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**Self-assembling supramolecular dendrimers for biomedical applications:
lessons learned from poly(amidoamine) dendrimers**

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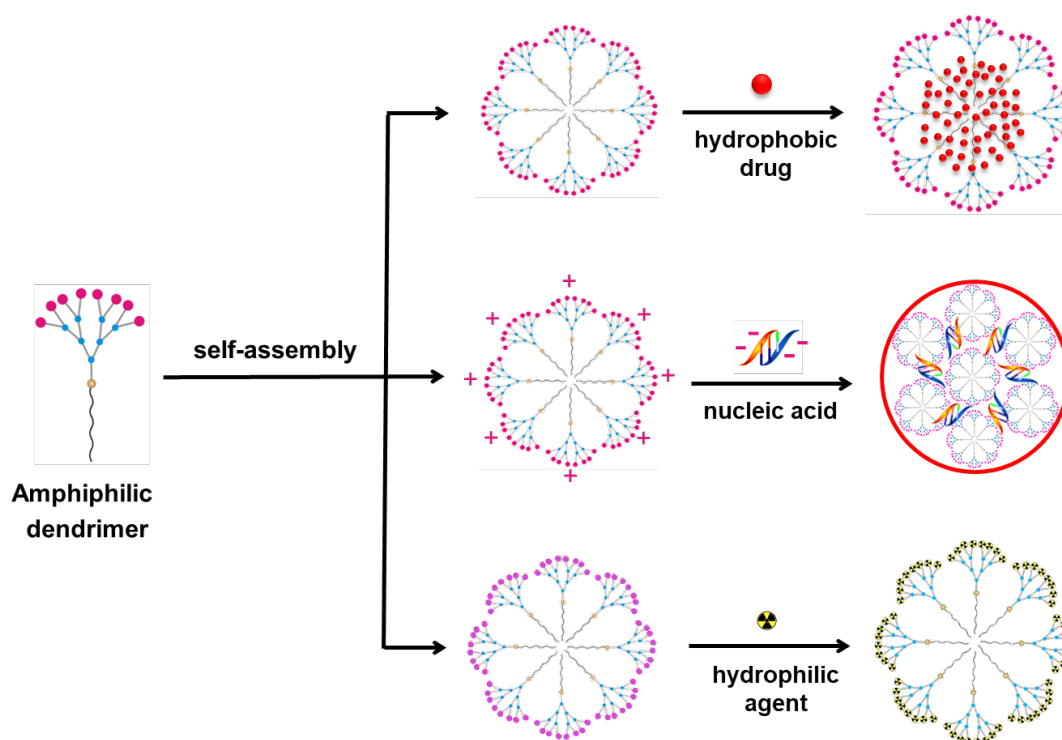
CONSPECTUS :

Dendrimers, notable for their well-defined radial structures with numerous terminal functionalities, hold great promise for biomedical applications such as drug delivery, diagnostics and therapeutics. However, their translation into clinical use has been greatly impeded by their challenging stepwise synthesis and difficult purification.

To circumvent these obstacles, we have pioneered a self-assembly approach to construct non-covalent supramolecular dendrimers using small amphiphilic dendrimer building units which can be easily synthesized and purified. By virtue of the amphipathic nature, the small amphiphilic dendrimers are able to self-assemble and generate large supramolecular dendrimers via non-covalent weak interactions such as van der Waals forces, H-bonds and electrostatic interactions etc. The so-created non-covalent dendrimers can mimic covalent dendrimers, not only in terms of radial structural feature emanating from a central core but also in their capacity to deliver drugs and imaging agents for biomedical applications. The non-covalent supramolecular dendrimers can be easily synthesized and modulated with regards size, shape, and properties by varying the nature of the hydrophobic and hydrophilic entities, as well as the dendrimer generation and terminal functionalities, ensuring their adaptability to specific applications. In particular, the dendritic structure of the amphiphilic building units permits the creation of large void spaces within the formed supramolecular dendrimers for physical encapsulation of drugs, while the large number of surface functionalities can be exploited for both physical and chemical conjugation of pharmaceutical agents for drug delivery.

Poly(amidoamine) (PAMAM) dendrimers are the most intensively studied for biomedical applications by virtue of their excellent biocompatibility imparted by their peptide-mimicking amide backbones and numerous interior and terminal amine functionalities. We present a short overview of our self-assembly strategy to construct supramolecular PAMAM

dendrimers for biomedical applications. Specifically, we start with the introduction of dendrimers and their synthesis, focusing on the innovative self-assembly synthesis of supramolecular dendrimers. We then detail the representative examples of the non-covalent supramolecular PAMAM dendrimers established in our group for the delivery of anticancer drugs, nucleic acid therapeutics and imaging agents, either within the dendrimer interior or at the dendrimer terminals on the surface. Some of the supramolecular dendrimer nanosystems exhibit outstanding performance, excelling the corresponding clinical anticancer therapeutics and imaging agents. This self-assembling approach to create supramolecular dendrimers is completely novel in concept yet easy to implement in practice, offering a fresh perspective for exploiting the advantageous features of dendrimers in biomedical applications.



KEY REFERENCES:

1. Chao Chen, Paola Posocco, Xiaoxuan Liu, Qiang Cheng, Erik Laurini, Jiehua Zhou, Cheng Liu, Yang Wang, Jingjie Tang, Valentina Dal Col, Tianzhu Yu, Suzanne Giorgio, Maurizio Fermeglia, Fanqi Qu, Zicai Liang, John J. Rossi, Minghua Liu, Palma Rocchi, Sabrina Pricl, Ling Peng, “Mastering dendrimer self-assembly for efficient siRNA delivery: from conceptual design to in vivo efficient gene silencing”, *Small* **2016**, *12*, 3667-3676.¹ *The first systemic study on the construction of non-covalent supramolecular PAMAM dendrimers to mimic the covalent dendrimers for the delivery of small interfering RNA (siRNA) and the impact of amphiphilicity on the self-assembly strategy to construct supramolecular dendrimers.*
2. Philippe Garrigue, Jingjie Tang, Ling Ding, Ahlem Bouhlef, Aura Tintaru, Erik Laurini, Yuanyu Huang, Zhenbin Lyu, Mengjie Zhang, Samantha Fernandez, Laure Balasse, Wenjun Lan, Eric Mas, Domenico Marson, Yuhua Weng, Xiaoxuan Liu, Suzanne Giorgio, Juan Iovanna, Sabrina Pricl, Benjamin Guillet, Ling Peng, “Self-assembling supramolecular dendrimer nanosystem for PET imaging of tumors”, *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, 11454-11459.² *This is the first report on the self-assembling of small amphiphilic dendrimer to generate supramolecular dendrimer for tumor imaging using positron emission tomography (PET), outperforming the clinical gold reference for PET imaging of tumor.*
3. Tuo Wei, Juan Liu, Chao Chen, Cheng Liu, Paola Posocco, Xiaoxuan Liu, Qiang Cheng, Shuidong Huo, Zicai Liang, Maurizio Fermeglia, Sabrina Pricl, Xing-Jie Liang, Palma Rocchi, Ling Peng, “Anticancer drug nanomicelles formed by self-assembling amphiphilic dendrimer to combat cancer drug resistance”, *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 2978–2983.³ *This is the first study on the concept of creating supramolecular dendrimer nanomicelles by self-assembling of amphiphilic dendrimer for the encapsulation and delivery of the anticancer drug doxorubicin to treat drug-resistant cancers with enhanced potency and reduced toxicity as well as overcome drug resistance.*
4. Yiwen Dong, Tianzhu Yu, Ling Ding, Erik Laurini, Yuanyu Huang, Mengjie Zhang, Yuhua Weng, Shuting Lin, Peng Chen, Domenico Marson, Yifan Jiang, Suzanne Giorgio, Sabrina Pricl, Xiaoxuan Liu, Palma Rocchi, Ling Peng, “A dual targeting dendrimer-mediated siRNA delivery system for effective gene silencing in cancer therapy”, *J. Am. Chem. Soc.* **2018**, *140*, 16264-16274.⁴ *This work reports on the self-assembling supramolecular dendrimer nanosystem for the targeted delivery of small interfering RNA (siRNA) for gene silencing and anticancer activity.*
5. Xiaoxuan Liu, Jiehua Zhou, Tianzhu Yu, Chao Chen, Qiang Cheng, Yuanyu Huang, Kheya Sengupta, Haitang Li, Cheng Liu, Yang Wang, Menghua Wang, Suzanne Giorgio, Fanqi Qu, Zicai Liang, Palma Rocchi, John J Rossi, Ling Peng, “Adaptive amphiphilic dendrimer based nanoassemblies as robust and versatile siRNA delivery systems”, *Angew. Chem. Int. Ed.* **2014**, *53*, 11822–11827.⁵ *This paper describes an adaptive amphiphilic dendrimer and its self-assembly into supramolecular systems for the delivery of small interfering RNA (siRNA) in vitro and in vivo*

1. Introduction

The first dendritic molecules, which are also called “cascade molecules”,⁶ were reported by Vögtle *et al.* in 1978. In 1985, Tomalia *et al.* synthesized poly(amidoamine) (PAMAM) dendrimers (**Figure 1A**),⁷ which constituted the first complete dendrimer family. This marked the beginning of the dendrimer era, and spurred unprecedented interest among the scientific community in all aspects of dendrimer science, including chemical synthesis, structural characterization, functional evaluation, and various applications.⁸

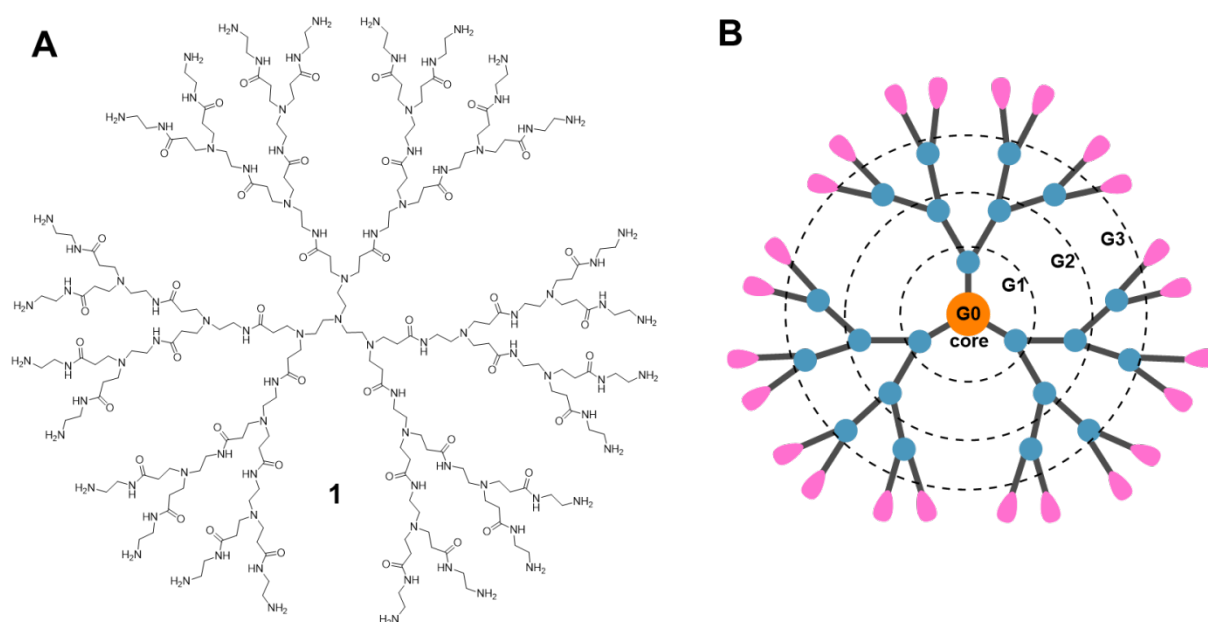


Figure 1: (A) Chemical structure of the first poly(amidoamine) (PAMAM) dendrimer (**1**). For clarity, only the dendrimer of generation 3 is presented. (B) Cartoon illustration of a dendrimer comprising a central core or generation 0 (G_0), repeating branching units forming consecutive levels or generations (G_1 , G_2 , and G_3), and the terminal groups on the dendrimer surface.⁹ Adapted with permission from ref 9, Copyright 2018 Science China Press.

By definition, a dendrimer is a synthetic molecule with a unique architecture bearing regular cascade-branched repeating units emanating from a focal point, and numerous terminal groups on its surface (**Figure 1**). From a structural perspective, dendrimers comprise three distinct parts: 1) a central core, where dendrimer growth begins; 2) repeated branching units that allow radially organized growth into consecutive layers or generations; and 3) numerous terminals, which can carry a large variety of functional groups and are exposed on the surface of the molecule (**Figure 1B**). These structural features endow dendrimers with unique structural properties and multivalent cooperativity, and also distinguish dendrimers from the three traditional architectural categories of polymers—i.e., linear, crosslinked, and simple branched—dendrimers belong to the 4th major architectural class of polymers.¹⁰

By virtue of their well-defined structure and multivalent cooperativity, dendrimers are particularly promising candidates for a wide variety of biomedical applications, such as drug delivery, diagnostics, and therapeutics for treating various diseases.¹¹⁻¹³ However, their successful implementation in clinical applications would require ready availability of the desired quantity with consistent levels of quality.¹⁴ Unfortunately, stepwise dendrimer synthesis is onerous, and the risk of structural defects increases with the number of generations. Such defective dendrimers have similar chemical compositions and physical properties to those of intact dendrimers, which makes dendrimer purification extremely difficult. This important drawback has resulted in the failure of a preclinical trial on the dendrimer-based delivery of an anticancer drug.¹⁵ Despite the recent successful clinical trials of VivaGel® and DEP®¹⁶—which are dendrimer-based drugs for the treatment of bacterial vaginosis and cancer, respectively—dendrimer synthesis, particularly of high-generation dendrimers, remains problematic.

To overcome these problems, we designed and spearheaded supramolecular dendrimer synthesis via the non-covalent self-assembly of small amphiphilic dendritic components,

whereby hydrophobic interactions, hydrogen bonds, and the hydrophilic/hydrophobic balance all play important roles (**Figure 2A**).^{1-5, 17} This approach is easy to implement in practice and circumvents laborious covalent dendrimer synthesis. The resulting non-covalent supramolecular dendrimers mimic covalent dendrimers in their dendritic structures, i.e., a core, branching units, and terminal groups (**Figure 2A**), and also in their capacity for drug delivery in biomedical applications (**Figure 2B**).

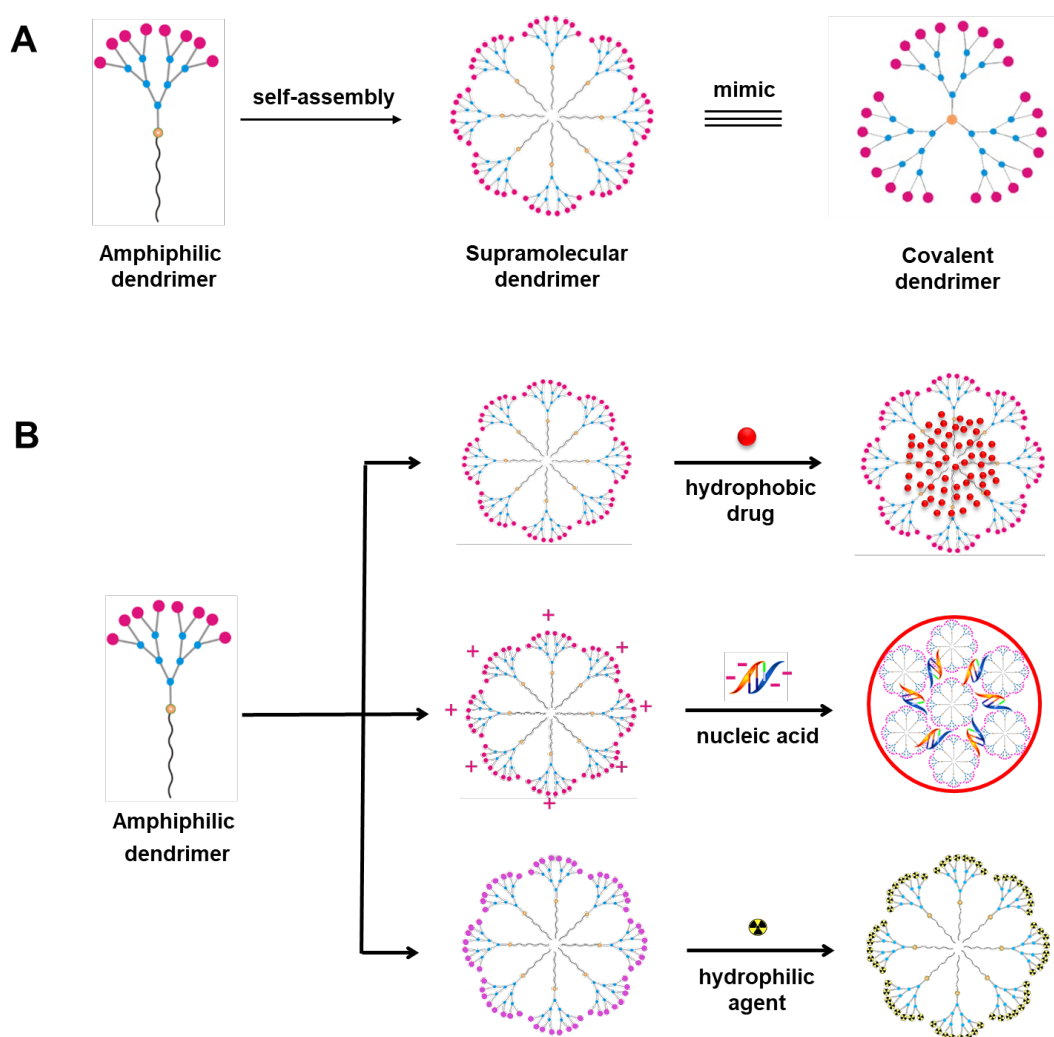


Figure 2: Cartoon representations of (A) the self-assembly of a small amphiphilic dendrimer into a supramolecular dendrimer that mimics a covalently constructed dendrimer;¹ and (B) the delivery of hydrophobic and hydrophilic pharmaceutical agents as well as negatively charged nucleic acid therapeutics. Adapted with permission from ref 1. Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA.

In particular, the dendritic structure of the amphiphilic building units permits the creation of ample voids within non-covalent supramolecular dendrimers for the physical encapsulation of drug molecules with high loading capacity. Furthermore, the numerous surface functionalities can be exploited for the physical and chemical conjugation of therapeutic agents for drug delivery in biomedical applications (**Figure 2B**). These non-covalently constructed supramolecular dendrimers are easy to prepare. Moreover, their size, shape, and properties can be altered by modifying the hydrophobic and hydrophilic entities, adjusting dendrimer generation, and changing the terminal groups, thereby ensuring adaptability to specific applications. By employing different small and simple amphiphilic building units, we have successfully constructed various supramolecular dendrimers for the delivery of drugs, nucleic acid therapeutics, and bioimaging agents.^{1-5, 18-27}

It is worth mentioning that amphiphilic dendrimers can also readily self-assemble into vesicle structures known as “dendrimersomes”.^{28, 29} The dendrimersome concept—which differs from the supramolecular dendrimer concept described in the present work—was inaugurated by Percec *et al.*,²⁸ and has been investigated extensively as a means of mimicking membranes in biological applications. State-of-the-art dendrimersomes have been reviewed elsewhere,²⁹ and will not be included in this account.

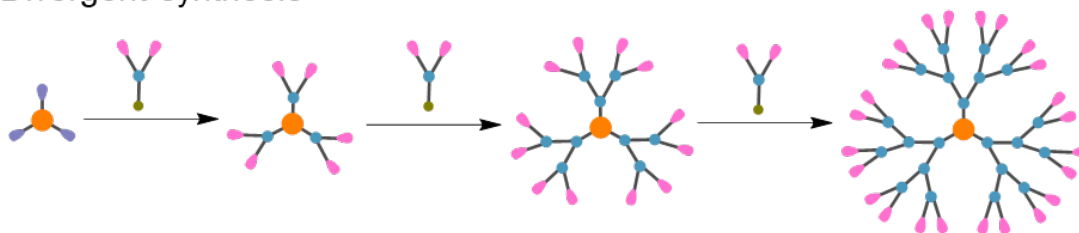
In the present account, we will give a brief overview of the supramolecular dendrimers developed by our group. We will begin by providing a general introduction to the common strategies used for dendrimer synthesis. We will then describe the synthesis of supramolecular dendrimers, and highlight their biomedical applications with representative examples that demonstrate their advantages and limitations. It should be noted that, in this account, the term “supramolecular dendrimers” refers to non-covalently constructed dendrimers that have the same structural features as covalent dendrimers—i.e., a central core, regular branching units,

and terminal groups. We will not include traditional covalent dendrimers that form supramolecular assemblies without dendritic structures.

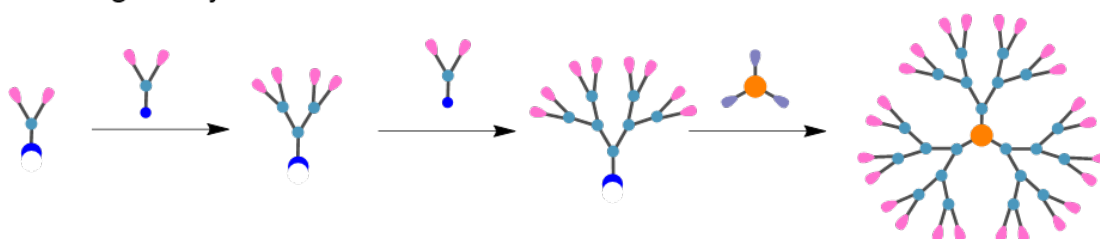
2. Dendrimer synthesis

Conventional dendrimer synthesis can be achieved using either a divergent (from a multifunctional central core and extending outwards; **Figure 3A**) or a convergent (inward-oriented synthesis from the dendrimer surface towards a central core; **Figure 3B**) approach, or a combination of the two (**Figure 3C**).^{8, 30} The combined divergent/convergent approach, which is also called the double-stage convergent approach, involves the synthesis of building blocks via a divergent approach followed by convergent dendrimer assembly (**Figure 3C**). In all these approaches, the dendrimers are constructed via stepwise covalent synthesis, which is burdensome and arduous. In particular, as the dendrimer grows, structural defects become more common owing to incomplete reactions or side-reactions caused by increasing steric hindrance, which reduces reactivity. It is difficult to separate the defective dendrimers from the intact dendrimers because they have very similar chemical and physical properties. Therefore, dendrimer purification is also very difficult and worrisome.

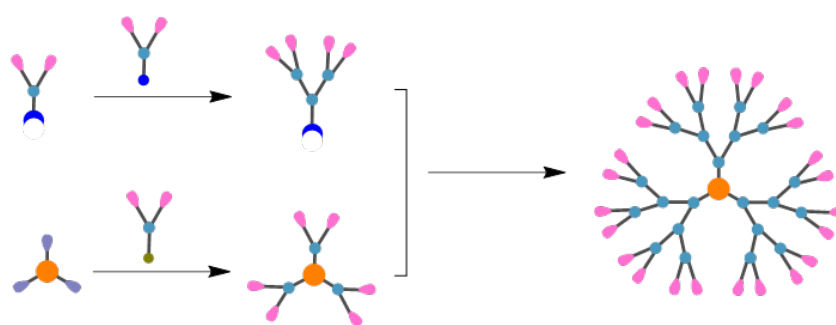
A. Divergent synthesis



B. Convergent synthesis



C. Combined divergent/convergent synthesis



D. Supramolecular self-assembly dendrimer

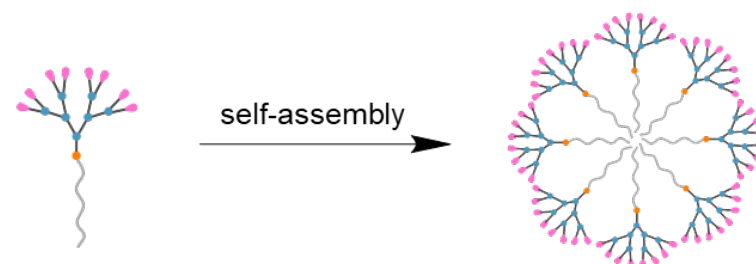


Figure 3: Cartoon illustration of the different approaches to dendrimer synthesis: (A) the divergent approach, (B) the convergent approach, and (C) the combined divergent and convergent approach for covalent dendrimer synthesis; and (D) the self-assembly approach for constructing non-covalent supramolecular dendrimers.¹⁷ Adapted with permission from ref 17, Copyright 2019 Elsevier.

To circumvent the limitations related to dendrimer synthesis via covalent means, we have pioneered the non-covalent synthesis of supramolecular dendrimers via the self-assembly of small amphiphilic dendritic components, which can be synthesized readily in pure form (**Figure 3D**).^{1-3, 5, 18, 19, 21-24} Self-assembly relies on the cumulative effects of multiple non-covalent interactions to assemble small molecular building blocks into supramolecular assemblies with weak interactions—such as van der Waals forces, hydrogen bonds, and electrostatic interactions—in a reversible, controllable, and specific way.^{31, 32} The non-covalent supramolecular dendrimers formed by self-assembly have the same structural features as covalent dendrimers—i.e., a distinctive core, repetitive branching units, and terminal groups. The concept of supramolecular dendrimer synthesis via self-assembly is new; it requires relatively little synthetic effort, yet offers new and specific properties for related applications.^{17, 33} Using PAMAM dendrimers as examples, we describe herein our recent work on the self-assembling synthesis of supramolecular dendrimers for biomedical applications, such as drug delivery, nucleic acid therapeutics, and imaging agents.

3. PAMAM dendrimers

Since the seminal report by Tomalia *et al.*,⁷ PAMAM dendrimers have been the most intensively studied of all dendrimers, and some are also commercially available. With numerous amide and tertiary amine functionalities in their interiors, and primary amine terminals on their surfaces, the very first PAMAM dendrimers developed by Tomalia *et al.* were originally envisioned to mimic proteins.⁷ These peptide-mimicking features endow PAMAM dendrimers with high water solubility and excellent biocompatibility, which explains their frequent use and their potential for various biomedical applications compared with other dendrimers.^{12, 34}

For example, we conceived and synthesized a structurally flexible PAMAM dendrimer bearing a triethanolamine (TEA) core³⁵ (**Figure 4**) to mimic the natural protein histone for DNA binding and DNA delivery.³⁶ The TEA constitutes an extended core offering ample space within the dendrimer to accommodate the branching units with structural flexibility. The positively charged terminal amine groups can interact electrostatically with the negatively charged nucleic acids, thereby protecting them from degradation and enabling their delivery to the desired destination.³⁶

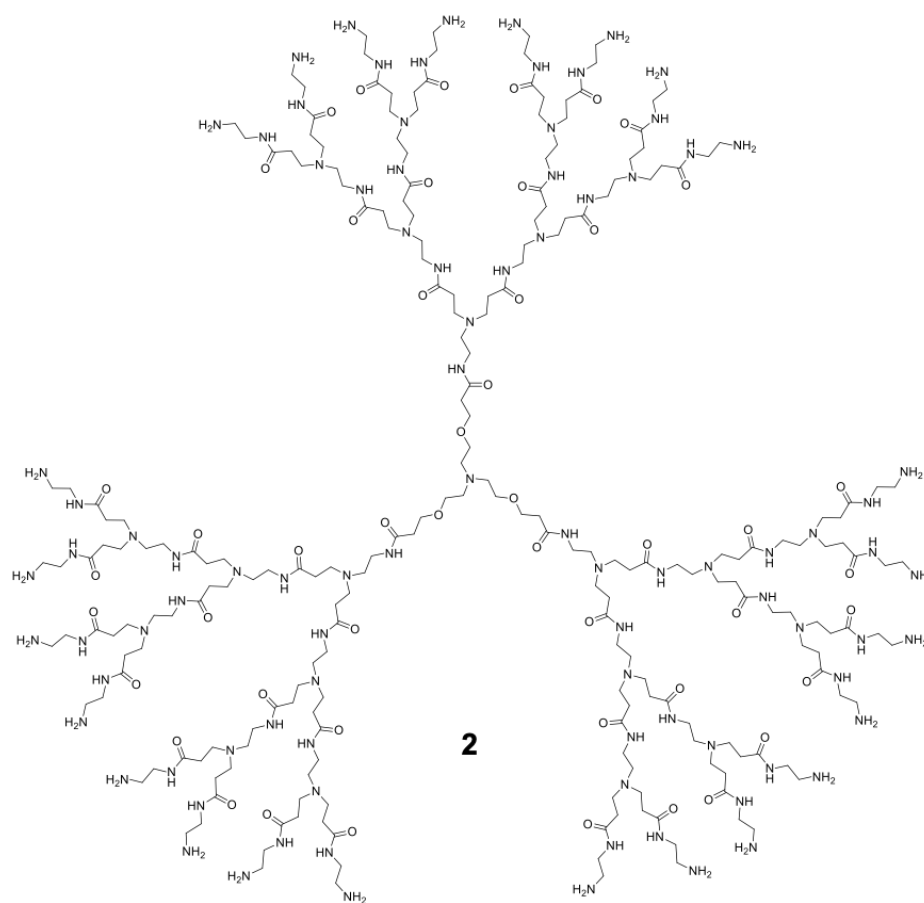


Figure 4: Chemical structure of poly(amidoamine) (PAMAM) dendrimer (**2**) bearing a triethanolamine (TEA) core developed by our group. Only the third-generation dendrimer is shown.³⁵ Adapted with permission from ref 35, Copyright 2005 Royal Society of Chemistry.

High-generation TEA-core dendrimers are particularly useful for delivering nucleic acid molecules such as DNA, small interfering RNA (siRNA), and small activation RNA (saRNA) for gene therapy in various disease models, both *in vitro* and *in vivo*.³⁷⁻⁴⁶ Our TEA-core fifth-generation dendrimer was scheduled to undergo clinical trials for the delivery of anticancer saRNA therapeutics to treat advanced liver cancer.⁴⁴ Unfortunately, because it fell short of the demands of good manufacturing practice (GMP), it was eventually substituted with a lipid vector formulation (Smarticles®),⁴⁷ the saRNA delivery performance of which is largely inferior to that of the TEA-core dendrimer. This definitely constitutes an unsuccessful example of clinical translation, although in general dendrimers have great potential for use in biomedical applications. However, their synthesis does present a significant challenge.¹⁴

4. Self-assembling supramolecular PAMAM dendrimers

We pioneered the self-assembly approach to constructing non-covalent supramolecular dendrimers to overcome the obstacles to implementing PAMAM dendrimers for biomedical applications.^{1-5, 18-27} Small amphiphilic dendrimers bearing hydrophobic alkyl chains and hydrophilic PAMAM dendrons can be readily synthesized in large quantities with high purity using either divergent or convergent methods or combination of both.^{1, 2, 5, 18-24} By virtue of their amphipathic nature, the hydrophobic interactions in their core regions, and the hydrogen bonds within their dendron shells, these small amphiphilic dendrimers can self-assemble into stable and robust non-covalent supramolecular dendrimers. It is easy to modify the length and nature of the hydrophobic chain and/or the generation of the hydrophilic PAMAM dendron, and to alter the different functionalities of the terminals. These changes provide incomparable structural flexibility and diversity for a wide range of potential biomedical applications. The following report is a brief summary of our studies in this direction. We present and discuss

the promising breakthroughs and challenges faced using representative examples of the delivery of nucleic acid therapeutics, anticancer drugs, and imaging agents.

Nucleic acid delivery

Nucleic acids have emerged as novel therapeutic agents for the treatment of various diseases, particularly since the discovery of RNA interference (RNAi).⁴⁸ RNAi involves the silencing of a targeted gene with siRNA, which sequence-specifically pairs with, and subsequently cleaves, the corresponding mRNA. The challenge facing siRNA therapeutics is delivery because siRNA is not stable and can be easily degraded. Furthermore, the negative charge and strong hydrophilicity of siRNA prevent it crossing cell membranes readily. Moreover, at high concentrations, naked siRNA can generate unwanted, and sometimes serious adverse effects. Consequently, suitable delivery systems that are able to prevent siRNA degradation, mask its negative charge, and ensure its safe and effective delivery to target cells for functional gene silencing are critical.⁴⁹

We have been actively engaged in developing dendrimer nanovectors for siRNA delivery. **Figure 5** illustrates the first amphiphilic dendrimer (**3**) and its derivatives (**4** and **5**), which were developed by our group to construct supramolecular dendrimers for siRNA delivery.^{1, 20-22} Each dendrimer bears a long hydrophobic alkyl chain of eighteen carbon atoms, and a small hydrophilic PAMAM dendron with terminals of primary amines, tertiary amines, and arginine residues (**Figure 5A**). They self-assemble into supramolecular dendrimers by virtue of their amphiphilicity, and form spherical nanomicelles to mimic covalent dendrimers. They can interact with the negatively charged siRNA via their positively charged terminals for siRNA binding and cellular uptake, followed by endosomal release via the “proton-sponge” effect for potent gene silencing (**Figure 5B**). **Figure 5C** shows the transmission electron microscope (TEM) images of the supramolecular dendrimer formed

with **3**,¹ which is similar in size to the high-generation TEA-core covalent PAMAM dendrimers. Most importantly, this supramolecular dendrimer can mimic TEA-core fifth-generation dendrimer **2** to deliver siRNA, with potent gene silencing at both the mRNA and protein levels (**Figures 5D** and **5E**) for effective anticancer activity in RNAi-based cancer treatment. The synthesis of **3** is much easier than that of TEA-core fifth-generation dendrimer **2** (**Figure 4**), and the creation of a supramolecular dendrimer comprising **3** is spontaneous in water.^{1,21} Our studies on dendrimer **3** constitute the exquisite demonstration of a supramolecular dendrimer created by self-assembling a small amphiphilic dendrimer. Such dendrimer is able to mimic covalently synthesized high-generation dendrimer in terms of size, shape, and siRNA delivery capability.

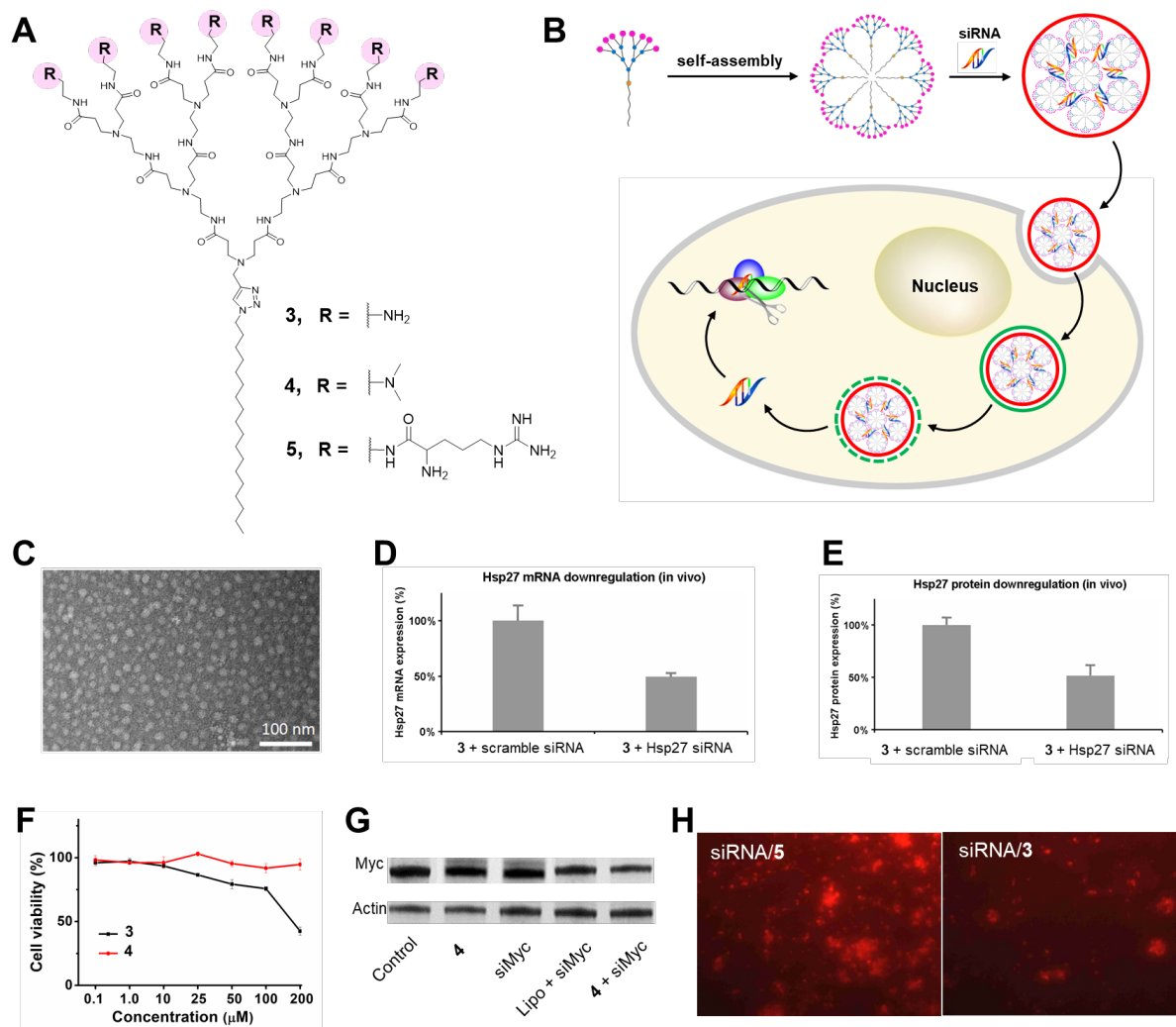


Figure 5: Amphiphilic dendrimers **3–5** and their self-assembly into supramolecular dendrimers for small interfering RNA (siRNA) delivery. (A) Chemical structures of **3–5** with primary amine, tertiary amine, and arginine residue terminals, respectively; (B) Cartoon illustration of the self-assembly of an amphiphilic dendrimer into a supramolecular dendrimer for siRNA binding and delivery, including cell uptake, endosome release, and subsequent gene silencing; (C) transmission electron microscope (TEM) images of the supramolecular dendrimer formed by **3**; gene silencing of Hsp27 at the mRNA level (D) and the protein level (E) in tumor-xenograft mice following treatment with siRNA delivered by a **3**-assembled supramolecular dendrimer; (F) toxicity profile of **4** in human embryonic kidney 293 (HEK293) cells compared with that of **3**; (G) reduced expression of the oncogene Myc at the protein level in patient-

derived cancer organoids following treatment with **4**, Myc siRNA, Myc siRNA delivered by the commercial vector Lipofectamine (Lipo), and by the supramolecular dendrimer formed with **4**; (H) cell uptake of siRNA delivered by the supramolecular dendrimer formed with **5** (left) was visibly higher than that delivered by the supramolecular dendrimer formed with **3** (right).^{1, 20-22} Reproduced and adapted with permission from ref 1, Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA; ref 20, Copyright 2020 Tsinghua University Press and Springer; ref 21, Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA; and ref 22, Copyright 2015 Royal Society of Chemistry.

It is worth noting that the supramolecular dendrimer formed with **4** is less toxic than that formed with **3** owing to the stronger tendency of the tertiary amine terminals towards ionization (**Figure 5F**);²⁰ furthermore, it effectively delivers siRNA to silence the oncogene Myc in patient-derived tumor organoids (**Figure 5G**), and has anticancer activity that exceeds that of the gold standard vector Lipofectamine 2000. Dendrimer **5** is able to mimic cell-penetrating peptides to promote cellular uptake,²² because the positively charged guanidinium terminals alongside the amine groups in the arginine residues are able to form divalent hydrogen bonds and electrostatic interactions with the negatively charged groups on the cell surface, thereby facilitating membrane penetration. Compared with non-arginine dendrimer **3**, the supramolecular dendrimer formed from **5** allows considerably enhanced cellular uptake of siRNA (**Figure 5H**), resulting in much more potent gene silencing. These studies demonstrate that simply by altering their terminal functionalities, it is possible to impart the specific activity and desired safety profile of the supramolecular dendrimers for nucleic acid delivery.

To investigate the impact of the amphiphilicity and hydrophobicity/hydrophilicity balance in self-assembled supramolecular dendrimers for siRNA delivery, we studied amphiphilic dendrimers **3a–e** derived from **3** with varying alkyl chain lengths, dendron **6**

without any chain, and dendrimer **7** with a hydrophilic oligo(ethyleneglycol) chain (**Figure 6**).^{1,21} Both **6** and **7** lack amphiphilicity, and are therefore unable to self-assemble into the supramolecular structure needed for siRNA delivery. This highlights the importance of the amphiphilicity of **3** and its ability to self-assemble into a robust supramolecular dendrimer structure, which enables it to mimic a covalent high-generation dendrimer with multivalent cooperativity for effective siRNA delivery.²¹ It is also important to note that dendrimers **3a–b** with short alkyl chains of C12 and C14 fail to form stable supramolecular dendrimers owing to insufficient hydrophobic interactions with the short alkyl chains, whereas the dendrimers with the corresponding hydrophobic chains of C16, C18, C20, and C22 generate stable supramolecular dendrimer nanomicelles with sizes increasing from 6.8 to 7.8 nm (**Figure 6B** and **Figure 5C**).¹ Taken together, these studies underline the impact of the alkyl chain length and the balance of amphiphilicity on the formation and size of stable supramolecular dendrimers.

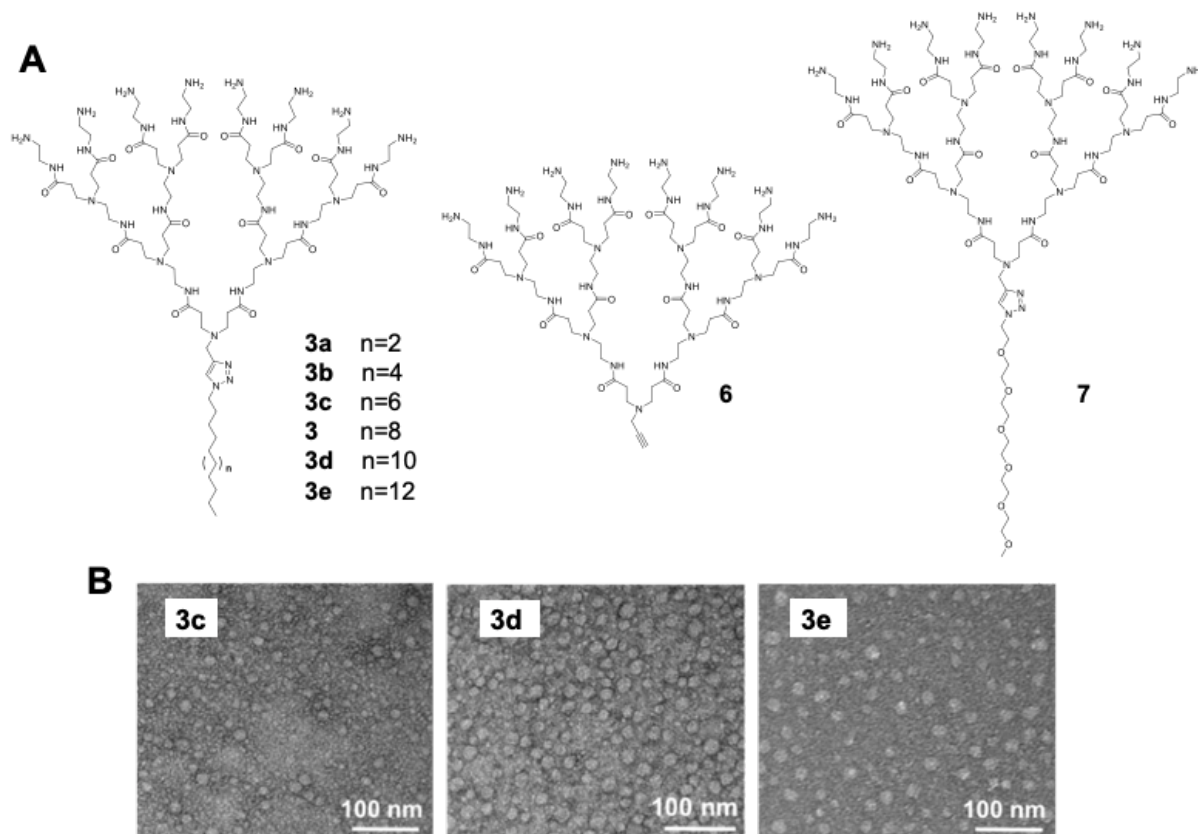


Figure 6: (A) Chemical structures of amphiphilic dendrimers **3a–e** derived from **3** with alkyl chains of differing lengths, dendron **6** without any chain, and dendrimer **7** bearing a hydrophilic oligo(ethyleneglycol) chain. (B) Transmission electron microscope (TEM) images of the supramolecular dendrimers formed by amphiphilic dendrimers **3c–e** with different chain lengths.^{1,21} Reproduced and adapted with permission from ref 1, Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA; and ref 21, Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA.

We further modified the hydrophobic part of **3** by appending two hydrophobic alkyl chains to synthesize dendrimer **8** (**Figure 7**).⁵ Remarkably, **8** can spontaneously self-assemble into vesicle-like dendrimersomes (**Figure 7B**) instead of the supramolecular dendrimers with micellar structures formed by **3**. Most interestingly, upon interaction with siRNA, the vesicle-like dendrimersomes formed by **8** are able to undergo concurrent structural rearrangement into small, spherical micelles (**Figure 7C**). This graceful vesicular-to-micellar structural transition allows **8** to maximally expose its positively charged terminal amine groups, ensuring stronger electrostatic interactions with the negatively charged siRNA. This enables the effective entrapment and condensing of siRNA into stable nanoparticles, thereby sheltering it from degradation before delivery to the desired destination. In addition, this dendrimer is able to capitalize on the delivery advantages of both lipid and dendrimer vectors. This allows it to effectively deliver siRNA to a variety of different cell types including primary and stem cells, as well as immune cells such as primary T cells (**Figure 7D**), natural killer cells (**Figure 7E**), and macrophages (**Figure 7F**), which are otherwise highly challenging for nucleic acid delivery.^{5, 24-26}

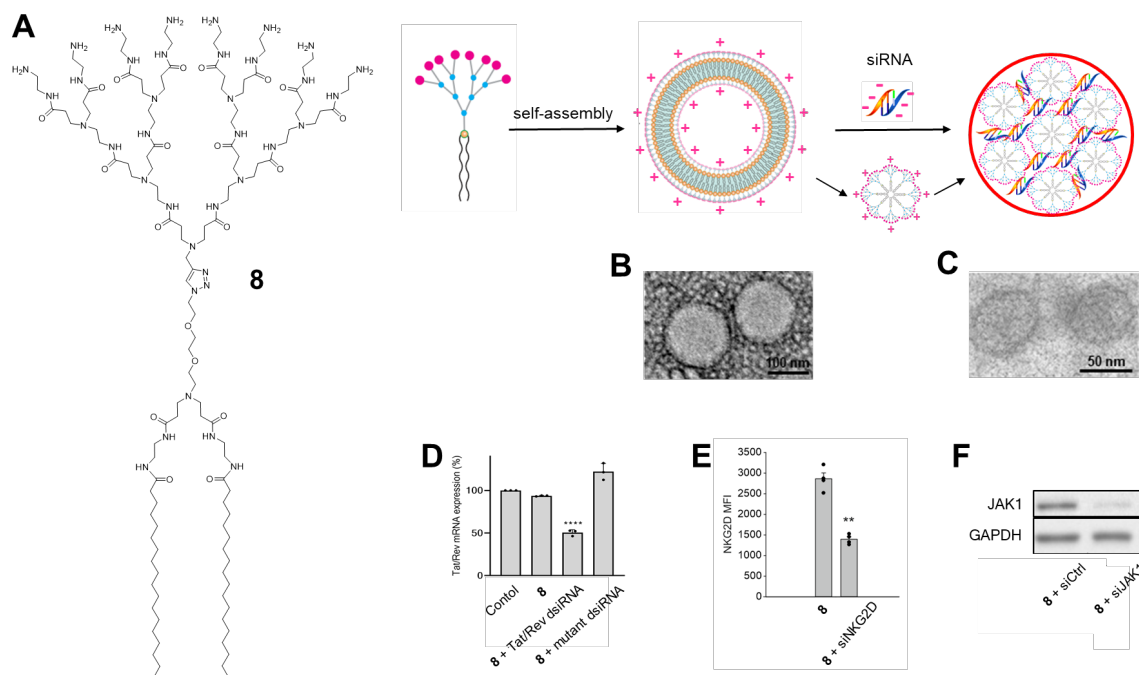


Figure 7: Amphiphilic dendrimer **8** and its self-assembling supramolecular structures for small interfering RNA (siRNA) delivery and gene silencing. (A) Chemical structure of **8** and cartoon illustration of the self-assembly of **8** into its supramolecular dendrimersome structure, before rearrangement into nanomicelles following siRNA binding for siRNA delivery. (B) Transmission electron microscope (TEM) images of the dendrimersome assembled from **8**. (C) TEM images of the siRNA/**8** complexes, in which the dendrimersomes formed from **8** have rearranged into supramolecular dendrimer nanomicelles following interaction with the negatively charged siRNA. (D) Tat/rev mRNA expression in T cells; (E) NKG2D expression in natural killer cells; and (F) JAK1 protein expression in macrophages following treatment with the corresponding siRNA molecules delivered by **8**, compared with the nontreatment control, the dendrimer alone, or scrambled siRNA delivered by **8**.^{5, 24} Reproduced and adapted with permission from ref 5, Copyright 2014 WILEY-VCH Verlag GmbH & Co. KGaA; and ref 24, Copyright 2020 Springer Nature.

To promote targeted delivery, we further established a supramolecular dendrimer delivery system based on **8** using a peptide harboring the dual-targeting warhead RGDK (**Figure 8**).⁴ RGDK is able to target tumor vasculature via the binding of the arginine–glycine–aspartic acid peptide (RGD) to the overexpressed integrin, while simultaneously enhancing cell penetration via RGDK binding to neuropilin-1 receptors on the cancer cell surfaces. In practice, the RGDK segment is incorporated at the terminal of the peptide E₁₆G₆RGDK. This peptide comprises a middle portion comprising oligo(glycine) G₆, which acts as the linker, and negatively charged oligo(glutamic acid) E₁₆ at the opposite terminal, which serves to promote the attachment to the positively charged siRNA/**8** complex via electrostatic interaction (**Figure 8A**). The resulting delivery system enables efficient and specific uptake by cancer cells owing to RGD/integrin and RGDK/neuropilin-1 receptor interaction (**Figure 8B**), giving rise to much more effective and potent gene silencing, and greatly improved anticancer activity (**Figure 8C**).⁴

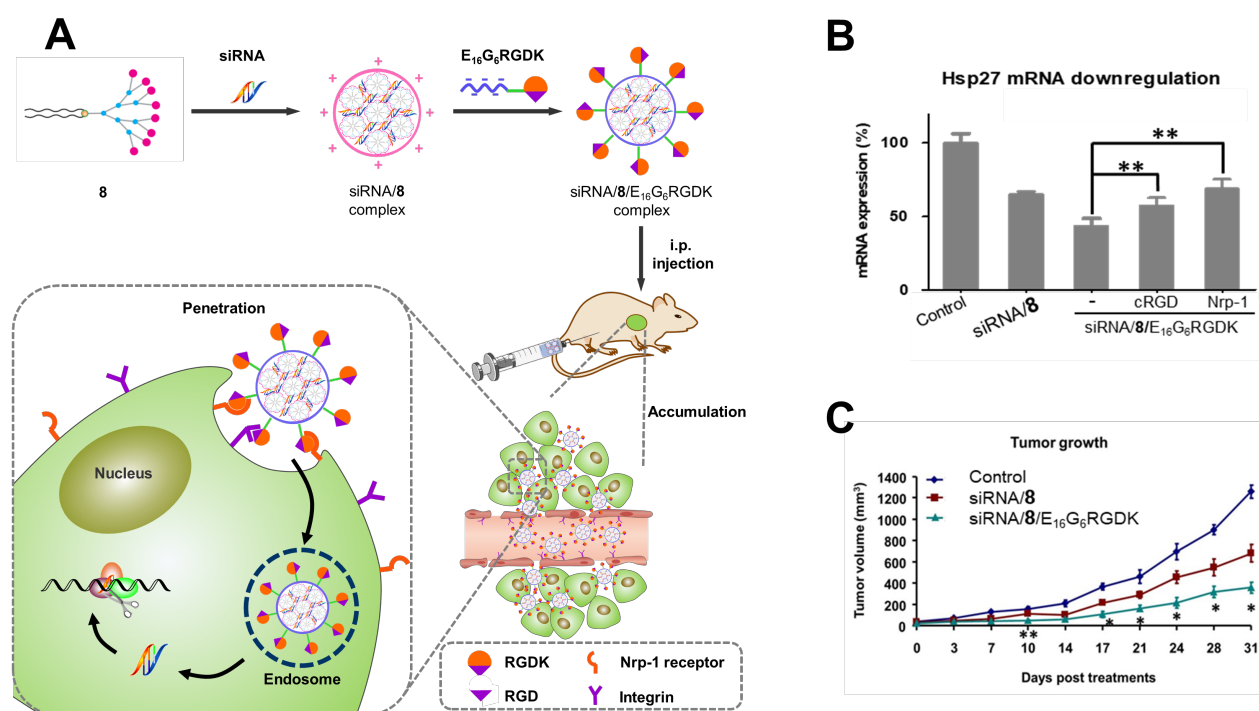


Figure 8: Targeted small interfering RNA (siRNA) delivery and gene silencing in cancer treatment using amphiphilic dendrimer **8**. (A) Cartoon illustration of the siRNA/**8** complex

decoration with the targeting peptide E₁₆G₆RGDK for targeted delivery. (B) Enhanced downregulation of the target gene Hsp27 at the mRNA level by siRNA/**8**/E₁₆G₆RGDK is reduced in the presence of either cRGD or anti-Nrp-1-receptor antibody. (C) Tumor growth in xenograft mice treated with phosphate-buffered saline (PBS), siRNA/**8**, and siRNA/**8**/E₁₆G₆RGDK.⁴ Reproduced and adapted with permission from ref 4, Copyright 2018 American Chemical Society.

The studies described above demonstrate the extraordinary ability of dendrimer **8** to undergo dynamic self-assembly and rearrangement in the presence of siRNA and the targeting peptide to form adaptive supramolecular assemblies. These unique and highly modular features, together with its ease of synthesis, make **8** a promising candidate for the functional delivery of siRNA and other nucleic acid therapeutics.^{4, 5, 24-26} Collectively, these studies highlight the potential of the self-assembling approach to the construction of modular and functional supramolecular dendrimers for siRNA delivery and gene silencing in biomedical applications.

Drug delivery

The aim of drug delivery is to maximize drug bioavailability to achieve optimal therapeutic efficacy while minimizing adverse effects.⁵⁰ Unlike covalently constructed dendrimers, self-assembled supramolecular dendrimers have a large void within their interior cores, which could potentially encapsulate drug molecules with high loading content (**Figure 2**). Therefore, we developed a supramolecular dendrimer system for drug delivery using **8** (**Figure 9A**), and determined its capacity to deliver drug with anticancer activity.³ Remarkably, the ample void within the supramolecular dendrimer formed by **8** is able to

effectively and stably encapsulate the anticancer drug doxorubicin (DOX) with up to 40% drug-loading efficiency (**Figure 9B**). The resulting DOX-loaded supramolecular dendrimer accumulates within the tumor lesion owing to the enhanced permeability and retention (EPR) effect (**Figure 9C**), and enters the cancer cells via macropinocytosis, allowing greatly enhanced cellular uptake while bypassing drug efflux. The DOX-loaded supramolecular dendrimer thus significantly increases the therapeutic potency of DOX (**Figure 9D**), overcomes doxorubicin resistance in various cancer models including drug-resistant cancers, and largely abolishes the toxicity associated with free DOX (**Figure 9D-E**). These results are extremely encouraging for the future of novel and effective supramolecular dendrimers for drug delivery in cancer therapy.

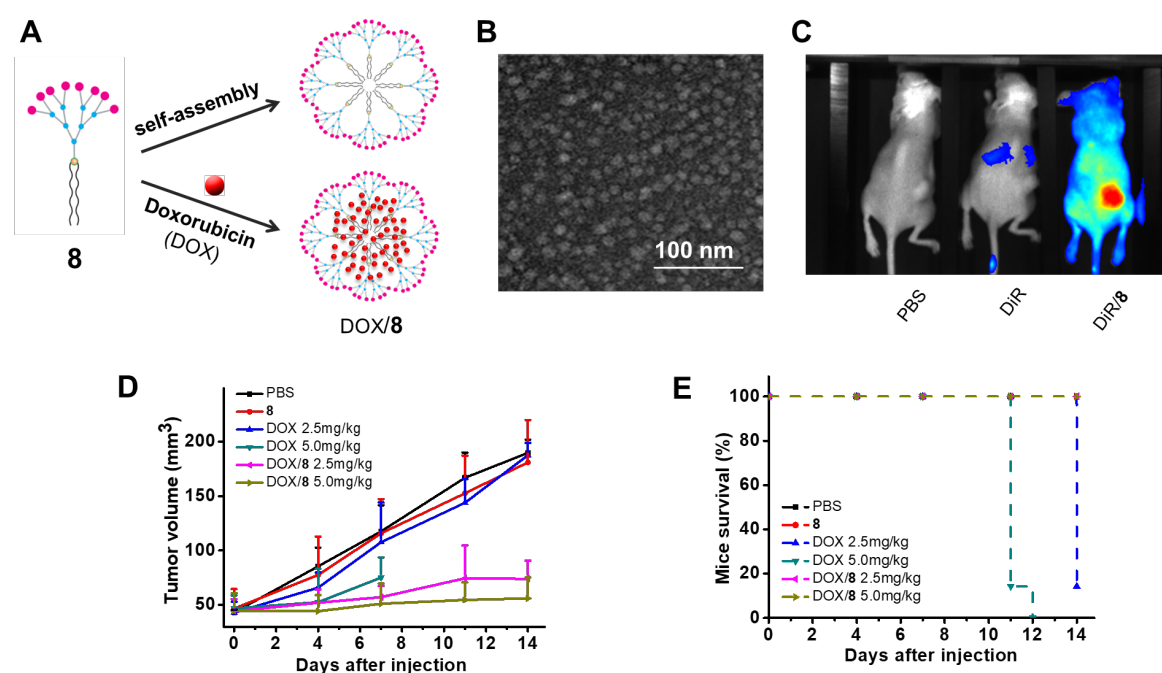


Figure 9: Self-assembling supramolecular dendrimer for drug encapsulation and drug delivery in cancer therapy. (A) Cartoon representation of the self-assembly of amphiphilic dendrimer **8** into supramolecular dendrimer nanomicelles, and the encapsulation of the anticancer drug doxorubicin (DOX) within its hydrophobic interior. (B) Transmission electron microscope (TEM) images of the DOX-loaded supramolecular dendrimers formed

from **8**. (C) Fluorescent imaging revealing the accumulation of the supramolecular dendrimers in tumors via the enhanced permeability and retention (EPR) effect. (D) Significant inhibition of tumor growth in mice, and (E) mouse survival rate following treatment with the DOX/**8** supramolecular dendrimer nanomicelles, compared with the control treatments of phosphate-buffered saline (PBS), dendrimer alone, and DOX alone.³ Reproduced and adapted with permission from ref 3, Copyright 2015 National Academy of Sciences.

Amphiphilic dendrimers can also be chemically conjugated with multiple copies of a bioactive molecule at the dendrimer terminals to construct self-assembling supramolecular dendrimers for drug delivery. Therefore, we created amphiphilic dendrimer **9** (**Figure 10**) by appending bioactive nucleoside derivatives to the dendrimer terminals via biodegradable ester bonds with a view to achieving responsive drug release following enzymic action.²⁷ Notably, the enzymatic hydrolysis of dendrimer conjugate **9** is considerably slower than that of the lower generation dendrimer **10** (**Figure 10**). This can be ascribed to enhanced steric hindrance with increasing dendron generation, which leads to reduced hydrolysis. This apparently negative dendritic effect could nevertheless be exploited in the tailored design of dendrimers for generation-dependent controlled drug release.

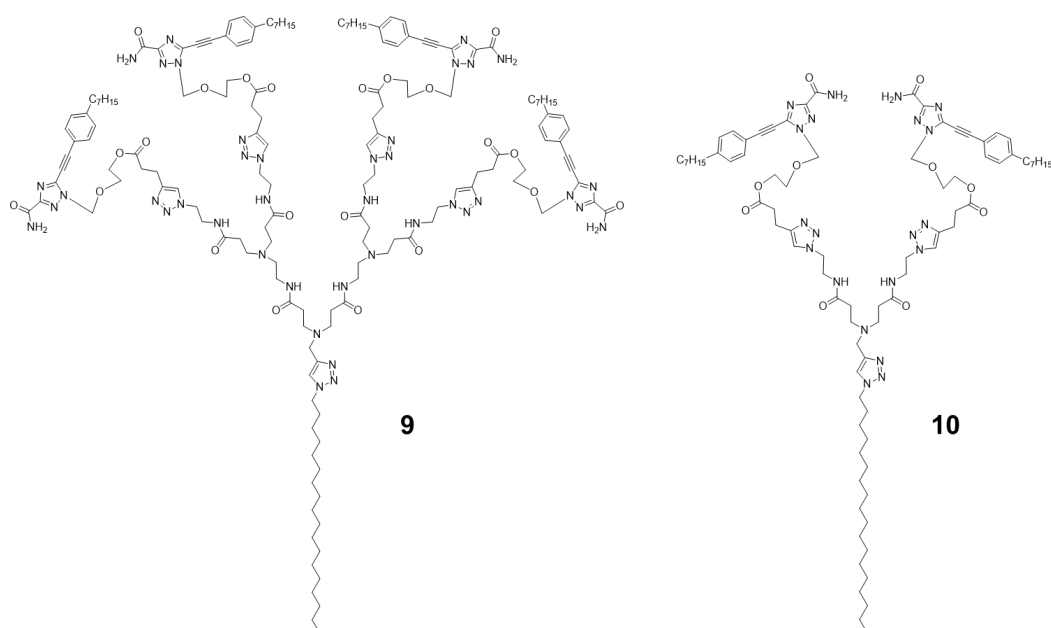


Figure 10: Amphiphilic dendrimers **9** and **10** bearing covalently conjugated bioactive nucleosides at the terminals, for drug delivery in response to enzyme hydrolysis.²⁷ Adapted with permission from ref 27, Copyright 2018 Royal Society of Chemistry.

Bioimaging

Biomedical imaging has revolutionized disease management by providing precise information for diagnosis and by supporting decision-making in therapy.⁵¹ Nanotechnology is expected to further improve the sensitivity and specificity of tumor imaging by allowing the accommodation of a large number of imaging agents within the nanoprobe, while exploiting the EPR effect for passive tumor targeting.^{52, 53} We have therefore developed tumor imaging nanoprobes using supramolecular dendrimers formed by the self-assembly of amphiphilic dendrimers.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have the highest sensitivity among the commonly used non-invasive imaging modalities, and can be used to quantitatively assess functional information for disease

diagnosis.⁵¹ Whereas PET offers higher sensitivity and resolution, SPECT is more readily accessible and less expensive for routine use. **Figure 11** illustrates amphiphilic dendrimers **11–12**, which we conceived and developed to construct supramolecular dendrimer nanoprobe for SPECT and PET imaging.^{2, 18, 19} The first ever supramolecular dendrimer nanoprobe for SPECT imaging was formed from **11**.¹⁸ This dendrimer is composed of a hydrophobic C18 alkyl chain and a hydrophilic PAMAM dendron with terminals bearing SPECT-reporting units comprising [¹¹¹In]In³⁺ chelated to a macrocyclic 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) ring. The DOTA cage is the “gold standard” chelator for many radionuclides including ¹¹¹In³⁺.⁵⁴ Because the DOTA macrocycle is large, and to avoid eventual synthetic trouble and vulnerability stemming from possible steric crowding of the dendrimer terminals, we synthesized dendrimer **11**, which has only four DOTA cages at its terminals. As expected, **11** self-assembled into small and uniform supramolecular dendrimer nanomicelles (**Figure 11B**, left panel), which accumulated effectively within the tumor via the EPR effect for SPECT imaging (**Figure 11C**, left panel). The representative SPECT image of a tumor-xenograft mouse obtained using the nanoprobe formed by **11** is presented in **Figure 11C** on the left, where the orange oval indicates the tumor position. However, this image also reveals considerable uptake in other organs such as the liver.¹⁸

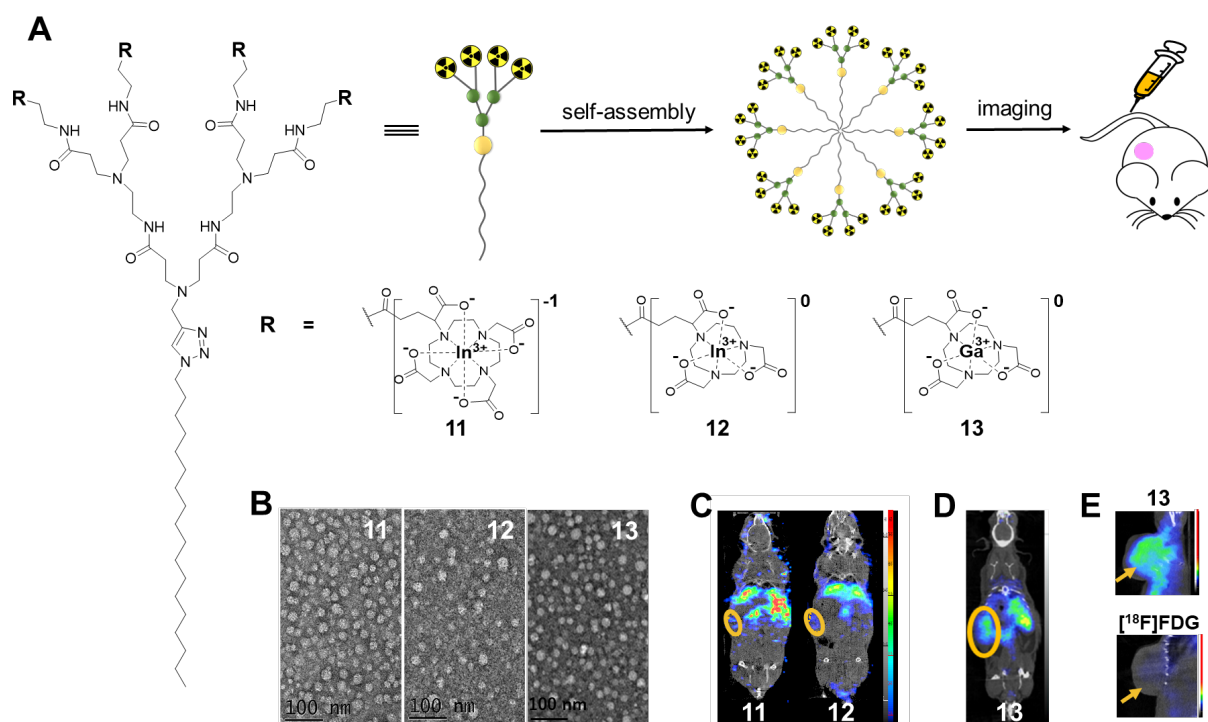


Figure 11: Amphiphilic dendrimers **11**, **12**, and **13** bearing the radionuclides $[^{111}\text{In}]\text{In}^{3+}$ or $[^{68}\text{Ga}]\text{Ga}^{3+}$ chelated to the macrocyclic cages 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) or 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) at the dendrimer terminals, for self-assembly into supramolecular dendrimer nanoprobes for tumor imaging based on single photon emission computed tomography (SPECT) and positron emission tomography (PET). (A) The chemical structures of **11**, **12**, and **13**, and a cartoon illustrating their self-assembly into supramolecular dendrimers for tumor imaging. (B) Transmission electron microscope (TEM) images of the supramolecular dendrimer probes formed from **11** (left), **12** (middle), and **13** (right). (C) Representative μ SPECT/CT maximum intensity projection images of tumor-xenograft mice 24 h after intravenous injection of the supramolecular dendrimer probes formed from **11** (left) and **12** (right). The orange ovals indicate the tumor positions. (D) Representative PET image of an orthotopic SOJ6-xenograft mouse 2 h after the intravenous injection of **13**. The orange ovals indicate the tumor positions. (E) Tumor uptake of **13** in an SOJ6-xenograft mouse (upper panel) compared with that of $[^{18}\text{F}]\text{FDG}$ (lower panel), the clinical gold reference for PET imaging in oncology. The orange

arrows indicate the tumor positions.^{2, 18, 19} Reproduced and adapted with permission from ref 2, National Academy of Sciences; ref 18, Copyright 2020 Wiley-VCH GmbH, and ref 19, Copyright 2020 Royal Society of Chemistry.

To reduce liver uptake, we developed dendrimer **12**, which bears the smaller macrocyclic 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) scaffold instead of the DOTA cage as the chelator for [¹¹¹In]In³⁺ (**Figure 11A**).¹⁹ The NOTA ring can form a neutral complex when chelating the trivalent metal ion In³⁺ (**12** in **Figure 11**), and it also generates more stable complexes with small metal ions such as In³⁺, Ga³⁺, and Cu²⁺ as it is smaller than the DOTA cage. Remarkably, the supramolecular dendrimer formed by **12** is similar in size to that formed by **11** (**Figure 11B**, middle panel), but gives rise to a highly favorable biodistribution profile with drastically reduced uptake by the liver and other organs, permitting a significantly improved tumor imaging contrast (**Figure 11C**, right panel). Thus, this study provided interesting perspectives for improving the biodistribution of supramolecular dendrimers for bioimaging.

Dendrimer **13** was established to construct supramolecular dendrimer nanoprobe for PET imaging (**Figure 11**).² We opted for [⁶⁸Ga]Ga³⁺ as the radioactive reporter for PET, because it can be conveniently generated for on-site and on-demand production. It is worth noting that the NOTA cage is an excellent chelator for [⁶⁸Ga]Ga³⁺, and has superior thermodynamic stability and kinetic inertness compared with the DOTA ring, owing to the perfect match in terms of size, geometry, and denticity between the NOTA cage and Ga³⁺.⁵⁴ Dendrimer **13** readily self-assembles into a supramolecular dendrimer measuring 15 nm in diameter (**Figure 11B**, right panel), which, via the EPR effect, accumulates in the tumor, as highlighted by the orange oval in the representative PET image in **Figure 11D**.² The nanoprobe formed from **13** also has a very favorable biodistribution. Most importantly,

the combination of dendrimer multivalence and EPR-mediated passive tumor targeting with supramolecular dendrimers permits superior imaging sensitivity and specificity compared with the clinical gold reference [^{18}F]FDG (2-fluorodeoxyglucose) for PET imaging. It is worth noting that this supramolecular dendrimer nanoprobe enables the detection of tumors (**Figure 11E**, upper panel) that are undetectable using [^{18}F]FDG (**Figure 11E**, lower panel).²

We also built imaging probes by encapsulating the hydrophobic imaging agents within the interior of the supramolecular dendrimers (**Figure 12A**), in the same manner as for the delivery of the anticancer drug DOX.³ For example, the fluorescent dye DiR has excellent near-infrared fluorescent (NIRF) properties for bioimaging but is insoluble in water, which largely limits its applications in biological studies. Upon encapsulation within the interior of the supramolecular dendrimer formed by **8**, the resulting DiR/**8** nanoprobe is readily soluble in water, while retaining the NIRF properties of DiR (**Figure 12B-C**). Furthermore, the DiR/**8** nanoprobe is spherical in form with a size of 15 nm (**Figure 12D**), and accumulates efficiently within tumors via the EPR effect for effective NIRF imaging (**Figure 12E**). Taken together, our studies demonstrate the promising advantages of self-assembling supramolecular dendrimers, which can be easily constructed, modulated, and adapted for various bioimaging modalities.

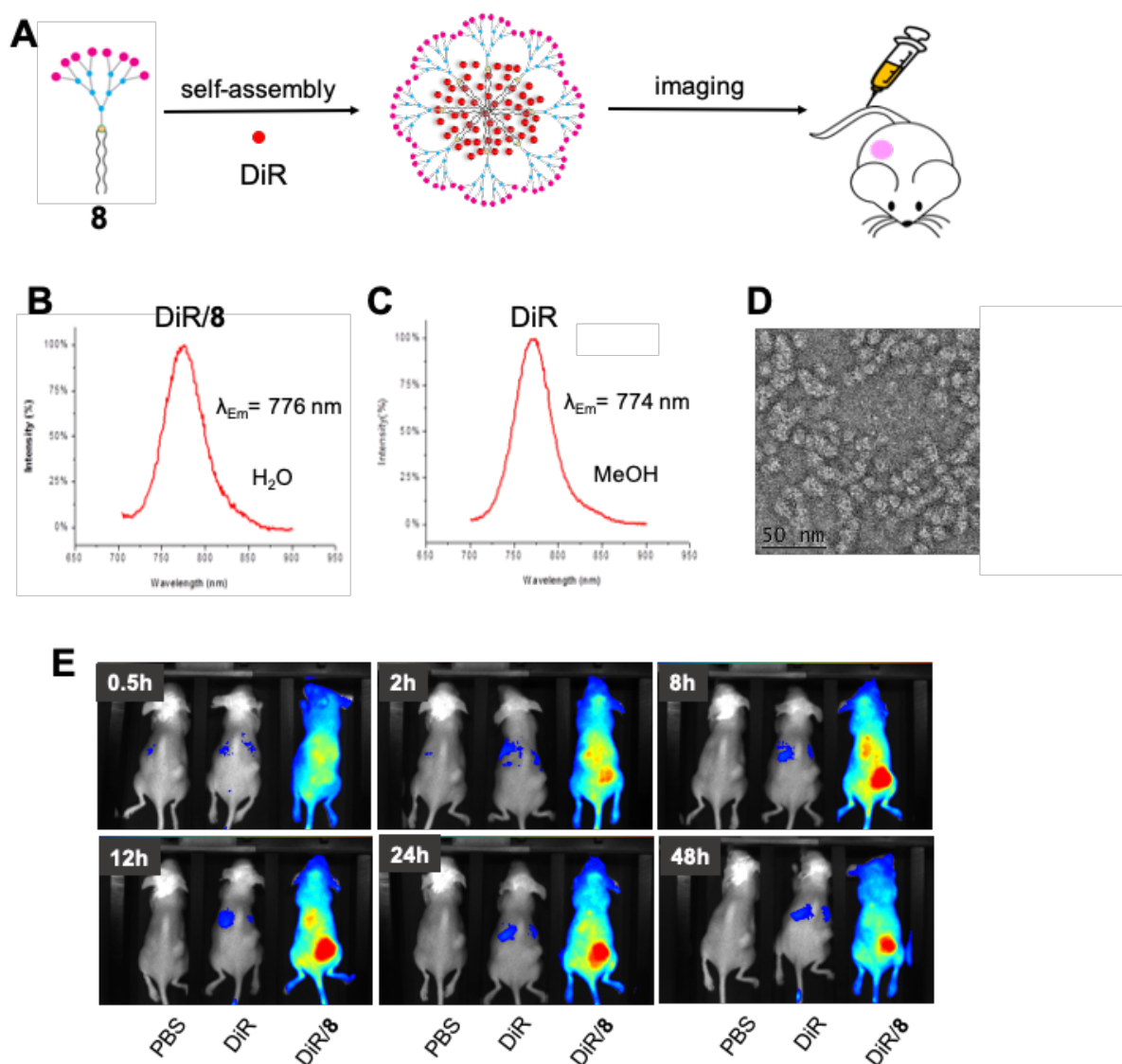


Figure 12: Encapsulation of the imaging agent within the interior of the self-assembling supramolecular dendrimer for bioimaging. (A) Cartoon illustration of the self-assembly of amphiphilic dendrimer **8** into the supramolecular dendrimer to encapsulate the fluorescent dye DiR for near-infrared fluorescent imaging of the tumor. Fluorescent emission spectra of DiR when (B) encapsulated within the supramolecular dendrimer formed by **8** in water, and (C) dissolved in organic solvent MeOH. (D) Transmission electron microscope (TEM) image of the DiR/**8** nanoprobe. (E) Representative fluorescent images of the tumor-xenograft mice 0.5, 2, 8, 12, 24, and 48 h after intravenous injection of the DiR/**8** nanoprobe in comparison with

that of DiR and PBS treatment.³ Reproduced with permission from ref 3, Copyright 2015 National Academy of Sciences.

5. Concluding remarks

This short account was written with the intention of providing out-of-the-box thinking about the construction of supramolecular dendrimers for biomedical applications. Completely different from conventional covalent synthesis, our proposed non-covalent synthesis by the self-assembly of small amphiphilic dendrimer building units is new in concept and easy to implement in practice, as well as adaptable and modular with regard to the various properties and functions required for particular applications. We have successfully implemented this approach to create non-covalent dendrimers that are able to mimic covalently constructed dendrimers for the delivery of drugs, nucleic acid therapeutics, and imaging agents, using PAMAM dendrimers as examples. PAMAM dendrimers are particularly appealing for biological applications by virtue of their biocompatibility, which arises from their peptide-mimicking amide backbones and numerous amine functionalities. The various supramolecular PAMAM dendrimers presented in this account have great potential for biomedical applications such as diagnosis, cancer therapy, gene therapy, and immune therapy. The self-assembly approach to dendrimer synthesis can also be easily applied to other dendrimers in general. It therefore constitutes a new and possibly key conceptual breakthrough, and an outside-the-box perspective for the biomedical application of dendrimers.

The translation of supramolecular dendrimers into real clinical interventions can be both promising and challenging. Despite amphiphilic dendrimers are much easier to synthesize than conventional PAMAM dendrimers, the reproducible scale-up of supramolecular dendrimers can be demanding, as with all other nanosystems for biomedical applications.⁵³ The stability of supramolecular dendrimers is also a key concern, because the self-assembly

process is driven by weak interactions; interactions with biomolecules in the body, or dilution in the blood may cause the disintegration of supramolecular dendrimers, leading to the premature release of the encapsulated cargo.⁵⁰ Moreover, as with any foreign particles that enter the body, supramolecular dendrimers may be recognized and eliminated by the reticuloendothelial system, resulting in limited efficacy.^{52, 55} All these obstacles must be circumvented for real clinical implementation. Fortunately, the experience and knowledge gained from the development of lipid- and supramolecular chemistry-based nanomedicines^{50, 55} will certainly benefit and accelerate the translation of supramolecular dendrimer nanosystems. We are actively pursuing this direction of research.

Biographies

Dr. Ling PENG is a research director at French National Research Center (CNRS), and working actively at the interface of chemistry and biology in conception, synthesis and evaluation of dendrimer nanosystems for biomedical applications as well as in chemical probes for understanding biological events and drug discovery.

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Abbreviations

DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DOX = doxorubicin; EPR = enhanced permeability and retention; NIRF = near-infrared fluorescent; NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid); PAMAM = poly(amidoamine); PBS = phosphate-buffered saline; PET = positron emission tomography; RNAi = RNA interference; saRNA = small activation RNA; siRNA = small interfering RNA; SPECT = single photon emission computed tomography; TEA = triethanolamine; TEM = transmission electron microscope

References

1. 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.
2. Garrigue, P.; Tang, J.; Ding, L.; Bouhleb, A.; Tintaru, A.; Laurini, E.; Huang, Y.; Lyu, Z.; Zhang, M.; Fernandez, S.; Balasse, L.; Lan, W.; Mas, E.; Marson, D.; Weng, Y.; Liu, X.; Giorgio, S.; Iovanna, J.; Pricl, S.; Guillet, B.; Peng, L., Self-assembling supramolecular dendrimer nanosystem for PET imaging of tumors. *Proc. Natl. Acad. Sci. USA* **2018**, *115* (45), 11454-11459.
3. Wei, T.; Chen, C.; Liu, J.; Liu, C.; Posocco, P.; Liu, X.; Cheng, Q.; Huo, S.; Liang, Z.; Fermeglia, M.; Pricl, S.; Liang, X.-J.; Rocchi, P.; Peng, L., Anticancer drug nanomicelles formed by self-assembling amphiphilic dendrimer to combat cancer drug resistance. *Proc. Natl. Acad. Sci. USA* **2015**, *112* (10), 2978-2983.
4. 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.
5. 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, X.; 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.* **2014**, *53* (44), 11822-11827.
 6. Buhleier, E.; Wehner, W.; Vögtle, F., "Cascade" and "nonskid-chain-like" syntheses of molecular cavity topologies. *Synthesis-Stuttgart* **1978**, *2*, 155-158.
 7. Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P., A new class of polymers: starburst-dendritic macromolecules. *Polym. J.* **1985**, *17* (1), 117-132.
 8. Tomalia, D. A.; Christensen, J. B.; Boas, U., *Dendrimers, dendrons, and dendritic polymers: discovery, applications, and the future*. Cambridge University Press: 2012.
 9. Ding, L.; Lyu, Z.; Dhumal, D.; Kao, C.-L.; Bernard, M.; Peng, L., Dendrimer-based magnetic resonance imaging agents for brain cancer. *Sci. China Mater.* **2018**, *61* (11), 1420-1443.
 10. Tomalia, D. A.; Fréchet, J. M., Discovery of dendrimers and dendritic polymers: a brief historical perspective. *J. Polym. Sci. Pol. Chem.* **2002**, *40* (16), 2719-2728.
 11. Lee, C. C.; MacKay, J. A.; Fréchet, J. M.; Szoka, F. C., Designing dendrimers for biological applications. *Nat. Biotech.* **2005**, *23* (12), 1517-1526.
 12. Kannan, R. M.; Nance, E.; Kannan, S.; Tomalia, D. A., Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. *J. Intern. Med.* **2014**, *276* (6), 579-617.
 13. Mintzer, M. A.; Grinstaff, M. W., Biomedical applications of dendrimers: a tutorial. *Chem. Soc. Rev.* **2011**, *40* (1), 173-190.
 14. Svenson, S., The dendrimer paradox – high medical expectations but poor clinical translation. *Chem. Soc. Rev.* **2015**, *44* (12), 4131-4144.
 15. Thomas, T. P.; Huang, B.; Choi, S. K.; Silpe, J. E.; Kotlyar, A.; Desai, A. M.; Zong, H.; Gam, J.; Joice, M.; Baker, J. R., Polyvalent dendrimer-methotrexate as a folate receptor-targeted cancer therapeutic. *Mol. Pharm.* **2012**, *9* (9), 2669-2676.
 16. <https://www.starpharma.com/>.
 17. Lyu, Z.; Ding, L.; Huang, A. Y. T.; Kao, C. L.; Peng, L., Poly(amidoamine) dendrimers: covalent and supramolecular synthesis. *Mater. Today Chem.* **2019**, *13*, 34-48.
 18. Ding, L.; Lyu, Z.; Tintaru, A.; Laurini, E.; Marson, D.; Louis, B.; Bouhleb, A.; Balasse, L.; Fernandez, S.; Garrigue, P.; Mas, E.; Giorgio, S.; Pricl, S.; Guillet, B.; Peng, L., A self-assembling amphiphilic dendrimer nanotracer for SPECT imaging. *Chem. Commun.* **2020**, *56* (2), 301-304.
 19. Ding, L.; Lyu, Z.; Louis, B.; Tintaru, A.; Laurini, E.; Marson, D.; Zhang, M.; Shao, W.; Jiang, Y.; Bouhleb, A.; Balasse, L.; Garrigue, P.; Mas, E.; Giorgio, S.; Iovanna, J.; Huang, Y.; Pricl, S.; Guillet, B.; Peng, L., Surface charge of supramolecular nanosystems for in vivo biodistribution: a microSPECT/CT imaging study. *Small* **2020**, doi:10.1002/sml.202003290.
 20. 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 toxicity profile. *Nano Res.* **2020**, doi: 10.1007/s12274-020-3216-8.
 21. 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.* **2012**, *51* (34),

8478-8484.

22. 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.
23. 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.
24. Chen, J.; Aleksandra, E.-M.; Garofalo, S.; Dey, A.; Tang, J.; Jiang, Y.; Clément, F.; Marche, P.; Liu, X.; Kaminska, B.; Santoni, A.; Limatola, C.; Rossi, J.; Zhou, J.; Peng, L., Synthesis and use of an amphiphilic dendrimer for siRNA delivery into primary immune cells. *Nat. Protoc.* **2020**, doi: 10.1038/s41596-020-00418-9.
25. 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.
26. 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* **2019**, *14* (18), 2441-2459.
27. Zhou, Z.; Cong, M.; Li, M.; Tintaru, A.; Li, J.; Yao, J.; Xia, Y.; Peng, L., Negative dendritic effect on enzymatic hydrolysis of dendrimer conjugates. *Chem. Commun.* **2018**, *54* (47), 5956-5959.
28. Percec, V.; Wilson, D. A.; Leowanawat, P.; Wilson, C. J.; Hughes, A. D.; Kaucher, M. S.; Hammer, D. A.; Levine, D. H.; Kim, A. J.; Bates, F. S.; Davis, K. P.; Lodge, T. P.; Klein, M. L.; DeVane, R. H.; Aqad, E.; Rosen, B. M.; Argintaru, A. O.; Sienkowska, M. J.; Rissanen, K.; Nummelin, S.; Ropponen, J., Self-assembly of janus dendrimers into uniform dendrimersomes and other complex architectures. *Science* **2010**, *328* (5981), 1009-1014.
29. Sherman, S. E.; Xiao, Q.; Percec, V., Mimicking complex biological membranes and their programmable glycan ligands with dendrimersomes and glycodendrimersomes. *Chem. Rev.* **2017**, *117* (9), 6538-6631.
30. Walter, M. V.; Malkoch, M., Simplifying the synthesis of dendrimers: accelerated approaches. *Chem. Soc. Rev.* **2012**, *41* (13), 4593-4609.
31. Lehn, J.-M., Toward self-organization and complex matter. *Science* **2002**, *295* (5564), 2400-2403.
32. Webber, M. J.; Appel, E. A.; Meijer, E. W.; Langer, R., Supramolecular biomaterials. *Nat. Mater.* **2016**, *15* (1), 13-26.
33. Cao, Y.; Liu, X.; Peng, L., Molecular engineering of dendrimer nanovectors for siRNA delivery and gene silencing. *Front. Chem. Sci. Eng.* **2017**, 663-675.
34. Lyu, Z.; Ding, L.; Dhumal, D.; Ya-Ting Huang, A.; Kao, C.-L.; Peng, L., CHAPTER 4 Poly(amidoamine) (PAMAM) dendrimers: synthesis and biological applications. In *Dendrimer Chemistry: Synthetic Approaches Towards Complex Architectures*, The Royal Society of Chemistry: 2020; pp 85-113.
35. Wu, J.; Zhou, J.; Qu, F.; Bao, P.; Zhang, Y.; Peng, L., Polycationic dendrimers interact with RNA molecules: polyamine dendrimers inhibit the catalytic activity of *Candida* ribozymes. *Chem. Commun.* **2005**, (3), 313-315.
36. Liu, X.; Wu, J.; Yammine, M.; Zhou, J.; Posocco, P.; Viel, S.; Liu, C.; Ziarelli, F.; Fermeiglia, M.; Pricl, S.; Victorero, G.; Nguyen, C.; Erbacher, P.; Behr, J.-P.; Peng, L., Structurally flexible triethanolamine core PAMAM dendrimers are effective nanovectors for DNA transfection in vitro and in vivo to the mouse thymus. *Bioconjugate Chem.* **2011**, *22* (12),

2461-2473.

37. Zhou, J.; Wu, J.; Hafdi, N.; Behr, J.-P.; Erbacher, P.; Peng, L., PAMAM dendrimers for efficient siRNA delivery and potent gene silencing. *Chem. Commun.* **2006**, (22), 2362-2364.
38. Liu, X.-x.; Rocchi, P.; Qu, F.-q.; Zheng, S.-q.; Liang, Z.-c.; Gleave, M.; Iovanna, J.; Peng, L., Pamam dendrimers mediate siRNA delivery to target Hsp27 and produce potent antiproliferative effects on prostate cancer cells. *ChemMedChem* **2009**, 4 (8), 1302-1310.
39. Zhou, J.; Neff, C. P.; Liu, X.; Zhang, J.; Li, H.; Smith, D. D.; Swiderski, P.; Aboellail, T.; Huang, Y.; Du, Q.; Liang, Z.; Peng, L.; Akkina, R.; Rossi, J. J., Systemic administration of combinatorial dsRNAs via nanoparticles efficiently suppresses HIV-1 infection in humanized mice. *Mol. Ther.* **2011**, 19 (12), 2228-2238.
40. Liu, X.; Liu, C.; Laurini, E.; Posocco, P.; Pricl, S.; Qu, F.; Rocchi, P.; Peng, L., Efficient delivery of sticky siRNA and potent gene silencing in a prostate cancer model using a generation 5 triethanolamine-core PAMAM dendrimer. *Mol. Pharm.* **2012**, 9 (3), 470-481.
41. Posocco, P.; Liu, X.; Laurini, E.; Marson, D.; Chen, C.; Liu, C.; Fermeglia, M.; Rocchi, P.; Pricl, S.; Peng, L., Impact of siRNA overhangs for dendrimer-mediated siRNA delivery and gene silencing. *Mol. Pharm.* **2013**, 10 (8), 3262-3273.
42. Kala, S.; Mak, A. S. C.; Liu, X.; Posocco, P.; Pricl, S.; Peng, L.; Wong, A. S. T., Combination of dendrimer-nanovector-mediated small interfering RNA delivery to target Akt with the clinical anticancer drug paclitaxel for effective and potent anticancer activity in treating ovarian cancer. *J. Med. Chem.* **2014**, 57 (6), 2634-2642.
43. Liu, X.; Liu, C.; Chen, C.; Bentobji, M.; Cheillan, F. A.; Piana, J. T.; Qu, F.; Rocchi, P.; Peng, L., Targeted delivery of Dicer-substrate siRNAs using a dual targeting peptide decorated dendrimer delivery system. *Nanomed.: Nanotechnol., Biol. Med.* **2014**, 10 (8), 1627-1636.
44. Reebye, V.; Sætrom, P.; Mintz, P. J.; Huang, K.-W.; Swiderski, P.; Peng, L.; Liu, C.; Liu, X.; Lindkær-Jensen, S.; Zacharoulis, D.; Kostomitsopoulos, N.; Kasahara, N.; Nicholls, J. P.; Jiao, L. R.; Pai, M.; Spalding, D. R.; Mizandari, M.; Chikovani, T.; Emara, M. M.; Haoudi, A.; Tomalia, D. A.; Rossi, J. J.; Habib, N. A., Novel RNA oligonucleotide improves liver function and inhibits liver carcinogenesis in vivo. *Hepatology* **2014**, 59 (1), 216-227.
45. Cui, Q.; Yang, S.; Ye, P.; Tian, E.; Sun, G.; Zhou, J.; Sun, G.; Liu, X.; Chen, C.; Murai, K.; Zhao, C.; Azizian, K. T.; Yang, L.; Warden, C.; Wu, X.; D'Apuzzo, M.; Brown, C.; Badie, B.; Peng, L.; Riggs, A. D.; Rossi, J. J.; Shi, Y., Downregulation of TLX induces TET3 expression and inhibits glioblastoma stem cell self-renewal and tumorigenesis. *Nat. Commun.* **2016**, 7 (1), 10637.
46. Huang, K.-W.; Reebye, V.; Czysty, K.; Ciriello, S.; Dorman, S.; Reccia, I.; Lai, H.-S.; Peng, L.; Kostomitsopoulos, N.; Nicholls, J.; Habib, R. S.; Tomalia, D. A.; Sætrom, P.; Wilkes, E.; Cutillas, P.; Rossi, J. J.; Habib, N. A., Liver activation of hepatocellular nuclear factor-4 α by small activating RNA rescues dyslipidemia and improves metabolic profile. *Mol. Ther. Nucleic Acids* **2020**, 19, 361-370.
47. Voutila, J.; Reebye, V.; Roberts, T. C.; Protopapa, P.; Andrikakou, P.; Blakey, D. C.; Habib, R.; Huber, H.; Saetrom, P.; Rossi, J. J.; Habib, N. A., Development and mechanism of small activating RNA targeting CEBPA, a novel therapeutic in clinical trials for liver cancer. *Molecular Therapy* **2017**, 25 (12), 2705-2714.
48. Setten, R. L.; Rossi, J. J.; Han, S.-p., The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Dis.* **2019**, 18 (6), 421-446.
49. Dong, Y.; Siegwart, D. J.; Anderson, D. G., Strategies, design, and chemistry in siRNA delivery systems. *Adv. Drug. Deliv. Rev.* **2019**, 144, 133-147.
50. Blanco, E.; Shen, H.; Ferrari, M., Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **2015**, 33 (9), 941-951.
51. James, M. L.; Gambhir, S. S., A molecular imaging primer: modalities, imaging agents,

and applications. *Physiol. Rev.* **2012**, *92* (2), 897-965.

52. Chen, H.; Zhang, W.; Zhu, G.; Xie, J.; Chen, X., Rethinking cancer nanotheranostics. *Nat. Rev. Mater* **2017**, *2* (7), 17024.

53. Shi, J.; Kantoff, P. W.; Wooster, R.; Farokhzad, O. C., Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* **2017**, *17* (1), 20.

54. Price, E. W.; Orvig, C., Matching chelators to radiometals for radiopharmaceuticals. *Chem. Soc. Rev.* **2014**, *43* (1), 260-290.

55. van der Meel, R.; Sulheim, E.; Shi, Y.; Kiessling, F.; Mulder, W. J. M.; Lammers, T., Smart cancer nanomedicine. *Nat. Nanotechnol.* **2019**, *14* (11), 1007-1017.