SUPPORTING INFORMATION

Polysarcosine-Functionalized Lipid Nanoparticles for Therapeutic mRNA Delivery

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Supporting data

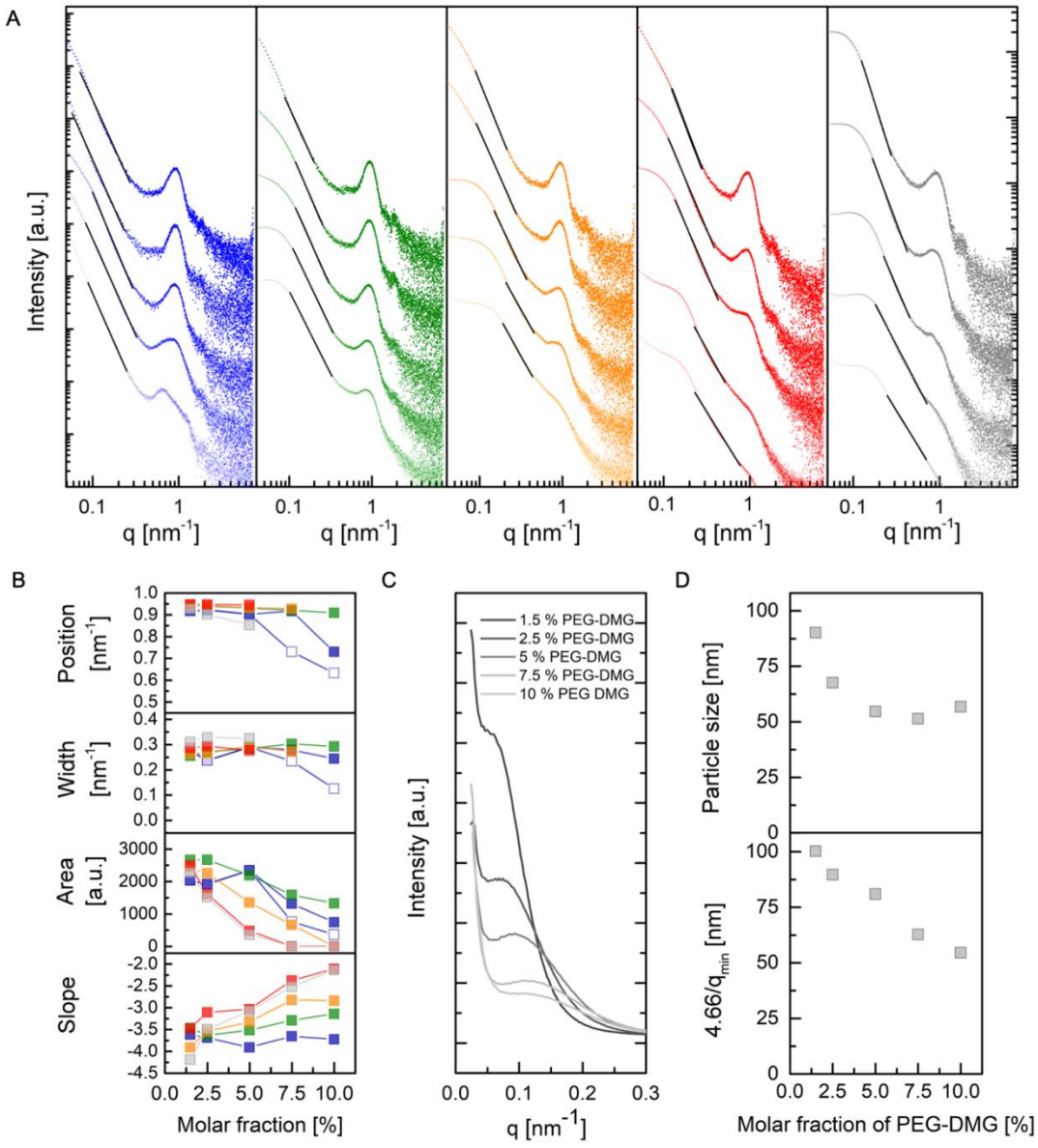

Figure S1 General overview of SAXS data from all systems. A) $pSar_x$ LNPs, from left to right $pSar_{11}$ (blue), $pSar_{23}$ (green), $pSar_{34}$ (orange), $pSar_{65}$ (red), and PEG-DMG (grey). For clarity the data sets are multiplied by constant factors. The fraction of grafted lipid increases from top to bottom (1.5, 2.5, 5, 7.5 and 10%). Slopes according to Porod power law (**Equation 4**) were fitted the q range between 0.02 and 0.6 nm^{-1} and are drawn as solid lines. B) Peak position, peak width, peak area and Porod slope as a function of the increased molar fraction of $pSar_x$, and PEG-DMG. For $pSar_{11}$ a peak splitting at higher molar fractions was considered (open and closed blue squares). C) Scattering curves from PEG-DMG formulations in the q range between 0 to 0.3 nm^{-1}. D) Particle size from dynamic light scattering measurements (top) and reciprocal q_{min} value, obtained from the curves in C, as a function of the PEG-DMG molar fraction. For ideal solid spheres the maximum and minimum positions would allow direct calculation of the diameter (**Equation 3**), for example the first minimum should be at r*q = 4.66.

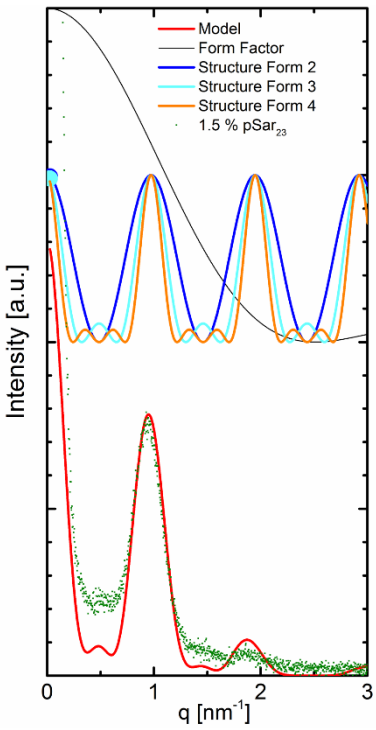

Figure S2 Simulation and experimental data in linear scale. Coloured curves on top show structure factors for a repeat distance of 6.45 nm with for 2 (blue), 3 (light blue) and 4 (orange) repeat units according to **Equation 8**. For clarity, data is scaled to the same maximum values. The upper black curve shows a form factor resulting from a uniform scatterer of 2.5 nm length according to **Equation 7**. For the red curve, the form factors for 2 and 3 units were averaged without weighing and multiplied by the structure factor. The green dotted data represents the measurement of the LNP formulated with 1.5% $pSar_{23}$. Several key aspects of the experimental curve shape, e.g. peak position and width are qualitatively represented by this simplified simulation.

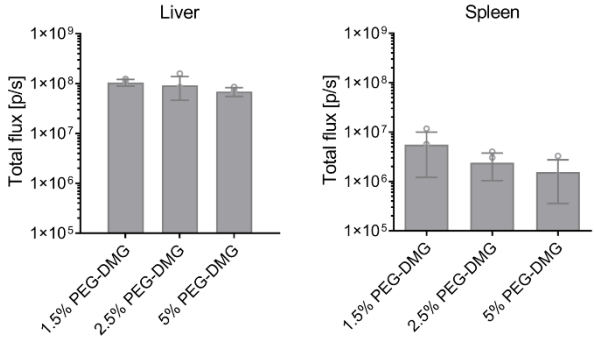

Figure S3. Luciferase expression upon IV injection of PEG LNPs. Transfection in Balb/C mice treated with a luciferase mRNA dose of 10 µg administered through an intravenous injection of PEG LNPs containing DODMA as the ionizable lipid. Luciferase expression in the liver and spleen 6 h post-injection is reported as the mean of total flux (p/s) ± standard deviation, for n=3. Increasing molar fractions of PEG lipid resulted in similar *in vivo* expression. The trend for slight decrease in *in vivo* performance in the spleen is not significant.

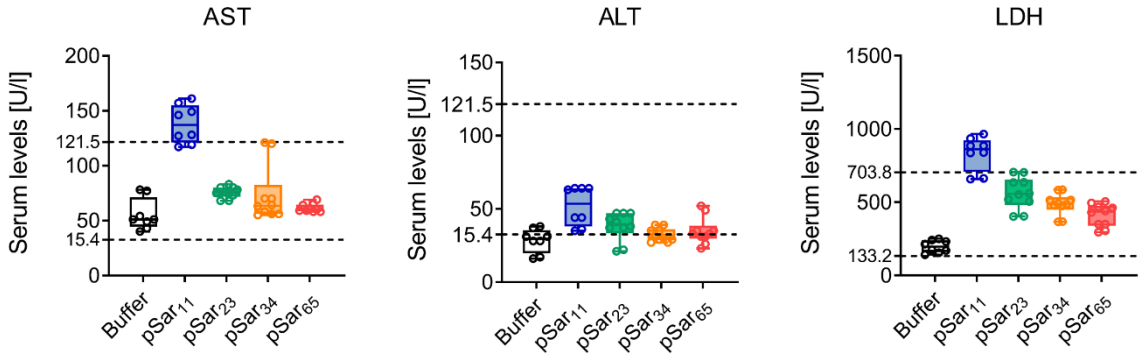

Figure S4. Effect of pSar chain length on liver enzyme release profile. AST, ALT and, LDH concentrations were measured 6 h post-injection of 10 µg of luciferase encoding-mRNA formulated in LNPs (DODMA) with 5 mol % pSar lipid with different chain length as indicated. Vehicle buffer (PBS) was used as control. Box plots show data as a mean ± standard deviation, for n=3. These results showed that upon administration of pSar LNPs, the enzymes levels are within the reference values obtained from several healthy mice (dot line). Only $pSar_{11}$ resulted in slightly higher liver enzyme values than the control groups.

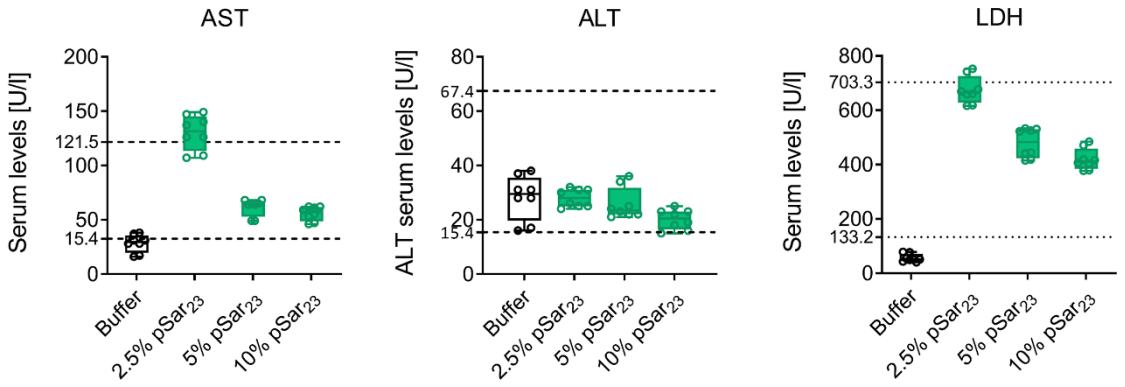

Figure S5. Effect of pSar molar faction on liver enzyme release profile. AST, ALT, LDH concentrations were measured 6 h post-injection of 10 µg of luciferase encoding-mRNA formulated within pSar-LNPs containing DODMA as the ionizable lipid. $pSar_{23}$-LNPs was intravenously administered with increased molar fraction (2.5-10%). Vehicle buffer (PBS) was used as control. Box plots show data as a mean with ± standard deviation, for n=3. These results showed that upon administration of pSar LNPs with varied molar fraction, the enzymes levels are within the reference values obtained from several healthy mice (dot line). Only in the case of 2.5 % of $pSar_{23}$ mediated delivery slightly higher AST values were observed compared to healthy mice.

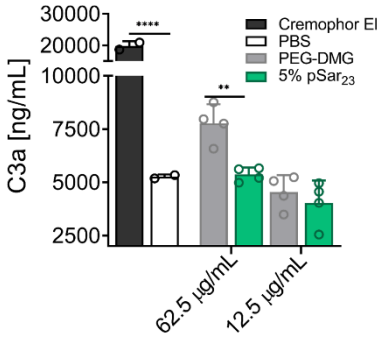

Figure S6. **Concentrations of C3a post incubation of $pSar_{23}$ and PEG LNPs. with human serum**. DODMA was used as the ionizable lipid for both LNP formulations. Data was taken 1 hour post-incubation at 37°C. LNPs concentrations were calculated as the nanoparticle theoretical plasma concentration, which derives from the ratio between the human dose and the human blood volume. This concentration provides a rough estimation of maximum concentration of LNP in the human blood. On average, a human with a body weight of 70 kg body weight has approximately 5.6 L of blood. Therefore, based on a human dose of 1 mg/kg, the theoretical plasma concentration (i.e., the *in vitro* testing concentration) is equivalent to an mRNA concentration of 12.5 µg/mL, with the 5 times higher concentration being equivalent to 62.5 µg/ml. Vehicle buffer (PBS) and Cremophor El were used as negative and positive control, respectively. Data is shown as mean ± standard deviation, n=2-3. Statistical significance was calculated with one-way ANOVA with multiple comparisons (* $p< 0.05$; ** $p<0.01$; *** $p<0.001$). LNP containing 5 % $pSar_{23}$ induced lower C3a levels when compared to PEG-DMG at high dose (62.5 µg/ml). This finding suggested that pSar LNP are likely less toxic than PEG LNP.

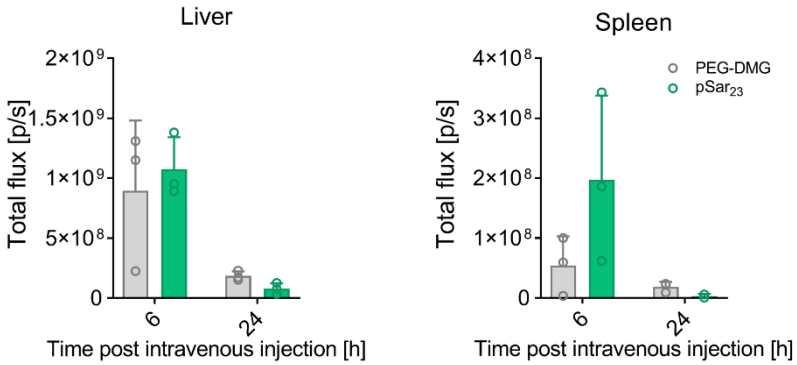

Figure S7. Luciferase expression of $pSar_{23}$ and PEG formulations containing DPL14 as the ionizable lipid. Luciferase expression in the liver and spleen 6 h post-injection of mRNA-loaded LNPs comprising of either 5% of $pSar_{23}$ or 1.5 % PEG-DMG at a dose of 2 µg mRNA. The mean of total flux (p/s) ± standard deviation for n=3 are reported. Total *in vivo* luciferase expression from pSar and PEG LNPs in liver and spleen is considered similar.

Table S1. Quantitative analysis of the peak as a function of pSar and PEG-DMG molar fraction.

Lipid Composition	Molar Composition [mol %]	Cationic lipid [mg]	Helper lipid [mg]	Chol [mg]	Stealth lipid [mg]	mRNA [mg]	N/P	Weight/weight ratio (ww)	Particle size [dm]	SD	PdI	mRNA accessibility [%]	SD
DODMA:Chol:DSPC:pSar11	40:48.5:10:1.5	0,84	0,27	0,63	0,05	0,113	4	15,9				70,4	4,7
DODMA:Chol:DSPC:pSar11	40:47.5:10:2.5	0,84	0,27	0,62	0,08	0,113	4	16,7	237,6	-	0,095	67,8	3,9
DODMA:Chol:DSPC:pSar11	40:45:10:5	0,84	0,27	0,59	0,17	0,113	4	16,5	134,3	9,8	0,251	74,2	2,7
DODMA:Chol:DSPC:pSar11	40:42.5:10:7.5	0,84	0,27	0,55	0,25	0,113	4	17,0	131,2	0,2	0,218	76,7	4,4
DODMA:Chol:DSPC:pSar11	40:45:10:10	0,84	0,27	0,52	0,34	0,113	4	17,4	127,0	26,9	0,257	79,5	1,8
DODMA:Chol:DSPC:pSar23	40:48.5:10:1.5	0,84	0,27	0,63	0,09	0,113	4	16,2	243,8	104,9	0,256	69,9	6,7
DODMA:Chol:DSPC:pSar23	40:47.5:10:2.5	0,84	0,27	0,62	0,16	0,113	4	16,7	134,9	27,5	0,242	74,3	5,6
DODMA:Chol:DSPC:pSar23	40:45:10:5	0,84	0,27	0,59	0,31	0,113	4	17,8	88,2	12,0	0,165	77,8	5,2
DODMA:Chol:DSPC:pSar23	40:42.5:10:7.5	0,84	0,27	0,55	0,47	0,113	4	18,9	80,5	15,8	0,213	78,8	6,9
DODMA:Chol:DSPC:pSar23	40:45:10:10	0,84	0,27	0,52	0,62	0,113	4	20,0	75,2	10,1	0,192	81,7	1,9
DODMA:Chol:DSPC:pSar34	40:48.5:10:1.5	0,84	0,27	0,63	0,13	0,113	4	16,6	159,0	59,5	0,272	74,5	10,0
DODMA:Chol:DSPC:pSar34	40:47.5:10:2.5	0,84	0,27	0,62	0,22	0,113	4	17,3	161,5	18,7	0,216	74,0	10,3
DODMA:Chol:DSPC:pSar34	40:45:10:5	0,84	0,27	0,59	0,44	0,113	4	19,0	92,1	15,6	0,202	83,1	2,5
DODMA:Chol:DSPC:pSar34	40:42.5:10:7.5	0,84	0,27	0,55	0,67	0,113	4	20,6	86,6	4,8	0,199	84,8	4,1
DODMA:Chol:DSPC:pSar34	40:45:10:10	0,84	0,27	0,52	0,89	0,113	4	22,3	65,9	7,5	0,187	85,1	4,5
DODMA:Chol:DSPC:pSar65	40:48.5:10:1.5	0,84	0,27	0,63	0,24	0,113	4	17,6	145,8	21,3	0,244	73,1	11,2
DODMA:Chol:DSPC:pSar65	40:47.5:10:2.5	0,84	0,27	0,62	0,41	0,113	4	18,9	106,0	15,3	0,283	77,8	7,4
DODMA:Chol:DSPC:pSar65	40:45:10:5	0,84	0,27	0,59	0,82	0,113	4	22,3	91,4	27,9	0,246	88,8	0,5
DODMA:Chol:DSPC:pSar65	40:42.5:10:7.5	0,84	0,27	0,55	1,22	0,113	4	25,6	84,7	28,1	0,231	90,7	0,4
DODMA:Chol:DSPC:pSar65	40:45:10:10	0,84	0,27	0,52	1,63	0,113	4	28,9	70,8	24,2	0,194	85,1	10,2
DODMA:Chol:DSPC:PEG-DMG	40:48.5:10:1.5	0,84	0,27	0,63	0,13	0,113	4	16,5	90,1	2,4	0,111	7,1	0,3
DODMA:Chol:DSPC:PEG-DMG	40:47.5:10:2.5	0,84	0,27	0,62	0,21	0,113	4	17,2	67,6	0,8	0,135	6,2	0,2
DODMA:Chol:DSPC:PEG-DMG	40:45:10:5	0,84	0,27	0,59	0,42	0,113	4	18,7	54,6	0,6	0,133	24,5	0,2
DODMA:Chol:DSPC:PEG-DMG	40:42.5:10:7.5	0,84	0,27	0,55	0,63	0,113	4	20,3	51,4	0,8	0,217	47,2	1,1
DODMA:Chol:DSPC:PEG-DMG	40:45:10:10	0,84	0,27	0,52	0,83	0,113	4	21,9	56,7	1,0	0,374	58,5	0,6

Table S2. Quantitative analysis of the peak as a function of pSar and PEG-DMG molar fraction

Lipid	Molar fraction [%]	Peak analysis														
		Position1 [x_c]	σ	Position2 [x_c]	σ	Width1 [w]	σ	Width2 [w]	σ	Area1 [A]	σ	Area2 [A]	σ	Adj. R-square	d-spacing [nm]	Corr length [nm]
$pSar_{11}$	1,5	0,917	1,34E-03			0,275	0,004			2024	20,2			0,96	6,85	7,3
	2,5	0,921	1,68E-03			0,236	0,005			1911	27,8			0,94	6,82	8,5
	5	0,902	1,70E-03			0,290	0,005			2340	30,5			0,95	6,97	6,9
	7,5	0,917	4,65E-03	0,72945	0,0052	0,191	0,278	0,23364	0,01401	1312,5	80,6	755,28	76,9	0,93	6,39	10,5
	10	0,729	1,71E-02	0,63181	0,0055	0,244	0,017	0,12493	0,02299	738,6165	157,6	359,29	136,0	0,91	6,41	12
$pSar_{23}$	1,5	0,944	1,04E-02			0,255	0,004			2661	30,1			0,96	6,66	7,9
	2,5	0,939	6,10E-03			0,271	0,005			2664	33,0			0,95	6,69	7,4
	5	0,930	1,76E-03			0,283	0,005			2187	28,9			0,94	6,76	7,1
	7,5	0,920	1,74E-03			0,302	0,005			1582	20,1			0,94	6,83	6,6
	10	0,908	2,17E-03			0,292	0,007			1321	21,6			0,92	6,92	6,9
$pSar_{34}$	1,5	0,943	1,48E-03			0,264	0,004			2397	28,4			0,96	6,66	7,6
	2,5	0,940	1,79E-03			0,266	0,005			2249	31,1			0,94	6,68	7,5
	5	0,932	1,94E-03			0,294	0,006			1344	19,2			0,93	6,74	6,8
	7,5	0,927	2,61E-03			0,272	0,008			661	14,0			0,87	6,78	7,3
	10									0						
$pSar_{65}$	1,5	0,947	1,66E-03			0,287	0,005			2486	30,5			0,95	6,63	7
	2,5	0,946	2,07E-03			0,293	0,006			1605	24,4			0,93	6,64	6,8
	5	0,945	2,83E-03			0,276	0,009			475	10,5			0,86	6,65	7,3
	7,5									0						
	10									0						
PEG-DMG	1,5	0,927	1,90E-03			0,310	0,006			2268	29,5			0,94	6,77	6,4
	2,5	0,901	2,11E-03			0,328	0,007			1508	21,3				6,97	6,1
	5	0,853	5,91E-03			0.324	0,020			358,4	16,2			0,58	7.37	6.2
	7,5									0						
	10									0						

Table S3. Physicochemical characterization of $pSar_{23}$-LNPs comprising DODMA, Dlin-MC3-DMA, and DPL14 ionizable cationic lipids.

Formulation		N/P	Weight/weight ratio (ww)	Particle Size [nm]	PdI	RNA accessibility [%]
Lipids	Molar fraction [%]					
DODMA:Chol:DSPC:$pSar_{23}$	40:45:10:5	4	17,8	84	0,151	79,8
MC3: Chol:DSPC:$pSar_{23}$	40:45:10:5	4	18,1	82,3	0,15	35,7
DPL14: Chol:DSPC:$pSar_{23}$	40:45:10:5	4	18,1	79,1	0,135	51,1

Formulation		N/P	Weight/weight ratio (ww)	Particle Size [nm]	PdI	Zeta Potential [µm.cm/V.s]	RNA accessibility [%]
Lipids	Molar fraction [%]						
DPL14:Chol:DSPC:PEG-DMG	40:48.5:10:1.5	4	16,8	87	0,196	2,48	5,6
DPL14: Chol:DSPC:$pSar_{23}$	40:45:10:5	4	18,8	85	0,166	2,6	47,9