Supporting Information for

Lipid nanoparticle topology regulates endosomal escape and delivery of RNA to the cytoplasm

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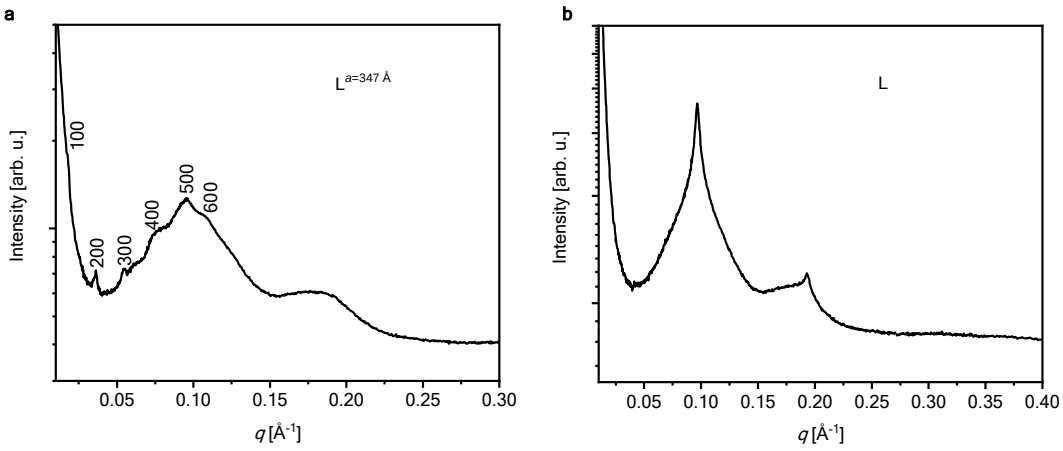

Fig. S1. Synchrotron SAXS data of LNP-RNA complexes with composition GMO/DOPC/DOTAP/DOPE-PEG. (**a**) LNP-siRNA complexes were formed with a lipid composition of GMO/DOPC/DOTAP/DOPE-PEG at a molar ratio 25/60/14/1 and charge ratio of 3. The complexes form a lamellar phase with a lattice parameter of 347 Å.(**b**) LNP-mRNA complexes were formed with a lipid composition of GMO/DOPC/DOTAP/DOPE-PEG at a molar ratio 25/60/14/1 and charge ratio of 3. The complexes form a lamellar phase but the exact indexing cannot be determined.

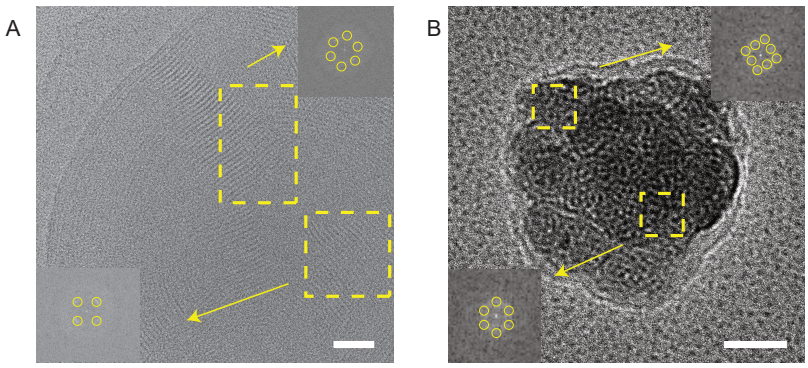

Fig. S2. Cryo-EM micrograph of LNP-RNA complexes prepared by thin film hydration methods are larger but conserve the nanostructure seen with microfluidics. Scale bar, 50 nm. The complexes were formed with thin film hydration method (**a.**) or microfluidic method (**b.**) with a lipid composition of GMO/DOTAP/DOPE-PEG at molar ratio 85/14/1 with mRNA. Inset represents the fast Fourier transform (FFT).

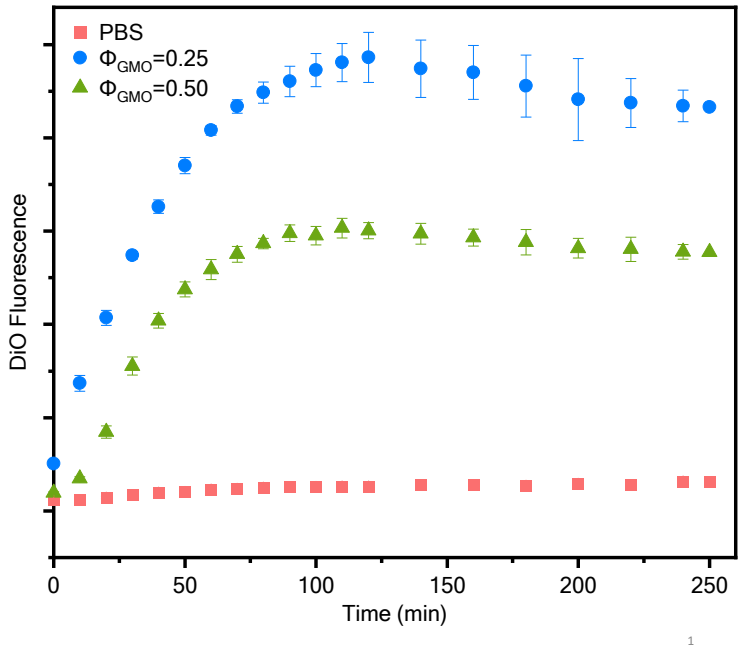

Fig. S3. FRET assay to evaluate membrane fusion between endosomes and LNP-siRNA complexes with GMO 50% molar percent and GMO 25% molar percent. The LNP-siRNA complexes with $\Phi_{GMO} = 0.50$ show significant fusion with endosomes. Bouxsein and co-workers (1) showed that siRNA delivered to cell culture by hexagonal bulk phases is efficient but regulated by direct fusion with the plasma membrane compromising its integrity. For that reason, having Q_{II} or Q_{II}/H_{II} is preferable (2) for the purpose of boosting endosomal escape.

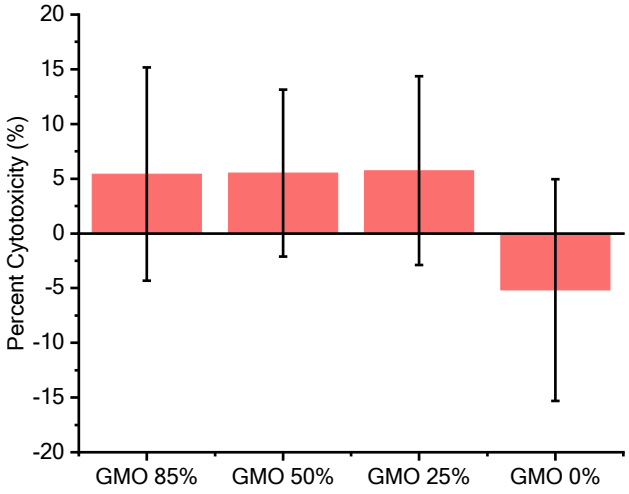

Fig. S4. Cytotoxicity of LNP-RNA complexes. Membrane integrity of HeLa cells is not compromised when treated with different LNP-RNA complexes.

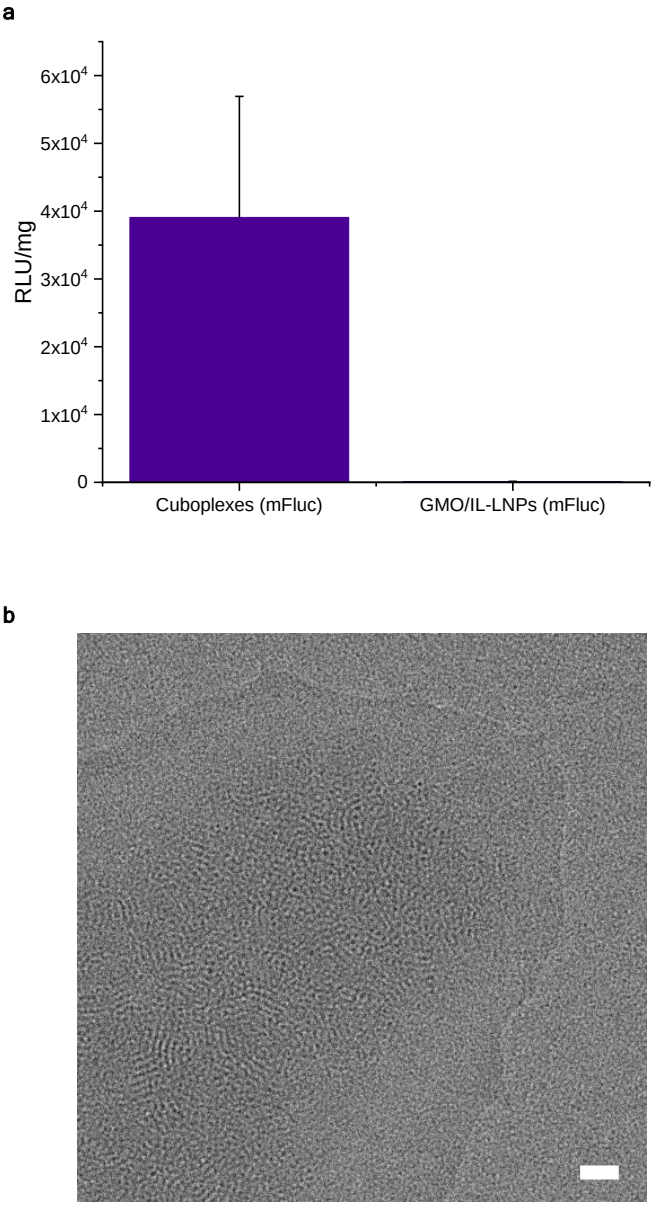

Fig. S5. Cuboplexes have a higher mRNA transfection efficiency compared to GMO-IL complexes. a. Luciferase activity of HeLa cells transfected with firefly luciferase mRNA using cuboplexes and LNP-mRNA complexes with composition of GMO:IL:DOPE-PEG 85:14:1 mol%. ($n = 6$, data presented as mean $\pm$ s.d.) DOTAP cuboplexes are more efficient than a GMO-based LNP where DOTAP is substituted by SM-102 IL. **b.**Cryo-EM micrograph of lipid-RNA complex with GMO and IL showing no well defined LNP nanostructure. Scale bar, 50 nm. The complex was formed with a lipid composition of GMO/IL/DOPE-PEG at molar ratio 85/14/1 and with mRNA at ρ=6.

Table S1. Sizes of LNP-RNA complexes measured by nanoparticle tracking analysis

GMO/DOPC/DOTAP/DOPE-PEG	RNA type	Average size (nm)
85/0/14/1	siRNA	205.1 $\pm$ 2.5 nm
50/35/14/1	siRNA	190.5 $\pm$ 3.1 nm
25/60/14/1	siRNA	244.9 $\pm$ 4.5 nm
0/85/14/1	siRNA	265.2 $\pm$ 3.5 nm
85/0/14/1	mRNA	151.8 $\pm$ 10.0 nm
50/35/14/1	mRNA	163.4 $\pm$ 8.3 nm
25/60/14/1	mRNA	153.9 $\pm$ 4.5 nm
0/85/14/1	mRNA	200.8 $\pm$ 2.1 nm

References

1. NF Bouxsein, CS McAllister, KK Ewert, CE Samuel, CR Safinya, Structure and Gene Silencing Activities of Monovalent and Pentavalent Cationic Lipid Vectors Complexed with siRNA. *Biochem.* **46**, 4785–4792 (2007).
2. J Rueben, A Jayaraman, MK Mahanthappa, C Leal, Near-Infrared-Triggered Reversible Transformations of Gold Nanorod-Laden Lipid Assemblies: Implications for Cellular Delivery. *ACS Appl. Nano Mater.* **5**, 710–717 (2022).