissolved in dried and degassed dichloromethane (10 mL). The mixture was then stirred at 40 °C for 1 h. The solvent was then evaporated and the residue dissolved in acetonitrile (12 mL) and stored at 6 °C overnight. The solution was then centrifugated two times (10 000 rpm, 15 min, 6 °C). The supernatant was collected and after solvent evaporation, the desired DSPE-PNMVA$_{53}$-XA (1.8 g) was obtained as a white powder and characterized by SEC in DMF using a PS calibration ($M_{n,\ SEC}$ = 5000 g/mol, $Đ$ = 1.12) and ^{1}H NMR in $CDCl_3$ ($M_{n,\ NMR}$ = 5300 g/mol, DP = 53). This procedure was adapted for the production of DSPE-PNMVA$_{35}$-XA.

Table S2. Synthesis and characteristics of DSPE-PNMVA-XA.

Final compound	SC-PNMVA precursor	$M_{n,\,NMR}$ [a] (g/mol)	$Đ$ [b]	DP [c]
DSPE-PNMVA$_{35}$-XA	SC-PNMVA$_{30}$-XA	3400	1.12	35
DSPE-PNMVA$_{53}$-XA	SC-PNMVA$_{40}$-XA	5300	1.12	53

Conditions : $[SC\text{-}PNMVA\text{-}XA]_0/[DSPE]_0/[NEt_3]_0$ = 1/1/4.2, 40 °C, 1 h. [a] $M_{n,\,NMR}$ of PNMVA determined by ^{1}H NMR in $CDCl_3$ on the purified polymer based on the relative intensities of the signal of the α-terminal methyl groups (at 0.87 ppm, 6H) and the methyl signal of PNMVA (2.5-2.9 ppm, 3H). [b] Determined by SEC DMF/LiBr (PS calibration) after purification of the polymer by dissolution in acetonitrile, centrifugation and solvent evaporation. [c] Degree of polymerization of the PNMVA segment deduced from the $M_{n,\,NMR}$.

Protocol 4. Synthesis of DSPE-PNMVA-H by removal of the terminal xanthate. The above mentioned DSPE-PNMVA$_{53}$-XA (1.2 g, 0.2 mmol) and LPO (33.5 mg, 0.084 mmol) were placed under inert atmosphere in a 10 mL round-bottom flask and dissolved in degassed isopropanol (5 mL). The mixture was then stirred at 80 °C for 8 h, a period during which LPO portions (16.8 mg, 0.042 mmol) were added every 2 h. The mixture was stirred for at 80 °C for an additional 16 h. The polymer was recovered by precipitation in *n*-hexane, dried under vacuum at 40 °C for 12 h and dialyzed through a 1 kDa porous membrane sequentially against an aqueous sodium chloride solution (0.3 M) for 12 h and against water for 12 h. After lyophilization, the desired DSPE-PNMVA$_{50}$-H was collected as a white powder (480 mg). The absence of the xanthate was confirmed by SEC-UV by the disappearance of the xanthate's characteristic absorption signal at 290 nm (**Figure S3**). This procedure was also applied to DSPE-PNMVA$_{35}$-XA leading to DSPE-PNMVA$_{24}$-H (**Table S3**).

Table S3. characteristics of DSPE-PNMVA after removal of the terminal xanthate.

Final compound	DSPE-PNMVA-XA precursor	$M_{n,\,SEC}$ [a] (g/mol)	$Đ$ [a]	$M_{n,\,NMR}$ [b] (g/mol)	DP [c]
DSPE-PNMVA$_{50}$-H	DSPE-PNMVA$_{53}$-XA	2800	1.27	5000	50
DSPE-PNMVA$_{24}$-H	DSPE-PNMVA$_{35}$-XA	1800	1.28	2400	24

Conditions : $[DSPE\text{-}PNMVA\text{-}XA]_0/[LPO]_0$ = 1/1.2, 80 °C in isopropanol, 24 h. [a] Determined by SEC DMF/LiBr (PS calibration) after purification of the polymer by precipitation in *n*-hexane and successive dialysis in water and 0.3 M NaCl. [c] $M_{n,\,NMR}$ of PNMVA determined by ^{1}H NMR in $CDCl_3$ on the purified polymer based on the relative intensities of the signal of the α-terminal methyl groups (at 0.87 ppm, 6H) and the methine signal of PNMVA (4.0-4.7 ppm, 1H). [c] Degree of polymerization of the PNMVA segment deduced from the $M_{n,\,NMR}$.

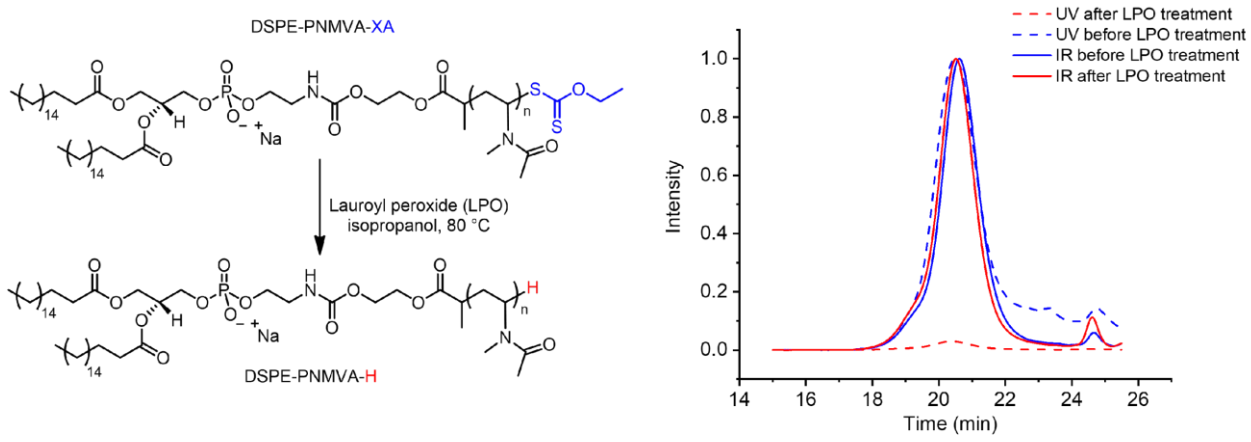


Figure S3. Overlay of the SEC-RI (solid traces) and SEC-UV (dashed traces, λ = 290 nm) of $DSPE\text{-}PNMVA_{53}$-XA before treatment with LPO (blue) and the resulting $DSPE\text{-}PNMVA_{50}$-H obtained after treatment.

Protocol 5. Synthesis of Et-PNMVA. Et-XA was synthesized according to previously reported procedures (*RSC Adv.*, **2015**, 5, 42388-42398 ; *Polymers*, **2022**, 14(4), 70). Then, Et-XA (302 mg, 1.36 mmol) and V70 (420 mg, 1.36 mmol) were placed under inert atmosphere in a Schenck tube

and added with distilled and degassed NMVA (6.75 g, 68.1 mmol). The reaction mixture was then stirred at 35 °C. After 5 h, the monomer conversion measured by ^{1}H NMR in CD_2Cl_2 reached 37 %. The mixture was diluted in dichloromethane followed by two precipitations in diethyl ether. The polymer was recovered by centrifugation (1000 rpm, 6°C, 10 min) and dried under vacuum at 40 °C for 12 h. The polymer was further purified by dialysis in milliQ water through a 500 Da porous membrane for 24 h followed by lyophilization. The desired Et-PNMVA$_{27}$-XA (434 mg) was recovered as a pale yellow powder and characterized by SEC in DMF using a PS calibration ($M_{n,\,SEC}$ = 7800 g/mol, $Đ$ = 1.23) and ^{1}H NMR in CD_2Cl_2 ($M_{n,\,NMR\ PNMVA}$ = 2700 g/mol , DP = 27).

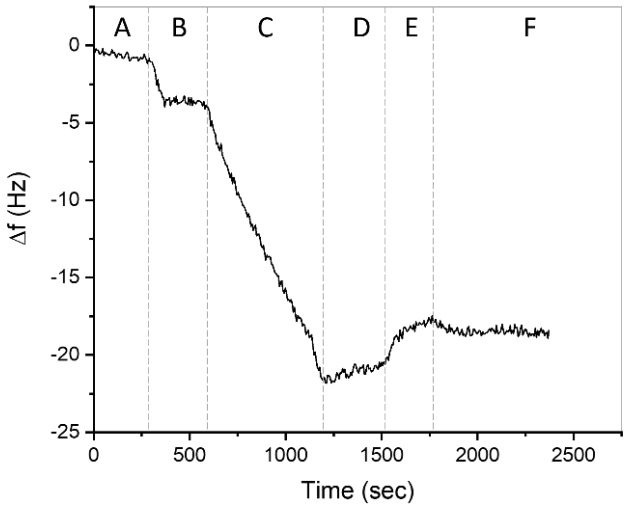


Figure S4. Typical QCM traces for the deposition of a DOTAP/Chol/DOPE (44.44%/33.33%/22.22%) membrane at 25 °C (7th harmonic): **A** = water baseline (300 µL/min), **B**-**D**-**F**: High salt BPS buffer baselines (300 µL/min), **C**: Liposomes introduction (50 µL/min), **E**: Low salt PBS buffer for liposome rupture (300 µL/min).

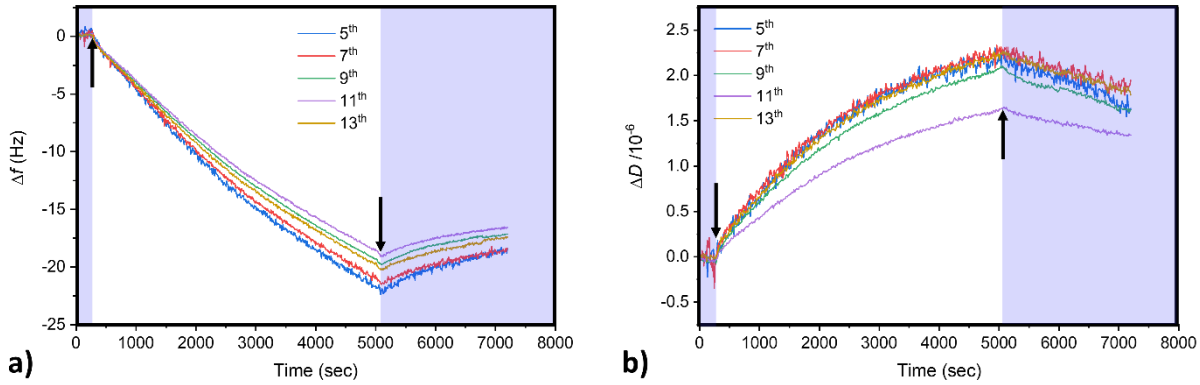


Figure S5. *Δf* (a) and *ΔD* (b) traces from QCM-D monitoring of interaction of 30 µM DSPE-PNMVA$_{24}$ with a DOTAP/Chol/DOPE(44.44%/33.33%/22.22%) membrane at 25 °C at different harmonics. Arrows designate the polymer solution addition and the final rising with buffer. Blue regions correspond to buffer elution periods.

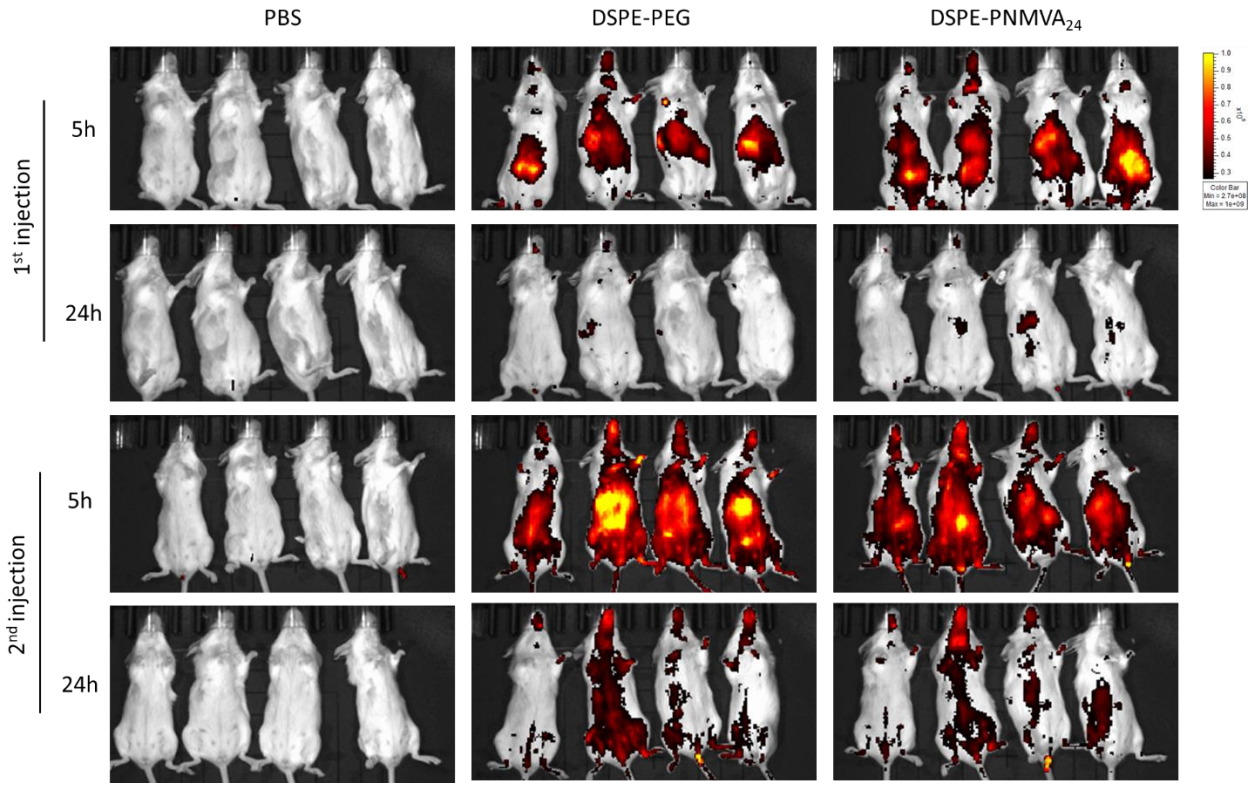


Figure S6. Mouse whole-body biodistribution imaging of DSPE-PEG and DSPE-PNMVA$_{24}$ grafted-lipoplexes and PBS injected mice after 5h and 24h of the first and the second injection.