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Dissecting the influence of cargo in the performance of polymeric nanoformulations for mRNA delivery

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Background: Messenger RNA (mRNA) therapies offer precise control over therapeutic protein production due to their transient nature and biodegradability. However, efficient cytoplasmic delivery remains challenging. Polymeric nanoparticles are promising mRNA delivery vehicles, valued for their biocompatibility and chemical versatility. While considerable attention has been given to the properties of the delivery vectors, the characteristics of the mRNA itself, such as length, may critically influence delivery efficiency. Here, we investigated how mRNA length impacts their delivery from polymeric nanoparticles.

Methods: Polymeric nanoparticles were prepared by electrostatic complexation of mRNA with a polymer synthesized via Michael addition, which was previously identified to effectively transfect fibroblasts [1]. Various mRNAs ranging from 1 to 9 kb, including Cre recombinase, GFP, Cas9 (with a guide RNA targeting GFP), as well as self-amplifying GFP mRNA, were complexed with the nanoparticles. Functional mRNA delivery to fibroblasts was evaluated by high-content imaging to compare GFP fluorescence. These nanoformulations were characterized with respect to their size, surface charge, mRNA entrapment and release.

Results: While size and surface charge did not change considerably with mRNA cargo, entrapment efficiency increased with mRNA length. These findings suggested a stronger affinity of longer mRNA molecules to the polymer due to electrostatic interactions. Longer mRNA molecules had lower biological efficacy, despite their enhanced capacity to undergo endosomal escape indicated by Galectin- 9 staining. We observed that longer mRNAs required more heparin to destabilize the nanoparticles, indicating poorer mRNA release.

Conclusion: Our findings highlight that mRNA length critically influences the efficiency of polymeric nanoparticles, which underscores the necessity of understanding the specific properties of the mRNA cargo to optimize nanoformulations for a wider variety of gene therapy indications.

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References: [1] A.F. Rodrigues, et al. Adv Sci. 2023, 10, 2205475.