Towards therapeutically relevant nanoparticle formulations: Influence of mRNA length

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To develop more efficient vaccines, immunotherapies and gene therapies, major efforts are being put into optimizing delivery systems for mRNA therapeutics. However, standard reporter mRNAs encoding for fluorescent proteins are rarely representative of their therapeutic counterparts. In fact, such therapeutic mRNAs, including the spike protein mRNA used in the COVID-19 vaccines, are often more than twice as long as common reporters used during development, directly influencing delivery efficiency. These issues stem from degradation, translation inefficiency, as well as encapsulation efficiency, meaning that to develop more predictable delivery vehicles and increase their clinical translation , better reporter mRNAs need to be developed.

Here, we have designed a set of mCherry-encoding constructs of varying lengths with retained protein encoding functionality when transfecting Jurkat T-cells. Using these constructs, we investigated the impact of mRNA length on both physicochemical and biofunctional properties of polymer-based nanotherapeutic delivery systems. The mRNA molecules were synthesised by in vitro transcription and purified using affinity-based chromatography. Their translational functionality into protein was confirmed in Jurkat T-cells using lab-grade , commercially available transfection reagent and flow cytometry analysis . For drug delivery purposes, the mRNAs were complexed with polymer-based nanoparticle systems , where physicochemical properties and influence of mRNA length was assessed , includ ing size, zeta potential, encapsulation efficiency and morphology .

Our results demonstrate that increasing the length of the mRNA constructs results in translational functionality, although with lower transfection efficiencies compared to the native optimized transcript when using transfection standard JetMessenger (Figure 1). Following complexation with cationic polymers, the hydrodynamic diameter increased proportionally to both RNA length and concentration, with a minimum size of 100nm. In conclusion, our designed mRNA constructs offer a biologically relevant and quantifiable in vitro readout for delivery system development.

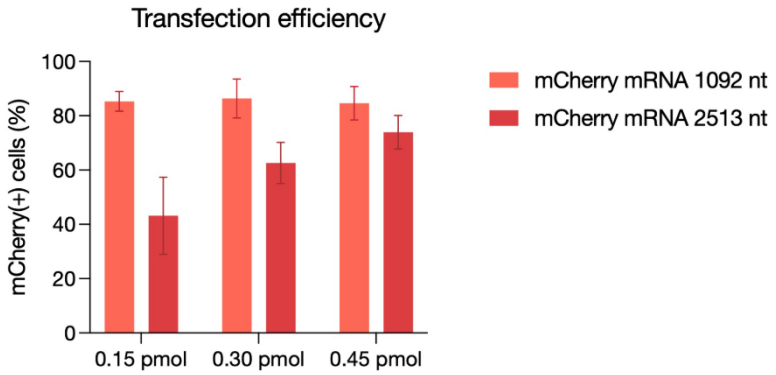

Figure 1: Transfection efficiency reported as percentage of fluorescence positive cells expressing 1092 nt and 2513 nt mCherry encoding mRNA. The mRNA was delivered to Jurkat T-cell line using JetMessenger transfection reagent and was evaluated by flow cytometry.