

Supplementary Materials

Table S1. Size, zeta-potential, polydispersity index (PDI), and encapsulation efficiency of LNP₅, LNP₁₀ and LNP₂₀.

	LNP ₅	LNP ₁₀	LNP ₂₀
Size (d.nm)	451.9 ± 364.1	122.6 ± 4.095	129.2 ± 3.39
PDI	0.994 ± 0.011	0.124 ± 0.002	0.268 ± 0.007
Zeta potential (mV)	11.11 ± 8.8	47.4 ± 7.55	49.4 ± 4.7
Encapsulation Efficiency (%)	n.d.	80.3	67.4

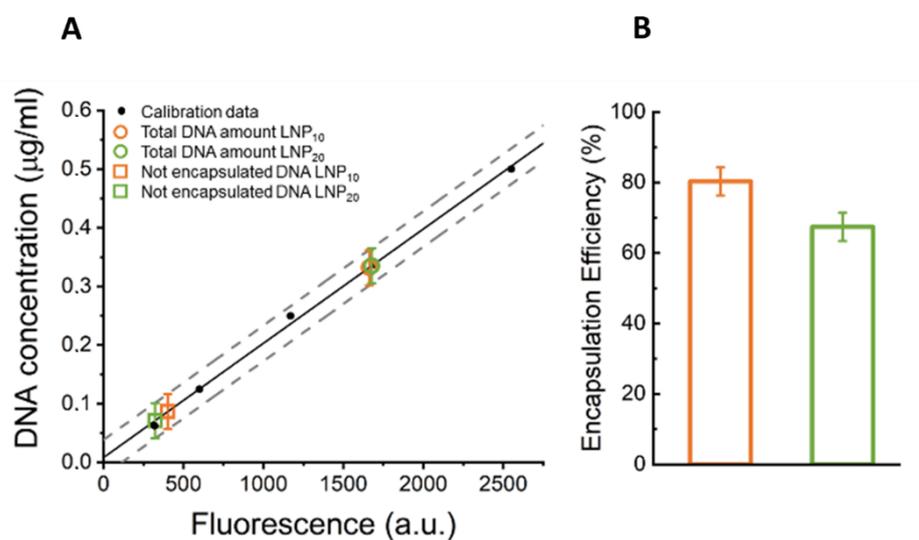


Figure S1. (A) Calibration curve (black points) obtained through fluorescence signal measurement of pDNA and the related fluorescence signals obtained from LNP₁₀ and LNP₂₀ before and after treatment with Triton X-100 for measuring non-encapsulated pDNA and total pDNA amounts. (B) Encapsulation efficiency of pDNA within LNP₁₀ and LNP₂₀ calculated as reported in the Materials and Methods section.

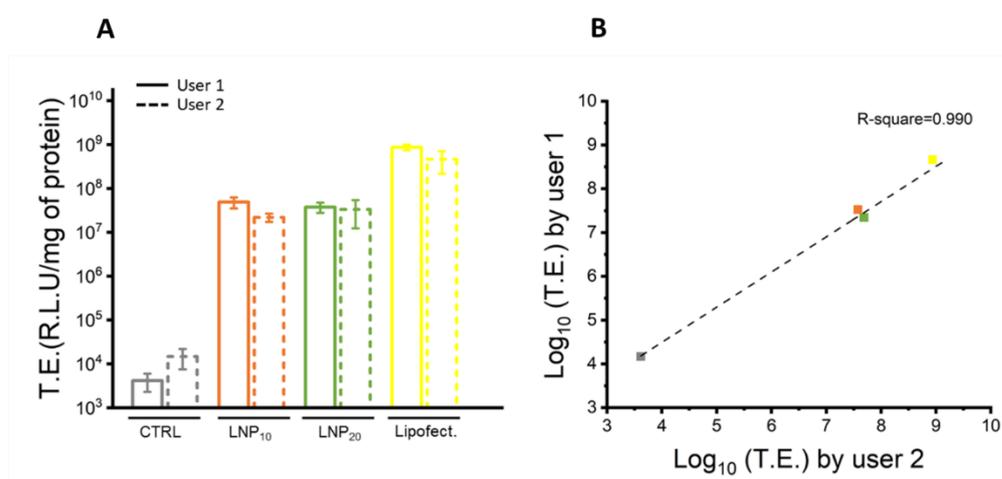


Figure S2. (A) Transfection efficiency (TE) of LNP₁₀, LNP₂₀, and LipofectamineTM3000 was measured by two users (indicated as “User 1” and “User 2”) in independent experiments. (B) High reproducibility of transfection data was demonstrated by linear regression of measured TE values.

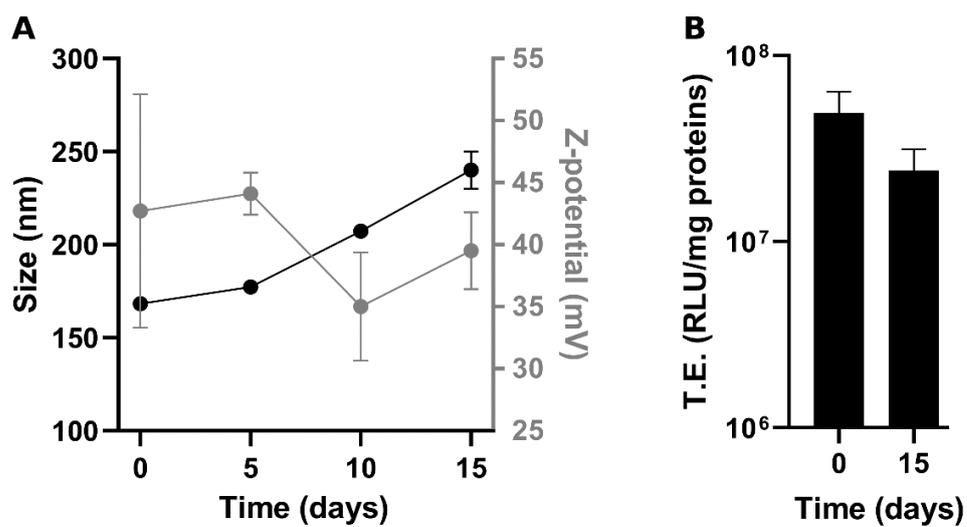


Figure S3. (A) Size (black) and Z-potential (grey) of LNP₁₀ measured at different time points (0, 5, 10, and 15 days). (B) Transfection efficiency (TE) of LNP₁₀ on HEK-293 cells measured at 0 and 15 days expressed as relative light units (RLU) per mg of proteins. .