DNA-organic molecular amphiphiles: Synthesis, self-assembly, and hierarchical aggregates

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Abstract
With the development of deoxyribonucleic acid (DNA) nanotechnology, various DNA nanostructures and DNA devices have been constructed, which exhibit potential applications in material science and biomedicine. Taking advantage of the programmability and biocompatibility of DNA, novel building block to chemically functionalize DNA with hydrophobic organic molecules has attracted more and more attention. Driving by amphiphilicity, DNA-organic molecular amphiphiles have been demonstrated to self-assemble or further induce hierarchically assemblies, providing novel-specific properties. In this minireview, we summarize the recent progress of DNA organic molecular amphiphiles including their synthesis, self-assembly behavior in aqueous solution, and the amphiphilic self-assembly based on hierarchical DNA nano-structures. We further briefly discuss the perspective of the application of the DNA-organic molecular amphiphiles.

KEYWORDS
DNA-organic molecular amphiphiles, hierarchical nanostructures, morphology control, self-assembly, synthesis

INTRODUCTION
Deoxyribonucleic acids (DNAs) are biomacromolecules that carry and code genetic information for living organisms. In 1980s, Seeman first proposed DNA nanotechnology,1 in which DNA were regarded as building blocks to construct hierarchical nanostructures. Based on their programmability, addressability, and biocompatibility, DNA could not only construct static nanostructures from one-dimensional (1D) to three-dimensional (3D),2 but also fabricate dynamic nanodevices.3 Recently, some of these DNA nanostructures and nanodevices have been utilized as drug delivery vehicles in the biomedicine and showed excellent efficiency.3 Moreover, DNA could be functionalized with different entities such as inorganic nanoparticles,3 organic molecules, and biologic macromolecules like peptides or proteins.3 Among them, DNA-organic molecular amphiphiles have attracted developing attention because they have provided novel building blocks, which could spontaneously assemble into various morphologies in aqueous solution, for example, spherical micelles, nanosheets, nanofibers, and vesicles. During last decades, many DNA-organic molecular amphiphiles7 have been designed and synthesized by conjugating different hydrophobic molecules including synthetic polymers, dendrons, and small organic molecules. When these component molecules possess intrinsic functionality, such as redox, photo physical, and biological properties, they would afford rationally designed functions into the final assemblies. It should also be noticed that DNA amphiphiles could further interact with or induce hierarchical nanostructures to form hierarchical nanostructures, bringing more specific proterties. While hydrophobic interaction, the driving force of the amphiphilic assembly, is nondirectional, the combination of the DNA and hydrophobic molecules...
would bring more addressability and programmability to the amphiphilic assembly process. Therefore, this minireview will introduce recent progress on these DNA-organic molecular amphiphiles. Herein, we will firstly summarize the coupling methods for covalent modification of DNA with hydrophobic molecules and then discuss the recent advances of their applications in self-assembly. The amphiphilic self-assembly based on hierarchical DNA nano-structures will also be introduced, followed by the conclusion and prospective.

SYNTHESIS OF DNA-ORGANIC MOLECULAR AMPHIPHILES

To prepare DNA-organic molecular amphiphiles, efficient conjugation strategies of hydrophobic molecules to DNA are necessary. Solution coupling and solid-phase synthesis are two general methods to prepare modified DNA conjugated by functional groups. However, solution coupling is widely utilized for the hydrophilic molecular conjugation and just applicable for some hydrophobic molecular conjugation in the mixture solvent because hydrophobic conjugation often results in low yields due to the solvent incompatibility. Therefore, solid-phase modification, which exhibits the reactions in organic solvents on the solid supports, is suitable for preparing DNA-organic molecule amphiphiles. Herein this section, we will briefly introduce the solid-phase modification, and moreover, the recent developed methods including polymerase chain reaction (PCR), post-polymerization, organic-phase reaction will also be discussed.

Solid-phase modification

Commercial oligonucleotides are automated prepared by iterative chemical synthesis on solid supports (controlled-pore glasses, [CPG]) using activated phosphoramidite materials. The four-step cycle includes (1) deblocking, (2) coupling, (3) capping, and (4) oxidation. A nucleoside can be attached through these four steps to complete a cycle, and then the next cycle can be initiated to attach the next nucleoside. Here, deblocking aims to remove the protective group so that hydroxyl is exposed and participates in the subsequent reaction. The coupling reaction is based on the phosphoramidite chemistry, which is reported to be very efficient with yield higher than 99% at each cycle. Capping is to remove the influence of unreacted hydroxyl groups attached to CPG after the coupling step on subsequent reactions. The purpose of oxidation is to change trivalent phosphorus into a more stable pentavalent phosphorus structure. In commercial synthesis, the DNA was synthesized 3′-end to 5′-end due to the standard procedure, but it should be mentioned that it is also possible to synthesize DNA from 5′ to 3′ with the special reagents.

Solid-phase functionalization is mainly based on the same phosphoramidite chemistry as illustrated in Figure 1A: the hydrophobic groups could be easily coupled with the 2-cyanoethyl-N,N-diisopropylphosphamidite group, which is then modified to the DNA on the solid support CPG. Due to the compatibility of different organic solvents in the solid-phase method, it is believed to be a universal method for hydrophobic functionalization. It should be noticed this coupling reaction is sensitive to water and oxygen, so it needs to be operated under anhydrous and anaerobic conditions. The solvent used in commercial synthesizer is anhydrous acetonitrile; therefore, molecules for conjugating with DNA possessing good solubility in acetonitrile would be ready to be applied to DNA synthesizer. When the hydrophobic groups exhibit low solubility in acetonitrile, the coupling could also be realized manually in any other available solvents. It also should be noted that the chemical modification of DNA by the solid-phase synthesis is not limited to the phosphoramidite chemistry. Amide formation from amino-terminated DNA and the carboxy-modified hydrophobic molecules (Figure 1B), Huisgen cycloaddition (copper catalyzed alkyn-azide cycloaddition) between azido terminated DNA and alkyne functionalized hydrophobic molecules (Figure 1C), Sonogashira coupling (Figure 1D) and the functionalization of 3′-terminus DNA with phosphite amide chemistry (Figure 1E) can also functionalize DNA at the end with hydrophobic molecules, which also broadens the application of solid-phase synthesis.

Recent developed modification methods

Modification based on PCR

Taking advantage of the standard synthesis of DNA and well-developed synthetic strategies, a lot reported DNA amphiphiles are prepared by solid-phase synthesis but the length of the DNA sequence is usually less than 50 mer. In order to extend nucleic acid segments, in 2007, Hermann and his colleagues utilized PCR protocol to generate well-defined double strands (ds) DNA block copolymers and ds DNA triblock architectures, in which the length of the DNA segments can extend from tens of to more than 1500 base pairs, as shown in Figure 2A. In addition, the polymers including polystyrene (PS), polypropylene oxide (PPO), poly(N-isopropylacrylamide) (PNIPAM), and poly(ethylene glycol) (PEG) could be tolerant in this PCR method.

Post-polymerization

Based on the solid-phase synthesis of DNA, post-polymerization is another method to synthesize DNA amphiphiles. Matyjaszewski and Das functionalized an atom-transfer radical polymerization (ATRP) initiator phosphoramidite on the DNA strand (23-mer) and obtained DNA-polymers through activators generated by electron transfer (AGET) ATRP in solution or even directly on the solid support, as shown in Figure 2B. This approach could provide conjugated polymer segments with well-defined molecular weight and narrow molecular weight distribution and could directly and rapidly purify the DNA conjugates through the solid-support method.

Organic-phase reaction

To overcome the incompatibility of DNA and hydrophobic molecules in the solution coupling, Herrmann reported an organic-phase reaction to achieve DNA modification
A. Phosphoramidite Chemistry

\[
\text{CPG} \quad \text{OH} \quad + \quad \text{NC} \quad \text{O} \quad \text{O} \quad \text{Hydrophobic molecule} \quad \rightarrow \quad 1. \text{Coupling} \quad 2. \text{Cleavage} \quad \text{CPG} \quad \text{O} \quad \text{P} \quad \text{O} \quad \text{Hydrophobic molecule}
\]

B. Amide Formation

\[
\text{CPG} \quad \text{NH}_2 \quad + \quad \text{HOOC} \quad \text{Hydrophobic molecule} \quad \rightarrow \quad 1. \text{Coupling} \quad 2. \text{Cleavage} \quad \text{CPG} \quad \text{NH} \quad \text{Hydrophobic molecule}
\]

C. Copper Catalyzed Alkyne-Azide Cycloaddition (CuAAC)

\[
\text{CPG} \quad \text{N}_2 \quad + \quad =\equiv \quad \text{Hydrophobic molecule} \quad \rightarrow \quad 1. \text{Coupling} \quad 2. \text{Cleavage} \quad \text{CPG} \quad \text{N} \quad \text{N} \quad \text{Hydrophobic molecule}
\]

D. Sonogashira Coupling

\[
\text{CPG} \quad + \quad =\equiv \quad \text{Hydrophobic molecule} \quad \rightarrow \quad 1. \text{Coupling} \quad 2. \text{Cleavage} \quad \text{CPG} \quad =\equiv \quad \text{Hydrophobic molecule}
\]

E. 3'-end functionalization of DNA with the modification of CPG

\[
\text{CPG} \quad \text{OH} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{Hydrophobic molecule} \quad \rightarrow \quad \text{DNA Synthesis} \quad \text{CPG} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{Hydrophobic molecule} \quad \rightarrow \quad 3' \quad \text{Cleavage} \quad \text{CPG} \quad \text{OH} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{Hydrophobic molecule}
\]

**Figure 1** Solid-phase synthesis of DNA-organic molecule amphiphiles. (A) Phosphoramidite Chemistry. Reproduced with permission: Copyright 2004, American Chemical Society.\(^{12}\) (B) Amide formation. Reproduced with permission: Copyright 2007, The Royal Society of Chemistry.\(^{13}\) (C) Copper catalyzed alkyne-azole cycloaddition. Reproduced with permission: Copyright 2010, The Royal Society of Chemistry.\(^{14}\) (D) Sonogashira coupling. Reproduced with permission: Copyright 2005, Wiley-VCH.\(^{15}\) (E) 3'-terminus functionalization of DNA with hydrophobic molecules. Reproduced with permission: Copyright 1995, American Chemical Society.\(^{16}\) Notably, in the reactions of b-d, chemical functionalities can be exchanged with each other in a particular case and polymerization.\(^{19}\) As illustrated in Figure 2C, DNA-surfactant complexes, formed by electrostatic interaction of oligonucleotides with the cationic surfactant in the aqueous phase, are soluble in organic solvents such as DMF, DMSO, THF, and CHCl\(_3\). These DNA-surfactant complexes permitted the terminal acylation of 3'-amine-modified oligonucleotides by various hydrophobic N-hydroxysuccinimide (NHS) esters, such as pyrene, triphenylphosphine, PPO, PS, and so on. Moreover, the polymerization of norbornene functionalized DNA-surfactant complex monomers allowed the generation of novel DNA side chain homopolymers. Subsequently, Zhang and his coworkers also utilized the organic-phase strategy.\(^{20}\) The norbornene-modified, protected DNAs were converted into different polymer-DNA nanostructures by the ring-opening metathesis polymerization. The obtained products included brush-type DNA graft polymers, DNA-poly(ethylene glycol) diblock graft copolymers, and DNA block copolymer micelles. These results illustrated that the organic-phase synthesis possessed the promising possibilities to extend DNA into material science.

**Micelle-templated approach**

Besides the modification of the pristine DNA strand and polymer segment, Sleiman and her coworkers developed a micelle-templated approach to enhance the reactivity of DNA with highly hydrophobic molecules (Figure 2D).\(^{21}\) First, DNA strands were conjugated to 1,12-dodecanediol phosphoramidite units (hexaethylene, or HE) to obtain DNA amphiphiles. Then the DNA amphiphiles self-assembled into monodisperse DNA micelles. By DNA hybridization, a complementary, non-hydrophobically modified DNA strand can orient its reactive group (amino group) toward the micelle core. Meantime, hydrophobic molecules functionalized with a NHS ester group were concentrated in the DNA micellar core and achieved the coupling reaction. The coupling efficiency is greatly improved. This DNA micelle-templated method will expand DNA-organic molecule hybrids in nanopore mimetics, oligonucleotide and drug delivery, and DNA nanotechnology.

**Self-assembly of DNA-organic molecule amphiphiles**

Due to the various molecular topologic structure and different size, the DNA amphiphiles could assemble into different nanostructures such as spherical micelles, nanofibers, vesicles, and nanosheets. These hydrophobic molecules mainly include synthetic polymers, dendrimers, and small molecules. The formed nanostructures usually could envelope hydrophobic molecules such as fluorescent molecules and drug molecules and anchor some other functional groups
or molecules through DNA hybridization, which has been demonstrated with potential application in nanoscience and biomedicine.

**Amphiphilic DNA block copolymers**

DNA is a kind of special polymer that possesses programmability and addressability. Combining DNA with various polymers will bring novel properties for the DNA block copolymers. Firstly, some hydrophilic DNA block copolymers were synthesized with high yield through solution coupling and were utilized in gene delivery and purification of the macromolecules. In 2001, Park et al firstly reported the synthesis of amphiphilic DNA block copolymer DNA-b-poly(D,L-lactic-co-glycolic acid) (PLGA) by amino modified DNA reacting with NHS activated PLGA in aqueous solution, which could self-assembled into spherical micelles with a diameter of 80 nm. Similar spherical micelles were also prepared in Mirkin’s group, which exhibited recognition properties defined by their DNA sequences.

In 2006, Herrmann and coworkers fabricated DNA-b-PPO spherical micelles and carried out some organic reactions on the DNA micellar scaffold, as shown in Figure 3A. Subsequently, the same group further explored the DNA-b-PPO systems as drug delivery systems. To enhance the cell internalization of the DNA block copolymer micelles, Mirkin et al prepared DNA-brush block copolymer micelles with a biodegradable polycaprolactone (PCL) core and a DNA strands corona. The DNA-brush block copolymer micelles possessed a higher surface density of DNA strands with more negatively charge, resulting in more effective cellular uptake and gene expression in vitro. In 2017, Zhang Chuan and Zhang Ke further explored the co-assembled micelles from DNA-b-PCL, PEG-b-PCL and the PCL homopolymer (Mn = 10.5 kDa). They demonstrated the relationship between structures and properties of this system, and it was found that these micelles successfully realized high cellular uptake and effective antisense gene regulation.

Besides the spherical micelles assembled from amphiphilic DNA block copolymers, amphiphilic DNA block copolymers can also assemble into other nanostructures, such as vesicles and helices. In 2014, Park et al reported a novel semiconductor-DNA amphiphiles, composed of 25-mer oligonucleotides and a π-conjugated polymer poly[3-(2,5,8,11-tetraoxatridecanyl)thiophene] (PTOTT), as shown in Figure 3B. The generated bio-conjugated copolymers possess both the recognition properties of DNA and excellent optoelectronic properties of semiconducting polymers. DNA-b-PTOTT can self-assemble into vesicles with controllable size in water but after mixing DNA-b-PTOTT with DNA-b-PEG, the system could self-assemble into nanoribbons, which demonstrated its adaptability and its possibility to offer more DNA-semiconducting polymer nanomaterials. In 2015, Jiang and colleagues covalent grafted DNA strands onto poly(propargylmethacrylate) (PPMA) to generate amphiphilic PPMA-g-DNA brush, which assembled into nanofibers and then spun into multi-strand helices. These...
nanofibers decorated with DNA strands at the periphery could position gold nanoparticles by DNA hybridization.

Smart DNA nanomaterials, response to stimuli, have also been well investigated due to their potential application in the biomedicine and nanotechnology. The stimuli include hybridization and enzyme, pH, light, and temperature response. In 2007, Herrmann et al hybridized different length of complementary DNA strands with DNA-b-PPO micelles to achieve the morphology control. Hybridization of the DNA-b-PPO spherical micelles with short complementary DNA could not change the morphology, but recognition with long DNA template induced a transition from sphere into rod-like micelles with more internalization.

Subsequently, Gianneschi et al reported the reversible shape control of amphiphilic DNA-brush copolymers upon DNA hybridization and selective enzymatic cleavage. In water, amphiphilic DNA-brush copolymers spontaneously assembled into spherical micelles. The adding of the DNAzyme, which cut the DNA strands at the specific site to generate a truncated ssDNA, induced a morphology change from sphere to cylindrical micelles. Upon further adding the complementary long strand of DNA, it reversibly turned back to spherical micelles. These results proved that the change of the ratio of hydrophobic segments and hydrophilic DNA could control the assembled morphology.

Amphiphilic DNA block copolymer aggregates could achieve the shape tuning in situ by external stimuli. As illustrated in Figure 3C, Liu et al reported a pH-responsive morphological control of DNA-b-PPO aggregates. DNA strands are cytosine-rich sequences, which could form compact bimolecular i-motif structures at pH 5 and exhibit flexible single strands at pH 8. Upon these properties, at pH 8 the DNA amphiphiles assembled into 20 nm spherical micelles, but at pH 5 they assembled into long nanofibers. Moreover, the shape change was reversible in situ, which was easily realized by regulating temperature and pH. These results illustrated that the conformational change of DNA probably impacts the assembled model. In 2016, Park et al investigated a thermal-responsive transformation of DNA-b-PNIPAM-b-PMA copolymers, as shown in Figure 3D. PNIPAM is hydrophilic below lower critical solution temperature (LCST) and turns hydrophobic above LCST. It was observed that DNA-b-PNIPAM-b-PMA formed spherical micelles at room temperature and turned into cylinder shape above LCST due to the conformational change of PNIPAM. These shapes can be transformed reversibly in situ by regulating temperature. In addition, the low temperature spherical morphology can be accessed by introducing long complementary DNA strands even above LCST. These results demonstrate the multidimensional morphology and multifunctions of the DNA amphiphilic polymers.

**Amphiphilic DNA-Dendron hybrids**

Due to the monodispersed and precisely branched molecular architectures, Dendron or dendrimers could be regarded...
as an interesting building block in material science.\textsuperscript{34} Compared with the linear and branched polymers, dendrimers are more spherical, and the number of end groups increases exponentially with the increase of generation, and they are easy to be further functionalized. Compared with small molecules, they are much larger, with nanoscale size and exhibiting supramolecular chemistry that can only be produced by the aggregation of many small organic molecules. At early stage, dendritic topology was introduced into the DNA nanostructure to obtain a series of nucleic-acid dendrimers.\textsuperscript{35} With the development of chemical modification of DNA, different size and topologic dendrons can be covalent attached to DNA sequences.\textsuperscript{36} Liu’s group has utilized a pH-driven DNA motor to control the association and disassociation of dendrons.\textsuperscript{37} In this system, two amphiphilic dendrons are covalently conjugated with a pH triggered DNA motor at the 3’ and 5’ ends. The DNA sequence is a 21-mer DNA containing four stretches of cytosine-rich sequence, which exhibits compact i-motif structure (closing state) at pH 5 and shows flexible (opening state) at pH 8. The results suggested that the DNA molecular motor may serve as a new platform to investigate nonspecific and specific macromolecular interactions on the molecular level.

Then the self-assembly of DNA-dendron amphiphiles has been investigated gradually. In 2010, Sleiman’s group first reported the assembly of DNA-dendron hybrids consisted of hydrophilic oligo ethylene glycol (OEG) dendron and DNA strand in organic solvent.\textsuperscript{38} As illustrated in Figure 4A, after hybridization with complementary DNA or DNA-Dendron, they formed well-defined nanofibers and further align into parallel rows, to produce highly ordered micrometer-sized surfaces. It is worth noting that the ds DNAs were stable at the core and OEG at the external. These results offered a new strategy to introduce hierarchical long-range ordering into DNA motifs and may expand their potential application such as scaffolds for 1D materials, templates for tissue growth.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Self-assembly of DNA-dendron amphiphiles. (A) The long nanofibers formed from DNA-Dendron. Reproduced with permission: Copyright 2010, American Chemical Society.\textsuperscript{38} (B) Functionalized nanofibers formed by G2Cl-18 hybridized with complementary DNA modified AuNPs. Reproduced with permission: Copyright 2011, The Royal Society of Chemistry.\textsuperscript{39a} (C) 2D DNA nanosheets formed from DNA-TPE Dendron and their application in the catalytical reactions. Reproduced with permission: Copyright 2018, The Royal Society of Chemistry.\textsuperscript{41}}
\end{figure}
Later, Liu’s group systematically studied the self-assembly behavior of a series of DNA–Dendron hybrids in aqueous solution.\(^{39}\) They included a highly hydrophobic dendron poly(phenylether) dendron peripherally modified with dichlorobenzene (G\(_2\)Cl) and a hydrophobic dendron with relative flexibility aliphatic polyether Dendron (PDMC). The former G\(_2\)Cl-DNA assembled into long nanofibers in aqueous solution, as shown in Figure 4B. The latter DNA-PDMC hybrids formed different nanostructures from spherical micelles to nanofibers, even to the irregular aggregates with the decrease of DNA length in water. After adding THF in water (THF/H\(_2\)O 1:10, v/v), DNA-PDMC hybrids all assembled into nanofibers. These assemblies could anchor gold nanoparticles or target functional molecules through the hybridization of shell DNA and encapsulate hydrophobic species at the dendron core. These results illustrated their potential application in the drug delivery and nanotechnology and offered detailed data for theoretical research. In 2015, the same group further functionalized DNA-Dendron fibers with carbohydrate to regulate Escherichia coli association,\(^{40}\) which illustrated their potential to be an easily modified multivalent supramolecular platform.

Recently, Varghese et al explored the assembly of DNA-tetraphenylethylene (TPE) dendron amphiphiles and their application.\(^{41}\) As shown in Figure 4C, DNA-TPE dendron amphiphiles can form 2D DNA nanosheets in aqueous solution. Addressable DNA decorated surface of the sheets could immobilize the catalytically active AuNPs. The catalytic activity of AuNPs positioned on the 2D nanosheets enhanced significantly in the reduction of nitroaromatics compared with that of discrete DNA functionalized NPs.

**DNA-small molecule amphiphiles**

Small molecules could also be conjugated with DNA at an arbitrary position (at the middle or at the end) and achieve their function in nucleic acid research, biosensing, light harvesting, diagnostic, and nanotechnology. However, due to the high hydrophilic/hydrophobic ratio, it is difficult for DNA-small molecule amphiphiles to form stable amphiphilic assemblies, for example, cholesterol or commercial fluorescent molecules.\(^{42}\) Recently, two kinds of small hydrophobic molecules, including lipids and π-molecules, have been successfully introduced into the DNA amphiphiles and realize their assembly.

Since 2006, a series of lipids have been covalently attached to DNA. Based on the different length and amount of alkane chains, the DNA-lipid amphiphiles can assemble into various assemblies\(^{43}\) such as vesicular aggregates, monodispersed micelles, and responsive liposomes. In addition, the conformational change of DNA strand will impact the stability of the DNA-lipid assemblies. Tan and coworkers developed stability-tunable DNA micelle flares from DNA-lipid amphiphiles (Figure 5A).\(^{44}\) The DNA strand contains a G-rich sequence. The intermolecular G-quadruplexes stabilized the DNA micelles against disruption by serum albumin. While exposed to light, the complementary DNA of G-rich sequences in the hairpin structure was released to hybridize with G-rich sequences and prevent the formation of G-quadruplex. This further induced the dissociation of micelles in serum albumin and subsequent cellular uptake.

With a similar strategy, Roelfes et al fabricated DNA-lipid micelles with G-quadruplex structures.\(^{45}\) The micelles collapsed by hybridization with complementary DNA strands and released the encapsulated cargos. These results should further the development of biocompatible DNA micelles for in vivo applications. Recently, Schnichels and Herrmann explored DNA-lipid micelles loaded with brimonidine to improve glaucoma treatment.\(^{46}\) These DNA micelles showed advantageous for retained release of the drug and exhibited excellent biocompatibility in vitro and in vivo.

Regarding the π-π stacking interaction in the hydrophobic molecules, several groups investigated some aromatic molecules covalently conjugated with oligonucleotides. In 2015, Häner et al developed that chimeric pyrene-DNA oligomers assembled into helical nanoribbons, driven by π-π stacking interactions among pyrene units.\(^{47}\) The individual nanoribbons aggregated into extended networks through DNA hybridization. Subsequently, they proved that the helical supramolecular assemblies from the chimeric oligomers were able to load cargo or gold nanoparticles, which showed their potential application in drug delivery. Moreover, Häner group investigated the coexistence of two independent excitonic states in aromatic stacks composed of two types of chromophores.\(^{48}\) In a DNA scaffold, an alternating fashion was arranged by pyrene and perylenediimide (PDI) molecules. The observation of electronic coupling between chromophores of the same type in alternating arrangements indicated that the pyrene H-aggregates and PDI coexist in p-stacked hetero-aggregates. These supramolecular DNA nanostructures may be relevant for the development of DNA-based smart materials, such as stimuli-responsive carriers of biologically active agents.

PDI, another class of attractive chromophores, has been applied in solar cells as dye sensitizers for the outstanding thermal, chemical, and photochemical stability and high fluorescence intensity. Recently, PDI molecules were also introduced to combine with DNA strand. Lewis and coworkers investigated the possible stacking structure of PDI molecules in the DNA-PDI system,\(^{49}\) such as a hairpin dimer structure from DNA-PDI-DNA, linear end-to-end supramolecular polymers from PDI-linker DNA dumbbell conjugates, which were driven by the hydrophobic interaction among PDI molecules. In 2019, a reversible morphology tuning of DNA-PDI assemblies through host-guest interaction was developed by Liu et al.\(^{50}\) As illustrated in Figure 5B, DNA-PDI firstly assembled into spherical micelles due to the strong π-π interaction of PDI units. The adding of cucurbit[10]uril lead to a morphological shifting from spheres to 2D DNA nanosheets and an obvious fluorescence enhancement at 675 nm. This change was result of the formation of a CB[10]-PDI\(_2\) inclusion and a J-type dimer of PDI units. Moreover, the competitive guest was able to induce the reversibility. The 2D nanosheets decorated with DNA strands could act as addressable template to organize several functional species. These results offered a novel strategy to achieve morphologic control by host-guest interaction and proved the potential application of DNA nanosheets in arraying functional molecules. Varghese’s group investigated a series of DNA-π molecular amphiphiles, which formed a variety of nanostructures, such as vesicles, nanosheets and helically twisted nanoribbons. In 2014, DNA-oligo(p-phenylene-ethynylene) (OPE) hybrids were synthesized through a general “click chemistry”-based
strategy and the amphiphiles assembled into vesicles with enhanced emission.\textsuperscript{51} They verified that the addressable surface of DNA-OPE vesicles could integrate gold nanoparticles by DNA hybridization. Further, ethynyl perylene-modified DNA (DNA-Pe), as a Förster resonance energy transfer acceptor, was introduced into the DNA-OPE vesicles to achieve a supramolecular light-harvesting antenna. These results illustrated that the DNA-chromophore aggregates have great promises in nanoelectronics, energy, and biomedical applications. In 2017, alkyl chains (−C_{10}H_{21}) tethered hexa-peri-benzocoronene (HBC) was covalently bind with DNA strand to obtain DNA-HBC amphiphiles.\textsuperscript{52} As shown in Figure 5C, driven by the strong π-stacking interaction and amphiphilicity, DNA-HBC hybrids assembled into crystalline 2D DNA nanosheets, which possess surface addressability to array functional molecules. Further, helically twisted nanoribbons from DNA-hexaphenylbenzene (HPB) were formed.\textsuperscript{53} As illustrated in Figure 5D, in the assembly process, the molecular chirality of ssDNA transferred into the HPB core and resulted in the bias of one of the chiral propeller conformations for HPB, which induced a helical twist and finally formed M-helical nanoribbons. These DNA nanoribbons could act as a reversible template to organize 1D chiral plasmonic nanomaterials.

**AMPHIPHILIC SELF-ASSEMBLY BASED ON HIERARCHICAL DNA NANO-STRUCTURES**

With the fast development of DNA nano-technology during last decades, arbitrary 2D and 3D complex structures...
have been easily constructed by the design of DNA.\textsuperscript{54} Taking advantage both of the addressability of DNA nano-structures and commercial modification strategy of DNA, hierarchical amphiphilic DNA structures could also be prepared. In the hierarchical amphiphilic DNA structures, the hydrophilic entities are the DNA nanostructures rather than the single or double stranded DNA. Comparing to the normal amphiphilic DNA hybrids, the hierarchical structures exhibit specific properties and novel assembly behaviors. Therefore, in this section, we will introduce the recent progress of the amphiphilic assembly based on hierarchical DNA nano-structures.

**Self-assembly of amphiphilic DNA nano-structures**

As discussed above, combined with the DNA modification strategies, the addressability of DNA nano-structures has allowed introducing any hydrophobic functional groups to prepare mono-dispersed amphiphilic DNA nano-structures. The composition, sequence, distribution, and orientation of hydrophobic entities can be easily tuned by the rational design of the DNA nano-structures, which can bring new assemblies that are not observed in normal DNA amphiphiles.

In 2013, Sleiman’s group reported a class of dendritic alkyl chain-based DNA amphiphiles (D-DNA) within a 3D DNA cube.\textsuperscript{55} These DNA-dendritic nanostructures exhibited amphiphilic properties but they are different from normal amphiphilic block copolymers due to the monodisperse, sequence defined and anisotropic properties. As illustrated in Figure 6A, spatial orientation, number, and chemical identity of hydrophobic moieties were rationally tuned, which was demonstrated to determine the assembly in either inter- or intramolecularly manner. For example, when four hydrophobic moieties were introduced on a DNA cube face, intermolecular dimerization was observed but when eight hydrophobic moieties were anchored on the DNA cube, the dendritic alkyl chains come together intramolecularly to form a micellar environment within the DNA cage. It should be noticed that these structures are monodisperse due to the large hindrance of the DNA nanostructures, and the structure can be formed below the Critical Micelle Concentration of the amphiphiles. Later, the same group synthesized a series of sequence-defined polymers and introduced them onto the DNA cube (Figure 6B).\textsuperscript{56} The sequence-defined hydrophobic polymers further provided orthogonal assembly modes to DNA cages, and new structures (dimers, trimers, tetramers handshake and doughnut-shaped) can be achieved by fine-tuning of the length of hydrophobic blocks, the sequence of the polymers, and the orientation of the polymers on the cages. These results are not only potentially useful for targeted drug delivery applications but also provide a possible route to understand the structural complexity and protein-inspired folding.

As the increased size of the DNA nano-structure, the “intra-structure” self-assembly or self-folding may become dominant when the hydrophobic moieties are introduced, which could benefit constructing hierarchical structures. Zhou et al reported a giant amphiphilic structure with DNA...
Amphiphilic DNA nano-structures interacted with amphiphilic membranes

While the self-assembly of amphiphilic DNA nanostructures are widely investigated, the interaction between the amphiphilic DNA nano-structures and other amphiphilic assemblies/membranes has also attracted increasing attention. Membrane is the structural basis of some typical amphiphilic assembly, including vesicles, lamellar structures, and tubes etc. Understanding the membrane is important to reveal the mechanism of amphiphilic assembly, which is also necessary to investigate many biological processes related to the natural lipid membrane. Amphiphilic DNA nano-structures have been demonstrated with the potential to reveal the membrane properties or benefit the understanding of membrane-related behaviors.

In 2012, an amphiphilic DNA nanochannel was constructed by introducing hydrophobic cholesterol at the end of a DNA origami barrel by Simmel et al (Figure 7A). Due to the hydrophobic interactions between cholesterol and lipid molecules, the DNA nanochannel could penetrate and span the lipid bilayer, which created an artificial pore onto the membrane. Through single-channel electrophysiological measurement, the channel could successfully distinguish DNA hairpins, G-quadruplex, or single DNA molecule mutations. Recently, based on similar idea, Howorka’s group developed smaller nanochannels by only a few DNA strands with an outer ethyl phosphorothioate hydrophobic belt, which simplified the design of nanostructures. In their design, the six-helix DNA nanopore could be closed by blocking the...
Direct the nondirectional amphiphilic assembly

Hydrophobic interaction, driven by the entropic effect, has been well known for its nondirectional property, which makes it a challenge to realize controllable amphiphilic assembly process in vitro. Recently, a novel frame-guided assembly strategy has been proposed. Taking advantage of the addressability of the DNA nanotechnology, some leading hydrophobic groups can be precisely positioned on to well-designed frames, which would guide the subsequent assembly of the free amphiphiles in the system. Therefore, the final morphology would be consistent with the pre-designed frames, which provide a strategy to direct the nondirectional amphiphilic assembly. In a successful example, as illustrated in Figure 8A, DNA-poly(aryl ether) dendron-oligos (ethylene glycol) were anchored on to the gold nanoparticles or gold nanorod as leading hydrophobic groups and subsequently recruited DNA-poly(aryl ether) dendron to form hetero-vesicles. Later, a variety of amphiphilic assemblies of defined size has been successfully constructed, including the thermally responsive PPO polymer system, small molecular SDS, and lipid system.
With the capability to direct the nondirectional amphiphilic assembly, DNA nanotechnology has been demonstrated to provide many delicate assembly structures to achieve irregular and asymmetric assemblies. For example, cuboid vesicles assemblies were successfully constructed by using the 3D DNA nanostructure with the same strategy. As illustrated in Figure 8B, DNA cuboid with a dimension of 20 nm × 20 nm × 40 nm was constructed, and the DNA-dendron conjugates were hybridized to the complementary DNA strands on the cuboid. The addition of other amphiphilic molecules could assemble around the frames and generate the cuboid vesicles along the frames, which is difficult for the traditional amphiphilic strategy. In 2014, Shih and Perrault developed a virus-inspired enveloped DNA nanostructure through similar method as illustrated in Figure 8C. In their design, the lipid molecules were anchored to the DNA nano-octahedron (DNO) and then mixed with the lipids and surfactants. Through dialysis procedure to remove the surfactant, the lipid was guided to assemble around the DNO by the handles to form the bilayer structures. The envelopment of DNA nanostructures within lipid bilayers conferred protection against nuclease digestion, and the immune activation was decreased two orders of magnitude below control. Comparing to 3D vesicles, 2D amphiphilic membranes are more difficult to prepare; however, with the assistance of hierarchical amphiphilic DNA structures, amphiphilic membrane could be also realized, which successfully overcomes the problem of thermodynamic instability of traditional 2D amphiphilic membrane in solution. Lin and his coworkers also prepared a DNA-origami ring as exoskeletal scaffold to construct liposomes with controllable size (Figure 8D). The hydrophobic lipid molecules were conjugated to the inner surface of the DNA-origami rings, and with a similar dialysis procedure describe above, the liposome would grow inside whose size was restricted by the DNA-origami rings. In their study, liposomes with different dimensions were successfully prepared, and the DNA ring-liposome complex could be successfully purified through a density gradient ultra-centrifugation. Later, taking advantage of the DNA nanotechnology, 3D DNA cages with changeable aspect ratio were designed, which could stack into tubular frameworks and successfully guided the formation of liposome tubes with defined diameters. When the DNA frames were designed with well-controlled mechanical properties, the programmed conformational changes were demonstrated to mediate membrane remodeling events, including fusion and membrane tubule bending.

CONCLUSION AND PERSPECTIVE

In summary, we have summarized the recent progress of the DNA amphiphiles, including their synthesis, self-assembly, and applications. In addition to the traditional solid-phase synthesis and solution coupling, novel strategies have been developed to prepare DNA amphiphiles, such as PCR protocol, post-polymerization, and organic-phase reaction, which have expanded the possibilities of DNA amphiphiles beyond the conventional gene delivery. Taking advantage of the rational design of topological structures of organic molecules, the self-assembly of the DNA amphiphiles based on DNA-block copolymers, DNA-Dendron hybrids, DNA-lipid and DNA-chromophore hybrids has also been discussed. Furthermore, the recent progress of the amphiphilic assembly based on hierarchical DNA nano-structures has also been highlighted due to the specific properties and novel assembly behaviors. It has been demonstrated that these DNA-organic amphiphile aggregates not only act as vehicles to load drug species or functional molecules in biomedicine and material science but also can mimic biosystems to facilitate us understanding the living system.

With the fast development of the amphiphilic DNA hybrids, it can be expected to be introduced into more complex DNA scaffolds to develop bio-inspired systems and smart nano-systems for biomedicine. Furthermore, it has the potential to be extended to dynamic DNA nanoarchitectures by using stimuli hydrophobic molecules. Combining with the responsive entities, stimuli-responsive biomaterials can also be prepared which shows great potential applications in biomedical fields including drug delivery and therapy. It can be expected that this field will bring more fundamental investigation on self-assembly and more applications in various fields.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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REFERENCES

1. N. C. Seeman, J. Theor. Biol. 1982, 99, 237.
2. (a) N. C. Seeman, Nature 2003, 421, 427; (b) P. W. K. Rothemund, Nature 2006, 440, 297; (c) D. R. Han, S. Pal, J. Nangreave, Z. T. Deng, Y. Liu, H. Yan, Science 2011, 332, 342; (d) S. M. Douglas, H. Dietz, T. Liedl, B. Högborg, F. Graf, W. M. Shih, Nature 2009, 459, 414.
3. (a) H. Yan, X. Zhang, Z. Shen, N. C. Seeman, Nature 2002, 415, 62; (b) Y. Krishnan, F. C. Simmel, Angew. Chem. Int. Ed. 2011, 50, 3124; (c) S. M. Douglas, I. Bachelet, G. M. Church, Science 2012, 335, 831.
4. Q. Hu, H. Li, L. Wang, H. Gu, C. Fan, Chem. Rev. 2019, 119, 6459.
5. C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, Nature 1996, 382, 607.
6. (a) B. Saccà, C. M. Niemeyer, Chem. Soc. Rev. 2011, 40, 5910; (b) J. R. Vieregg, M. Lueckeheide, A. B. Marcelj, L. Leon, A. J. Bologna, J. R. Rivera, M. V. Tirrell, J. Am. Chem. Soc. 2018, 140, 1632.
7. (a) T. Schnitzler, A. Herrmann, Acc. Chem. Res. 2012, 45, 1419; (b) S. K. Albert, M. Golla, N. Krishnan, D. Perumal, R. Varghese, Acc. Chem. Res. 2020, 53, 2668; (c) C. J. Whitfield, M. Zhang, P. Winterwerber, Y. Zhou, D. Y. W. Ng, T. Weil, Chem. Rev. 2021, https://doi.org/10.1021/acs.chemrev.0c00174.
8. (a) A. Jaschke, J. P. Furste, E. Nordhoff, F. Hillenkamp, D. Cech, V. A. Erdmann, Nucleic Acids Res. 1994, 22, 4810. (b) J. H. Jeong, S. W. Kim, T. G. Park, Bioconjugate Chem., 2003, 14, 473.
9. R. B. Fong, Z. L. Ding, C. J. Long, A. S. Hoffman, P. S. Stayton, Bioconjugate Chem. 1999, 10, 720.
10. (a) J. H. Jeong, T. G. Park, Bioconjugate Chem. 2001, 12, 917. (b) S. A. Bell, M. E. McLean, S. - K. Oh, S. E. Tichy, W. Zhang, R. M. Corn, R. M. Crooks, E. E. Simanek, Bioconjugate Chem. 2003, 14, 488.
11. M. H. Caruthers, Acc. Chem. Res. 1991, 24, 278.
12. Z. Li, Y. Zhang, P. Fullhart, C. A. Mirkin, Nano Lett. 2004, 4, 1055.
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