Optimization of phospholipid chemistry for improved lipid nanoparticle (LNP) delivery of messenger RNA (mRNA)

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Supporting Information Figures

Figure S1. Characterization of four-component LNPs. (A) RNA encapsulation results for 4A3-SC8:cholesterol:phospholipid (see x-axis):DMG-PEG2000 (molar ratio = 38:5:30:30:1.5) LNPs (n=4). (B) Surface charge, (C) hydrodynamic diameter, and (D) polydispersity index was also measured for the series of LNPs (n=3).
Figure S2. LNPs formulated with different phospholipids are stable at room temperature and 4 °C. 4A3-SC8 LNPs were formulated with different phospholipids using the molar ratios 4A3-SC8:Chol:DMG-PEG:PL 38.5:30:3:30 (%mol/mol) and a molar ratio of lipid to mRNA of 10000:1 (weight ratio 23:1). The size and PDI were monitored for 72h at room temperature (A-B) and 4°C (C-D).

Figure S3. LNPs formulated with different PLs encapsulated mRNA. LNPs were formulated with different phospholipids to encapsulate Cy5-labeled mRNA. The gel was imaged using an IVIS Lumina to obtain Cy5 fluorescence emission.
**Figure S4. Transmission Electron Microscopy (TEM) images of LNPs with different phospholipids.** LNPs were formulated with different phospholipids and imaged using TEM. The molar ratio used for the formulations was 4A3-SC8:Chol:Phospholipid:DMG-PEG = 38.5:30:30:1.5 (mol/mol); the mol ratio of 4A3-SC8 to mRNA was 10000:1 and the weight ratio of 4A3-SC8 to mRNA was 23:1.

**Figure S5.** DOPE phospholipid increases C12-200 LNP mediated mRNA delivery. Results for luciferase protein activity following delivery of firefly luciferase mRNA (A) Hek293T and (B) HeLa cells. N=4 ± stdev, one-way ANOVA **** p<0.0001, Turkey’s test ** p<0.01, * p<0.05.
**Figure S6. Uptake of LNPs is not affected by phospholipid identity.** Distribution of mCherry fluorescence (A), percentage of mCherry positive cells (B), and mean fluorescence intensity of each LNP (C) obtained by flow cytometry after 48 h incubation of Hek293T cells with mCherry mRNA-LNPs (250 ng). Uptake of LNP was assessed after 48 h incubation of Hek293T cells with Cy5-labeled mRNA-LNP (250 ng) and flow cytometry was used to determine (D) distribution of Cy5 fluorescence, (E) percentage of Cy5 positive cells, and (F) mean fluorescence intensity. N=4 ± stdev, one-way ANOVA **** p<0.0001, Turkey’s test ** p< 0.001, * p<0.01.
Figure S7. DOPE-LNP enhances endosomal escape. Larger size confocal images from Figure 3A collected of Cy5-mRNA (290 ng) delivered by DOPE-LNP (top images) and DSPC-LNP (bottom images) after 24h incubation and staining with LysoTracker green.
Figure S8. Lysosome and Cy5-mRNA colocalization. Additional confocal images collected of Cy5-mRNA (290 ng) delivered by DOPE-LNP (top images) and DSPC-LNP (bottom images) after 24h incubation and staining with LysoTracker green. These images were used to calculate the Pearson coefficient values.

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<th>Pearson’s Coefficient</th>
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Figure S9. Tabulated Pearson’s Coefficients. Colocalization coefficients obtained from the images in Figure 3 and Figure S4 using ImageJ’s PSC colocalization plugin.
Figure S10. Molar ratios and percentages for SORT LNPs. SORT LNPs were prepared in 40:1 weight ratio of total lipids to mRNA and injected IV at 0.1 mg/kg.

Figure S11. Characterization of five-component SORT LNPs. (A) RNA encapsulation results for 4A3-SC8:DOPE:DMG:phospholipid (see x-axis) (molar ratio = 15:15:30:30:3:x, where x was varied from 5% to 40% (x=3.315, 7, 15.75, 27, 42)) (n=4). (B) Surface charge, (C) hydrodynamic diameter, and (D) polydispersity index was also measured for the series of LNPs (n=3).

Notes and references