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Literature Reference

LR2022021086

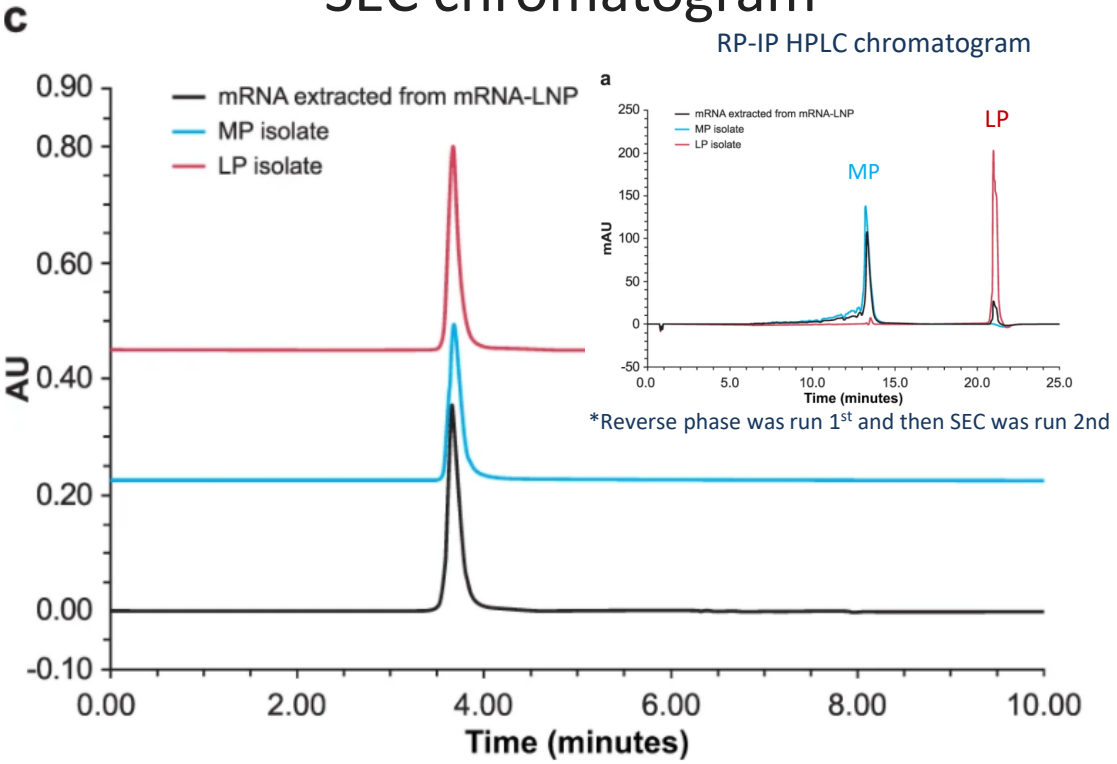
mRNA-LNP (Lipid Nanoparticle) on Sepax Analytical SEC

A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems

SEC was used to rule out if tertiary mRNA structures (i.e. aggregates) were the cause of the Late Peak

Column	Zenix SEC-300 4.6x 150mm Part Number: 213300-4615
Mobile Phase	100mM Tris acetate/2.5mM EDTA pH 8
Flow Rate/Detection	0.25 mL/min, UV 260nm
Instrument	Waters H-Class UPLC
Sample Notes	mRNA extracted from formulated mRNA-LNP
Length of Sample	~2500-3000 nucleotides
mRNA: Lipid prep	mRNA was extracted from the mRNA-LNP formulation by IPA precipitation. (IPA and then NH4-Acetate) Dry-vacuo and then resuspended in RNase-free H2O
MP and LP prep	RP-IP HPLC on extracted mRNA from LNP's and fractionated Generating purified MP and LP fractions MP and LP fractions re-injected onto RP-IP HPLC and SEC

SEC chromatogram



*Reverse phase was run 1st and then SEC was run 2nd

The SEC profile of the extracted mRNA vs. MP (main peak) vs. LP (late peak) were identical, thus eliminating aggregation as the origin of the late peak. This implicates other chemical reactions occurring to cause the generation of the late peak.

ModeRNA Therapeutics

Packer, Meredith, et al. "A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems." Nature communications 12.1 (2021): 1-11