

Supplementary Materials: Microfluidic Formulation of DNA-Loaded Multicomponent Lipid Nanoparticles for Gene Delivery

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Table S1. Chemical and physical characterization of LNP₂ and LNP₈.

	LNP ₂	LNP ₈
Size (d.nm)	145.3 ± 1.6	178.2 ± 2.7
Zeta potential (mV)	18.5 ± 1.4	18.4 ± 2.1
PdI	0.113 ± 0.001	0.362 ± 0.002
Encapsulation Efficiency	76.8	72.7

Table S2. Chemical and physical characterization performed on three different batches of PEGylated LNP₂.

	Batch 1	Batch 2	Batch 3
Size (d.nm)	145.3 ± 1.6	137.5 ± 0.67	145.6 ± 1.78
Zeta potential (mV)	18.5 ± 1.4	21.1 ± 2.4	14.5 ± 3.4
PdI	0.113 ± 0.001	0.238 ± 0.002	0.225 ± 0.002

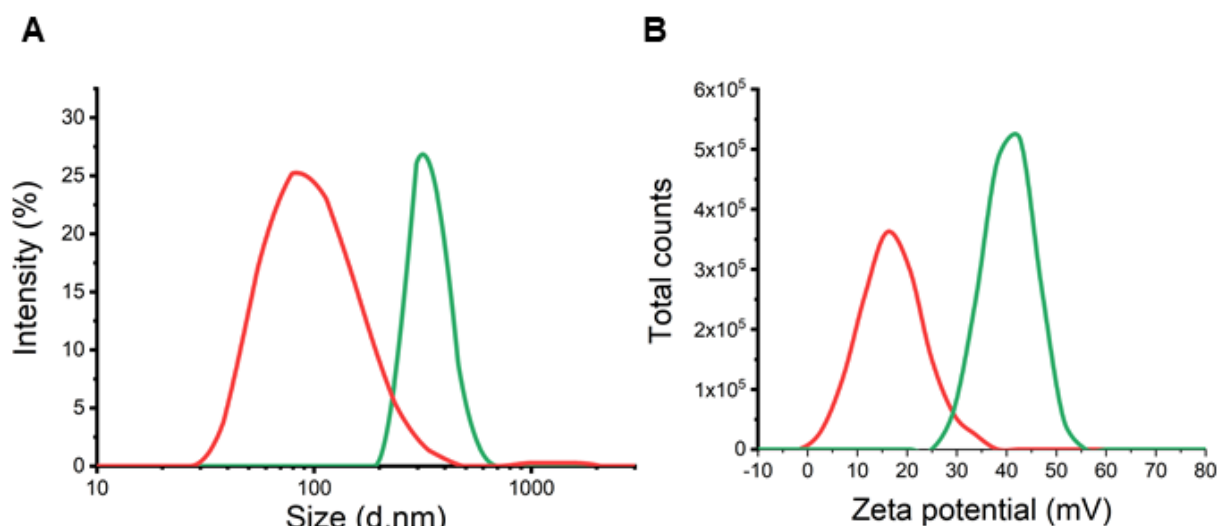


Figure S1. Size (panel A) and zeta potential (panel B) of LNP₂ concentrated 5× after synthesis (red lines) and LNP₂ derived from 5× concentrated lipid and pDNA solutions (green lines).

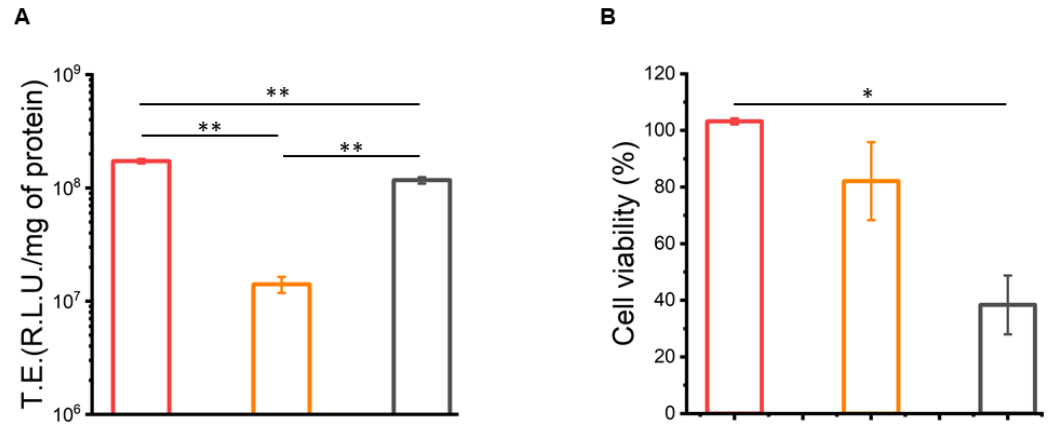


Figure S2. Transfection efficiency (T.E.) expressed as Relative Light Unit (R.L.U.) to milligrams of proteins (A) and cell viability, expressed as percentage with respect to untreated cells, of LNP₂ at 0.5 mg/mL (red histograms) and LNP₂ at 0.1 mg/mL (orange histograms), towards HEK-293 cells. All values are compared to Lipofectamine (grey histograms). Statistical significance was evaluated using Student's t-test: * $p < 0.05$; ** $p < 0.01$ (no asterisk means lack of significance).

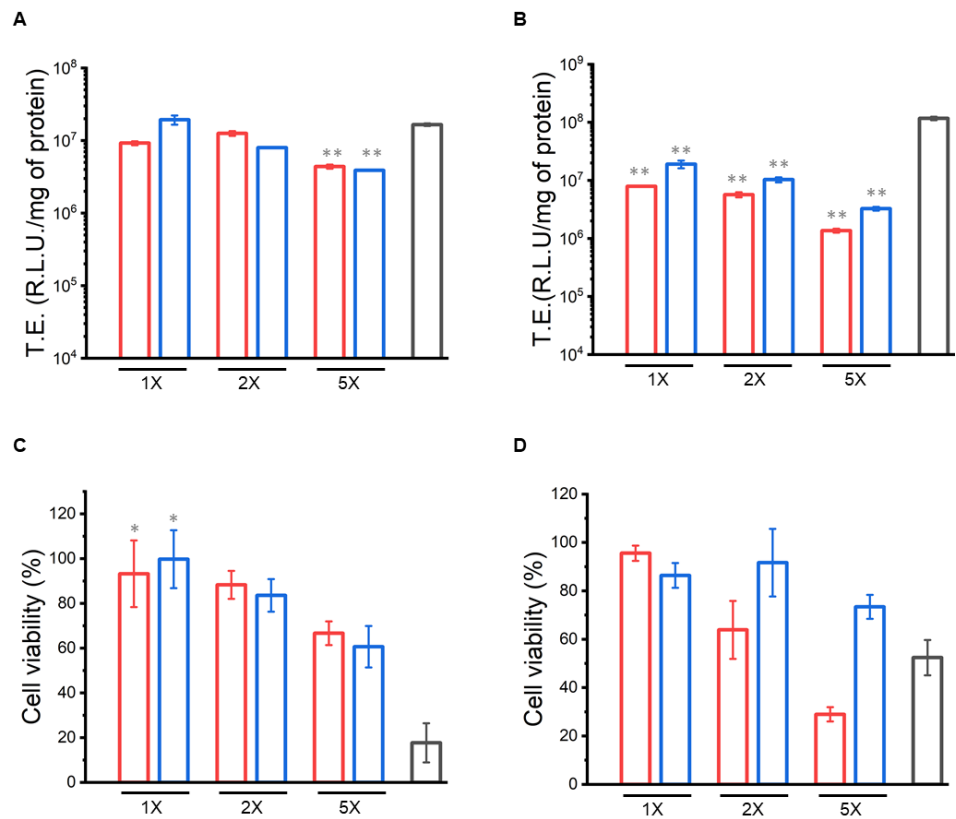


Figure S3. LNP₂ (red histograms) and LNP₈ (blue histograms) transfection efficiency (TE) expressed as Relative Light Unit (R.L.U.) to milligrams of proteins, towards HaCaT cells (A) and CaSki cells (B) compared to T.E. of LipofectamineTM 3000 (grey histograms). Cell viability of HaCaT cells (C) and CaSki cells (D) after treatment with LNP₂, LNP₈ and LipofectamineTM 3000, expressed as percentage with respect of untreated cells. Statistical significance was evaluated using Student's t-test: * $p < 0.05$; ** $p < 0.01$ (grey asterisk represents significance with respect of LipofectamineTM 3000, no asterisk means lack of significance).

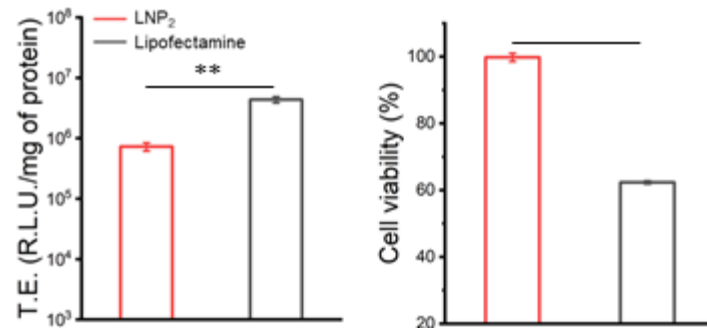


Figure S4. LNP₂ (red histograms) and LipofectamineTM 3000 (grey histograms) transfection efficiency (TE) expressed as Relative Light Unit (R.L.U.) to milligrams of proteins, towards N/TERT cells (A). Cell viability of N/TERT cells (B) after treatment with LNP₂ and LipofectamineTM 3000, expressed as percentage with respect of untreated cells. Statistical significance was evaluated using Student's t-test: ** $p < 0.01$ (no asterisk means lack of significance).