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i-LipoP® technology

Application Note

Physicochemical properties

i-LipoP® transfection kit is based on high-quality lipoparticles only made of poly-lactic acid and lipid corona (no stabilizer, no surfactant). i-LipoP® exhibits controlled size around 220 nm, homogeneity in size distribution, positive surface charge, high reproducibility and spherical shape (fig. 1).

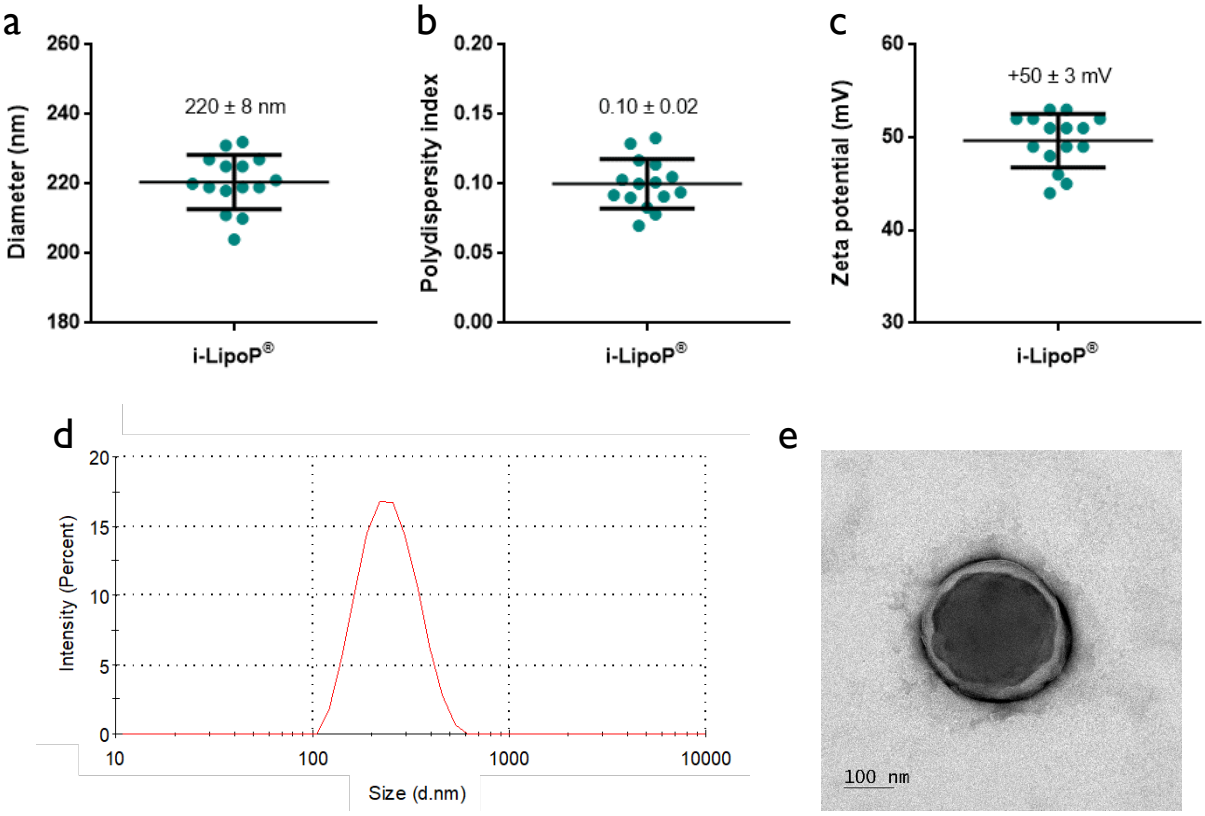
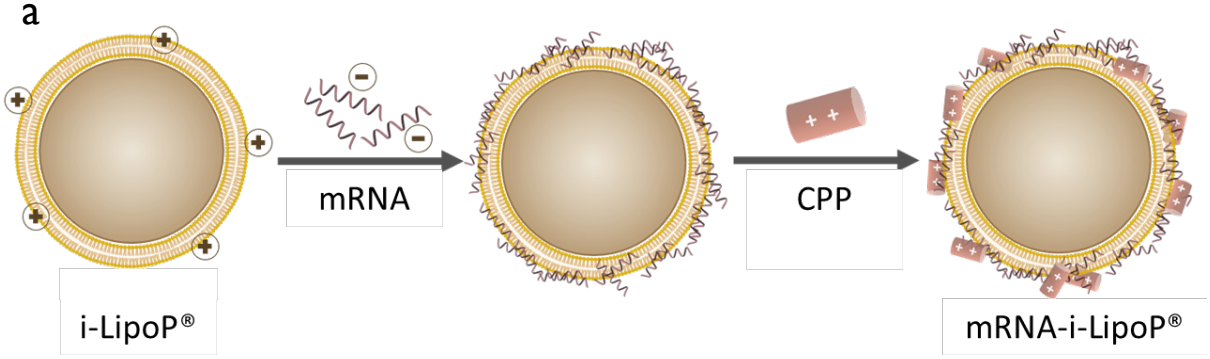


Figure 1. Physicochemical properties of 15 independent i-LipoP® batches characterized for i) their diameter (a), ii) their polydispersity index (b) using Dynamic Light Scattering, iii) their surface charge through zeta potential (c) obtained by Electrophoretic Light Scattering, iv) their size distribution with example of one batch (d) and v) their morphology observed by Transmission Electron Microscopy (e).

mRNA vectorization

i-LipoP® enables mRNA vectorization of a wide range of size, from 300 bp to several kb, by successive adsorption of mRNA and cell-penetrating peptide (fig. 3). After mRNA formulation, i-LipoP® diameter and polydispersity index slightly increased. Zeta potential reversed twice to reach final positive value. The formulation is stable up to 48h before use. Also, formulation process did not degrade mRNA and enabled its total complexation on i-LipoP®.

a



b

Formulation	Diameter (nm)	PdI	Zeta pot. (mV)	Stability
i-LipoP®	226 ± 2	0.12 ± 0.01	+52 ± 2	> 6 months
mRNA-i-LipoP®	262 ± 5	0.21 ± 0.01	+37 ± 1	> 48 h

c

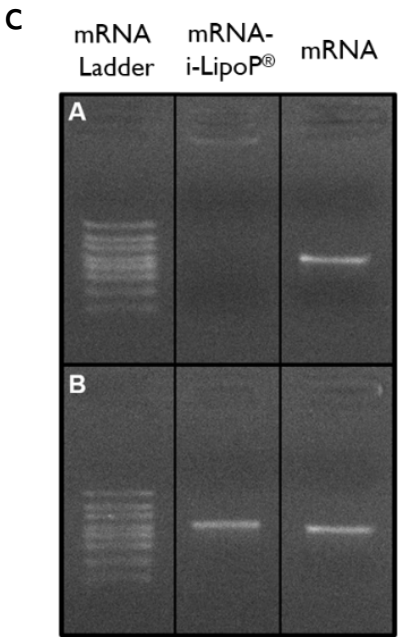


Figure 3. Schematic representation of the formulation strategy used to successively adsorb mRNA and CPP on i-LipoP® (a). Note that the scale proportions are not respected. Physicochemical properties and stability of mRNA-loaded i-LipoP® (b). Agarose gel electrophoresis analysis of mRNA-based formulations (A) without and (B) with mRNA desorption treatment (c). For desorption treatment, samples were incubated at room temperature for 30 min and then with heparin (to desorb mRNAs and peptides) and proteinase K (to degrade peptides) at 56°C for 15 min.

mRNA-loaded i-LipoP® shows higher transfection efficiencies in different cell types including difficult-to-transfect cells like Epithelioma papulosum cyprini line and CHSE-214 cell line compared to TransIT® and Lipofectamine® transfection kits (fig. 4).

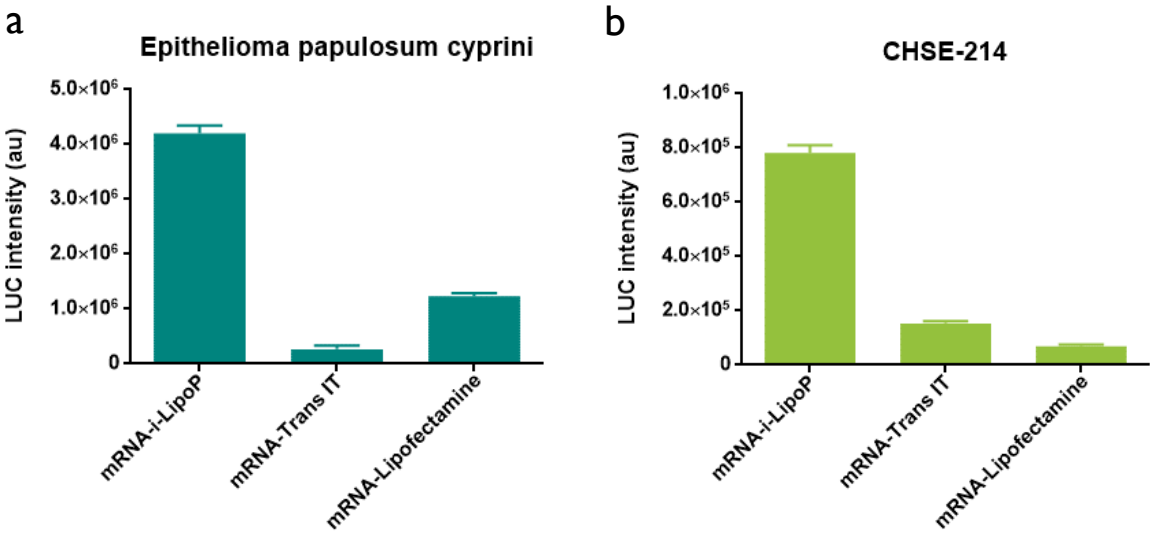


Figure 4. *In vitro* evaluation at 24 h of transfection efficiency through measurement of fluorescent intensity (Bright-Glo luciferase Assay) obtained after transfection of mRNA formulated either in i-LipoP®, TransIT® or Lipofectamine® in Epithelioma papulosum cyprini line (a) and CHSE-214 cell line (b).

Toxicity

mRNA-loaded i-LipoP® are biocompatible with different cell types such as in Oncorhynchus tshawytscha and HeLa cells (fig. 5).

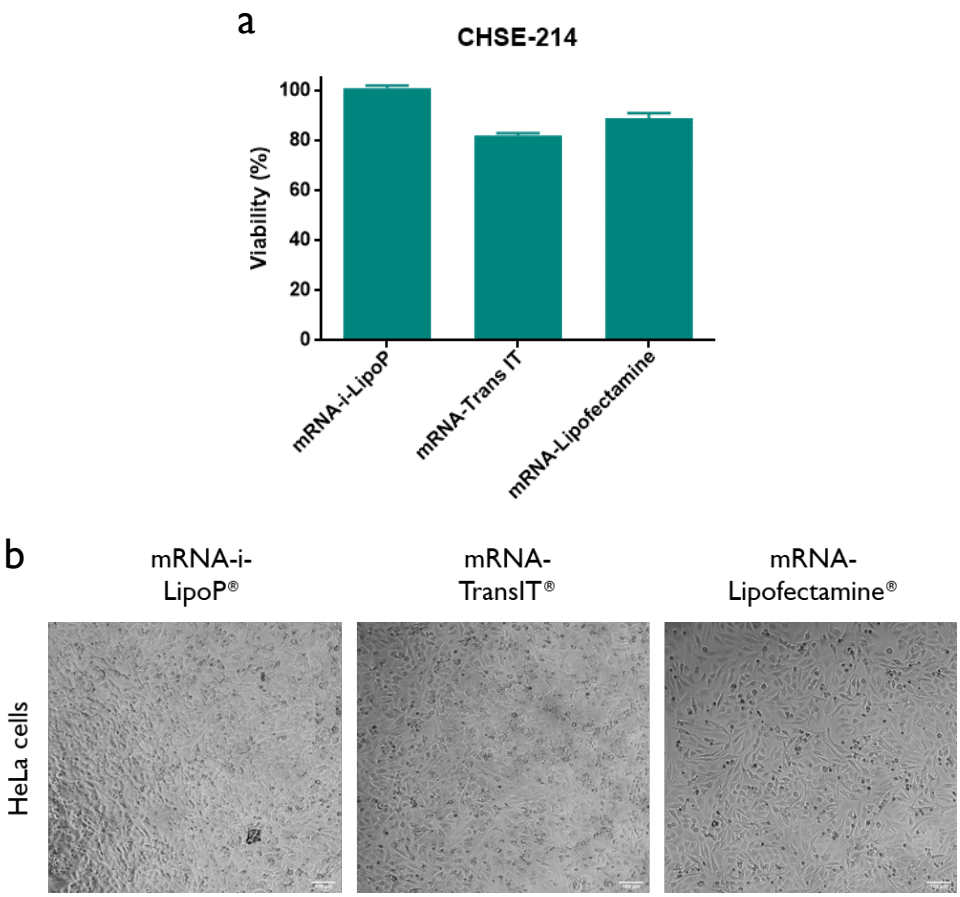


Figure 5. *In vitro* assessment at 24 h of cytotoxicity (normal phase mode) of mRNA formulated either in i-LipoP®, TransIT® or Lipofectamine® in CHSE-214 cell line (a) and HeLa cells (b).

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