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Supplemental information

Lipid nanoparticle mRNA systems containing high levels of sphingomyelin engender higher protein expression in hepatic and extra-hepatic tissues

Nisha Chander, Genc Basha, Miffy Hok Yan Cheng, Dominik Witzigmann, and Pieter R. Cullis

Supplemental Information

Table S1. LNP luc mRNA systems containing high ESM helper lipid contents can be formulated using ethanol dilution/rapid mixing techniques and exhibit high mRNA encapsulation efficiencies and low polydispersity indices.

Sample	Lipid composition (mol%)				%EE	Sizing data	
	MC3	Cholesterol	Helper lipid (ESM)	Peg DMG		Number (nm)	PDI
ESM-10	50	38.5	10	1.5	98 ± 1.1	52 ± 3.9	0.04
ESM-20	44.25	34.25	20	1.5	96 ± 2.0	48 ± 2.1	0.11
ESM-30	38.5	30	30	1.5	95 ± 2.1	47 ± 2.7	0.09
ESM-40	33	25.5	40	1.5	97 ± 1.1	44 ± 1.6	0.08
ESM-50	27.25	21.25	50	1.5	88 ± 4.2	52 ± 4.2	0.10
ESM-55	24.5	19	55	1.5	53 ± 3.1	69 ± 2.2	0.19

Table S2. Formulation at 10% and 40% DSPC as helper content at the expense of ionizable lipid and cholesterol (keeping the ionizable lipid/cholesterol ratio constant) on mRNA encapsulation efficiencies and LNP size.

Sample	Lipid composition (mol%)				%EE	Sizing data	
	MC3	Cholesterol	Helper lipid (DSPC)	Peg DMG		Number (nm)	PDI
DSPC-10	50	38.5	10	1.5	99 ± 1.2	54 ± 2.1	0.09
DSPC-40	33	25.5	40	1.5	97 ± 2.2	43 ± 2.0	0.09

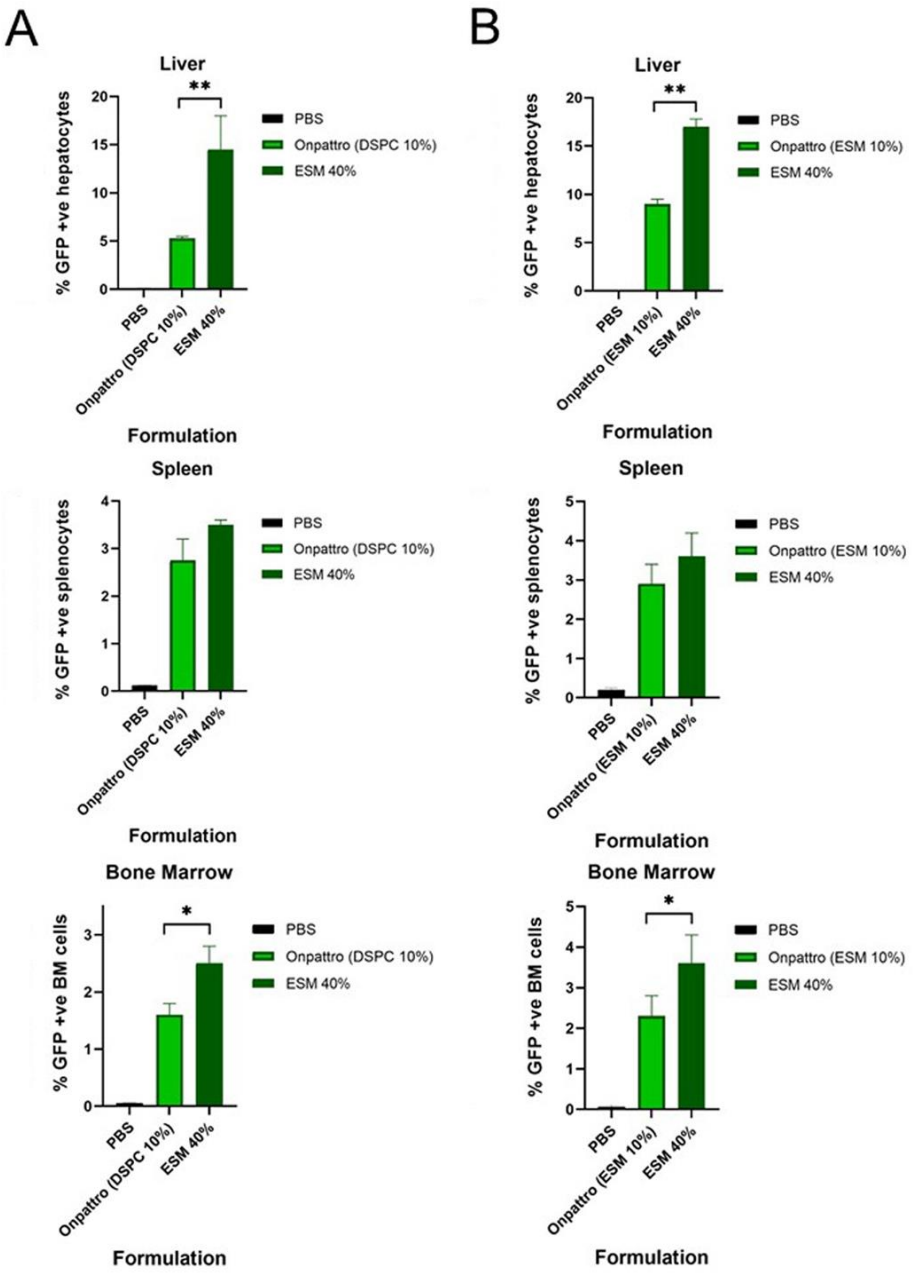


Figure S1. **LNP GFP mRNA systems containing 40 mol% ESM exhibit superior gene expression in target organs at 4 h post-injection as compared to systems containing 10 mol% DSPC or ESM.**

C57Bl6 mice were divided into 2 groups and received intravenous (i.v.) injection of GFP mRNA delivered with LNPs based on Onpattro formulation comprising 10 mol% DSPC or 10 mol% ESM, and 40% ESM including PBS as a negative control. LNPs entrapping GFP mRNA were labelled with 0.2 mol% DiD as fluorescent lipid marker. Mice received 3 mg/kg mRNA and 4 hrs post injection, phenotypic detection of hepatocytes was performed and cellular uptake and GFP expression was detected in hepatocytes, splenocytes and bone marrow cells. LNP-mRNA delivery or transfection efficacy were assessed based on the relative mean fluorescence intensity of DiD^{+ve} or GFP^{+ve} cells, respectively, measured on histograms obtained from gated cell populations. **A**:

Flow cytometry data indicating GFP expression in indicated tissues 4 hour post injection following administration of DSPC 10% and 40% ESM and **B:** ESM 10% and 40% ESM. Bar graphs represent the arithmetic mean ± SD of the percentage of GFP^{+ve} cells. BM: bone marrow. * P<0.05; **P< 0.01.

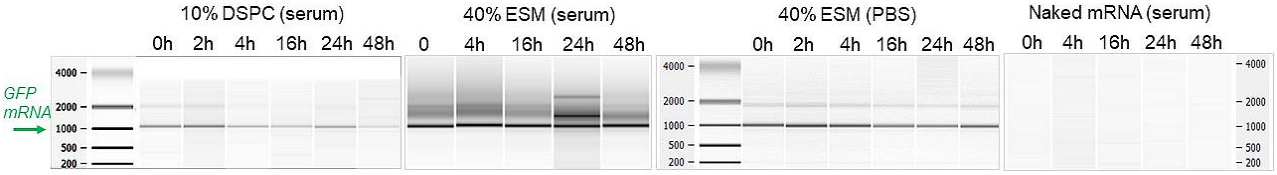
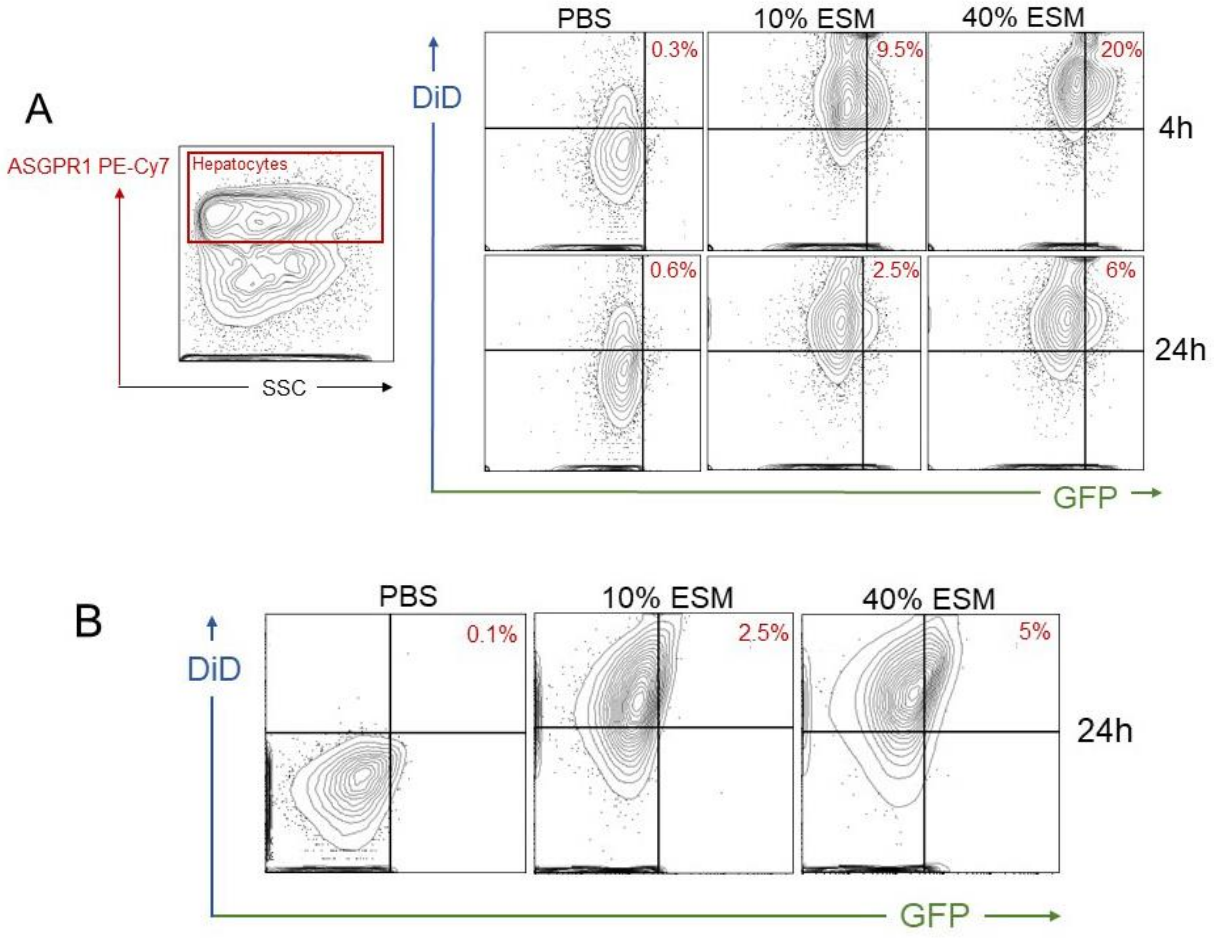


Figure S2.
Image of an Agilent Bioanalyzer gel showing the mRNA integrity through the migration following incubation of GFP-mRNA encapsulated in 10% DSPC (MC3-50%) and 40% ESM (MC3-33%) in 50% fetal bovine serum (FBS) at indicated times. Naked GFP-mRNA and 40% ESM (MC3-33%) were incubated in 50% FBS and PBS respectively and acted as controls. mRNA integrity was determined by automated electrophoresis using Agilent 2100 Bioanalyzer. The data are representative of 2 different experiments.



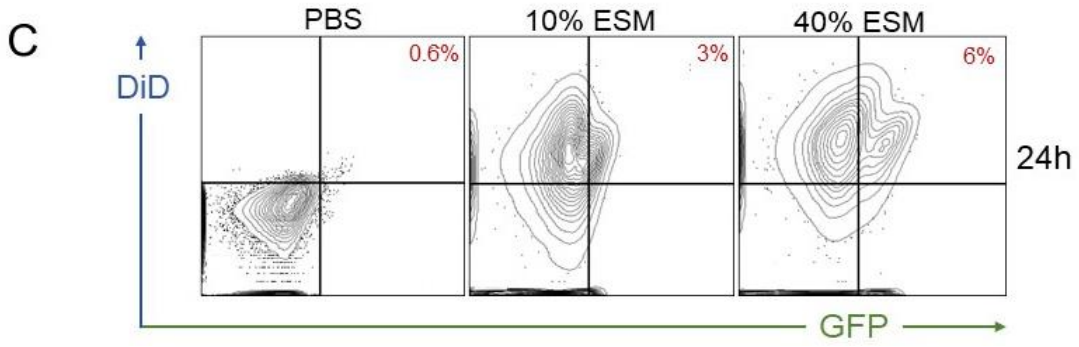
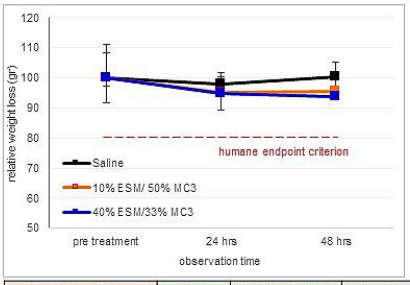


Figure S3. Contour plots display the percentage of (A) DiD+ hepatocytes and DiD+/GFP+ hepatocytes indicating LNP uptake and GFP translation in DiD+ cells as determined by co-expression of DiD and GFP. (B) LNP transfection efficiency and GFP translation in DiD+ splenocytes. The 24 hr time point only is shown. (C) LNP transfection efficiency and GFP translation in DiD+ bone marrow cells. The 24 hr time point only is shown.

A



B

Liver panel					
	24 hours	av	48 hours	av	range
Saline	ALT (U/L)	24.6	ALT (U/L)	34	18-71
	AST (U/L)	69.6	AST (U/L)	99.7	45-182
	ALP (U/L)	155	ALP (U/L)	115.3	37-249
	SDH (U/L)	10.1	SDH (U/L)	16.5	
	Bilirubin (mg/dl)	43.2	Bilirubin (mg/dl)	37.2	20-60
					range
MC3 50%/ESM 10%	ALT (U/L)	67.3	ALT (U/L)	30	18-71
	AST (U/L)	114.3	AST (U/L)	94	45-182
	ALP (U/L)	130	ALP (U/L)	105.7	37-249
	SDH (U/L)	29.2 *	SDH (U/L)	14.3	
	Bilirubin (µmol/L)	33.6	Bilirubin (µmol/L)	39.6	20-60
					range
MC3 33%/ESM 40%	ALT (U/L)	87.7	ALT (U/L)	33	18-71
	AST (U/L)	270.3 *	AST (U/L)	97	45-182
	ALP (U/L)	97.0	ALP (U/L)	80.3	37-249
	SDH (U/L)	35.6 *	SDH (U/L)	16.0	
	Bilirubin (µmol/L)	33.6	Bilirubin (µmol/L)	29.4	20-60

C

Blood Chemistry (48 hours)	Saline	MC3 50%/ESM 10%	MC3 33%/ESM 40%
Glucose mmol/L	12.8	15.5	10.8
IDEXX SDMA µg/dL	1.0	1.3	1.0
Creatinine µmol/L	14.7	14.7	11.3
Urea (BUN) mmol/L	10.3	8.3	7.9
BUN Creatinine Ratio	194.0	143.3	173.3
Phosphorus mmol/L	3.9	4.1	3.3
Calcium mmol/L	2.9	2.9	2.8
Sodium mmol/L	150.3	150.0	150.0
Potassium mmol/L	7.9	9.1	8.8
Na: K Ratio	19.7	16.7	17.3
Chloride mmol/L	107.7	107.7	108.7
TCO2 (Bicarbonate) mmol/L	22.0	24.0	26.3
Anion Gap	29.0	27.7	24.0
Total Cations mmol/L	158.7	159.3	159.0
Total Anions mmol/L	129.7	131.7	135.0
Total Protein g/L	48.7	47.7	44.7
Albumin g/L	31.3	29.0	25.7
Globulin g/L	17.3	18.7	19.0
Albumin:Globulin Ratio	1.8	1.6	1.3
Creatine Kinase U/L	391.0	345.3	185.7
Osmolality mmol/kg	317.3	319.7	314.0
Hemolysis Index	+++	++	+
Normal Icterus Index	Normal	Normal	Normal
Lipemia	Normal	Normal	Normal

D

Cytokine panel (48 hours)	Saline	MC3 50%/ESM 10%	MC3 33%/ESM 40%	range
GM-CSF (pg/mL)	<16	<16	<16	16-10.000
INFγ (pg/mL)	9.48	<3.2	<3.2	3.2-10.000
IL-1α (pg/mL)	39.5	667.4 *	262.4	16-10.000
IL-1β (pg/mL)	<16	<16	<16	16-10.000
IL-2 (pg/mL)	<3.2	<3.2	<3.2	3.2-10.000
IL-4 (pg/mL)	<3.2	<3.2	<3.2	3.2-10.000
IL-6 (pg/mL)	9.5	10.9	71.8 *	3.2-10.000
IL-10 (pg/mL)	<16	<16	<16	16-10.000
IL-12 (p70) (pg/mL)	<16	<16	<16	16-10.000
TNFα (pg/mL)	<16	<16	<16	16-10.000

Figure S4. Blood chemistry and cytokine multiplex panel analysis measured after a single dose regimen of 3.0 mg/kg LNP-mRNA intravenously. The data show the weight monitoring (A), the analytes measured that include biomarkers of liver health (B), blood chemistry (C) and the multiplex cytokine panel. Measures were taken from serum harvested via saphenous bleeding and cardiac puncture performed on animals during the observation period and following euthanasia 48 hours post iv administration of Onpattro-based and new LNP-mRNA formulations including saline control. The dose was given at 3.0 mg of LNP-mRNA per kg of animal body weight. Results reflect the mean of three replicate animals ± SD. *: $P<0.05$.