Imparting the unique properties of DNA into complex material architectures and functions

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While the remarkable chemical and biological properties of DNA have been known for decades, these properties have only been imparted into materials with unprecedented function much more recently. The inimitable ability of DNA to form programmable, complex assemblies through stable, specific, and reversible molecular recognition has allowed the creation of new materials through DNA’s ability to control a material’s architecture and properties. In this review we discuss recent progress in how DNA has brought unmatched function to materials, focusing specifically on new advances in delivery agents, devices, and sensors.

Introduction
DNA is a remarkable biopolymer with molecular recognition properties far surpassing any synthetic analog. The predictability and ease of DNA–DNA binding, the creation of algorithms for the de novo design of new assembled structures, and the ability to synthesize DNA oligonucleotides with an almost unlimited range of sequences and functional end-groups has allowed the production of frameworks for the rational design of materials with programmable shape, size, and function. In this review, we examine some key examples in which the unique qualities of DNA have enabled the creation of an entire new set of devices and sensors with previously unmatched complexity and function. For example, not only have numerous 2D and 3D nano- and mesoscale DNA architectures been designed and synthesized to highlight the power and generality of DNA self-assembly; these architectures have also been employed for the delivery of therapeutics and the study of chemical reactions. In addition, the creation of nanoparticle-oligonucleotide conjugates can serve as building blocks for controlled nanoparticle assemblies ranging from simple dimers to complex 3D heterostructures as a framework for fabricating new device architectures. Finally, the reversible, single-molecule interactions of DNA aptamers and DNAzymes with specific analytes can be transduced into a variety of detection signals for new materials for sensors and in vivo imaging agents.

Assembly of DNA into programmed discrete structures in two and three dimensions
DNA is composed of four nucleotide bases, adenosine (A), guanine (G), thymine (T), and cytosine (C), where hybridization between A–T and C–G produces a double helix consisting of anti-parallel complementary strands. Once hybridized, the persistence length increases from ~1 nucleotide for single stranded DNA (ssDNA) to about 100 bases for double stranded DNA (dsDNA), and this rigidity allows self-assembled DNA structures to hold their shape upon assembly. The optimization of solid-phase synthesis has facilitated the production of synthetic oligonucleotides with almost any sequence, providing materials engineers with an unlimited selection of designs for different self-assembled 2- and 3D DNA architectures. In more recent years, the expansion of design rules into computer algorithms has led to a virtual explosion in the number of DNA scaffolds that can be created for a wide variety of applications that include nanomaterial assembly, biosensors [1], drug delivery [2], computation [3,4], and biomolecular actuation [5–7]. In their pioneering work, Seeman

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and co-workers provided the initial guidelines for forming tetrameric complexes in solution from immobile DNA junctions [8,9]. Four different strands of DNA cooperated to form a junction in which each arm contained free ssDNA that could hybridize to an adjacent junction, resulting in a square-like, branched-DNA unit (Fig. 1a). A key aspect of this assembly is the design of DNA strands that each bind simultaneously to different complementary partners, allowing the joining of multiple strands into complex architectures.

This basic motif has since been elaborated into larger and more complex 2- and 3D assemblies tuned toward addressing specific applications in materials science. For example, targeted delivery of bioactive cargo is of importance for treating cancer and other diseases, but monodisperse vehicles with predictable delivery profiles have been difficult to create. A 3D DNA tetrahedron has been demonstrated as a potential carrier for this application: the precisely sized scaffold can encapsulate proteins [10] (Fig. 1b) or peptides [11], while the superstructure can be easily transported to the target thanks to its innate biocompatibility. These multivalent DNA structures can also be easily modified with additional functional oligonucleotides [12] added to their exteriors or can be specifically shaped [13] to provide resistance to nuclease degradation and facilitate cell uptake. Because of these unique attributes, the DNA tetrahedron structure has been successfully used for the in vivo delivery of siRNA for cancer targeting and therapy; a truly remarkable achievement [14]. Mimicking the well-defined and homogeneous structure of these DNA carriers with synthetic systems is difficult, but DNA allows for the specific programming of the orientation and number of modifications per delivery platform for optimal delivery efficacy, whereas polymer particles and assemblies typically created through bulk emulsification or precipitation lack the same precise control over size and surface.

DNA nanostructures have also been incorporated into and used to form active hydrogels, which can be applied as novel biomaterials for tissue engineering and scaffolds for ‘smart’ delivery and sensing. For example, simple, branched DNA nanostructures ligated together under controlled conditions to form cross-linked structures exhibit the familiar viscoelastic mechanical properties of hydrogels, allowing facile programming of such properties over the degree of crosslinking, strength of crosslinking, and length between junctions [15]. Control over hydrogel properties is extremely important for tissue engineering in particular, as the cellular growth scaffold must mimic that of the desired tissue or growth environment. By tuning the initial DNA branched structure, gel-loading (e.g. with DNA-binding drugs, insulin, and even mammalian cells), and environmental conditions, the material’s swelling profile, tensile modulus, and degradation rate (drug release rate) may be controlled accordingly. In addition, an exciting variation of DNA hydrogels, ‘meta-hydrogels,’ has been engineered to act as a mechanical metamaterial that can exhibit liquid-like properties when outside of water but solid-like properties when immersed in water, acting as a water-induced switch (Fig. 1c) [16].

Another significant breakthrough in DNA nanotechnology, called ‘DNA origami,’ was made by Rothemund, in which ssDNA isolated from the M13 bacteriophage was folded into compact 2D DNA architectures [17]. Rather than assembling many short strands into a larger structure, the long viral ssDNA is allowed to react with many short oligonucleotides, or ‘staples’, producing a vast library of different DNA configurations ranging from simple geometrical shapes to complex maps of the world (Fig. 1d) in only
a few steps. Following Rothemund’s initial work, a diverse range of DNA origami structures have been synthesized and applied as active materials or templates for various research areas. For example, Sugiyama and co-workers applied ‘window’-shaped DNA origami as a platform to study the interaction of EcoRI protein with DNA strands placed under tension [18]. Similarly, Gothelf and co-workers used rectangular DNA scaffolds as breadboards for running different chemical reactions for nanoscale combinatorial chemistry [19]. DNA origami has also been applied toward engineering complex 3D systems; for example, Yan and co-workers recently created exquisite 3D DNA shapes with large curvature (Fig. 1e) [20]. Highly novel examples of 3D origami systems designed for biological applications include the fabrication of stimuli-responsive DNA boxes and cages. These 3D structures can be controlled to ‘lock’ and ‘unlock’ the accessibility of oligonucleotide [21] or protein [22] targets, allowing the controlled release of a payload from within the heart of the structure. Similar to the aforementioned DNA tetrahedron, the ability to engineer multivalent 3D, stimuli-responsive origami structures that can be easily modified with nanoscale precision may have a significant impact on applications such as controlled release or active transport in both in vitro and in vivo settings.

**DNA-mediated assembly of nanomaterials for device fabrication**

The advent of nanotechnology has brought to existence a diverse set of nanomaterials possessing unique electronic, optical, or magnetic properties. One of the grand challenges associated with engineering functional materials or devices from such nanoscale objects has been how to integrate and assemble them into hierarchical arrays with minimal defects. However, as demonstrated in initial groundbreaking work by Alivisatos and Mirkin [23,24], these particles and surfaces are generally easily modified with oligonucleotides that can direct the organization of nanomaterials into different packing densities and arrangements for a variety of applications. By varying the length and sequence of the DNA on the nanoparticles, core-satellite structures [25] or discrete geometrical organizations [26,27] have been formed in which the DNA controls not only the distance between particles [28] but also the angle of packing [29]. Particle distances may also be adjusted within an already assembled structure through dynamic hybridization schemes such as hairpin structures [30]. Beyond discrete nanoparticle clusters, Mirkin and Gang recently reported methods to create well-ordered bulk nanoparticle solids (Fig. 2a) [31,32]. Specifically, tuning hybridization between two particles led to gold nanoparticle (AuNP) superlattices with either face-centered-cubic (FCC) or body-centered-cubic (BCC) orientation. Since their first demonstration, further studies have clarified the effect of particle size, hydrodynamic size, and length of DNA on assembly [33], and similar techniques have been applied to create mixed-particle systems of AuNPs and quantum dots [34]. It is worth noting that this degree of tunability in nanoparticle ordering, relative distance, and packing cannot be easily achieved with synthetic polymers or small molecule systems, nor is it trivial to obtain thermodynamically stable nanoparticle superlattices through simple thermal annealing.

Anisotropic nanomaterials may also be organized with similar tools. The size and shape of metal nanostructures determine their plasmon behavior, and an understanding of the different facets on a given structure can allow face-selective DNA functionalization for engineering new optically active materials. For example, Mirkin and co-workers recently created a hexagonal close-packed superlattice of Au nanorods by using different DNA sequences and lengths (Fig. 2b) [35], and Mann and co-workers described a similar example in which nanorods were assembled in 2D along their long axes to maximize DNA overlap and hybridization [36].

**FIGURE 2**

(a) Amorphous and crystalline 3D gold nanoparticle arrays assembled through DNA interactions. $T_{\text{pre}}$ and $T_{\text{m}}$ encode for DNA premelting and melting temperatures, respectively [32]. (b) Schematic illustration and TEM image of superlattices with ordered (i) nanorod (55 nm length, 14 nm width), (ii) nanoprim (140 nm edge length), (iii) rhombic (64 nm diameter) and (iv) octahedra (83 nm diameter) nanoparticles [35]. (c) DNA origami mediated assembly of gold nanoparticles on lithographically patterned surfaces [43].
DNA has also guided the assembly of various anisotropic nanoparticles such as nanoprisms and rhombic dodecahedra. In these cases, the inherent difference in surface energies in anisotropic colloidal particles allowed selective functionalization of the sides of the particles for building unique hybrid structures [37,38]. Furthermore, since DNA hybridization is thermally reversible, the plasmon responses of DNA-nanorod assemblies can be easily and controllably tuned through simple temperature changes [39].

DNA-guided assembly directly on surfaces has also been shown as a promising method for the creation of nanoelectronic, nanophotonic, or optoelectronic solid-state devices, as such materials often require control over nanomaterial placement, organization, and orientation in both two and three dimensions. The overall strategy of these methods is to use complementary and/or orthogonal sequences in both surface-bound and particle-bound DNA to direct the assembly of particles both to a surface and to each other. DNA is deposited onto a surface through either physical interaction with a hydrophilic surface (typically patterned from a hydrophobic resist) or covalent conjugation through an end group on the DNA. For example, 2D DNA origami has been deposited onto areas etched by either e-beam lithography or photolithography, followed by hybridization and deposition of DNA-AuNPs onto the etched surface [40–42]. Because of the mesoscale size regime of DNA origami, methods to merge top-down lithography with bottom-up self-assembled DNA have been developed to produce precise ordered arrays of 5 nm AuNPs (Fig. 2c) [43,44]. These DNA origami can also in principle be extended to organizing other nanomaterials on surfaces, including carbon nanotubes (CNTs) [45] or biomolecules (Fig. 3a,b) [46–48]. Although CNTs are typically very difficult to disperse in aqueous media without the addition of surfactants [49] or chemical modification of the nanotube surface [50], ssDNA can solubilize CNTs through π–π stacking of the DNA bases to the CNT sidewall [51]. Specific DNA oligonucleotides have also been shown to bind to specific CNT widths and chiralities [52], empowering DNA for both CNT purification and assembly (Fig. 3c,d) [53,54].

In addition, cost-effective printing methods have been developed to produce patterned domains of large-area close-packed nanoparticle films on substrates. For example, nanoparticle superlattices with long range order were obtained within DNA arrays patterned by traditional micro-contact printing or inkning-subtraction-printing [55], by use of thermal annealing to promote hybridization between the DNA-AuNPs and the surface DNA [56]. Similar techniques have been applied to create 3D particle assemblies on substrates with surface strands used to promote interparticle hybridization [57]. The ability to organize nanoparticle arrangements on surfaces through simple tuning of DNA hybridization exemplifies the power of a self-recognizing polymer such as DNA for controlling particle packing in multiple dimensions. The typical approaches for engineering ordered thin films of nanoparticles on substrates to date have been to use electrostatic interactions [58], spatial confinement [59], or air–liquid interfaces [60]. Using DNA interactions to drive particle ordering provides a framework to assemble a diverse set of nanoparticle sizes and compositions while circumventing barriers to manufacturing such as needing high nanoparticle concentrations or slow evaporation. Furthermore, because DNA interactions can be programmed to include flexible, compressible sections, polydisperse nanoparticle batches may also be assembled into well-ordered arrangements [33–35].

**Tunable DNA switches as general platforms for sensitive and specific analyte detection**

The unique ability of DNA to bind to itself or other analytes has also been employed to create powerful new diagnostics. A typical clinical sample contains only a minute amount of a particular

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**FIGURE 3**

DNA origami is used to pattern (a) gold nanoparticles of different sizes [47] and (b) silver nanoparticles in specified locations [48]. Carbon nanotubes aligned by DNA on (c) Si substrates [53] and (d) across gold electrodes [54].
biomarker in the presence of many other proteins, peptides, small molecules, and cells, so sensors are needed that boast high sensitivities and specificities, stability in a range of conditions, and favorable cost-to-benefit ratios. DNA has already been utilized heavily in oligonucleotide detection and gene sequencing, and DNA’s self-complementarity and library of different sequences have made it an ideal choice for labeling and identifying particular biomarkers through a ‘biobarcode’ that can be amplified and sequenced for multiplexed detection [61–63]. However, in this review we will focus specifically on the ways in which tuning the energies of DNA binding with itself or other analytes has made DNA a powerful tool for engineering sensors that actuate a unique response to analyte binding.

In the simplest scenario, one or more detection strands are made complementary to a nucleic acid analyte, and the act of binding causes the emission of a detectable signal or change in signal. In a common example, changes in fluorescence can be observed via quenching or Förster Resonance Energy Transfer (FRET) by utilizing the DNA-analyte exchange to change the distance between a donor and acceptor dye or quencher. Other common detection schemes include colorimetric via plasmon shifts in aggregated nanoparticles (Fig. 4a) [64,65], electrochemical via changes in current and voltage [66], magnetic via changes in T2 relaxivity with iron oxide nanoparticles [67], and, more recently, Surface Enhanced Raman Spectroscopic (SERS), in which DNA recognition brings the Raman active reporter directly into the ‘hot spot’ between two metallic nanoparticles [68]. In order to sense analytes other than nucleic acids, aptamers of DNA or RNA have been found that bind with high specificity to substrates such as small molecules, peptides, or proteins. To generate a signal upon aptamer–ligand binding, a sensing strand is displaced from its complement to bind to a more strongly binding non-DNA analyte; this displacement mechanism has been employed for fluorescence sensing (Fig. 4b) [69], MRI contrast agents (Fig. 4c) [70], and SERS sensors (Fig. 4d) [71]. Another interesting use of this type of displacement mechanism is to change the properties of DNA–polymer networks. For example, Tan and co-workers have designed hydrogels composed of polymers crosslinked with hybridized DNA–aptamers, which become soluble after the analytes cause the decrosslinking of the network [72–74]. For in vivo applications, the aptamers’ affinities for both soluble and bound biomarkers can bias the accumulation of contrast agents into diseased sites [75] or even actuate changes in imaging signal [76]. In one example of the latter, the presence of elevated levels of thrombin, such as those found in a growing blood clot, caused a gas-filled microbubble to change its response and contrast under ultrasound (Fig. 4e); an aptamer displacement mechanism similar to that described for the aforementioned hydrogels caused the decrosslinking of a stiff exterior shell then in turn changed the mechanical properties of the bubble [77].

Other DNA sequences known as DNAzymes are able to undergo structural conformational changes upon binding to induce catalytic covalent cleavage of specific substrates. DNAzymes are useful because they possess lower cost, higher thermal tolerance, and

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**FIGURE 4**

(a) Color changes are caused by aggregation of GNP aggregation or dispersion due to aptamer binding of cocaine [64]. (b) A tripartite, fluorescence-quenching assembly [69]. (c) An adenosine-responsive gadolinium MRI contrast agent [70]. (d) Gold nanoparticles (GNP) with adsorbed thrombin binding aptamer (TBA) and SERS active dye will decrease their Raman signal in the presence of thrombin [71]. (e) Contrast enhanced sonography of TBA crosslinked microbubbles. Acoustic signal will increase in presence of thrombin as shell becomes more fluid [77].
smaller size than their protein enzyme counterparts while preserving much of their efficacy. For example, researchers have also been able to isolate DNAzymes that require specific metal ions to catalyze hydrolysis of a ribonucleic acid bond. These DNAzymes can thus sense for their required ion cofactor; examples of which include Cu\(^{2+}\) [78], Mg\(^{2+}\) [79], Zn\(^{2+}\) [80], UO\(_2^{2+}\) [81], and Pb\(^{2+}\) (Fig. 5a) [82]. Liu and co-workers in particular have shown success with a Pb\(^{2+}\)-dependent DNAzyme with detection methods that include a fluorophore/quencher pair [83], electrochemical detection [84], and gold nanoparticle-based aggregation methods [85].

As with detection antibodies in ELISAs, DNAzymes can also be conjugated to a variety of different biomolecular or synthetic substrates to amplify signal generated from a detected analyte such as DNA [86], microRNA [87], or other biomolecules [88]. For instance, the horseradish peroxidase mimic DNAzyme (HRP–DNAzyme) folds into a stable G-quadruplex to complex a molecule of hemin, which in the presence of hydrogen peroxide generates a colorimetric or chemiluminescent signal from 2,2'-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) (ABTS) or luminol, respectively [89,90]. This DNAzyme was utilized in an assay in which carcinoembryonic antigen (CEA) was captured to antibody-conjugated paramagnetic microspheres, followed by detection with gold nanoparticles decorated with both anti-CEA and HRP–DNAzyme complexes to produce a chemiluminescent signal detectable down to CEA concentrations of 4.1 pg mL\(^{-1}\) (Fig. 5b) [91]. In another example, proteins at the very tip of the filamentous M13 bacteriophage were designed to bind to an analyte, while the remainder of the phage was functionalized with many copies of HRP–DNAzyme; this methodology produced detectable signal down to 125 fmol with hydrogen peroxide and ABTS [66].

A major benefit of employing DNAzymes and aptamers for sensing materials is that they are able to do much of the work of enzymes and antibodies but with more favorable cost and thermal stability. In addition, the substantial degree of control over analyte or substrate binding, as well as the ability to switch binding on or off depending on the application, allows DNA-based sensing materials to be joined with many different types of assays and instruments, improving their utility further.

**Conclusions and future prospects**

The unique properties of DNA have enabled the generation of many different types of structures and materials with unprecedented design and function. Such materials include 2- and 3D nano- and mesoscale DNA architectures, precise assemblies of nanoparticles in solution and on surfaces, and switchable systems for biomolecule detection. The variability of DNA sequences and binding energies, its tunability in flexibility or stiffness, and the ease of synthesis and production have made it one of the most unique and exquisite macromolecular systems from which to build new materials. Moving forward, we imagine that DNA alone or in combination with other molecular structures will be engineered into new functional materials that demonstrate increasing complexity in structure and optimal function.

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