

Development and applications of peptide barcoded nanoparticles for high-throughput screening of mRNA delivery materials *in vivo*

mRNA lipid nanoparticles (LNP) present a promising class of therapeutics, with broad applications in protein replacement therapy, gene editing, immunotherapy, and vaccines, owing to their versatility and precise nature. While recent years have seen dramatic improvements in the safety and efficacy of mRNA therapeutics, their functional delivery to target tissues and cells *in vivo* remains challenging, partly due to the lack of predictive power of *in vitro* assays and the low-throughput and costly nature of *in vivo* screening approaches. Thus, there is still a need for safe, specific, and potent mRNA delivery materials, as well as higher throughput *in vivo* screening methods.

In this work, we developed a novel *in vivo* nanoparticle screening platform that relies on LC-MS/MS based detection of peptide barcodes translated from barcoded mRNAs in transfected cells, allowing for a readout of functional delivery that is directly proportional to protein production effected by each nanoparticle within a pooled library. We showed that this approach has high sensitivity and accuracy in both cultured cells *in vitro* and in tissues *in vivo* and demonstrated the applicability of this approach to *in vivo* screening of LNPs by developing and optimizing the formulation of a biodegradable LNP, RM133-3-21, for potent mRNA delivery to the liver. We then screened a large library of ionizable lipids for their ability to deliver mRNA to the lung and optimized both the structure and formulation of the lead compound. The resulting LNP, C15-21, is highly potent and is able to transfect up to 80% of lung endothelial cells after a single dose. In addition, we demonstrated that C15-21 is able to efficiently deliver Cas9 mRNA and sgRNA for targeted gene disruption in the lung, resulting in up to 7.5% gene editing in lung endothelial cells. Finally, we also developed materials and formulations that show high specificity for splenocytes *in vivo*.

Taken together, the work presented in this thesis contributes to the field of mRNA therapeutics by increasing the throughput of LNP testing *in vivo* and by introducing novel delivery materials.