Opportunities and Challenges in DNA-Hybrid Nanomaterials

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ABSTRACT: Nature has inspired the development of many life-like materials. Although still simplistic, key biological functionalities have been incorporated, enabling a wide variety of applications. DNA-based systems, in particular, show high promise due to their ability to merge specific Watson–Crick base pairing with unique properties that are also programmable, scalable, or dynamic. By combining the fields of DNA-based covalent polymers, DNA origami, and DNA-functionalized supramolecular polymers, new frontiers in next-generation DNA-based hybrid materials that can outperform current bioartificial systems will be realized. Many challenges must still be overcome before this emerging technology can be materialized.

The cell and the cellular environment are an inspiration for many scientists. Complex tasks are performed in highly crowded spaces that are often prone to interference, yet these processes are regulated with high spatial and temporal control. For many years, scientists have attempted to understand cellular processes, and despite great success, much is yet to be discovered. In order to understand these complex phenomena and to improve our ability to manipulate them, artificial mimics are currently being developed, although they are simplistic compared to those occurring naturally (Figure 1). Within synthetic biology, where biological components are used to build novel functional biological systems, the field of DNA nanotechnology is rapidly expanding.

The structure of DNA opens up numerous possibilities in the biomedical field, due to DNA’s ability to bind sequences specifically and programmably through canonical Watson–Crick base pairing of its nucleobase substituents. DNA is able to be manipulated, for example, into various morphologies and implemented as complementary cross-linkers to form hydrogels.1,2 Because these interactions depend on multivalent noncovalent hydrogen bonding, reversibility is readily obtained, for example, by changes in salt concentration, temperature, or complementary deoxyoligonucleotide displacement strands. In order to retain the programmable properties of DNA with high spatial precision, DNA by itself has also been used as a building block to construct various elegant but complex structures known as DNA origami.

Although DNA holds great potential, the negative charge on the structure restricts cell internalization, and the backbone is prone to hydrolysis and enzymatic degradation. In order to increase stability and to include new functionalities that are not obtained by DNA alone, a wide variety of DNA-based materials have been developed. These materials incorporate a range of bioconjugates, from nucleobases and nucleosides that include the universal DNA hydrogen-bonding recognition array to long deoxyoligonucleotides that also have regular helicity due to the presence of the phosphate backbone.3-5

In this Perspective, we highlight the challenges and opportunities of DNA-based materials, with a focus on the fields of covalent polymers, DNA origami, and supramolecular polymers. It has become clear that these fields complement each other and could potentially be combined in next-generation DNA-hybrid materials for future biomedical applications (Figure 2). We review the steps already taken toward the development of DNA-hybrid materials and the potential for future developments.

DNA-Based Covalent Polymers. Covalent polymers are synthesized by the polymerization of small monomers (e.g., acrylates and methacrylates), which is initiated by radicals, cations, or anions. Aside from homopolymers, random copolymers and block copolymers can be fabricated by seedung monomers with different substitutions. Covalent polymerization is ideal to form long and stable structures and is, hence, highly scalable (Figure 2). Typically, the molecular weight distribution, polydispersity, and chain-end functionality can be controlled using living radical polymerization techniques. Aside from controlling structural properties, the degradation of covalent polymers can be tuned by the incorporation of ester groups.6 Diverse DNA-based covalent polymers have already been developed, although they are simplistic compared to those occurring naturally (Figure 1). Within synthetic biology, where biological components are used to build novel functional biological systems, the field of DNA nanotechnology is rapidly expanding.

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been developed, ranging from single nucleobases to oligonucleotide grafts. The latter includes designs where oligonucleotide sequences are grafted to, from, or through, creating polymers with various architectures.

Although covalent polymers show promising developments, some living radical polymerization techniques, including atom transfer radical polymerization (ATRP), require a metal catalyst to initiate the polymerization reaction. These metals can potentially chelate with nucleotides, and remnants are difficult to remove, which may result in cell toxicity in biomedical applications. Moreover, overall synthesis yields tend to be low due to solubility issues, and only partial functionalization is obtained when using a postpolymer modification strategy. In order to circumvent these limitations, reversible addition-fragmentation chain-transfer (RAFT) polymerization strategies can be utilized with prefunctionalized protected nucleotide monomers. Using this technique, bulky biofunctional groups may still cause steric hindrance during polymerization; however, this effect can be minimized by tuning the linker length.

A major disadvantage of covalent polymers is that it is difficult to achieve spatial functionalization and to implement dynamics that allow local rearrangements in the backbone. Moreover, covalent polymers tend to form random coils or single-chain nanoparticles in solution, shielding biofunctional group availability and, hence, sterically hindering host–guest binding.

Regardless of these intrinsic shortcomings, DNA-based covalent polymers have already found applications in sensors, DNA and drug delivery, cancer therapy, stimuli-response hydrogels, logic gates, and actuators. Recent efforts have demonstrated free radical polymerization in situ within living cells, enabling intracellular manipulation, control, and tracking, paving the way toward unprecedented biomedical applications. Although exciting results have been obtained, all of these applications remain in their infancy due to low signal sensitivity and slow response rate, limiting accurate quantification and single-base mismatch discrimination. Our understanding of the interactions between DNA-based covalent polymers and cells, including other biomolecules, cellular components, and vesicles, is incomplete, and further research will be required to extend their application potential.

**DNA Origami Noncovalent Structures.** Hierarchical structure and spatial control is readily obtained with DNA origami (Figure 2).1 By folding a long bacteriophage-derived single-stranded DNA into a desired structure, any two-
dimensional (2D) or three-dimensional (3D) morphology can be fabricated by including hundreds of short staple strands forming crossovers by so-called Holliday junctions. Nanometer spatial control over functionalized groups can be implemented by designing staple strands with a “toehold”. Functionalized moieties with a complementary sequence to this toehold can then be coupled. By tuning the length of the complementary sequence, functionalized moieties can be tightly anchored or be dynamic and, hence, in equilibrium with the aqueous solvent. This tunability and binding equilibrium ($k_{on}$ and $k_{off}$) has shown to improve fluorescence microscopy down to sub-nanometer resolution, identified as DNA point accumulation for imaging in nanoscale topography (DNA-PAINT) super-resolution microscopy.8

DNA-origami-based structures have already found promising applications from molecular robots and nanoreactors to drug delivery and cell internalization.1,9 High control over the dimensions, functionality, and manipulation, however, comes with the sacrifice of poor scalability.10 Furthermore, hundreds of staple strands are required for one origami structure, which means that building more complex multicomponent structures and preparing larger quantities is prone to high costs and assembly errors. Lastly, error quantification and structural purification are still challenging. Currently, various techniques are being developed to overcome this limitation, including hierarchical assembly using interaction motives and multi-scaffold designs.11,12 Recently, Dietz et al. developed rings and polyhedra that span megadalton (MDa) and even gigadalton (GDa) sizes, approaching dimensions of 450 nm in diameter (Figure 3A),11 illustrating the potential of this approach.

**DNA-Functionalized Supramolecular Polymers.** Moving closer to life-like materials, supramolecular polymers mimic natural processes in the sense that they are modular, tunable, adaptable, and responsive.13 Due to a combination of weak noncovalent interactions, usually in the form of hydrophobic effects, hydrogen bonding, and $\pi-\pi$ interactions, the small molecular building blocks self-assemble into one-dimensional (1D) fibrous structures in which the monomers can migrate both within and between fibrous assemblies, providing these structures with intrinsic dynamic properties (Figure 2).14 These structures require a precisely balanced hydrophilic-to-hydrophobic ratio; otherwise, spherical micelles are obtained. Moreover, extending monomers with bulky biofunctional groups, such as peptides and proteins, can interfere with self-assembly due to an imbalance in the hydrophilic-to-hydrophobic ratio and/or noncovalent interactions, such as hydrophobic and hydrogen bonding. Aggregate stability can be tuned by improving noncovalent interactions and, hence, the packing between monomers. However, monomers could potentially be released from their supramolecular polymeric support by pulling forces exerted by cells.

Figure 3. DNA-based nanomaterials. (A) DNA origami structures are assembled into a 1.2 GDa hierarchical polyhedral using interaction motives. Adapted and modified with permission from ref 11. Copyright 2017 Springer Nature. (B) Supramolecular DNA–peptide amphiphiles self-assemble into reversible intertwined superstructures. Adapted and modified with permission from ref 17. Copyright 2018 American Association for the Advancement of Science. (C) DNA platform is folded into a DNA tube upon which polymers are grafted using atom transfer radical polymerization. Corresponding atomic force microscopy images are depicted to the right. Adapted and modified with permission from ref 29. Copyright 2018 The Royal Society of Chemistry.
Interesting supramolecular polymers containing deoxyligo-nucleotides have found applications in tissue engineering, protein recruitment, and logic gates, showing life-like and responsive properties not found in covalent polymers and DNA origami. Hänner and co-workers observed that supramolecular assemblies functionalized with single nucleotides formed 2D sheets as opposed to 1D fibers when longer deoxynucleoside linkers were incorporated. This finding highlights that molecular design plays a significant role in the aggregate morphology that supramolecular polymers form. The importance of molecular design was also illustrated in extensive studies by Stupp and co-workers using complementary DNA–peptide amphiphiles. Spherical micelles obtained by the individual components formed rapid intertwined superstructures upon mixing (Figure 3B) that were shown to be reversible upon the addition of displacement deoxynucleotide strands. In supramolecular polymers, the intrinsic dynamic property aids in backbone rearrangements for increased order and optimal host–guest binding. However, as a result, sequence control and, hence, programmability are difficult to achieve. In addition, because supramolecular polymerization is prone to fall into kinetic traps, protocols that govern self-assembly are critical and fundamental understanding of their pathway complexity is required.

Toward DNA-Hybrid Materials. The field of DNA nanotechnology has been advanced by combining major developments in the fields of biology, physics, chemistry, material science, and computer science. Incorporating small organic and inorganic molecules into DNA origami structures has already improved stability and rigidity, as compared to DNA-only counterparts. In addition, fewer DNA staple strands are required, enabling the creation of more complex structures that are better suited for in vivo applications. As well as the four standard DNA nucleobases, a handful of DNA derivatives have been developed that show improved stability and lower immunogenicity. This development increases the DNA toolbox and opens up further possibilities to build structurally diverse materials with highly programmable and spatially addressable properties.

By taking advantage of the self-assembly of amphiphilic structures, Sleiman and co-workers developed nanocages with grafted polymers. Upon self-assembly, these cages formed higher-order structures containing hydrophobic cores, in which the cage aggregation number was dependent on the hydrophobic chain length. Therapeutic molecules or nanoparticles could be encapsulated within these hydrophobic cores, nanocages decorated with cholesterol can form nanoparticles in membranes, and DNA patterns can also be printed on gold nanoparticles using sequence-grafted DNA nanostructures.

More complex hybrid materials could potentially be achieved by merging the fields of covalent polymers, DNA origami, and supramolecular polymers. Interests hybrid DNA-based materials have already been developed in the group of Tanja Weil. Using a DNA origami template, polymers were grafted in a pattern, which enabled the fabrication of patterned polymers with nanometer resolution. This approach was extended to fabricate polymer-coated DNA tubes (Figure 3C) containing DNAzymes in the interior of the tube. The obtained polymer tube was shown to support catalytic activity and to provide protection against nuclease digestion of these DNAzymes. This approach opens up the possibility of delivering labile cargo and spatially functionalizing either the inside or outside of DNA tubes with nanometer precision; such high-order control over covalent polymers has not been achieved elsewhere. These exciting examples set the stage for nanometer hierarchically controlled micro- and macrostructures that have not been possible until now.

CONCLUSIONS AND PROSPECTS

DNA-based covalent polymers, DNA origami, and DNA-functionalized supramolecular polymers have shown emergent properties in a wide variety of applications. Yet these materials are still simple compared to naturally occurring biomolecules, restricting their potential for sophisticated applications. Knowledge and techniques from multiple fields are required to design more complex and functional DNA-hybrid materials to advance the field forward. Detailed fundamental understanding of nucleotide–material interactions is also essential to equip polymers with full DNA functionalities. Novel exciting DNA-hybrid materials are already emerging that enable more control over nanometer spatial resolution, moving the field closer to real-life properties and unprecedented biomedical applications, such as in designing artificial materials that can direct organogenesis, drug delivery, enzymatic activity, and gene editing with remarkably high precision. By merging the fields of covalent polymers, DNA origami, and supramolecular polymers, DNA-hybrid materials that are scalable, programmable, and possess intrinsic dynamic properties can potentially be realized, providing combined properties that cannot be obtained by any of these materials individually.

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Notes

The authors declare no competing financial interest.

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