Application note Short summary

Integrating High-Purity mRNA Synthesis and Scalable LNP Formulation: AMCAP™ and TAMARA for RNA-LNP Development

The successful development of RNA therapeutics relies on both high-quality mRNA synthesis and reproducible lipid nanoparticle (LNP) formulation. This application note highlights a collaborative workflow combining **Anemocyte's AMCAP™ ONE-POT capping technology** for efficient production of high-purity 5' CAP-1 mRNA with **Inside Therapeutics' TAMARA microfluidic platform** for controlled and scalable LNP formulation.

In this study, two AMCAP™ mRNA constructs encoding EGFP and β-galactosidase were formulated into SM-102-based LNPs using TAMARA under different flow conditions. Particle size and PDI were assessed before and after dialysis, and biological activity was evaluated in HEK293T cells. The resulting mRNA-LNPs exhibited controlled particle size, low polydispersity, and efficient in vitro protein expression.

Together, **AMCAP™** and **TAMARA** offer complementary technologies for efficient mRNA production and reproducible RNA-LNP formulation.

AMCAP™ and TAMARA provide a streamlined and reproducible workflow for robust RNA-LNP development, from mRNA synthesis to microfluidic formulation.

This work was carried out by Dr Cristina Chiriaco and Dr Marco Pupo at Anemocyte, in collaboration with Dr Yannick Mousli, Dr Sezen Gul, and Robin Oliveres from Inside Therapeutics.

Physicochemical characterization of mRNA-LNPs

Two AMCAP™ ONE-POT mRNA constructs encoding Enhanced Green Fluorescent Protein (EGFP; $mRNA_1$) and β-galactosidase (β-GAL; $mRNA_2$) were formulated into SM-102-based LNPs using the TAMARA microfluidic platform. The effects of three flow rate ratios (FRRs; 2, 3, and 4) and three total flow rates (TFRs; 1, 5, and 10 mL/min) on particle size and PDI were evaluated by DLS before and after dialysis.

Key findings

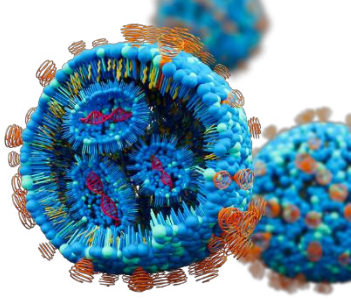
- → At a fixed FRR of 3, **increasing the TFR from 1 to 10 mL/min** reduced particle size for both $mRNA_1$-LNPs and $mRNA_2$-LNPs, while maintaining low PDI values, indicative of homogeneous nanoparticle populations.
- → At a fixed TFR of 5 mL/min, increasing the **FRR from 2 to 4** had a more modest effect on particle size than TFR, with a general trend toward smaller particles. Low PDI values were maintained across all formulation conditions.
- → **All formulations exhibited controlled particle size and low PDI**, demonstrating robust and reproducible mRNA-LNP production across the evaluated process conditions.

Combined with AMCAP™-generated mRNA, TAMARA enabled the reproducible production of well-defined mRNA-LNPs with controlled physicochemical properties.

Tables 1 & 2: *Formulation parameters and DLS characterization of $mRNA_1$-LNPs and $mRNA_2$-LNPs before and after dialysis.*

	Process parameters		Pre-dialysis analysis		Post-dialysis analysis	
Sample ID	**FRR**	**TFR (mL/min)**	**Z-Average (nm)**	**PDI**	**Z-Average (nm)**	**PDI**
$mRNA_{1A}$-LNPs	3	1	128.3	0.08	121.9	0.06
$mRNA_{1B}$-LNPs	3	5	81.3	0.17	92.3	0.15
$mRNA_{1C}$-LNPs	3	10	61.1	0.25	85.5	0.17
$mRNA_{1D}$-LNPs	4	5	80.2	0.15	115.4	0.06
$mRNA_{1E}$-LNPs	2	5	96.0	0.11	89.6	0.08

	Process parameters		Pre-dialysis analysis		Post-dialysis analysis	
Sample ID	**FRR**	**TFR (mL/min)**	**Z-Average (nm)**	**PDI**	**Z-Average (nm)**	**PDI**
$mRNA_{2A}$-LNPs	3	1	133.8	0.06	128.9	0.06
$mRNA_{2B}$-LNPs	3	5	84.6	0.11	97.6	0.15
$mRNA_{2C}$-LNPs	3	10	70.0	0.20	101.4	0.12
$mRNA_{2D}$-LNPs	4	5	80.9	0.11	90.6	0.11
$mRNA_{2E}$-LNPs	2	5	88.6	0.10	101.0	0.08



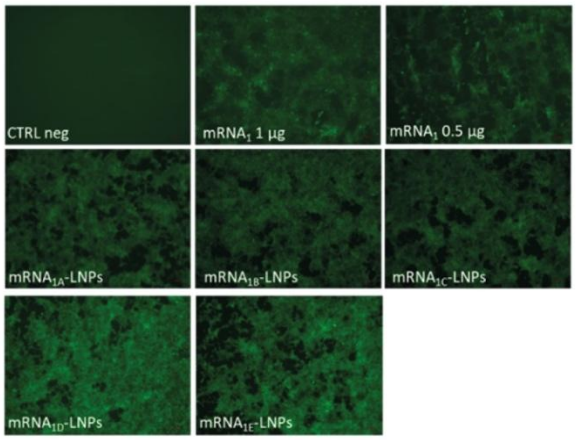
In vitro delivery and protein expression

The biological performance of the formulated mRNA-LNPs was evaluated in HEK293T cells. $mRNA_1$-LNPs (EGFP) were assessed by fluorescence microscopy and flow cytometry, while $mRNA_2$-LNPs (β-GAL) were evaluated using a luminescence-based BETA-GLO assay following 72 h incubation with increasing mRNA doses. AMCAP™ EGFP mRNA and β-GAL mRNA transfected using JetMessenger (Polyplus) served as positive controls.

Key findings

- → **All $mRNA_1$-LNP formulations achieved high transfection efficiency,** with **>90% EGFP-positive cells,** comparable to or slightly exceeding the positive control.
- → Across the TFR/FRR conditions evaluated, **$mRNA_{1A}$-LNPs and $mRNA_{1B}$-LNPs** showed the highest EGFP expression at the maximum dose tested, while **$mRNA_{1C}$-LNPs, $mRNA_{1D}$-LNPs, and $mRNA_{1E}$-LNPs** also maintained strong protein expression.
- → A clear **dose-dependent increase in EGFP expression** was observed across all formulations.
- → **β-GAL mRNA-LNPs** also demonstrated efficient intracellular delivery, achieving consistently high β-galactosidase activity comparable to that of the highest-dose positive control.

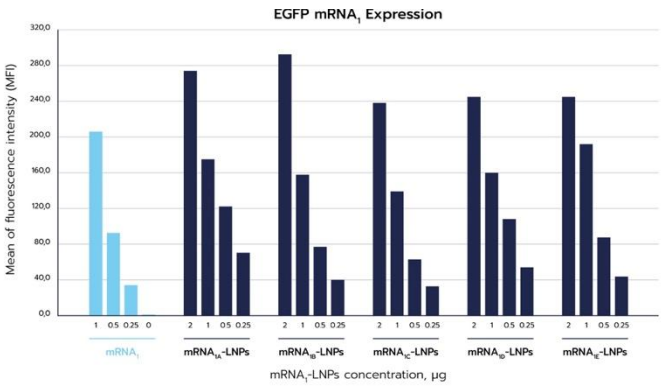
A



The combination of AMCAP™ mRNA and TAMARA-formulated LNPs enabled efficient intracellular delivery and robust *in vitro* protein expression for both mRNA constructs.

Figure 1. *Biological validation of TAMARA-formulated AMCAP™ mRNA-LNPs.* ***(A)*** *Representative fluorescence microscopy images of HEK293T cells following transfection with 2 µg/well of EGFP mRNA-LNPs.* ***(B)*** *Dose-dependent EGFP expression measured by flow cytometry.* ***(C)*** *β-GAL activity following transfection with different doses of β-GAL mRNA-LNPs.*

B



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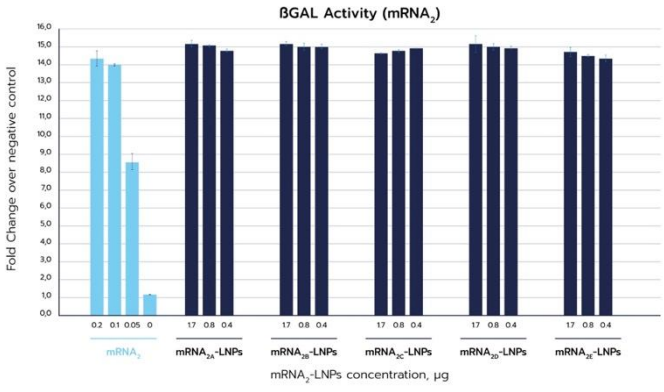

Conclusion

This application note demonstrates the complementary value of AMCAP™ for **high-purity mRNA synthesis** and TAMARA for **reproducible RNA-LNP formulation**.

- → **AMCAP™ ONE-POT capping technology** enabled rapid production of high-quality 5' CAP-1 mRNA, simplifying the mRNA manufacturing workflow while reducing processing time and costs.
- → **TAMARA microfluidic platform** generated reproducible mRNA-LNPs with controlled particle size and low PDI, supporting robust formulation optimization and efficient intracellular delivery.

Together, AMCAP™ and TAMARA provide a streamlined workflow for mRNA-LNP development, from high-quality mRNA production to reproducible LNP formulation, supporting the development of high-performance RNA therapeutics.

Read the full Application Note on https://insidetx.com

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