ACS Partner Journal

ACCOUNTS of materials research



pubs.acs.org/amrcda

Article

# Amphiphilic Dendrimer Vectors for RNA Delivery: State-of-the-Art and Future Perspective

Jiaxuan Chen, Dandan Zhu, Xiaoxuan Liu, and Ling Peng*

Cite This: *Acc. Mater. Res.* 2022, 3, 484−497

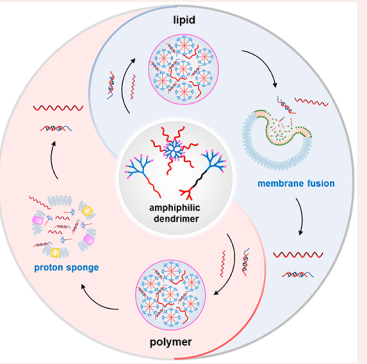
Read Online

ACCESS | Metrics & More | Article Recommendations



**CONSPECTUS:** Dendrimers, a special family of polymers, are particularly promising materials for various biomedical applications by virtue of their well-defined dendritic structure and cooperative multivalency. Specifically, in this Account, we present state-of-the-art amphiphilic dendrimers for nucleic acid delivery.

Ribonucleic acid (RNA) molecules are fast becoming an important drug modality, particularly since the recent success of mRNA vaccines against COVID-19. Notably, RNA therapeutics offer the unique opportunity to treat diseases at the gene level and address "undruggable" targets. However, RNA therapeutics are not stable and have poor bioavailability, imposing the need for their protection and safe delivery by vectors to the sites-of-action to allow the desired therapeutic effects. Currently, the two most advanced nonviral vectors are based on lipids and polymers, with lipid vectors primarily exploiting the membrane-fusion mechanism and polymer vectors mainly endocytosis-mediated delivery. Notably, only lipid vectors have been advanced through to their clinical use in the delivery of, for example, the first siRNA drug and the first mRNA vaccine.




The success of lipid vectors for RNA delivery has motivated research for further innovative materials as delivery vectors. Specifically, we have pioneered lipid/dendrimer conjugates, referred to as amphiphilic dendrimers, for siRNA delivery with the view to harnessing the delivery advantages of both lipid and polymer vectors while enjoying the unique structural features of dendrimers. These amphiphilic dendrimer vectors are lipid/dendrimer hybrids and are thus able to mimic lipid vectors and exploit membrane-fusion-mediated delivery, while simultaneously retaining the multivalent properties of polymer vectors that allow endocytosis-based delivery. In addition, they have precisely controllable and stable nanosized chemical structures and offer nanotechnology-based delivery.

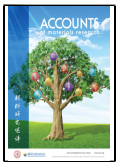
Effective amphiphilic dendrimer vectors share two important elements: chemical hydrophilic entities to bind RNA and RNA complex-stabilizing hydrophobicity. These two combined features allow the encapsulation of RNA within a stable complex before its release into the cytosol following endocytosis. This hydrophilic/hydrophobic balance permitted by the structural features of amphiphilic dendrimers plays a determining role in RNA delivery success.

In this Account, we provide a conceptual overview of this exciting field with the latest breakthroughs and key advances in the design of amphiphilic dendrimers for the delivery of siRNA and mRNA. Specifically, we start with a short introduction to siRNA- and mRNA-based therapeutics and their delivery challenges. We then outline the pioneering and representative studies on amphiphilic dendrimer vectors to highlight their historical development and promising features that offer to facilitate the once challenging RNA delivery. We conclude by offering perspectives for the future of amphiphilic dendrimer vectors for nucleic acid delivery in general.

## 1. INTRODUCTION

Dendrimers, a special family of synthetic polymers with well-defined radial structures and cooperative multivalency (Figure 1),[1] are promising materials for developing biosensors, diagnostics, drugs, and drug delivery systems including nucleic acid delivery.[2,3] Specifically, nucleic acid molecules are emerging as extremely appealing therapeutics, which provide treatment options for diseases that are beyond the reach of traditional approaches.[4] In particular, small interfering RNA (siRNA) can sequence-specifically and efficiently silence any disease-associated gene, offering a unique capacity to address "undruggable" targets and provide precision medicine.[5] On the other hand, mRNA therapeutics can be used to transiently generate desired functional proteins for therapeutic and prophylaxis purposes.[6,7] Both siRNA- and mRNA-based therapeutics can be rapidly and reliably designed on the basis of gene sequence, offering fast and personalized precision medicine. An excellent example is the mRNA-based vaccines

**Received:** December 14, 2021
**Revised:** February 27, 2022
**Published:** May 3, 2022







https://doi.org/10.1021/accountsmr.1c00272
*Acc. Mater. Res.* 2022, 3, 484−497

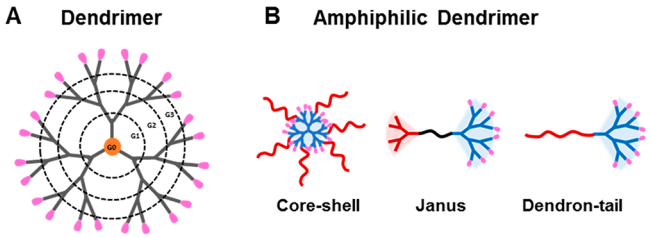



**Figure 1.** Cartoon illustrations of (A) a dendrimer and (B) amphiphilic dendrimers. (A) The dendrimer structure is composed of a central core or generation 0 ($G_0$), repeat branching units forming consecutive levels or generations (G1, G2, and G3) and terminal groups on the surface (shown in pink). (B) Amphiphilic dendrimers of core−shell, Janus, and dendron-tail structures composed of distinct hydrophobic (red) and hydrophilic (blue) entities.

against the devastating and evolving COVID-19 pandemic. These mRNA vaccines have been successfully developed with unprecedented speed and have shown excellent efficacy.

However, both siRNA and mRNA therapeutics suffer instability issues and have poor bioavailability. They thus require delivery systems to protect them from degradation and deliver them to sites of action to allow their desired therapeutic effects.[4] Lipids and polymers are the two most studied nonviral vectors and possess distinct delivery mechanisms.[8,9] In order to capitalize on the delivery advantages of both lipid and polymer vectors while preserving the unique structural features of dendrimers, various lipid/dendrimer conjugates, also termed amphiphilic dendrimers (Figure 1B), have been explored for siRNA delivery.[10−15] These amphiphilic dendrimers are lipid/dendrimer hybrids and can be classified as core−shell, Janus, and dendron-tail structures (Figure 1B). They mimic lipids, thus offering the delivery features of lipid vectors, while retaining the multivalent properties of polymers and their advantages as polymer vectors.[10,12,14,16] In addition, they have precisely controlled structures and form nanosized complexes with RNA molecules and hence offer all the advantages of nanotechnology-based delivery. Noteworthy, the chemical composition and the structural geometry as well as the hydrophilic/hydrophobic balance of amphiphilic dendrimer vectors play determining roles in RNA delivery.

In this Account, we present a holistic overview of amphiphilic dendrimer vectors for siRNA delivery and mRNA delivery, considering the evolution of this subject through key developments and advances, latest breakthroughs, and future perspectives. We start with a brief description of siRNA and mRNA therapeutics and their delivery challenges.

## 2. SIRNA AND MRNA THERAPEUTICS AND CHALLENGES WITH THEIR DELIVERY

Nucleic acid therapeutics offer the unique opportunity to treat diseases at the gene level and address "undruggable" targets, and they are emerging as the third drug modality alongside small molecule and protein drugs. Currently, there are over 3000 clinical trials for nucleic acid therapeutics, and several have been approved including eight antisense oligonucleotides (ASOs), four siRNA drugs, and two mRNA vaccines.[4] Different nucleic acid therapeutics such as DNA, mRNA, siRNA, and ASO have specific modes of action and discrete sequences. Moreover, they have distinct physicochemical properties such as size and molecular weight, single- or double-stranded structure, and conformational flexibility. This Account is primarily focused on the nucleic acid therapeutics based on siRNA and mRNA.

Since the discovery of RNA interference (RNAi) in 1998, there has been tremendous interest in developing siRNA therapeutics to treat various diseases.[5] siRNA are small, double-stranded RNA molecules composed of ∼19−25 base pairs. Once inside the cell, the antisense strand of siRNA binds to the target mRNA via complementary Watson−Crick base pairing and cleaves the mRNA within the RISC complex to inhibit gene expression, leading to gene silencing (Figure 2). This process of gene silencing is catalytic, yet highly efficient and specific, offering unique advantages over traditional antisense oligonucleotides not only in therapeutic efficacy but also in duration of effect. In addition, siRNA therapeutics can be rationally designed on the basis of the primary gene sequence and thus are able to address undruggable targets and provide precision and personalized medicine.

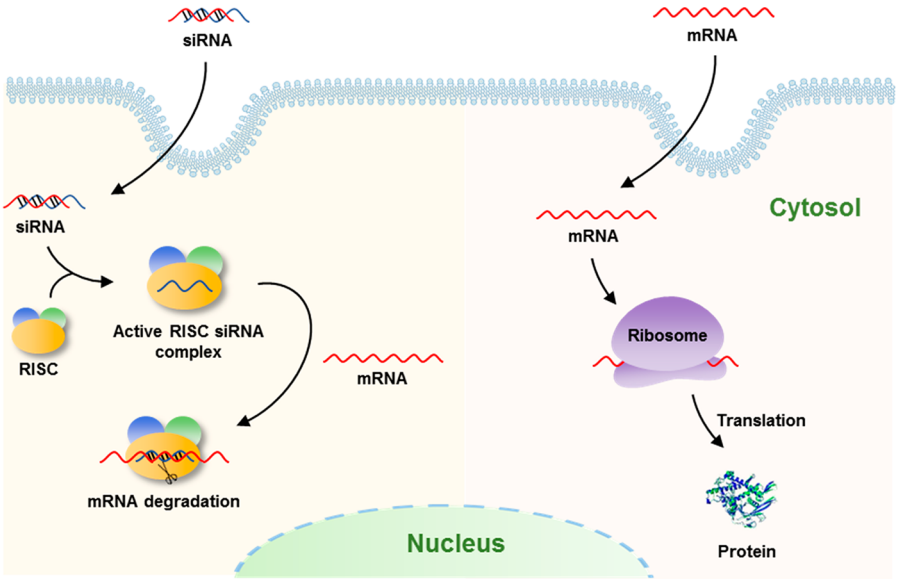



**Figure 2.** siRNA and mRNA as therapeutics and their modes of action.



https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

In contrast to siRNA, mRNA is a large and single-stranded nucleic acid carrying protein-coding information from the genome. Once introduced into cells and having reached ribosomes, an mRNA-based therapeutic is translated to produce the desired functional protein for medicinal purposes (Figure 2). Therapeutic mRNAs can be designed and applied for vaccination against cancer and infectious diseases like COVID-19, protein-replacement therapy, and gene editing.[6,7] The inherent advantages of mRNA therapeutics include low genome integration probability, transient expression, simple and rapid in vitro transcription preparation, and large-scale production. Unlike DNA-based gene therapy that requires entry into nucleus, mRNA therapeutics need only to reach the cytoplasm to produce functional proteins, hence reducing the risks of insertional mutagenesis. In addition, when compared to direct protein therapy, mRNA therapeutics are easier and more flexible to design, have a more cost-effective preparation, and are less risky with regards biosafety.

Despite the advantages of siRNA- and mRNA-based therapeutics, they are, unfortunately, unstable and readily degraded via chemical hydrolysis and enzymatic action because of their labile phosphodiester linkages. In addition, their dense negative charge and high hydrophilicity hamper movement across cell membranes. Exogenous RNA molecules can also be easily cleared by the reticuloendothelial system or detected by the innate immune system for triggering immune responses.[4,17] Therefore, suitable delivery systems that protect these RNA molecules from degradation and promote their cellular uptake and delivery to the desired sites of action are crucial for practical applications of siRNA and mRNA therapeutics.

Both viral and nonviral vectors have been developed for nucleic acid delivery.[4] Viral vectors have the advantage of offering high transfection efficiency but bear serious safety concerns with respect to their high immunogenicity and risk of insertional mutagenesis. Additional drawbacks of viral vectors include low packaging capacity and high production costs. Nonviral vectors with their better safety profile, easy packaging of both large and small nucleic acids and simple and low-cost production therefore represent an extremely attractive alternative. The first siRNA drug Patisiran and the first mRNA vaccine both used nonviral vectors. In particular, the recent success of mRNA vaccines against COVID-19 has greatly fueled and intensified research interest in nonviral delivery platforms.

It should be mentioned that chemical modification and ligand conjugation of the nucleic acids themselves has been attempted and successfully implemented in siRNA delivery to achieve stability, efficacy, and specificity, leading to three clinically approved GalNAc-siRNA drugs Givosiran, Inclisiran, and Lumasiran. In addition, base modifications in certain nucleosides such as pseudouridine, $N^1$-methylpseudouridine, and 5-methylcytidine in mRNA can further suppress immune responses, while improving mRNA translation.[4,6,18] For example, $N^1$-methylpseudouridine was used in mRNA vaccines against COVID-19. The chemistry for ligand conjugation and nucleobase/nucleoside/oligonucleotide modification has been extensively reviewed elsewhere,[4,19,20] and falls outside the scope of the present Account focused on the nonviral delivery of siRNA and mRNA.

## 3. LIPID AND POLYMER VECTORS

A myriad of nonviral vectors have been explored for nucleic acid delivery, such as lipids, polymers, peptides, proteins, inorganic materials, etc.[4] Among them, lipids and polymers are the most advanced nonviral vectors because of their safety, flexibility, and efficiency.[8,9,21,22] For example, the first in-human trial of siRNA therapeutics was performed using a cyclodextrin-based polymeric vector,[23] whereas the first approved siRNA drug Patisiran and the first mRNA vaccine employed lipid nanoparticles.[9,21]

Lipid vectors for nucleic acid delivery have evolved from liposomes, through lipoplexes to lipid nanoparticles (LNPs).[9] Liposomal delivery involves encapsulating nucleic acids within liposomes formed with neutral lipids and exploits the membrane-fusion mechanism for delivery into the cell. However, liposomal delivery is plagued with low encapsulation efficiency. To overcome this problem, lipoplexes based on cationic lipids were developed. These cationic lipids interact with negatively charged nucleic acids to yield high encapsulation and delivery efficiency. Nevertheless, lipoplexes suffer from high toxicity caused by the cationic lipids. Besides, the positive charge of cationic lipids could result in their rapid plasma clearance and short circulation time. Different from liposomes and lipoplexes, LNPs are prepared from ionizable lipids that are positively charged at acidic pH (enabling nucleic acid binding) and neutral at physiological pH (eliminating toxicity caused by cationic lipids).[24] LNPs enter into cells via endocytosis, and within acidic endosomes, the ionizable lipids are protonated. This protonation allows the lipids to readily interact with negatively charged endosomal membranes and thereby induce the fusion process for endosome disruption and nucleic acid release, leading to effective delivery (Figure 3A). It should be mentioned that LNPs are multicomponent systems encompassing ionizable lipid, phospholipid, cholesterol, and pegylated lipid. The phospholipids are used as "helper lipids" to enhance endosome release and transfection efficiency, the cholesterol to enhance stability, and the pegylated lipids to promote the formation of homogeneous LNPs, which increase the stability and circulation time by preventing the interactions with blood components and reticuloendothelial clearance. Importantly, the ratio of these components affects the delivery efficacy, and this ratio can be varied and tailored for delivery of distinct nucleic acids such as siRNA and mRNA.

Besides lipid vectors, cationic polymers are also frequently used nonviral vectors.[8,22] The advantages of polymer vectors are the immense structural diversity and the ability to make functional modifications. These vectors also form robust, small, and uniform complexes, which improve transfection efficiency. Cationic polymers pack negatively charged nucleic acids through electrostatic interactions into polyplexes, which enter cells via endocytosis before undergoing endosome release possibly via the "proton-sponge" effect for effective delivery (Figure 3B). Poly-L-lysine (PLL) was the first cationic polymer investigated for nucleic acid delivery.[25] Subsequently, Behr and co-workers reported polyethylenimine (PEI) with excellent efficiency for DNA transfection.[26] The high transfection efficiency of PEI can be attributed to the buffering capacity of the abundant amino groups within PEI, generating a high positive charge density under physiological and acidic conditions. This feature expedites PEI binding to negatively charged nucleic acids via strong electrostatic interactions under physiological conditions, while, at the same time, facilitating endosome escape of nucleic acid cargos by endosomal buffering via the "proton-sponge" effect.[27,28] PEI has therefore become a gold standard for in vitro nucleic acid transfection, but unfortunately, it cannot be used in clinical applications due


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

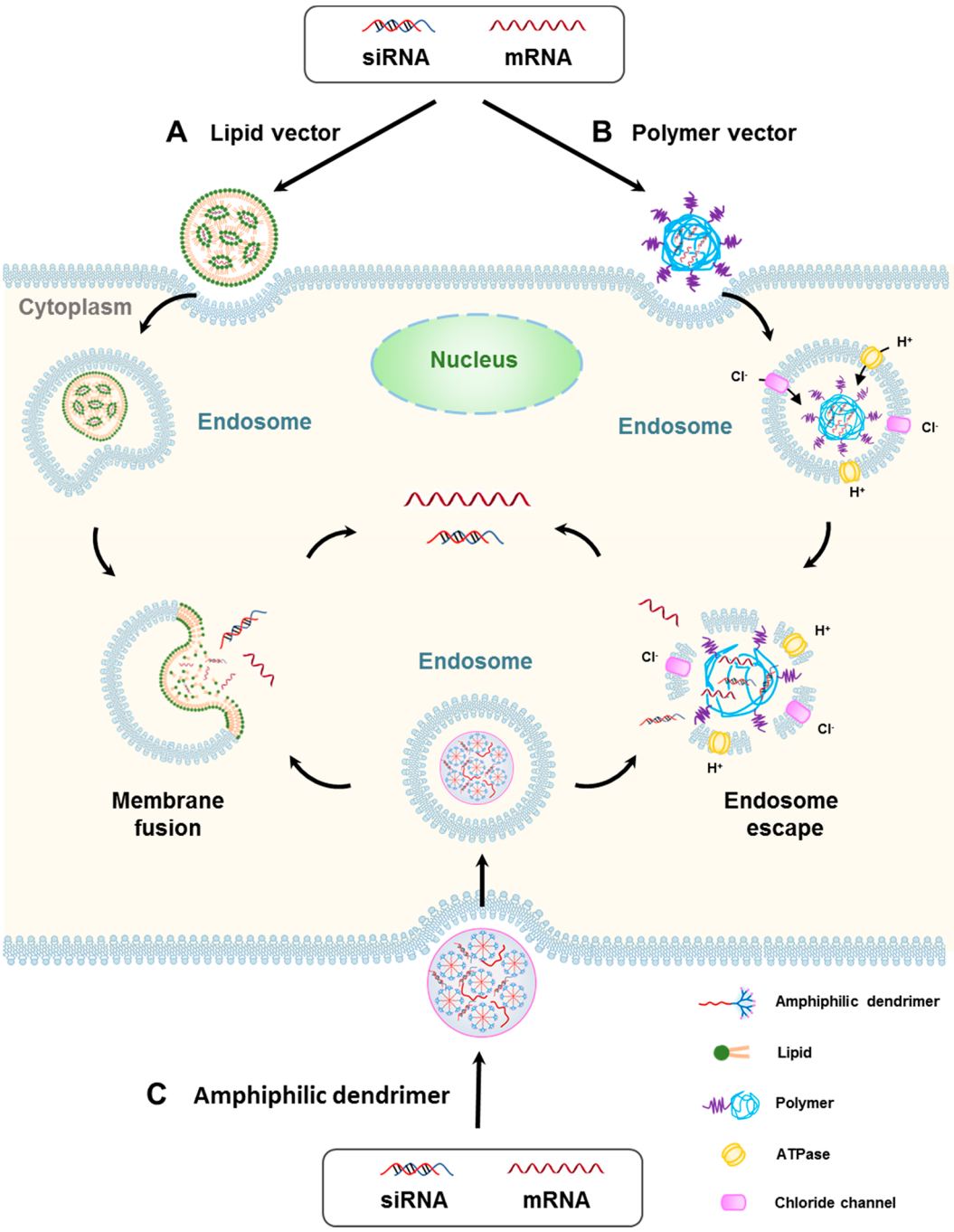



**Figure 3.** Nucleic acid delivery mediated by nonviral vectors prepared with (A) lipids, (B) polymers, and (C) amphiphilic dendrimers.

to its high toxicity. Therefore, great efforts have been pursued to develop various polymer vectors for safe and effective nucleic acid delivery.[8,22]

Although lipids and polymers are the most advanced and frequently used nonviral vectors for nucleic acid delivery, they unfortunately have limitations. Indeed, while lipid vectors have limited functional modification, poor stability, rapid clearance, and cause inflammatory or anti-inflammatory responses, polymer vectors usually lack precise control of their chemical compositions and structures. In addition, endosome release is crucial for RNA delivery and yet only a few delivery complexes can escape from the endosome.[29] Lipid vectors essentially exploit the membrane-fusion mechanism for endosome release, whereas polymer vectors often use the "proton-sponge" effect (Figure 3). Therefore, the search continues for innovative materials as more effective vectors and is of paramount importance for nucleic acid therapeutics.

## 4. AMPHIPHILIC DENDRIMER VECTORS

Dendrimers are extremely appealing materials for developing RNA vectors by virtue of their well-defined radial structure and the cooperative multivalency confined within a nanosized dimension. They are composed of three distinct structural components: (1) the core, (2) the repetitive branching layers (also referred to as "generation"), and (3) the abundant terminal groups (Figure 1A). Different from the conventional polymers, dendrimers have precisely controlled chemical compositions and uniquely well-defined structures.[30]



https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

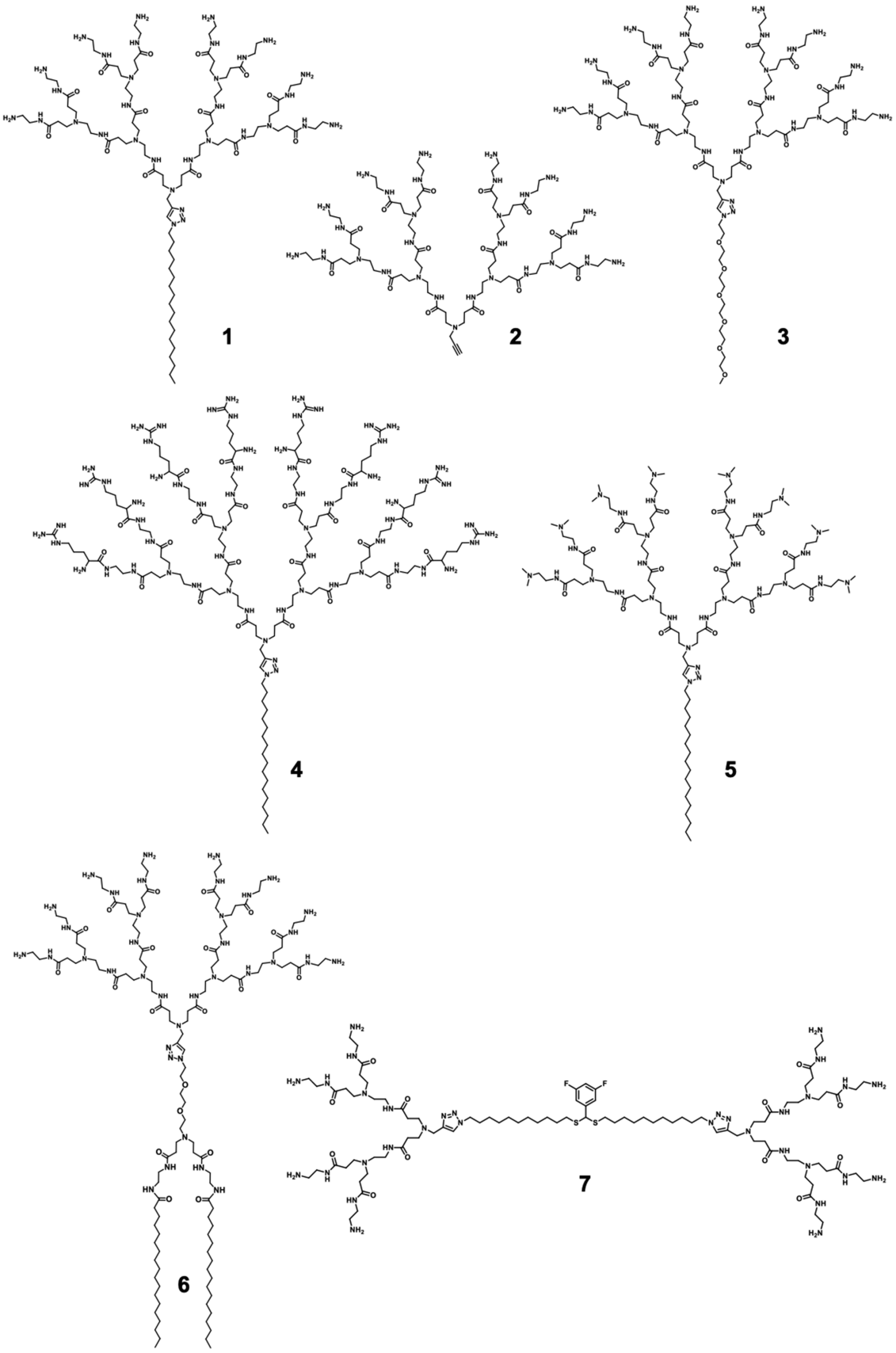



**Figure 4.** Examples of dendron-tail amphiphilic PAMAM dendrimers developed in our group for siRNA delivery.[10,12,14,16,31,32] Adapted with permission from ref 10 (Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA), ref 12 (Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA), ref 14 (Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA), ref 16 (Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA), ref 31 (Copyright 2015 Royal Society of Chemistry), and ref 32 (Copyright 2021 Tsinghua University Press and Springer).



https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

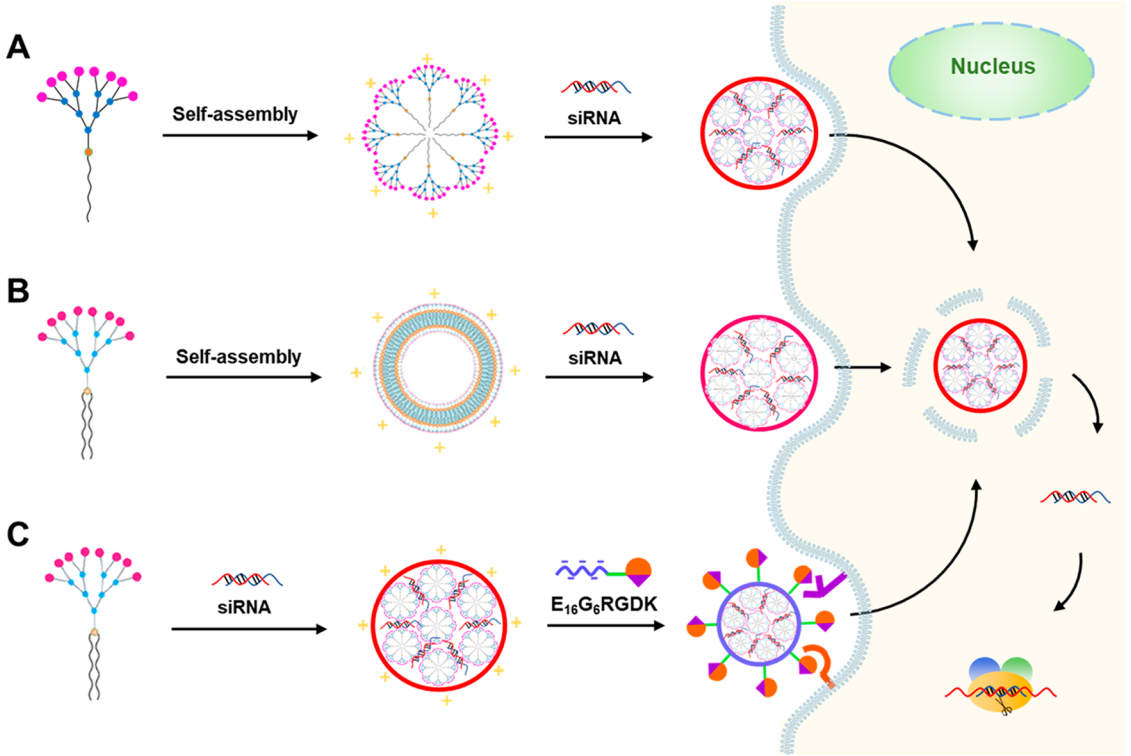



**Figure 5.** Self-assembly of amphiphilic dendrimers for siRNA delivery.[10,14,33] (A) Self-assembly of **1** into nanomicelles for siRNA delivery. (B) Self-assembly of **6** into nanovesicles for siRNA delivery. (C) Peptide-decorated siRNA/**6** nanoparticles for targeted siRNA delivery in cancer cells. Adapted with permission from ref 10 (Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA), ref 14 (Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA), and ref 33 (Copyright 2018 American Chemical Society).

With the aim of harnessing the structural features of dendrimers together with the delivery advantages of both lipid and polymer vectors while overcoming their limitations, scientists have developed elaborate lipid/dendrimer conjugates, also termed amphiphilic dendrimers (Figure 1B), for nucleic acid delivery. These amphiphilic dendrimers form nanosized complexes with nucleic acid molecules thus allowing their nanotechnology-based delivery via endocytosis-mediated cell uptake (Figure 3C). These precisely controlled dendritic structures harbor multivalent cooperativity and can exploit membrane-fusion-based endosome release by mimicking lipid vectors, while simultaneously retaining the "proton-sponge"-mediated endosome release of polymer vectors (Figure 3).[10,12,14] We present below representative examples to highlight the pioneering and exemplary studies of amphiphilic dendrimers for the delivery of siRNA and mRNA by retracing their development, challenges faced, and scientific breakthroughs so far.

As briefly mentioned above in the Introduction, amphiphilic dendrimers can be classified into three groups (Figure 1B): core−shell, Janus, and dendron-tail. While core−shell amphiphilic dendrimers are mostly prepared on the basis of traditional hydrophilic spherical dendrimers with hydrophobic motifs introduced via surface modifications, Janus amphiphilic dendrimers are constructed with two different dendrons bearing distinct hydrophobic and hydrophilic entities. Dendron-tail amphiphilic dendrimers have straightforward design and ease of synthesis and are the most studied for nucleic acid delivery. In this Account, we use the term amphiphilic dendrimers to collectively refer to all three types.

### 4.1. siRNA Delivery

Our group has pioneered studies on dendron-tail amphiphilic dendrimers for siRNA delivery with the view to capitalize on the advantageous delivery features of both lipid and dendritic polymer vectors. In 2012, we reported the first amphiphilic dendrimer **1** (Figure 4) for functional siRNA delivery in vitro and in vivo.[10] This dendrimer vector is composed of a simple hydrophobic alkyl chain and a hydrophilic poly(amidoamine) (PAMAM) dendron. By virtue of its amphiphilicity, this dendrimer spontaneously self-assembles into nanomicelles (Figure 5A).[16] At physiological pH, these nanomicelles are positively charged at their amine terminals and thereby bind negatively charged siRNA via electrostatic interactions to form stable nanoparticles which protect the siRNA from degradation and facilitate its cellular uptake via endocytosis. Once internalized in the cell, **1**-mediated delivery exploits both the lipid-based membrane-fusion mechanism and the polymer-based proton-sponge effect for endosomal release (Figure 3), resulting in excellent siRNA delivery and potent gene silencing of heat shock protein 27 (Hsp27) in a castration-resistant prostate cancer model.

Importantly, the amphiphilic feature of **1** is crucial for siRNA delivery, because neither the hydrophilic dendron **2** alone without the hydrophobic chain nor dendrimer **3** with a hydrophilic PEG chain (Figure 4) was able to achieve functional siRNA delivery.[10,16] Additionally, amphiphilic dendrimers of lower generations[10] or with either a longer or shorter hydrophobic chain had no delivery activity:[16] an amphiphilic dendrimer with a too short hydrophobic chain could not form stable nanomicelles, while one with a too long hydrophobic chain formed very stable nanomicelles that bound


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

siRNA but did not readily release it for gene silencing. The best amphiphilic dendrimer **1** bears a hydrophobic alkyl chain of 18 carbons and a PAMAM dendron of generation 3, highlighting the importance of an intricate balance between hydrophilicity and hydrophobicity for efficient siRNA delivery.[16]

To further enhance delivery efficiency, amphiphilic dendrimer **4** bearing arginine terminals was constructed (Figure 4) in order to mimic arginine-rich cell-penetrating peptides for favorable cellular uptake.[31] Cell-penetrating peptides bearing positively charged arginine residues are known to interact with negatively charged cell membranes through electrostatic interactions and divalent H-bonds involving the guanidinium moieties in arginine residues and the phosphate/carboxylate/sulfate groups on the cell surface. Such interactions facilitate membrane penetration.[34] The arginine-decorated dendrimer **4** exhibited substantially enhanced cell uptake of siRNA when compared with that of **1**, leading to more performant siRNA delivery and stronger gene silencing in both human prostate cancer PC3 cells and hematopoietic $CD34^+$ stem cells.[31] This study highlighted that conjugation of arginine amino acids to the dendrimer terminals constitutes a promising strategy to increase the efficiency of siRNA delivery.

It should be mentioned that the arginine-decorated dendrimer **4** has permanently charged positive terminals, which may generate potential cytotoxicity via strong interaction with the negatively charged cell membrane. In addition, the permanently positive charges may also result in rapid plasma clearance and short circulation time. Aiming to overcome these drawbacks, the ionizable amphiphilic dendrimer **5** carrying tertiary amine terminals (Figure 4) was created for siRNA delivery.[32] Tertiary amines can be less basic than primary amines because of the steric hindrance that can impede the protonation of the nitrogen atom. Indeed, **5** was much less protonated than **1** at pH 7.4 and its siRNA complex had also much less surface potential than that formed with **1**. Consequently, **5** showed much lower toxicity than **1** and **4**. Nevertheless, **5** was sufficiently charged at pH 7.4 to interaction with the negatively charged siRNA and formed stable siRNA complexes for siRNA delivery, while at acidic pH 5.0 of the endosome, **5** was almost fully charged, interacting with and destabilizing the negatively charged endosomal membrane for effective siRNA release, and leading to consequently gene silencing. Therefore, **5** maintained effective siRNA delivery similar to that of **1**.

This observed relationship between delivery efficacy and toxicity of amphiphilic dendrimers is similar to that observed for cationic lipid vectors and ionizable lipid vectors: cationic dendrimer vectors are more effective for delivery but also show greater toxicity than ionizable dendrimers. Therefore, the trade-off between activity and toxicity should be carefully evaluated to maximize the delivery activity while minimizing the toxicity profile. This trade-off is often critically impacted by the hydrophilic and hydrophobic balance as well as the apparent $pK_a$ values of the ionizable amine functionalities in the delivery vector.[35]

In searching for more effective delivery systems, we also altered the hydrophobic entity in **1** and constructed the amphiphilic dendrimer **6** harboring two hydrophobic C18 alkyl chains (Figure 4).[14] Unlike the nanomicelles formed following self-assembly of **1**, **6** self-assembles spontaneously into nanovesicles in water (Figure 5B). This interesting finding can be explained by the increased chain number in **6**, enhancing the volume of the hydrophobic entity, which alters the hydrophilic/hydrophobic ratio and thus the packing parameter of **6** for forming vesicles. Remarkably, the positively charged vesicles formed by **6** were observed to rearrange into nanomicelles upon interaction with the negatively charged siRNA, giving rise to highly ordered spherical nanoparticles of siRNA/**6** complexes that enter into cells via macropinocytosis and allow endosomal release through the combined features of lipid and polymer vectors. Moreover, **6** exhibited excellent siRNA delivery, leading to much better gene silencing than **1** in various cell lines including stem cells and primary cells as well as in vivo in different disease models.[14,36−38] Also remarkably, it enabled siRNA delivery to primary immune cells otherwise known to be particularly sensitive, fragile, and difficult to transfect. Indeed, the **6**-mediated siRNA delivery system allowed functional gene silencing in primary T cells, natural killer cells, macrophages, and microglia,[38] offering a promising alternative to modulate the function of immune cells for immunotherapy.

Targeted delivery to specific cells is an important concern with regards efficiency and safety. To address this, the **6**-mediated siRNA delivery system was decorated with targeting ligands for effective delivery to cancer cells in tumor lesions. Specifically, positively charged siRNA/**6** complexes were coated with a negatively charged peptide bearing a RGDK segment, which simultaneously targets integrins (via RGD) and neuropilin-1 receptors (via RGDK), both overexpressed on cancer cells (Figure 5C).[33] The targeted delivery gave rise to enhanced siRNA delivery and stronger gene silencing of Hsp27 in prostate cancer PC-3 cells; the resulting increase in anticancer potency allowed a more than 10-fold reduction in siRNA dose compared to that required with nontargeted delivery in PC-3 xenograft mice. Consequently, **6** constitutes an extremely promising vector for siRNA delivery in biomedical applications.

Continued efforts to improve targeted delivery led to the construction of a bola-amphiphilic dendrimer **7** for redox-responsive siRNA delivery specifically to cancer cells.[12] As cancer cells are constantly under stressful survival conditions, they possess high levels of reactive oxygen species (ROS). This feature can be exploited for ROS-responsive and cancer cell-specific delivery. Different from conventional lipid/dendrimer conjugates, bola-amphiphilic dendrimer **7** is a dumbbell-shaped molecule with two hydrophilic PAMAM dendrons connected via a hydrophobic core, which harbors a ROS-sensitive thioacetal functionality at the focal point. This dendrimer binds siRNA to form a stable siRNA/**7** complex. After cellular uptake, the high ROS level in cancer cells generated rapid degradation of the thioacetal group in **7** and resulted in the dissociation of the siRNA/**7** complexes and subsequent siRNA release, leading to effective gene silencing of Hsp27 and potent anticancer activity. Notably, this bola-dendrimer also simultaneously exploits the delivery characteristics of lipid and dendrimer vectors.[12]

Appealing with regard to their biocompatibility and biodegradability, peptide dendrons have also been studied for their use within amphiphilic dendrimers for siRNA delivery. For example, Guan and co-workers developed a panel of bola-type amphiphilic peptide dendrimers for siRNA delivery.[11] These dendrimers have two polylysine dendrons connected to a linear core composed of either a hydrophobic alkyl chain or a fluorinated chain via a disulfide linkage (**8** and **9** in Figure 6).


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

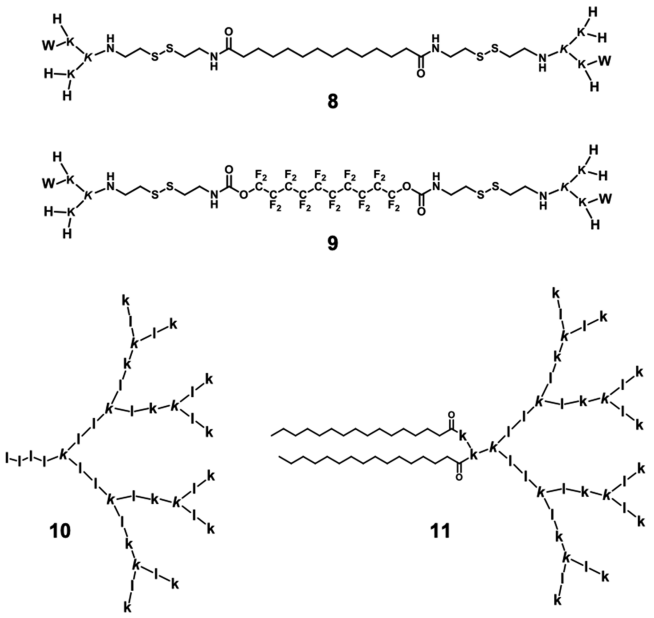



**Figure 6.** Amphiphilic peptide dendrimers **8**−**11** developed for siRNA delivery.[11,40] One-letter codes are used here to present the amino acids: K for L-lysine, H for L-histidine, W for L-tryptophan, k for D-lysine, and l for D-leucine. Branching lysine units are in italics. Adapted with permission from ref 11 (Copyright 2015 American Chemical Society) and ref 40 (Copyright 2019 American Chemical Society).

Tryptophan was appended onto the dendron terminals to favor siRNA binding, and histidine also to promote endosomal escape through the pH-responsive imidazole group. The disulfide linkages were designed to respond to the intracellular high level of glutathione, thereby further boosting endosome release. Noteworthy, the dendrimer bearing the fluorocarbon chain (**9**) exhibited the most powerful and robust functional siRNA delivery even under high serum concentration conditions. The excellent performance of **9** suggests that the fluorine−fluorine interaction may play a crucial role in stabilizing assembly and siRNA delivery. A similar "fluorocarbon effect" has previously been reported for DNA delivery.[39]

Interestingly, Reymond and co-workers constructed amphiphilic dendrimers composed of peptide dendrons bearing various amino acid residues for siRNA delivery.[40] Their structure−activity relationship study indicated that efficient transfection depends on a favorable ratio and arrangement of hydrophobic and cationic amino acid residues. For example, the two best performing dendrimers **10** and **11** each bear a peptide dendron with alternate hydrophilic lysine branching units and hydrophobic isoleucine residues alongside respectively either a hydrophobic D-leucine oligopeptide or a two C18 chain conjugation (Figure 6). In addition, both **10** and **11** were able to aggregate in a stereoselective process involving intermolecular $\beta$-sheet cross-links to form siRNA/dendrimer nanoparticles, which, under acidic conditions, rearranged to $\alpha$-helical conformations favoring endosome escape and siRNA release. Notably, peptide dendrimers composed of unnatural amino acids with D-configurations exhibited higher stability than their corresponding natural (i.e., L-configuration) amino acid counterparts and, at the same time, better siRNA delivery activity for silencing GADPH in HeLa cells.

Recently, we also elaborated a biodegradable amphiphilic dendrimer **12** (Figure 7) bearing poly(aminoester) dendron (**12** in Figure 7).[41] Poly(aminoester) skeleton is widely employed for constructing biodegradable materials thanks to

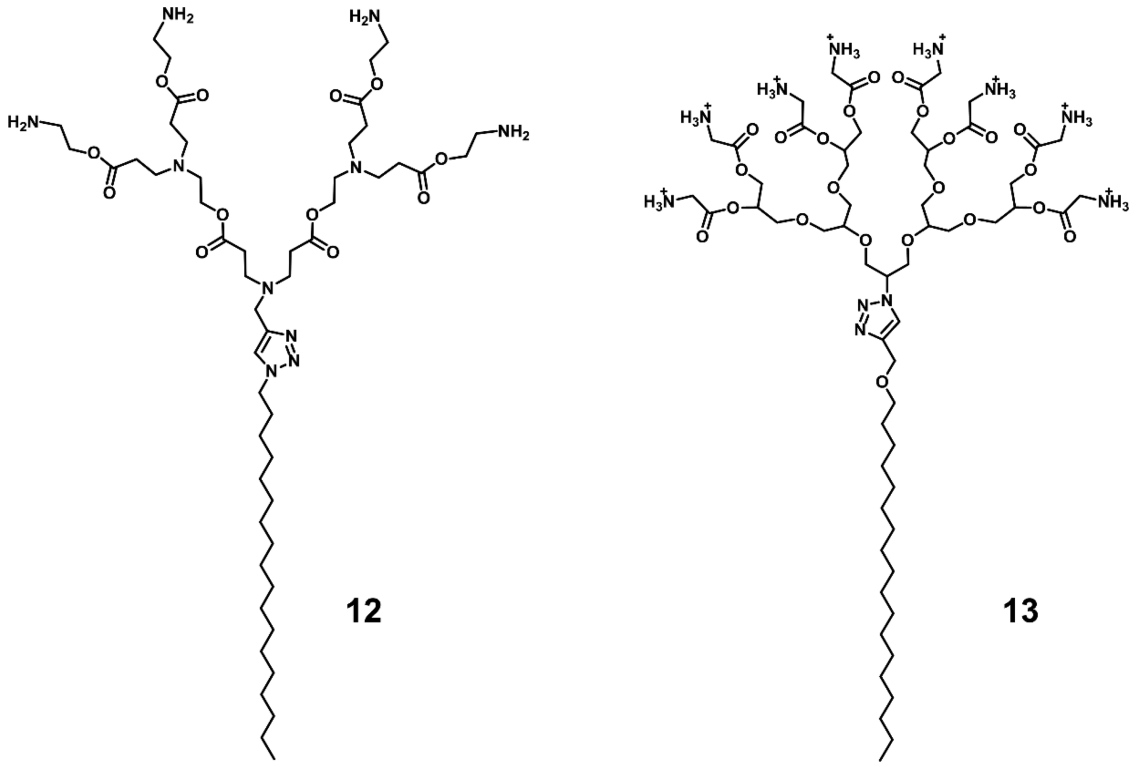



**Figure 7.** Amphiphilic dendrimers **12** and **13** bearing poly(aminoester) and glycerol dendrons, respectively, for siRNA delivery.[41,42] Adapted with permission from ref 41 (Copyright 2022 Royal Society of Chemistry) ref 42 (Copyright 2012 American Chemical Society).


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

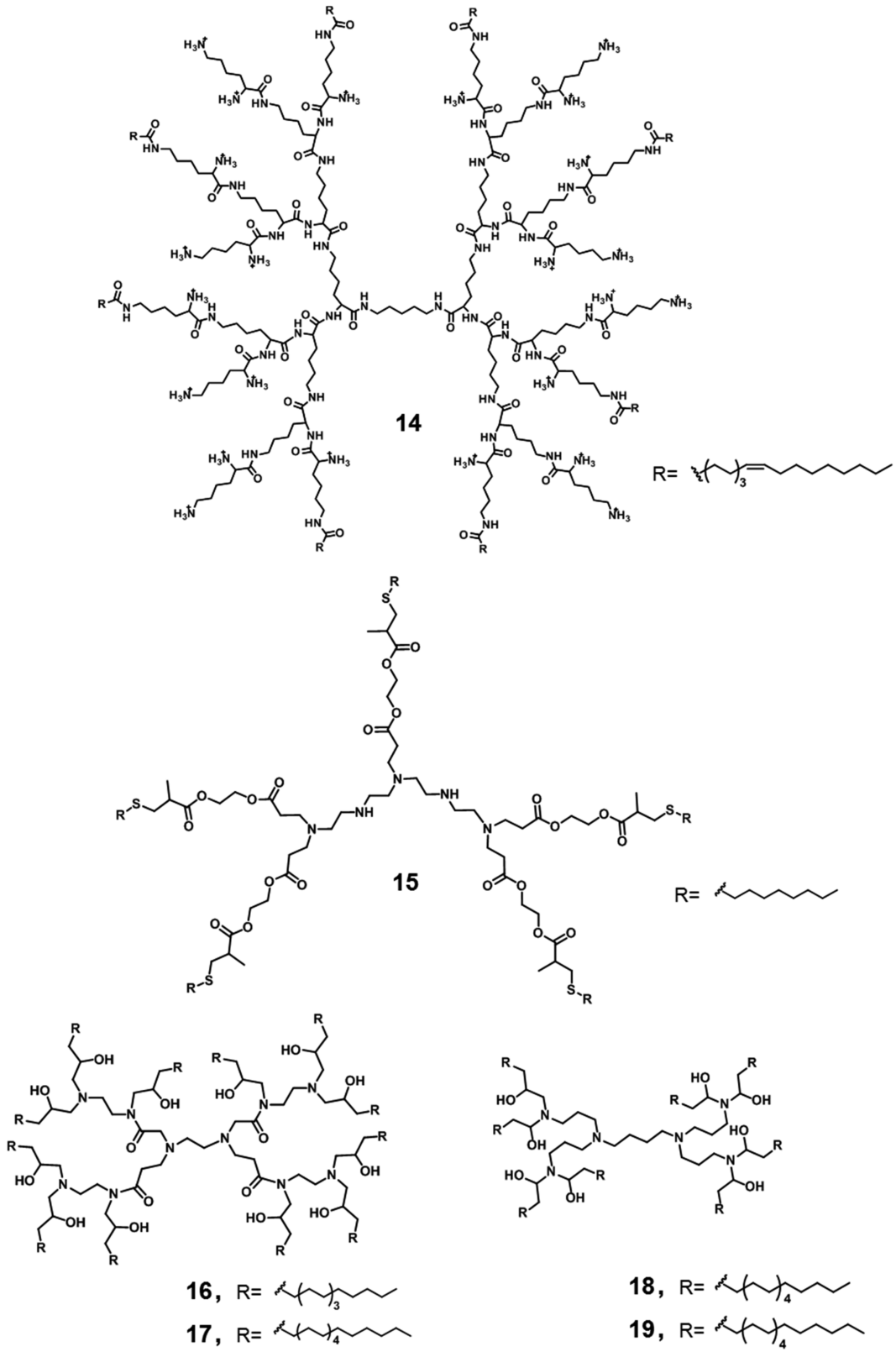



**Figure 8.** Examples of core−shell type amphiphilic dendrimers established for siRNA delivery.[13,15,43,45] Adapted with permission from ref 13 (Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA), ref 15 (Copyright 2016 National Academy of Sciences), ref 43 (Copyright 2007 American Chemical Society), and ref 45 (Copyright 2015 American Chemical Society).

its multiple advantageous features: first, the ester linkage is acid-, base- and enzyme-labile, thus degradable via ester hydrolysis; second, the amine functionality can serve as the buffer to neutralize the acid generated from the ester hydrolysis, rending the degradation process within a benign and nonaggressive environment. Indeed, **12** was readily disintegrated upon enzymatic action and exhibited more


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

effective siRNA delivery with a better safety profile than its PAMAM dendrimer counterpart.

In a further stride toward developing improved biocompatible dendrimer vectors, Haag and co-workers synthesized amphiphilic dendrimers bearing glycerol dendrons for siRNA delivery (**13** in Figure 7).[42] Glycerol dendrimers have polyether backbones composed of glycerol repeating units designed to mimic the neutral and biocompatible features of polyethylene glycol (PEG). Glycine residues were added to the dendrimer terminals via ester linkages (**13** in Figure 7) to provide, at physiological pH, positively charged groups for binding to the negatively charged siRNA. Unfortunately, these glycerol dendrimers did not perform well at delivering siRNA although they are not toxic, even at high doses. One plausible explanation is the absence of tertiary amine functionalities within the chemical structures of these dendrimers that, while permitting the formation of stable nanoparticles with siRNA, may not therefore enable the proton-sponge effect for endosomal release.

In addition to the above-mentioned dendron-tail amphiphilic dendrimers, core−shell type amphiphilic dendrimers have also been developed for siRNA delivery. In 2007, Rana conjugated oleic acids on the surface of a polylysine peptide dendrimer to construct **14** (Figure 8), which led to efficient siRNA delivery and gene silencing without any significant cytotoxicity or immune response.[43] Later, Cheng and co-worker constructed a library of >300 surface-engineered core−shell dendrimers in order to perform structure−activity relationship analysis on core−shell dendrimers for siRNA delivery.[44] They introduced various hydrophilic and hydrophobic groups such as alkyls and fluoroalkyls, amino acids, boronic acids, guanidines, and aromatic and heterocyclic rings on the surface of PAMAM dendrimers. Their structure−activity relationship study revealed that surface modification containing hydrophobic functionalities such as alkyls, fluoroalkyls, boronic acid, etc. is essential for achieving efficient siRNA delivery.

Also, Zhou et al. studied a library of more than 1500 core−shell-type amphiphilic poly(aminoester) dendrimers with a large chemical diversity of cores and peripheries.[15] After extensive in vitro screening and structure−activity relationship analysis, effective delivery was associated with dendrimers possessing a siRNA binding dendron core and an RNA complex-stabilizing hydrophobic periphery. Further in vivo evaluation led to the identification of the lead dendrimer **15** (Figure 8), a lipid-like molecule composed of a polyamine core and a hydrophobic periphery of alkyl chains. This dendrimer exhibited very high delivery efficacy yet exceptionally low toxicity with predominant biodistribution in liver and cancerous liver. It holds therefore great promise as an RNA vector for treating liver cancer and liver diseases. It is good to mention that this dendrimer vector must be coformulated with cholesterol, phospholipid, and pegylated lipid as multicomponent lipid nanoparticles (LNPs) for effective siRNA delivery.

To address cell-specific delivery, Khan et al. coformulated active core−shell amphiphilic dendrimers with cholesterol and pegylated lipid for specific siRNA delivery to different cell subpopulations in liver and lung.[13,45] For example, by reducing the amount of cholesterol and pegylated lipid, the corresponding delivery formulations made with **16** and **17** (Figure 8) exhibited an increased specificity and potency for delivery to liver endothelial cells when compared with that of hepatocytes.[13] Interestingly, by altering the ratio of **17**/cholesterol/pegylated lipid from 98/1/1 to 97/1/2, the obtained nanoformulation achieved potent and specific siRNA delivery to endothelial cells in lung instead of liver. Similarly, coformulation of the amphiphilic polypropylimine (PPI) dendrimers **18** and **19** (Figure 8) with cholesterol and pegylated lipid also achieved specific siRNA delivery to endothelial cells in lung.[45] The exact mechanism behind this cell-specific delivery remains unclear. The different formulations may form distinct "protein coronas" with serum proteins, the effect of which impacts and directs a specific delivery. Noteworthy is the specific delivery to endothelial cells in lung, which could be explored for fighting diseases associated with lung infections and inflammation such as COVID-19.

### 4.2. mRNA Delivery

Notably, advancements in the development of mRNA delivery systems have been greatly accelerated by the experience and knowledge obtained from siRNA delivery.[21] Indeed, both siRNA and mRNA are made of the same chemical building blocks and require delivery to the cytoplasm. However, siRNA and mRNA differ in size, overall structure (i.e., double vs single-stranded) and mechanism of action. Consequently, an effective vector for siRNA delivery may not be suitable for mRNA delivery. For example, the amphiphilic dendrimers developed in our group for siRNA delivery are minimally effective for mRNA delivery. Thus, specific efforts to identify effective vectors for mRNA delivery are required.

In 2018, Siegwart and co-workers reported the amphiphilic dendrimer-based LNPs for mRNA delivery.[46] The candidate dendrimer was the previously established amphiphilic dendrimer **15** (Figure 8), which was identified and validated for siRNA delivery when coformulated with phospholipids, cholesterol, and a pegylated lipid as LNPs.[15] Notably different from siRNA delivery, the formulations based on **15** for mRNA delivery required significantly fewer ionizable cationic lipids and more zwitterionic phospholipids. Compared to the shorter siRNA molecules, the long mRNA molecules may require weaker electrostatic association (hence less ionizable lipid) but a stronger capacity to escape from the endosome (hence more zwitterionic phospholipid). This study highlights the importance of distinct formulations for different RNA therapeutics, even when the main delivery vector is the same. It also provides a rational design guideline for optimizing the dendrimer-based LNP formulations for adaptive nucleic acid delivery.

Further in-depth investigations on **15**-based delivery formulations led to the discovery of the selective organ targeting (SORT) strategy for mRNA delivery.[47] By addition of either cationic or anionic SORT lipid molecules such as 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) or 1,2-dioleoyl-*sn*-glycero-3-phosphate (18PA) as the fifth component in the **15**-based LNPs and varying their percentages, completely selective delivery to liver, spleen, and lung was achieved for functional mRNA delivery and CRISPR-Cas gene editing. This SORT strategy was also applicable to other LNPs and may extend to other nucleic acid therapeutics, providing a modular and general strategy for achieving organ-selective delivery, which remains a major obstacle for clinical applications of nucleic acid therapeutics.

Noteworthy, Anderson and co-workers established a mRNA vaccine platform using the amphiphilic dendrimer **20** (Figure 9) coformulated with pegylated lipids for functional delivery of


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

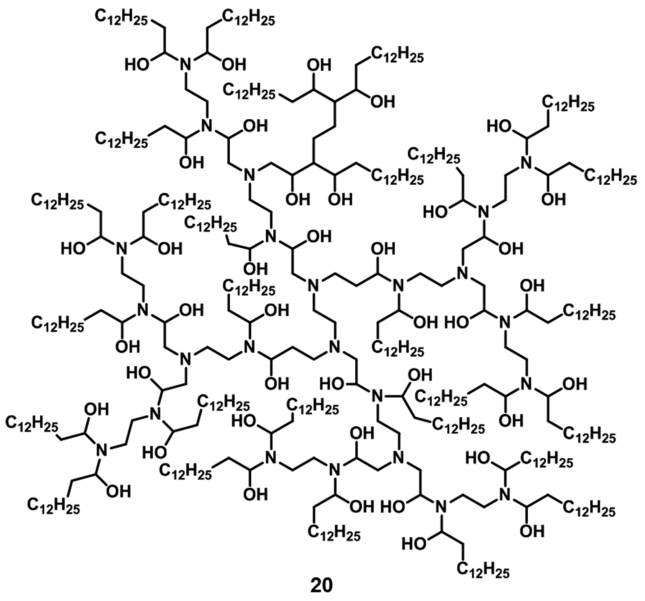



**Figure 9.** Amphiphilic dendrimer **20** developed for the delivery of mRNA vaccines.[48] Adapted with permission from ref 48 (2016 National Academy of Sciences).

replicon mRNA vaccines.[48] This dendrimer delivery system was devoid of toxicity and immunogenicity, while effectively delivering self-replicating mRNA vaccines and generating protective immunity. A single dose of the so-formed vaccines via intramuscular administration elicited rapid and robust CD8+ T-cell and antibody responses that fully protected against lethal infectious agents such as Ebola, H1N1 influenza, and *Toxoplasma gondii*. In addition, the vaccine can be made with multiple antigen-expressing mRNAs, and the preparation of the mRNA/dendrimer complexes was shown to be easy, fast, and reliable using a microfluidic method. The formed mRNA/dendrimer complexes are very stable, protect mRNA from degradation, and maintain immunization activity even after a long shelf life. This flexible, safe, and efficient mRNA vaccine platform could offer promising perspectives for elaborating vaccines responding rapidly to evolving and emerging pathogens or sudden outbreaks such as COVID-19.

Although the above dendrimer-based multicomponent LNPs were effective for mRNA delivery, they have limitations such as instability, short shelf life, and possibly reduced delivery efficacy due to pegylation. To challenge the multicomponent delivery systems, Percec and co-workers recently explored ionizable Janus amphiphilic dendrimers (Figure 10) as one-component vectors for mRNA delivery.[49] The delivery complexes were constructed using a simple injection method to assemble amphiphilic dendrimers with mRNA into stable vesicular dendrimersomes, which were stable for over 135 days at 5 °C. Several Janus dendrimers exhibited excellent activity for mRNA delivery, even outperforming the FDA-approved LNP formulation. This study highlights the potential of amphiphilic dendrimers as simple, one-component vectors for mRNA delivery.

Of note, active dendrimers screened from cell-based experiments for mRNA delivery did not match those identified from in vivo evaluation. For example, although dendrimer **21** showed excellent mRNA delivery performance in a cell-based assay, it performed poorly in mice. On the other hand, dendrimer **22** performed very well in the animal model but showed almost no delivery activity in cell-based experiments.[49] Similar paradoxical findings have previously been reported for other vectors,[46] and presumably arise because the cell experimental conditions do not faithfully reproduce the

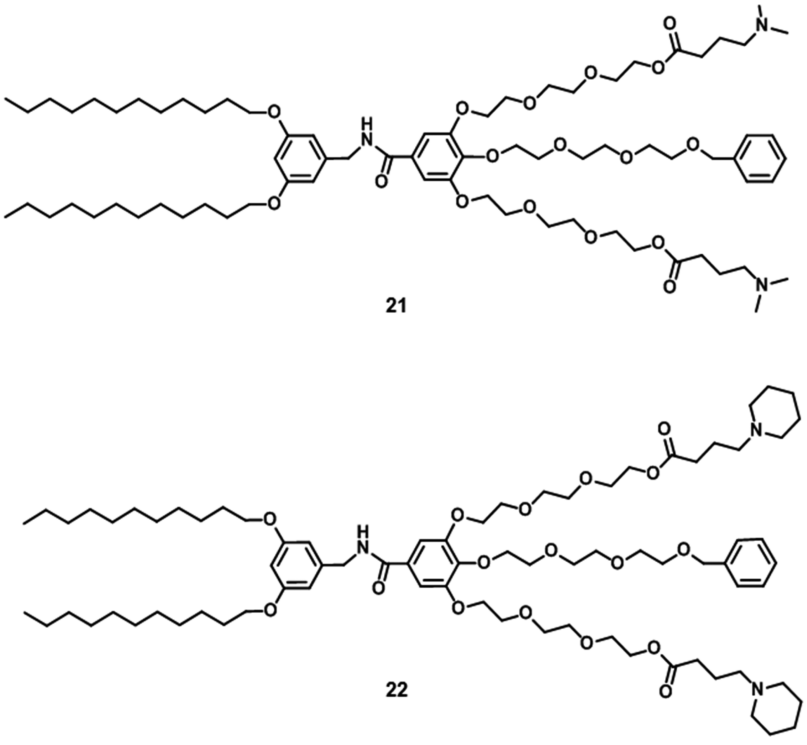



**Figure 10.** Ionizable amphiphilic Janus dendrimers **21** and **22** developed for mRNA delivery.[49] Adapted with permission from ref 49 (Copyright 2021 American Chemical Society).


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

physiological environment encountered during delivery in an animal model.

Remarkably, some of these Janus amphiphilic dendrimers demonstrated selective mRNA delivery in lung or in liver and spleen after structural modification.[49,50] Although the exact mechanism for the observed targeted delivery remains unclear, it is very likely that each RNA/dendrimer complex has distinct physicochemical properties and may form particular protein coronas that allow selective organ targeting. Moreover, all active dendrimer vectors identified in this study are of low generation. Thus, they are essentially lipid derivatives that can be easily prepared and scaled up to amplify production. In summary, these amphiphilic dendrimers constitute promising vectors for mRNA delivery for future biomedical applications by virtue of their delivery efficacy, small size, easy synthesis, and simple one-component formulation.

## 5. SUMMARY AND OUTLOOK

In this Account, we have presented a holistic view of key advances in the development of amphiphilic dendrimer vectors for siRNA delivery and mRNA delivery. Specifically, amphiphilic dendrimers are lipid−dendrimer hybrids that mimic lipid vectors for membrane-fusion-mediated delivery while simultaneously retaining the properties of polymer vectors that permit exploitation of the proton-sponge effect. In addition, amphiphilic dendrimers can be easily synthesized and have precisely controlled chemical structures that allow multiple functionalities, providing nanotechnology-based delivery systems.

In the past decade, remarkable efforts have led to the development of amphiphilic dendrimers with improved safety and performance for RNA delivery. The chemical composition, structural features, and hydrophilic/hydrophobic balance of amphiphilic dendrimers all play critical roles in the delivery. In particular, the balance between hydrophilicity and hydrophobicity often impacts significantly both delivery capacity and the toxicity profile, with a frequently encountered dilemma of high-performing vectors exhibiting high toxicity. A fine balance between hydrophilicity and hydrophobicity to achieve maximum delivery efficiency with minimal toxicity is crucial and requires careful consideration.

Most structure−activity relationship studies thus far concern amphiphilic polyamine dendrimers bearing ionizable amine terminals. Alternatively, amphiphilic dendrimers with arginine terminals can mimic arginine-rich cell-penetrating peptides to improve movement across the membrane and thus cellular uptake. In addition, introducing stimulus-responsive and biodegradable functional groups imparts capacity for on-demand and specific delivery to distinct cell populations and related tissue microenvironments. Further development of amphiphilic dendrimers carrying new and ingenious chemical structures with biodegradation and targeting capacity will certainly enrich the RNA delivery toolbox.

Selective cell- and organ-specific delivery of RNA therapeutics constitutes a formidable challenge. Amphiphilic dendrimers can achieve such targeted delivery via the appending of targeting functionalities to their delivery complexes.[33] In addition, both amphiphilic dendrimers alone[49] and amphiphilic dendrimer-based LNPs[13,45,47] can achieve cell- and organ-specific RNA delivery. Although the exact mechanisms behind such specific delivery of these systems are not clear, they represent promising alternatives for future biomedical applications. Before this, further investigations on the physicochemical features and biointerface interactions of these selective delivery systems may illuminate the underlying targeting mechanisms and provide insight into allowing the rational design and creation of highly efficient, safe, specific targeted delivery systems of the future. Additional incorporation of imaging agents within the delivery system will enable theranostic approaches in precision medicine including tracing, staging, and real-time assessment to ensure precise delivery to specific organs, tissue, cells and subcellular organelles.

It should be mentioned that, although both siRNA and mRNA are made of the same chemical building blocks and require delivery to the cytoplasm, they differ in overall structure (i.e., double vs single-stranded) and length as well as the mechanism of action. Consequently, specific vectors are required for their delivery. Future research will also look to using amphiphilic dendrimers for the delivery of other types of RNA therapeutics such as antisense oligonucleotides for gene silencing, small activating RNA for gene activation and mRNA plus single guide RNA for gene editing. In addition, the development of vectors for local and oral delivery would further facilitate the clinical implementation of RNA therapeutics. Collectively, these will greatly enrich and expand the RNA therapeutics landscape. We look forward to the new advancements in this fast growing and exciting field.

## AUTHOR INFORMATION


### Corresponding Author

**Ling Peng** − *Aix Marseille Université, CNRS, Center Interdisciplinaire de Nanoscience de Marseille, CINaM UMR 7325, "Equipe Labellisée Ligue Contre le Cancer", 13288 Marseille, France;* orcid.org/0000-0003-3990-5248; Email: ling.peng@univ-amu.fr

### Authors

**Jiaxuan Chen** − *Aix Marseille Université, CNRS, Center Interdisciplinaire de Nanoscience de Marseille, CINaM UMR 7325, "Equipe Labellisée Ligue Contre le Cancer", 13288 Marseille, France; State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Drug Discovery, Center of Advanced Pharmaceuticals and Biomaterials, China Pharmaceutical University, 210009 Nanjing, P. R. China*

**Dandan Zhu** − *State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Drug Discovery, Center of Advanced Pharmaceuticals and Biomaterials, China Pharmaceutical University, 210009 Nanjing, P. R. China*

**Xiaoxuan Liu** − *State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Drug Discovery, Center of Advanced Pharmaceuticals and Biomaterials, China Pharmaceutical University, 210009 Nanjing, P. R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/accountsmr.1c00272


### Notes

The authors declare no competing financial interest.

### Biographies

**Jiaxuan Chen** received his PhD from both Aix-Marseille University in France and China Pharmaceutical University in China under the joint



https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

supervision of Dr. Ling Peng and Prof. Xiaoxuan Liu. Currently, he is a postdoctoral fellow at China Pharmaceutical University. His research interests focus on medicinal chemistry and nucleic acid delivery systems.

**Dandan Zhu** received her PhD from China Pharmaceutical University under the codirection of Dr. Ling Peng and Prof. Xiaoxuan Liu. She is now a postdoctoral fellow in the Institute of Pharmaceutical Sciences at China Pharmaceutical University. Her research interest is to study functional amphiphilic dendrimers for siRNA delivery and the related delivery mechanisms.

**Xiaoxuan Liu** is a professor at China Pharmaceutical University. Her research interests focus on the development of functional dendrimer-based nanosystems as delivery platforms for nucleic acid therapeutics and their applications in cancer and inflammation diseases.

**Ling Peng** is a research director at the French National Scientific Research Center (CNRS) and a distinguished member of French Chemical Society. She is working actively at the interface of chemistry and biology in conception, synthesis, and evaluation of dendrimer nanosystems for biomedical applications, in particular for the delivery of drug, nucleic acid, and imaging agent in disease treatment and diagnosis.

## ■ ACKNOWLEDGMENTS

We are grateful for financial support from the Ligue Nationale Contre le Cancer (L.P.), Campus France PHC program CAI YUANPEI (L.P., X.L.), the French National Research Agency for funding of the Era-Net EURONANOMED European Research projects "Target4Cancer", "NANOGLIO", "iNANO-GUN" (L.P.), the European Union's Horizon 2020 research and innovation program H2020 NMBP "SAFE-N-MED-TECH" (No. 814607, L.P., X.L.), the China Scholarship Council (J.C.), Campus France bourse Eiffel (J.C.), CNRS, and Aix-Marseille Université.

## ■ REFERENCES

(1) Tomalia, D. A.; Fréchet, J. M. J. Discovery of dendrimers and dendritic polymers: A brief historical perspective. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40* (16), 2719−2728.
(2) Lee, C. C.; MacKay, J. A.; Frechet, J. M.; Szoka, F. C. Designing dendrimers for biological applications. *Nat. Biotechnol.* **2005**, *23* (12), 1517−26.
(3) Kannan, R. M.; Nance, E.; Kannan, S.; Tomalia, D. A. Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. *J. Int. Med.* **2014**, *276* (6), 579−617.
(4) Kulkarni, J. A.; Witzigmann, D.; Thomson, S. B.; Chen, S.; Leavitt, B. R.; Cullis, P. R.; van der Meel, R. The current landscape of nucleic acid therapeutics. *Nat. Nanotechnol.* **2021**, *16* (6), 630−643.
(5) Setten, R. L.; Rossi, J. J.; Han, S. P. The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discovery* **2019**, *18* (6), 421−446.
(6) Sahin, U.; Kariko, K.; Tureci, O. mRNA-based therapeutics-developing a new class of drugs. *Nat. Rev. Drug Discovery* **2014**, *13* (10), 759−80.
(7) Weng, Y.; Li, C.; Yang, T.; Hu, B.; Zhang, M.; Guo, S.; Xiao, H.; Liang, X. J.; Huang, Y. The challenge and prospect of mRNA therapeutics landscape. *Biotechnol. Adv.* **2020**, *40*, 107534.
(8) Kumar, R.; Santa Chalarca, C. F.; Bockman, M. R.; Bruggen, C. V.; Grimme, C. J.; Dalal, R. J.; Hanson, M. G.; Hexum, J. K.; Reineke, T. M. Polymeric Delivery of Therapeutic Nucleic Acids. *Chem. Rev.* **2021**, *121* (18), 11527−11652.
(9) Zhang, Y.; Sun, C.; Wang, C.; Jankovic, K. E.; Dong, Y. Lipids and Lipid Derivatives for RNA Delivery. *Chem. Rev.* **2021**, *121* (20), 12181−12277.
(10) Yu, T.; Liu, X.; Bolcato-Bellemin, A. L.; Wang, Y.; Liu, C.; Erbacher, P.; Qu, F.; Rocchi, P.; Behr, J. P.; Peng, L. An amphiphilic dendrimer for effective delivery of small interfering RNA and gene silencing in vitro and in vivo. *Angew. Chem., Int. Ed. Engl.* **2012**, *51* (34), 8478−8484.
(11) Zeng, H.; Johnson, M. E.; Oldenhuis, N. J.; Tiambeng, T. N.; Guan, Z. Structure-Based Design of Dendritic Peptide Bolaamphiphiles for siRNA Delivery. *ACS Cent Sci.* **2015**, *1* (6), 303−312.
(12) Liu, X.; Wang, Y.; Chen, C.; Tintaru, A.; Cao, Y.; Liu, J.; Ziarelli, F.; Tang, J.; Guo, H.; Rosas, R.; Giorgio, S.; Charles, L.; Rocchi, P.; Peng, L. A fluorinated bola-amphiphilic dendrimer for on-demand delivery of siRNA, via specific response to reactive oxygen species. *Adv. Funct. Mater.* **2016**, *26* (47), 8594−8603.
(13) Khan, O. F.; Zaia, E. W.; Yin, H.; Bogorad, R. L.; Pelet, J. M.; Webber, M. J.; Zhuang, I.; Dahlman, J. E.; Langer, R.; Anderson, D. G. Ionizable amphiphilic dendrimer-based nanomaterials with alkyl-chain-substituted amines for tunable siRNA delivery to the liver endothelium in vivo. *Angew. Chem., Int. Ed. Engl.* **2014**, *53* (52), 14397−14401.
(14) Liu, X.; Zhou, J.; Yu, T.; Chen, C.; Cheng, Q.; Sengupta, K.; Huang, Y.; Li, H.; Liu, C.; Wang, Y.; Posocco, P.; Wang, M.; Cui, Q.; Giorgio, S.; Fermeglia, M.; Qu, F.; Pricl, S.; Shi, Y.; Liang, Z.; Rocchi, P.; Rossi, J. J.; Peng, L. Adaptive amphiphilic dendrimer-based nanoassemblies as robust and versatile siRNA delivery systems. *Angew. Chem., Int. Ed. Engl.* **2014**, *53* (44), 11822−11827.
(15) Zhou, K.; Nguyen, L. H.; Miller, J. B.; Yan, Y.; Kos, P.; Xiong, H.; Li, L.; Hao, J.; Minnig, J. T.; Zhu, H.; Siegwart, D. J. Modular degradable dendrimers enable small RNAs to extend survival in an aggressive liver cancer model. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (3), 520−525.
(16) Chen, C.; Posocco, P.; Liu, X.; Cheng, Q.; Laurini, E.; Zhou, J.; Liu, C.; Wang, Y.; Tang, J.; Col, V. D.; Yu, T.; Giorgio, S.; Fermeglia, M.; Qu, F.; Liang, Z.; Rossi, J. J.; Liu, M.; Rocchi, P.; Pricl, S.; Peng, L. Mastering dendrimer self-assembly for efficient siRNA delivery: From conceptual design to in vivo efficient gene silencing. *Small* **2016**, *12* (27), 3667−3676.
(17) Dowdy, S. F. Overcoming cellular barriers for RNA therapeutics. *Nat. Biotechnol.* **2017**, *35* (3), 222−229.
(18) Kariko, K.; Buckstein, M.; Ni, H.; Weissman, D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* **2005**, *23* (2), 165−75.
(19) Ku, S. H.; Jo, S. D.; Lee, Y. K.; Kim, K.; Kim, S. H. Chemical and structural modifications of RNAi therapeutics. *Adv. Drug. Delivery Rev.* **2016**, *104*, 16−28.
(20) Roberts, T. C.; Langer, R.; Wood, M. J. A. Advances in oligonucleotide drug delivery. *Nat. Rev. Drug Discovery* **2020**, *19* (10), 673−694.
(21) Hou, X.; Zaks, T.; Langer, R.; Dong, Y. Lipid nanoparticles for mRNA delivery. *Nat. Rev. Mater.* **2021**, *6*, 1078.
(22) Piotrowski-Daspit, A. S.; Kauffman, A. C.; Bracaglia, L. G.; Saltzman, W. M. Polymeric vehicles for nucleic acid delivery. *Adv. Drug. Delivery Rev.* **2020**, *156*, 119−132.
(23) Davis, M. E.; Zuckerman, J. E.; Choi, C. H.; Seligson, D.; Tolcher, A.; Alabi, C. A.; Yen, Y.; Heidel, J. D.; Ribas, A. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* **2010**, *464* (7291), 1067−70.
(24) Jayaraman, M.; Ansell, S. M.; Mui, B. L.; Tam, Y. K.; Chen, J.; Du, X.; Butler, D.; Eltepu, L.; Matsuda, S.; Narayanannair, J. K.; Rajeev, K. G.; Hafez, I. M.; Akinc, A.; Maier, M. A.; Tracy, M. A.; Cullis, P. R.; Madden, T. D.; Manoharan, M.; Hope, M. J. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem., Int. Ed. Engl.* **2012**, *51* (34), 8529−33.
(25) Laemmli, U. K. Characterization of DNA condensates induced by poly(ethylene oxide) and polylysine. *Proc. Natl. Acad. Sci. U. S. A.* **1975**, *72* (11), 4288−92.
(26) Boussif, O.; Lezoualc'h, F.; Zanta, M. A.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J. P. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo:


https://doi.org/10.1021/accountsmr.1c00272
Acc. Mater. Res. 2022, 3, 484−497

polyethylenimine. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92* (16), 7297−7301.
(27) Behr, J. P. The proton sponge: A trick to enter cells the viruses did not exploit. *Chimia* **1997**, *51* (1−2), 34−36.
(28) Sonawane, N. D.; Szoka, F. C., Jr; Verkman, A. S. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. *J. Biol. Chem.* **2003**, *278* (45), 44826−31.
(29) Gilleron, J.; Querbes, W.; Zeigerer, A.; Borodovsky, A.; Marsico, G.; Schubert, U.; Manygoats, K.; Seifert, S.; Andree, C.; Stoter, M.; Epstein-Barash, H.; Zhang, L.; Koteliansky, V.; Fitzgerald, K.; Fava, E.; Bickle, M.; Kalaidzidis, Y.; Akinc, A.; Maier, M.; Zerial, M. Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. *Nat. Biotechnol.* **2013**, *31* (7), 638−46.
(30) Tomalia, D. A.; Nixon, L. S.; Hedstrand, D. M. The Role of Branch Cell Symmetry and Other Critical Nanoscale Design Parameters in the Determination of Dendrimer Encapsulation Properties. *Biomolecules* **2020**, *10* (4), 642.
(31) Liu, X.; Liu, C.; Zhou, J.; Chen, C.; Qu, F.; Rossi, J. J.; Rocchi, P.; Peng, L. Promoting siRNA delivery via enhanced cellular uptake using an arginine-decorated amphiphilic dendrimer. *Nanoscale* **2015**, *7* (9), 3867−3875.
(32) Dhumal, D.; Lan, W.; Ding, L.; Jiang, Y.; Lyu, Z.; Laurini, E.; Marson, D.; Tintaru, A.; Dusetti, N.; Giorgio, S.; Iovanna, J. L.; Pricl, S.; Peng, L. An ionizable supramolecular dendrimer nanosystem for effective siRNA delivery with a favorable safety profile. *Nano Res.* **2021**, *14* (7), 2247−2254.
(33) Dong, Y.; Yu, T.; Ding, L.; Laurini, E.; Huang, Y.; Zhang, M.; Weng, Y.; Lin, S.; Chen, P.; Marson, D.; Jiang, Y.; Giorgio, S.; Pricl, S.; Liu, X.; Rocchi, P.; Peng, L. A dual targeting dendrimer-mediated siRNA delivery system for effective Gene silencing in cancer therapy. *J. Am. Chem. Soc.* **2018**, *140* (47), 16264−16274.
(34) Nakase, I.; Akita, H.; Kogure, K.; Graslund, A.; Langel, U.; Harashima, H.; Futaki, S. Efficient intracellular delivery of nucleic acid pharmaceuticals using cell-penetrating peptides. *Acc. Chem. Res.* **2012**, *45* (7), 1132−9.
(35) Patel, P.; Ibrahim, N. M.; Cheng, K. The Importance of Apparent pKa in the Development of Nanoparticles Encapsulating siRNA and mRNA. *Trends Pharmacol. Sci.* **2021**, *42* (6), 448−460.
(36) Ellert-Miklaszewska, A.; Ochocka, N.; Maleszewska, M.; Ding, L.; Laurini, E.; Jiang, Y.; Roura, A. J.; Giorgio, S.; Gielniewski, B.; Pricl, S.; Peng, L.; Kaminska, B. Efficient and innocuous delivery of small interfering RNA to microglia using an amphiphilic dendrimer nanovector. *Nanomedicine (Lond)* **2019**, *14* (18), 2441−2458.
(37) Garofalo, S.; Cocozza, G.; Porzia, A.; Inghilleri, M.; Raspa, M.; Scavizzi, F.; Aronica, E.; Bernardini, G.; Peng, L.; Ransohoff, R. M.; Santoni, A.; Limatola, C. Natural killer cells modulate motor neuron-immune cell cross talk in models of Amyotrophic Lateral Sclerosis. *Nat. Commun.* **2020**, *11* (1), 1773.
(38) Chen, J.; Ellert-Miklaszewska, A.; Garofalo, S.; Dey, A. K.; Tang, J.; Jiang, Y.; Clement, F.; Marche, P. N.; Liu, X.; Kaminska, B.; Santoni, A.; Limatola, C.; Rossi, J. J.; Zhou, J.; Peng, L. Synthesis and use of an amphiphilic dendrimer for siRNA delivery into primary immune cells. *Nat. Protoc.* **2021**, *16* (1), 327−351.
(39) Wang, M.; Liu, H.; Li, L.; Cheng, Y. A fluorinated dendrimer achieves excellent gene transfection efficacy at extremely low nitrogen to phosphorus ratios. *Nat. Commun.* **2014**, *5*, 3053.
(40) Heitz, M.; Javor, S.; Darbre, T.; Reymond, J. L. Stereoselective pH Responsive Peptide Dendrimers for siRNA Transfection. *Bioconjugate Chem.* **2019**, *30* (8), 2165−2182.
(41) Ma, C.; Zhu, D.; Lin, W.; Li, Y.; Huang, Y.; Zhu, H.; Ye, M.; Wang, Y.; Peng, L.; Liu, X. A biodegradable amphiphilic poly-(aminoester) dendrimer for safe and effective siRNA delivery. *Chem. Commun. (Camb.)* **2022**, *58*, 4168.
(42) Malhotra, S.; Bauer, H.; Tschiche, A.; Staedtler, A. M.; Mohr, A.; Calderon, M.; Parmar, V. S.; Hoeke, L.; Sharbati, S.; Einspanier, R.; Haag, R. Glycine-terminated dendritic amphiphiles for nonviral gene delivery. *Biomacromolecules* **2012**, *13* (10), 3087−3098.
(43) Baigude, H.; McCarroll, J.; Yang, C. S.; Swain, P. M.; Rana, T. M. Design and creation of new nanomaterials for therapeutic RNAi. *ACS Chem. Biol.* **2007**, *2* (4), 237−41.
(44) Liu, H.; Chang, H.; Lv, J.; Jiang, C.; Li, Z.; Wang, F.; Wang, H.; Wang, M.; Liu, C.; Wang, X.; Shao, N.; He, B.; Shen, W.; Zhang, Q.; Cheng, Y. Screening of efficient siRNA carriers in a library of surface-engineered dendrimers. *Sci. Rep.* **2016**, *6*, 25069.
(45) Khan, O. F.; Zaia, E. W.; Jhunjhunwala, S.; Xue, W.; Cai, W.; Yun, D. S.; Barnes, C. M.; Dahlman, J. E.; Dong, Y.; Pelet, J. M.; Webber, M. J.; Tsosie, J. K.; Jacks, T. E.; Langer, R.; Anderson, D. G. Dendrimer-Inspired Nanomaterials for the in Vivo Delivery of siRNA to Lung Vasculature. *Nano Lett.* **2015**, *15* (5), 3008−16.
(46) Cheng, Q.; Wei, T.; Jia, Y.; Farbiak, L.; Zhou, K.; Zhang, S.; Wei, Y.; Zhu, H.; Siegwart, D. J. Dendrimer-Based Lipid Nanoparticles Deliver Therapeutic FAH mRNA to Normalize Liver Function and Extend Survival in a Mouse Model of Hepatorenal Tyrosinemia Type I. *Adv. Mater.* **2018**, *30* (52), 1805308.
(47) Cheng, Q.; Wei, T.; Farbiak, L.; T, J. L.; Dilliard, S. A.; Siegwart, D. J. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. *Nat. Nanotechnol.* **2020**, *15* (4), 313−320.
(48) Chahal, J. S.; Khan, O. F.; Cooper, C. L.; McPartlan, J. S.; Tsosie, J. K.; Tilley, L. D.; Sidik, S. M.; Lourido, S.; Langer, R.; Bavari, S.; Ploegh, H. L.; Anderson, D. G. Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (29), No. e4133-42.
(49) Zhang, D.; Atochina-Vasserman, E. N.; Maurya, D. S.; Huang, N.; Xiao, Q.; Ona, N.; Liu, M.; Shahnawaz, H.; Ni, H.; Kim, K.; Billingsley, M. M.; Pochan, D. J.; Mitchell, M. J.; Weissman, D.; Percec, V. One-Component Multifunctional Sequence-Defined Ionizable Amphiphilic Janus Dendrimer Delivery Systems for mRNA. *J. Am. Chem. Soc.* **2021**, *143* (31), 12315−12327.
(50) Zhang, D.; Atochina-Vasserman, E. N.; Maurya, D. S.; Liu, M.; Xiao, Q.; Lu, J.; Lauri, G.; Ona, N.; Reagan, E. K.; Ni, H.; Weissman, D.; Percec, V. Targeted Delivery of mRNA with One-Component Ionizable Amphiphilic Janus Dendrimers. *J. Am. Chem. Soc.* **2021**, *143* (43), 17975−17982.


https://doi.org/10.1021/accountsmr.1c00272
*Acc. Mater. Res.* 2022, 3, 484−497