Single-Polynucleotide Lipid Nanoparticles: Ultrafast Assembly and Multiscale Structure

Maxime Guichonnet [1], Johanna Mirande-Ney [1], Sebastien Teychene [1] and Kevin Roger [1]

[1] Laboratoire de Génie Chimique, Université de Toulouse, CNRS, Institut National Polytechnique de Toulouse, Toulouse 31400, France

maxime.guichonnet@toulouse-inp.fr

Lipid nanoparticles (LNPs) have emerged as key carriers for the delivery of polynucleotides such as mRNA, and their two-step synthesis involving nanoprecipitation followed by dialysis is under intense scrutiny in the scientific literature. Despite their widespread use, the mechanisms governing LNP formation remain poorly understood, involving complex internal structures, multiple particle populations, and strong coupling between composition, assembly pathway, and delivery performance. This complexity makes it difficult to establish clear structure–function relationships and calls for approaches yielding more homogeneous and well-defined objects. Here, we show that LNP assembly can be directed toward the formation of smaller and more structurally defined nanoparticles. Using small-angle X-ray and neutron scattering (SAXS/SANS) and cryogenic transmission electron microscopy (cryo-TEM), we demonstrate that the resulting nanoparticles encapsulate a single polynucleotide and quantify their multiscale organization. Direct in situ measurements reveal that these particles assemble through a rapid and cooperative co-assembly process occurring on the millisecond timescale [1,2], consistent with a kinetically driven pathway far from equilibrium controlled by molecular diffusion rather than colloidal aggregation [3]. We further show that the final structure is highly responsive to its environment, including ionic strength and pH, highlighting the role of physicochemical conditions in tuning nanoparticle organization [3–5]. Despite this responsiveness, the nanoparticles remain stable upon storage at 4 °C while maintaining efficient transfection. Overall, this work establishes a direct link between assembly pathway, structure, and function, and provides new insights for the rational design of lipid-based nanocarriers.

Keywords:
Lipid nanoparticle, kinetics, co-assembly

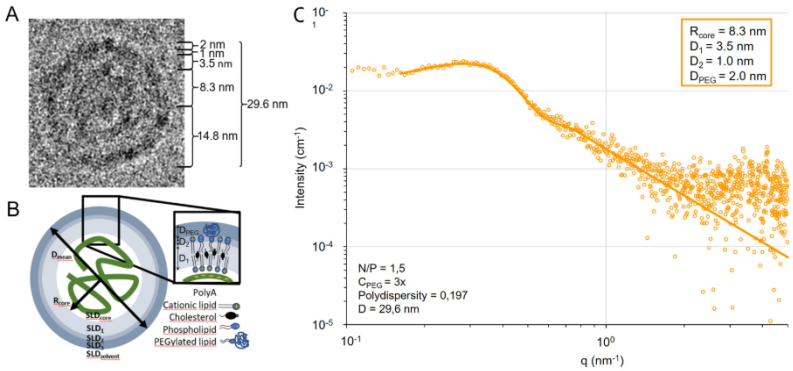

Fig.1: (A) Representative cryo-TEM image of PEGylated LNP, revealing the internal structural features and characteristic length scales within the particles. (B) Proposed structural model of the LNP architecture. (C) SAXS profile of PEGylated LNPs fitted using the structural model shown in panel (B)

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