

## Structural DNA Nanotechnology: State of the Art and Future Perspective

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**ABSTRACT:** Over the past three decades DNA has emerged as an exceptional molecular building block for nanoconstruction due to its predictable conformation and programmable intra- and intermolecular Watson–Crick base-pairing interactions. A variety of convenient design rules and reliable assembly methods have been developed to engineer DNA nanostructures of increasing complexity. The ability to create designer DNA architectures with accurate spatial control has allowed researchers to explore novel applications in many directions, such as directed material assembly, structural biology, biocatalysis, DNA computing, nanorobotics, disease diagnosis, and drug delivery. This Perspective discusses the state of the art in the field of structural DNA nanotechnology and presents some of the challenges and opportunities that exist in DNA-based molecular design and programming.

### 1. INTRODUCTION

Self-assembly is a remarkable process that Nature uses to organize chemical systems composed of nonliving components into living, biological systems. Nature accomplishes this incredible feat by adding information to matter and by guiding the self-assembly process to create functional structures. Toward the goal of engineering biomimetic, bioinspired, or biokleptic components that can communicate, regulate, and actuate in artificial molecular networks, information-coding polymers such as DNA, RNA, and proteins have been used as ideal building blocks in the assembly of designer nanoarchitectures. This Perspective will concentrate on the most recent and inspiring advances in structural DNA nanotechnology and present an outlook of the future of this rapidly expanding field. More comprehensive reviews that provide very detailed descriptions of the state of the art in this field can be found elsewhere in the literature.<sup>1–5</sup>

### 2. RECENT DEVELOPMENTS IN STRUCTURAL DNA NANOTECHNOLOGY

DNA, Nature's molecule of choice for storing and transmitting genetic information, is an excellent nanoscale building block because of its specific three-dimensional (3D) conformation, chemical addressability, and predictable Watson–Crick base-pairing. Structural DNA nanotechnology, derived from Seeman's innovative proposal that DNA could be used as a physical material for the self-assembly of nanoscale structures<sup>6</sup> (Figure 1), has developed with astounding speed over the past 30 years. The most significant underlying concept is the application of immobile, branched DNA junctions, together

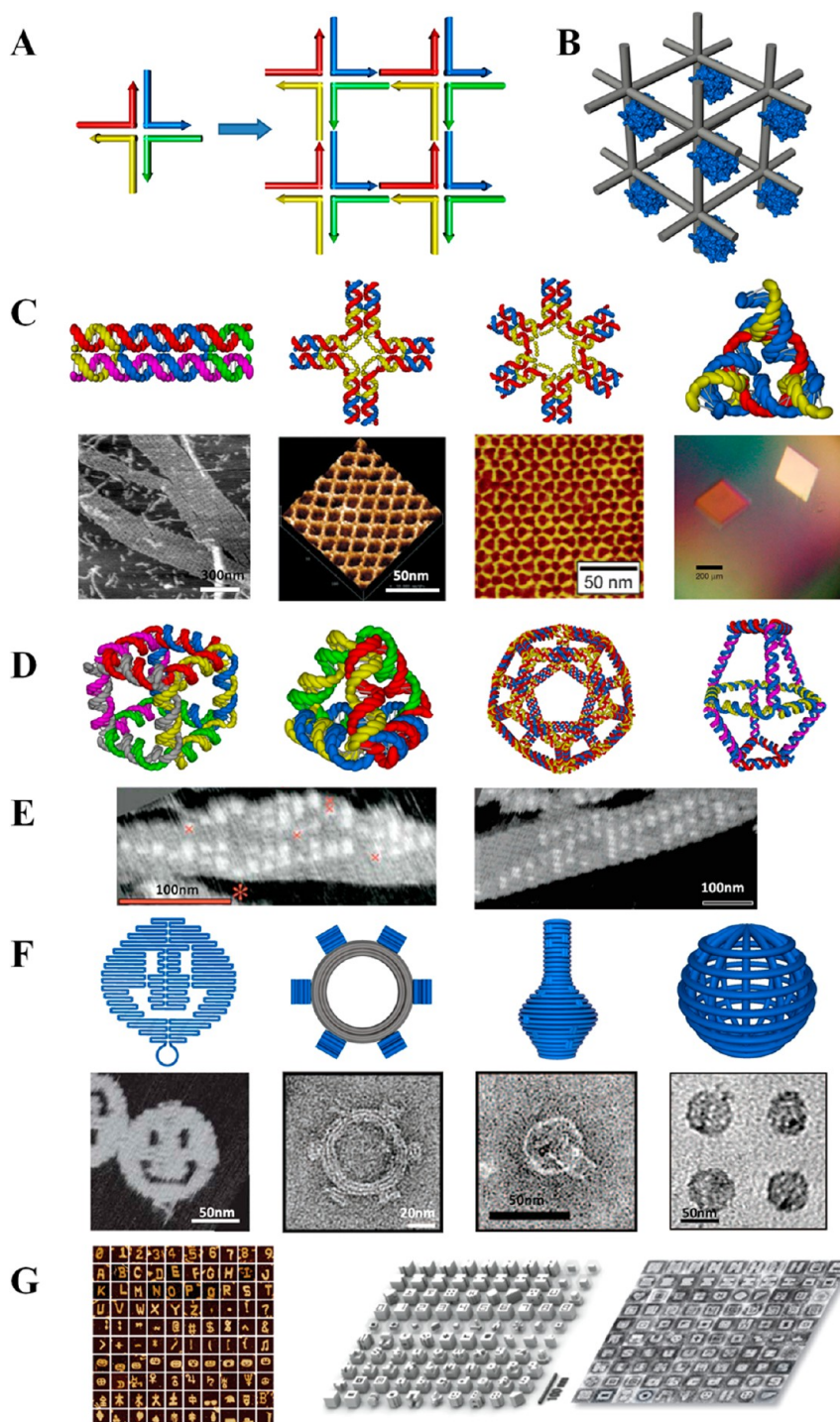
with sequence-specific sticky end associations, to create self-assembling arrays, objects, and devices (Figure 1A).

**2.1. Methods of DNA-Based Construction.** Over the past several decades, researchers have established a collection of convenient methods to construct DNA nanostructures that exhibit significant geometric and topological complexity. Designing and predicting the 3D conformation of these nanostructures is now routine thanks to several user-friendly software interfaces that have been developed.<sup>7–12</sup> A number of 2D and 3D lattices assembled from small, repeating DNA nanostructure motifs were produced<sup>13–20</sup> (Figure 1C), and several discrete polyhedral objects were constructed from fixed numbers of DNA junction motifs<sup>21–27</sup> (Figure 1D). In 2009, Seeman's group was the first to assemble 3D DNA crystals from deliberately designed sticky-end connections<sup>16</sup> (Figure 1C, right) rather than through simple, nonspecific base stacking. They used self-assembling tensegrity triangle motifs to create 3D crystals with various unit dimensions. This work represents a milestone in fulfilling Seeman's original vision of using 3D DNA lattices as hosts to organize guest protein molecules and facilitate protein crystallography<sup>6</sup> (Figure 1B). Several researchers encoded algorithms into DNA nanostructure components to direct the assembly of particular 2D lattice arrays and had some initial success<sup>28,29</sup> (Figure 1E); however, scaling-up algorithmic assembly to realize more complex patterns remains a challenge, mainly because of the errors that accumulate during assembly. If error correction mechanisms<sup>30,31</sup> could be implemented, it would represent a ground-breaking advance in this field.

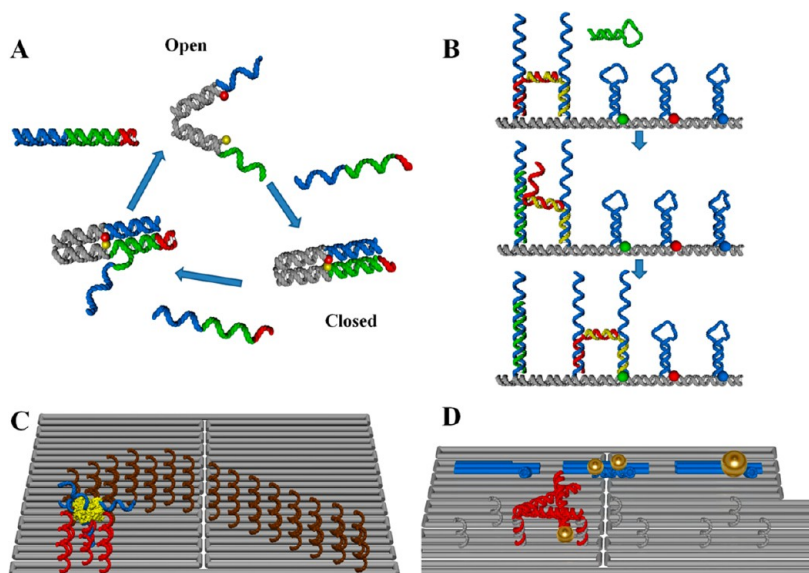
In 2006, the emergence of DNA origami<sup>32</sup> transformed the landscape of structural DNA nanotechnology. The DNA origami method uses a number of short single-stranded DNA (ssDNA) oligonucleotides to direct the folding path of a long ssDNA "scaffold" strand. Rothemund used genomic ssDNA from the M13 phage as the scaffold strand (7249 nucleotides) and designed a set of short "staple" strands to selectively bind to distant regions of the scaffold and fold it into a predesigned shape.<sup>32</sup> This assembly method results in near-quantitative yield for most 2D designs (Figure 1F), even with unpurified staples. Several groups successfully extended DNA origami fabrication to 3D<sup>33–36</sup> and to the assembly of twisted<sup>37</sup> and curved<sup>38</sup> 3D objects (Figure 1F). Other research groups have focused their attention on scaling-up DNA origami using the following methods: edge-to-edge base-stacking interactions between individual origami units,<sup>39</sup> sequence-specific sticky end cohesion between individual units,<sup>40</sup> "super origami,"<sup>41</sup> and use of longer scaffolds for origami construction.<sup>42,43</sup>

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**Figure 1.** Structural foundations of structural DNA nanotechnology and representative examples (each panel described left to right). Seeman's original proposals to use immobile DNA junctions to create self-assembling arrays (A)<sup>6</sup> and self-assembled 3D DNA lattices (B) as scaffolds to organize macromolecules into crystalline lattices.<sup>6</sup> (C) DNA nanostructure motifs used to create periodic 2D arrays and 3D crystal (top, helical structures of the motifs; bottom, AFM images of the assembled 2D arrays and optical image of the 3D crystal): double-crossover DNA tile,<sup>13</sup> 4×4 DNA tile,<sup>14</sup> 6×4 DNA tile,<sup>15</sup> and tensegrity triangle DNA tile.<sup>16</sup> (D) Polyhedral DNA nanostructures: molecular models of a DNA cube,<sup>21</sup> DNA tetrahedron,<sup>22</sup> DNA dodecahedron,<sup>23</sup> and DNA biprism.<sup>24</sup> (E) Algorithmic self-assembly based on double-crossover tiles: Sierpinski triangles<sup>28</sup> and binary counter.<sup>29</sup> (F) DNA origami nanostructures (top, schematic drawings of the structures; bottom, corresponding AFM or TEM images): 2D DNA origami smiley face,<sup>32</sup> 3D DNA origami in the shape of a gear,<sup>37</sup> curved single-layer 3D origami in the shape of a vase,<sup>38</sup> and DNA origami gridiron.<sup>114</sup> (G) Complex nanostructures produced using the single-stranded DNA tile strategy.<sup>45,46</sup> Images reproduced with permission: (C) ref 13, 1998 Nature Publishing Group (NPG); ref 14, 2003 American Association for the Advancement of Science (AAAS); ref 15, 2006 American Chemical Society (ACS); ref 16, 2009 NPG; (E) ref 28, courtesy of P. Rothmund; ref 29, 2005 ACS; (F) ref 32, 2006 NPG; ref 37, 2009 AAAS; ref 38, 2011 AAAS; ref 114, 2013 AAAS; and (G) ref 45, 2012 NPG; ref 46, 2012 AAAS.



**Figure 2.** Representative dynamic DNA nanostructures. (A) DNA tweezer based on DNA strand displacement technique.<sup>48</sup> (B) Autonomous DNA walker catalyzed by metastable DNA hairpin fuel.<sup>63</sup> (C) Movement of DNA spider on a prescribed landscape.<sup>62</sup> (D) DNA assembly line: DNA walker will transport gold nanoparticles to a different product formation station with instructions from DNA strand displacement.<sup>66</sup>

More recently, Yin and co-workers synthesized a variety of 1D,<sup>44</sup> 2D,<sup>45</sup> and 3D<sup>46</sup> DNA nanostructures from single-stranded DNA tiles (SSTs). The platform that they developed is based on a series of interlocking local connections between SSTs. Collections of SSTs form 2D sheet or 3D block canvases that can be selectively engraved to create different shapes and patterns by simply including or omitting specific SSTs (Figure 1G).

**2.2. Dynamic DNA Nanodevices.** Natural biological devices are designed to operate in dynamic conditions, responding to subtle biological cues to realize their functions. The structural properties of DNA that allow it to serve as a versatile construction material have been exploited to create dynamic nanodevices (Figure 2A) ranging from small switchable structures<sup>47–52</sup> and reconfigurable systems<sup>53–58</sup> to structures that display complex movements such as rolling,<sup>59</sup> rotating,<sup>60</sup> and walking.<sup>61–67</sup>

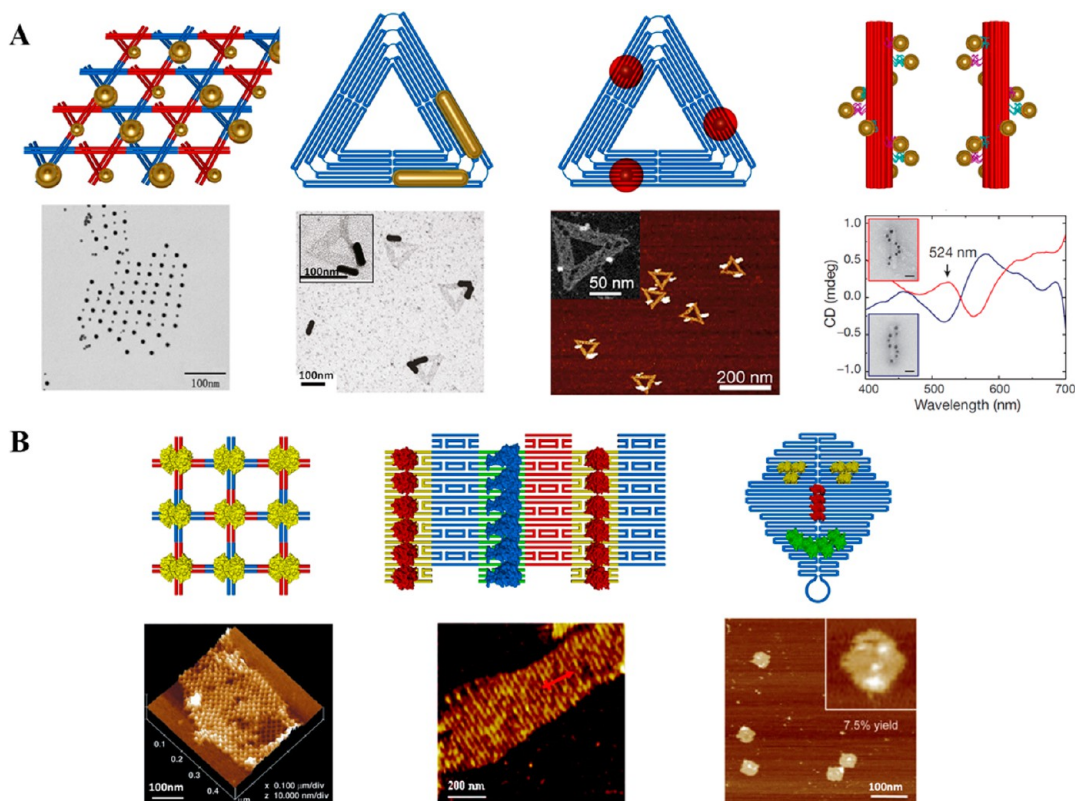
Protein molecular motors transform chemical energy into mechanical energy to facilitate a variety of biological functions from cell division, transport, and motility to enzymatic activity. DNA nanotechnologists have long envisioned programming DNA walker molecules to mimic the ability of natural motor proteins to walk along intracellular tracks and achieve controlled motion. Imparting directionality to DNA walkers could be realized by means of successively adding DNA fuels, by coordinating conformational changes between different components of the walker, by leading the walker through selective track modifications, or by pairing their motion to unidirectional reaction cycles. Researchers have already demonstrated unidirectional motion by DNA walkers through prescribed tracks<sup>63,65</sup> and landscapes<sup>62</sup> (Figure 2B,C). On the basis of this technology, it is possible to develop walkers that are programmed to travel a certain path by encoding the directions into the nucleotide sequences of the walker itself and into the corresponding landscape. For example, Seeman's group reported a DNA-based robot that manufactured structures on a nanoscale assembly line<sup>66</sup> (Figure 2D). Their DNA walker traveled through three fixed modules that were individually programmed to selectively incorporate a gold nanoparticle into

the final product, resulting in eight possible outcomes. Recently, researchers demonstrated that DNA walkers can also be used to mediate multistep organic synthesis,<sup>67</sup> pointing to the possibility of programming chemical reactions with dynamic DNA nanodevices.

### 2.3. Applications of Structural DNA Nanotechnology.

As structural DNA nanotechnology transitions from adolescence into adulthood, the need to demonstrate potential applications is of the utmost importance. We must improve our ability to engineer and program complex molecular systems and prove that designer DNA nanostructures can be employed in real-world applications. If we continue to exploit the programmability of DNA nanostructures to accurately template functional molecules, materials, and probes, we will be able to organize these external elements into practical devices and engineer molecular sensors, circuits, and actuators.

Inorganic nanomaterials such as quantum dots, nanowires and nanorods, and metal nanoparticles have attracted much attention because of their unique optical and electronic properties that can be used in solar cells, phototransistors, laser diodes, light-emitting diodes, and other optoelectronic devices.<sup>68</sup> However, a better understanding of the photophysical behavior of these materials is necessary to use them in such devices. Researchers have successfully used DNA nanoscaffolds to organize metallic nanoparticles, semiconductor nanocrystals<sup>69–74</sup> (Figure 3A), and organic chromophores<sup>75</sup> into well-defined architectures. These hybrid DNA nanostructure complexes have enabled systematic investigation of distance-dependent interactions between photonic elements.<sup>76–78</sup> In one example, Liedl and co-workers constructed a spiral, nanoscale staircase on which gold nanoparticles were arranged at regular intervals and with chiral geometries<sup>72</sup> (Figure 3A). This work demonstrates how DNA scaffolding can be used to control the precise structural arrangement of metal nanoparticles, enabling researchers to tailor surface plasmon resonance and the interaction with visible light. In another example, DNA nanostructures were used to organize various organic chromophores into artificial light-harvesting complexes with control over cascading, unidirectional energy transfer.<sup>75</sup>



**Figure 3.** Representative examples of DNA nanostructure-directed assembly of inorganic and protein molecules (top, schematics; bottom, corresponding TEM or AFM images). (A) Left to right: gold nanoparticles organized by a 2D DNA tile array,<sup>69</sup> gold nanorod dimers with controlled angles between the nanorods organized by DNA origami,<sup>70</sup> DNA origami-directed quantum dot architectures,<sup>71</sup> and DNA origami-directed gold nanoparticles in a chiral arrangement and the induced circular dichroic effect.<sup>72</sup> (B) Left to right: Organization of streptavidin proteins by a 2D DNA nanoarray,<sup>14</sup> protein arrays templated by a 2D DNA nanostructure through aptamer–protein interactions,<sup>79</sup> and orthogonal Snap-tag- and His-tag-mediated decoration of DNA origami.<sup>80</sup> Images reproduced with permission: (A) ref 69, 2006 ACS; ref 70, 2011 ACS; ref 71, 2012 ACS; ref 72, 2012 NPG; and (B) ref 14, 2003 AAAS; ref 79, 2007 ACS; ref 80, 2010 Wiley.

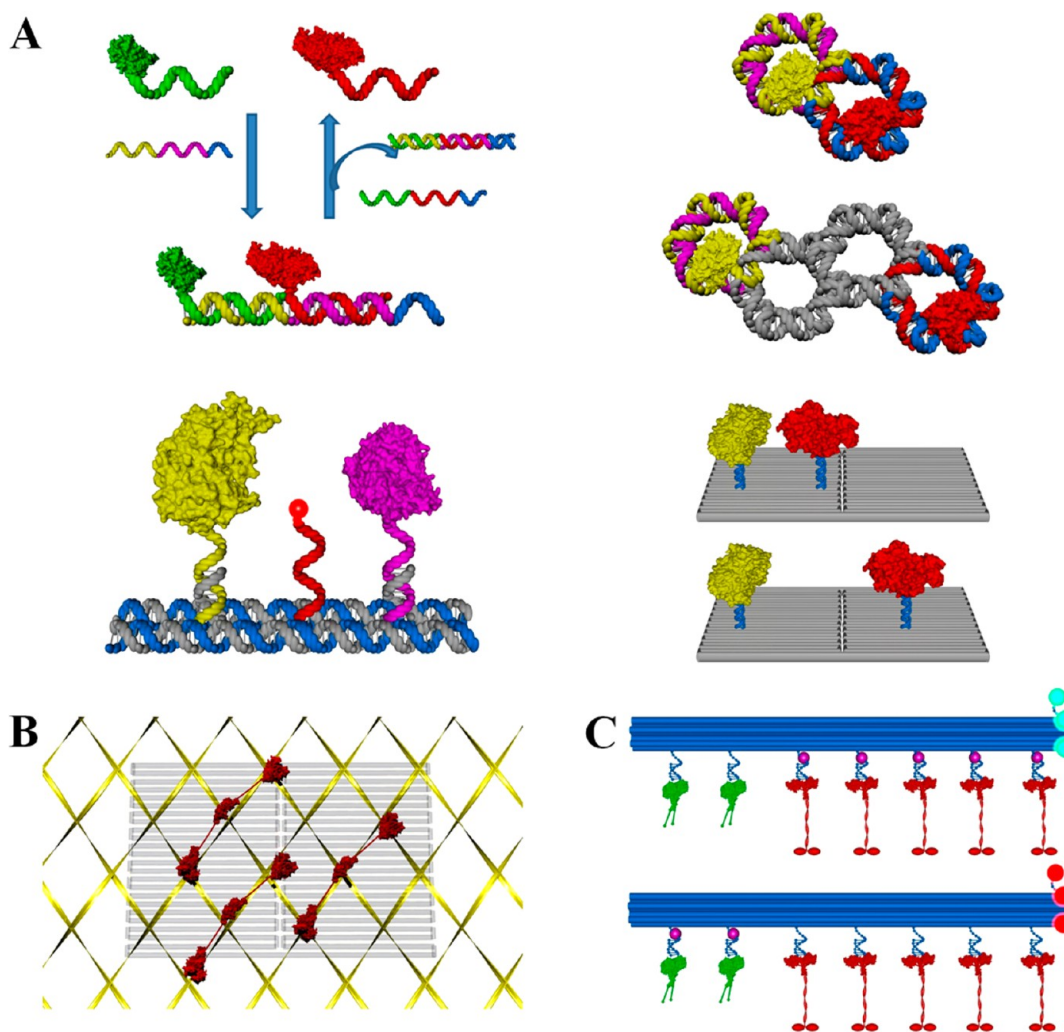
As we previously mentioned, one of the initial goals of structural DNA nanotechnology was to use 3D DNA lattices as hosts to organize guest protein molecules and facilitate protein crystallography. Although this vision has yet to be realized, scientists have already begun to use DNA nanostructures as chaperones to align and organize protein molecules using different strategies, including ligand–protein (such as biotin–streptavidin) interactions,<sup>14</sup> aptamer–target interactions,<sup>79</sup> and ligand-engineered (tagged) protein interactions<sup>80</sup> (Figure 3B). Shih and co-workers recently designed DNA origami nanotube liquid crystals to provide the appropriate “alignment environment” for determining the previously unknown structure of a membrane protein by nuclear magnetic resonance (NMR).<sup>81</sup> Turberfield and co-workers used periodic 2D DNA tile arrays as templates to arrange proteins and subsequently used cryo-EM to solve their structures.<sup>82</sup>

Proteins, some of Nature’s most powerful agents, are large macromolecules that perform a wide assortment of functions required to sustain life, including metabolic catalysis, DNA replication, and molecular transport. In order to better understand the governing dynamics in complex protein systems, we need control over the number, orientation, and arrangement of the constituents. Nucleic acid scaffolds afford this level of control, and researchers have already used RNA and DNA platforms to engineer a number of enzyme cascades<sup>83–87</sup> (Figure 4A). For example, Silver and co-workers used a bacterial host to transcribe RNA and assemble intracellular

RNA nanoscaffolds for spatial organization of metabolic elements for hydrogen production.<sup>83</sup> Willner and co-workers organized a glucose oxidase and horseradish peroxidase enzyme cascade by 2D DNA lattices.<sup>85</sup> More recently, Yan and co-workers conducted substrate channeling in a multienzyme cascade by using an artificial DNA swinging arm.<sup>87</sup>

DNA origami scaffolds have also been used to organize motor proteins and study their spatially dependent motility<sup>88</sup> (Figure 4B). Understanding how motors cooperate productively, and compete antagonistically, is important for understanding how intracellular transport is regulated. Researchers recently demonstrated this “molecular tug-of-war” by displaying different numbers of dynein and kinesin motor proteins from a DNA origami structure.<sup>89</sup> By controlling the number, distance, and orientations of the two types of biological motors, they were able to systematically study coordinated motor behavior (Figure 4C).

Structural DNA nanotechnology has also emerged as a useful tool for biological and medicinal applications (Figure 5). The intrinsic biocompatibility, nanoscale dimensions, programmability, and ability for functionalization of DNA nanostructures are virtually unrivaled by existing techniques. In particular, the addressable configuration of DNA origami lends itself to detection of gene expression<sup>90</sup> and single nucleotide polymorphism.<sup>91</sup> The Sugiyama group developed DNA origami frames and rulers to investigate biomolecular interactions such as protein–DNA binding events and homologous recombination processes in real time at the single molecule level<sup>92–94</sup> (Figure 5A).



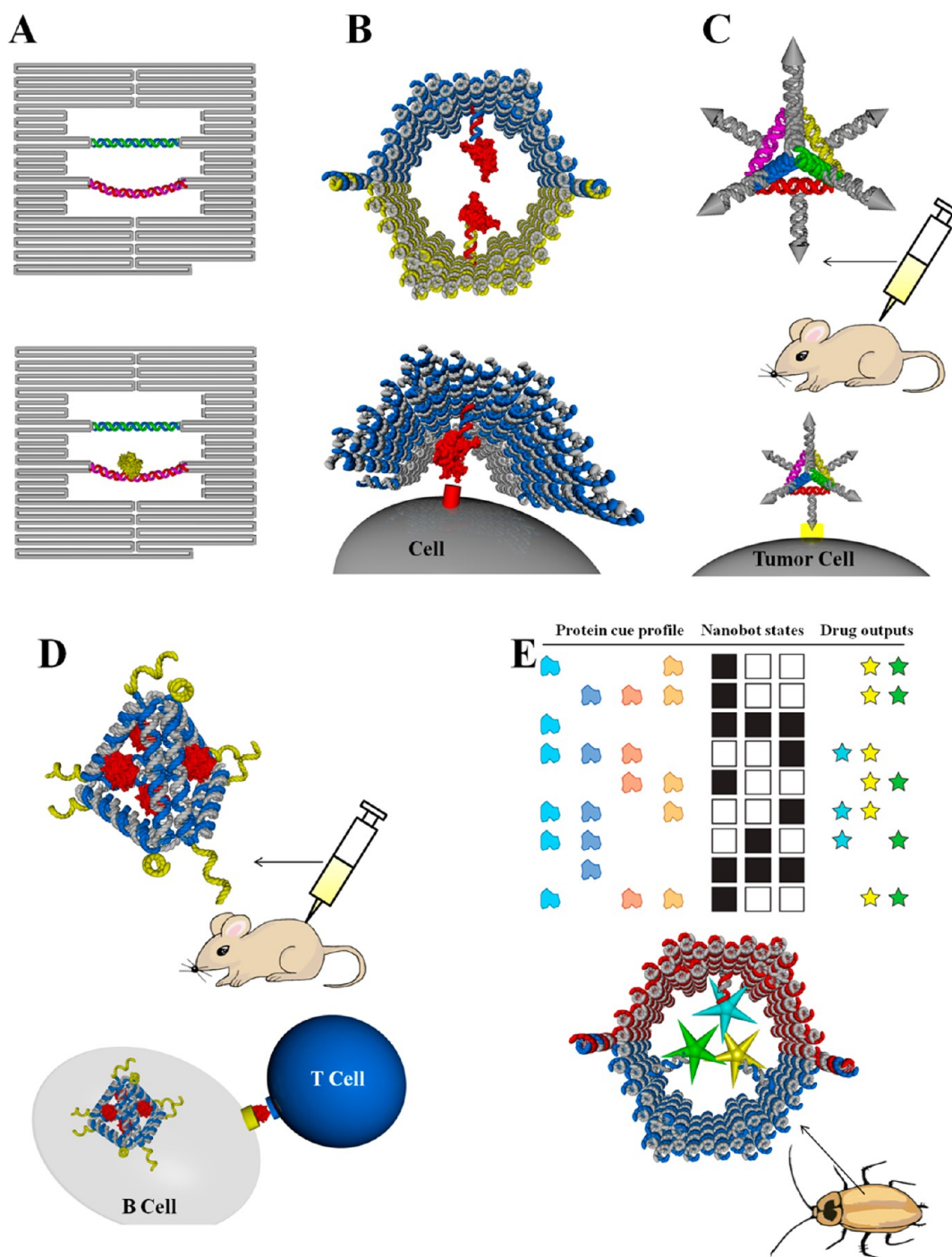
**Figure 4.** Representative examples of DNA nanostructure-directed assembly of protein molecules for functional structures. (A) Upper left, assembly and disassembly of holoenzymes mediated by DNA strand displacement;<sup>84</sup> upper right, glucose oxidase (yellow) and horseradish peroxidase (red) enzyme cascade organized by 2D DNA lattices;<sup>85</sup> lower left, substrate channeling in a multienzyme cascade by an artificial DNA swinging arm;<sup>87</sup> and lower right, glucose oxidase (yellow) and horseradish peroxidase (red) enzyme cascade organized on DNA origami with distance control.<sup>86</sup> (B) Rectangular DNA origami travels on a cellular actin network through the binding and action of myosin lever arms.<sup>88</sup> (C) Molecular tug-of-war between two motor proteins displayed from a 12-helix DNA bundle.<sup>89</sup>

Further, the spatial addressability and multivalent properties of DNA nanostructures make them promising vehicles for targeted drug delivery. For example, Douglas and co-workers demonstrated a barrel-shaped nanorobot that releases Fab antibody fragments in the presence of target cells.<sup>95</sup> In their system, two ssDNA aptamer locks are opened by specific markers present on the surface of cells (Figure 5B). After opening, the payload molecules inside the barrel are exposed, inducing a particular cellular signaling pathway. Anderson and co-workers used a DNA tetrahedron to deliver small interfering RNA *in vivo* to target and suppress gene expression in a mouse model<sup>96</sup> (Figure 5C). Programmable DNA nanostructures have also been used as synthetic vaccine platforms.<sup>97,98</sup> Yan, Chang, and co-workers used a DNA tetrahedron to coassemble model antigens and CpG adjuvants into nanoscale complexes with precise control of the valency and spatial arrangement of each component<sup>98</sup> (Figure 5D). Tests on immunized mice demonstrated that antigen–adjuvant–DNA complexes induced stronger and longer-lasting antibody responses against the antigen, without stimulating a reaction to the DNA nanostructure itself, as compared to an unstructured mixture of antigen and CpG

molecules. More recently, Amir et al. showed that DNA origami robots can dynamically interact with each other and perform logic computations in a living animal, opening up opportunities to develop smart theranostic nanodevices<sup>99</sup> (Figure 5E).

### 3. FRONTIERS OF STRUCTURAL DNA NANOTECHNOLOGY

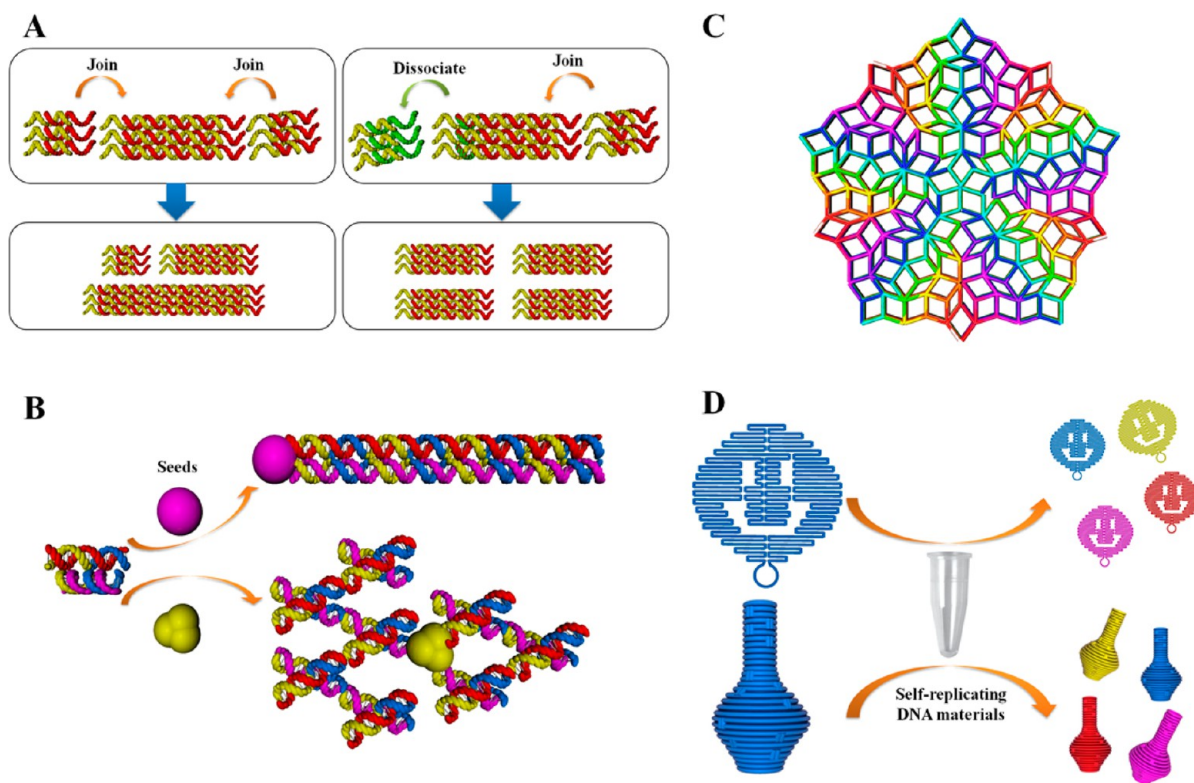
The interdisciplinary nature of DNA nanotechnology crosses the traditional boundaries of physics, chemistry, biology, and engineering and allows scientists to connect and integrate their unique perspectives in pursuit of solutions to the most pressing problems in medicine, technology, and more. From the earliest DNA junction motifs to the most recently developed DNA nanostructures of incredible complexity, the field has started to explore various novel applications, including directed material assembly, structural biology, biocatalysis, DNA computing, nanorobotics, disease diagnosis, and drug delivery, as we mentioned briefly in the previous section. Each of these applications is made possible by the ability of DNA nanostructures to direct molecular species with nanoscale precision while maintaining the utmost



**Figure 5.** Biological applications of DNA-directed assembly. (A) DNA origami frames to investigate protein–DNA binding events in real time at the single molecule level.<sup>92</sup> (B) Barrel-like DNA nanorobot programmed to be open in the presence of target cells and expose Fab antibody fragment cargo.<sup>95</sup> (C) Six siRNA duplexes and folic acid tags (gray) chaperoned by a DNA tetrahedron are injected into mice; the tetrahedra bind to tumor cells by targeting folate receptors expressed on the tumor cell surface.<sup>96</sup> (D) DNA tetrahedron–adjuvant–antigen vaccine complex. CpG ODN adjuvant molecules (curved yellow ribbons) and the model streptavidin antigen (red) bind specifically to B cells and are subsequently presented to T cells to activate B cell response and antibody production.<sup>98</sup> (E) Three different drugs carried by a DNA nanorobot can be released in a programmed fashion by undergoing complex DNA computation in a living cockroach.<sup>99</sup> Image (E) reproduced with permission from ref 99, 2014 NPG.

structural integrity. DNA nanotechnology is progressing with such incredible speed that it is becoming more and more difficult to predict from which areas the next breakthroughs will occur. Next, we are merely providing our opinion about the critical challenges that the field faces and which directions we believe researchers should pursue to help structural DNA nanotechnology reach its full potential.

We have divided the remaining outlook into three main areas: Design and Assembly, which will include discussions of dynamic, developmental, quasicrystal lattice, 3D periodic crystal lattice, scaffolded, surface-mediated, algorithmic, and topological assembly; Future Applications, which will include discussions of structural DNA nanotechnology for molecular scaffolds, sensors, robotics, and computing; and From Nano to



**Figure 6.** Schematic illustrations of (A) dynamic DNA self-assembly with simultaneous joining in one end and dissociation in the other end, (B) developmental DNA self-assembly, in which the assembly process may grow into different final products in response to different external cues, (C) an example of a possible quasicrystal (2D penrose tiling) using DNA tile self-assembly, and (D) self-replication of DNA nanostructures.

Angstrom Technology, in which we conceive of potential directions the field might explore over the longer term.

**3.1. Design and Assembly.** *3.1.1. Dynamic Assembly.* George Whitesides once wrote, “Although much of current understanding of self-assembly comes from the examination of static systems, the greatest challenges, and opportunities, lie in studying dynamic systems. Perhaps the most important justification for studying self-assembly is its central role in life.”<sup>100</sup> Dynamic self-assembly processes underlie many forms of adaptive and intelligent behaviors in natural systems; however, very little is known about the principles that govern them. One of the most intriguing, dynamic self-assembly processes in living cells is the polymerization of cytoskeletal biopolymers such as microtubules. Microtubule polymerization is characterized by two unique phenomena, referred to as tread-milling<sup>101</sup> and dynamic instability.<sup>102</sup> Tread-milling is said to occur with the net addition of tubulin monomers at one end of the microtubule and simultaneous net loss of tubulin at the opposite end. Dynamic instability is characterized by switching between phases of relatively slow and rapid shortening of the microtubules at their ends. Although these phenomena were once thought to be incompatible, it is now known that both behaviors coexist in near-steady-state conditions in cells.<sup>101,102</sup>

It would be quite interesting if we could use the desirable properties of DNA nanostructures to recapitulate these phenomena and ultimately dissect the governing dynamics of microtubule polymerization (Figure 6A). DNA tiles could be designed such that the rate of assembly equaled the rate of disassembly, resulting in steady-state tread-milling and fixed-length nanotubes. Further, if tiles with bi- or tridirectional growth were utilized, the resulting arrays would have defined shapes. The intrinsic conformational flexibility and rigidity of

different DNA building blocks could be exploited to mimic dynamic instability, where polymerization of flexible DNA tiles can be induced through seeded growth on a rigid tile and depolymerization of the flexible tiles can be initiated by removing the rigid tile protection cap. When the association and dissociation reactions reach equilibrium, the input of additional rigid tiles will catalyze the polymerization of released flexible tiles. Studying the association and dissociation kinetics of model DNA tile species with variable flexibility is absolutely essential to recreating this or similar dynamic self-assembling systems.

*3.1.2. Developmental Assembly.* The creation of new life depends on a set of extraordinary developmental processes including stem cell growth, differentiation, and morphogenesis. These processes rely on Nature’s ability to precisely control the spatial and temporal relationship between cellular components and signaling pathways. It would be extremely interesting if we could create synthetic DNA systems that mimic this kind of spatiotemporal development. DNA tiles have the potential to develop into unique patterns through instructions embedded in the building blocks or by external stimuli such as fuel strands that trigger new growth pathways (Figure 6B). Metastable DNA nanostructures could be designed and used to serve as nucleation seeds and/or catalysts to increase the growth and development of particular pathways. Multivalency and/or cooperativity within DNA nanostructures could be exploited for nucleation and initiation of alternative assembly paths.

Researchers have already begun implementing certain aspects of developmental assembly. For example, Pierce and co-workers recently reported the dynamic assembly of DNA nanostructures through a seeded cascade of hybridization chain reactions based on toehold-mediated strand displacement.<sup>103</sup> Strand

displacement circuits have also been used to trigger DNA tile assembly and control their growth into DNA tubes.<sup>104</sup> There are several key challenges to implementing toehold-mediated strand displacement in dynamic DNA systems, including leakage, slow reaction rates, and the necessity for high salt conditions. Researchers are currently trying to address each of these problems. Zhang and co-workers reportedly designed toehold exchange probes and optimized the specificity of DNA hybridization so that their system can detect single-base changes.<sup>105</sup> Designing robust self-assembling DNA platforms to mimic developmental systems will also certainly require a thorough understanding of the thermodynamics and kinetics of DNA self-assembly.

**3.1.3. Quasicrystal Lattice Assembly.** In 2011, the Nobel Prize in Chemistry was awarded to Dan Shechtman for his discovery of quasicrystals, a finding that fundamentally changed how chemists understand solid matter. Prior to his report,<sup>106</sup> scientists believed that the atoms in a crystal were always packed into symmetric patterns that repeated periodically. We have since come to understand that it is possible to form packed crystals from nonrepeating patterns, an arrangement of molecules now referred to as “quasicrystalline”. The distinctive properties of quasicrystals, as well as their unique structures, have intrigued scientists ever since their discovery;<sup>106–108</sup> however, very little is currently known about the properties exhibited by synthetic and naturally occurring quasicrystals. Scientists have yet to determine what guides quasiperiodic rather than periodic growth and what factors result in the unique properties that quasicrystals display.<sup>109,110</sup> One of the biggest challenges facing researchers today is the lack of plausible systems from which to assemble quasicrystals and enable further studies. DNA platforms are promising candidates for the controlled, programmable growth of synthetic quasicrystals (Figure 6C). Interacting DNA building blocks can potentially be programmed to assemble into 2D and 3D quasicrystal patterns, allowing us to investigate the still unknown mechanisms of quasicrystal growth and providing a means to organize other materials for engineering pursuits.

**3.1.4. Periodic 3D Crystal Lattice Assembly.** Realizing 3D DNA lattices as hosts to organize guest protein molecules and facilitate protein crystallography necessitates that 3D DNA crystals can themselves be reliably assembled and characterized. Researchers successfully demonstrated the assembly of a 3D DNA crystal in which the triangular unit tiles were connected by sticky ends and solved its structure to  $\sim 4$  Å resolution using X-ray crystallography.<sup>16</sup> However, most DNA crystals only diffract to 7–10 Å, leaving scientists trying to determine why rationally designed DNA crystals do not diffract with better resolution. There are several possible explanations, including defects that arise during crystallization, impurities in the synthetic DNA, and the presence of bulky solvent molecules in the large cavities of the DNA lattices.

Crystal defects may be caused by the limited rigidity of DNA unit motifs, where any over- or under-twisting of the tiles causes inter-tile mismatches that are detrimental to the integrity of the crystal lattice. We surmise that imparting flexibility to certain domains of the DNA building blocks may allow the unit tiles to more reliably accommodate their neighbors and reach a lower energy state for crystal lattice formation, thereby improving the overall quality of the crystal. The Sleiman group pioneered DNA junctions with metal complex modifications that combine rigidity within the core of the junction with intrinsic flexibility in the arms.<sup>111</sup> This type of modified DNA unit motif has the

potential to improve the quality of DNA crystals but has yet to be exploited for crystallization applications.

Reducing the volume of solvent present in the lattice cavities by inserting sequence-specific binding proteins may improve the diffraction quality, but sequence-independent methods to orient proteins within the DNA cavities still need to be developed. This strategy is particularly attractive, as some have already demonstrated that RNA-binding proteins are useful chaperones for RNA crystallization. Piccirilli and co-workers derived RNA-specific antibodies using synthetic phage display libraries and showed that the antibody fragments promoted crystallization of RNA molecules.<sup>112</sup> Similarly, DNA tile binding antibodies could be identified through *in vitro* evolution and used for coassembly of the DNA units and proteins into designed 3D crystals.

Recent developments in free electron laser (FEL) X-ray nanocrystallography have the potential to revolutionize the field of structural biology by providing highly focused coherent X-ray beams with a peak brilliance that is  $10^9$  higher than the X-ray beams at the most powerful synchrotron facilities.<sup>113</sup> Obtaining high-quality diffraction patterns using FEL X-ray requires micrometer-sized nanocrystals; it might be possible to program the growth of 3D DNA lattices into finite nanocrystals with suitable dimensions by designing a 3D box that acts as a scaffold to nucleate the growth of a periodic lattice of DNA tiles. Growing 3D crystals with designed crystal morphologies and dimensions is undoubtedly an interesting topic in itself.

**3.1.5. Scaffolded Assembly.** The development of scaffolded DNA origami represents a milestone in structural DNA nanotechnology.<sup>32</sup> While the complexity and robustness of 2D and 3D DNA origami objects has increased over the past few years, researchers still lack basic understanding of the thermodynamics and kinetics of scaffolded assembly. Understanding the minutia of DNA origami formation will allow us to guide the design of more complex DNA nanostructures, optimize annealing protocols, and manipulate functionalized DNA nanostructures more effectively. Structurally speaking, we are still a long way from being able to weave a scaffold strand along arbitrary paths within a DNA origami structure, although some progress has been made in this direction. Recently, Yan and co-workers developed a novel strategy to fold gridiron-like DNA origami structures.<sup>114</sup> In that work, interconnected four-arm junctions were used as vertices within a network of DNA fragments, and measured distortion of the junctions from relaxed conformations allowed the scaffold strand to traverse through individual vertices in several directions. Despite this initial success, interlacing the scaffold strand through the vertices of multiarm junctions remains a challenge that, if achieved, would dramatically improve our ability to form aperiodic tiling patterns and polyhedral 3D structures using the DNA origami technique. Besides increasing complexity, scaling up the size of DNA origami and reducing the cost of staple strand synthesis are also important issues facing DNA nanotechnologists. Various strategies to address these limitations have been explored, including the use of longer single-stranded scaffolds,<sup>42</sup> double-stranded scaffolds,<sup>115</sup> origami of origami (super-origami),<sup>41</sup> and enzymatic production of staple strands on microarray chips,<sup>116</sup> which has the added benefit of greater fidelity than chemical synthesis. Researchers are relentlessly pushing forward to achieve more robust DNA origami technology.

**3.1.6. Surface-Mediated Assembly.** DNA origami has shown great success in directing the assembly of nanoelectronic



and photonic elements and has been used as a lithographic mask to etch nanoscale patterns on silicon and graphene substrates.<sup>117</sup> For practical device applications, it is highly desirable to achieve robust patterning of self-assembled DNA nanostructures on inorganic surfaces, and several groups have developed unique strategies to organize DNA origami nanostructures on solid substrates.<sup>118–120</sup> The next logical step is to generate chemically functional surface features to facilitate patterning of DNA origami nanostructures into spatially addressable arrays. Surface-mediated assembly may be the key to scaling-up DNA nanostructure assemblies into wafer-size arrays. Researchers have already shown that mica and silicon dioxide surfaces will mediate the assembly of small DNA tiles into millimeter-range periodic 2D lattices.<sup>121</sup> The buffer conditions, especially the concentration and species of the ions present, may play a critical role in surface-mediated diffusion of DNA nanostructures, an important factor that remains to be explored.<sup>122</sup> It would also be interesting to use fluidic 2D surfaces such as lipid bilayers to improve the surface-mediated diffusion of DNA nanostructures.<sup>123</sup>

**3.1.7. Algorithmic Assembly.** In mathematics and computer science, an algorithm describes a set of simple instructions for solving a problem. However, if you look beyond their traditional context in mathematics, you will see that algorithms can be used to describe the process of self-assembly in the natural world. Consider the self-assembly of lipids into membranes, or viral proteins into capsids, or even just amino acids into intricately folded protein structures, each process involves the spontaneous, or automatic, assembly of small components into larger, more complex structures. The process by which these structures grow can be described as algorithmic. In each example, a limited number of molecular building blocks grow into higher order structures by following the growth rules encoded into the building blocks themselves. DNA tiles are information-rich building blocks ideally suited for implementing algorithmic self-assembly. Originally proposed by Winfree, algorithmically self-assembled DNA nanostructure patterns have been experimentally demonstrated. For example, Winfree and co-workers showed that DNA double-crossover tiles could be programmed to compute and grow into Sierpinski triangle<sup>28</sup> and binary counter-assemblies.<sup>29</sup> They also showed that prescribed DNA origami displaying sticky-end-capture probes function as effective nucleation seeds to grow algorithmic arrays while suppressing spurious nucleation, which is a major source of errors during algorithmic assembly.<sup>124</sup> The design of novel nucleation frames could improve the fidelity and robustness of algorithmic assemblies of DNA tiles. Other errors arise from sticky end mismatches between different tiles that share certain sticky end sequences. The kinetics of tile–tile association between the algorithmic building blocks should be carefully investigated to promote the desired computations and reduce any undesirable mismatches. Also, tile sets could be expanded beyond the typical double-crossover DNA tiles to more complex or optimal geometries to facilitate multivalent and cooperative binding between the tiles and allow for improved understanding of the constraints that limit the scope of algorithmic assembly.

**3.1.8. Topological DNA Nanostructures.** In biological systems, there is a clear relationship between the specific structure of a biomolecule and its function. In particular, biopolymers are important molecules whose structure supports the organization and functionality of cells. The topology of biopolymers can be exploited to facilitate tasks such as packing information-bearing DNA molecules into tiny compartments

within cells. Molecular topology is a fascinating and technically challenging topic that DNA nanotechnology is ideally suited to examine. Seeman and co-workers were the first to show that topological structures such as knots and Borromean rings could be self-assembled from DNA by combining right-handed B-form and left-handed Z-form DNA together to create positive and negative nodes.<sup>125</sup> Yan and co-workers later used the DNA origami method to construct Möbius strip topological structures that could be reconfigured into catenanes and twisted topological ribbons through toehold-mediated strand displacement.<sup>56</sup> More recently, Willner and co-workers developed strategies to interlock DNA rings into multiring catenanes.<sup>126</sup> Weizmann and co-workers just reported the assembly of complex knots and links by specifically configuring four-way DNA junctions.<sup>127</sup> Despite these interesting examples, the area of DNA-based topological nanostructures is underdeveloped compared to the geometric structures that have been reported over the past decade. New construction strategies and topological targets should be identified to push the frontiers of DNA-based molecular topology forward.

**3.1.9. Self-Replicating DNA Nanostructures.** Self-replication is an astounding process by which a molecule in a dynamic system makes an identical copy of itself. Biological cells, provided they have a suitable environment, reproduce by cell division. During cell division, linear DNA autonomously undergoes replication by enzyme-mediated processes and is transmitted to offspring. It is a considerable challenge to design and construct autonomous structures that mimic the action of nucleic acid polymerases and are capable of replicating entire synthetic DNA systems nonenzymatically (Figure 6D). The first development in this direction was reported by Seeman's group in 2011.<sup>128</sup> They constructed a seven-tile seed and successfully generated several generations of progeny in a step-by-step manner. Winfree and co-workers recently showed that mechanically induced scission of 2D DNA crystals can accurately replicate self-assembled DNA nanopatterns by creating new fronts of crystal growth.<sup>129</sup> However, constructing autonomous self-replicating systems that do not require external manipulation remains a significant challenge. Pierce and co-workers demonstrated autocatalytic DNA duplex formation by way of a cross-catalytic circuit,<sup>63</sup> yet extending this concept to independent formation of sophisticated DNA nanopatterns needs additional development.

**3.2. Future Applications.** The successful design and assembly of the DNA nanosystems discussed above will undoubtedly lead to many new opportunities and innovative applications. The information-rich character of self-assembling DNA nanostructures in particular will create many new frontiers for the application of designer DNA nanostructures as molecular scaffolds, sensors, computers, and robots. In the following sections we will discuss the potential of DNA nanostructures to serve as scaffold for functional nanoelectronic and nanophotonic devices, to regulate protein interactions, and to create sense–compute–actuate elements for molecular medicine. However, these examples are in no way limiting, and the field has already demonstrated a tendency to grow in unexpected directions, surprising even the sage of researchers.

**3.2.1. Molecular Scaffolds for Nanophotonics or Nanoelectronics.** One of the most obvious applications of a DNA nanostructure is to direct the assembly of other, less controllable materials, as was discussed in the previous sections. We have seen several examples of spatially addressable DNA origami structures being used to organize nanoelectronic and

photonic components. However, we have not yet seen concrete examples of DNA nanostructures in functional nanoelectronic and photonic devices, where bottom-up, DNA-directed assembly is interfaced with top-down, lithographic methods of micro- and macroscale patterning. The latest developments in surface-mediated self-assembly and site-specific control of chemical properties could enable more precise arrangement of these nanophotonic or nanoelectronic elements into regular, large-scale patterns that can be integrated with macroscopic systems.

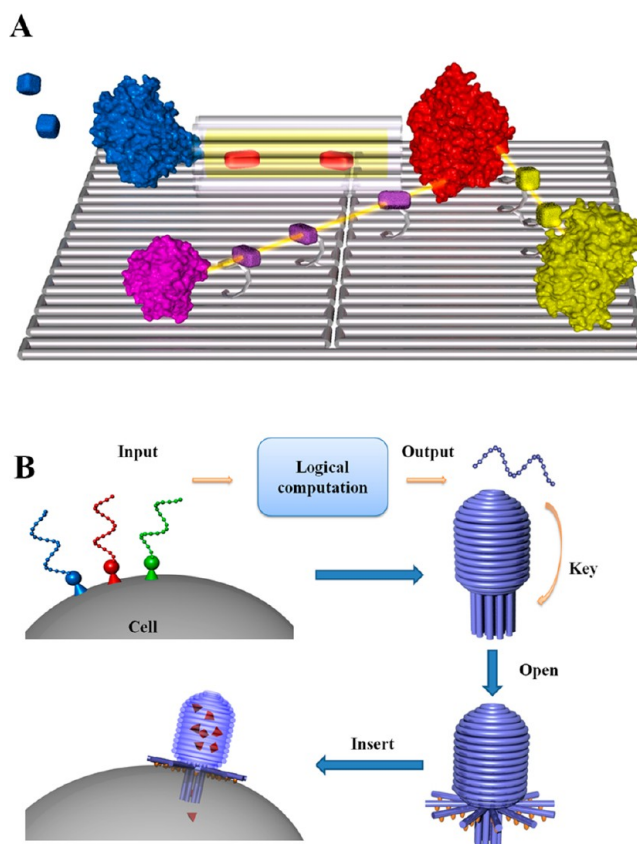
**3.2.2. Molecular Scaffolds for Enzyme Cascades.** DNA-directed assembly of complex protein arrays is another area of development to watch for in the future. Enzymes, marvels of natural evolution, are intramolecular organizations of proteins that are capable of recognition, capture, and activation of molecules and regulation of biochemical processes. These protein complexes act as the central functional components of metabolism and reproduction in living systems.<sup>130</sup> The binding sites for substrates and cofactors are chemically specific, while the active sites are stereospecific and highly sensitive to conformational rearrangement. Inspired by Nature, researchers have pursued a variety of strategies to regulate and control the catalytic activities of enzymes, as well as to understand the mechanism of enzyme function and pathways.<sup>131–135</sup> Compared to most conventional techniques, DNA nanotechnology is a highly efficient and controllable strategy to achieve structural programmability and reconfigurability through rational design and construction.

Assembling enzymes and cofactors on DNA nanostructure scaffolds has already allowed researchers to probe the essential parameters for modulating catalysis, such as intermolecular distance and relative spatial position.<sup>85–87,136–138</sup> One example of controlling the activity of an individual enzyme using DNA was reported in 2013, where the authors achieved mechanical regulation of the enzyme luciferase by attaching a DNA spring.<sup>139</sup> In the same year, a DNA tweezer-actuated enzyme nanoreactor was successfully constructed.<sup>140</sup>

An even loftier and more valuable goal is to engineer highly programmed cascading enzyme pathways on DNA nanostructure platforms with control of input and output sequences. Achieving this goal not only would allow researchers to mimic the elegant enzyme cascades found in Nature and attempt to understand their underlying mechanisms of action but also would facilitate the construction of artificial cascades that do not exist in Nature (Figure 7A).

One major challenge in integrating multiple proteins into DNA nanostructures is to precisely define their relative orientation and position. A set of reliable and general methods for site-specific conjugation of proteins with oligonucleotides must be established in order to accommodate the diversity of proteins of interest. In an ideal system, a single protein with multiple coupling sites would be conjugated to unique DNA sequences to enable absolute orientational control of the protein relative to the DNA nanostructure. In this way, the active sites of the enzymes, in a multienzyme cascade for example, could be precisely oriented to facilitate substrate–intermediate–product transfer, and the overall enzymatic activity of the cascade could be optimized.

**3.2.3. Molecular Sense–Compute–Actuate Devices.** A far-reaching goal of structural DNA nanotechnology is to develop smart molecular machines that perform sense–compute–actuate mechanisms based on intrinsically information-rich DNA molecules and structures (Figure 7B). For example, the development of “smart molecular doctors” would revolutionize

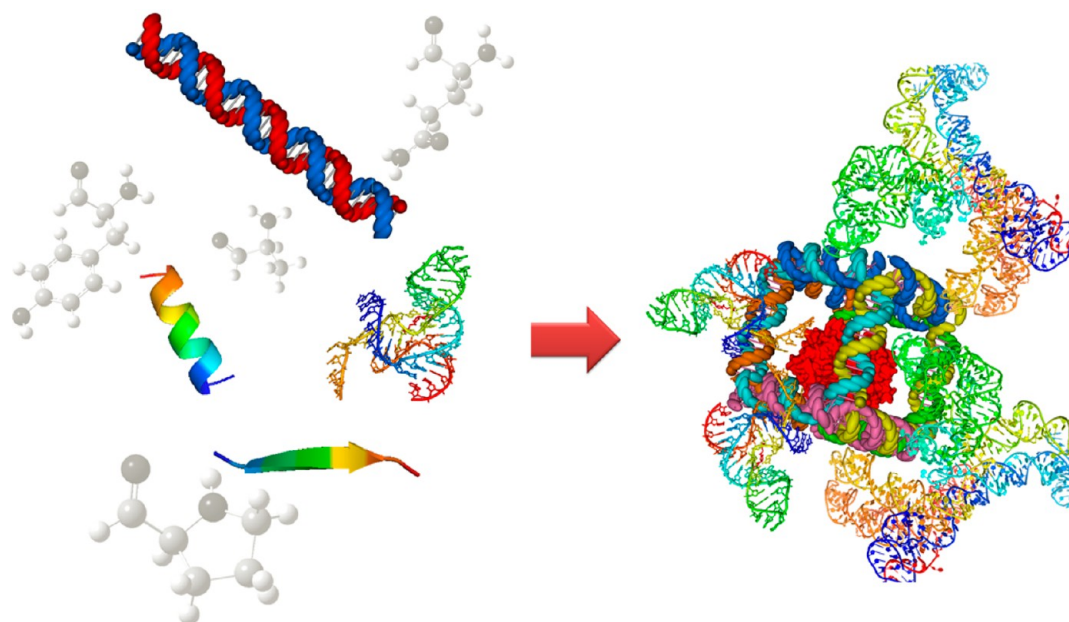


**Figure 7.** Illustration of potential applications of DNA nanotechnology. (A) Programming biochemical pathways with controlled input and output. (B) Design and implementation of theranostic nanodevices on targeted cell surfaces that carry out functions such as compute, sense, release signal, trigger activation, and deliver therapeutic molecules across the cell membrane.

the field of personalized medicine. A smart molecular doctor would have the same responsibilities as a real (human) doctor, including diagnostic and therapeutic roles, but would operate entirely at the cellular level. Directly treating individual diseased cells to cure them on the single-cell level offers improved therapeutic efficiency and fewer side effects since smaller drug doses are required compared to conventional therapies.

Other targeted drug delivery systems based on multifunctional liposomes, polymersomes, and nanoparticles have already been developed.<sup>141</sup> DNA is an attractive material for theranostic applications, not only because of its inherent design modularity, structural programmability, and biocompatibility but also because DNA molecules of a particular sequence or with certain modifications can selectively bind, distinguish, and communicate with target cells to trigger drug release. Researchers have made strides toward constructing DNA-based drug containers and DNA nanostructures that can be embedded into lipid bilayers,<sup>142</sup> particularly after the establishment of the DNA origami method. The first DNA origami box with a responsive lid that recognized a specific oligonucleotide key and subsequently opened was reported in 2009.<sup>34</sup> More recently, researchers developed a DNA nanobarrel with two single-stranded aptamer locks that were opened by the presence of target cells *in vitro*.<sup>95</sup>

Performing DNA computation directly on the surface of cells, or in cellular environments, will facilitate *in vivo* targeting and drug release. Recently, Rudchenko, Stojanovic, and colleagues engineered DNA strand displacement cascades that



**Figure 8.** From nano- to angstrom-level control: engineering molecular toolboxes composed of DNA, RNA, peptide, and protein molecules and their unnatural derivatives to extract new design rules and create complex, self-assembling structures with angstrom-level spatial control.

detected the presence of certain cell markers on the surface of cells.<sup>143</sup> In another report, Hemphill and Deiters successfully engineered oligonucleotide logic gates to detect specific microRNA inputs in live mammalian cells.<sup>144</sup> As more complex and robust DNA-based computing systems are developed, it may be possible to integrate them into cellular systems to control and trigger cellular functions such as gene expression or to interfere with the metabolic pathways.<sup>145</sup> Recently, researchers reported the construction of a consensus network that distinguishes between two different input signals and reports the majority signal.<sup>146</sup> By combining DNA computation-based target cell detection with reconfigurable DNA-based drug containers, it may be possible to create a DNA nanorobot that can interface and communicate with living cells (Figure 7B).

There are a number of critical issues that must be addressed before DNA nanorobots can be used for drug delivery *in vivo*. Researchers must find a way to protect DNA nanostructures from degradation by the intra- and extracellular nucleases and liver metabolism over long periods. Compact DNA nanostructures generally display relative stability against DNA nucleases for a short time (a few hours).<sup>147,148</sup> In the future it will be important to increase resistance to biodegradation by using methods such as chemical cross-linking of selected DNA strands or designated DNA backbone modifications. Identifying the mechanisms by which DNA nanostructures enter cells without being damaged, and escape endosomal processing,<sup>149</sup> is also a critical point. Other issues such as immunogenicity<sup>150</sup> and tissue distribution should also be considered.

The biggest obstacles to transforming DNA nanostructures from mere curiosities into real-world solutions are the cost of synthetic DNA, small production scales, typically low yield of complex 3D structures, and sensitivity of DNA to ionic strength, temperature, and nucleases. Researchers have already begun to address these issues by optimizing origami design and folding strategies to increase assembly yields<sup>151</sup> and shorten assembly times<sup>152</sup> and by developing suitable purification strategies for large-scale synthesis.<sup>153</sup> It is also important to develop biocompatible conditions for efficiently folding DNA

nanostructures, rather than by thermal annealing under high magnesium concentrations.<sup>154,155</sup>

**3.3. From Nano to Angstrom Technology.** Living cells are information-rich, sophisticated machines that display angstrom-level organizational precision. Although DNA nanostructures are exquisitely programmable, they are only able to regulate biological molecules at a relatively coarse level compared to Nature. If we want additional control, we must push the boundaries of nanoscale fabrication to the angstrom level. In contrast to DNA, RNA and proteins have more refined architectures with angstrom-level features. These aspects of their organization have attracted increasing attention in the past decade. For example, several rationally designed RNA nanostructure have been constructed.<sup>156,157</sup> Methods for engineering designed proteins and nanostructured complexes using proteins have also begun to emerge.<sup>158,159</sup> The progress of characterization techniques such as cryo-EM, X-ray diffraction, and NMR supports the development of angstrom technology. In particular, the most recent developments in cryo-EM techniques allow crystallization-free structural determination of large-sized proteins that is comparable to X-ray methods.<sup>160,161</sup> Using DNA origami frames both as structural hosts and as references, the structure of DNA and RNA binding proteins may now be determined to angstrom-level resolution by cryo-EM.<sup>162</sup> This advance will provide researchers with atomic-level structural information (in conjunction with the structural solutions obtained from X-ray crystallography) that can be fed back into the design pipeline, elevating the field to unimaginable heights (Figure 8).

In summary, after more than 30 years of growth, structural DNA nanotechnology is transitioning from adolescence into adulthood. The field is crossing the boundaries of physics, chemistry, biology, and engineering and is poised to generate unique approaches and solutions to real-world challenges in science and technology. In the next phase of structural DNA nanotechnology, novel interactions between DNA, RNA, and proteins could be used to facilitate angstrom technology, representing the major challenges and opportunities in molecular design, assembly, computing, and programming.

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## Notes

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