Supporting Information

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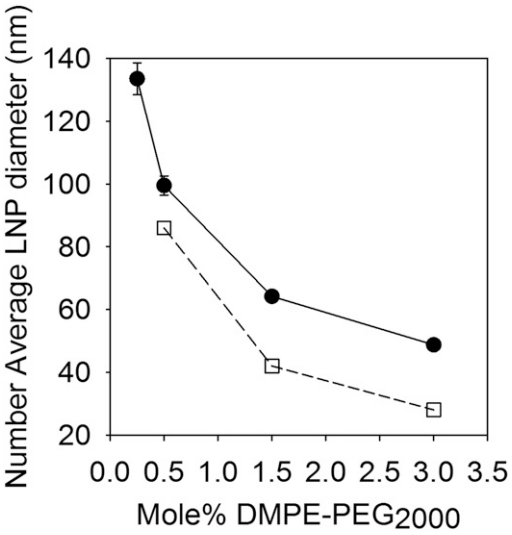

Fig. S1. LNP size for different amounts of PEG lipids. Size of mRNA–LNPs (closed circles) and empty LNPs (open squares), expressed as the number averaged LNP diameter, as a function of the mole percent of DMPE-PEG_{2000}. Lines are to guide the eye. Values are mean ± SEM (n = 3).

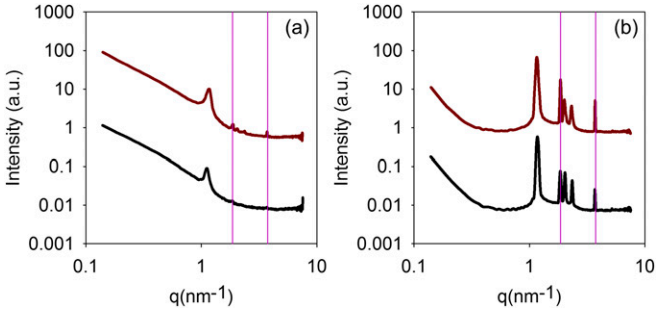

Fig. S2. Characterization of LNP bulk phase using SAXS. SAXS data for DLin-MC3-DMA:Chol mixtures in 50:38.5 (red curve) and 50:28.5 (black curve) mole ratio in the (*A*) presence of polyA and (*B*) absence, which have been dialyzed against buffer pH 3:ethanol 3:1 volume mixture. The pink vertical lines correspond to cholesterol monohydrate crystals. The intensity of the samples has been offset for clarity.

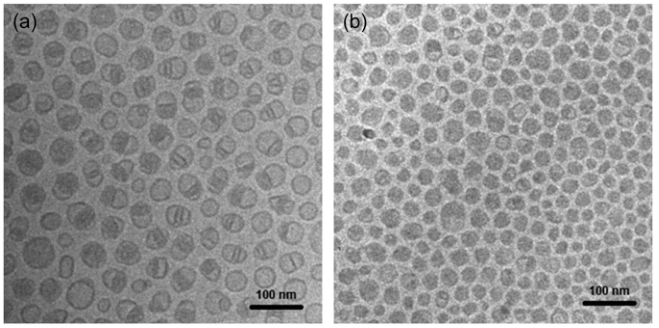

Fig. S3. Cryo-TEM characterization of LNPs in pH 3 and 25% ethanol. Cryo-TEM images of LNPs with lipid molar compositions of DLin-MC3-DMA:DSPC:Chol:DMPE-PEG_{2000} in the ratio 50:10:38.5:1.5 in buffer pH 3:ethanol 3:1 volume mixture in the (*A*) absence and (*B*) presence of mRNA.

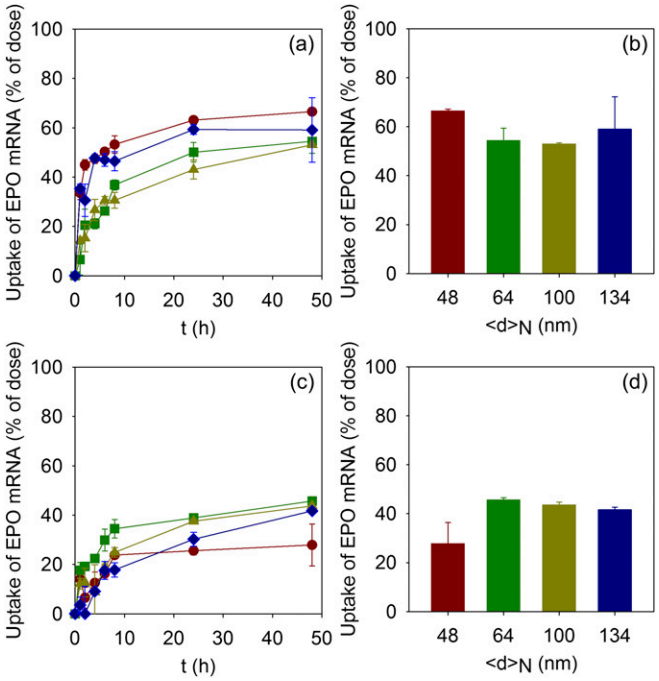

Fig. S4. Cellular uptake of LNPs. (*A* and *C*) Uptake of LNPs expressed as the percent of EPO mRNA dosed as a function of time in (*A*) adipocytes and (*C*) hepatocytes for LNPs with constant surface composition and different size: $\langle d \rangle_N$ = 47 nm (red circles), $\langle d \rangle_N$ = 64 nm (green squares), $\langle d \rangle_N$ = 99 nm (yellow triangles), and $\langle d \rangle_N$ = 133 nm (blue diamonds). Lines are to guide the eye. (*B* and *D*) Uptake of LNPs expressed as the percent of EPO mRNA dosed after 48 h postdosing (*B*) adipocytes and (*D*) hepatocytes for LNPs with the same lipid composition as *A* and *C*. The experiments were done in the presence of 1% human serum. Values are means ± SEM (n = 3).

Table S1. Molecular volume and neutron and X-ray SLD of the LNPs' main components

Component	Molecular volume ($\times 10^3$ nm^3)	SLD neutron ($\times 10^{-4}$ nm^{-2})*	SLD X-ray ($\times 10^{-4}$ nm^{-2})
Water	30	−0.56/6.4	9.5
DLin-MC3-DMA	1,290[†]	0.09	7.9
Cholesterol	630[‡]	0.21/1.4	9.7
DSPC	1,322[§]	0.18/6.7	9.4
DMPE	1,023[§]	0.31	9.7
PEG unit	670[¶]	0.62	13
RNA	325[#]	3.6/4.5	16

*The components with two SLD values correspond to the hydrogeneous and deuterated form, respectively, whereas the molecules with only a single value refer to the standard hydrogeneous form.
[†]Determined from the measured density.
[‡]Taken from Greenwood et al. (1).
[§]Taken from Armen et al. (2).
[¶]Calculated from the density given by Cheng et al. (3).
[#]Estimated average nucleotide volume from the average nucleotide molar mass and the density calculated by Voss and Gerstein (4).

1. Greenwood AI, Tristram-Nagle S, Nagle JF (2006) Partial molecular volumes of lipids and cholesterol. *Chem Phys Lipids* 143:1–10.
2. Armen RS, Uitto OD, Feller SE (1998) Phospholipid component volumes: Determination and application to bilayer structure calculations. *Biophys J* 75: 734–744.
3. Cheng G, et al. (2008) Small angle neutron scattering study of conformation of oligo(ethylene glycol)-grafted polystyrene in dilute solutions: Effect of the backbone length. *Macromolecules* 41:9831–9836.
4. Voss NR, Gerstein M (2005) Calculation of standard atomic volumes for RNA and comparison with proteins: RNA is packed more tightly. *J Mol Biol* 346:477–492.