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Spatially-Interactive Biomolecular Networks Organized by Nucleic Acid Nanostructures

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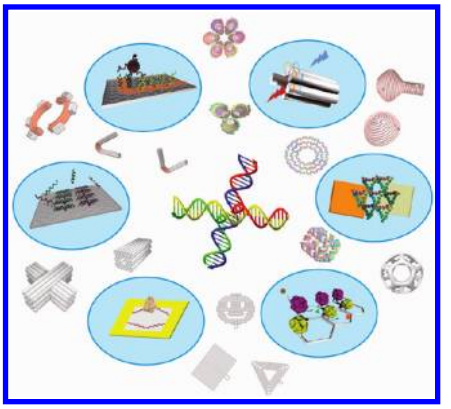

Living systems have evolved a variety of nanostructures to control the molecular interactions that mediate many functions including the recognition of targets by receptors, the binding of enzymes to substrates, and the regulation of enzymatic activity. Mimicking these structures outside of the cell requires methods that offer nanoscale control over the organization of individual network components. Advances in DNA nanotechnology have enabled the design and fabrication of sophisticated one-, two- and three-dimensional (1D, 2D, and 3D) nanostructures that utilize spontaneous and sequence-specific DNA hybridization. Compared with other self-assembling biopolymers, DNA nanostructures offer predictable and programmable interactions and surface features to which other nanoparticles and biomolecules can be precisely positioned.

The ability to control the spatial arrangement of the components while constructing highly organized networks will lead to various applications of these systems. For example, DNA nanoarrays with surface displays of molecular probes can sense noncovalent hybridization interactions with DNA, RNA, and proteins and covalent chemical reactions. DNA nanostructures can also align external molecules into well-defined arrays, which may improve the resolution of many structural determination methods, such as X-ray diffraction, cryo-EM, NMR, and super-resolution fluorescence. Moreover, by constraint of target entities to specific conformations, self-assembled DNA nanostructures can serve as molecular rulers to evaluate conformation-dependent activities.

This Account describes the most recent advances in the DNA nanostructure directed assembly of biomolecular networks and explores the possibility of applying this technology to other fields of study. Recently, several reports have demonstrated the DNA nanostructure directed assembly of spatially interactive biomolecular networks. For example, researchers have constructed synthetic multienzyme cascades by organizing the position of the components using DNA nanoscaffolds *in vitro* or by utilizing RNA matrices *in vivo*. These structures display enhanced efficiency compared with the corresponding unstructured enzyme mixtures. Such systems are designed to mimic cellular function, where substrate diffusion between enzymes is facilitated and reactions are catalyzed with high efficiency and specificity. In addition, researchers have assembled multiple choromophores into arrays using a DNA nanoscaffold that optimizes the relative distance between the dyes and their spatial organization. The resulting artificial light-harvesting system exhibits efficient cascading energy transfers. Finally, DNA nanostructures have been used as assembly templates to construct nanodevices that execute rationally designed behaviors, including cargo loading, transportation, and route control.

Introduction

Biological systems use complex macromolecular nanostructure networks to mediate a range of cellular functions, such as biomolecular synthesis, signal transduction, and gene expression and regulation, all with high efficiency and specificity. Many of these macromolecular systems have evolved through the spontaneous self-assembly of components into highly organized spatial structures, where the position and orientation of molecules are precisely controlled to facilitate functionality. For example, the multienzyme cascades[1] found in biochemical synthesis pathways and the light harvesting system in photosynthetic reaction centers[2] both rely on very specific arrangements of components.

Over the past few decades, molecular self-assembly processes have been exploited to construct various nanostructures including vesicles, nanofibers, and nanotubes from

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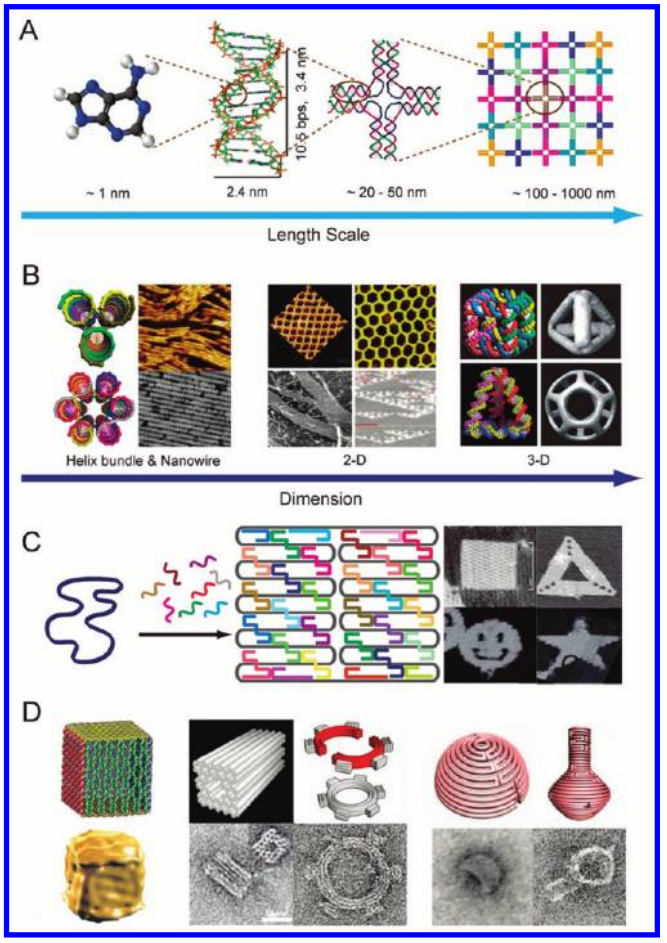


FIGURE 1. Introduction to structural DNA nanotechnology. (A) Self-assembly of nanostructures based on cDNA base pairing. (B) DNA helix bundles (left),[9,10] 2D arrays (middle),[11,12] and 3D objects (right).[13−15] (C) DNA origami for constructing 2D nanostructures[18] and (D) 3D architectures: hollow box (left),[19] multilayer monolith and square-toothed gear (middle),[5,22] and semisphere and nanoflask (right).[23] Panel B, left and part of the middle image, reproduced with permission from refs 9−11. Copyright 2005 and 1999 American Chemical Society. Parts of panel B, middle and right, and panel D, right and part of middle image, reproduced with permission from refs 12, 14, 22, and 23. Copyright 2003, 2005, 2009, and 2011 AAAS. Part of panel B, right, panel C, panel D, left, and part of panel D, middle, reproduced with permission from refs 5, 13, 15, 18, and 19. Copyright 2009, 1991, 2008, 2006, and 2009 Nature Publishing Group.

self-assembling lipids, peptides, nucleic acids, and polysaccharides.[3] However, it remains a challenge to accurately arrange multiple heterogeneous components into geometric patterns with nanometer precision, as in natural systems. Additional challenges include the development of novel assembly algorithms to increase structural complexity and improve the fidelity and yield of the assembly process.

DNA is among the most promising biomolecules for the construction of complex biomolecular networks.[4] DNA is a self-assembling biopolymer that is directed by canonical Watson−Crick base pairing to form predictable, double helical secondary structures, which are stabilized by hydrogen-bonding, $\pi-\pi$ stacking, and hydrophobic interactions. B-form DNA double helices have well-defined structural characteristics, including a helical repeat of ~3.4 nm, helical diameter of ~2.0 nm, and ~34.3° twist angle between base-pairs in solution (Figure 1A).[5] The use of double helical DNA molecules for nanoscale engineering pursuits began with Seeman's construction of artificial branched DNA tiles, where four rationally designed oligomeric nucleic acid strands self-assembled into an immobile four-way junction.[6] Double-crossover (DX) DNA tiles,[7] with increased structural rigidity compared with four-way junction tiles, were developed later and were suitable for assembling more complex periodic nanostructures through sticky end interactions.[8] Tile-based DNA assembly has been demonstrated through the construction of a number of unique nanostructures, ranging from multihelix bundles, nanotubes[9,10] and 2D lattice arrays[11,12] to 3D geometric shapes including a cube,[13] a tetrahedron,[14] and a buckyball (Figure 1B).[15]

An important milestone in structural DNA nanotechnology was the creation of aperiodic patterns using a scaffolding strategy. Early reports include the organization of DX tiles into 2D lattice barcode patterns, directed by a long ligated DNA strand,[16] and the assembly of a 3D octahedron, directed by a 1.7 kb DNA strand.[17] In 2006, Paul Rothemund made a breakthrough in scaffold-directed DNA nanostructure assembly; in the method he developed, referred to as DNA origami, a long single-stranded DNA scaffold (e.g., 7429-nt M13 phage genome DNA) is folded into arbitrary 2D shapes by following predetermined folding paths that are specified by a collection of short oligonucleotide "staple" strands (Figure 1C).[18] Many 2D nanostructures including a square, rectangle, smiley face, triangle, and star have been demonstrated using the DNA origami method. One of the most attractive properties of DNA origami structures is the addressability of the surface, the result of the unique sequence at each oligonucleotide staple position. Thus, various patterns can be displayed by selectively modifying staple strands at desired locations with single-stranded probe extensions. The DNA-origami method has several advantages over "tile-based" assembly approaches: (1) scaffolded DNA can be folded into nearly any symmetric or asymmetric structure; (2) well-formed nanostructures are generated with high yield using unpurified oligonucleotides, because the scaffold imposes the correct stoichiometry between strands; (3) spatially addressable assembly is achieved with a resolution of ~6 nm. The DNA-origami

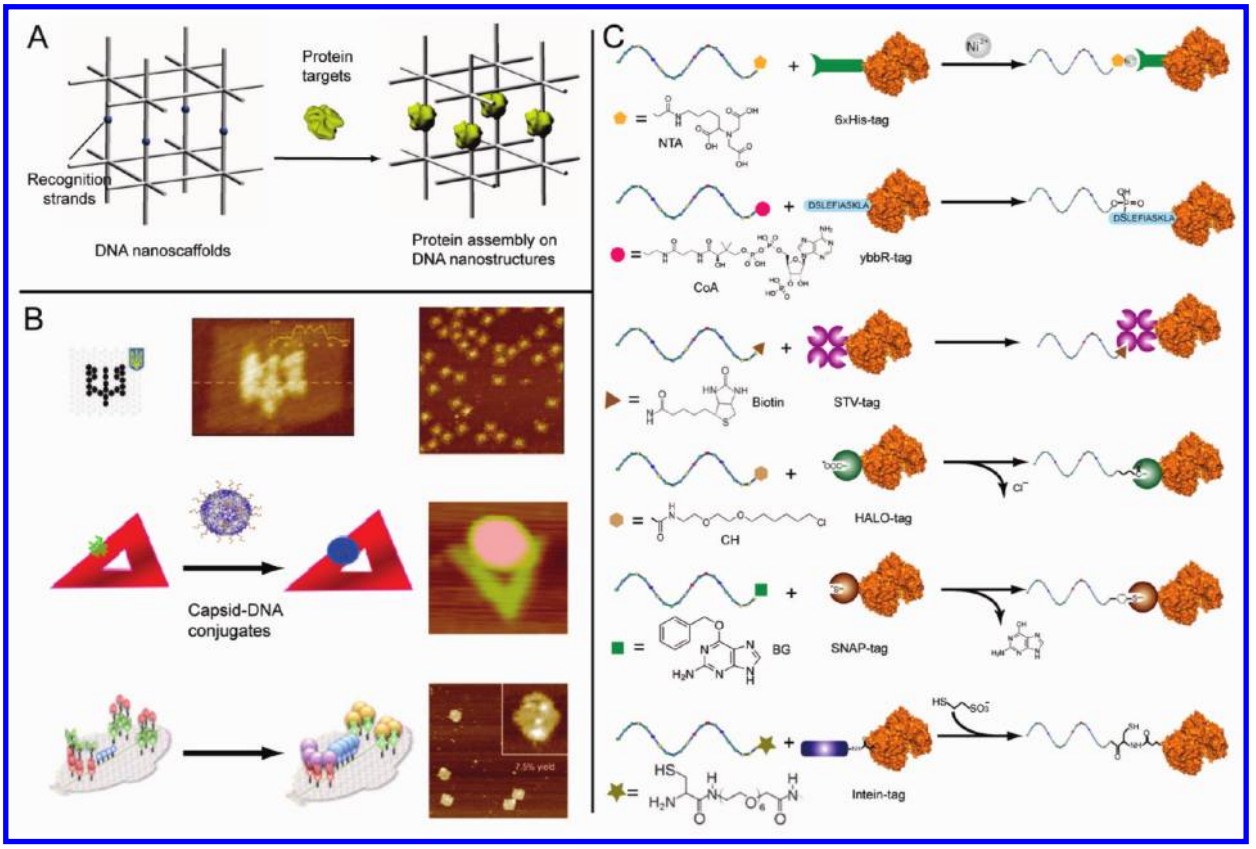


FIGURE 2. DNA-directed assembly. (A) Seeman's proposal to organize macromolecules within a DNA nanoscaffold. (B) Patterning macromolecules on DNA origami: streptavidin (top),[29] virus capsid (middle),[32] and orthogonal protein decoration (bottom).[36] (C) Site-specific protein−oligo conjugation using His-tag, ybbR-tag, STV-tag, HALO-tag, SNAP-tag, and Intein-tag (from top to bottom). Panel B, top, reproduced with permission from ref 29. Copyright 2009 IOP Publishing. Panel B, middle, reproduced with permission from ref 32. Copyright 2010 American Chemical Society. Panel B, bottom, reproduced with permission from ref 36. Copyright 2010 Wiley.

approach was further developed for the construction of 3D nanostructures. The Gothelf group assembled a hollow DNA box by joining six distinct (though connected by the scaffold) origami sheets through the action of staple strands bridging the edges (Figure 1D, left).[19] The Shih group introduced a method to construct solid 3D shapes by packing scaffolded DNA double helices into pleated layers, constrained to a honeycomb or square lattice.[5,20,21] Twisted and curved 3D objects were further developed through insertion or deletion of base pairs at selected positions within the helical layers (Figure 1D, middle).[22] The Yan group recently developed a strategy to construct DNA nanostructures with complex curvatures by nesting a collection of concentric DNA rings of decreasing circumference to generate the rounded contours of various 3D objects (Figure 1D, right).[23] In addition to these reports, several computational tools including caDNAno[24] and CanDo[25] have been developed to facilitate the design of DNA nanostructures, making structural DNA nanotechnology more accessible to researchers from other fields.

In addition to DNA, RNA nanotechnology has recently emerged as an attractive method to construct nanostructures with functional diversity.[26] In order to form the variety of loops and structural motifs that are required for functionality, RNA nanostructures rely on the self-complementarity of single strands. One of the attractive features of RNA-based nanostructures is the potential for *in vivo* assembly, because single-stranded RNA molecules are readily transcribed in cells. DNA/RNA hybrid nanostructures are likely to have a synergistic potential that combines the predictability of DNA assembly with the functional diversity of RNA.[27]

DNA nanostructures are reliable directors in the organization of heterogeneous nanoscale entities such as peptides,[28] proteins,[29] and nanoparticles.[30] Supermolecular networks of molecules that are scaffolded by DNA nanostructures exhibitwell-controlled intercomponent distances and relative numbers. This characteristic presents exciting opportunities for fundamental studies of distance-dependent molecular interactions and for practical applications including biosensing, molecular biophysics, biocatalysis, drug delivery, and responsive nanodevices. Herein, we describe the progress that has been made in DNA-directed assembly of biomolecular networks.

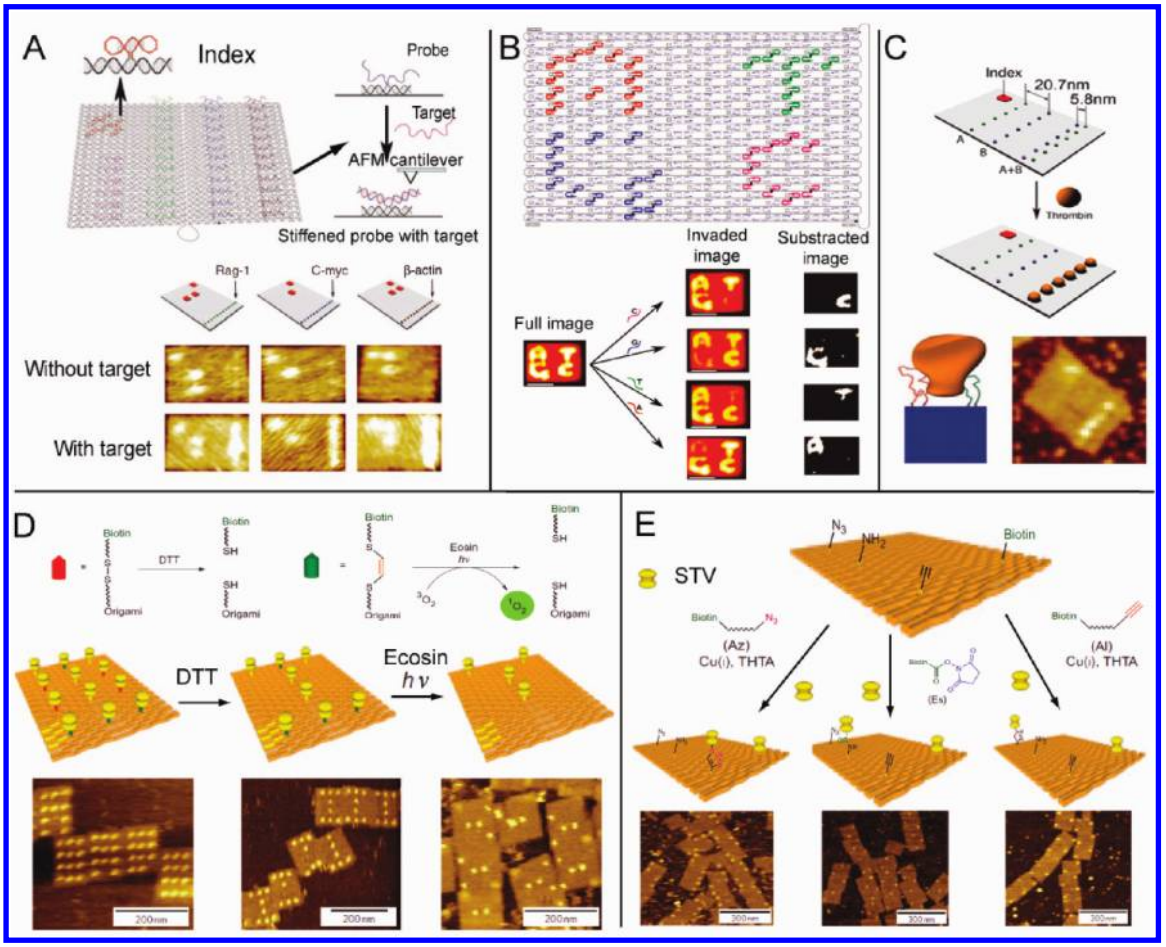


FIGURE 3. DNA nanostructures as a template for label-free detection of bimolecular interactions: (A) RNA hybridization assay; reproduced from ref 40, copyright 2008 AAAS. (B) SNP detection; reproduced from ref 41, copyright 2011 American Chemical Society. (C) Spatially dependent multivalent ligand-protein binding; reproduced from ref 42, copyright 2008 Nature Publishing Group. (D) Chemical bond formation and (E) bond cleavage; reproduced from ref 46, copyright 2010 Nature Publishing Group.

DNA-Directed Self-Assembly

In Seeman's original proposal, he suggested that a DNA nanolattice could be used as a framework to organize proteins into 3D crystals, where the position and orientation of each protein could be controlled by elements of the DNA nanostructure (Figure 2A).[6] Since that time, the sequence specificity of DNA hybridization has been exploited to assemble external biomolecules at specific positions on addressable DNA nanostructures. Hybridization between the DNA functionalized biomolecules and single-stranded probe extensions of the DNA nanostructures generate networks of molecules with controlled intermolecular distances and ratios. This approach was demonstrated by organizing smaller biomolecules, including aptamers[31] and peptide,[28] as well as larger macromolecules, including proteins[29] and virus capsids,[32] on DNA nanostructures (Figure 2B).

Critical to DNA-directed assembly efforts are the development of efficient oligonucleotide–biomolecule coupling methods. One of the attractive features of DNA scaffolds is that the constituent oligonucleotides can be modified with a variety of different functional groups for subsequent cross-linking reactions with other biomolecules;[33] amino and thiol modifications are among the most common. Despite their versatility, one of the drawbacks of conventional cross-linking methods is a lack of control over the conjugation site and stoichiometry of coupling. The presence of multiple lysine and cysteine residues on the surface of most proteins makes it difficult to generate a site-specific protein conjugation, which is required for certain applications.[34] Genetic modification of proteins with reactive tags (His-tag and ybbR-tags, for example) and the use of fusion domains (such as streptavidin, intein, SNAP, and HALO) are alternative approaches to achieve site-specific protein–oligo conjugation with very high efficiency (Figure 2C).[34,35] In addition to covalent coupling approaches, noncovalent binding between proteins and specific ligands can also be used for

assembling protein nanoarrays.[12,28,31] Orthogonal display of several proteins on DNA origami was demonstrated by employing three site-specific coupling strategies: SNAP-tag, HALO-tag, and biotin–streptavidin interactions.[36] The use of strong domain interactions (zinc-finger domains, for example) is another approach for site-specific protein–oligo coupling and can be achieved by tethering one binding domain to a protein and the other binding domain to an oligonucleotide.[37,38] The versatility of DNA nanostructures has also been used to improve the binding affinity between molecules. The assembly of a multivalent ligand complex was achieved by combining several individual ligands on a DNA nanostructure, where the distance between ligands was carefully controlled. The resulting binding affinity (K_d) between binding partners was in the low nanomolar range.[39] It should be possible to achieve more precise control over the orientation of biomolecules by combining site-specific conjugation strategies with 3D DNA nanostructures that have specifically tailored cavities or cages to constrain the guest molecule through steric interactions.

Label-Free Detection of Bimolecular Interactions

Label-free detection is becoming more and more attractive for biological assays, where bimolecular interactions (e.g., mass, dielectric, and morphology) are characterized without the need for sample modification. DNA nanostructures have several features that make them promising agents for label-free detection. As water-soluble nanoscale "chips", DNA nanostructures are capable of displaying multiple probes from their surface for the detection of various bimolecular interactions. For example, the interaction between probe extensions and target molecules can result in a measurable change in surface morphology (height), which can be distinguished by atomic force microscopy (AFM). This detection strategy was used to demonstrate label-free RNA hybridization on a DNA origami chip.[40] As shown in Figure 3A, multiple nucleic acid probes were designed to target specific RNA sequences and were precisely patterned on an underlying DNA origami scaffold. Before RNA hybridization, the single-stranded DNA probes were quite flexible and not clearly visible by AFM. Detection of specific RNA targets by the DNA probes resulted in the formation of double helical DNA–RNA V-shaped junctions with characteristic features that were obviously identified by AFM analysis. In addition, DNA origamis were decorated with "barcode loops" so that several nucleic acid detection tiles could be differentiated, allowing for simultaneous, multiple-target analysis. Beyond external molecule detection, DNA origamis have been used to demonstrate the detection of genomic single nucleotide polymorphisms (SNPs) by direct AFM readout (Figure 3B).[41] Letters corresponding to each of the four DNA nucleotides (A, T, C, and G) were patterned on a DNA origami tile. Each letter was composed of a collection of single-stranded DNA that was bound to probes that were extended from the DNA origami surface, creating an obvious topography that was easily detected by AFM. The single-stranded DNA in each letter contained distinct binding sites that were complementary to the particular nucleotide they represented. In the presence of a DNA sequence containing the target nucleotide variation, the strands that represented the corresponding character were displaced, resulting in the disappearance of the underlying letter pattern.

Addressable DNA nanostructures, with the ability to organize a variety of ligands into specific spatial patterns, provide an opportunity to study the factors that govern protein–ligand binding. The distance-dependent binding of a multivalent aptamer–protein complex was characterized using a DNA origami platform. Two aptamers were placed at several distances from a target protein to determine the spacing that resulted in the strongest binding (Figure 3C).[42] By organizing bispecific linkers, multifunctionalized DNA nanostructures can bring different cells into close proximity and induce specific cell–cell interactions for therapeutic applications.[43] Distance-dependent ligand binding can also be used to probe the internal arrangement of protein domains. The ideal spatial arrangement of two tandem SH2 domains of Syk kinase was determined by organizing the domains with a double-stranded DNA nanoscaffold. The nanoscaffold displayed the two domain-binding ligands at various distances and flexibilities.[44]

DNA origami has been used to visualize chemical reactions on the single molecule level. In Figure 3D,E, origami structures act as addressable supports to monitor chemical formation and cleavage reactions with readout of chemical reactions achieved via biotin–streptavidin complexes. When biotin linkers were cleaved by disulfide bond reduction or photogenerated singlet oxygen, the biotin–streptavidin conjugates were released from the origami surface. Using a similar approach, bond formation was also detected; incoming functional groups were linked to biotin, and the incorporation of each group was visualized by the addition of streptavidin. Three functional groups commonly used for bioconjugation reactions were studied: alkyne, amine, and azide.[45,46]

Conformational Biophysics

Nearly 30 years after the initial proposal of creating self-assembling 3D crystals using DNA junction structures,[6] Seeman and co-workers demonstrated a 4 Å resolution

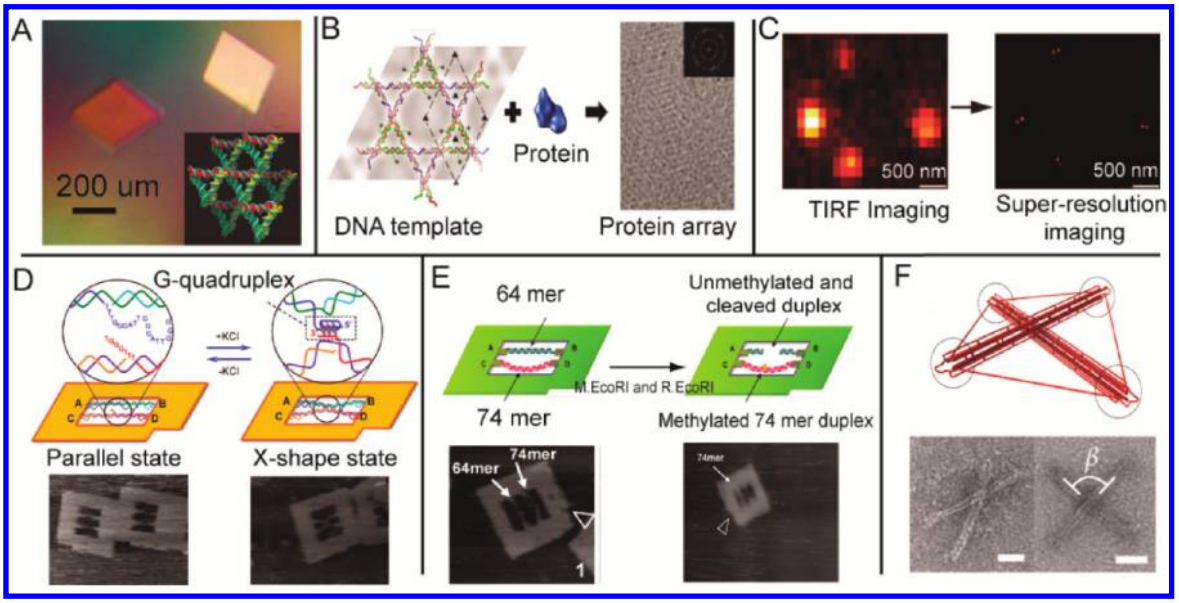


FIGURE 4. DNA nanostructures as biophysical study tools: (A) X-ray diffraction (reproduced with permission from ref 47, copyright 2009 Nature Publishing Group), (B) cyro-EM (reproduced with permission from ref 48, copyright 2011 American Chemical Society), and (C) super-resolution imaging (reproduced with permission from ref 51, copyright 2009 Wiley). Conformational studies of (D) G-quadruplex formation (reproduced with permission from ref 52, copyright 2010 American Chemical Society), (E) DNA methylation (reproduced with permission from ref 53, copyright 2010 American Chemical Society), and (F) constrained intermolecular forces (reproduced with permission from ref 55, copyright 2010 Nature Publishing Group).

crystal structure of a self-assembled DNA tensegrity triangle (Figure 4A).[47] In addition to X-ray diffraction experiments, DNA nanostructures have also been used to align and localize macromolecules in particular nanoenvironments for other structural determination methods. Self-assembled DNA nanoaffinity templates were used to facilitate data collection in single-particle electron cryomicroscopy (cryo-EM) by creating dense and nonoverlapping arrays of protein molecules (Figure 4B).[48] Detergent-resistant, DNA-nanotube liquid crystals were employed to introduce weak alignment of membrane proteins for their structural determination by NMR.[49] This method was recently used for NMR structural determination of mitochondrial uncoupling protein 2 (UCP2).[50] Reconstruction of super-resolution fluorescence images was reported, where DNA origami served as a molecular ruler to locally organize several fluorophores for imaging calibration (Figure 4C).[51]

Beyond structural determination methods, DNA nanostructures can be used to study conformation-dependent biological activities by constraining macromolecules to specific environments with controlled arrangements and molecular forces. Real-time observations of a G-quadruplex, a structure that is associated with the telomeric region of chromosomes, were facilitated by stretching two corresponding G-strands across the inner cavity of a DNA origami frame structure (Figure 4D).[52] The formation and disruption of the G-quadruplex structure was visualized by fast-scaning AFM through the addition or removal of K^+ ion. In Figure 4E, conformation-dependent DNA methylation was also studied by using a DNA origami frame structure to control the tension of two double helical substrates (64 nt and 74 nt).[53] AFM images of enzyme–substrate binding and cleavage revealed that enzyme-catalyzed methylation occurs more frequently for the structurally relaxed 74 nt substrate. Other DNA nanostructures have also been designed to measure the biophysical properties of molecules. DNA-based nanomechanical scissors were used to measure the force experienced when MutS binds to unpaired and bulged bases. In this system, the MutS binding force was determined by analyzing the interruption of sticky-end hybridization.[54] Toward exploiting intermolecular forces such as tensional integrity to perform work, prestressed 3D tensegrity DNA nanostructures in which rigid bundles of double helices resist compressive forces were assembled. The forces generated by the prestressing mechanism may be be used to bend DNA bundles or actuate enzymatic cleavage at specific sites (Figure 4F).[55]

Organization of Multienzyme Reaction Pathways

The metabolism of living systems involves complex synthetic pathways with numerous multistep reactions that possess extraordinary yields and specificities. Many of the enzyme systems carrying out these reaction pathways are

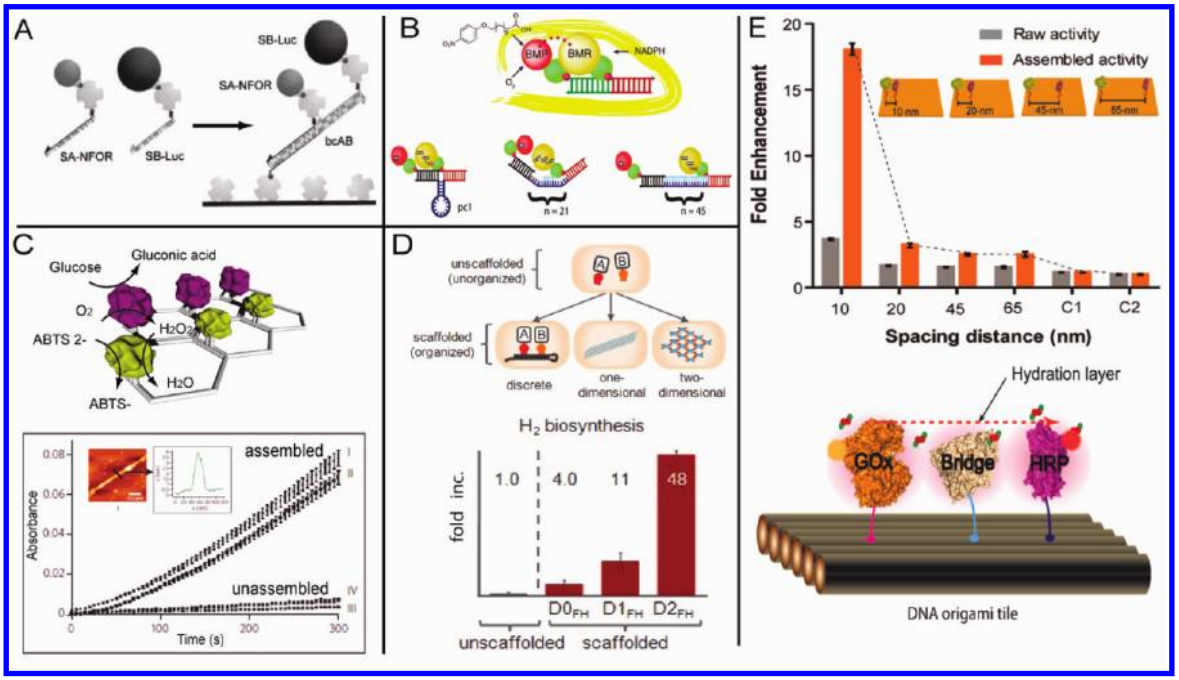
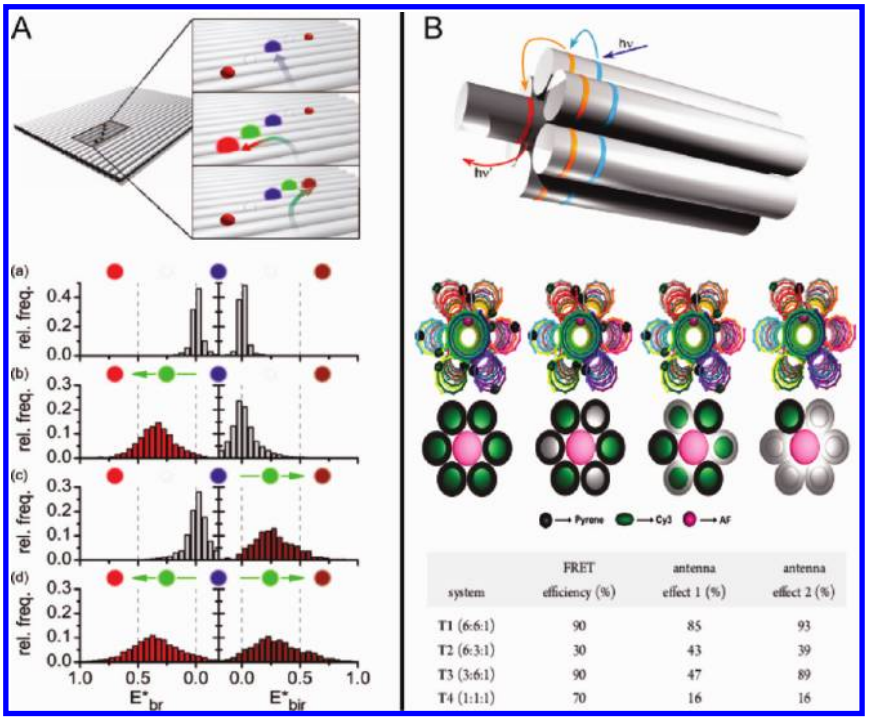


FIGURE 5. DNA/RNA nanostructures for engineering multienzyme systems. A linear double-stranded DNA scaffold for (A) assembling an enzyme cascade, NAD(P)H:FMN (NFOR) oxidoreductase and luciferase (Luc) (reproduced with permission from ref 59, copyright 2002 Wiley), and (B) evaluating the distance-dependent activity of cytochrome P450 BM3 by varying the spacing between the BMR reductase domain and the BMP porphyrin domain (reproduced with permission from ref 60, copyright 2011 American Chemical Society). (C) Two-dimensional DNA strip for organizing GOx/HRP cascades (reproduced with permission from ref 61, copyright 2009 Nature Publishing Group). (D) *In vivo* assembly of RNA nanostructures to organize the [FeFe]-hydrogenase and ferredoxin enzyme pathway for improved hydrogen production (reproduced with permission from ref 62, copyright 2011 AAAS). (E) Organization of a GOx/HRP cascade on DNA origami tiles with controlled spatial positions (top), and a protein bridge for facilitating surface-limited intermediate diffusion between enzymes (bottom) (reproduced with permission from ref 63, copyright 2012 American Chemical Society).

highly organized complexes with precisely controlled enzyme positions and orientations, facilitating efficient diffusion of substrates between the enzymes.[1] Artificial synthesis of these multienzyme systems is generally achieved by genetic fusion,[56] chemical cross-linking, and coimmobilization;[57] however, precise control over spatial organization of components is lacking for these methods.

With DNA nanostructures as assembly scaffolds, it has become feasible to organize multiple enzymes with controlled spacing in linear as well as 2D or 3D geometric patterns, which enables the study of cascade activity.[58] One of the first demonstrations was the assembly of a bioenzymatic NAD(P)H:FMN oxidoreductase and luciferase cascade on a double-stranded DNA scaffold with an observed ~3-fold increase in activity compared with the corresponding unassembled enzyme pair (Figure 5A).[59] This strategy was later applied to probing the distance-dependent activity of multidomain complexes of cytochrome P450 BM3 by varying the length of spacing scaffolds between the BMR reductase domain and the BMP porphyrin domain (Figure 5B).[60] Two-dimensional DNA nanostructures provide an even greater opportunity to organize multienzyme systems into more complicated geometric patterns. There was a report of the self-assembly of a glucose oxidase (GOx) and horseradish peroxidase (HRP) enzyme cascade on 2D hexagonal DNA strips, with the distance between the two enzymes controlled by the underlying nanostructure (Figure 5C).[61] A greater than 10-fold activity enhancement was observed compared with the corresponding unstructured enzymes. In addition to *in vitro* assembly, multienzyme pathways can also be organized by introducing nucleic acid nanostructures as assembly scaffolds *in vivo*, an approach facilitated by recent advances in RNA nanotechnology.[26] This idea was demonstrated by the assembly of an intracellular reaction pathway ([FeFe]-hydrogenase and ferredoxin) for enhancing bacterial hydrogen production.[62] In Figure 5D, discrete, 1D, and 2D RNA scaffolds were assembled *in vivo* through the incorporation of aptamers for capturing the target enzyme cascade. Remarkably, a 48-fold enhancement of hydrogen production was observed for the RNA-templated [FeFe]-hydrogenase and ferredoxin network. This study suggests that a metabolic engineering approach can be used to introduce structural nucleic acid



system	FRET efficiency (%)	antenna effect 1 (%)	antenna effect 2 (%)
T1 (6:6:1)	90	85	93
T2 (6:3:1)	30	43	39
T3 (3:6:1)	90	47	89
T4 (1:1:1)	70	16	16

FIGURE 6. Energy-transfer within DNA nanostructures. (A) Four-color FRET[65] and (B) artificial light-harvesting network.[66] Reproduced with permission from refs 65 and 66. Copyright 2011 American Chemical Society.

nanostructures inside cells for the organization of multienzyme reaction pathways. Recently, a GOx/HRP cascade was organized on DNA origami tiles with precisely controlled spatial positions, which was applied to investigating the distance-dependent interenzyme substrate diffusion (Figure 5E).[63] The study revealed that substrate transfer between enzymes might occur at the connected hydration shells for closely paced enzymes and demonstrated this idea by constructing a protein bridge to facilitate the intermediate transfer across protein surfaces.

Light-Harvesting Networks

In natural photosynthesis, light is harvested by antenna systems that consist of networks of spatially organized chromophores to facilitate unidirectional energy transfer to a redox center.[64] In artificial systems, DNA nanostructures can be used to arrange multiple pairs of fluorescence donors and acceptors into precise geometric patterns to achieve efficient energy transfer. In Figure 6A, a DNA origami tile was used to organize several distinct fluorophores into closely packed linear arrangements to achieve multicolor energy transfer, observable at the single-molecule level.[65] Energy transfer was directed along a path from a blue to red dye or from a blue to IR dye by placing a "jumper dye" between the primary donor and the final acceptor. As shown in Figure 6B, an artificial light-harvesting antenna was constructed by assembling multiple donor–acceptor pairs on a seven-helix DNA bundle.[66] Steady-state and time-resolved fluorescence spectroscopy was used to measure the efficiency of energy transfer for networks with various ratios of donor to acceptor dyes.

Responsive Nanodevice

In 1966, the science fiction movie "Fantastic Voyage" described a shrunken micrometer-sized submarine that could be injected into the human circulatory system to search for and destroy a threatening blood clot in the brain. The enormous potential of DNA nanotechnology is bringing us closer to this dream. Autonomous DNA walkers are early demonstrations of functional nanorobots, where the motion of the legs is coordinated and driven by either strand displacement[67] or deoxyribozyme (DNAzyme)–substrate binding and cleavage.[68] Recent advances in DNA origami make it possible to construct integrated nanosystems that combine walkers, cargo, tracks, and drive mechanisms to achieve complex motions on 2D or 3D surfaces. There was a report of an integrated system that executed cargo loading, transportation, and destination control functions.[69] In Figure 7A, the hands of the DNA walker bound to specific nanoparticle cargo when the cassette was switched from an "OFF" to "ON" state. Fuel strands were employed to initiate the walker's stepwise movement, with a 120° rotation for

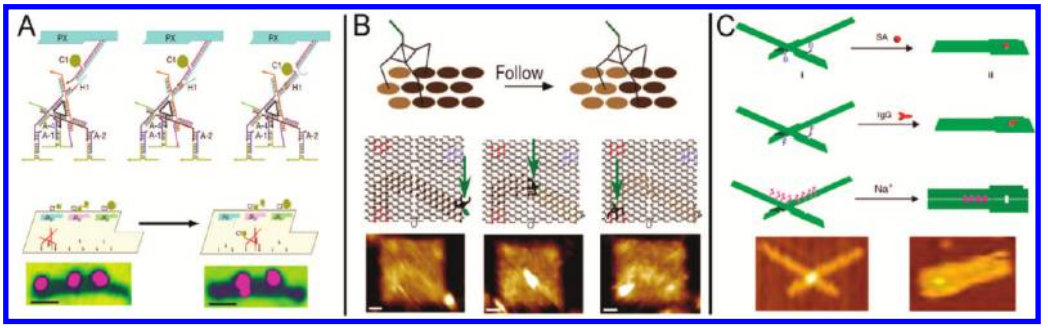


FIGURE 7. Responsive DNA nanodevices: (A) a cargo transportation system consisting of an assembly template, cargo loading apparatus, and DNA walker,[69] (B) walker movement along a 2D deoxyribonucleotide substrate surface,[70] and (C) forceps for sensing various noncovalent interactions.[74] Reproduced with permission from refs 69, 70, and 74. Copyright 2010 and 2011 Nature Publishing Group.

each step. The cargo-transportation system was programmed to reach eight different destinations by controlling the states of the three loading cassettes and the movement along the tracks. In parallel, a spider-like molecular walker was developed with the ability to travel along a 2D oligonucleotide substrate track assembled on a DNA origami tile.[70] The walker was composed of an inert streptavidin protein body with three catalytic DNAzyme legs and a single capture leg for loading the molecular spider on the surface of the origami (Figure 7B). For movement along a predetermined path, the molecular walker was first loaded at the START position via hybridization of the capture leg to a partially complementary probe extended from the DNA origami surface. The walker was subsequently released by the addition of a 27-nt single-stranded DNA trigger that was fully complementary to the START probe, displacing the capture leg and allowing the walker to move to the substrate track. The catalytic action of the DNAzyme legs, binding to and cleaving the underlying DNA substrate track, drove the spider toward uncleaved substrate until it reached a STOP site, where further movement was inhibited by strong binding between a noncleavable probe and the DNAzyme legs.

In addition to walkers, other responsive DNA nanodevices such as tweezers,[71] I-motif switches,[72] and hybridization-chain-reaction systems[73] have been developed. These devices are capable of sensing the presence of specific DNA, changes in pH, and mRNA expression. Recently, origami-based forceps with the ability to switch between "open" and "closed" positions were reported. The action of the forceps was triggered by noncovalent interactions including metal ion–nucleotide, biotin–streptavidin, and antigen–antibody binding interactions (Figure 7C).[74]

Future Perspective

Self-assembled DNA nanostructures can now be used to organize a variety of heterogeneous elements into precise patterns on rationally designed 2D and 3D nanoarchitectures. Future challenges include identifying how to harness this power to construct functional, spatially interactive biomolecule complexes. Here, we identify several potential applications of DNA nanotechnology in constructing artificial bionanosystems.

Bottom-up Engineering of Multicomponent Complexes. Translating biochemical reaction pathways to noncellular environments is of great scientific interest. Exerting control over these pathways beyond nature's repertoire would enable enzyme-catalyzed production of novel molecules and energy conversion optimized for ambient and extreme environments. Engineering functional multienzyme complexes requires a method to reliably organize the individual protein components with control over the relative position, orientation, and quantity of the participating molecules. The combination of self-assembled DNA nanostructures and common bioconjugation strategies makes it possible to rationally design and organize multiprotein pathways, as well as modulate the local environment and influence the corresponding chemical reactions (Figure 8A). For example, the direct transfer of a substrate from one enzyme to a proximal enzyme (substrate channeling), is one of the primary ways that natural systems facilitate highly efficient enzyme activity.[75] Similar channeling effects can be replicated in a DNA nanostructure system by optimizing the relative position and orientation of the catalytic components. Directed diffusion over longer distances can be achieved by modifying the environment between two enzymes with specific properties (polarity or hydrophobicity) that encourage substrate diffusion. It is also possible to constrain the diffusion between two enzymes by constructing DNA cavities or nanotubes. Further, enzyme pathway feedback mechanisms may be realized by constructing branched reaction pathways, where the catalytic activities are regulated by activation or deactivation of a specific pathway.

Artificial Macromolecular Photosynthetic Complex. Natural photosynthetic systems harvest light energy and

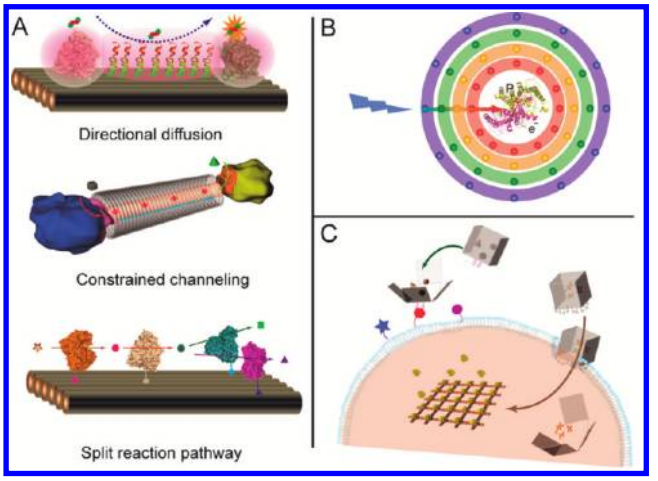


FIGURE 8. (A) Engineering enzyme pathways to achieve directional substrate diffusion (top), constrained substrate tunneling (middle), and split enzyme pathways as feedback mechanisms (bottom). (B) Schematic illustration of an artificial photosynthesis system that couples light-harvesting and charge-separation components within a multilayer DNA nanostructure. (C) DNA nanocontainer for target-specific drug delivery and *in vivo* regulation of cellular activities.

convert it into chemically useful forms. Artificial photosynthetic complexes that execute light harvesting and charge separation have been constructed by incorporating chromophores and electron donors or acceptors into supramolecular structures.[76] However, these systems exhibit little spatial control and often involve complex synthetic chemistry. DNA nanostructures can serve as scaffolds for the assembly of biohybrid systems, where efficient light-harvesting apparatus can be coupled with charge-separation complexes with nanometer-scale precision (Figure 8B). In particular, an artificial light-harvesting complex must be capable of wide-spectrum absorbance and contain an efficient energy transfer pathway, both of which can be satisfied by DNA nanostructure directed assembly. DNA nanostructures can be used to arrange multiple chromophores into 2D or 3D patterns with optimized stoichiometric ratios and intercomponent distances. Units of charge separation can be held in close proximity to the light-harvesting components for efficient conversion of light energy.

***In Vivo* Delivery and Regulation.** Nanotechnology has been applied to target-specific drug delivery, *in vivo* regulation, visualization, and sensing. Structural DNA nanotechnology may be used to construct more effective drug-delivery vehicles through the implementation of complex control mechanisms to sense specific targets, respond to environmental conditions, release molecular payloads, and trigger additional responses to regulate biological functions that impede disease progression. DNA-based nanocontainers, such as DNA boxes with switchable lids that open and close[19] and nanocages with the ability to encapsulate or release nanoparticles,[77] have demonstrated potential as drug-delivery vehicles. An autonomous DNA nanorobot controlled by an aptamer-encoded logic gate was recently reported to transport molecular payloads to cells, sense cell surface inputs for triggered activation, and transform its structure for payload delivery.[78] However, additional research is needed to improve these DNA nanodevices. First, new structural switching mechanisms (rather than strand displacement) should be implemented to control drug release in specific biological conditions. One possibility is to use structural-switching aptamers[79] to introduce a locking mechanisms to DNA nanocontainers,[78] which are triggered by aptamer-target binding. Second, the resistance of DNA nanostructures to the components of serum and cell lysate must be increased so that they may withstand *in vivo* delivery conditions. A recent study has shown that certain DNA origami structures maintain their structural integrity after incubation with cell lysate for 12 h, a significant increase in stability compared with natural single- and double-stranded DNA.[80] Finally, it is a challenge to transfer DNA nanostructures across biological membranes, since most cell membranes will only permit free passage of small molecules. Some recent studies have shown that DNA nanostructures modified with CPG[81,82] or aptamers[83] can be taken up by cells. The display of certain ligands (amphiphilic molecules, for example) from the surface of a DNA nanostructure may facilitate tissue penetration and cellular uptake of DNA nanodevices. Combining DNA/RNA nanotechnology with molecular biology may result in the development of novel ways to regulate cellular response. It may be feasible to construct artificial intracellular or extracellular nanomatrices that are designed to influence gene expression or modulate biological pathways (Figure 8C).

Concluding Remarks

Self-assembled DNA nanostructures are excellent scaffolds to direct the assembly of highly organized, spatially interactive biomolecule networks with enhanced functionality. Combining the promise of structural DNA nanotechnology with biology, chemistry, computer science, physics, and materials science will likely result in the emergence of new and exciting discoveries beyond the limited scope discussed here.

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FOOTNOTES

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