Optimized untranslated region and Kozak motif design of synthetic mRNA improves *in vivo* protein replacement therapy

Authors

Renzo Knol, Tahmina Fariaby, Jeroen Bussmann, Alexander Kros, Bram Slütter

Abstract oral presentation

Synthetic mRNA to express theoretically any protein of interest has recently received immense attention since the global success of the SARS-CoV-2 mRNA vaccines[1]. Moreover, several dozens of mRNA-based therapeutics with a broad range of applications are currently undergoing clinical trials, highlighting the enormous potential of mRNA as a therapeutic agent[2]. Besides delivery efficiency, mRNA therapeutics' efficacy largely depends on protein translation levels reached in target cells. This is determined by the synthetic mRNA's ability to utilize the endogenous translation machinery without eliciting high inflammatory responses and before being degraded by abundant nucleases. Besides the protein-coding sequence (CDS), endogenous mRNAs contain a 5' cap, 3' poly(A) tail, and untranslated regions (UTRs) surrounding the CDS that play pivotal roles in overall mRNA stability and translatability. Furthermore, in eukaryotes, the 5' UTR contains a highly conserved sequence motif known as the Kozak motif, which includes the start codon and is crucial for translation initiation. Optimizing these UTRs greatly impacts the mRNA's therapeutic effect. Several previous studies focused on UTRs from highly expressed genes or stable mRNAs. Recently, massive parallel *in silico* studies utilizing machine learning have generated synthetic UTRs potentially more potent than endogenous UTRs[3]. However, a small subset of architectures was often only tested *in vitro* and focused on either the 5' UTR or the 3' UTR. Here we have compared translation levels of EGFP surrounded by combinations of synthetic and endogenous UTRs *in vitro* and in zebrafish embryos. It was observed that Kozak motif design has a profound impact on translation level regardless of UTR design and that the addition of an optimal Kozak motif to dengue virus 5' UTR resulted in significantly higher EGFP translation levels compared to all other tested 5' UTRs. Subsequently, a selection of UTR and Kozak motif designs were compared in mice expressing firefly luciferase. Finally, the best-performing design outperformed the BioNTech SARS-CoV-2 mRNA vaccine UTR design in a protein replacement therapy expressing low-density lipoprotein receptor (LDLR) mRNA in LDLR-deficient mice delivered by lipid nanoparticles.

References (*max 3*)

1. Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2020).
2. Wang, Y. S. *et al.* mRNA-based vaccines and therapeutics: an in-depth survey of current and upcoming clinical applications. *J. Biomed. Sci.* **30**, 1–35 (2023).
3. Cao, J. *et al.* High-throughput 5′ UTR engineering for enhanced protein production in non-viral gene therapies. *Nat. Commun.* **12**, (2021).