



RNA delivery systems

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Between December 2020 and December 2021, COVID-19 vaccines prevented over 14 million deaths (1). Two of these vaccines were lipid nanoparticles (LNPs) formulated to carry mRNA. Other LNP-RNA drugs, including those that edit (2) or silence (3–8) genes *in vivo*, have generated excellent data in patients. At first glance, these results underscore an exciting concept: RNA therapies have arrived.

Yet, this concept comes with a big asterisk: If an RNA drug cannot enter the right target cell, it will not work. Since RNAs are large and anionic, they cannot efficiently cross the cell bilayer on their own and therefore usually require a drug delivery system (9). One bright spot in the field is clinical delivery to hepatocytes, which has already been reported (2–8). In a leading example, Alnylam Pharmaceuticals earned five FDA approvals between 2018 and 2023; ongoing clinical trials suggest that liver programs from other companies are also going well.

These data strongly indicate that many clinical programs could result from improving liver targeting or unlocking a single new (i.e., non-liver) cell type. As a result, academic and company labs across the world work on improving delivery. In this Special Feature, a subset of these efforts is shared via four perspectives and seven research articles. Highlighting the diverse approaches scientists are taking to improve delivery, these articles span topics as disparate as protein engineering, polyanion design, DNA barcoding, and self-reporting RNA nanosystems. The variety of scientific approaches described here is consistent with the fact that drug delivery is a complex, interdisciplinary process.

The four perspectives come from labs that approach RNA therapeutics and drug delivery in distinct ways. In one example, scientists highlight the advantages of non-viral delivery systems over viral delivery systems such as adeno-associated virus and describe how to think about therapeutics that rely on CRISPR genome editing (10). Within this context, the authors outline diverse non-viral systems including nanoparticles, enveloped delivery vehicles, and virus-like particles. Complementing this perspective is one describing chemical structures used in LNP formulations (11). The authors subsequently highlight the intersection between high-throughput chemistry and careful, rational design before describing approaches to test LNPs. These same approaches can be used to study other chemistries, including dynamic structures (12). The authors outline how nanoparticles can be designed to respond when they are exposed to changes in their environment, including pH. In many cases, these tunable responses can lead to payload release. The structures and chemistries discussed in these perspectives lead to a fourth perspective describing what happens when nanoparticles reach a target cell (13). This paper details the biology of endosomal escape, why it has been difficult for nanoparticles to escape endosomes historically, and efforts to change this. The authors pay especially close attention to the different

pathways used for endocytosis and, importantly, detail the advantages and limitations of scientific techniques used to study these pathways.

The topics addressed in these perspectives are also reflected in the research articles within this special issue. In the first example, authors describe a self-reporting theranostic nanoparticle (14). The system is designed to silence target gene expression yet also monitor the functional activity of targets further downstream. The authors demonstrate the utility of this system in a xenograft mouse model of ovarian cancer. Using genetics in another way, authors show that a system derived from human paraneoplastic antigen Ma2 (PNMA2) can be used as an mRNA delivery system in mammalian cell lines (15). After finding that PNMA2 forms icosahedral capsids but does not naturally encapsulate nucleic acids, the authors use cryo-electron microscopy and protein engineering to design a new construct with encapsulating properties. Using chemistry instead of genetics, scientists report that LNPs can adsorb biologically relevant polyanions (16). By designing these layered LNPs, the scientists create delivery systems that transfect cells *in vitro* as well as in adult mice. The study above demonstrates how molecules that bind to the surface of a nanoparticle can affect its behavior, which leads to another manuscript focusing on a magnetic separation method to isolate and characterize biomolecules that bind LNPs (17). The authors use magnetic iron oxide-loaded LNPs to separate the LNPs and corona from biological media, which allows them to analyze the components without compromising particle integrity. It is likely that systems to understand the LNP corona will be useful for the last three papers in the Special Feature, since all three report non-liver LNP delivery *in vivo*. In the first example, LNP delivery to maternal organs is demonstrated in pregnant mice via several routes of administration (18). The authors report

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delivery to trophoblasts, endothelial cells, and immune cells, also noting a relationship between the structure of the ionizable lipid and efficacy. These data provide a glimpse into the ways nanoparticles can be designed for use during pregnancy. In the second example, authors use DNA barcodes to test many different LNPs in vivo (19). The authors report that cationic charge on cholesterol leads to different tropism than cationic charge on helper lipids, then subsequently shows delivery to the heart endothelial cells as well as lung stem cells in mice. Finally, the authors report successful delivery to photoreceptors and the retinal pigment epithelium in non-human primates, which are considered a gold-standard preclinical model (20). The LNP that leads to delivery relies on thio-lipids synthesized via the Gewald reaction. Notably, the authors did not observe any acute toxicity, which further supports the investigation of this chemical space in large animals.

Although the work contained in this Special Feature is exciting, it is important to acknowledge unanswered questions

that limit the broad adoption of nanoparticles as therapeutic vectors. For example, it is unclear how frequently mRNA therapies can be readministered when elevated (i.e., non-vaccine) doses are used. A related question is how easily emerging nanoparticle classes can be manufactured reproducibly at human scale. Both questions will depend on the cargo, which brings up a third limitation: It remains unclear how cargo impacts delivery vehicle design. For example, scientists are developing mRNA constructs that express more protein or express protein for longer durations that could alter the dose or dosing schedule in humans. Finally, it will be important to understand how all these traits in one preclinical system change as a function of the model (e.g., does delivery in mice predict delivery in nonhuman primates?). Yet, despite these limitations, we believe the work contained in this Special Feature conveys one critical lesson: sustained, interdisciplinary efforts are positioned to help RNA therapeutics reach their scientific and clinical potential.

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