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DNA Materials: Bridging Nanotechnology and Biotechnology

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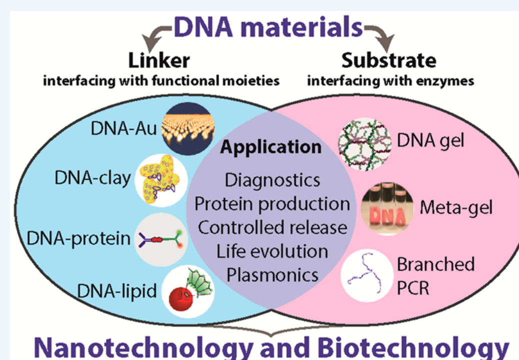

CONSPECTUS: In recent decades, DNA has taken on an assortment of diverse roles, not only as the central genetic molecule in biological systems but also as a generic material for nanoscale engineering. DNA possesses many exceptional properties, including its biological function, biocompatibility, molecular recognition ability, and nanoscale controllability. Taking advantage of these unique attributes, a variety of DNA materials have been created with properties derived both from the biological functions and from the structural characteristics of DNA molecules. These novel DNA materials provide a natural bridge between nanotechnology and biotechnology, leading to far-ranging real-world applications.

In this Account, we describe our work on the design and construction of DNA materials. Based on the role of DNA in the construction, we categorize DNA materials into two classes: substrate and linker. As a substrate, DNA interfaces with enzymes in biochemical reactions, making use of molecular biology's "enzymatic toolkit". For example, employing DNA as a substrate, we utilized enzymatic ligation to prepare the first bulk hydrogel made entirely of DNA. Using this DNA hydrogel as a structural scaffold, we created a protein-producing DNA hydrogel via linking plasmid DNA onto the hydrogel matrix through enzymatic ligation. Furthermore, to fully make use of the advantages of both DNA materials and polymerase chain reaction (PCR), we prepared thermostable branched DNA that could remain intact even under denaturing conditions, allowing for their use as modular primers for PCR. Moreover, via enzymatic polymerization, we have recently constructed a physical DNA hydrogel with unique internal structure and mechanical properties. As a linker, we have used DNA to interface with other functional moieties, including gold nanoparticles, clay minerals, proteins, and lipids, allowing for hybrid materials with unique properties for desired applications. For example, we recently designed a DNA–protein conjugate as a universal adapter for protein detection. We further demonstrate a diverse assortment of applications for these DNA materials including diagnostics, protein production, controlled drug release systems, the exploration of life evolution, and plasmonics.

Although DNA has shown great potential as both substrate and linker in the construction of DNA materials, it is still in the initial stages of becoming a well-established and widely used material. Important challenges include the ease of design and fabrication, scaling-up, and minimizing cost. We envision that DNA materials will continue to bridge the gap between nanotechnology and biotechnology and will ultimately be employed for many real-world applications.

■ INTRODUCTION

The impact of nanotechnology can already be felt across many disciplines, including chemistry, biology, physics, and medicine, and furthermore has resulted in the emergence of new fields such as nanobiotechnology. Operating at the intersection between nanotechnology and biotechnology, nanobiotechnology seeks to solve biological problems using nanotechnology and vice versa.[1] To this end, DNA, the central genetic material in biological systems, can also serve as a generic material for nanoscale engineering,[2−8] providing a natural bridge between nanotechnology and biotechnology. Here, using examples drawn primarily from our own work, we will show that DNA can be used as both a genetic and a generic material to merge the burgeoning fields of nanotechnology and biotechnology.

The traditional conception of DNA has emphasized its role as a genetic, inheritable molecule, but an alternative view of using DNA as a generic construction material has been recently developed. Compared with synthetic polymers, DNA possesses many unique properties, including its biological function, biocompatibility, molecular recognition capacity and nanoscale controllability. To date, scientists have utilized DNA beyond its genetic role to prepare a myriad of materials associated with a wide range of applications.[5,7]

The unique properties of DNA render it an excellent building block for the construction of DNA materials. The most important attribute of DNA is its programmable molecular recognition capability, enabling rational design of materials with nanoscale precision. In general, two complementary single

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stranded DNAs (ssDNAs) are hybridized into a double stranded helix (dsDNA) following the Watson−Crick base-pairing rules: adenine (A) with thymine (T) and cytosine (C) with guanine (G). This regularity along with the predictability of DNA structures exceeds that of the other common biomolecules, which cannot currently be engineered with a similar level of control. A second key advantage of DNA is the rich toolkit of enzymes available for the manipulation of DNA, which allows researchers to harness the power of millions of years of evolution. Furthermore, the rigidity and flexibility of DNA can be easily designed and tailored through the combination of ssDNA and dsDNA. In particular, the persistence length of dsDNA is about 50 nm, and DNA strands shorter than this length can be considered as rigid rods. On the other hand, ssDNA is much more flexible than dsDNA, with a persistence length of only 1 nm, and therefore can easily form looped and curved structures. Finally, DNA molecules are inherently biocompatible, making them suitable materials for both *in vitro* and *in vivo* applications. In fact, no other materials can provide all of the aforementioned advantages, making DNA an attractive, versatile, and easily manipulable building block for the construction of novel nanoscale materials.

In this Account, we are focusing on our research into the construction of DNA materials and their application in biomedical and nanotechnology fields. We divide DNA materials into two categories by the role of DNA in the construction: substrate and linker. Substrate specifically refers to the role of DNA in biochemical reactions involving enzymes. On the other hand, in the role of linker, DNA interfaces with a functional moiety, which may be a protein, a nanoparticle, or DNA itself. In some cases, DNA may even play active roles as both substrate and linker. Overall we demonstrate that DNA materials bridge nanotechnology and biotechnology together with important real-world applications.

CONSTRUCTION

Substrate: Interfacing with Enzymes

DNA Hydrogel. Molecular biology has provided a vast assortment of enzymes for the synthesis, manipulation, and modification of DNA.[9] By interfacing with specific enzymes, DNA acts as a substrate to generate a variety of DNA materials with different formats such as branched nanostructures and hydrogels. Among these materials, DNA hydrogel has attracted particular attention because of its bulk scale and wide practical applications.[10−17] In terms of structure, DNA hydrogels can be categorized into two types: chemical and physical gels. The network of chemical gels is formed through the cross-linking of subunits via covalent chemical bonds. In contrast, physical gels are cross-linked or entangled via noncovalent interactions such as hydrogen bonding and van der Waals forces.

In 2006, our group was the first to use DNA as the building block to prepare an all-DNA chemical hydrogel via an enzymatic reaction (Figure 1).[10] In this process, DNA served both as linker and as substrate. To form the DNA hydrogel, branched DNA motifs, such as X-, T-, or Y- shaped DNA (termed as X-DNA, T-DNA, and Y-DNA), were first prepared through the self-assembly of rationally designed ssDNAs. For example, to fabricate a Y-DNA, three ssDNAs with partially complementary sequences were designed: each ssDNA contained short palindromic ssDNA regions at the end of each arm of the Y-DNA to serve as a connector between the motifs (i.e., sticky-ends). Through the hybridization of the

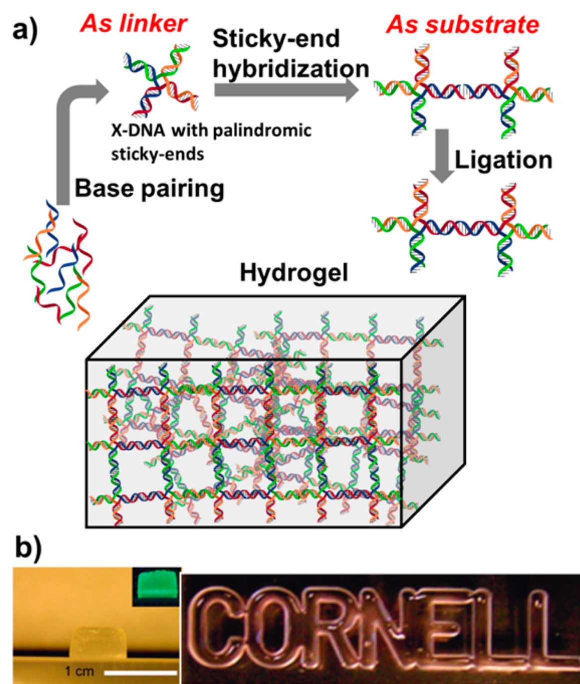

Figure 1. Chemical DNA hydrogel. (a) Scheme of the synthesis route; (b) left, an X-DNA hydrogel at centimeter scale; inset shows fluorescence image of stained DNA hydrogel; right, DNA hydrogel molded into a pattern.

sticky-ends, these branched motifs were first connected together to form a 3-D network. A further step as a substrate of a ligation enzyme (T4 ligase) was carried out to seal the nicks in the DNA, resulting in a covalently linked dsDNA chain. Notably, unlike most other hydrogels, this cross-linking process could be performed under physiological conditions, which allowed proteins, drugs, and live cells to be encapsulated within the DNA hydrogel *in situ*, enabling a variety of applications such as controlled drug delivery, tissue engineering, and 3-D cell culture.

The morphological and mechanical properties of the DNA chemical hydrogel were similar to those of most common hydrogels. In contrast, the physical DNA hydrogel that we recently created had a unique cross-linking network, which exhibited unusual microstructures and unexpected mechanical properties (Figure 2).[16] The formation process was based on interfacing with Φ29 DNA polymerase through a combination of rolling circle amplification (RCA) and multiprimed chain amplification (MCA) processes. After running the RCA and the subsequent MCA, a continuous chain reaction was established, creating extremely long DNA molecules and resulting in a physically linked DNA hydrogel (Figure 2a). Remarkably, this novel physical gel possessed unique microscale internal structure and unexpected macroscale properties (termed "metaproperties"; the gel was a "meta-hydrogel"). SEM images of the gel revealed that this gel had a hierarchical structure, consisting of densely packed birdnest-like structures woven together throughout the gel, which had never been observed in other hydrogels (Figure 2b). The storage-loss modulus (G') of the meta-hydrogel was extremely low (about 10 Pa; i.e., extremely soft) (Figure 2c). As a result, the gel exhibited a mechanical metaproperty: when the gel was removed from water (not the same as drying), it was deformed by surface tension and gravity, behaving just like liquid; but when it was

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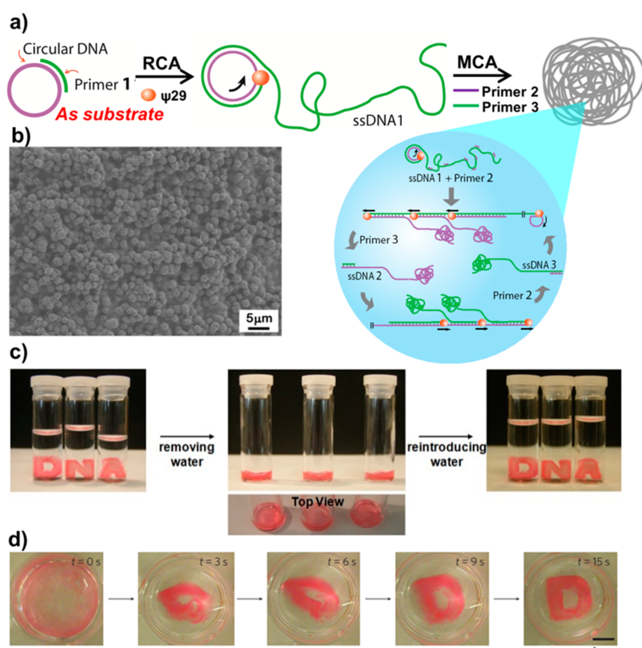

Figure 2. Physical DNA hydrogel. (a) Scheme of the synthesis route. (b) SEM image of internal structures of the gel. (c) Hydrogels with D, N, and A shapes and their metaproperty upon removing and reintroducing water. (d) The process of DNA hydrogel returning to its original shape after reintroducing water.

returned to solution, the gel recovered (i.e., "remembered") its original shape, showing solid-like properties (Figure 2d). This meta-hydrogel opened a new strategy for DNA hydrogel formation using polymerase as a key processor and has potential applications including sustained drug release, cell culture and therapy, DNA immune-therapy, electric switches, and flexible circuits.

Branched PCR. One of the most important and powerful enzymatic processes in biotechnology is the polymerase chain reaction (PCR). Unfortunately, the high temperatures involved in PCR would normally disrupt the fragile hydrogen bonds that maintain dsDNA and branched DNA nanostructures, thus preventing DNA architectures from being used in PCR. In order to create more stable DNA structures that are capable of withstanding elevated temperatures, DNA can be chemically modified to improve its thermal stability. For example, DNA–organic molecule hybrids have been utilized for the construction of DNA structures and networks.[18−20] In addition, other workers have used psoralen, a DNA-intercalating chemical capable of forming covalent cross-links between the two hybridized strands of dsDNA, to create DNA structures with enhanced thermal stability.[21,22] Making use of this cross-linking, we recently used chemical treatment with psoralen to prepare thermostable branched DNA that remained intact even under high temperature.[23] To accomplish this, we constructed branched DNA junctions that were composed of a double-stranded core region (which provided a defined structure) and single-stranded primer regions (which conferred biological activity). Upon psoralen treatment, we found that the double-stranded core regions were chemically stabilized, while the single stranded primer region retained intact substrate function for DNA processing enzymes such as Taq DNA polymerase, which is commonly employed in PCR (Figure 3a). As a result, these structures were used in PCR and interfaced with enzyme such as Taq DNA polymerase as direct replacements of

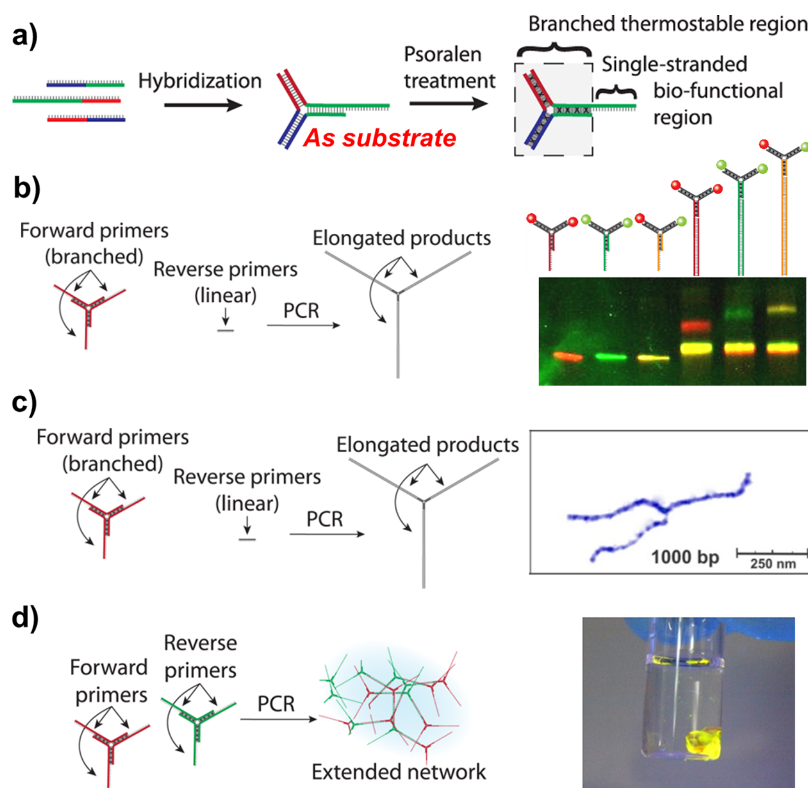

Figure 3. Branched PCR. (a) Illustration of branched thermostable DNA as PCR primers. (b) Modular primers with multiple labels. (c) Elongation of Y-DNA by PCR. (d) Formation of DNA hydrogel by PCR.

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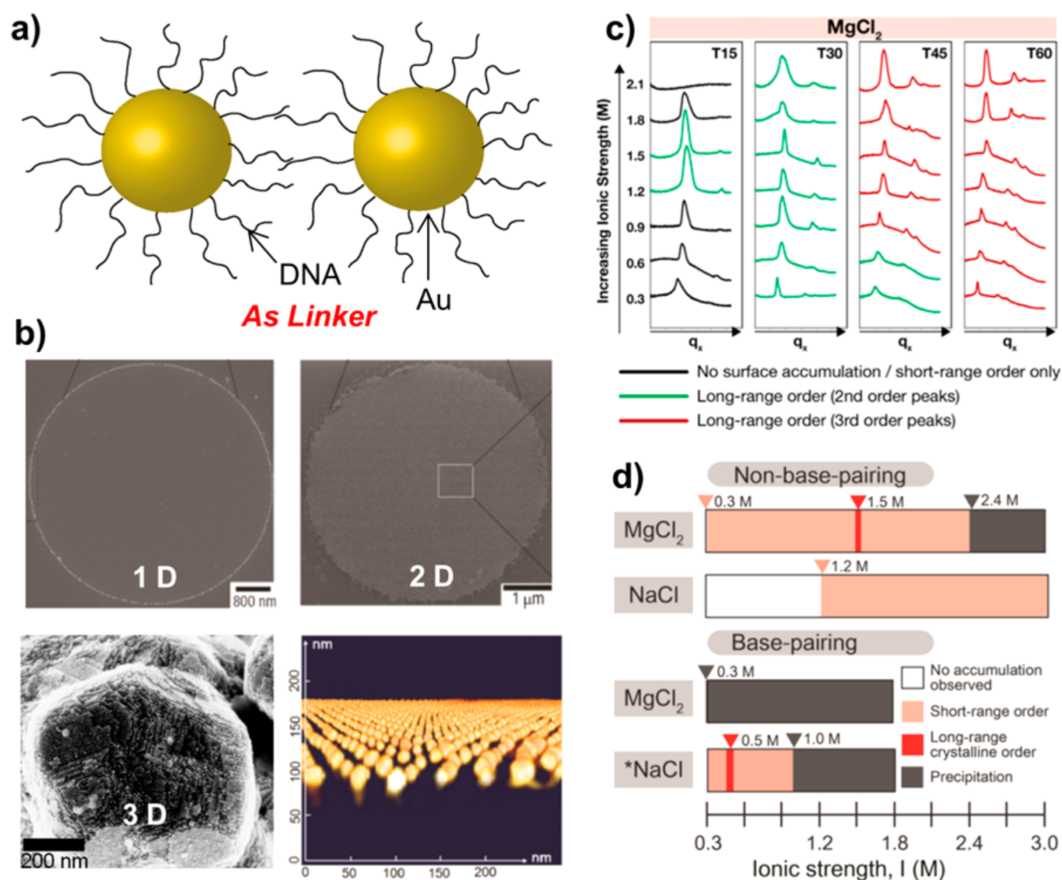

Figure 4. DNA-mediated assembly of AuNPs. (a) Scheme of DNA-linked AuNPs. (b) SEM images of a 1-D circle, 2-D circular sheet, and 3-D supracrystal of AuNPs, and STEM image of a freestanding ultrathin AuNP membrane. (c) Scattering spectra for non-base-pairing DNA−NPs in $MgCl_2$ with different lengths of DNA linkers. (d) Interfacial behavior of base-pairing and non-base-pairing DNA−NPs for different ionic strengths.

conventional linear primers and the single-stranded primer regions were subsequently extended (Figure 3c). Our branched and thermal-stable DNA primers allowed nucleic acid targets to be simultaneously amplified and labeled with multiple fluorophores, enabling potential applications in multiplexed and branched PCR for detection (Figure 3b). Furthermore, branched DNA with multiple primers on each arm was utilized in the PCR reaction to create branched DNA networks and hydrogels (Figure 3d), allowing for novel biotechnological applications, such as a genetically encoded gel for protein production. This technology provides a versatile platform to construct functional DNA materials with a wide range of applications such as nucleic acid detection and synthetic biology.

Linker: Interfacing with Functional Moieties

As demonstrated above, although DNA can be used as a substrate for enzymes as well as programmable construction building blocks to generate a variety of materials, DNA can also be used as linkers when DNA is interfaced with functional moieties such as inorganic nanoparticles, organic molecules, or biomolecules. As a result, new hybrid materials can be created that combine the advantages of both the DNA and the new functional moieties.

DNA-Linked Nanoparticles. Assembly of nanoscale building blocks such as metal and semiconductor nanoparticles, into highly ordered superlattices can generate new classes of bulk scale materials with tunable optical, magnetic, and electronic properties arising from the collective oscillations of the closely packed nanoparticles.[6,24−26] To this end, our group used DNA as a nanoparticle ligand (or a linker) to control the drying mediated ("dewetting") crystallization of DNA-capped gold nanoparticles (DNA−AuNPs).[27−32] The construction of DNA−AuNPs was realized by thiol−gold conjugation using thiol modified DNA and AuNPs (Figure 4a). The control of nanoparticle crystallization was achieved by combining both bottom-up and top-down nanotechnology. More specifically, polydimethylsiloxane (PDMS) micromolds with various geometries and dimensions were patterned and used to sandwich drying DNA−AuNP droplets onto a solid silicon substrate to obtain "molded microdroplets" with specific geometries, positions, and contact line boundaries. By variation of the molding pressure, the dewetting dynamics were precisely controlled. For example, by drying DNA−AuNPs in a micromold under a low pressure, edge dewetting was promoted resulting in 1-D DNA−AuNP corrals (Figure 4b). Conversely, under high pressure, center dewetting was promoted resulting in 3-D DNA−AuNP "supracrystals". Further tunability was imparted by tuning the aspect ratio (depth/diameter) of the micromold, resulting in an intermediate drying phase that formed 2-D microdiscs. Moreover, when DNA−AuNPs were dried on a microhole surface, free-standing nanoparticle superlattices were readily achieved. These highly ordered free-standing, ultrathin nanoparticle membranes (FUN-membranes) retained the geometry of the microhole over several micrometers and were successfully formed on several holey substrates such as copper, silicon nitride, and carbon. Using DNA ligands

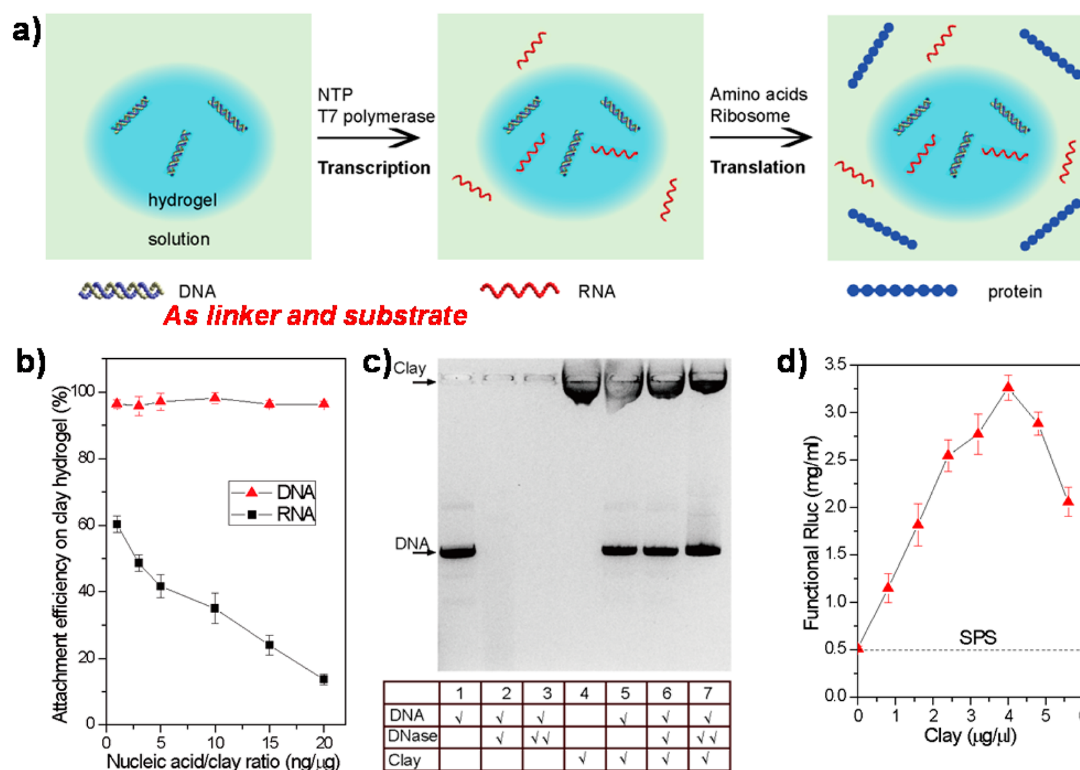

Figure 5. DNA-linked clay minerals. (a) Scheme of transcription and translation reactions in the clay hydrogel environment. (b) Attachment efficiency of DNA/RNA on clay hydrogel. (c) Protection of DNA in clay hydrogel environment against DNase. (d) Protein production was enhanced in the clay hydrogel environment.

of various lengths with and without specific base paring, free-standing superlattices can be formed with varying degrees of order and interparticle spacing, resulting in hexagonal packing symmetries spanning several hundred nanometers.

These examples illustrate the importance of the drying process in the self-assembly of DNA–AuNPs. In order to fully tap the potential of DNA as a linker, this complex process must be fundamentally understood. To this end, we performed real-time in situ small-angle X-ray scattering (SAXS), and the results indicated that, upon drying, base-pairing DNA–AuNPs formed ordered crystallites in the early stages of drying, while non-base-pairing DNA–AuNPs demonstrated crystallization at the later stages of the drying process.[29] Notably, drying of highly concentrated nanoparticle solution can lead to ordered, faceted nanoparticle “supracrystals”.

Of equal intrigue are the solution phase crystallization processes mediated by DNA. In order to better characterize these phenomena, we developed a SAXS technique to probe nanoparticle order at the water–air interface, whereby a microliter scale drop of a DNA–AuNP solution was positioned on a silicon substrate.[32] Remarkably, the SAXS results indicated that the non-base-pairing DNA–AuNPs assembled into highly ordered superlattices over a much wider range of ionic strengths compared with base-paring DNA–AuNPs (Figure 4c). Of particular importance was the resistance to high ionic strengths in both monovalent (NaCl) and divalent ($MgCl_2$) salts. Highly ordered superlattices (as defined by the presence of third order peaks) were achieved in the ionic strength range of 0.3 to 2.1 M, with $MgCl_2$ achieving crystallite grain sizes almost three times that achieved using the base-pairing DNA–AuNPs (Figure 4d). To facilitate the understanding as well as rational design of such highly ordered nanoparticle arrays, we developed a semiempirical model to accurately predict the behavior of base-pairing and non-base-pairing nanoparticles in both NaCl and $MgCl_2$. A crucial parameter of our modified Daoud–Cotton model is the correction for the variation of nanoparticle ligand densities for the various ssDNA ligand lengths. Once the ligand densities were obtained by using a previously established fit for DNA height in NaCl,[33] using values extracted from the SAXS experiments, we were able to correctly model the DNA brush height as a function of both ionic strength and ligand length.[32]

DNA-Linked Clay Minerals. Unlike synthesized nanoparticles (e.g., AuNPs), the unique properties of natural nanomaterials such as clay have been shown to have interesting and unique DNA interfacing properties. Clay minerals, composed mostly of phyllosilicate and formed by the chemical weathering of rocks, have been present on Earth for approximately 3.7 billion years. The long time scale in which these minerals have been widely distributed on Earth raises questions regarding what role clay materials may have played in the evolution of life. Interestingly, we found that clay spontaneously and instantaneously formed hydrogels in the presence of ionic buffers such as ocean water.[34] More importantly, DNA, the central molecule of early evolution, attached to the clay hydrogel through electrostatic interactions (as linker). We also demonstrated through experiments that the clay hydrogel inhibited enzymes that are associated with DNA degradation but enhanced transcription and translation (as substrate, Figure 5). On the basis of these experimental results, we proposed that clay hydrogels may have provided a protective environment for early life evolution.

DNA-Linked with Proteins and Lipids. DNA is also an excellent material for use as a linker with other biomolecules,

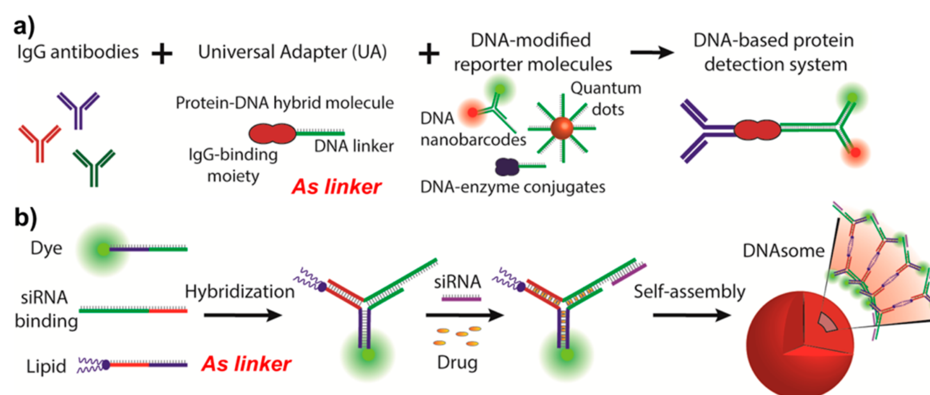

Figure 6. Scheme of constructing DNA−protein (a) and DNA−lipid (b) hybrid materials.

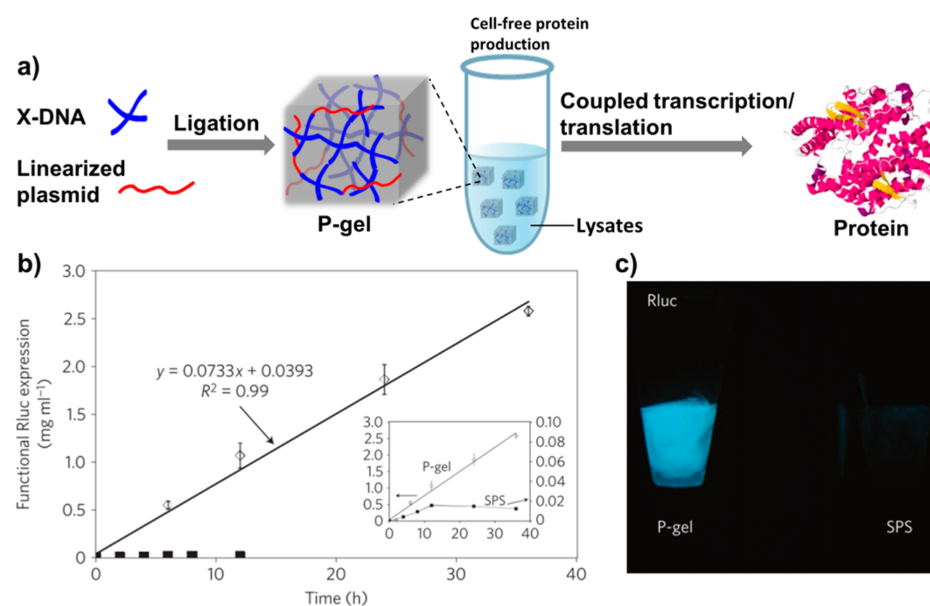

Figure 7. Cell-free protein-producing DNA hydrogel (P-gel). (a) Scheme of P-gel. (b) Protein production from P-gel (open diamonds) over SPS (filled squares). (c) Bioluminescence image of Rluc protein expressed from P-gel over SPS.

such as proteins and lipids. DNA−protein conjugation methods allow for the unique advantages of both DNA and proteins to be combined within a single hybrid structure. For example, our group recently developed a versatile DNA-based protein detection system by using a universal adaptor (UA), a bifunctional protein−DNA hybrid molecule, to interface between IgG antibodies and DNA-modified reporter molecules (Figure 6a).[35] Our system successfully employed a DNA molecule to replace secondary antibodies, which are typically used for the labeling and read-out of most protein-based diagnostic assays, further expanding protein detection via DNA-based molecules. In addition, we have also reported on lipid−DNA conjugates, which formed a liposome-like structure (termed "DNAsome"). Our DNAsomes can carry multiple drugs simultaneously and may find clinical utility as a precisely engineered vector for delivery of multidrugs (Figure 6b).[36]

APPLICATIONS

As a substrate and linker, the predictability of DNA hybridization and assembly combined with the ease of functionalization makes DNA nanostructures ideal for addressing biotechnological, biomedical, and nanotechnological challenges. In our group, we have strived to realize the utility of DNA materials by developing real-world applications including protein production, diagnostics, controlled drug release systems, and plasmonics.

Biotechnological and Biomedical Applications

Protein Production. Protein production is of critical importance for biotechnology such as protein engineering and also for biomedical research and development such as drug discovery. Traditional cell-based protein production systems are generally labor intensive, costly, and time-consuming. In addition, many proteins are difficult to produce through cell-based systems due to cytotoxicity, inclusion bodies, or other issues, severely limiting the availability for obtaining valuable proteins. As an alternative, cell-free protein production systems can overcome many of the difficulties associated with traditional cell-based systems.[37,38]

Almost all cell-free systems are solution phase systems (SPS), in which the template DNA (e.g., plasmid DNA) is dispersed in solution. Typically the yields are low (<1 μg/mL). Various strategies have been explored to improve the yield and efficiency, such as tuning the components of reactions, but improvement was still limited.[39] As an alternative approach to enhance protein production, the gene template itself can be immobilized to a scaffold in order to increase the local

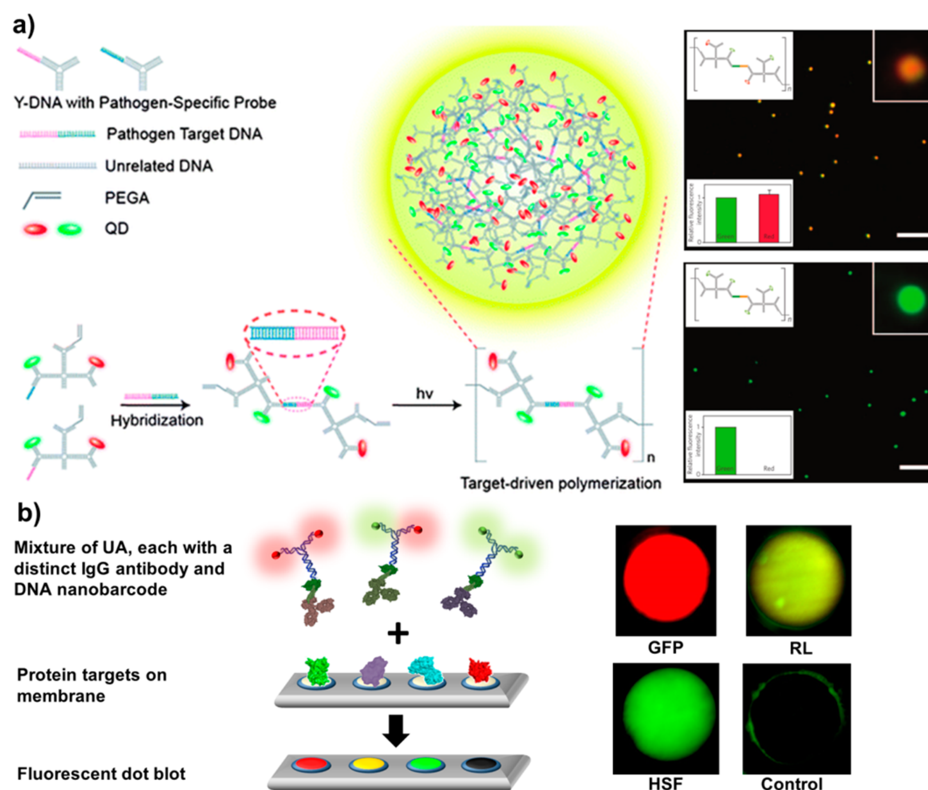

Figure 8. DNA-based diagnostics. (a) Detection of pathogen DNA via target driven polymerization. Fluorescence images show the resulting aggregates. The fluorescence color ratios of the aggregates were controlled by the design of the ABC monomers. (b) Detection of multiple proteins using a universal adapter (UA). Three different proteins were detected in a multiplexed reaction: green fluorescent protein (GFP), *Renilla* luciferase (RL), and heat shock factor (HSF).

concentration of genes. We used genetic DNA as a substrate and covalently linked genes onto the DNA hydrogel matrix through enzymatic ligation (Figure 7).[12] As a result, not only was this protein-producing DNA hydrogel (termed P-gel) successful in producing active proteins, but the protein yield was 300-fold higher than that of the commercially available cell-free systems.[12,40] The design of the P-gel DNA sequence was almost the same as that of the aforementioned DNA chemical hydrogels. The most critical scheme was that the X-DNA possessed complementary and palindromic sticky-ends to the overhangs of the linearized plasmid DNA (gene). Enhanced protein production was attributed to three mechanisms related to our DNA material.[12] First, the plasmid was covalently linked to the DNA hydrogel, offering the protection of genes from degradation and denaturation. As a result, gene stability was greatly enhanced, and protein production was prolonged. Second, the immobilization of plasmid with DNA hydrogel provided a much higher local gene concentration in comparison to SPS. And third, due to the close proximity among and the lack of diffusion of plasmids, the enzymatic turnover rate increased dramatically, facilitating a very high rate of protein production. This P-gel system has successfully produced more than 16 different proteins so far, including reporter proteins, membrane proteins, kinases, and toxic proteins.[12] Our P-gel provides a DNA hydrogel-based, high efficiency cell-free platform for engineering and producing desirable proteins, which might lead to a paradigm shift in protein manufacturing and pharmaceutics.

Diagnostics. Nucleic acid based diagnostics are growing in importance due to the prevalence of PCR-based methods as well as the rise of next generation sequencing technologies.[41] The utilization of branched DNA structures further expanded the potential and versatility of current nucleic acid-based detection methodologies. One prominent example is the branched DNA assay, which achieves sensitive detection of pathogen nucleic acid through branching probes and chemiluminescent labels.[42] On the other hand, multiplexed detection requires simultaneous detection of multiple bio-markers from a single sample. Our group created DNA-based nanobarcodes to address these challenges including high specificity, superior sensitivity, and multiplexity.[43] Specifically, using DNA strands with partially complementary sequences, branched tree-like structures were created with nonpalindromic sticky overhangs, which permitted the attachment of specific multiple fluorescent tags. The predictability of DNA hybridization and self-assembly allowed the structure of the DNA to be controlled and a specific ratio of two fluorescent dyes to be incorporated. These "nanobarcodes" not only exhibited high specificity to a target using a complementary capture probe, but were also able to identify multiple targets simultaneously using encoding with a unique ratio of fluorescence intensities.

We further made use of fluorescence encoding and nucleic acid engineering for the target-driven polymerization of branched DNA monomers to achieve a useful, enzyme-free platform for diagnostics (Figure 8a).[44] Using X-DNA as a core acceptor and Y-DNA as donor, we first constructed anisotropic, branched, and cross-linkable building blocks (ABC monomers). ABC monomers were labeled with various moieties including photo-cross-linkable groups, fluorophores, and sticky-ends that were complementary to a specific pathogen DNA. Only in the presence of a specific pathogen DNA, these ABC monomers formed dimers. With exposure to UV illumination, these dimers

polymerized into DNA aggregates. However, if the target pathogen DNA was absent, polymerization did not occur. In other words, our polymerization was driven to complete only in the presence of a target; hence the detection system realized "target-driven" polymerization to achieve specific signal amplification, all without using any enzymes. Because these DNA aggregates exhibited specificity for a target and enabled identification with a unique fluorescent ratio, the ABC monomer system was ideal for the multiplexed detection of pathogens, especially in point-of-care (POC) conditions.

Protein-based detection has been the workhorse of diagnostics for many years and is among the most used methods in basic research laboratories and clinics. Most protein-based diagnostic assays, such as enzyme-linked immunosorbent assay (ELISA), Western blot, or immunohistochemistry, typically rely on primary antibodies for initial target recognition, followed by secondary antibodies for labeling and read-out. However, because there are only limited types of secondary antibodies available, it is a challenge to select primary/secondary antibody pairs. To address this issue, we recently developed a DNA-based protein detection system via a universal adapter (UA), which did not require secondary antibodies and allowed multiplexed detection by using multiple primary antibodies.[35] As mentioned above, we constructed the UA first, which was a protein–DNA hybrid molecule consisting of a universal IgG binding protein and a ssDNA binding moiety. The ssDNA moiety was designed to specifically interface with reporter molecules (Figure 8b). The key feature of our system was to employ the UA to replace secondary antibodies; UA linked IgG primary antibodies and reporter molecules to generate a modular library of prelabeled primary IgG antibodies for protein detection. This system not only excludes the use of secondary antibodies but also provides a versatile platform for protein detection with modularity, high capacity, and multiplexed capability.

Controlled Drug Release System. DNA hydrogels are an ideal platform as a controlled drug release system with unique advantages including biocompatibility, biodegradability, high efficiency cross-linking under physiological conditions, and *in situ* drug encapsulation. We demonstrated that camptothecin (CPT) and porcine insulin were successfully coencapsulated inside a DNA hydrogel *in situ* with high encapsulation efficiencies close to 100%.[10] *In vitro* release curves showed that the encapsulated CPT and insulin were released in a controlled manner from DNA hydrogels without any initial burst release; the release profile was controlled by the internal structures and degradation rates of the DNA hydrogels, which could be both readily tuned by adjusting the initial concentrations and types of branched DNA building blocks. Similarly, another type of DNA hydrogel, the DNA physical meta-hydrogel, showed controlled drug release profiles of both insulin and doxorubicin.[16] In related but independent work by Takakura and his collegues, a similar DNA hydrogel consisting of CpG motifs was demonstrated as an ideal system for sustained release of both immunostimulatory signals and anticancer agents, which was used for highly effective tumor chemo/immunotherapy.[15]

In addition to DNA hydrogels, our group also designed a DNA–lipid amphiphile that self-assembled into novel, DNA-based liposome-like core–shell structures, which were termed DNAsomes (Figure 6b). Due to natural base-pairing between DNA and RNA sequences, our DNAsomes readily carried large amounts of small interfering RNA (siRNA) that was released in a controlled fashion, enabling efficient co-delivery of siRNA and other drugs to mammalian cells.[36]

Nanotechnological Applications

Plasmonics. The understanding that plasmons from neighboring nanoparticles can interact and hybridize in a manner similar to electronic wave functions opens up the possibility to engineer designer nanoparticle assemblies with novel optical and electronic properties.[45] As we demonstrated above, DNA is an ideal linker to construct such highly ordered nanoparticle assemblies; we have realized both solid and solution phase ordered assemblies of nanoparticles via DNA.[27,32] The interparticle spacing can be easily and precisely tuned by changing either the DNA ligand length or ionic strength, which greatly facilitates the construction of plasmonic assemblies. As a general rule, plasmon coupling will not occur until the edge-to-edge interparticle spacing-to-particle diameter ratio is less than 2.5, which can be obtained by selecting appropriate DNA length or particle diameter. In building free-standing DNA–AuNP nanoparticle superlattices, using various DNA ligand lengths, we tuned this interparticle spacing from ~1 to 20 nm, a much wider range than can be achieved with typical alkyl ligands.[28] The resulting plasmon coupling was clearly visible under white light illumination. An obvious plasmon peak shift was detected: at the smallest edge-to-edge interparticle spacing, the plasmon peak shift was nearly 60 nm; while at the longest spacing, the peak shift was 20 nm. The resulting free-standing plasmonic superlattice sheets present a viable approach for the bottom-up fabrication of solid-state plasmonic nanodevices. Based on the plasmon peak shift, it can be conjectured that the other DNA–NP superlattices can be similarly included into potential nanodevices. Indeed, the edge-to-edge interparticle spacings of ~8 nm for the patterned nanoparticle superlattices could lead to plasmon peak shifts up to 20 nm.[27] The variety of substrates that these superlattices were patterned on (e.g., copper, carbon, silicon nitride) will further facilitate any potential device integration. Similar great potential can be envisioned for our solution state Gibbs' monolayers, where interparticle spacing as low as ~2 nm was reported, which, like the free-standing superlattices, could demonstrate plasmon peak shifts of up to ~60 nm.[30] The remarkable stability of this system to ionic strengths of both monovalent and divalent salts was crucial to potential plasmonic detection schemes for biomolecules, where buffered systems composed of various salts were necessary to retain the native state of the analyte.[32]

■ CONCLUSION AND OUTLOOK

DNA materials have already proven their utility for achieving versatile control and manipulation of matter on the nanometer scale. To attract a wider range of applications, we will need to develop DNA materials for use in real-world applications outside the laboratory setting. One of the most direct and immediate applications for DNA nanostructures is in the area of molecular diagnostics. Given the prominent role of DNA in biology's central dogma, DNA materials may act as a substrate for enzymes to carry out many of life's most important natural processes. For example, one intriguing avenue for research in DNA materials may aim to develop materials with properties of "artificial life" (e.g., artificial cells capable of protein production). In addition, the role of DNA as a linker not only is applicable to static assembly but also allows for dynamic and responsive materials (e.g., DNA-based circuits or

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responsive DNA hydrogels), which can add another layer of controllability for system biology or material applications. We envision that through collective efforts, DNA materials will continue to bridge the gap between nanotechnology and biotechnology and will ultimately be employed for a myriad of real-world applications.

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