Supporting Information: Lipid Nanoparticles Containing siRNA Synthesized by Microfluidic Mixing Exhibit an Electron-Dense Nanostructured Core

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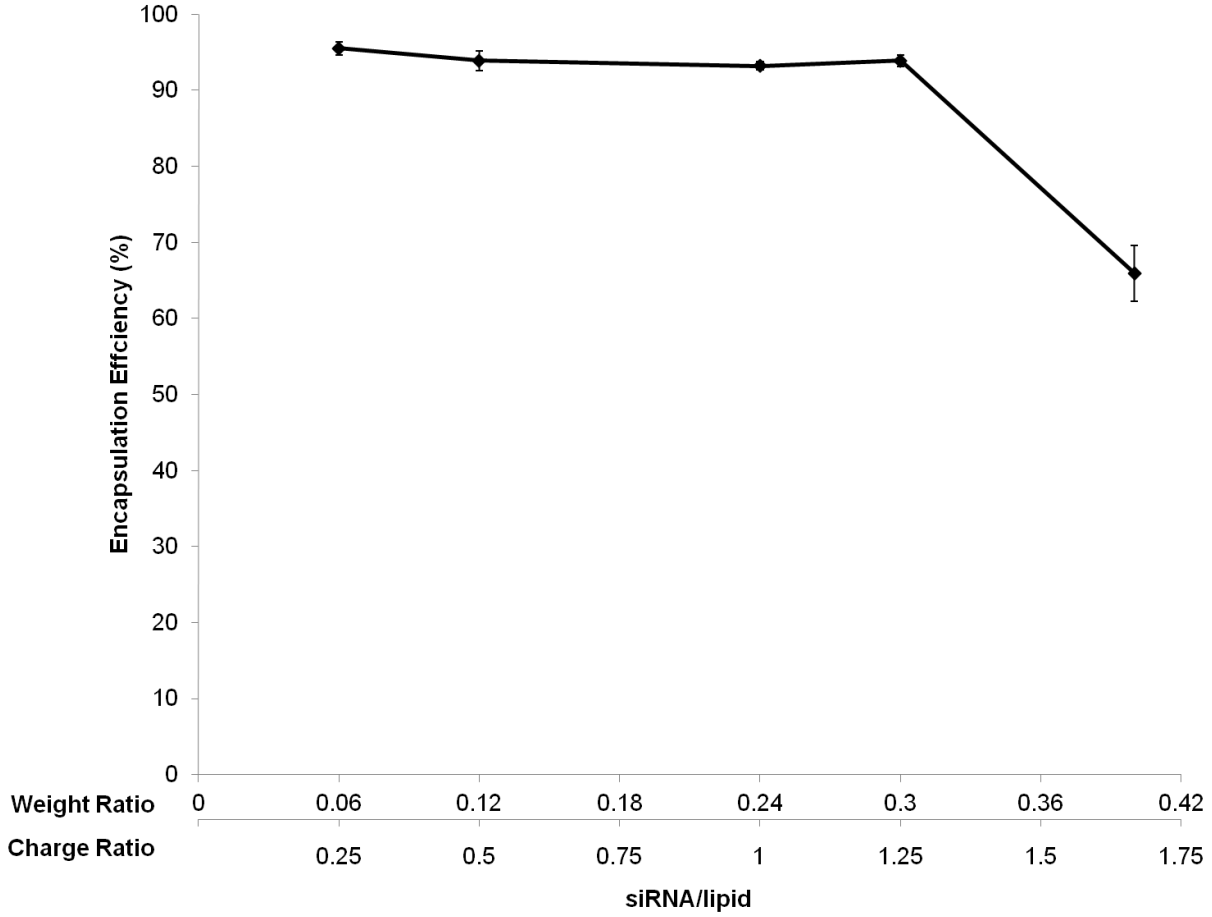
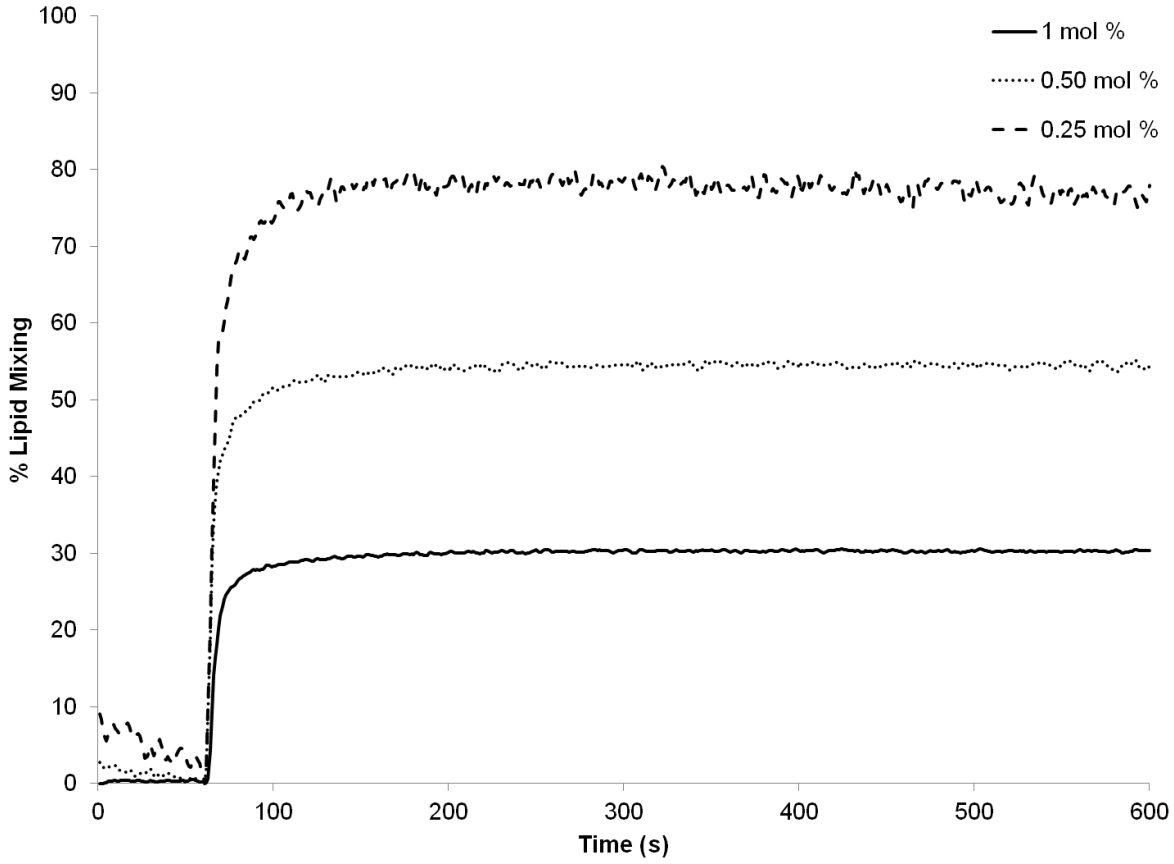
SUPPORTING INFORMATION FIGURE 1



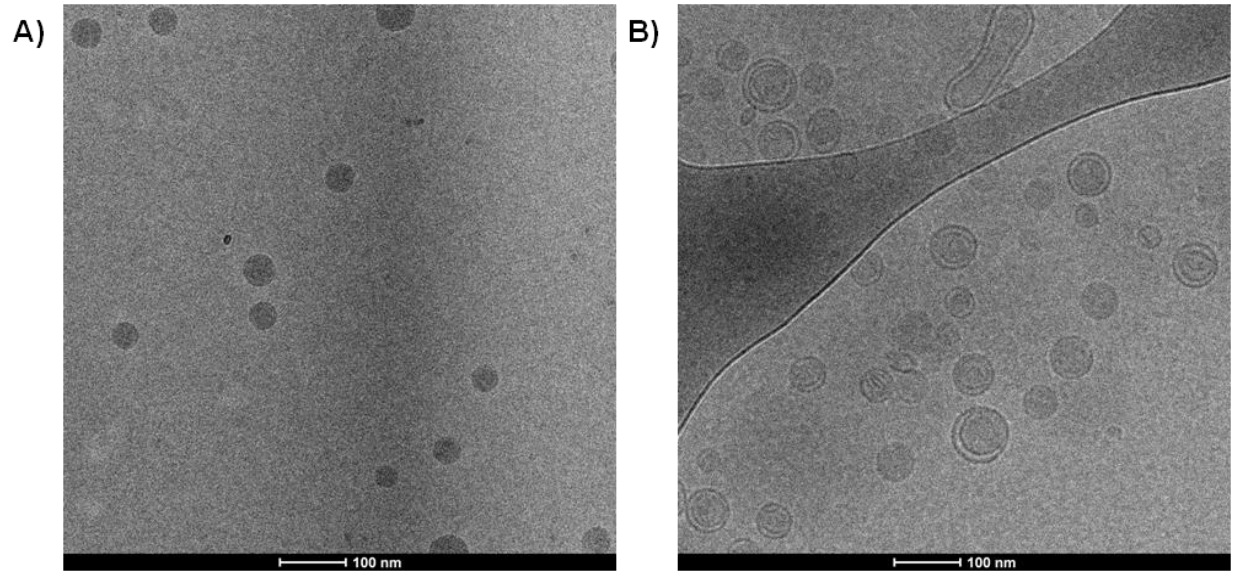
Supporting Information Figure 1: siRNA encapsulation efficiency of LNP siRNA systems prepared by microfluidic mixing at various siRNA/lipid ratios. LNP siRNA with the lipid composition DLinKC2-DMA/DSPC/Chol/PEG-lipid (40/11.5/47.5/1; mol/mol) were prepared using microfluidic mixing as described in Methods. Measurements of trapping efficiency were made as indicated in Methods. Data points and error bars represent the average encapsulation efficiencies and standard deviations, respectively, measured from three independently prepared LNP formulations.

SUPPORTING INFORMATION FIGURE 2



Supporting Information Figure 2: Lipid mixing as measured by the FRET assay is a function of the concentration of FRET pairs incorporated into the DOPS vesicles. The assay was performed as described in Methods for an LNP system comprised of DLinKC2-DMA/DSPC/Chol/PEG-lipid (40/11.5/47.5/1; mol/mol) prepared by microfluidic mixing in the absence of siRNA. The reaction was initiated by injecting the LNP (at t = 1 min) into a stirred cuvette containing anionic DOPS/NBD-PE/Rh-PE liposomes prepared at 98:1:1 mol% (solid line), 99:0.5:0.5 mol% (dotted line) and 99.5:0.25:0.25 mol% (dashed line).

SUPPORTING INFORMATION FIGURE 3



Supporting Information Figure 3: LNP containing different ratios of cationic lipid and DSPC can exhibit different structures. (A): Cryo-TEM micrograph obtained from LNP siRNA with the lipid composition DLinKC2-DMA/DSPC/Chol/PEG-lipid (40/11.5/47.5/1; mol/mol) and siRNA at an siRNA/cationic lipid charge ratio of 0.25. (B): Cryo-TEM micrograph obtained from LNP siRNA with lipid composition DLinKC2-DMA/DSPC/Chol/PEG-lipid (20/31.5/47.5/1; mol/mol) at the same siRNA/cationic lipid charge ratio as in (A). The encapsulation efficiencies achieved for both formulations were in excess of 90% as determined using the RiboGreen assay described in Methods.