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 seeding 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

Figure 1. Complex natural structures as compared to simple, human-made artificial materials.

Figure 2. DNA-based covalent polymers, DNA origami, and DNA-functionalized supramolecular polymers with their advantages and disadvantages. Merging these fields enables one to incorporate the best properties of all three fields into next-generation DNA-hybrid materials.

77 been developed, ranging from single nucleobases to
78 oligonucleotide grafts.^{3–5} The latter includes designs where
79 oligonucleotide sequences are grafted to, from, or through,
80 creating polymers with various architectures.

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

97 A major disadvantage of covalent polymers is that it is
98 difficult to achieve spatial functionalization and to implement
99 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.³ Recent efforts have
demonstrated free radical polymerization *in situ* within living
cells, enabling intracellular manipulation, control, and tracking,
paving the way toward unprecedented biomedical applica-
tions.⁶ 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 quantifi-
cation and single-base mismatch discrimination. Our under-
standing 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

Hierarchical structure and spatial control is readily obtained with DNA origami.

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 π - π 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.

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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.

174 Interesting supramolecular polymers containing deoxyoligo-
175 nucleotides have found applications in tissue engineering,
176 protein recruitment, and logic gates, showing life-like and
177 responsive properties not found in covalent polymers and
178 DNA origami.¹⁵ Häner and co-workers observed that supra-
179 molecular assemblies functionalized with single nucleotides
180 formed 2D sheets as opposed to 1D fibers when longer
181 deoxyoligonucleotide linkers were incorporated.¹⁶ This finding
182 highlights that molecular design plays a significant role in the
183 aggregate morphology that supramolecular polymers form. The
184 importance of molecular design was also illustrated in extensive
185 studies by Stupp and co-workers using complementary DNA–
186 peptide amphiphiles.¹⁷ Spherical micelles obtained by the
187 individual components formed rapid intertwined super-
188 structures upon mixing (Figure 3B) that were shown to be
189 reversible upon the addition of displacement deoxynucleotide
190 strands. In supramolecular polymers, the intrinsic dynamic
191 property aids in backbone rearrangements for increased order
192 and optimal host–guest binding. However, as a result,
193 sequence control and, hence, programmability are difficult to
194 achieve. In addition, because supramolecular polymerization is
195 prone to fall into kinetic traps, protocols that govern self-
196 assembly are critical and fundamental understanding of their
197 pathway complexity is required.^{18,19}

198 **Toward DNA-Hybrid Materials.** The field of DNA
199 nanotechnology has been advanced by combining major
200 developments in the fields of biology, physics, chemistry,
201 material science, and computer science.^{20,21} Incorporating
202 small organic and inorganic molecules into DNA origami
203 structures has already improved stability and rigidity, as
204 compared to DNA-only counterparts.²² In addition, fewer
205 DNA staple strands are required, enabling the creation of more
206 complex structures that are better suited for *in vivo*
207 applications. As well as the four standard DNA nucleobases,
208 a handful of DNA derivatives have been developed that show
209 improved stability and lower immunogenicity.^{23,24} This
210 development increases the DNA toolbox and opens up further
211 possibilities to build structurally diverse materials with highly
212 programmable and spatially addressable properties.

213 By taking advantage of the self-assembly of amphiphilic
214 structures, Sleiman and co-workers developed nanocages with
215 grafted polymers.²⁵ Upon self-assembly, these cages formed
216 higher-order structures containing hydrophobic cores, in which
217 the cage aggregation number was dependent on the hydro-
218 phobic chain length. Therapeutic molecules or nanoparticles
219 could be encapsulated within these hydrophobic cores,
220 nanocages decorated with cholesterol can form nanopores in
221 membranes, and DNA patterns can also be printed on gold
222 nanoparticles using sequence-grafted DNA nanostructures.^{26,27}

223 More complex hybrid materials could potentially be
224 achieved by merging the fields of covalent polymers, DNA
225 origami, and supramolecular polymers. Interesting hybrid
226 DNA-based materials have already been developed in the
227 group of Tanja Weil. Using a DNA origami template, polymers
228 were grafted in a pattern, which enabled the fabrication of
229 patterned polymers with nanometer resolution.²⁸ This
230 approach was extended to fabricate polymer-coated DNA
231 tubes (Figure 3C) containing DNazymes in the interior of the
232 tube. The obtained polymer tube was shown to support
233 catalytic activity and to provide protection against nuclease
234 digestion of these DNazymes.²⁹ This approach opens up the
235 possibility of delivering labile cargo and spatially functionaliz-
236 ing 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 under-
standing 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, program-
mable, 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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