SUPPLEMENTARY INFORMATION

TITLE

Analysis of PEG-lipid Anchor Length on Lipid Nanoparticle Pharmacokinetics and Activity in a Mouse Model of Traumatic Brain Injury

AUTHOR LIST

Lauren E. Waggoner^{a,‡}, Katelyn F. Miyasaki^{b,‡}, and Ester J. Kwon^{b,*}

AUTHOR ADDRESS

Departments of ^aNanoengineering and ^bBioengineering, University of California, San Diego, La Jolla, CA, 92122, USA

AUTHOR INFORMATION

*Address correspondence to ejkwon@ucsd.edu

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Supplementary Figure S1: Lipid Structures. (a) Structure of DLin-MC3-DMA purchased from BioFine International. Structures of (b) DSPC, (c) cholesterol, (d) DMG-PEG(2000), (e) DSPE-PEG(2000), and (f) DSPE-PEG(2000)-Cy7 purchased from Avanti Polar Lipids.



Supplementary Figure S2: Average size and polydispersity of mRNA LNPs with varying **PEG-lipid compositions.** DLS analysis of (a) Z-average size and (b) polydispersity (PdI) of LNPs formulated with mRNA used in the studies (mean \pm SD, n=2).



Supplementary Figure S3: Characterization and activity of siRNA LNPs with varying PEGlipid compositions. DLS analysis of (a) size distributions and (b) zeta potentials of LNPs formulated with siRNA. (c) Percent encapsulation of siRNA in LNPs.



Supplementary Figure S4: siRNA LNP Activity. Knockdown of luciferase activity in OVCAR-8-Luc expressing cells after 24 hours of treatment with LNPs formulated with siRNA against luciferase at various siRNA doses. Percent knockdown was calculated by normalization to untreated cells (mean \pm SD, n=3). The siRNA cargo (sequence: CUUACGCUGAGUACUUCGAdTdT) was purchased from IDT.



Supplementary Figure S5: Fluorescent surface scans of mRNA LNPs formulated with Cy7labeled PEG-DSPE. Surface organ scan of representative organs of mRNA LNPs formulated with Cy7-labeled PEG-DSPE 4 hours post-administration in a mouse model of TBI. Triplicate scans are quantified in Figure 3.







Supplementary Figure S7: Analysis of mRNA LNP area distribution after convection enhanced delivery. Distribution of Cy5-labeled (a) 0.1%, (b) 0.5%, (c) 1.0% DSPE-PEG mRNA LNPs after CED administration in brain sections 0, 0.5, and 1 mm away from the injection site (scale bar = 1 mm). (d) LNP area distribution in brain slices 0, 0.5, and 1 mm rostral from the injection site (mean ± SEM, n=3).



Supplementary Figure S8: Cell colocalization of mRNA LNPs after convection enhanced delivery. Colocalization, indicated by white arrows, of Cy5-labeled 0.1% and 0.5% DSPE-PEG mRNA LNPs after CED administration with (a) microglia (Iba1) and (b) astrocytes (GFAP) located in the striatum in brain sections 0.5 mm away from the injection site (nuclei, blue; scale bar = 50 μ m).