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Recent advances in DNA nanotechnology

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DNA is a powerful guiding molecule to achieve the precise construction of arbitrary structures and high-resolution organization of functional materials. The combination of sequence programmability, rigidity and highly specific molecular recognition in this molecule has resulted in a wide range of exquisitely designed DNA frameworks. To date, the impressive potential of DNA nanomaterials has been demonstrated from fundamental research to technological advancements in materials science and biomedicine. This review presents a summary of some of the most recent developments in structural DNA nanotechnology regarding new assembly approaches and efforts in translating DNA nanomaterials into practical use. Recent work on incorporating blunt-end stacking and hydrophobic interactions as orthogonal instruction rules in DNA assembly, and several emerging applications of DNA nanomaterials will also be highlighted.

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Introduction

DNA nanotechnology has provided a powerful tool to create objects with arbitrary control of size and shape as well as high templating potential [1•]. From a structural perspective, the rigid, well-defined DNA duplex makes it suitable as a building block for nanoscale to microscale constructions with high spatial resolution. Importantly, the hybridization between DNA single strands is sequence-dependent and highly selective, allowing the programmable connection of DNA duplexes into a user-defined pattern. The unique sequences of DNA strands that constitute a DNA object also provide a precise 'address' to site-specifically position functional materials with high precision on the nanometer scale.

It is of note that Watson–Crick base-pairing is employed as the only assembly language in most of DNA nanomaterials. Because it is limited to four DNA bases, more complicated designs require a large number of unique DNA sequences for their construction. In addition to issues of cost, scalability and complexity for *in vivo* applications, this can result in increased assembly errors, potentially lowering product yields. As chemists, a powerful approach to address this limitation is to combine the toolbox of supramolecular chemistry with DNA nanotechnology. This has expanded the repertoire of DNA assembly languages and resulted in new structural and functional complexity without the need to increase the number of assembly components [1•,2].

Over the past 30 years, the fascinating aspects of DNA nanomaterials altogether have allowed their use to greatly advance research in many fields. The scope of this review is to provide a brief overview of the most recent trends in DNA assembly's design and scalability, followed by the recent progress in incorporating additional supramolecular interactions (blunt-end stacking and hydrophobic interactions) as orthogonal assembly languages in DNA assembly. In the last section, some selected applications of DNA nanotechnology will be covered.

Structural DNA assembly

New approaches for DNA nanostructure assembly

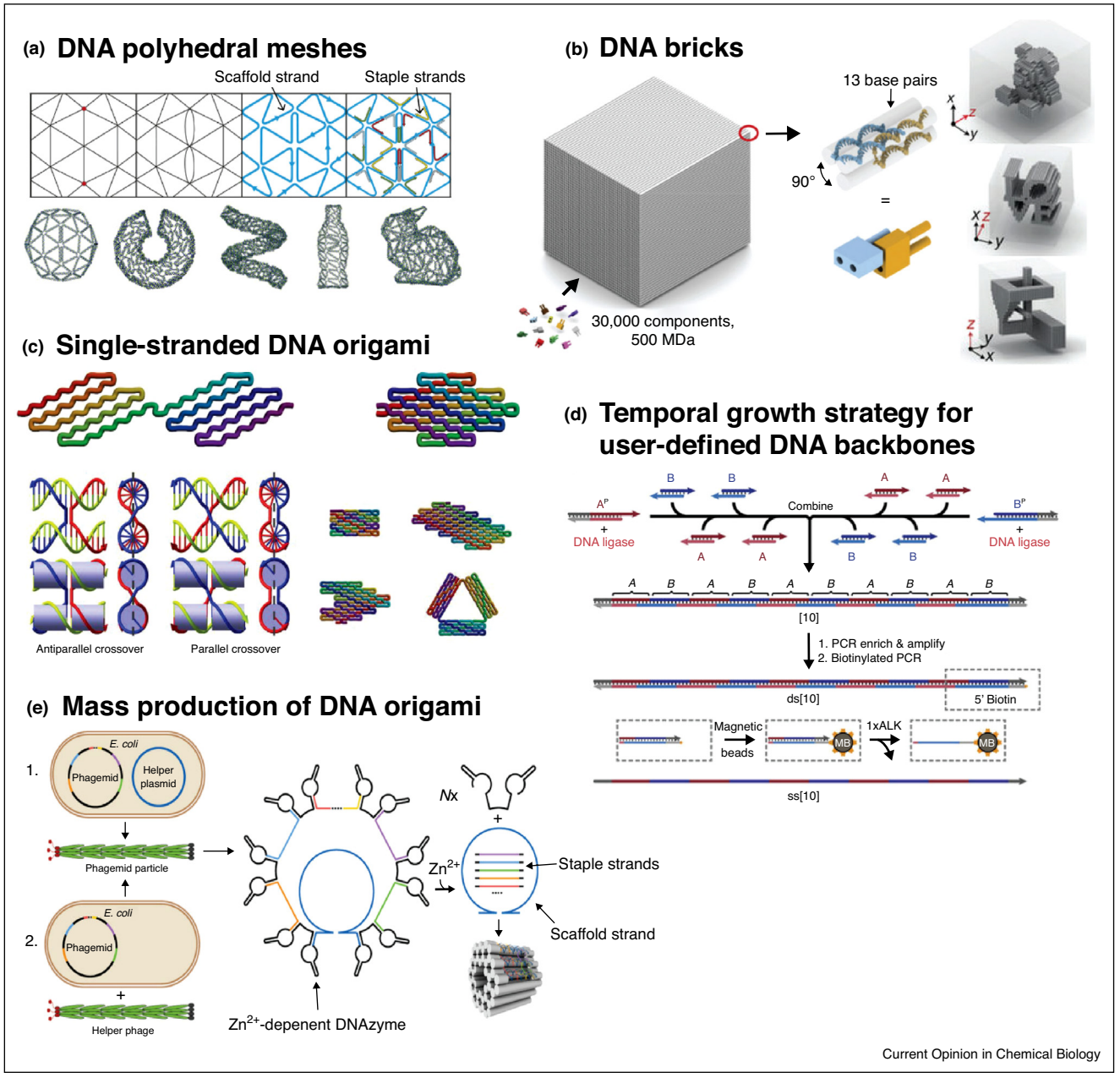
Following the lead of the first DNA objects reported by Seeman and co-workers in 1991 [3], an enormous library of well-defined DNA structures ranging from monodisperse discrete DNA nanostructures to extended DNA arrays has been generated, along with the development of new DNA assembly approaches. Recent trends have attempted to expand the complexity of an object that one can create. We direct the readers who are interested in a detailed progress to the more comprehensive reviews [1•,4•].

The invention of DNA origami by Rothemund in 2006 has revolutionized the field by offering an opportunity to create large, arbitrary 2D DNA-dense nanostructures [5••]. This approach is based on the folding of a long DNA single strand into a specific shape, guided by hundreds of short DNA single strands. Since then, progress towards 3D construction has been made, which significantly increased the complexity of DNA nanostructures and allowed DNA origami to be one of the most widely used approaches by many research groups [4•]. As an interesting example of new folding strategies, Högberg and co-workers applied graph theory to create wireframe DNA origami, where a 3D desired object (e.g., a Stanford bunny) was converted into a polyhedral triangulated

mesh [6]. A long DNA scaffold was routed on this 3D mesh, which was then folded by multiple short, unique DNA single strands, thus replacing all polygonal edges by DNA helices (Figure 1a).

Yin and co-workers introduced a scaffold-free concept called DNA bricks assembly [7•] and they recently demonstrated that up to 1 giga-dalton DNA nanostructures can be assembled from ~10^4 unique brick components, the highest complexity achieved to date [8]. These bricks are DNA single strands with four binding domains designed to form staggered duplexes with neighboring DNA bricks, creating 3D DNA arrays (Figure 1b). Analogous to Lego bricks, this approach allows a user to create an object by selecting a set of DNA bricks that define the overall shape of the target structure.

Figure 1



Structural DNA assembly. **(a)** Scaffold-based assembly of 3D wireframe origami based on polyhedral-mesh strategy [6]. **(b)** Scaffold-free 3D assembly by using multiple unique DNA brick components [8]. **(c)** The folding of 2D DNA origami from a long DNA single strand, following the parallel crossover design principle [9]. **(d)** Temporal growth strategy for the production of long DNA single strands with user-defined sequence patterns [15]. **(e)** Bacteriophage-based mass production of DNA-strand components for DNA origami assembly [21•].

The concept of single-stranded DNA origami was recently reported by Yin, Yan and co-workers. It is based on a unimolecular folding of a long DNA single strand into a user-defined shape [9]. Unlike others, this approach relied on parallel crossovers rather than antiparallel crossover junctions to prevent kinetic traps caused by knotting, thus promoting the efficiency of unimolecular folding process (Figure 1c).

It is of note that DNA sequences in these structures are all unique, which significant increase the templating capability of these DNA nanomaterials. However, high complexity in DNA nanomaterials is not always necessary in some applications when considering the synthetic cost and assembly yield. By contrast to the DNA-intensive origami approach, our group and others introduced modular methods to create wireframe DNA nanostructures, where essential DNA components for structural and functional roles are retained [10–14]. The DNA-economical nature of these structures and their stability under physiological conditions enable their potential applications as cellular probes, molecular imaging agents and drug delivery vehicles. Our group recently reported an enzymatic approach, called temporal growth, to prepare a long DNA strand with desired sequence patterns from a small number of repeating building blocks with identical DNA sequences (Figure 1d). This long DNA backbone was used to guide the size-defined assembly of DNA nanotubes [15] and DNA tile arrays [16].

Similar to its DNA counterpart, RNA is an emerging building block for nanoscale construction, due to the diversity of RNA interactions and their 3D secondary structures [17•]. Andersen and co-workers demonstrated the co-transcriptional folding of RNA tiles, where a RNA single strand folds itself into shape while being transcribed. These RNA tiles can further assemble into hierarchical 2D lattices mediated by kissing–loop interactions [18•]. Very recently, the largest RNA nanostructures were generated by the single-stranded DNA origami approach discussed earlier (Figure 1c) [9]. It is foreseeable that these nascent RNA nanostructures will become powerful candidates for biological applications, given the numerous biological roles of RNA as compared to DNA.

Scaling-up and optimization of DNA nanomaterials

Scalability is a major challenge in translating DNA nanomaterials into practical uses. As such, a large-scale production of DNA nanomaterials though biotechnological processes is currently underway. Voigt and co-workers used reverse-transcriptase enzymes to express DNA single strands in bacteria, which can self-assemble into DNA crossover nanostructures inside the cells [19]. These DNA single strands could also be purified and used for further assembly. Building upon an enzymatic method developed by the Högberg group [20], Dietz and co-workers produced DNA single-stranded components of DNA origami by using a combination of bacteriophage and enzyme-free DNA cleavage [21•]. The 'staple' genes consist of individual staple sequences punctuated by Zn^{2+}-dependent self-excising DNAzyme moieties, which could break down a long DNA strand into individual staple strands after Zn^{2+} treatment (Figure 1e). The scaffold strand could also be generated during staple strand production. Strikingly, they could obtain a hundred-milligram quantity of folded DNA origami by using a laboratory-scale bioreactor. Yin, Yan and co-workers also synthesized long DNA scaffold single strands by applying molecular cloning techniques in bacteria, and used the resulting strands for folding single-stranded DNA origami [9].

In many DNA origami objects, the majority of strand components serves purely for structural purpose, and only some are used for functionality. Douglas and co-workers addressed this by redesigning a scaffold strand that could be folded with the repetitive binding of as few as 10 unique staple sequences, which significantly simplified the design and reduced synthesis cost [22]. In addition, the assembly of more complex DNA nanostructures typically requires high Mg^{2+} concentration and extremely long folding times (up to a week). To overcome this, several application-friendly conditions were examined such as an assembly in Na^{+}-based solution [23] and a rapid isothermal assembly [24–26]. Therefore, it can be envisaged that these production techniques can bring DNA nanotechnology into affordable industrial-scale applications.

Supramolecular DNA assembly

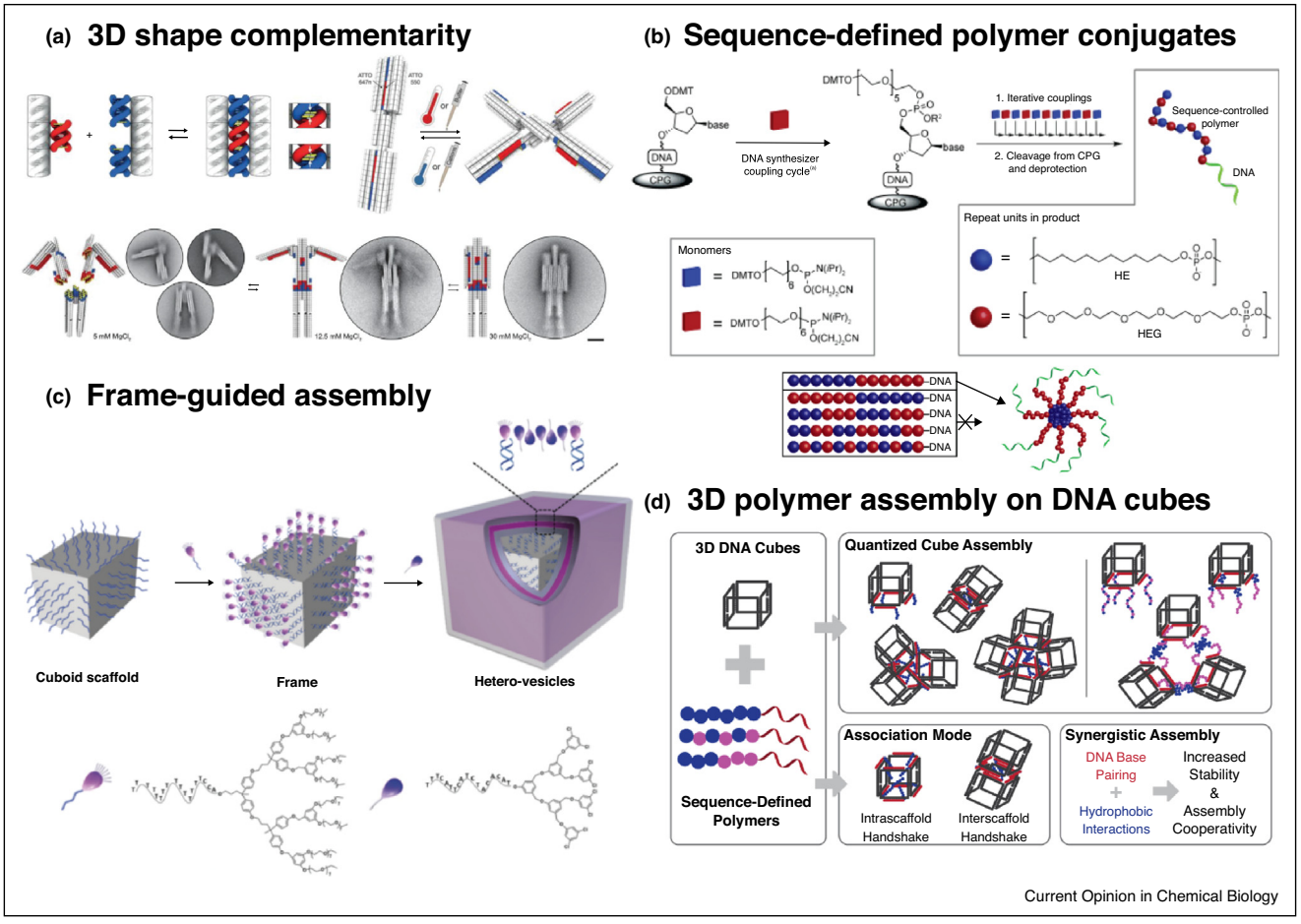
Blunt-end stacking in DNA assembly

Blunt-end stacking through π–π stacking of the terminal base-pairs between two DNA duplexes has recently become another main toolbox for selective DNA assembly. A systematic engineering of blunt-end stacking on DNA origami was investigated by Woo and Rothemund, showing that the stacking bond is sequence-dependent and number-dependent, and possesses stacking polarity, a similar concept to the polarity of DNA strands [27••]. 3D hierarchical assembly mediated by shape complementarity through blunt-end stacking was demonstrated by Dietz and co-workers [28•]. Dynamic conformational switching could be selectively achieved by merely altering cation concentration and temperature (Figure 2a). They were recently able to program the shape complementary to generate hierarchical DNA structures of up to 1.2 giga-dalton in high yields [29]. Furthermore, blunt-end stacking has been exploited to control 2D hierarchical assembly of DNA nanostructures such as extended networks of DNA origami and DNA tiles on supported lipid bilayers [30,31].

Assembly of amphiphilic DNA nanomaterials

DNA modification with hydrophobic moieties is an attractive approach to merge the programmability and

Figure 2



Supramolecular DNA assembly. **(a)** Reversible, hierarchical 3D assembly of DNA nanostructures governed by blunt-end stacking-mediated shape complementarity of their 3D components [28•]. **(b)** Sequence-dependent assembly of monodisperse, sequence-defined DNA–polymer conjugates synthesized by stepwise solid phase synthesis [34•]. **(c)** Templated heterovesicle formation from the assembly of DNA–dendron conjugates on DNA origami frame [44]. **(d)** Scaffold-directed assembly of sequence-defined polymer conjugates using 3D DNA cubes [46•].

anisotropy provided by DNA with the hierarchical and long-range organization of hydrophobic interactions [32•]. The synthesis of DNA–polymer conjugates typically involves attaching a whole polymer chain to a DNA strand. A new strategy functionalized a DNA strand with an initiator group, which was then used as a macroinitiator in the polymerization process to generate DNA–polymer conjugates [33]. Another efficient strategy developed by our group is to use a stepwise solid-phase synthesis to prepare monodisperse, sequence-defined DNA–polymer conjugates [34•,35]. This process is based on phosphoramidite chemistry and is highly compatible with an automated DNA synthesizer. The chemical nature, number and sequence order of monomer units, which were important parameters to determine their self-assembly behavior in solution, could be easily changed (Figure 2b). Our group recently demonstrated that self-assembled DNA micelles from these conjugates could be used as nanoreactors for hydrophobic modifications of DNA strands [36] and delivery vehicles of anticancer drugs [37].

In recent years, several research groups have focused on using DNA nanoscaffolds to site-specifically arrange hydrophobic moieties, direct their association modes, and obtain greater control on their hierarchical assembly [38–41. Di Michele, Cicuta and co-workers could induce the assembly of highly flexible four-way DNA junctions into 3D crystals by cholesterol modifications [42•]. Liu and co-workers used the hydrophobicity of DNA conjugates functionalized with hydrophobic poly(benzyl ether) dendron to create amphiphilic surface coating on DNA origami sheets [43]. They later used 3D DNA origami as a frame to prepare amphiphilic heterovesicles [44]. In this strategy, the DNA–dendron conjugates were hybridized to the complementary DNA strands on the origami frames, resulting in hydrophobically-driven

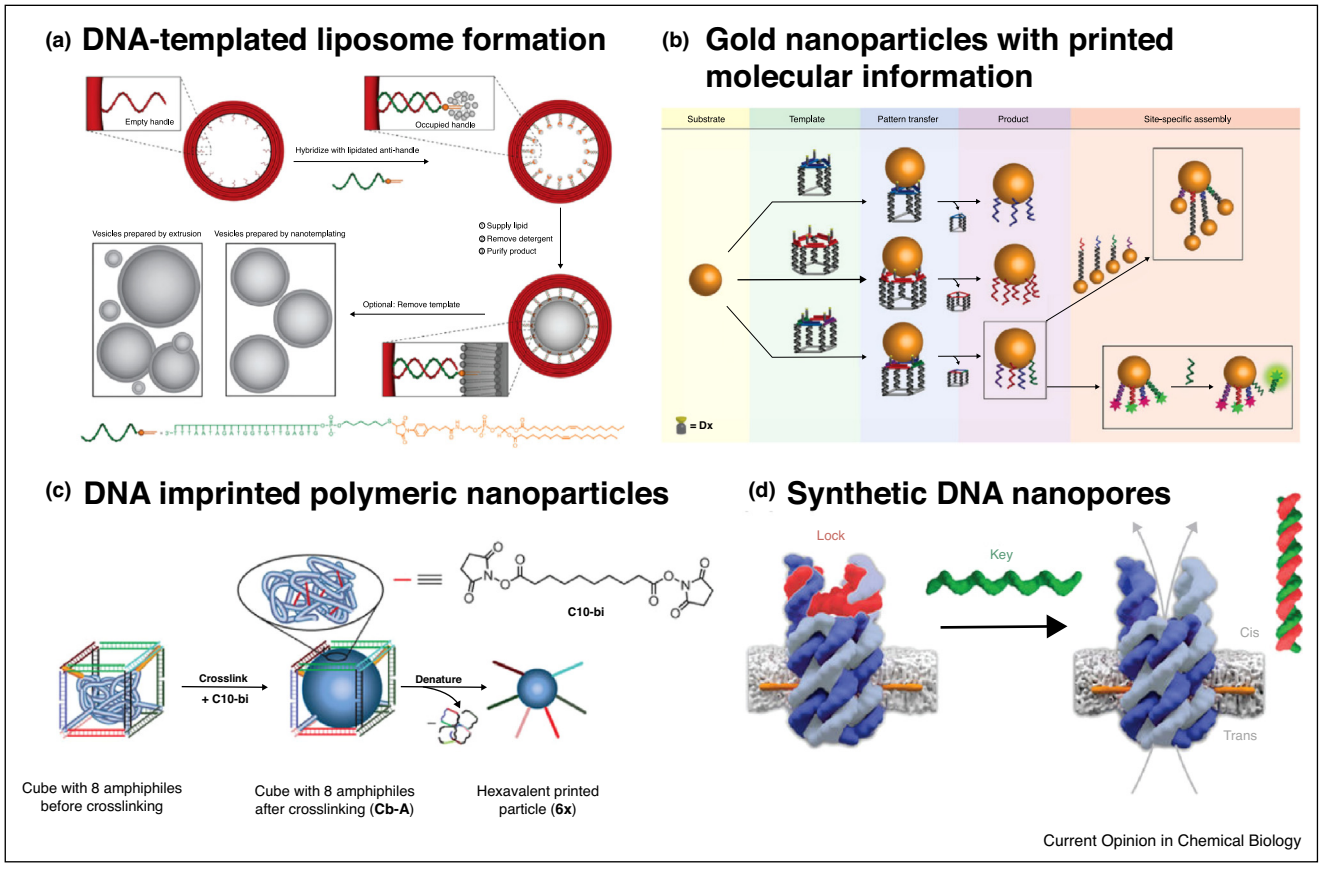
precipitation. An addition of non-complementary DNA–dendron conjugates or other amphiphilic molecules could re-disperse the precipitates and generate asymmetric vesicles along the frames (Figure 2c). Very recently, Mao and co-workers organized phospholipid–DNA conjugates in the cavity of a 'barrel-shaped' DNA origami, creating a confined hydrophobic environment for the reconstitution of individual integral membrane proteins [45].

Inspired by protein folding, our group demonstrated a directional assembly mode of sequence-defined polymers on a 3D DNA cube (Figure 2d) [46•]. When four hydrophobic oligo–alkyl conjugates were arranged on one face of DNA cube, an intermolecular association of these chains resulted in 'quantized cube assemblies', where chain length defined the number of DNA cubes that can be organized around a hydrophobic core. When eight conjugates were organized on both faces of DNA cube, an intramolecular association of these chains occurred inside the cube, forming a monodisperse micelle inside the cube ('DNA-micelle cube'), which could encapsulate hydrophobic guest molecules. Interestingly, unprecedented 'doughnut-shaped DNA cube-ring structures', where DNA cubes were organized into rings, formed when using specific sequences of amphiphilic polymers composed of alkyl chains and oligo(ethylene glycol) spacer chains. These assembly modes are a direct consequence of using monodisperse, sequence-defined DNA–polymer conjugates and would be challenging to achieve with classical, polydisperse polymers.

Selected emerging applications of DNA nanotechnology

With advances in DNA synthesis, DNA can now be routinely generated in almost any sequence and length. Combined with excellent knowledge in design principles and software developments, numerous applications of DNA nanomaterials have been demonstrated, particularly for the arbitrary organization of functional materials and for biomedical applications [47]. As an example, a DNA tetrahedron has been widely explored as an addressable platform for electrochemical/electrochemiluminescence biosensing [48,49] and intracellular fluorescence detection of cancer biomarkers [50,51]. In this

Figure 3



Emerging applications of DNA nanotechnology. **(a)** DNA-templated liposome formation by sized-controlled DNA nanorings [53•]. **(b)** 2D DNA pattern transfer from DNA cube 'nano-stamps' to gold nanoparticles [55•]. **(c)** Core-crosslinking of DNA-micelle cubes to generate DNA-imprinted polymeric nanoparticles with controlled DNA valency and positions [58•]. **(d)** Synthetic DNA nanopore for controlled transport of charged molecules across the lipid membranes [60•].

section, focus will be given to the most recent and emerging applications of DNA nanotechnology.

DNA nanomaterials have been applied as casting molds to transfer their shapes to both inorganic and organic materials. Bathe, Yin and co-workers designed a hollow, rigid DNA origami objects and attached gold seeds inside the cavity [52]. Silver nanoparticles with a shape defined by the dimension and geometry of the mold were subsequently grown from the gold seeds. As a more recent example, highly monodisperse and size-defined liposomes were generated by DNA templating as demonstrated by Lin and co-workers [53•]. This involved the binding of DNA–lipid conjugates to rigid DNA rings, serving as nucleation sites where templated-confined vesicle formation was then initiated by lipid addition (Figure 3a). This strategy was later applied to engineer designer shapes and dynamics of liposomes by the hierarchical assembly of DNA-cage templates [54].

Molecular information from DNA nanostructures can be transferred to other structures to create highly asymmetric materials. Our group [55•] and the Fan [56] group applied DNA 'nano-stamps' to transfer specific DNA patterns onto the surface of gold nanoparticle. These were used as building blocks for site-specific and hierarchical assembly of discrete gold clusters (Figure 3b). In a different approach, Eritja and co-workers used a DNA 'nano-stamp' to transfer DNA 'ink' patterns on a flat gold surface, an analogous process to photolithography [57]. Our group recently reported a templating approach to generate DNA-imprinted polymer nanoparticles. An amino-functionalized hydrophobic core of a DNA-micelle cube was covalently-crosslinked by multiple bifunctional linkers. After removing the DNA cube template, monodisperse DNA micelles with defined numbers and positions of DNA strands could be obtained, which can be used as anisotropic building blocks for further hierarchical assembly (Figure 3c) [58•].

Another promising application of DNA nanotechnology is its interface with lipid bilayers towards protein-membrane mimicry [59•]. Howorka and co-workers recently reported a design of cholesterol-modified DNA nanopore composed of six concatenated DNA strands with a 'lock' DNA strand functioning as a gate. Addition of the key strand will release the lock strand, thus opening the pore and allowing small molecule diffusion across the membrane (Figure 3d) [60•]. A recent work by Song, Castro and co-workers used DNA objects as an addressable interfacial platform by demonstrating programmed cell–cell adhesion mediated by the higher-order assembly of DNA origami functionalized on different cell surfaces [61]. Additionally, the long-range organization of DNA nanostructures has been a recent focus on supported lipid bilayers [30,31]. The fluidity of lipid bilayers could increase the order and result in long-range DNA assemblies over microns, which could be useful for surface patterning on bilayers.

Conclusions

DNA nanotechnology has presented a great opportunity to efficiently create designer structures, leading to their exploration as programmable materials in many research fields [47]. Recent developments on structural DNA nanotechnology include new design strategies that are suitable for creating complex and large DNA nanostructures, some of which are applicable for scale-up production by biotechnological processes. The optimization of DNA assembly process is also of particular focus to bring DNA nanotechnology into industry-friendly use. Futhermore, the incorporation of supramolecular interactions orthogonal to DNA base-pairing in DNA nanomaterials provides new design parameters by bringing new assembly modes and functions. Although blunt-end stacking and hydrophobic interactions are briefly covered in this review, other supramolecular interactions have also been introduced in DNA nanotechnology as well [2]. As such, more design variation and broader application scope of DNA nanomaterials can be envisaged in the future. The field has just started to progress towards the practical applications of DNA nanomaterials. This will require collaborative effort from multiple disciplines to overcome some issues including scalability and stability of DNA nanomaterials, as well as a balance between design simplicity and functional complexity, particularly for *in vivo* applications of DNA nanomaterials.

Conflict of interest statement

Nothing declared.

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