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Review

# What's Next after Lipid Nanoparticles? A Perspective on Enablers of Nucleic Acid Therapeutics

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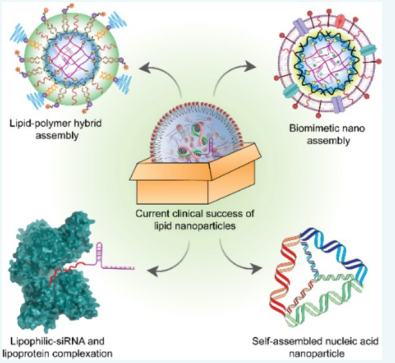
**ABSTRACT:** Recent success of mRNA-based COVID-19 vaccines have bolstered the strength of nucleic acids as a therapeutic platform. The number of new clinical trial candidates is skyrocketing with the potential to address many unmet clinical needs. Despite advancements in other aspects, the systemic delivery of nucleic acids to target sites remains a major challenge. Thus, nucleic acid based therapy has yet to reach its full potential. In this review, we shed light on a select few prospective technologies that exhibit substantial potential over traditional nanocarrier designs for nucleic acid delivery. We critically analyze these systems with specific attention to the possibilities for clinical translation.

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## INTRODUCTION

Nucleic acid therapies have emerged as a potential cure-all solution for many diseases owing to the possibility of specific alteration of misfiring genes (such as gene editing and inhibition).[1,2] Recent advancements in the understanding of disease pathogenesis have created demands for gene therapy as a promising alternative to traditional medicine, especially in hard-to-cure diseases.[3] The increased rate in the clinical approval of nucleic acid therapeutics and innumerable candidates in different phases of clinical trials demonstrate the potential of nucleic acids as therapeutics. The clinical success of the oligonucleotide-based drug Spinraza (Eondys 51) followed by the Federal Drug Administration (FDA, United States) approval of the first siRNA drug Onpattro (patisiran, ALN-TTR02)[4] brought the spotlight onto the potentiality of gene therapies. The recent approvals of various nucleic acid based COVID-19 vaccines (Pfizer−BioNTech, Moderna, and Johnson & Johnson) by the FDA and European Medicines Agency have instigated a new wave of nucleic acid based therapies.[5−8]

Although the therapeutic success of nucleic acid based vaccines is recognized, there are a few delivery barriers that limit the recognition of nucleic acids as viable therapeutics in broader biomedical applications. First, nucleic acids with their high residual negatively charged backbone are impermeable to the plasma membrane.[3,9] Second, unmodified nucleic acids are systemically unstable because of the presence of nucleases in bodily fluids.[10] Both of these factors have necessitated the development of smart strategies to efficiently deliver functional nucleic acids.[9−11] Utilization of a delivery vector often provides protection against nucleases alongside efficient intracellular delivery across the cell membrane.[10−16,18] For intracellular delivery, viral vector platforms show promise, but these have some drawbacks such as immunogenicity, difficulty of assembly, cytotoxicity, and inflammatory responses.[17−19] Nonviral delivery platforms hold the potential to address some of these shortcomings.[14−16,20−24] Among nonviral delivery systems, lipid nanoassemblies are considered especially promising.[11,23,25−28] The recent success of lipid nanoparticle (LNP)-based mRNA vaccines brought the spotlight onto self-assembled lipid nanostructures as "go-to" delivery vehicles for therapeutic nucleic acids.

Despite the clinical success of LNPs, there is still significant room for improvement. In general, major bottlenecks in lipid-based nanoassemblies include poor long-term stability,[29,30] low cytosolic delivery,[31] and lack of active targeting for site-specific delivery. Explicit attention to address these pitfalls presents an enormous opportunity. In this review, we survey and critically analyze the ongoing research of nucleic acid drugs (Figure 1) with a specific focus on unmet needs, pitfalls, and potential scopes for improvement.

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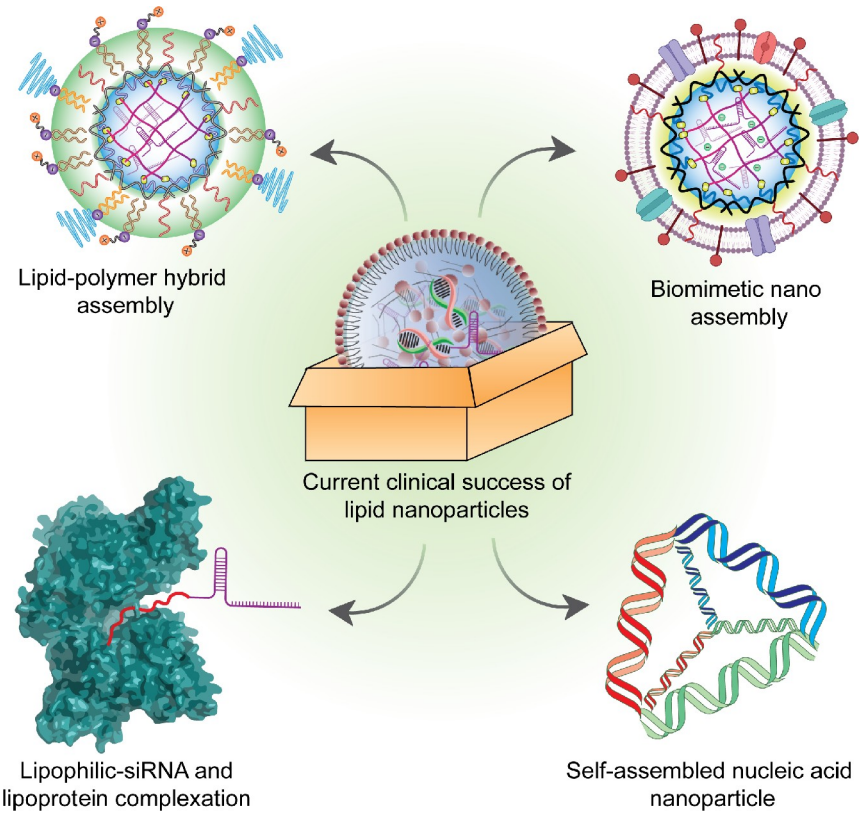


**Figure 1.** Four different methodologies for nucleic acid delivery with potential clinical adaptability for future nucleic acid therapeutics.

## SUCCESS JOURNEY OF LIPID NANOPARTICLES FOR GENE DELIVERY

Recent success with mRNA vaccines against COVID-19 highlights the prospect of LNPs as delivery vectors for gene therapies.[32] Here, we list a few noteworthy candidates that are progressing in clinical trials for a variety of applications (Table 1). For example, Arcturus Therapeutics has presented an interesting nucleic acid approach toward COVID-19 vaccines. They use a self-replicating mRNA encapsulated in their LUNAR LNP that is based on a proprietary ionizable lipid, DSPC, cholesterol, and PEG2000-DMG.[33] Their self-replicating RNA is comprised of the replicase genes *nsP1*−*nsP4* of the Venezuelan equine encephalitis virus along with the prefusion Spike protein of SARS-CoV-2. This candidate has shown exciting results in preclinical trials because of its self-amplifying capability, which is due to the replicase genes *nsP1*−*nsP4*, allowing for increased expression of the spike protein when compared to the prefusion spike protein alone. *nsP4* encodes for an RNA polymerase that is primarily responsible for the spike protein to replicate, causing the increased expression, whereas *nsP1* and *nsP2* aid in the stability of the mRNAs that are produced through capping and a polyA tail. This self-replicating RNA vaccine is double-stranded, which can cause an adaptive immune response through a type I IFN (interferon) response. Another mRNA-based COVID-19 vaccine, CureVac, is in the clinical pipeline with a commercial name CVnCoV. The lipid-based formulation, which encapsulates the mRNA encoding the spike protein, is composed of an ionizable amino lipid, a phospholipid, cholesterol, and a PEGylated lipid.[34] These two formulations suggest the overlapping themes that are seen in many successful LNP formulations, that is, using a combination of ionizable lipids with a PEGylated lipid and a phospholipid, along with a certain percentage of cholesterol.

In addition to LNPs, BioNTech also uses RNA−lipoplexes (RNA−LPX) in their large number of mRNA-based formulations in the pipeline. Another successful candidate, BNT113, utilizes the RNA−LPX platform containing a cationic liposome consisting of *N*-[1-(2,3-dioleyloxy) propyl]-*N*,*N*,*N*-trimethylammonium chloride and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine.[35] This BNT113 is a vaccine formulation against human papillomavirus (HPV), a virus that has been linked to causing cancer. Similarly, MiNA, a company focused on small activating RNAs (saRNA), has developed a formulation for liver cancer that is in a phase 1 clinical trial. The saRNA here is designed to upregulate CCAAT/enhancer-binding protein alpha (CEBPA) that is encapsulated in a proprietary liposomal nanoparticle, NOV340 SMARTICLES.[36] These ionizable liposomes are anionic or neutral that become slightly cationic in the tumor environment to enhance cellular internalizations.[37] This charge-conversion strategy presents a new direction in designing delivery vectors for targeted therapeutics.[38]

Lipid nanoparticles, liposomes, and other lipid conjugation strategies have become a popular choice for clinical gene therapy. Though a significant number of clinical candidates have already been approved or are being evaluated, as exemplified in Table 1, there remains significant room for improvement. In the next section, we discuss a few next-generation strategies that promise to overcome some of the stumbling blocks in the current generation of clinical candidates.


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**Table 1. List of Major Nucleic Acid Candidates Currently in Clinical Trials**

| name | company | formulation | nucleic acid | disease | target/encoded protein | clinical trial phase | ID number |
|---|---|---|---|---|---|---|---|
| ARCT-154 | Arcturus Therapeutics, Inc. | LUNAR (LNP) | self-replicating mRNA | COVID-19 vaccine | Spike protein | phase 3 | NCT05012943 |
| ARCT-810 | Arcturus Therapeutics, Inc. | LUNAR (LNP) | mRNA | ornithine transcarbamylase deficiency | ornithine transcarbamylase (OTC) | phases 1 and 2 | NCT04442347 |
| ARCoV | Walvax Biotechnology, Abogen Biosciences | ionizable lipid | mRNA | COVID-19 vaccine | receptor binding domain (amino acids 319−541) | phase 3 | NCT04847102 |
| mRNA-1647 | Moderna | ionizable LNP | mRNA | cytomegalovirus infection | CMV gH pentamer complex and herpesvirus glycoprotein (gB) protein | phase 3 | NCT05085366 |
| mRNA-1345 | Moderna | ionizable LNP | mRNA | respiratory syncytial virus | RSV fusion protein | phases 2 and 3 | NCT05127434 |
| mRNA-4157 | Moderna−Merck | ionizable LNP | mRNA | solid tumors/melanoma | tumor-associated antigens | phase 2 | NCT03313778 |
| mRNA-3705 | Moderna | ionizable LNP | mRNA | methylmalonic acidemia | human methylmalonyl-coenzyme A mutase (hMUT) | phases 1 and 2 | NCT04899310 |
| mRNA-2416 | Moderna | ionizable LNP | mRNA | relapsed/refractory solid tumor malignancies lymphoma, ovarian cancer | OX40 ligand (OX40L) | phases 1 and 2 | NCT03323398 |
| mRNA-1273.617 | Moderna | ionizable LNP | mRNA | COVID-19 delta variant | N/A | phase 2 | NCT04927065 |
| mRNA-1273 | Moderna | ionizable LNP | mRNA | COVID-19 | Spike protein | phase 3 | NCT04860297 |
| mRNA-5671 | Moderna−Merck | LNP | mRNA | neoplasms | KRAS | phase 1 | NCT03948763 |
| BNT112 | BioNTech | RNA−lipoplex | mRNA | prostate cancer | prostatic acid phosphatase (PAP), prostate-specific antigen (PSA), 3 antigens | phases 1 and 2 | NCT04382898 |
| BNT111 | BioNTech | RNA−lipoplex | mRNA | advanced melanoma | NY-ESO-1, MAGE-A3, and TPTE | phase 2 | NCT04526899 |
| BNT113 | BioNTech | RNA−lipoplex | mRNA | human papillomavirus 16 (HPV16)+ head and neck cancer | HPV-16 E6 and E7 | phase 2 | NCT04534205 |
| BNT122 | BioNTech−Genentech | RNA−lipoplex (RNA−LPX) | mRNA | 1L melanoma/adjuvant colorectal cancer | N/A | phase 2 | NCT04486378 |
| BNT151 | BioNTech | LNP | mRNA | solid tumors | IL-2 | phases 1 and 2 | NCT04455620 |
| BNT161 | BioNTech−Pfizer | LNP | mRNA | seasonal influenza | influenza antigens | phase 1 | N/A |
| BNT162 | BioNTech−Pfizer | LNP | mRNA | COVID-19 | Spike protein | phase 4 | NCT05168709 |
| CVnCoV | CureVac AG | ionizable LNP | mRNA | COVID-19 vaccine | Spike protein | phase 3 | NCT04652102 |
| CV7202 | CureVac AG | LNP | mRNA | rabies | rabies lyssavirus glycoprotein (RABV-G) | phase 1 | NCT03713086 |
| INT-1B3 | InteRNA | LNP | miRNA | solid tumors | miR-193a-3p | phase 1 | NCT04675996 |
| MiNA | MTL-CEBPA | NOV340 SMARTICLES | saRNA | liver cancer | CCAAT/enhancer-binding protein alpha (CEBPA) | phase 1 | NCT02716012 |
| Onpattro (patisiran) | Alnylam | LNP | siRNA | ATTR amyliodosis-expansion | transthyretin | phase 3 | NCT03997383 |



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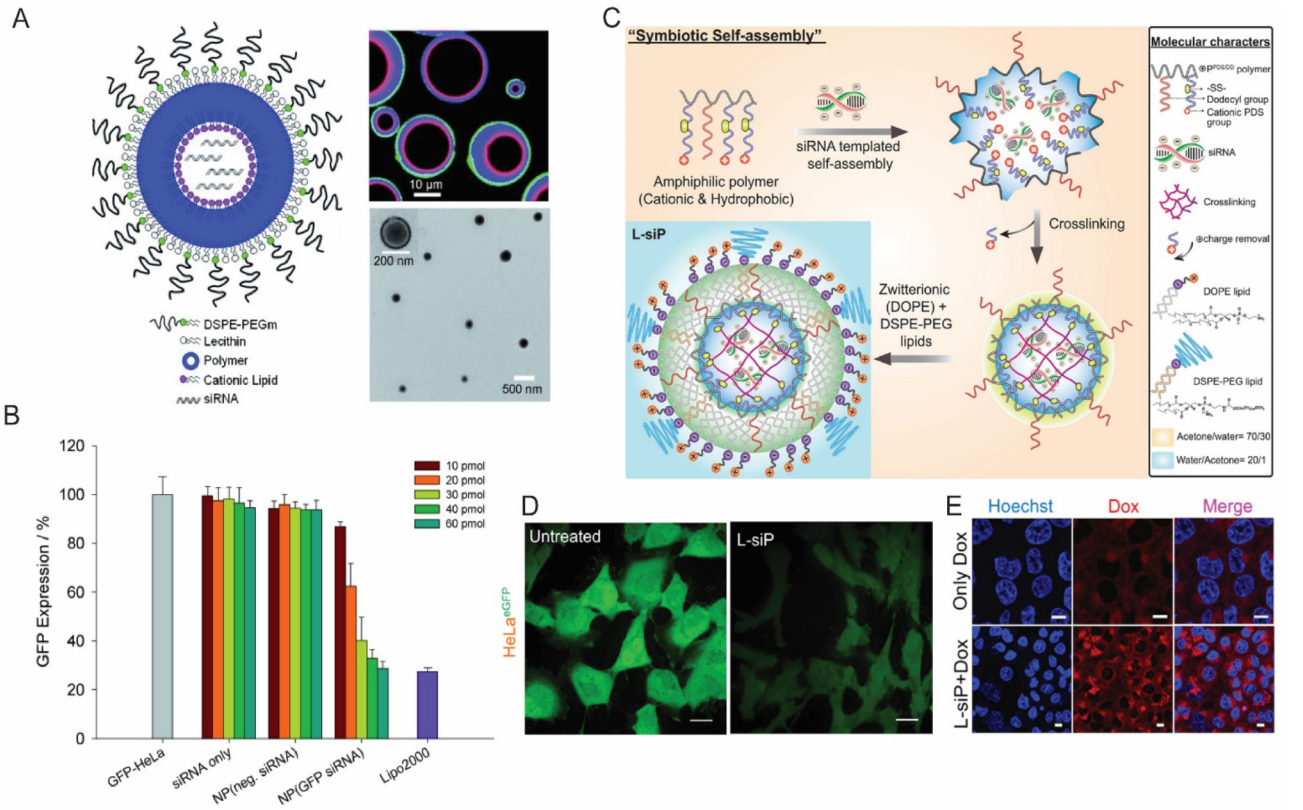


**Figure 2.** (A) Schematic illustration of core/shell lipid−polymer−lipid hybrid nanomaterial, with confocal microscopy image (right top) and TEM image (right bottom) confirming the core/shell morphology. (B) Flow cytometry data showing dose-dependent GFP knockdown in HeLa cells. (Reproduced with permission from ref 54. Copyright 2011 Wiley-VCH Verlag GmbH & Co.) (C) Schematic illustration depicting the formulation procedure of "virus mimicking" lipid−polymer hybrid particles. (D) Confocal microscopy data showing efficient knockdown of GFP in HeLa cells; scale bar, 20 $\mu$m. (E) Effect of treating NCI-ADR/RES cells with MDR1 siRNA using lipid−polymer hybrid nanoassemblies; scale, 10 $\mu$m. (Reproduced with permission from ref 61. Copyright 2019 American Chemical Society.)

## NEXT-GENERATION MATERIALS FOR EFFICIENT NUCLEIC ACID DELIVERY

An array of synthetic materials ranging from polymeric nanoparticles[39,40] to lipid assemblies[25,41,42] and cell-penetrating peptides[20,21] have been reported as alternatives for viral gene delivery vectors for efficient and nontoxic nucleic acid delivery. The success of LNPs in the clinical setting has generated more confidence in nonviral technologies than ever before. Consequently, new technologies that can address the pitfalls of lipid nanoassemblies are emerging. In this review, we focus on a select few strategies with the potential for clinical translation of nucleic acids therapeutics.

## CORE−SHELL LIPID−POLYMER HYBRID ASSEMBLIES

Lipid-based nanocarriers, such as liposomes, solid−lipid nanoparticles, and other nanostructured lipid vectors, offer advantages in features such as bioavailability, trapping efficiency, and low production costs.[43,44] These advantages are also accompanied by some liabilities, often arising from poor encapsulation stability and high polydispersity.[45,46] On the other hand, polymeric nanoassemblies are known to exhibit excellent encapsulation stability, low polydispersity, and convenient synthetic procedures.[47,48] However, their poor bioavailability has been a major stumbling block in their clinical translation.[47,49] Lipid−polymer hybrids are being developed as the next-generation formulation that strives to include the best of both lipid and polymeric systems.

Structurally, lipid−polymer hybrids consist of two functional components: (i) a hydrophobic polymer/lipid core to facilitate stable encapsulation of the cargo; (ii) a stealth lipid layer for enhanced biocompatibility of the carrier.[50−52] This bicomponent approach often offers significant tunability in carrier design. The independent structural tunability of each of these modules opens the scope of parametric optimizations that are critical for maximizing on-target delivery efficacy of functional biologics. Despite the success of this concept in efficient delivery of hydrophobic drugs,[52,53] encapsulation of highly charged and hydrophilic nucleic acids poses a significant barrier. To this end, a differentially charged hollow core−shell approach has been employed with a lipid−polymer−lipid hybrid design. GFP siRNA was encapsulated in a cationic lipid inner core surrounded by a hydrophobic poly(lactic-*co*-glycolic acid) (PLGA) shell and a neutral lipid encasing to form a stealth surface layer (Figure 2A).[54,55] Following the success of the initial concept, an improved version of the lipid−polymer hybrid using lipidoid G0-C14 in the nanoparticle core showed a significant increase in stability with a release half-life of ∼9 days, compared to ∼8 h for lipofectamine$_{2000}$.[55,56] *In vivo* validation of the hybrid formulations in delivering PHB1 siRNA established the potential of the nanodelivery platform with an ∼76% decrease in PHB1 expression in the treated group.[56] Inspired by such promise, scientists have explored several structural variations in polymer−lipid combina-


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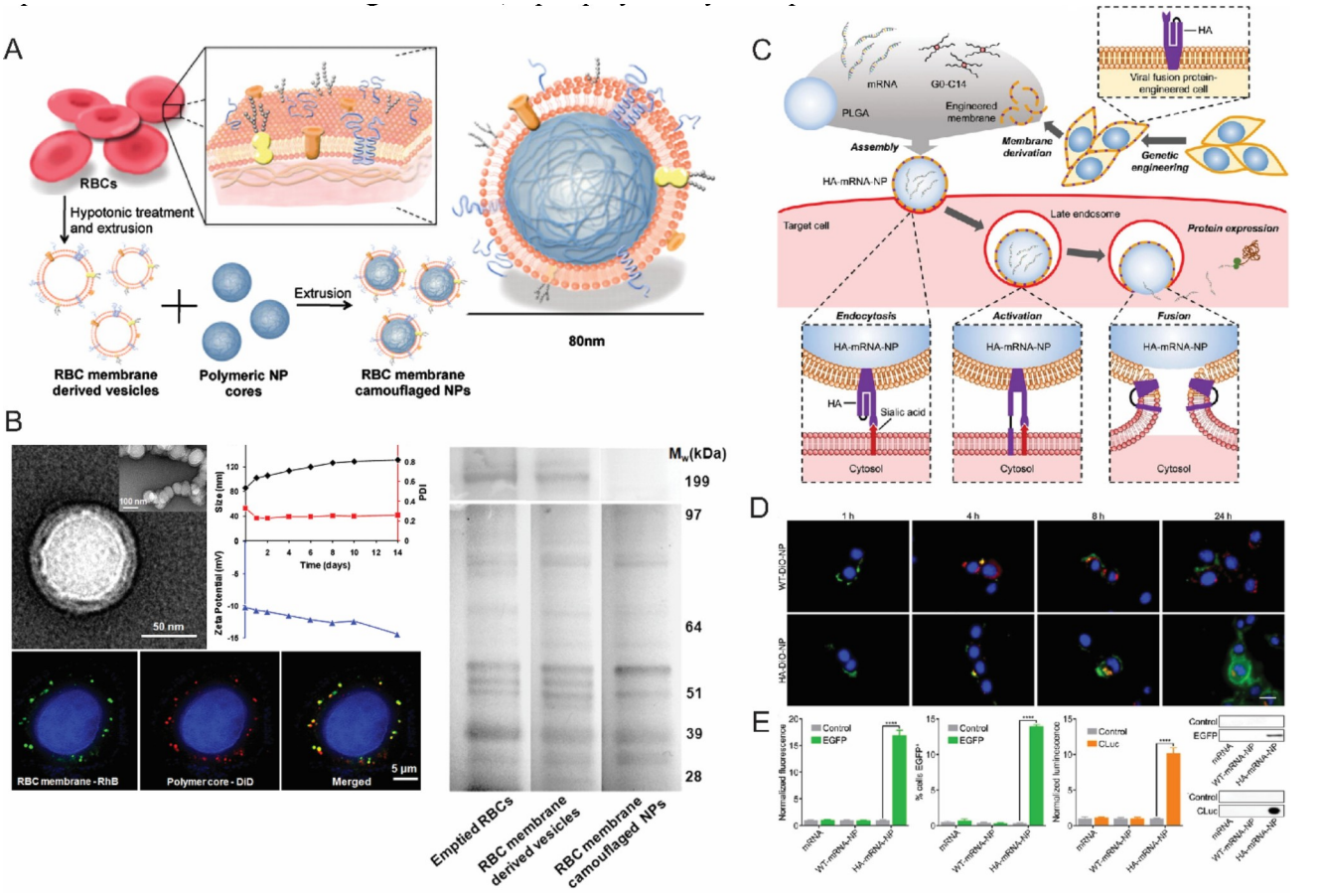


**Figure 3.** (A) Schematic illustration of RBC-membrane-coated PLGA nanoparticles; (B) TEM and confocal microscopy characterization of RBC-membrane-coated nanoparticles (left); size and zeta potential data showing the stability of nanoparticles; SDS-PAGE data (right) show the similarity of protein expression of the RBC-derived membranes with native RBC membranes. (Reproduced with permission from ref 65. Copyright 2011 The National Academy of Sciences of the USA.) (C) mRNA delivery strategy utilizing genetically modified cells expressing viral fusion protein hemagglutinin (HA). (D) Endosomal escape and cytosolic localization of DiO-labeled nanoparticles in B16-WT cells; scale bar, 20 $\mu$m. (E) Expression of eGFP and cLUC in B16-WT cells, confirmed with flow cytometry and Western blots. (Reproduced with permission from ref 83. Copyright 2022 Wiley-VCH Verlag GmbH & Co.)

tions.[57,58] Among them, charge-reversing polymers appeared to be particularly interesting because of their ability to switch the surface charge from cationic to anionic or vice versa, on demand, which mitigates the cation-mediated toxicity observed with traditional polymeric transfection agents.[38,58] To this end, our group envisaged a polymer system that uses a "bait-and-switch" strategy to incarcerate siRNA in a tightly packed polymeric core using a novel ad hoc electrostatic encapsulation process that eliminates cationic charges.[59,60] To further improve the system by harnessing the advantages of a lipid coating, the formulation was completed by encasing a zwitterionic lipid shell using hydrophobic alkyl chains on the polymer as handles to obtain "virus-inspired" symbiotic self-assemblies (Figure 2C).[61] These nanoparticles show efficient silencing of three different genes—*GFP*, *PLK1*, and *MDR1*—with negligible cytotoxicity compared to commercial transfection agents (Figure 2D,E). A prime utility of the tunability design is the scope it provides to modulate the nanoparticles with specific targeting abilities. For instance, the role of surface coating in such lipid−polymer hybrids has been investigated with a specific focus on penetration across the blood−brain barrier (BBB).[62] In one such study, a PLGA core was coated with DSPE-PEG alongside four discrete lipids, viz., polysorbate 80 (PS 80), poloxamer 188 (Pluronic F-68), DSPE-PEG-glutathione (GSH), and DSPE-PEG-transferrin (Tf). These formulations were then used to study the effect of each individual components in facilitating BBB penetration of nanoparticles. Among all the variations, GSH-NP and PS 80-NPs showed the maximum amount of penetration through an intact BBB. With further optimization, PS 80-NPs showed an impressive 90% decrease in luciferase expression in Neuro-2a cells. The BBB penetration ability of PS 80-NPs were further evaluated in a weight-drop-induced traumatic brain injury model. Dy677-siRNA-loaded PS 80-NPs showed 5-fold and 3-fold higher fluorescence intensity in brain tissue compared to free siRNA and PEG-NPs. Finally, Tau-siRNA-loaded PS 80-NPs showed approximately 70% downregulation of Tau expression levels in primary neuron cells followed by 50% blocking of Tau expression in a mice model, which explicitly demonstrates the potential of this tunable design. Overall, lipid−polymer hybrids present a robust delivery platform with enhanced stability and extensive structural tunability. This indeed opens new avenues for optimizing delivery efficiency and potential translation to the clinical setting.

## BIOMIMETIC NANOASSEMBLIES

Bioinspired nanoparticles have gained popularity in nucleic acid delivery. Although advancements in the field of nano-


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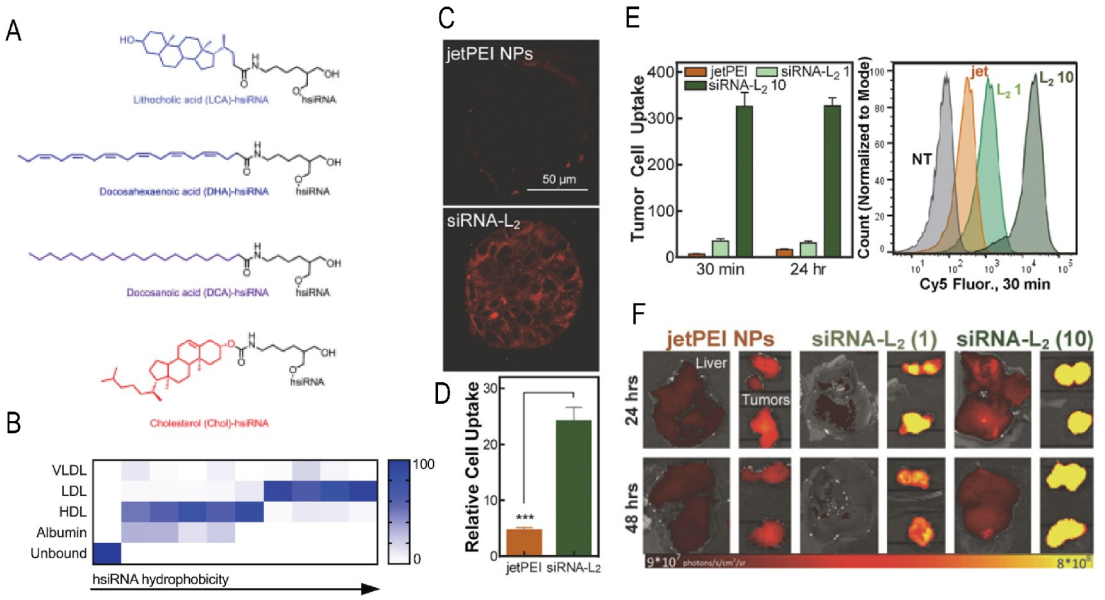


**Figure 4.** (A) Different chemical structures conjugated to hsiRNA to understand the effect of hydrophobicity toward lipoproteins. (B) The summary of the effect of hsiRNA hydrophobicity on protein binding. (Reproduced with permission from ref 90. Copyright 2018 Oxford University Press.) (C) Confocal microscopy showing the tumor spheroid penetrations and internalizations of siRNA-$L_2$. (D) Comparative cellular uptake of Cy-5-conjugated siRNA-$L_2$ and jetPEI NPs with Cy5 siRNA in MCF-7 spheroids. (E) Internalization of jetPEI NPs (1 mg/kg) and siRNA-$L_2$ (1 and 10 mg/kg) in tumor cells isolated from a xenograft mouse model 1 h postinjection. (F) Accumulations of jetPEI NPs and siRNA-$L_2$ in the liver and tumor. (Reproduced with permission from ref 93, Copyright 2017 The National Academy of Sciences of the USA.)

technology have aided nanoparticle-mediated biomacromolecular delivery platforms, *in vivo* performance of nanoparticles remains underwhelming. Thus, there is significant interest in using biomimetic nanoparticles (BNP) to address critical shortcomings in purely synthetic nanomaterials.[63] Owing to their similarity to the surface functionalities of the parent cell, BNPs provide a wide range of functions, such as low immunogenic response, long circulation time, and innate disease relevant targeting abilities (Figure 3A,B).[63−65] BNPs can be divided into two broad categories: (i) endogenous membrane debris such as cell membrane fragments, exosomes, and microvesicles, with the latter two generally classified together as extracellular vesicles (EVs); (ii) cell-membrane-coated nanoparticles, where a synthetically prepared nanoparticle is wrapped in cell membrane camouflage.

A variety of cell types like red blood cells (RBC),[66,67] immune cells,[68,69] cancer cells,[64,70] or stem cells[71−73] have been exploited in the past for their unique targeting capabilities. For example, owing to their "self-markers" (e.g., CD47 proteins, various peptides, and glycans), RBC-based nanoparticles can evade immune clearance effectively, which prolongs the circulation half-life.[74,75] Vectors designed from red blood cells have shown promising results in delivering therapeutic cargo ranging from small drug molecules to much larger biomolecules, such as proteins and nucleic acids.[65,75] In one example, rapamycin-loaded PLGA particles camouflaged in an RBC-membrane envelope show successful delivery to atherosclerotic plaques with minimal macrophage-mediated phagocytosis.[74] The potential of BNPs is not just limited to evading immune clearance and having excellent targeting capabilities. In past few years, advancements in the BNP engineering process have gained attention in many cumbersome targets, such as translocating therapeutics across the BBB. For instance, GAPDH and BACE1 siRNA were delivered specifically to neurons, microglia, and oligodendrocytes across the BBB through endogenous EVs isolated from primary dendritic cells expressing LAMP2b, which is specifically known to fuse with neuronal RVG peptides.[76] In general, the modularity offered in BNP systems is unparalleled because of the compatibility with widespread selection of cell types and characteristics.[77−80] For instance, blood cell membranes are usually used for prolonged circulation, whereas immune cells and tumor cells provide explicit targeting capabilities.[63,66,67,71] Interestingly, engineered cell membranes and hybrid cell membranes were also reported as precursors for the vector design process.[81,82] Unlike vectors derived from a single cell type, these hybrids are not limited to the native characteristics of the source cells; that is, these vectors can be conferred with a wide range of functions. An example of that is the use of genetically engineered B16F10 cells that express a viral fusion protein hemagglutinin (HA) to mimic the viral mechanism of payload delivery (Figure 3C).[83] HA expression on the surface of influenza viruses has been reported to cause escape from endosomal entrapment.[84] HA proteins consist of two subunits. Whereas the HA1 subunit facilitates the attachment of the virus on the cell surface, the HA2 subunit undergoes a conformational change triggered by low pH in the late endosomal stage, causing fusion of the viral envelope with the endosomal membrane, resulting in endosomal escape of the viral payload.[85−87] These engineered nanoparticles were used to deliver EGFP and CLuc mRNA into cytosol (Figure 3D), which indeed exhibits a superior endosomal escape ability and significantly better transfection (Figure 3E). Overall, the robustness, modularity, and structural similarities with cellular membranes make BNPs an attractive choice for therapeutic gene delivery with potential for clinical success.

## LIPOPHILIC CONJUGATES LINKED TO siRNA

Another well-explored approach involves lipoprotein particles, formed by the complexation between lipophilic-siRNA and


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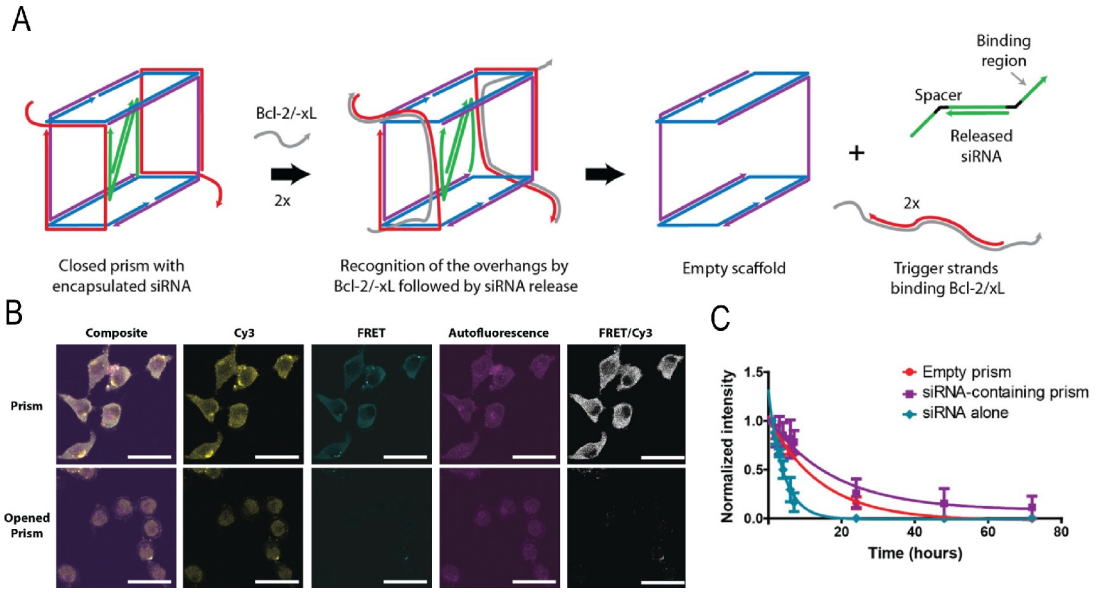


**Figure 5.** (A) Schematic representation of an siRNA-encapsulating prism and siRNA release mechanism. (B) Spectral images of a prism in fixed cells before and after the release of cargo. (C) Stability of an siRNA-encapsulated prism in serum. (Reproduced with permission from ref 106. Copyright 2016 American Chemical Society.)

lipophilic proteins. Hydrophobic conjugation of siRNA with cholesterol, lipids, and fatty chain conjugates have shown to increase the stability, biodistribution, and gene-silencing efficiency.[88−90] These lipophilic siRNAs have affinity toward lipoproteins that are rich in phospholipids and cholesterol. The journey started with covalent conjugation of cholesterol to siRNA, which facilitated the cellular internalizations and efficient gene silencing.[91] In this example, a mechanistic aspect of mRNA degradation by cholesterol-conjugated *apoB*-siRNA (Chol-*apoB*-siRNA) was studied. The modified siRNA could silence the *apoliprotein B* (*apoB*) gene *in vivo* in a transgenic mouse model.[71] In another example, cholesterol, bile acids, and long-chain fatty acids were conjugated to siRNA and the delivery mechanism of these lipophilic siRNAs were studied *in vivo*.[92] In this study, the preassembled formulation of cholesterol−siRNA with lipoprotein (high-density lipoprotein) was ∼8 to ∼15 times more effective at silencing apoB protein expression than the same amount of unbound cholesterol−siRNA. The length of fatty acid chains and the binding affinity of these conjugates to lipoproteins were found to be crucial for efficient and selective internalizations. The longer chain siRNA conjugates (docosanyl and stearoyl) were more effective at *apoB* gene silencing *in vivo* than the shorter chain conjugates (lauroyl and myristoyl) because the former showed strong interaction with high-density lipoproteins. In another study, the preferential association of lipophilic siRNAs with lipoproteins based on their hydrophobicity was evaluated *in vivo*. Higher hydrophobic siRNA (hsiRNA) conjugates spontaneously associated with low-density lipoprotein, whereas the lesser hydrophobic conjugates preferred high-density lipoproteins (Figure 4A,B).[90] Spontaneous binding with lipoproteins improved the plasma half-life of these hsiRNAs and showed slower clearance. The cell-specific distribution and gene-silencing efficacies of these lipoprotein−hsiRNA complexes were determined by *Ppib*mRNA level quantification in different organs of mice.

Recently, diacyl lipid-conjugated siRNA was found to bind albumin, which significantly increased the blood circulation time with increased tumor penetration and accumulation (Figure 4C,D).[93] The serum has an abundance of albumin (>40 mg/mL) with ∼20-day circulation half-life.[94] Albumin has been used extensively as a carrier of therapeutics and a few formulations, such as Abraxane, Levemir, and Optison, have already been approved for clinical applications.[95,96] The diacyl lipid-modified siRNA (siRNA-$L_2$) showed a 5.7-fold increased circulation half-life and 8.6-fold increased bioavailability compared to free siRNA. Also, in comparison to the commercial siRNA nanocarrier jetPEI, siRNA-$L_2$ showed 19-fold better tumor accumulations and a 46-fold increase in per-tumor-cell uptake in a triple-negative breast cancer mouse model (Figure 4E,F). The albumin hitchhiking of lipophilic siRNA conjugates is (i) a synthetically simple strategy, (ii) shows improved tumor cellular uptake with enhanced gene-silencing efficacy, and (iii) has better pharmacokinetics and is safe at a high dose. Therefore, this approach possesses the potential for clinical translation in RNAi-based cancer therapy.

## SELF-ASSEMBLED NUCLEIC ACID NANOPARTICLES

Exploring the base pair complementarity driven self-assembly to create nucleic acid based nanosystems for delivery is another interesting direction in this area. DNA nanotechnology has been exploited to develop delivery vehicles where DNA self-assembles through the Watson−Crick base pairing.[97,98] The potential for this platform arises from their (i) well-defined shape and easily tunable size with monodispersity, (ii) biocompatibility, (iii) convenient functionalization with desired chemical conjugations, and (iv) programmability to introduce stimuli-responsive release of cargo.[99−101] This technology has been explored for intracellular drugs[102,103] and protein delivery[104] and has the potential to be extended to nucleic acids. For example, a self-assembled DNA tetrahedral nanostructure was reported for siRNA delivery, which showed effective gene silencing *in vivo* in a nude mouse bearing a KB xenograft tumor.[105] The tetrahedron was decorated with cancer-targeting folate ligands, and it showed >50% firefly


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luciferase gene silencing in HeLa cells. The study showed the importance of the number and spatial orientation of these ligands on the surface of the particle. The precise control in the size of these nanoparticles (∼28.6 nm) avoided renal filtrations and showed improved blood circulation time (half-life, ∼24.2 min) compared to that of the siRNA itself (half-life, ∼6 min). These nanostructures can be programmed to be responsive to external and internal stimuli. Many studies are shown to use endogenous stimuli to release the cargo. In one example, a DNA "nanosuitcase" has been shown to stably encapsulate siRNA and release it only in the presence of the endogenous recognition sequence (oligonucleotide trigger) (Figure 5A).[106] The flexibility with this system offers AND-gated responses for both targeting and synergistic therapies. The DNA cage showed controlled release of siRNA upon recognition of two trigger strands (Bcl-2 and Bcl-xL) related to anti-apoptotic genes. The successful release of the cargo in fixed HeLa cells was monitored via FRET studies. The strategic placement of Cy3 and Cy5 dyes in the nanosuitcase exhibited a FRET signal, and the successful release of cargo was monitored by the disappearance of the signal (Figure 5B). In another example, a spherical micellar particle was synthesized from monodispersed sequence-specific DNA−polymer conjugates.[107] This spherical nucleic acid (SNA) showed stable encapsulation of an 18-mer antisense oligonucleotide with a triggered release of cargo via strand displacement. The hydrophobic core and DNA corona formed stable micellar particles in aqueous media. SNA could stably encapsulate phosphorothioated antisense oligonucleotide and efficiently downregulate luciferase in HeLa cells. In essence, the ease of synthesis, high specificity toward stimuli, and ability to introduce AND-gated strategies[108] make nucleic acid based nanoassemblies potential candidates for delivery.

## ■ DISCUSSION AND PERSPECTIVE

In this review, we have discussed a few strategies for nucleic acid delivery that have shown exciting potential during the initial stages of investigations. These systems share conceptual similarities to address major drawbacks of traditionally utilized lipid-based nanoassemblies. Despite achieving initial success, these technologies are far from perfect. As mentioned previously, the stability of nanocarriers has been one of the key hurdles faced by the traditional lipid-based systems. In this context lipid−polymer hybrids offer the potential to address this pitfall due to the extent of tunability in both the polymer design and selection of lipid components. Careful design of the polymer core can ensure stable long-term encapsulation of the nucleic acid for a long period of time, whereas systematic variation in the lipid shell can optimize delivery requirements.[54,61] Despite the ingenuity from a materials standpoint, significant hurdles remain before their adaptation in the clinic. For this system, scalability must be addressed by simplifying formulation procedures. Although microfluidic platforms have aided the production ability of these platforms,[109] these technologies are at a relatively rudimentary stage and further understanding of manufacturing procedures is critical to ensure clinical success. In addition, biomimetic nanoparticles inherit intrinsic characteristics of the source cells, which in theory can mitigate targeting requirements to deliver genetic payloads. Interestingly, the clinical translation of BNPs is significantly hindered because of their heterogeneous properties depending on the source cells and preparation methods that can impact their performance.[78−80,110] On the other hand, the innate characteristics of the source cells might also cause immunogenic responses. For instance, immune-cell-derived vectors have been shown to generate immune responses via pro-inflammatory M1-macrophage polarization.[77] Likewise, lipophilic RNAs showed excellent pharmacokinetics profiles with promising gene silencing, but the modification process also affects its potency. Previously, GalNAc−siRNA conjugates have shown targetability toward liver hepatocytes;[111] however, lipophilic conjugates exhibit limitations toward preferential accumulation in other desired disease sites. Lesser hydrophobic conjugates preferentially bind to high-density lipoproteins (HDL), which accumulate in the kidney, whereas higher hydrophobic chains prefer low-density lipoproteins (LDL), which accumulate in the liver.[112] Therefore, further studies are needed for a detailed understanding of the relationship between conjugate structures and preferential tissue accumulation *in vivo* for successful clinical adoption of RNA conjugates. Local injection can be adopted as the solution to targetability in some isolated cases, but a mechanistic understanding of intracellular trafficking and their retention time is still lacking. More importantly, the controlled release chemistry of these conjugates is not yet realized. For example, a cleavable linker can be installed to enhance pharmacokinetics and systemic stability. Similarly, nucleic acid nanoassembly platforms are relatively new, and the investigations are limited to proof-of-concept demonstrations. The structural integrity of the nucleic acid nanostructure upon cellular internalization is well-studied, but the effect of physiochemical properties toward pharmacokinetic bioavailability needs exploration. Alongside, understanding the effect of nucleic acid engineering on biodegradability and biocompatibility is also essential. Success here will impinge addressing potential immunogenicity and dose-dependent toxicity, as well as optimizing pharmacokinetic and clearance mechanisms. In summary, given the clinical success of COVID-19 vaccines, there is a lot of excitement around the use of nucleic acid based therapeutics in areas other than vaccines. Developing strategies that mitigate the shortcomings of promising delivery platforms and innovating new ones to harness the potential of nucleic acid therapeutics are forthcoming.

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#R.D. and P.K. contributed equally to this work.

### Notes

The authors declare no competing financial interest.

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