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DNA Self-assembly for Nanomedicine ☆

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ABSTRACT

Self-assembling DNA nanostructures based on rationally designed DNA branch junction molecules has recently led to the construction of patterned supramolecular structures with increased complexities. An intrinsic value of DNA tiles and patterns lies in their utility as molecular pegboard for deterministic positioning of molecules or particles with accurate distance and architectural control. This review will discuss the state-of-art developments in self-assembled DNA nanostructural system. Biomedical aspects of information guided DNA nanostructures will also be summarized. We illustrate both the use of simple DNA artworks for sensing, computation, drug delivery and the application of more complex DNA architectures as scaffolds for the construction of protein and nanoparticle arrays.

Contents

1. Introduction

Watson and Crick's 1953 understanding of nucleic acid structure [1] has led to more inquiries about nature's complex biomolecular infrastructure. One profound question is how biomolecules interact at cellular and molecular level, which is key to all biological processes. A simple way to address this question would be to keep the biomolecules in close proximity and study the interactions between them. In solving the puzzle, the major challenge is to place the biomolecules within the optimal distances that mimic nature's biomachinery.

Biomimetic self-assembly is a bottom-up approach which utilizes biomolecules as a template to arrange matter in a precisely defined manner. Among these DNA is a promising candidate due to its remarkable molecular recognition capabilities. Seeman's vision of using DNA as a structural material in 1982 laid the foundation of 'Structural DNA Nanotechnology' [2]. He proposed a set of basic rules to design branched DNA architectures that can self-assemble into periodic lattices. Since then, a variety of DNA nanostructures have been designed with well-defined periodicities and internal features [3–9]. Sequences of the DNA strands are rationally designed to self-assemble into complex architectures through complementary base-pairing interactions. DNA self-assembly opens up a new paradigm to study inter- and intra-molecular interactions. In this review, we will focus on DNA self-assemblies that generate nanometer scale

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architectures, form simple to complex DNA patterns that hold diagnostic and therapeutic potentials in nanomedicine. Recent progresses towards these goals and unmet future challenges will also be discussed.

2. DNA Self-Assembly

2.1. Property of DNA as a material

What makes DNA a fascinating molecule for self-assembly lies in its predictable base specific interactions where 'A' pairs with 'T' and 'G' pairs with 'C'. Apart from this inherent molecular recognition property, DNA is also a well-characterized polymer with well-structured conformation and geometry. Double stranded B-DNA is a nanometer scale object, with a diameter of ~ 2 nm and a helical repeat of 3.4 nm for every 10.5 base pairs. Any arbitrary sequence of DNA can be conveniently synthesized in the laboratory by automated phosphoramidite chemistry. A range of functional groups can be covalently attached to the DNA at the end or in the middle using simple attachment chemistries and this greatly enhances the applicability of DNA as a functional sensor or reporter molecule. Duplex DNA behaves as a rigid rod with a persistence length of 50 nm, while single stranded DNA adds flexibility to a design with a persistence length of 1-2 nm. The combination of stiffness with flexibility allows deliberately designed two and three-dimensional (3D) structures to form. Lastly, a collection of enzymes, such as restriction enzymes commonly used in biotechnology, is available for manipulating DNA. These enzymes not only provide a toolbox to confirm and examine the formation of the desired structure but also can be used to modify the structure for various applications.

2.2. DNA Nanoarchitectures

The fabrication of DNA nanostructures begins with the self-assembly of single stranded DNA into small building block materials called tiles. DNA tiles bearing complementary sticky ends are then able to further self-assemble into larger arrays with distinct topological and geometric features. Fig. 1 illustrates some of the DNA nanostructures (DNA tiles). The programmability of the sticky end sequences governs the assembly of tiles in a particular fashion to realize a complex architecture. In general, the process of DNA self-assembly can be divided into four categories: 1) the *quick mix approach* - where a set of unique sequenced basic unit strands are designed with unique sticky ends. During the self-assembly, these strands associate at particular positions to form the entire architecture [10–17]; 2) *hierarchical self-assembly* – an approach to minimize the unique set of strands required to generate individually formed multi-tile DNA structure, thus sequences can be reused by generating the unique tiles separately and mix them in a particular order [6–20]; 3) *algorithmic self-assembly* - the tiles are programmed with built-in instructions which code for the next layer of tiles to self-assemble on the previous layer following algorithmic rules [21–23]; 4) *nucleated self-assembly* - a longer strand acts as a nucleation site for the attachment of the associated strands to generate a complex pattern [24–26]. The architectures generated up to date range from simple one-dimensional (1D) structures [25–32] to highly complex two-dimensional (2D) structures [10–25]. Some three-dimensional (3D) objects have also been successfully constructed [25,33–36].

Domains of 2D DNA lattices with size up to millimeters in dimension (containing trillions of DNA tiles connected into a single piece of array) was recently achieved by Mao and co-workers utilizing the concept of sequence symmetric DNA tile designs [37–39] (Fig. 2a). A variety of designs have also led to the self-assembly of 2D DNA nanoarrays with finite dimensions [18–20,26]. Among these, there has been a strong desire to use less DNA material to assemble DNA nanoarrays with fixed dimension and boundary. For example, Liu et al. utilized the concept of geometric symmetry to reduce the number of tiles that are required to self-assemble finite size DNA nanoarrays [19] (Fig. 2b). Park et al. designed a finite, addressable, 16 tile system using a multi-step hierarchical self-assembly process, which also requires fewer tiles with fewer unique sticky ends to build up such arrays [20] (Fig. 2c). In another elegant design of hierarchical self-assembly, Jaeger and coworkers constructed modular RNA motifs called 'tecto-RNAs' that were used to self-assemble into addressable RNA nanoarchitectures [40]. All these examples demonstrate the exquisite programmability of nucleic acids as nanoscale building blocks (Fig. 2d).

Algorithmic DNA self-assembly, originally proposed by Erik Winfree, is another unique way of generating complex nanoscale DNA patterns by encoding computational information and algorithmic rules into DNA tiles and sticky-ends. Experimental implementations of algorithmic DNA self-assembly started to show its exciting success in recent years, led by Winfree's group. They have demonstrated that complex patterns such as Sierpinski triangles [21] can be achieved by using only a small set of algorithmic tiles (Fig. 2e). Algorithmic self-assembly has also been used to perform simple computations such as binary counting [22] and cumulative XOR [23]. It is envisaged that algorithmic self-assembly could be used as computational cassette for smart drug delivery and disease diagnostic by reporting a set of triggering events through cooperative assembly process.

Another cost effective strategy to self-assemble finite sized DNA nanoarrays with increased complexity is through a process called nucleated self-assembly. For example, Yan et al. [24] designed an aperiodic patterned DNA lattice (barcode lattice) formed by nucleating shorter synthetic DNA oligos along a longer ligated scaffolding DNA strand. The sequence of the long strand was designed such that it encoded for barcode information and the resulting arrays have a designed boundary (Fig. 2f). In a ground- breaking work, Rothemund used nucleated self-assembly to create a variety of complex patterns and shapes known as scaffolded DNA Origami [26]. In this case a single stranded viral genome was used as a nucleation strand and over 200 short "staple" strands, complementary to multiple regions of the long scaffolding strand, were assembled in a single step of annealing to fold the viral genome into arbitrary shapes of pre-determined folding path (Fig. 2g). Prior to this remarkable success, Shih and Joyce demonstrated that 3D DNA Origami can be achieved by folding a long piece of DNA by a few short "staple" strands into the geometry of DNA octahedron [25] (Fig. 4e). Since the sequences and positions of the "staple" strands in the DNA Origami are known, each position can act as potential probe site to direct the assembly of other molecules (e.g. nanoparticles or proteins) into spatially addressable arrays. This has important implications in the areas of nanoelectronics, nanophotonics and biomedical applications.

2.3. DNA Nanostructures as Scaffolds for Directed Self-assembly

Owing to the fact that DNA can be functionalized by various chemical conjugation strategies, the potential of DNA to pattern molecules ranging from biologic to inorganic origins has been exploited. DNA nanoarrays carry high density of probes at specific distances defined by deliberately designed internal features. These probes capture molecules of diagnostic and therapeutic significance and thus potentially, can be useful in biomedical applications.

Three general strategies have been explored for displaying protein molecules onto self-assembled DNA nanoscaffolds.

The first method relies on ligand-protein interactions that a DNA strand in a tile structure can be chemically modified by a ligand molecule which has specific affinity to its protein targets. For example, Park et al. [41] utilized the biotin-streptavidin interactions to pattern streptavidin protein molecules at nanometer spatial resolutions. By selectively functionalizing DNA tiles with biotin, 2D arrays of streptavidin with programmable inter-protein spacings were achieved (Fig. 3a). Using

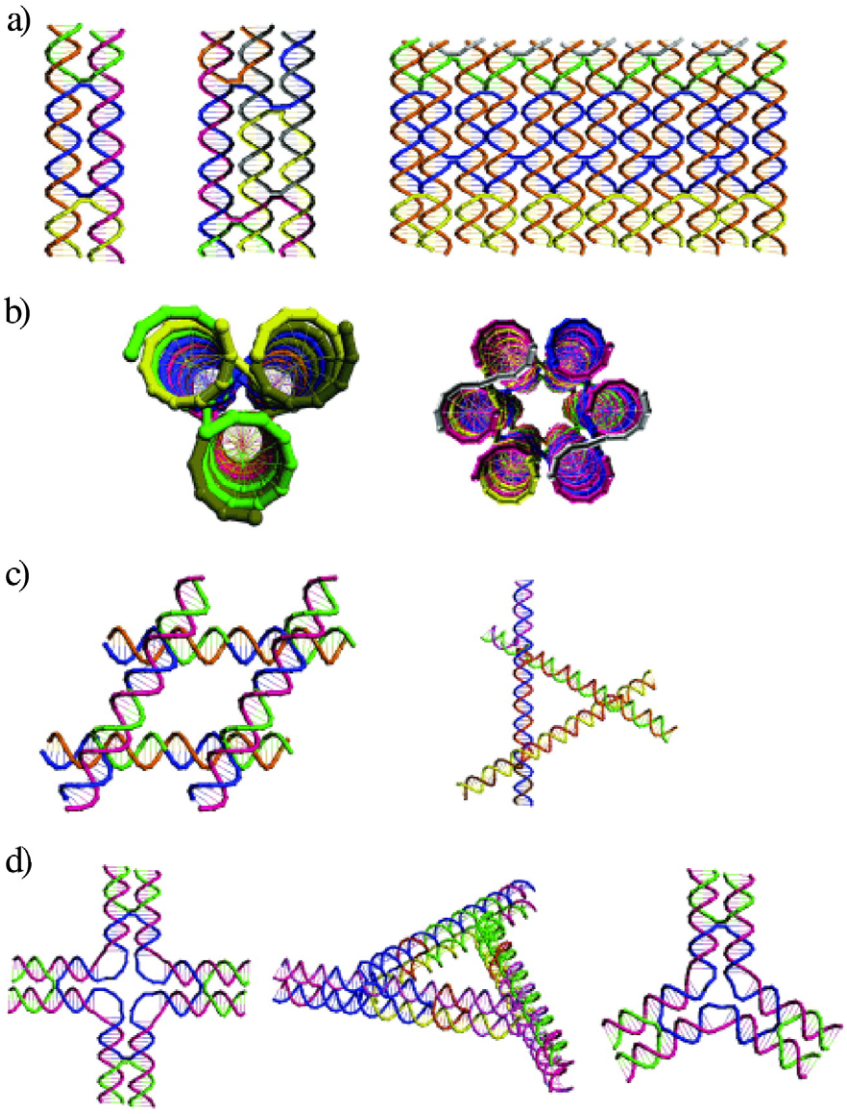

Fig. 1. The basic structural elements in DNA nanotechnology; a) DX, TX, and 12 helix DNA tile; b) three and six helix bundles; c) a parallelogram DNA tile with 4 four arm junctions and a triangle DNA tile with 3 four arm junctions; d) cross shaped tile, a DX based triangular DNA tile, 3 point star motif (adapted from ref. [4]).

the ligand-protein recognition approach, Mao and coworkers constructed antibody arrays with a vision of potential applications in immunodiagnostics or catalysis [42]. As a model system, the grid like scaffold was used to conjugate fluorescein which binds to anti-fluorescein antibody to form periodic arrays of antibodies with a periodic spacing of ~ 20 nm (Fig. 3b).

The second strategy for DNA directed protein assembly is to covalently fuse a single stranded DNA oligo to a short peptide through bi-functional linker and subsequently hybridize the DNA sequence to a DNA probe that protrudes out of the DNA tile surface. Although multiple chemical steps are involved in preparing such DNA-peptide fusion molecules, this strategy could be highly programmable through combinatorial synthesis. In an experimental demonstration, Williams et al. [43] showed that Myc-epitope peptide was covalently conjugated to DNA oligonucleotide and upon self-assembly, peptides were displayed on the DNA scaffold at periodic distances. Functionality of the assembled peptide array was demonstrated by addition of anti-myc antibody which binds to the peptides displayed on the DNA scaffold (Fig. 3c). The conclusion that the peptide, after being displayed onto self-assembled DNA architectures, still preserves its function creates new opportunities to devise spatial combinatorics of peptides for high affinity ligand nanodisplays.

The third strategy of displaying protein molecules on DNA nanoscaffolds is by introducing DNA or RNA aptamers into DNA tiles and utilizes the specific aptamer-protein binding properties to achieve a programmable assembly of various protein targets. Aptamers are DNA or RNA molecules that can be selected from random pools based on their ability to bind other molecules [44,45]. Aptamers have been selected to bind nucleic acid, proteins, small organic compounds, and even entire organisms [46,47]. Aptamers that exhibit sub-nanomolar affinities for a wide range of protein targets have been identified [48]. It is possible to generate a virtually unlimited number of specific ligand-aptamer pairs, so that each class of spatially-displayed aptamer will interact with high affinity to its specific ligand. Due to the ease of synthesizing nucleic acid aptamers and their compatibility with DNA nanostructures through simple strand hybridization, the aptamer-protein binding approach has proven to be a highly programmable way for DNA directed self-assembly of protein nanoarrays. For example, Chhabra et al. [49] recently demonstrated that spatially addressable multi-protein nanoarrays can be constructed by incorporating different aptamer sequences into complex DNA nanostructures (Fig. 3d).

Another important value of self-assembled DNA nanoarchitectures as scaffolding elements lies in their potential to organize various

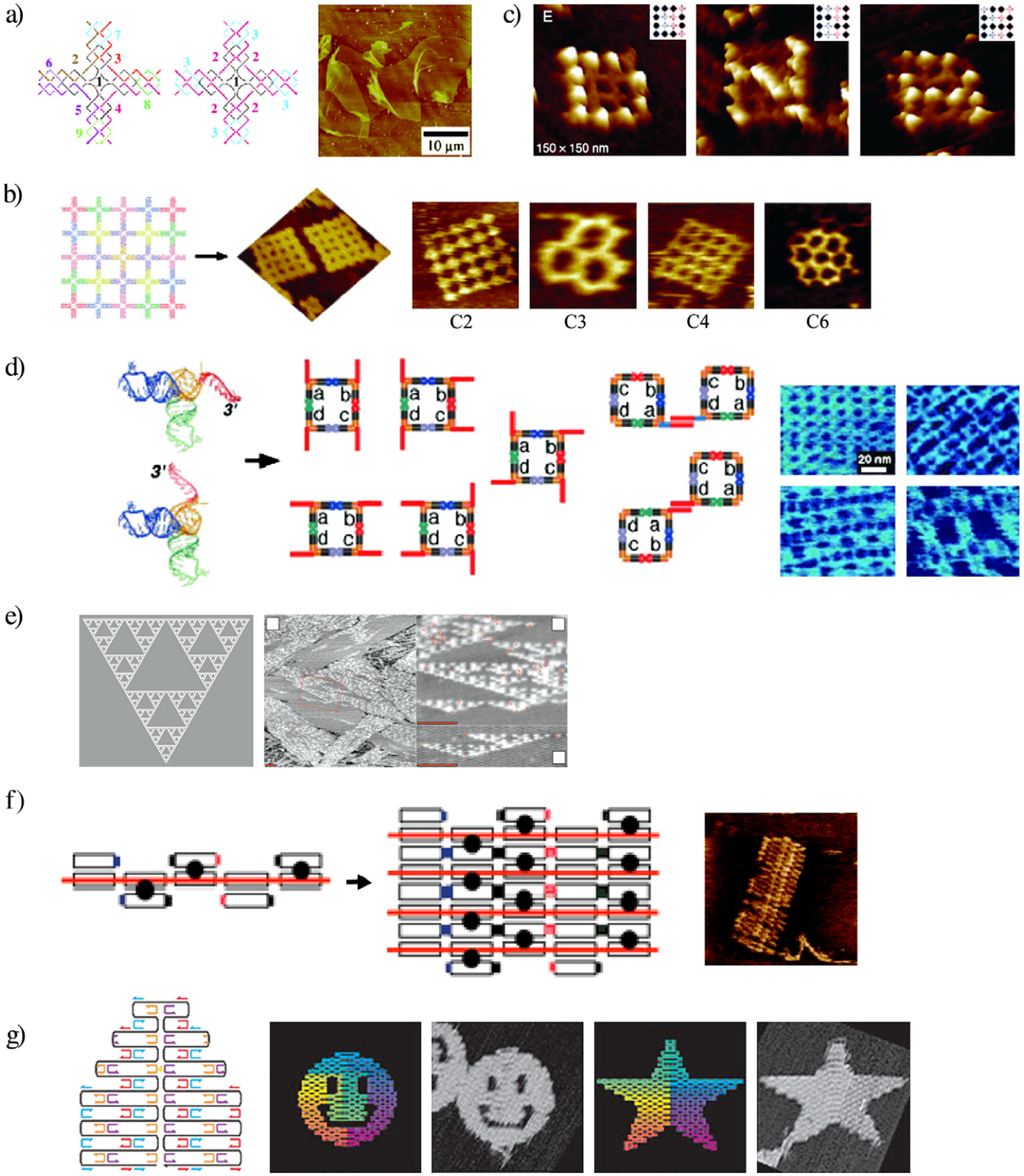

Fig. 2. Examples of DNA nanostructures of different types and complexities; a) sequence symmetric arrays formed from cross-shaped tiles (adapted from ref. [37]); b) symmetric finite sized DNA arrays with C2, C3, C4 and C6 symmetries (adapted from ref. [19]); c) addressable finite-sized DNA scaffold, generated by hierarchical self-assembly process (adapted from ref. [20]); d) tecto-RNA assemble into tectosquares that form finite sized and two dimensional architectures with varying complexities (adapted from ref. [40]); e) Sierpinski triangles, generated by algorithmic self-assembly process (adapted from ref. [21]); f) a DNA barcode lattice, assembled by nucleated self-assembly process (adapted from ref. [24]); g) DNA origami, generated by nucleated self-assembly process, formed complex architectures (adapted from ref. [26]).

nanoparticles (NPs) into discrete structures. Indeed, it is still a grand challenge in nanotechnology and material science to achieve: 1) mono-functionalization of NPs and multi-functionalization of NPs that each bear unique composition and spatial-addressability and 2) self-assembled multi-component NP-superstructures. These challenges could be met by integrating the exquisite addressability of structural DNA nanotechnology

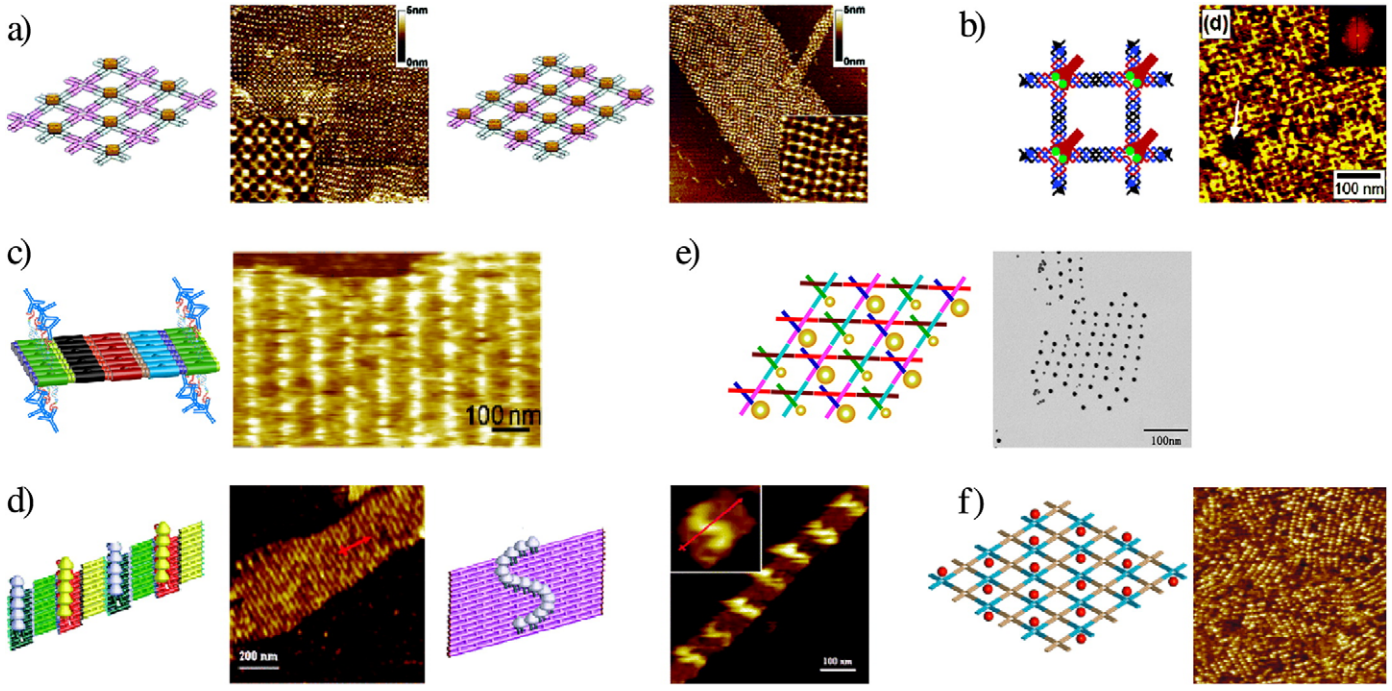

Fig. 3. DNA nanostructures as scaffolds for Templating biomolecules and inorganic metallic nanoparticles, a) assembling streptavidin in a programmable manner by biotinylated DNA arrays (adapted from ref. [41]); b) Ordered arrays of antigens showing organization of antibodies in a periodic fashion (adapted from ref. [42]);c) Peptide arrays templated by DNA scaffold and antibody arrays (adapted from ref. [43]); d) aptamers directed complex and periodical multiprotein arrays, thrombin organized in 'S' manner by aptamer decorated complex DNA origami tile (adapted from ref. [49]);e) Multi-component system patterned by DNA scaffolds (adapted from ref. [52]); f) Two dimensional organization of gold nanoparticles templated by DNA scaffolds (adapted from ref. [53]).

and the powerful optical properties of a NP system. Structural DNA nanotechnology has matured to the stage that finite-sized fully addressable 'molecular pegboards' or 'DNA origami' can now be readily constructed in both two- and three-dimensions for nano-scale positioning of molecules. The application of structural DNA nanotechnology in NP research will create unprecedented opportunities in nanoscale multiplexing for a wide range of applications in nanomedicine. For example, discrete nanoscale optical barcodes with the combination of various quantum dots and metallic nanoparticles could be utilized for single cell analysis and imaging. Although we are still far from realizing these potentials, recent research of using periodic DNA tile arrays to direct the assembly of metallic nanoparticles into designed nanoarchitectures [50–57] have demonstrated the organizational power of DNA tile arrays for this purpose (Fig. 3f).

2.4. DNA Supramolecular Frameworks

Besides the DNA octahedron construction mentioned in the previous section, a variety of other types of 3D DNA nanoobjects have been realized using different strategies. The first 3D DNA object, a topologically closed DNA cube structure, was realized by Seeman and co-workers through enzymatic ligations [33] (Fig. 4a). Later on, a truncated DNA octahedron was achieved using step-wise solid phase synthesis [34] (Fig. 4b). Shih and coworkers constructed a nanoscale octahedron from a single-stranded DNA by folding upon itself (Fig. 4d) [25]. Recently, Turberfield and coworkers created both tetrahedral [35] and bi-pyrimid [36] shaped DNA objects using a one-step multi-strand DNA hybridization strategy (Fig. 4c and e). They have also demonstrated that such DNA cages can be used to encapsulate protein molecules [58]. Using rigid organic molecules as vertices, Sleiman et al. [59] have generated a series of complex 3D DNA ensembles for example triangular prisms, cube, pentameric prisms, biprisms, etc (Fig. 4f). Mao et al. [60] have demonstrated that discrete 3D DNA objects can also be constructed by hierarchical self-assembly process from smaller number of symmetric building blocks. They designed a three point star motif as a building block which upon self-assembly generated tetrahedra, dodecahedra and buckyballs containing four, twenty and sixty blocks respectively (Fig. 4h). The concept of hierarchical one-pot assembly has a potential to construct a range of relatively complex 3D nanoarchitectures. More recently, Andersen et al. [61] have constructed a DNA origami-based addressable nanoscale box with a controllable lid that responds to the specific 'DNA' keys. This DNA box has internal cavity that has the capacity to encage a ribosome or a poliovirus. The DNA box holds the potential to carry and deliver the payload with a lid that can be controlled at will (Fig. 4i).

In the future, these supramolecular 'nanocontainers' could be used as models for advanced drug delivery and biosensing. Due to the inherent molecular recognition properties of DNA combined with the ease of attachment chemistry, drugs, proteins and small molecules could be linked to the DNA nanocontainers and translocated to the desired site of delivery.

Another interesting type of DNA supramolecular framework called dendrimer-like (DL) DNA was elegantly designed and demonstrated by Luo and coworkers [62] which had been shown to have useful applications both as sensing elements and biomaterials. DL-DNA is a three dimensional network of Y-shaped DNA motif (Y-DNA) formed by controlled-assembly process followed by enzymatic ligation. The resulted DL-DNA are stable, monodispersed and robust in nature. Luo et al. [63] also designed and synthesized DNA hydrogels using various DL-DNA motifs (Fig. 4g). These gels have the ability to produce proteins in a cell-free environment as shown by Luo et al. [64] in their recent endeavor. The DNA hydrogel consisted for X-shaped DNA as crosslinks with actual gene components as monomers. A total of 16 different proteins were produced using hydrogels with improved efficiencies and yields much higher than those of currently employed solution phase protein producing systems. This work opens up great opportunities in applications ranging from tissue engineering to drug delivery.

2.5. Self-assembled DNA Nanoarchitectures as Biosensing Materials

Besides the organizational power of self-assembled DNA nanoarchitectures as spatially addressable scaffolds, other intrinsic value of self-assembling DNA nanoarray system lies in the fact that 1) The arrays are

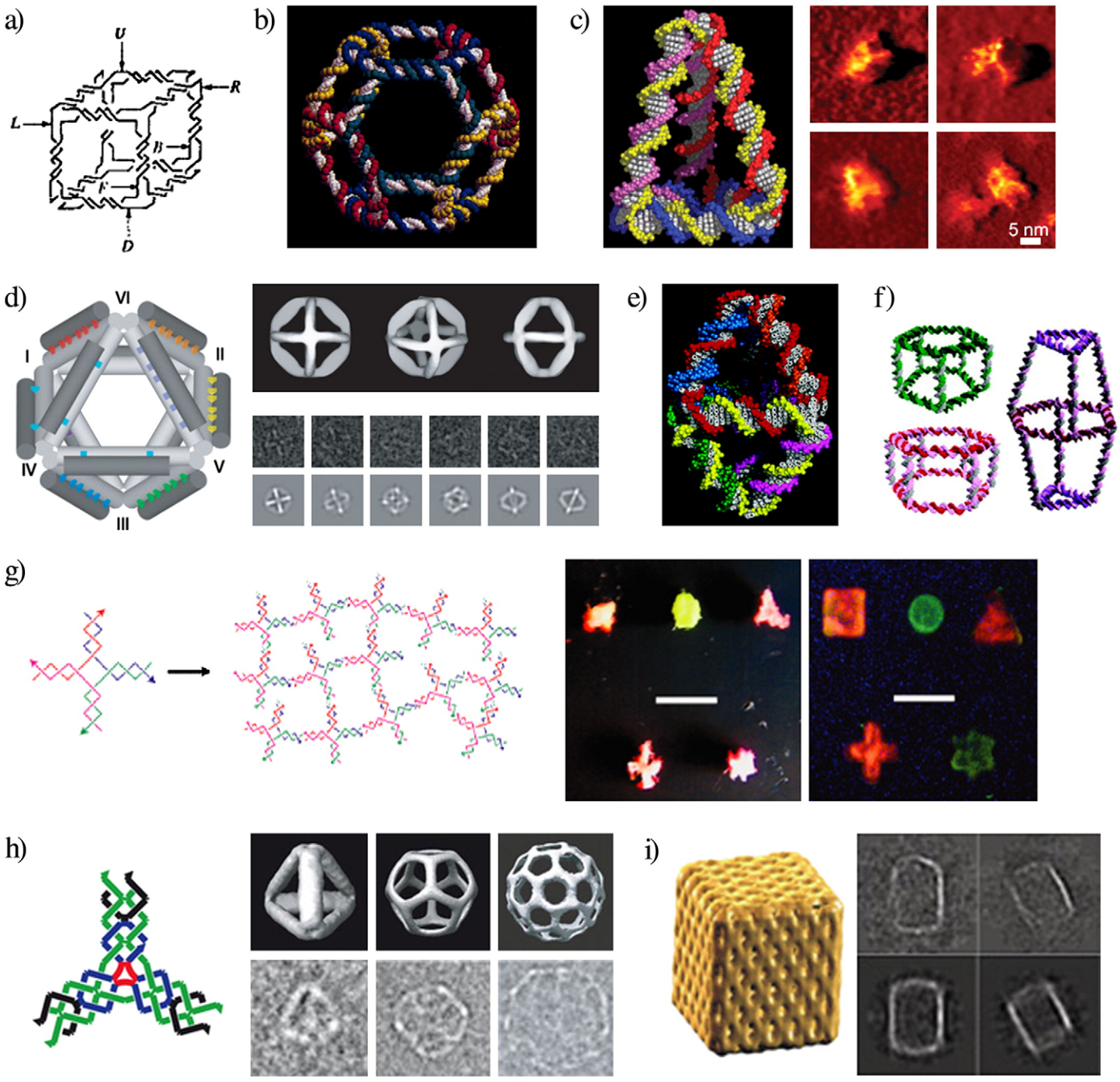

Fig. 4. Examples of 3D DNA objects and DNA supramolecular frameworks; a) schematic of the DNA cube generated through enzymatic ligations (adapted from ref. [33]); b) A DNA truncated octahedron (adapted from ref. [34]); c) DNA tetrahedron (adapted from ref. [35]); d) DNA octahedron generated by nucleated self-assembly process (adapted from ref. [25]); e) DNA bipyramid (adapted from ref. [36]); f) 3D DNA construction of pentaprisms, heptaprisms and biprisms (adapted from ref. [59]); g) a DNA based hydrogel self-assembled from X-DNA, based on DNA monomer type, different shape and size of gels can be assembled (adapted from ref. [63]); h) Hierarchical self-assembly of tetrahedral, dodecahedra and buckyball from a three point star motif (adapted from ref. [60]); i) A 3D DNA box with controllable lid (adapted from ref. [61]).

water-soluble, avoiding surface chemistry problems common for chip based micro-arrays and bio-conjugation steps necessary for bead-based technology. The molecular recognition events for the detection happen in solution, thus are more efficient compared to diffusion at solid/liquid interface; 2) The process of the array formation is through self-assembly, thus it is massively parallel and highly efficient; 3) A rich set of molecular probes (aptamers, DNA, RNA) can be easily engineered modularly into the combinatorial array for multiplex detection of a wide range of molecules, from proteins, DNA, RNA to small molecules. Taking advantage of these unique properties, Luo and coworkers employed dendrimer like DNA to generate a multiplexed detection system [65]. In this work, Y-DNA was used as construction elements and different numbers of fluorescent dyes were inserted to address each DL-DNA with a unique label. Multiple DNA oligos of pathogenic origin were detected simultaneously with attomolar detection limit (Fig. 5a). More recently, Luo et al. [66] have developed a modular, anisotropic multifunctional system using X- and Y-DNA, called 'ABC monomers' for ultrasensitive detection of pathogens. Upon target binding, the ABC monomers form ABC dimers which can further photo-crosslink leading to the formation of detectable polymeric spheres. These polymeric spheres served as multi-drug delivery vectors due to their anisotropy and were internalized by cells with little cytotoxicity. The ABC monomers have the

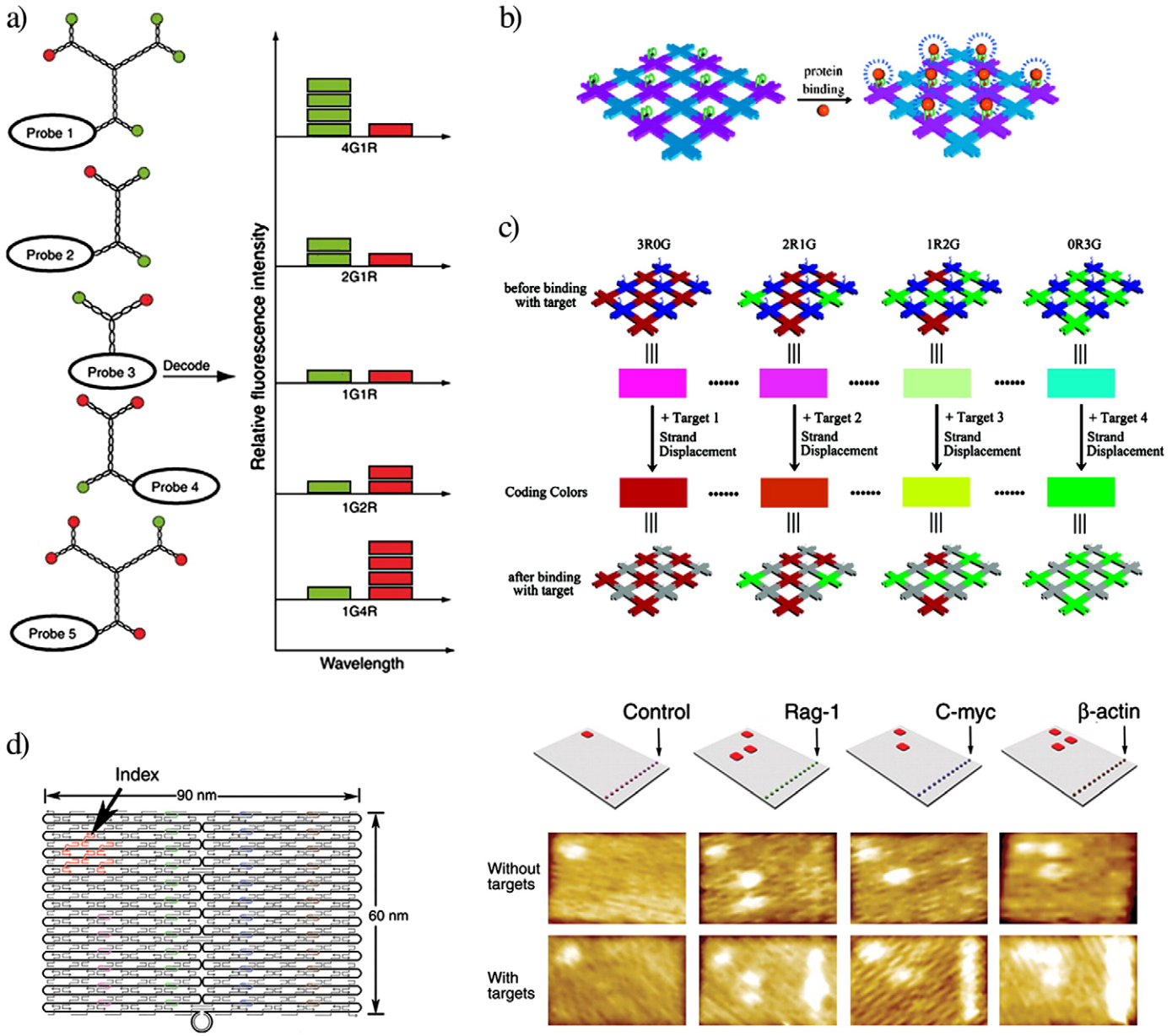

Fig. 5. Self-assembled DNA nanostructures with biosensing capabilities, a) Fluorescence barcoded DL-DNA for multiplexed detection of pathogenic DNA (adapted from ref. [65]); b) schematic of the aptamer tagged DNA arrays with built-in signal for protein detection (adapted from ref. [67]); c) Combinatorial barcoded DNA arrays for multiplexed detection of pathogens and small molecules (adapted from ref. [68]); d) DNA origami based nucleic acid probe tiles for the label free detection of RNA (adapted from ref. [69]).

potential of creating multifunctional nanoarchitectures with drug delivering abilities.

Yan and coworkers utilized self-assembled signaling aptamers to detect various proteins in solution [67]. The signal was incorporated in the interior design of the aptamers by replacing one nucleotide with the fluorescent analogue in the active site (Fig. 5b), which displays an increase of fluorescence upon binding with the target. Upon self-assembly, high-density signaling aptamer arrays were generated with a detection limit of 5-20 nM. By employing combinatorial design of self-assembled barcoding nanoarrays, multiplexed biosensing using aptamer bearing DNA tile arrays has been achieved [68]. Different fluorescent color schemes were used as barcodes and multiple DNA targets, such as SARS virus and HIV virus, and protein targets, such as thrombin and ATP were analyzed simultaneously in the same solution (Fig. 5c). Recently, Yan et al. [69] employed DNA origami-based self-assembled DNA nanoarrays for label-free detection of RNA. Trillions of rectangular-shaped nucleic acid probe-tiles were constructed in one step that carried single-stranded DNA as probe sequences (Fig. 5d). Upon hybridization with target RNA, the increase in stiffness and height can be readily imaged by Atomic Force Microscopy. Rag 1, C-myc and β-actin were simultaneously analyzed with a detection limit of 200 pM. This technology has shown the potentials of probing biomolecular interactions involving multiple components [70].

3. Challenges and Future Perspective

As discussed above, recent developments in the design, construction and self-assembly of DNA based nanostructures have stimulated much excitement for combining the exquisite power of sequence addressablilty and structural integrity for deterministic positioning of biomolecular and nanoelectronic/photonic elements, as well as designing a water-soluble detection array system that overcomes many of the disadvantages of bench-top micro-array chip technology. The field is seeing rapid progress with brain powers from scientists across disciplinary. A wonderful example of such progress is the

recent use of self-assembled DNA nanotubes to help structural determination of membrane proteins. In this work, Shih and co-workers [71] demonstrated that Origami DNA nanotube forms liquid crystals to align membrane proteins in orders that can facilitate its structural characterization by NMR. It is anticipated that more examples of such developments will come out in the near future.

A few challenges still need to be met to realize the full potential of the DNA self-assembly. First, chemistry needs to be developed to protect the self-assembled DNA nanostructures from being degraded and to facilitate their membrane penetration so that they can be used for in-vivo applications such as drug delivery vehicles to across cell membranes. Since interactions between constituting DNA oligonucleotides are mostly based on Watson-Crick base pairing rules and a minor alteration in the ambient environment may result in the disassembly of DNA nanostructures, certain photoactive or light actuated cross linker strategies may be used to covalently crosslink the strands with each other. Nuclease resistant modification of DNA could also be achieved by using 2'-methoxy or 2'-fluoro modified RNA bases. Certain surfactants may render the delivery of nucleic acid structures more favorable by neutralizing the negative charge. Second, robust attachment chemistry needs to be developed to link various molecules to DNA so that a multi-component nanostructural system can be realized. Among these, stable linkage between nanoparticles and DNA needs to be developed and high throughput protocols for covalent fusion of proteins/peptides with DNA oligos are of immediate needs for constructing protein nanoarrays for proteomics applications. Scaffolded DNA Origami has proven to be a very robust method to produce finite size spatially addressable DNA nanoarrays with essentially close to 100% yield but its scalability of using longer genomic sequences as scaffolds is still unclear and needs to be explored. Also, implementing methods to reduce the error rates in DNA self-assembly is another important topic which is actively pursued by many groups in the field. Last but not least, it is a formidable task to bridge the gap between bottom-up DNA self-assembly with top-down nano- and micr-fabrication techniques to make high throughput nanodevices. Recent progress in this direction may pave the way toward this important goal [72].

As discussed in previous sections, DNA self-assembly offers aqueous solution based detection system, compatible to the biomolecular interactions in the cellular environments. Using massive parallelism of DNA self-assembly process, probes for different disease markers can be conjugated to DNA scaffold for a high throughput assay of the diseased state. The output may be read by fluorescence change or by coupling with appropriate transducer elements. It is anticipated that in the long run, molecular computers will be assembled with built-in information, encoded in the sequences, of drug loading, delivery, targeting and ultimately release of the pay load. Following simple algorithmic rules with the integration of logic gates, molecular computers can be coded for autonomous detection of multiple analytes in a matrix and respond to the outcome based on the result of diagnostic computations. We anticipate that with collective efforts of researchers from different disciplines, highly complex autonomous systems would be realized with potential applications in therapeutics, drug screening and molecular diagnostics.

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