Table S1. Particle size and zeta potential of lipoplexes and LNPs with incorporated lipophilic dye DiO, at an N/P ratio of 5. Data reported as mean ± SD (n = 3).

Formulation	Loading N/P value	Particle size (d.nm) ± SD	PdI	Zeta potential (mV) ± SD	Measured N/P value
Lipoplex incorporated with DiO	5	116.7 ± 0.6	0.09	16.1 ± 0.6	4.5
LNP incorporated with DiO	5	100.1 ± 1.2	0.07	27.8 ± 2.7	5.7

Notes: Lipoplexes were slightly larger in size than LNPs. The zeta potential of LNPs was more positively charged than that of lipoplexes.

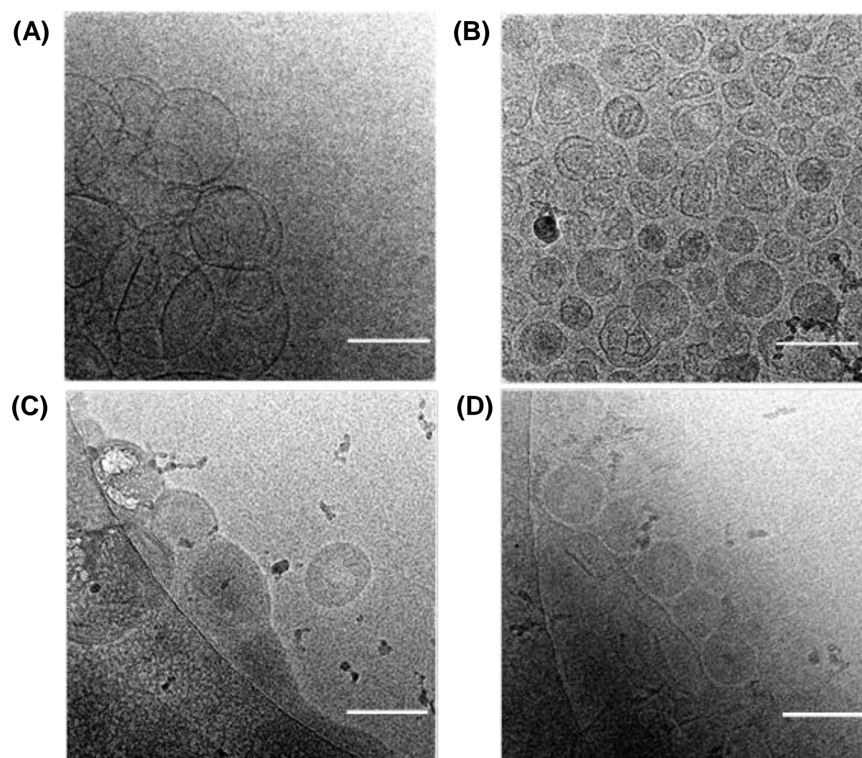

Figure S1 Cryo-TEM micrographs of lipoplexes and LNPs. (**A**) Lipoplex (N/P = 10); (**B**) LNPs (N/P = 10); (**C**) Lipoplex (N/P = 5); and (**D**) LNPs (N/P = 5). Bar = 100 nm.

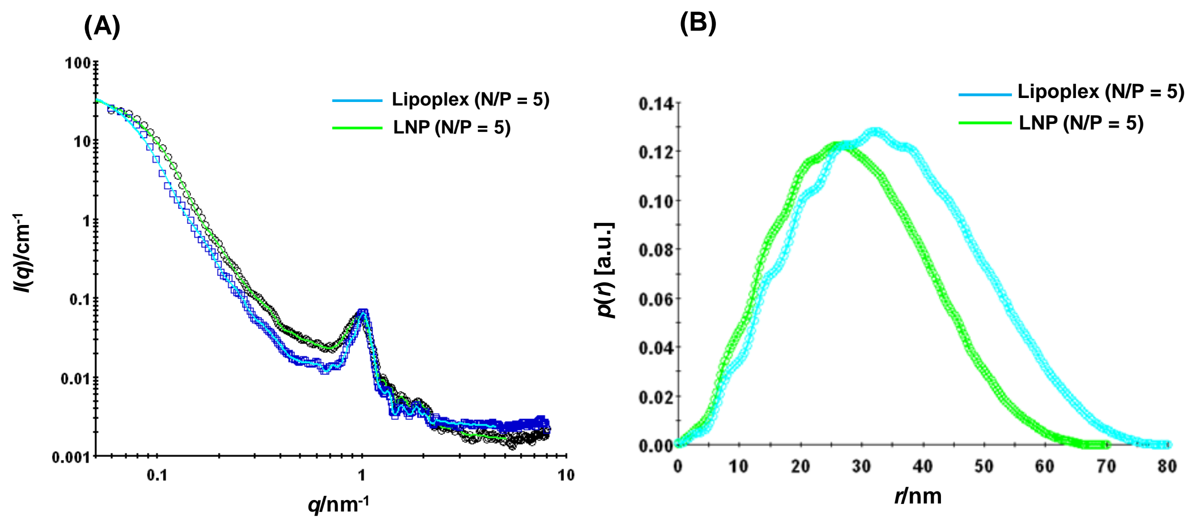

Figure S2 Structural characterization of the lipoplex and the LNP (N/P = 5) as obtained by SAXS. (**A**) Small-angle X-ray scattering intensities on an absolute scale. (**B**) The pair-distance distribution functions, *p(r)*.

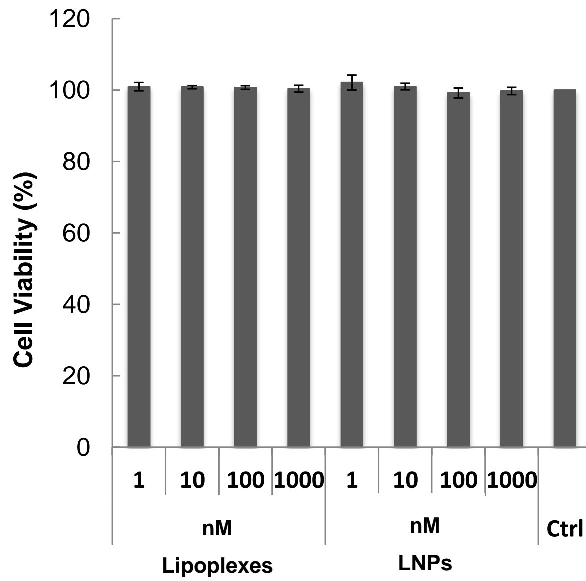

Figure S3 WST-8 assay of lipoplexes and LNPs (N/P = 5) in HeLa cells. Cells were incubated with lipoplexes and LNPs at doses from 1 to 1000 nM siRNA for 24 h. After washing twice with DPBS, cell viability was tested using the WST-8 assay kit (Dojindo Molecular Technologies, Inc., Osaka, Japan). No significant differences in cytotoxicity were observed between lipoplexes and LNPs when compared with that of untreated control cells. DMEM was used as a control (Ctrl). Data shown are mean ± SD (n = 5).

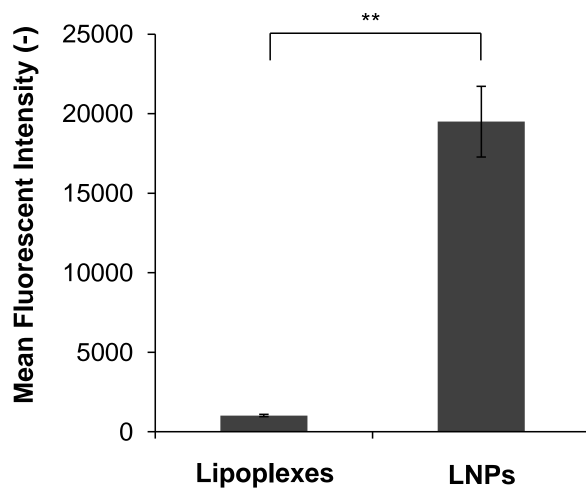

Figure S4 Comparison of intracellular uptake of Alexa647 labeled siRNA with lipoplexes and LNPs. HeLa cells were incubated with lipoplexes and LNPs at an N/P ratio of 5 and a dose of 100 nM siRNA for 1 h. Mean fluorescence intensity was quantified by flow cytometry. Aspin-Welch test was performed. **P < 0.01.

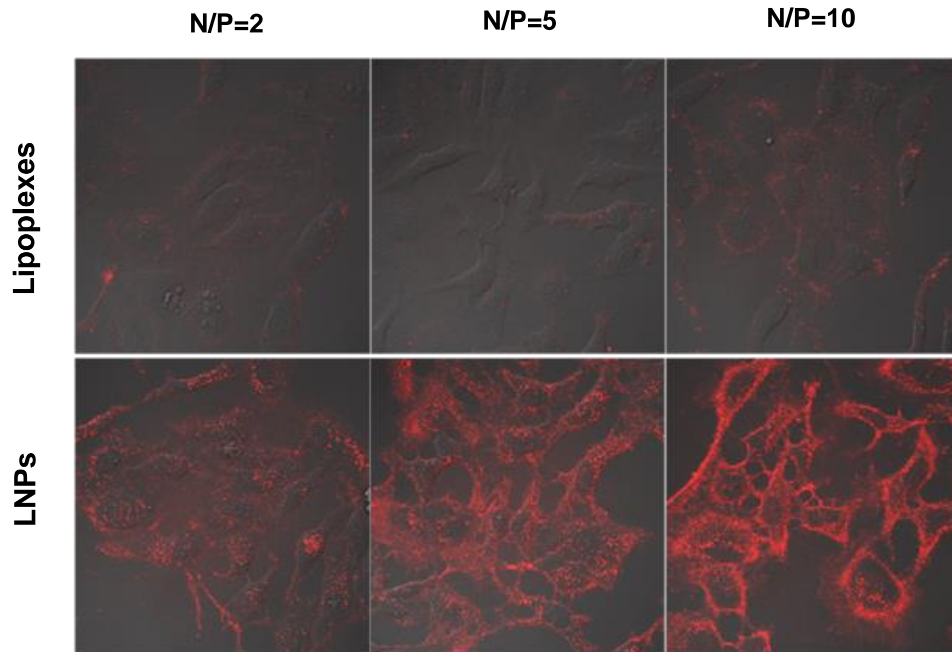

Figure S5 Cellular uptake of Alexa647-labeled siRNA carried by lipoplexes or LNPs at each N/P ratio. HeLa cells were treated with lipoplexes and LNPs encapsulating fluo-siRNA at a dose of 100 nM siRNA for 1 h.

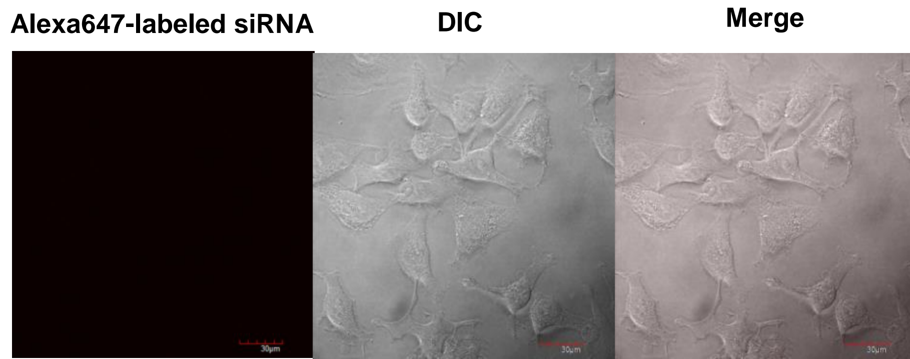

Figure S6 Cellular uptake of Alexa647-labeled siRNA. HeLa cells were treated with Alexa647-labeled siRNA at a dose of 100 nM siRNA for 1 h. DIC; Differential Interference Contrast

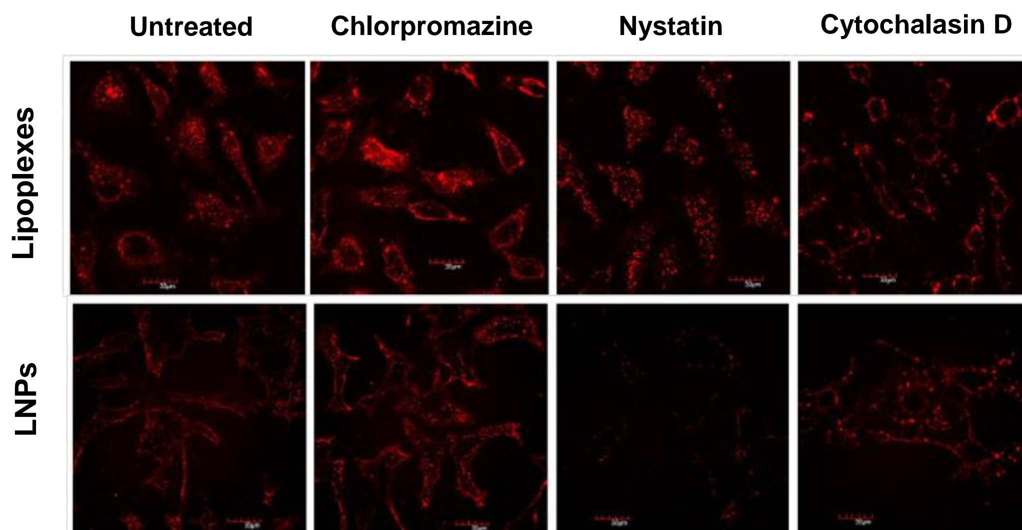

Figure S7 Inhibitory studies of endocytosis in cellular uptake of lipoplexes and LNPs at an N/P ratio of 5. HeLa cells were pretreated with chlorpromazine, nystatin or cytochalasin D (All concentration were 10 μg/mL) for 30 min followed by treatment of cells with lipoplexes (siRNA: 1 μM) or LNPs (siRNA: 100 nM) for 1 h in the presence of inhibitors. Alexa647-siRNA was monitored by fluorescence microscopy.

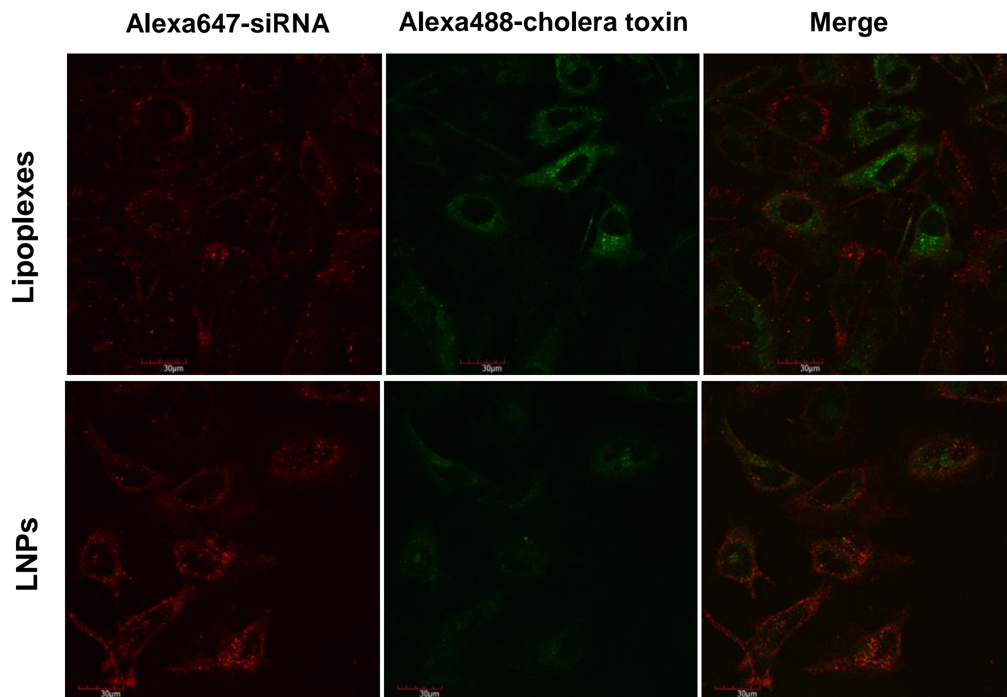

Figure S8 Colocalization studies in cellular uptake of lipoplexes and LNPs at an N/P ratio of 5. HeLa cells were treated with lipoplexes (siRNA: 1 μM) or LNPs (siRNA: 100 nM) for 1 h together with Alexa488-labeled cholera toxin B. Alexa647-siRNA and Alexa488-cholera toxin was monitored by fluorescence microscopy. The red color represents Alexa647-siRNA and the green color represents Alexa488-cholera toxin. In the case of LNPs, the intracellular colocalization of Alexa488-labeled cholera toxin with Alexa647-labeled siRNA suggests that internalization of LNPs was driven by caveolae-mediated endocytosis. On the other hand, in the lipoplexes case, the intracellular localization of siRNA and cholera toxin was not completely merged, indicating that lipoplexes were partially taken up by caveolae-mediated endocytosis.