Review Article

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# Bio-surface engineering with DNA scaffolds for theranostic applications

https://doi.org/10.1515/nanofab-2018-0001
Received December 25, 2017; accepted March 12, 2018

**Abstract:** Biosensor design is important to bioanalysis yet challenged by the restricted target accessibility at the biomolecule-surface (bio-surface). The last two decades have witnessed the appearance of various "art-like" DNA nanostructures in one, two, or three dimensions, and DNA nanostructures have attracted tremendous attention for applications in diagnosis and therapy due to their unique properties (e.g., mechanical flexibility, programmable control over their shape and size, easy and high-yield preparation, precise spatial addressability and biocompatibility). DNA nanotechnology is capable of providing an effective approach to control the surface functionality, thereby increasing the molecular recognition ability at the bio-surface. Herein, we present a critical review of recent progress in the development of DNA nanostructures in one, two and three dimensions and highlight their biological applications including diagnostics and therapeutics. We hope that this review provides a guideline for bio-surface engineering with DNA nanostructures.

**Keywords:** DNA nanostructures, theranostic application, diagnostics, therapeutics, bio-surface programmable, biosensor

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## 1 Introduction

Since Seeman's pioneering work in the early 1980s [1], DNA nanotechnology has attracted increasing attention because of the unparalleled ability of DNA molecules in self-assembly. Owing to the excellent physicochemical stability and the high specificity of Watson-Crick base-pairing, DNA has emerged as a powerful nanoscale building block for the construction of nanostructures with well-defined geometry. DNA is comprised of four canonical bases with A-T and G-C pairings, hence providing a precise means of programing DNA hybridization processes *via* rational design of the sequence. Over the past several decades, by exploiting the unique self-recognition properties of DNA molecules, researchers have created a collection of 'art-like' DNA nanostructures [1-6], molecular computers [7-9] and nanomachines [10-14].

The versatile chemistry of DNA allows for easy functionalization with a variety of molecules and nanoparticles [15], therefore offering a promising approach for building artificial functional systems using DNA nanostructured scaffold in combination with various decorations. For instance, Yan *et al.* [16] designed and constructed a tweezer-like DNA nanodevice with the ability to actuate the inhibition and activation of a G6pDH/$NAD^+$ enzyme/cofactor pair in response to external stimuli. In addition, many of the DNA or DNA nano-structures have been utilized as linkers or templates to mediate the assembly of metal nanoparticles (NPs) into complex plasmonic architectures [17-19]. As a proof of concept, Ding group designed a 2D rectangular DNA origami sheet, in which AuNPs could be assembled due to its well-controlled positions and particle spacing [20], and 3D plasmonic chiral nanostructures were achieved by rolling the 2D origami template. In addition to its important role as building blocks in nanodevices, DNA also serves as an excellent molecular recognition element in biosensors. A critical challenge in DNA nanostructure-based biosensors lies in controlling the structure of the self-assembled DNA film at the biosensing interface, which plays an important role in the performance of

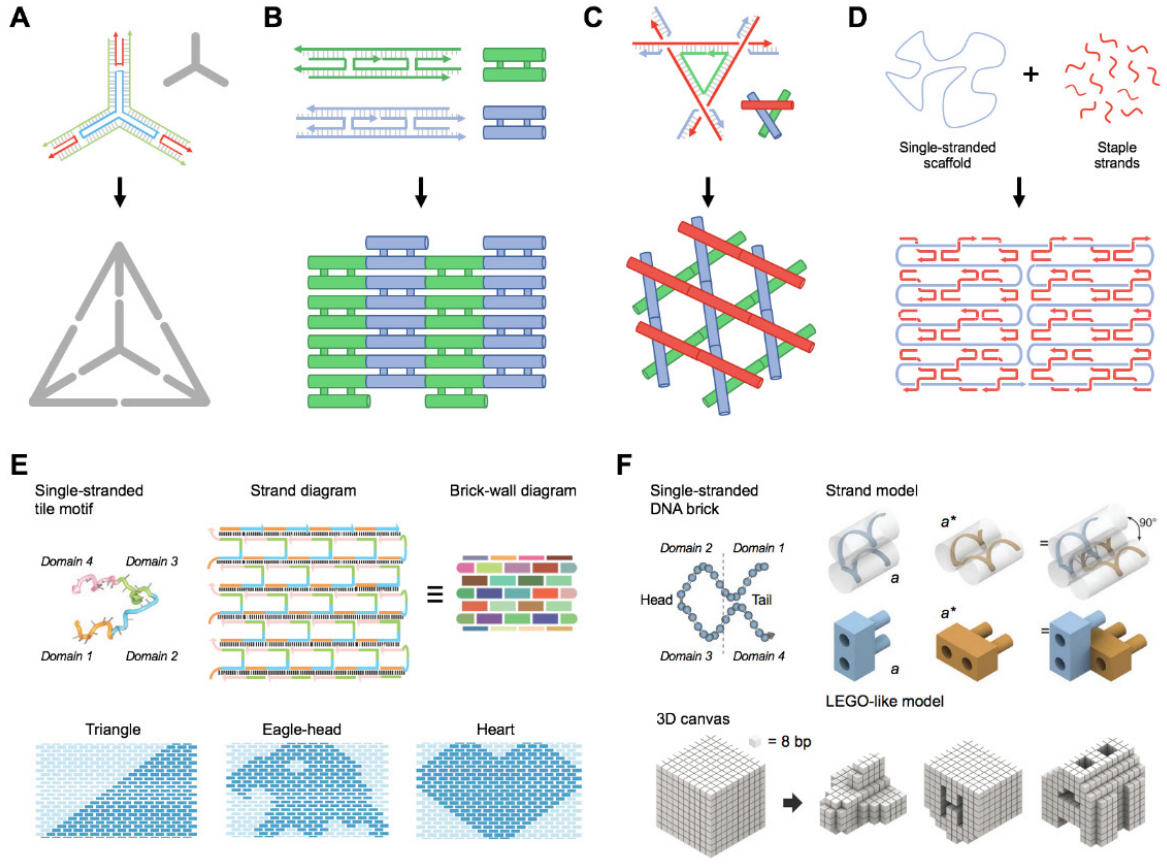


**Figure 1** (A) Design of a DNA tetrahedron assembled from a 3-point-star motif (adapted from He *et al*. 5). (B) Design of DX tiles arranging into 2D lattices (adapted from Winfree *et al*. 2). (C). A tensegrity triangle motif assembling into a designed 3D lattice (adapted from Zheng *et al*. 45). (D) The DNA origami strategy to fold DNA into desired shapes (adapted from Rothemund *et al*. 3). (E) Shapes self-assembled using single-stranded DNA tiles (adapted from Wei *et al*. 59). (F) Design of DNA brick structures (adapted from Ke *et al*. 60).

biosensors. It is thus of paramount importance to obtain an in-depth understanding of the physical structure of immobilized DNA layers. Given the biological nature of DNA nanostructures, they have shown good cellular biocompatibility with minimal cytotoxicity. Therefore, DNA nanostructures have attracted much interest in theranostic applications [21]. In this review, we summarize recent progress in the development of DNA nanostructures in one, two and three dimensions, with a highlight on their biological applications, particularly diagnostics and therapeutics. We apologize for not being able to include all important works in this field due to limitation of space.

## 2 Assembly of DNA nanostructures

Researchers have constructed a variety of DNA objects (an example shown in Figure 1A). Some examples include cubes [Chen, 1991 #61][22,23], tetrahedra [24,25], octahedra [26,27], icosahedra [28,30], dodecahedra [25], prisms [30] and buckyballs [25][He, 2008 #65]. DNA-based objects can also be tailored to contain a release mechanism, thereby making them useful for drug delivery and theranostics [31-34].

A variety of rigid DNA motifs have been designed for robust self-assembly of nanostructures based on DNA. For example, the double crossover (DX) motif was used to demonstrate the assembly of the first two dimensional crystalline DNA arrays (Figure 1B) [2]. Other motifs used in the creation of 2D lattices are the triple-crossover (TX) motif [35], a cross-shaped double-decker tile [36], a tensegrity triangle with duplex [37] and double crossover edges [38], a three-point-star motif where each arm is a four-way junction [39], a four-point-star (cross) motif [40] and a six-point-star motif [41]. Recently, a motif based on paranemic crossover (PX) DNA was used to create 2D arrays [42,43]. Such 2D arrays are useful as programmable scaffolds for the organization of nanoparticles and biomolecules [44].

Seeman and colleagues constructed the first designed three dimensional array using the tensegrity triangle

motif (Figure 1C) [45]. The tensegrity triangle is a rigid DNA motif formed from three double helices connected at the vertices by four arm junctions [37]. The triangle motif contains seven DNA strands (or three unique strands in a threefold symmetric version). The ends of the duplex edges contain unpaired nucleotides that are complementary to those on the other end of the edge, thereby allowing one triangle to connect to six such triangles via sticky ends. This assembly continues infinitely in three directions leading to the formation of an infinite periodic lattice (i.e. a crystal) [45]. The edges of the tensegrity triangle motif can be designed to contain desired number of helical turns thus leading to crystals with varying cavity sizes. For example, crystals have been designed and assembled using tensegrity triangle motifs containing 2, 3 or 4 double helical turns of DNA [45,46]. These designed DNA crystals have been used to host a triplex-forming oligonucleotide [47], a polyaniline molecule [48], a color changing strand displacement-based device [49] and as a system to study torsionally stressed DNA [50]. The goal of such designed DNA crystals is to host external molecules (specifically macromolecules such as hard-to-crystallize proteins) and aid in structure determination of the guests.

A more frequently used method to create DNA nanostructures is DNA origami, where a long single stranded scaffold DNA (usually the viral genome M13) is folded by hundreds of short complementary staple strands into any desired shape (Figure 1D) [3]. The highlight of the DNA origami method is that it does not require purification or a stoichiometric mixture of component strands like tile-based assembly. This strategy has been used in the construction of twisted bundles [51], three-dimensional multilayer structures [52] and hollow 3D structures such as boxes [53], spheres and flasks [54]. Recently, the folding pathways of the scaffold strand were designed to result in complex wireframe and mesh-like objects [55,56]. Moreover, cross-shaped origami structures tailed by sticky ends on the edges have been used to create long range two-dimensional arrays [57].

Single stranded DNA tiles containing four domains have been designed to assemble into well-defined nanostructures [58]. The tile is composed entirely of concatenated sticky ends that bind to four local neighbors during self-assembly. Adjacent DNA single stranded tiles connect to each other by pairing up with complementary domains, and continue to form DNA lattices composed of parallel DNA helices. Single strand tile-based approach is an alternative to scaffold DNA-based construction to create larger DNA nanostructures in both 2D and 3D. For example, in the "molecular canvas" strategy (Figure 1E), a pool of DNA strands (single stranded tiles) are designed to connect to each other, forming a rectangular canvas, where each tile serves as a pixel [59]. For a specific shape to be drawn on the canvas, only a subset of tiles corresponding to pixels of the target shape are chosen and annealed to create the desired pattern. This strategy was further expanded to create three dimensional shapes using single stranded "bricks" (Figure 1F) [60].

Besides, an amount of work has shown that using amphiphilic DNA hybrids (e.g. DNA block copolymers and lipid-DNA) is also an effective approach for the assembly of DNA nanostructures [61-66]. Liu *et al.* [67] introduced a hydrophobic DNA-surfactant complex in an organic phase for nucleic acid modification and polymerization, producing various amphiphile-DNA nanostructures and DNA side chain polymers. Andreas Herrmann group exploited a DNA tetrahedron with pendant poly (N-isoproylacrylamide) segments for assembling a precisely defined composite nanoparticles [68].

# 3 Surface engineering of macro-surface with DNA nanostructures

## 3.1 1D DNA probes

1D DNA probes are single-stranded DNA (ssDNA) comprising a sequence of at least 15 nucleotides. They are widely used in constructing most DNA sensors and devices due to a lack of secondary structure. The ssDNA with end-tethered thiol can self-assemble onto Au surfaces via the well-known thiol-gold chemistry [69] with a typical surface coverage of $10^{11}$-$10^{13}$ molecules/cm$^2$. This self-assembly has become a paradigm for the design of DNA recognition layers. Ideally, thiolated DNA is expected to pack well into an ordered monolayer at Au surfaces by a single-point attachment, exhibiting an upright orientation that is favorable for target hybridization. However, in reality, the DNA layer has a rather complicated structure due to the existence of significant interactions between the DNA bases and the Au surface such as electrostatic repulsion between strands, interactions between nitrogen atoms of bases and the Au surface, and strong inter-strand entanglement (Figure 2A) [70]. For example, Herne and Tarlov [69] discovered that thiolated ssDNA (SH-ssDNA) is not only specifically adsorbed onto the Au via the Au-S bond but also nonspecifically adsorbed via Au-N interactions. Moreover, the discrepancy between the actual thickness of SH-ssDNA monolayer with the expected maximum thickness suggests that the SH-ssDNA monolayer is not tightly packed and that the DNA chains

are not oriented perpendicularly to the surface. Grant *et al.* [71] revealed that the attached DNA could extend further into the solution when repulsive electrostatic fields were applied onto the surface. In addition, several groups discovered that short DNA probes with a high surface density may take upright conformations, which however jeopardizes target accessibility [72,73].

The ideal configuration for target hybridization is to functionalize the surface with a low density of DNA probes that possess an upright orientation. Tarlov *et al.* [74] developed a two-step assembly strategy, which involves mercaptohexanol (MCH) passivation that fills the unoccupied space and significantly removes nonspecifically adsorbed DNA (Figure 2B). The DNA probes are projected out into the solution with a favorable upright orientation [75], owing to the repulsion between the net negative dipole of the alcohol terminus and the negatively charged DNA backbones. This two-step sequential adsorption protocol has been extensively applied in developing various types of DNA-based biosensors and nanodevices [76]. When coupled with signal amplification by enzymes or nanomaterials, highly sensitive DNA sensors with detection limit down to low femtomolar concentrations have been achieved, which allows for the detection of a broad range of pathogens or tumor targets. Pei group designed a stochastic DNA walker powered by exonuclease III, which can autonomously move on a spherical nucleic acid (SNA)-based 3D track (Figure 2C) [77]. By studying the morphological effect of the 3D track on the nuclease activity, they found that the performance of the DNA walker depends on the DNA density and the track conformation. Despite the success of two-step sequential adsorption protocol in improving the surface assembly of DNA as well as the biosensing ability, it remains challenging to eliminate inter-strand interactions owing to the flexible nature of ssDNA. For instance, Ye *et al.* [78] revealed the existence of many defects of DNA monolayers at Au surface and aggregation patches of DNA using electrochemical atomic force microscopy.

## 3.2 2D structured DNA probes

It is well-established that ssDNA strands with internal complementary sequences can form secondary structures. This feature can be exploited for constructing pseudo-2D probes in most situations due to their simple structures. In addition, such probes exhibit relatively rigid and ordered structures, which can effectively prevent inter-strand entanglement and thus greatly improve the orderliness of DNA layers at the surface. Accordingly, these 2D structured DNA probes broaden the application in biological analysis.

In 2003, Fan *et al.* [79] developed a new type of electrochemical DNA (E-DNA) sensor by attaching 2D

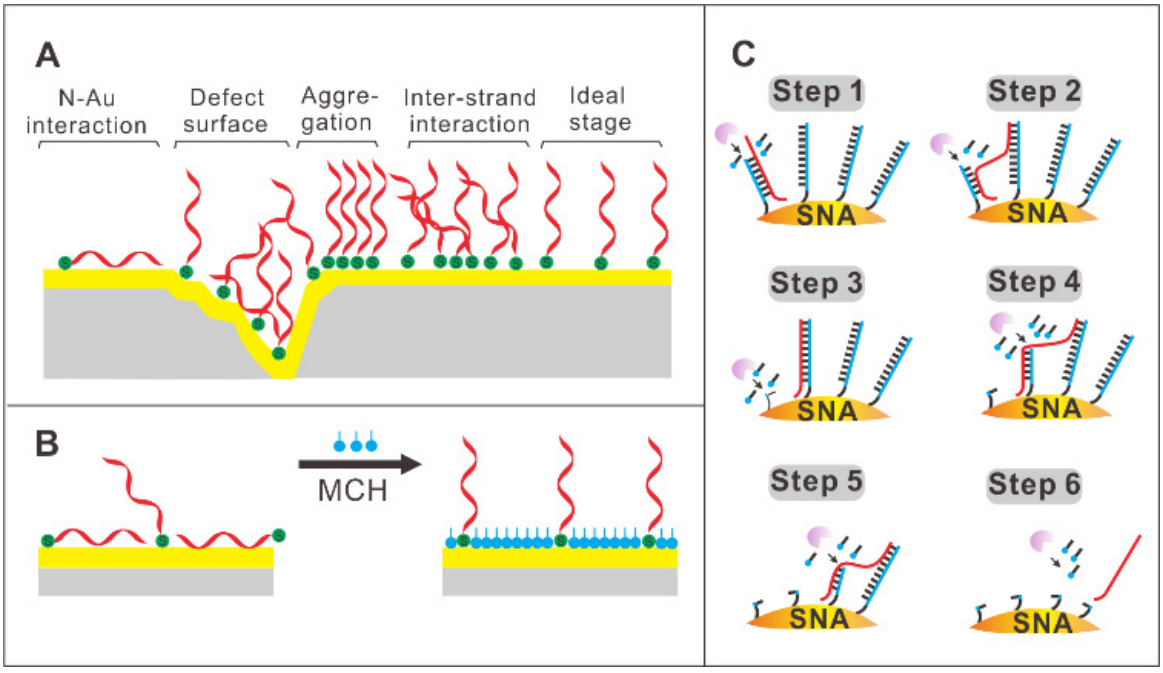


**Figure 2** (A) Schematics for different states of ssDNA probes binding on a gold surface. (B) The formation of a mercaptohexanol (MCH) monolayer preventing contacts between the DNA backbone and the substrate (adapted from Tarlov *et al.*74). (C) Schematic illustration of Exo III-powered stochastic DNA walkers moving on spherical nucleic acid (SNA) surfaces (adapted from Qu *et al.*77).

DNA probes with a hairpin (stem-loop) structure on Au electrodes. Such E-DNA sensors detect electrochemical signals by means of target-induced conformational changes of 2D DNA structures analogous to the optical molecular beacon (MB) approach. The DNA stem-loop structure is tagged with a thiol and an electroactive ferrocene at the 3' end and 5' end, respectively (Figure 3A). The 2D hairpin probe then self-assembles onto an Au electrode via the facile gold-thiol chemistry. In the absence of a target, the ferrocene is close to the Au electrode surface, generating redox current. Hybridization with a complementary target sequence results in the opening of the stem and the extension of the loop into a linear duplex. Hence, the distance between the ferrocene and the Au electrode is significantly increased, leading to prominent variations in electron transfer and in turn producing readily measurable changes in electrochemical signals with a detection limit of 10 pM. In contrast to existing electrochemical approaches, this E-DNA sensor allows for sensitive and selective detection of oligonucleotides without the use of exogenous reagents. This E-DNA thus provides a cost-effective and efficient technique for reagent-less and reusable detection of picomolar DNA, which holds great potential in medical and military applications. By using the E-DNA scheme, Lai *et al.* [80] achieved sequence-specific detection of unpurified amplification products of the *gyr*B gene of *Salmonella typhimurium*, which may open the path toward effective and field-portable sample-to-answer pathogen identification.

While the initial design of E-DNA sensor was based on a 'signal-off' scheme that is susceptible to false-positives, many efforts have been taken to design 'signal-on' E-DNA sensors. In 2005, Bockisch *et al.* [81] developed an alternative 'signal-on' sensor for the detection of nucleic acid targets using stem-loop probes, which exploited variation in the steric effect due to hybridization-induced conformational change. Later, Liu *et al.* [82] developed an enzyme-based signal-on E-DNA sensor that further increased the sensitivity up to low femtomolar range (Figure 3B). Wei *et al.* [83] then reported a similar electrochemical sensor that was used to detect mRNA targets with 0.4 fM sensitivity in salivary samples.

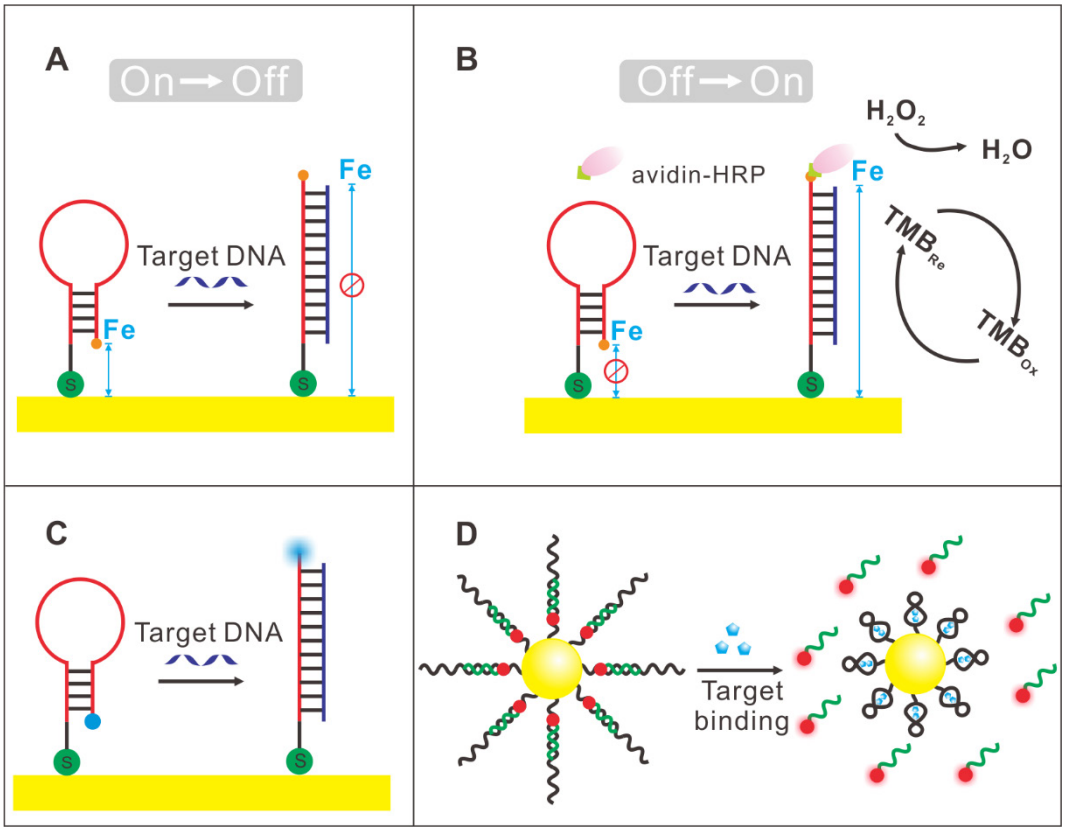


**Figure 3** Schematic illustration of 2D structured DNA probes. Electrochemical DNA (E-DNA) sensor: (A) stem-loop structured DNA probe with ferrocene (Fe) as signal molecule (adapted from Fan *et al.*79); (B) stem-loop structured DNA probe with enzyme-based signal amplification (adapted from Liu *et al.*82). Fluorescent DNA (F-DNA) sensor: (C) stem-loop structured DNA probe with fluorescent molecule for signal generation (adapted from Du *et al.*84); (D) Aptamer nano-flares with fluorescent reporters (adapted from Mirkin *et al.*87).

An alternative approach is to use detection schemes based on fluorescent DNA sensors. Du *et al.* [84, 85] developed label-free biological sensors based on fluorescence of DNA hairpins immobilized on metal surfaces. Hybridization efficiency was found to be sensitive to hairpin secondary structure, as well as to the surface distribution of DNA hairpins on the substrate (Figure 3C). Such a sensor can be easily adapted to microarrays and scalable to sensing of different biological molecules.

In a different line of studies, functional nucleic acids with pseudo-2D structures, such as DNA or RNA aptamers and DNAzymes, can also be employed to develop electrochemical sensors for non-nucleic acids targets. For example, Zuo *et al.* [86] designed an electrochemical sensor for adenosine triphosphate (ATP) by employing an anti-ATP aptamer. The tertiary aptamer structure is stabilized in the presence of the target ATP, which responsively denatures the duplex and releases the complementary DNA. This structural switching brings the ferrocene moiety close to the Au electrode surface, generating measurable electrochemical signals. For a similar purpose, Mirkin *et al.* [87] developed an aptamer 'nanoflare' probe by functionalizing AuNPs with a dense monolayer of nucleic acid aptamers with a high affinity for ATP (Figure 3D). These nanoconjugates can be used for the intracellular quantification of small molecules or proteins in living systems owing to the good cellular uptake ability of AuNPs.

## 3.3 3D structured DNA probes

Although 2D probes have shown significantly improved biosensing ability compared to 1D probes, it should be pointed out that 2D probes are usually not robust enough to survive on highly crowded surfaces. It is therefore of paramount importance to precisely control the surface density and orientation of grafted DNA probes on the surface to achieve high-performance sensors. 3D DNA nanostructures are expected to address this issue.

In 2010, Pei *et al.* [88] developed the first 3D DNA structure-based electrochemical sensor by using exquisite tetrahedron-structured DNA probes (TSPs). The TSPs were self-assembled from three thiolated 55-base oligonucleotides and one 80-base oligonucleotide with corresponding complementary regions that fold them into a tetrahedral structure (Figure 4A). The tetrahedron assembly process is extremely fast and efficient with a high yield of ~85%. This 'pyramidal'-structured probe is expected to self-assemble onto Au surfaces with a three-point attachment via the thiol-gold interaction and to leave a pendant probe at the top vertex. Due to the three thiols at the base of the pyramid, TSP is rapidly and strongly anchored at the Au surface within only 2 min, which was monitored in real time by both quartz crystal microbalance and surface plasmon resonance. The surface density was determined as ~$4.8 \times 10^{12}$ TSP $cm^{-2}$, corresponding to a probe-to-probe spacing of ~4 nm.

By employing this DNA nanostructure-based TSP platform, Pei *et al.* [88] constructed an E-DNA sensor with a conventional sandwich hybridization strategy. The performance of E-DNA sensor was first evaluated by the detection of biotinylated target DNA. The formation of the sandwich pair through the conjugation of avidin-HRP (horseradish peroxidase) with biotin produces intense electrochemical signal via the substrate catalysis of HRP. This non-optimized TSP-based sensor allows for the detection of low picomolar concentrations (~1 pM), and improves the sensitivity by approximately 250 times compared with that of 1D and 2D probes employing similar HRP-based signal transduction approach. In addition, this TSP-based sensor can sensitively discriminate single-base mismatched DNA, surpassing that of ssDNA probe-based sensors by 25-100 fold in the discrimination factors. Optimization of the TSP-based sensor using interfacial engineering and multienzyme amplification could further improve the sensitivity [89]. For example, Wen *et al.* [89] employed poly-HRP80, a polymerized streptavidin-HRP conjugate with up to 400 HRP molecules per conjugate, to further amplify the electrochemical signal, which allows for the detection of miRNAs with an ultralow limit of 10 aM, corresponding to ~600 molecules in 100 μL (Figure 4B). It is worthwhile to note that these TSPs provide a promising platform to study DNA hybridization thermodynamics and kinetics with nanometer precision [90]. The detection limit of such DNA sensors can also be programmed and improved by using macroscopic Au electrodes patterned with differently sized TSPs.

The TSP-based surface has proven to be a versatile platform for the detection of virtually any target by programmed configuration of the four edges of the pendant. For example, Pei *et al.* [91] constructed various TSP-based logic gates (INH, XOR, AND, OR, and half-adder) by incorporating different dynamic sequences into one or two edges. Such logic gates showed sensitive response to a broad range of targets, including protons, metal ions ($Hg^{2+}$), small molecules (ATP), and complementary DNA strands. In addition to the edges, the pendant probe can also be replaced with different functional nucleic acids for the detection of a variety of biomolecules. For instance, replacement of the pendant probe with an aptamer turns the E-DNA sensor into an immunological sensor for proteins. Pei *et al.* [88] constructed a thrombin sensor by

replacing the top vertex with an anti-thrombin aptamer sequence, which allowed for the detection of thrombin as low as 100 pM. In another example, Wen *et al.* [92] developed an electrochemical sensor for the detection of cocaine by incorporating a corresponding split aptamer. This sensor exhibited excellent sensitivity toward cocaine, with a detection limit of 33 nM, which is lower than previously reported sensors by three orders of magnitude. In addition, Pei *et al.* [93] established a highly sensitive and regenerative electrochemical immunological sensor (iTSP sensor) by anchoring DNA-conjugated antibodies on the TSP surface. This TSP surface is well suited for various immunological assays as well as DNA-programmed protein microarrays for proteomic studies. Qu *et al.* [94] developed a rapid, DNA nanostructure-supported aptamer pull-down (DNaPull) assay with convective flux in a glass capillary, which allows for analyzing the targets in droplets with nanoliter or picoliter volumes. Moreover, they exploited this DNaPull assay to quantify intracellular ATP level in a discrete number of HNE1 cells to be 3.4 mM (Figure 4C). Additionally, Qu *et al.* [95] constructed a DNA nanostructured microarray (DNM) with a tubular three-dimensional sensing surface and an ordered nanotopography, and used it for rapid and sensitive multiplexed detection of heavy-metal ions (Figure 4D).

Along a different line, tetrahedral-structured DNA also offers unprecedented opportunities to construct functional plasmonic nanostructures by incorporating

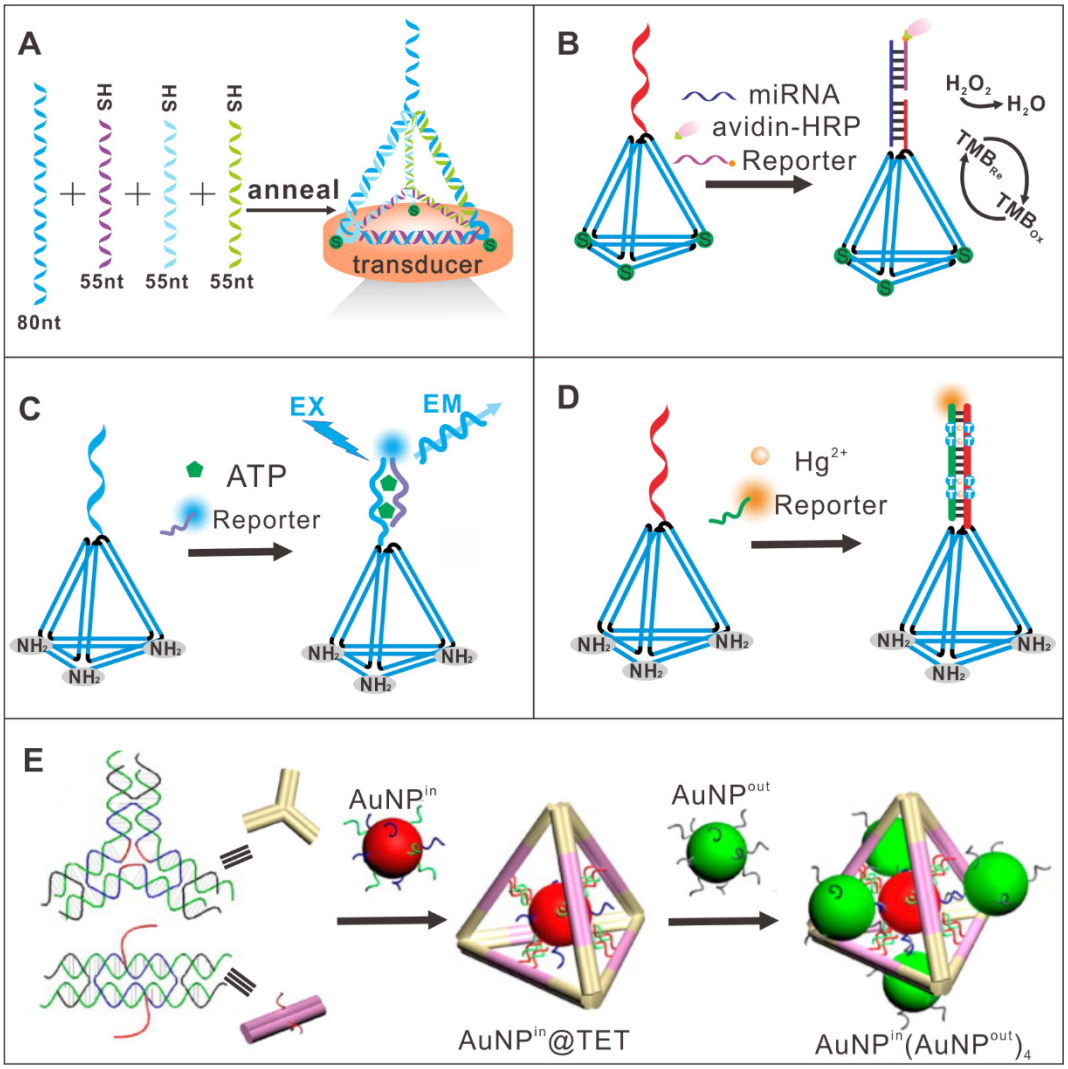


**Figure 4** Schematic illustration of 3D structured DNA probes. Electrochemical DNA (E-DNA) sensor: (A) tetrahedron-structured DNA probe (adapted from Pei *et al.*88); (B) tetrahedron-structured DNA probe with enzyme-based signal amplification (adapted from Wen *et al.*89). Fluorescent DNA (F-DNA) sensor: (C) tetrahedron-structured DNA probe for small molecule detection (adapted from Qu *et al.*94); (D) tetrahedron-structured DNA probe for sensing metal ions ($Hg^{2+}$) (adapted from Qu *et al.*95). (E) Self-assembly of methane-like NP-molecules (adapted from Li *et al.*97).

metal nanoparticles (NPs). By positioning four different metal and/or semiconductor NPs in the corners of the tetrahedral-structured DNA, Yan *et al.* [96] fabricated a family of hetero-particle chiral pyramids with a yield approaching 80%. These chiral pyramids possessed excellent optical activity with *g*-factors significantly higher than those of traditionally optically active materials and tunable circular dichroism signals in the range of 350–550 nm. Such DNA directional self-assembly approach provides a rapid and high yield synthesis of chiral materials, which might facilitate the applications of chiral materials in life science and metamaterials. Later, Li *et al.* [97] developed a general strategy for building molecule-like NP architectures (NP-molecule) analogous to the atom-molecule relationship (Figure 4E). The AuNPs are encapsulated into a DNA tetrahedron, which then serve as a guiding agent for further assembly. A rich variety of NP-molecules, such as $CH_4$, $SF_6$, $W(CH_3)_6$, and $C_2H_6$-like structures have been constructed. Such NP-molecules hold great potential for exploring new nanomaterials with emergent functions that do not belong to the individual component NPs.

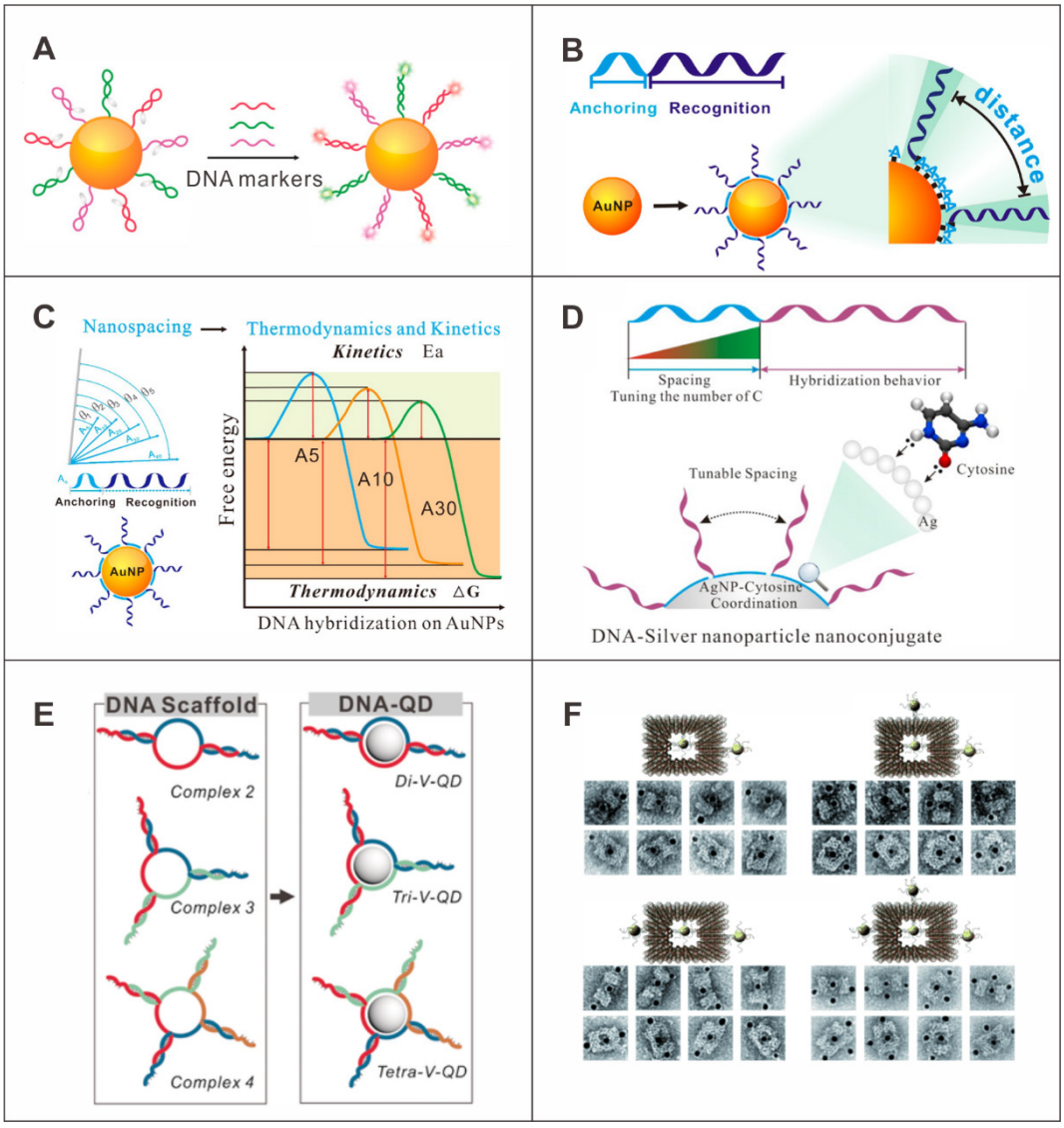


**Figure 5** DNA nanostructures with NPs. (A) Multicolor nanobeacons (adapted from Song *et al.*104). (B) Polyadenine (polyA) blocks for adjusting the DNA probe conformations on Au nanoparticles (adapted from Pei *et al.*107). (C) Modulating the thermodynamics and kinetics of DNA hybridization though different length of PolyA blocks (adapted from Zhu *et al.*108). (d) Adjusting the DNA probe conformations on Ag nanoparticles though Polycytosine (polyC) blocks (adapted from Zhu *et al.*114). (E) DNA scaffolds self-assembled from different sets of chimeric DNA strands for valence-engineered QDs. (adapted from Shen *et al.*116). (F) DNA origami nanocage to encapsulate AuNPs (adapted from Zhao *et al.*118).

## 3.4 Engineering of nano-surfaces with DNA nanostructures

AuNPs have unique optical and electronic properties, and therefore have shown great potential as a building block for an extensive range of applications that include diagnostics [98], drug delivery [99], and plasmonics [100-103]. It is therefore of great interest to create effective DNA recognition layers on the AuNP surface for various applications. Analogous to the functionalization on the macroscopic surface of Au electrode, Song *et al.* [104] constructed multicolor nanoscale MBs by immobilizing three stem-loop oligonucleotides labeled with different fluorophores on the surface of 15 nm AuNPs (Figure 5A). Three probes labeled with FAM, Cy5 and Rox were designed to target three types of tumor-suppressor genes (exon segments of *p*16, *p*21 and *p*53). Hybridization tests were carried out in solutions spiked with a mixture of three targets, and the nanoscale MBs respond only to the specific target with negligible crosstalk. Since DNA-AuNP conjugates show highly efficient cellular uptake, later Li *et al.* [105] demonstrated a multicolor fluorescence nanoprobe based on nanoflares, that can simultaneously detect and image three intracellular tumor-related mRNAs in living cells. The fluorescence intensity was found to correlate well with the concentration of tumor-related mRNA in living cells, which provides a useful means to monitor the stage of tumor progression.

Surface functionalization of oligonucleotides is typically carried out via the well-established thiol-gold chemistry. Although this approach has proven to be very useful on the macroscopic surface, it is much more difficult to modulate the orientation and conformation of DNA probes on the surface of AuNPs. Poly adenine (PolyA)-mediated self-assembly strategies have been developed as a promising means for precisely controlling the DNA distribution on the NP surface in a quantitative manner. Whitman *et al.* [106] first demonstrated the use of PolyA-mediated method for tuning grafting density of ssDNA on a planar Au surface. Their study showed that adenines can preferentially adsorb onto Au surfaces with high intrinsic affinity, making it possible to use PolyA sequences as the anchoring block through multipoint attachment, in contrast to single contact point using thiolated DNA. Based on similar principles, Pei *et al.* [107] developed a general approach for spatially controlled functionalization of the highly curved surface of AuNPs (Figure 5B). They demonstrated that the multipoint attachment of PolyA block can effectively minimize the nonspecific interactions between other bases and Au, hence pushing the appended block upwards that as a result facilitates DNA hybridization. The lateral spacing and conformation of DNA probes on Au surfaces can also be well-controlled by varying the length of the PolyA blocks. Zhu *et al.* [108] applied developed polyA-based assembly strategy to investigate the thermodynamics and kinetics of DNA hybridization on gold nanoparticle. They found that the thermodynamic properties are similar to that of duplex in solution and fast hybridization rate is observed with the increasing length of polyA (Figure 5C). This work facilitates constructing high-efficiency and high-speed biosensors for DNA analysis. DNA-AuNP conjugates fabricated using PolyA-mediated method exhibit high and tunable hybridization ability and are therefore well-suited for constructing complex nanostructures and nanodevices [109]. For instance, they constructed a rapid plasmonic sensor for the sensitive detection of DNA. Later, the same group further employed the PolyA-mediated approach for facile assembly of CpG-AuNP conjugates for cost-effective drug delivery, and modulated the immunostimulatory activity of CpG-AuNPs by varying the length of the PolyA block [110]. In another study, Zhao *et al.* [111] synthesized core-shell gold nanostructures with PolyA-mediated interior nanogaps as SERS nanotags for both the multiplex analysis of DNA markers of diseases and the simultaneous analysis of a variety of bioactive molecules. Recently, by employing the same method, He *et al.* [112] fabricated a series of core-satellite assemblies, including symmetric Au-Au NPs and asymmetric Ag-Au NP-decorated silicon wafers. The surface density of AuNPs on core NP surface can be systematically modulated by adjusting the length of the PolyA block as well. Their study demonstrated that such core-satellite NPs can serve as high-performance surface-enhanced resonance Raman scattering (SERRS)-based sensing platform for various applications [113]. Similar to PolyA-mediated assembly, Zhu *et al.* [114] discovered that poly cytosines (PolyC) are also able to mediate the assembly of DNA oligonucleotides on AgNPs [115] and exploited the unique anisotropic plasmonic scattering behavior of these DNA-AgNP conjugates (Figure 5D).

In addition to ssDNA strands, the assembly of complex DNA nanostructures onto the NP surface has also attracted great attention. Shen *et al.* [116] proposed a DNA programmed general strategy for valence-control of QDs in a single step with near quantitative yield (>95%) (Figure 5E). In addition, Lo *et al.* [117] constructed DNA nanotubes with longitudinally alternating large and small capsules, which can selectively encapsulate AuNPs into the large capsules, producing 'nanopeapod' particle lines. These nanotubes can release their NP cargo in response to specific DNA strands, which might find applications

in gene-triggered selective drug delivery and biological sensing. Moreover, DNA origami provides more functional sites via staple strand modification and possesses higher rigidity than ssDNA, therefore offering more flexibility in designing complex metal nanostructures. Zhao *et al.* [118] demonstrated the application of spatially addressable DNA origami nanocages to encapsulate AuNPs of various sizes (Figure 5F). The encapsulation efficacy was found to decrease with larger AuNPs due to the severe deformation when accommodating 15 nm AuNPs. More significantly, the programmability of the DNA origami cages provides a promising opportunity to interrupt the symmetry of spherical NPs and allows for the complex spatial arrangement of AuNPs.

## 3.5 Surface engineering of live cell with DNA nanostructures

While the utilization of DNA nanostructures in biosensors and nanodevices has been extensively explored in the past few decades, their applications in diagnostics and therapeutics in living systems are beginning to emerge. In 2010, Walsh [119] and Li [120] independently discovered that DNA tetrahedron can cross the cellular membrane without the aid of transfection reagents. The DNA tetrahedron showed high resistance toward nuclease degradation and remained substantially intact within cells for at least several hours [120]. Given the noncytotoxic nature and hollow structure of DNA tetrahedron, it holds

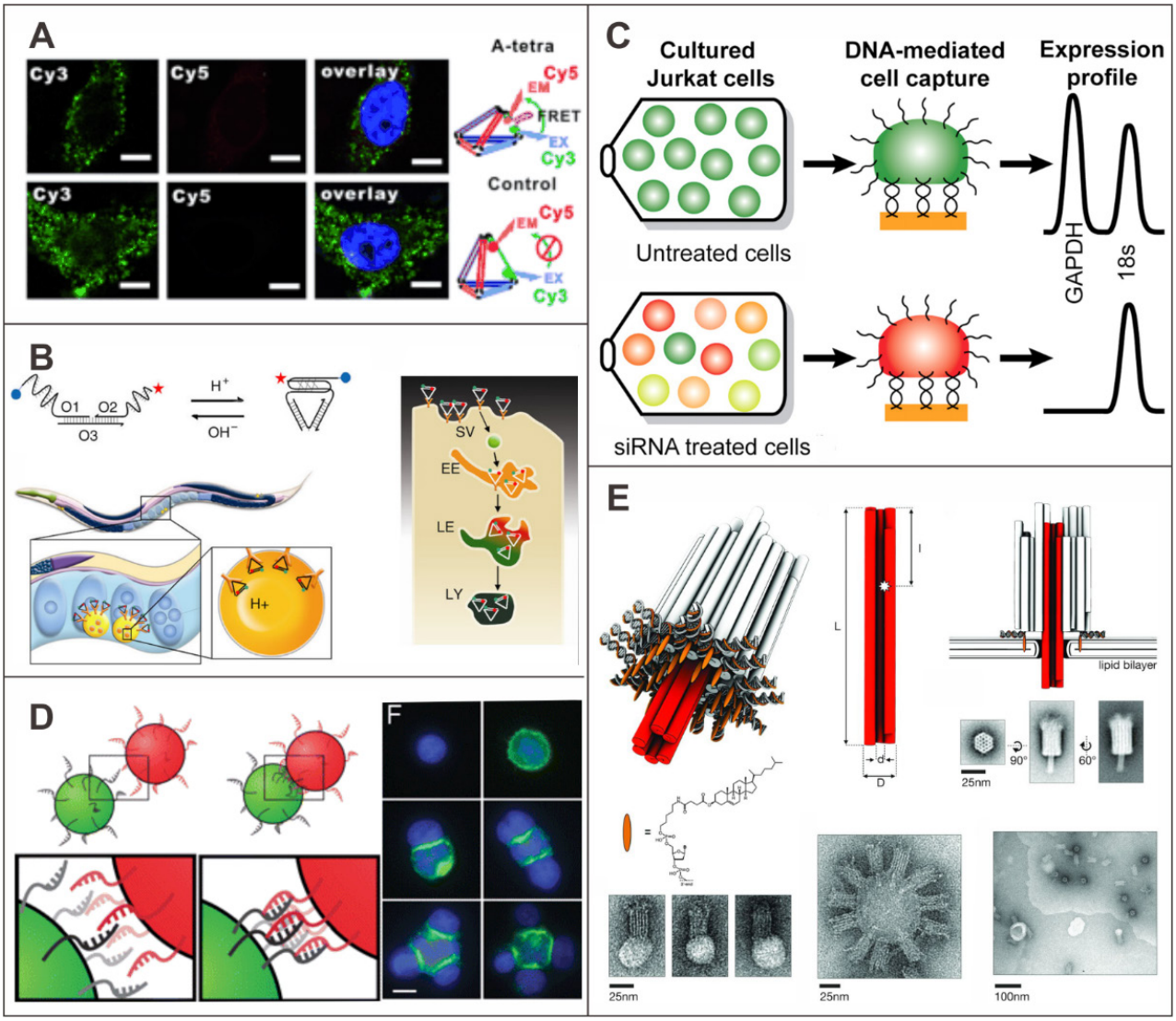


**Figure 6** (A) Using reconfigurable DNA tetrahedron to detect intracellular ATP in living cells (adapted from Pei *et al.* 91). (B) Probing the functionality of a DNA nanomachine in coelomocytes of C. elegans (adapted from Surana *et al.*123). (C) Overview of single-cell gene silencing assay (adapted from Toriello *et al.*129). (D) Oligonucleotides directing the synthesis of 3-dimensional multicellular structures (adapted from Gartner *et al.*132). (E) Synthetic DNA membrane channels (adapted from Langecker *et al.*134).

great potential for applications in cellular imaging and targeted drug delivery. For example, Pei *et al.* [91] designed a series of 3D DNA nanostructure-based logic gates using tetrahedral DNA nanostructures and applied the logic operations for in vivo sensing and imaging of ATP in living cells (Figure 6A). The controllable shape of tetrahedron also offers a useful means to logically control drug release in cells. By appending unmethylated CpG motifs to the tetrahedron, Li *et al.* [120] demonstrated that functional tetrahedrons can be applied as a new type of noninvasive delivery system for CpG oligodeoxynucleotides (ODNs). The DNA tetrahedral nanostructures were found to significantly increase the efficacy of CpG ODNs without sacrificing biocompatibility. Another work reported by Lee *et al.* [121] constructed DNA/siRNA tetrahedral nanostructures using six ssDNA strands and six double-stranded siRNAs for targeted delivery in vivo. The design of such DNA nanostructures allows for full control over the nanostructure size, spatial orientation of the siRNA, and the locations and density of cancer-targeting ligands, which provides a useful means to develop highly efficient, long-lived and target-specific nanocarriers for delivering therapeutics into living systems. Interestingly, they found that at least three folate ligands per tetrahedron are required for optimal delivery of the siRNAs into cells, and gene silencing efficacy can be dramatically affected by the orientation and location of ligands. A variety of different DNA nanostructures have also been exploited for in vivo applications. For example, Modi *et al.* [122] constructed a DNA nanomachine called the I-switch, which shows response to protons and functions as a pH sensor based on fluorescence resonance energy transfer (FRET). They then demonstrated the operation of this pH-triggered DNA nanomachine for mapping spatial and temporal pH changes associated with endosome maturation in live cells. Later, the same group demonstrated that this synthetic DNA nanomachine can also function autonomously in a multicellular living organism, which provides a powerful tool to interrogate complex biological phenomena (Figure 6B) [123].

DNA nanostructures also provide a very powerful approach to control cell behavior, including cell adhesion, cell orientation, cell motility, and intracellular signaling pathways, through the nanoscale engineering of cell surface interface. It has been demonstrated that cells are inherently sensitive to local mesoscale, microscale, and nanoscale patterns of chemistry and topography [124]. In 2006, Chandra *et al.* [125] reported a novel strategy for engineering cell adhesion events by employing a DNA-based approach. ssDNA was attached to the plasma membranes of living cells, and upon hybridization with complementary sequences tagged on the materials surfaces, cells can be captured and patterned into arrays. This DNA-based approach offers the ability to engineer cells to a much higher level of complexity than traditional approaches typically involving the cell's natural repertoire of adhesion receptors, such as laminin or RGD peptides [126, 127]. By exploiting this method, they demonstrated the formation of complex cell patterns [128], the capture of single cells for gene expression analysis (Figure 6C) [129], and the attachment of living cells to AFM tips for force measurement [130]. Later, the same group improved this technique by using NHS-DNA conjugates, which allows for capturing many new types of cells that are generally incompatible with integrin-targeting approaches, and significantly shortens the processing time to less than 1hr [131]. Similarly, by exploiting the hybridization between DNA-coated cells (Figure 6D), the same group demonstrated the bottom-up synthesis of microtissues composed of multiple cell types with programmed connectivity [132]. They then constructed a paracrine signaling network in these isolated artificial 3D microtissues. In addition to patterning cells, another study reported by Coyle *et al.* [133] has shown that a DNA-based strategy can also be used to mediate the assembly of proteins into defined heterodimers or higher-order oligomers on supported membranes, which can then be used to direct the molecular composition of cell membrane receptor clusters. Moreover, instead of using simple ssDNA strands, Langecker *et al.* [134] used scaffolded DNA origami to create lipid membrane channels composed of a stem that penetrated and spanned a lipid membrane, as well as a barrel-shaped cap that adhered to the membrane (Figure 6E). They have demonstrated that such synthetic channels can be used to discriminate single DNA molecules.

## 4 Conclusion and outlook

This review summarizes the unique properties of DNA nanostructures including strong capability for biomolecular recognition on the bio-surface, and such rationally designed DNA nanostructures show innovative applications in biosensing for analysis of various proteins, nucleic acids, and small molecules. Moreover, DNA nanostructures with good permeability present well controlled logical responses to environmental stimuli, which allows for their use in drug delivery. Hence, it is possible to achieve in vivo diagnosis and controlled release of drugs by taking advantage of DNA-based nanocarrier systems with embedded intelligent sensors. Given its

versatility, it is envisioned that DNA nanostructures hold great promise as a solid tool in theranostics.

**Acknowledgments:** This work was supported by the National Science Foundation of China (grant numbers 21722502, 21505045, 21705048), the Shanghai Pujiang Talent Project (16PJ1402700), China Postdoctoral Science Foundation (2015M581565, 2017T100283).

## References

[1] Seeman N., Nucleic acid junctions and lattices, J. Theor. Biol., 1982, 99, 237-247.
[2] Winfree E., Liu F.R., Wenzler L.A., Seeman N.C., Design and self-assembly of two-dimensional DNA crystals, Nature, 1998, 394, 539-544.
[3] Rothemund P.W.K., Folding DNA to create nano- scale shapes and patterns, Nature, 2006, 440, 297-302.
[4] Yin P., Choi H.M.T., Calvert C.R., Pierce N.A., Programming biomolecular self-assembly pathways, Nature, 2008, 451, 318-322.
[5] He Y., Ye T., Su M., Zhang C., Ribbe A.E., Jiang W., Mao C., Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra, Nature, 2008, 452, 198-201.
[6] Douglas S.M., Dietz H., Liedl T., Hoegberg B., Graf F., Shih W.M., Self-assembly of DNA into nanoscale three-dimensional shapes, Nature, 2009, 459, 414-418.
[7] Adleman L.M., Molecular computation of solutions to combinatorial problems, Science, 1994, 266, 1021-1024.
[8] Barish R.D., Schulman R., Rothemund P.W.K., Winfree E., An information-bearing seed for nucleating algorithmic self-assembly, Proc. Natl. Acad. Sci. U.S.A., 2009, 106, 6054-6059.
[9] Seelig G., Soloveichik D., Zhang D.Y., Winfree E., Enzyme-free nucleic acid logic circuits, Science, 2006, 314, 1585-1588.
[10] Yurke B., Turberfield A.J., Mills A.P., Simmel F.C., Neumann J.L., A DNA-fuelled molecular machine made of DNA, Nature, 2000, 406, 605-608.
[11] Lund K., Manzo A.J., Dabby N., Michelotti N., Johnson-Buck A., Nangreave J., Taylor S., Pei R., Stojanovic M.N., Walter N.G., Winfree E., Yan H., Molecular robots guided by prescriptive landscapes, Nature, 2010, 465, 206-210.
[12] Zhong R., Xiao M., Zhu C., Shen X., Tang Q., Zhang W., Wang L., Song S., Qu X., Pei H., Wang, C., Li L., Logic catalytic interconversion of G-molecular hydrogel, ACS Appl. Mater. Inter., 2018, 10, 4512–4518.
[13] Xiao M., Man T., Zhu C., Pei H., Shi J., Li L., Qu X., Shen X., Li J., $MoS_2$ nanoprobe for microRNA quantification based on duplex-specific nuclease signal amplification, ACS Appl. Mater. Inter., DOI: 10.1021/acsami.7b18984.
[14] Gu H., Chao J., Xiao S.-J., Seeman N.C., A proximity-based programmable DNA nanoscale assembly line, Nature, 2010, 465, 202-205.
[15] Voigt N.V., Torring T., Rotaru A., Jacobsen M.F., Ravnsbaek J.B., Subramani R., Mamdouh W., Kjems J., Mokhir A., Besenbacher F., Gothelf K.V., Single-molecule chemical reactions on DNA origami, Nat. Nanotechnol., 2010, 5, 200-203.
[16] Liu M., Fu J., Hejesen C., Yang Y., Woodbury N.W., Gothelf K., Liu Y., Yan H., A DNA tweezer-actuated enzyme nanoreactor, Nat. Commun., 2013, 4, 3127.
[17] Kuzyk A., Schreiber R., Fan Z., Pardatscher G., Roller E.-M., Hoegele A., Simmel F.C., Govorov A.O., Liedl T., DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response, Nature, 2012, 483, 311-314.
[18] Thacker V.V., Herrmann L.O., Sigle D.O., Zhang T., Liedl T., Baumberg J.J., Keyser U.F., DNA origami based assembly of gold nanoparticle dimers for surface-enhanced Raman scattering, Nat. Commun., 2014, 5, 4448.
[19] Chao J., Lin Y., Liu H., Wang L., Fan C., DNA-based plasmonic nanostructures, Mater. Today, 2015, 18, 326-335.
[20] Shen X., Song C., Wang J., Shi D., Wang Z., Liu N., Ding B., Rolling up gold nanoparticle-dressed DNA origami into three-dimensional plasmonic chiral nanostructures, J. Am. Chem. Soc., 2011, 134, 146-149.
[21] Pei H., Zuo X.L., Zhu D., Huang Q., Fan C.H., Functional DNA nanostructures for theranostic applications, Acc. Chem. Res., 2014, 47, 550-559.
[22] Chen J., Seeman N.C., Synthesis from DNA of a molecule with the connectivity of a cube, Nature, 1991, 350, 631.
[23] Zhang C., Ko S.H., Su M., Leng Y., Ribbe A.E., Jiang W., Mao C., Symmetry controls the face geometry of DNA polyhedra, J. Am. Chem. Soc., 2009, 131, 1413-1415.
[24] Goodman R.P., Schaap I.A.T., Tardin C.F., Erben C.M., Berry R.M., Schmidt C.F., Turberfield A.J., Rapid chiral assembly of rigid DNA buildingblocks for molecular nanofabrication, Science, 2005, 310, 1661.
[25] He Y., Ye T., Su M., Zhang C., Ribbe A.E., Jiang W., Mao C., Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra, Nature, 2008, 452, 198.
[26] Zhang Y., Seeman N.C., Construction of a DNA-truncated octahedron, J. Am. Chem. Soc., 1994, 116, 1661-1669.
[27] Shih W.M., Quispe J.D., Joyce G.F., A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron, Nature, 2004, 427, 618.
[28] Bhatia D., Mehtab S., Krishnan R., Indi S.S., Basu A., Krishnan Y., Icosahedral DNA nanocapsules by modular assembly, Angew. Chem. Int. Edit., 2009, 48, 4134-4137.
[29] Zhang C., Su M., He Y., Zhao X., Fang P.A., Ribbe A.E., Jiang W., Mao C.D., Conformational flexibility facilitates self-assembly of complex DNA nanostructures, Proc. Natl. Acad. Sci. U.S.A., 2008, 105, 10665-10669.
[30] Aldaye F.A., Sleiman H.F., Modular access to structurally switchable 3D discrete DNA assemblies, J. Am. Chem. Soc., 2007, 129, 13376-13377.
[31] Chandrasekaran A.R., Levchenko O., DNA nanocages, Chem. Mater., 2016, 28, 5569-5581.
[32] Schnitzler T., Herrmann A., DNA block copolymers: functional materials for nanoscience and biomedicine, Accounts Chem. Res., 2012, 45, 1419-1430.
[33] Zhong R., Tang Q., Wang S., Zhang H., Zhang F., Xiao M., Man T., Qu X., Li L., Zhang W., Pei H., Self-assembly of enzyme-like nanofibrous G-molecular hydrogel for printed flexible electrochemical sensors, Adv. Mater., 2018, 1706887.https://doi.org/10.1002/adma.201706887.
[34] Rodríguez-Pulido A., Kondrachuk A.I., Prusty D.K., Gao J., Loi M.A., Herrmann A. Light-triggered sequence-specific cargo

release from DNA block copolymer–lipid vesicles, Angew. Chem. Int. Ed., 2013, 125, 1042-1046.
[35] LaBean T.H., Yan H., Kopatsch J., Liu F., Winfree E., Reif J.H., Seeman N.C., Construction, analysis, ligation, and self-assembly of DNA triple crossover complexes, J. Am. Chem. Soc., 2000, 122, 1848-1860.
[36] Majumder U., Rangnekar A., Gothelf K.V., Reif J.H., LaBean T.H., Design and construction of double-decker Tile as a route to Three-Dimensional periodic assembly of DNA, J. Am. Chem. Soc., 2011, 133, 3843-3845.
[37] Liu D., Wang M., Deng Z., Walulu R., Mao C., Tensegrity: construction of rigid DNA Triangles with flexible four-arm DNA junctions, J. Am. Chem. Soc., 2004, 126, 2324-2325.
[38] Zheng J., Constantinou P.E., Micheel C., Alivisatos A.P., Kiehl R.A., Seeman N.C., Two-Dimensional nanoparticle arrays show the organizational power of robust DNA motifs, Nano Lett., 2006, 6, 1502-1504.
[39] He Y., Chen Y., Liu H., Ribbe A.E., Mao C., Self-assembly of hexagonal DNA Two-Dimensional (2D) Arrays, J. Am. Chem. Soc., 2005, 127, 12202-12203.
[40] He Y., Tian Y., Chen Y., Deng Z., Ribbe A.E., Mao C., Sequence symmetry as a tool for designing DNA nanostructures, Angew. Chem., 2005, 117, 6852-6854.
[41] He Y., Tian Y., Ribbe A.E., Mao C., Highly connected Two-Dimensional crystals of DNA six-point-stars, J. Am. Chem. Soc., 2006, 128, 15978-15979.
[42] Shen W., Liu Q., Ding B., Shen Z., Zhu C., Mao C., The study of the paranemic crossover (PX) motif in the context of self-assembly of DNA 2D crystals, Org. Biomol. Chem., 2016, 14, 7187-7190.
[43] Shen W.L., Liu Q., Ding B.Q., Zhu C.Q., Shen Z.Y., Seeman N.C., Facilitation of DNA self-assembly by relieving the torsional strains between building blocks, Org. Biomol. Chem., 2017, 15, 465-469.
[44] Chandrasekaran A.R., Programmable DNA scaffolds for spatially-ordered protein assembly, Nanoscale, 2016, 8, 4436-4446.
[45] Zheng J.P., Birktoft J.J., Chen Y., Wang T., Sha R.J., Constantinou P.E., Ginell S.L., Mao C.D., Seeman N.C., From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal, Nature, 2009, 461, 74-77.
[46] Nguyen N., Birktoft J.J., Sha R., Wang T., Zheng J., Constantinou P.E., Ginell S.L., Chen Y., Mao C., Seeman N.C., The absence of tertiary interactions in a self-assembled DNA crystal structure, J. Mol. Recognit., 2012, 25, 234-237.
[47] Rusling D.A., Chandrasekaran A.R., Ohayon Y.P., Brown T., Fox K.R., Sha R., Mao C., Seeman N.C., Functionalizing designer DNA crystals with a triple-helical veneer, Angew. Chem., 2014, 53, 3979-3982.
[48] Wang X., Sha R.J., Kristiansen M., Hernandez C., Hao Y.D., Mao C.D., Canary J.W., Seeman N.C., An organic semiconductor organized into 3D DNA arrays by "bottom-up" rational design, Angew. Chem. Int. Edit., 2017, 56, 6445-6448.
[49] Hao Y.D., Kristiansen M., Sha R.J., Birktoft J.J., Hernandez C., Mao C.D., Seeman N.C., A device that operates within a self-assembled 3D DNA crystal, Nat. Chem., 2017, 9, 824-827.
[50] Hernandez C., Birktoft J.J., Ohayon Y.P., Chandrasekaran A.R., Abdallah H., Sha R.J., Stojanoff V., Mao C.D., Seeman N.C., Self-assembly of 3D DNA crystals containing a torsionally stressed component, Cell. Chem. Biol., 2017, 24, 1401-1406.
[51] Dietz H., Douglas S.M., Shih W.M., Folding DNA into twisted and curved nanoscale shapes, Science, 2009, 325, 725-730.
[52] Douglas S.M., Dietz H., Liedl T., Hogberg B., Graf F., Shih W.M., Self-assembly of DNA into nanoscale three-dimensional shapes, Nature, 2009, 459, 414-418.
[53] Andersen E.S., Dong M., Nielsen M.M., Jahn K., Subramani R., Mamdouh W., Golas M.M., Sander B., Stark H., Oliveira C.L.P., Pedersen J.S., Birkedal V., Besenbacher F., Gothelf K.V., Kjems J., Self-assembly of a nanoscale DNA box with a controllable lid, Nature, 2009, 459, 73-U75.
[54] Han D.R., Pal S., Nangreave J., Deng Z.T., Liu Y., Yan H., DNA origami with complex curvatures in Three-Dimensional space, Science, 2011, 332, 342-346.
[55] Zhang F., Jiang S.X., Wu S.Y., Li Y.L., Mao C.D., Liu Y., Yan H., Complex wireframe DNA origami nanostructures with multi-arm junction vertices, Nat. Nanotechnol., 2015, 10, 779-784.
[56] Benson E., Mohammed A., Gardell J., Masich S., Czeizler E., Orponen P., Hogberg B., DNA rendering of polyhedral meshes at the nanoscale, Nature, 2015, 523, 441-444.
[57] Liu W.Y., Zhong H., Wang R.S., Seeman N.C., Crystalline Two-Dimensional DNA-origami Arrays, Angew. Chem. Int. Edit., 2011, 50, 264-267.
[58] Yin P., Hariadi R.F., Sahu S., Choi H.M.T., Park S.H., LaBean T.H., Reif J.H., Programming DNA tube circumferences, Science, 2008, 321, 824-826.
[59] Wei B., Dai M.J., Yin P., Complex shapes self-assembled from single-stranded DNA tiles, Nature, 2012, 485, 623-626.
[60] Ke Y.G., Ong L.L., Shih W.M., Yin P., Three-Dimensional structures self-assembled from DNA bricks, Science, 2012, 338, 1177-1183.
[61] Kwak M., Herrmann A., Nucleic acid/organic polymer hybrid materials: synthesis, superstructures, and applications, Angew. Chem. Int. Ed., 2010, 49, 8574-8587.
[62] Kwak M., Herrmann, A., Nucleic acid amphiphiles: synthesis and self-assembled nanostructures, Chem. Soc. Rev., 2011, 40, 5745-5755.
[63] Kwiat M., Elnathan R., Kwak M., de Vries J.W., Pevzner A., Engel Y., Burstein L., Khatchtourints A., Lichtenstein A., Flaxer E., Herrmann A., Patolsky F., Non-covalent monolayer-piercing anchoring of lipophilic nucleic acids: preparation, characterization, and sensing applications, J. Am. Chem. Soc., 2012, 134, 280-292.
[64] Liu K., Chen D., Marcozzi A., Zheng L., Su J., Pesce D., Zajaczkowski W., Kolbe A., Pisula W., Müllen K., Clark N.A., Herrmann A., Thermotropic liquid crystals from biomacromolecules, P. Natl. Acad. Sci. U.S.A., 2014, 111, 18596-18600.
[65] Liu K., Varghese J., Gerasimov J.Y., Polyakov A.O., Shuai M., Su J., Zajaczkowski W., Marcozzi A., Pisula W., Noheda, B. Palstra T. T.M., Clark N. A. Herrmann A., Controlling the volatility of the written optical state in electrochromic DNA liquid crystals, Nat. Commun., (2016), 7, 11476.
[66] Liu K., Zheng L., Ma C., Göstl R., Herrmann A., DNA–surfactant complexes: self-assembly properties and applications, Chem. Soc. Rev., 2017, 46, 5147-5172.
[67] Liu K., Zheng L., Liu Q., de Vries J.W., Gerasimov J.Y., Herrmann A., Nucleic acid chemistry in the organic phase: from functionalized oligonucleotides to DNA side chain polymers, J. Am. Chem. Soc., 2014, 136, 14255-14262.
[68] Wilks T.R., Bath J., de Vries J.W., Raymond J.E., Herrmann A., Turberfield A.J., O'Reilly R.K., "Giant surfactants" created by

the fast and efficient functionalization of a DNA tetrahedron with a temperature-responsive polymer, ACS Nano, 2013, 7, 8561-8572.
[69] Herne T.M., Tarlov M.J., Characterization of DNA probes immobilized on gold surfaces, J. Am. Chem. Soc., 1997, 119, 8916-8920.
[70] Kimura-Suda H., Petrovykh D.Y., Tarlov M.J., Whitman L.J., Base-dependent competitive adsorption of single-stranded DNA on gold, J. Am. Chem. Soc., 2003, 125, 9014-9015.
[71] Walker H.W., Grant S.B., Conformation of DNA block copolymer molecules adsorbed on latex particles as revealed by hydroxyl radical footprinting, Langmuir, 1995, 11, 3772-3777.
[72] Charreyre M.T., Tcherkasskaya O., Winnik M.A., Hiver A., Delair T., Cros P., Pichot C., Mandrand B., Fluorescence energy transfer study of the conformation of oligonucleotides covalently bound to polystyrene latex particles, Langmuir, 1997, 13, 3103-3110.
[73] Petrovykh D.Y., Perez-Dieste V., Opdahl A., Kimura-Suda H., Sullivan J.M., Tarlov M.J., Himpsel F.J., Whitman L.J., Nucleobase orientation and ordering in films of single-stranded DNA on gold, J. Am. Chem. Soc., 2006, 128, 2-3.
[74] Levicky R., Herne T.M., Tarlov M.J., Satija S.K., Using self-assembly to control the structure of DNA monolayers on gold: A neutron reflectivity study, J. Am. Chem. Soc., 1998, 120, 9787-9792.
[75] Qu X., Li M., Zhang H., Lin C., Wang F., Xiao M., Zhou Y., Shi J., Aldalbahi A., Pei H., Chen H., Li L., Real-time continuous identification of greenhouse plant pathogens based on recyclable microfluidic bioassay system, ACS Appl. Mater. Inter., 2017, 9, 31568-31575.
[76] Liu D.S., Bruckbauer A., Abell C., Balasubramanian S., Kang D.J., Klenerman D., Zhou D.J., A reversible pH-driven DNA nanoswitch array, J. Am. Chem. Soc., 2006, 128, 2067-2071.
[77] Qu X., Zhu D., Yao G., Su S., Chao J., Liu H., Zuo X., Wang L., Shi J., Wang L., Huang W., Pei H., Fan C., An exonuclease III-powered, on-particle stochastic DNA walker, Angew. Chem., 2017, 56, 1855-1858.
[78] Josephs E.A., Ye T., A Single-Molecule View of Conformational Switching of DNA Tethered to a Gold Electrode, J. Am. Chem. Soc., 2012, 134, 10021-10030.
[79] Fan C.H., Plaxco K.W., Heeger A.J., Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA, Proc. Natl. Acad. Sci. U.S.A., 2003, 100, 9134-9137.
[80] Lai R.Y., Lagally E.T., Lee S.H., Soh H.T., Plaxco K.W., Heeger A.J., Rapid, sequence-specific detection of unpurified PCR amplicons via a reusable, electrochemical sensor, Proc. Natl. Acad. Sci. U.S.A., 2006, 103, 4017-4021.
[81] Bockisch B., Grunwald T., Spillner E., Bredehorst R., Immobilized stem-loop structured probes as conformational switches for enzymatic detection of microbial 16S rRNA, Nucleic Acids Res., 2005, 33.
[82] Liu G., Wan Y., Gau V., Zhang J., Wang L., Song S., Fan C., An enzyme-based E-DNA sensor for sequence-specific detection of femtomolar DNA targets, J. Am. Chem. Soc., 2008, 130, 6820-6825.
[83] Wei F., Wang J., Liao W., Zimmermann B.G., Wong D.T., Ho C.-M., Electrochemical detection of low-copy number salivary RNA based on specific signal amplification with a hairpin probe, Nucleic Acids Res., 2008, 36.
[84] Du H., Disney M.D., Miller B.L., Krauss T.D., Hybridization-based unquenching of DNA hairpins on Au surfaces: Prototypical "molecular beacon" biosensors, J. Am. Chem. Soc., 2003, 125, 4012-4013.
[85] Du H., Strohsahl C.M., Camera J., Miller B.L., Krauss T.D., Sensitivity and specificity of metal surface-immobilized "molecular beacon" biosensors, J. Am. Chem. Soc., 2005, 127, 7932-7940.
[86] Zuo X., Song S., Zhang J., Pan D., Wang L., Fan C., A target-responsive electrochemical aptamer switch (TREAS) for reagentless detection of nanomolar ATP, J. Am. Chem. Soc., 2007, 129, 1042-1043.
[87] Zheng D., Seferos D.S., Giljohann D.A., Patel P.C., Mirkin C.A., Aptamer Nano-flares for Molecular Detection in Living Cells, Nano Lett., 2009, 9, 3258-3261.
[88] Pei H., Lu N., Wen Y., Song S., Liu Y., Yan H., Fan C., A DNA nanostructure-based biomolecular probe carrier platform for electrochemical biosensing, Adv. Mater., 2010, 22, 4754-4758.
[89] Wen Y., Pei H., Shen Y., Xi J., Lin M., Lu N., Shen X., Li J., Fan C., DNA Nanostructure-based Interfacial engineering for PCR-free ultrasensitive electrochemical analysis of microRNA, Sci. Rep., 2012, 2:867.
[90] Lin M., Wang J., Zhou G., Wang J., Wu N., Lu J., Gao J., Chen X., Shi J., Zuo X., Fan C., Programmable engineering of a biosensing interface with tetrahedral DNA nanostructures for ultrasensitive DNA detection, Angew. Chem. Int. Edit., 2015, 54, 2151-2155.
[91] Pei H., Liang L., Yao G., Li J., Huang Q., Fan C., Reconfigurable Three-Dimensional DNA nanostructures for the construction of intracellular logic sensors, Angew. Chem. Int. Edit., 2012, 51, 9020-9024.
[92] Wen Y., Pei H., Wan Y., Su Y., Huang Q., Song S., Fan C., DNA nanostructure-decorated surfaces for enhanced aptamer-target binding and electrochemical cocaine sensors, Anal. Chem., 2011, 83, 7418-7423.
[93] Pei H., Wan Y., Li J., Hu H., Su Y., Huang Q., Fan C., Regenerable electrochemical immunological sensing at DNA nanostructure-decorated gold surfaces, Chem. Commun., 2011, 47, 6254-6256.
[94] Qu X., Zhang H., Chen H., Aldalbahi A., Li L., Tian Y., Weitz D.A., Pei H., Convection-driven pull-down assays in nanoliter droplets using scaffolded aptamers, Anal. Chem., 2017, 89, 3468-3473.
[95] Qu X., Yang F., Chen H., Li J., Zhang H., Zhang G., Li L., Wang L., Song S., Tian Y., Pei H., Bubble-Mediated Ultrasensitive Multiplex Detection of Metal Ions in Three-Dimensional DNA nanostructure-encoded microchannels, ACS Appl. Mater. Inter., 2017, 9, 16026-16034.
[96] Yan W., Xu L., Xu C., Ma W., Kuang H., Wang L., Kotov N.A., Self-assembly of chiral nanoparticle pyramids with strong R/S optical activity, J. Am. Chem. Soc., 2012, 134, 15114-15121.
[97] Li Y., Liu Z., Yu G., Jiang W., Mao C., Self-assembly of molecule-like nanoparticle clusters directed by DNA nanocages, J. Am. Chem. Soc., 2015, 137, 4320-4323.
[98] Rosi N.L., Mirkin C.A., Nanostructures in biodiagnostics, Chem. Rev., 2005, 105, 1547-1562.
[99] Rosi N.L., Giljohann D.A., Thaxton C.S., Lytton-Jean A.K.R., Han M.S., Mirkin C.A., Oligonucleotide-modified gold nanoparticles for intracellular gene regulation, Science, 2006, 312, 1027-1030.

[100] Li L., Hutter T., Finnemore A.S., Huang F.M., Baumberg J.J., Elliott S.R., Steiner U., Mahajan S., Metal oxide nanoparticle mediated enhanced raman scattering and its use in direct monitoring of interfacial chemical reactions, Nano Lett., 2012, 12, 4242-4246.
[101] Li L., Hutter T., Steiner U., Mahajan S., Single molecule SERS and detection of biomolecules with a single gold nanoparticle on a mirror junction, Analyst, 2013, 138, 4574-4578.
[102] Li L., Steiner U., Mahajan S., Single nanoparticle SERS probes of ion intercalation in metal-oxide electrodes, Nano Lett., 2014, 14, 495-498.
[103] Li L., Hutter T., Li W.W., Mahajan S., Single nanoparticle-based heteronanojunction as a plasmon ruler for measuring dielectric thin films, J. Phys. Chem. Lett., 2015, 6, 2282-2286.
[104] Song S., Liang Z., Zhang J., Wang L., Li G., Fan C., Gold-nanoparticle-based multicolor nanobeacons for sequence-specific DNA analysis, Angew. Chem. Int. Edit., 2009, 48, 8670-8674.
[105] Li N., Chang C., Pan W., Tang B., A multicolor nanoprobe for detection and imaging of tumor-related mRNAs in living cells, Angew. Chem. Int. Edit., 2012, 51, 7426-7430.
[106] Opdahl A., Petrovykh D.Y., Kimura-Suda H., Tarlov M.J., Whitman L.J., Independent control of grafting density and conformation of single-stranded DNA brushes, Proc. Natl. Acad. Sci. U.S.A., 2007, 104, 9-14.
[107] Pei H., Li F., Wan Y., Wei M., Liu H., Su Y., Chen N., Huang Q., Fan C., Designed diblock oligonucleotide for the synthesis of spatially isolated and highly hybridizable functionalization of DNA-gold nanoparticle nanoconjugates, J. Am. Chem. Soc., 2012, 134, 11876-11879.
[108] Zhu D., Song P., Shen J., Su S., Chao J., Aldalbahi A., Zhou Z., Song S., Fan C., Zuo X., Tian Y., Wang L., Pei H., PolyA-mediated DNA assembly on gold nanoparticles for thermodynamically favorable and rapid hybridization analysis, Anal. Chem., 2016, 88, 4949-4954.
[109] Chen L., Chao J., Qu X., Zhang H., Zhu D., Su S., Aldalbahi A., Wang L., Pei H., Probing cellular molecules with polyA-based engineered aptamer nanobeacon, ACS Appl. Mater. Inter., 2017, 9, 8014-8020.
[110] Chen N., Wei M., Sun Y., Li F., Pei H., Li X., Su S., He Y., Wang L., Shi J., Fan C., Huang Q., Self-Assembly of poly-adenine-tailed CpG oligonucleotide-gold nanoparticle nanoconjugates with immunostimulatory activity, Small, 2014, 10, 368-375.
[111] Zhao B., Shen J., Chen S., Wang D., Li F., Mathur S., Song S., Fan C., Gold nanostructures encoded by non-fluorescent small molecules in polyA-mediated nanogaps as universal SERS nanotags for recognizing various bioactive molecules, Chem. Sci., 2014, 5, 4460-4466.
[112] Zhu Y., Jiang X., Wang H., Wang S., Wang H., Sun B., Su Y., He Y., A poly adenine-mediated assembly strategy for designing surface-enhanced resonance raman scattering substrates in controllable manners, Anal. Chem., 2015, 87, 6631-6638.
[113] Qi L., Xiao M., Wang F., Wang L., Ji W., Man T., Aldalbahi A., Naziruddin Khan M., Periyasami G., Rahaman M., Alrohaili A., Qu X., Pei H., Wang C., Li L., Poly-cytosine-mediated nanotags for SERS detection of $Hg^{2+}$, Nanoscale, 2017, 9, 14184-14191.
[114] Zhu D., Chao J., Pei H., Zuo X., Huang Q., Wang L., Huang W., Fan C., Coordination-mediated programmable assembly of unmodified oligonucleotides on plasmonic silver nanoparticles, ACS Appl. Mater. Inter., 2015, 7, 11047-11052.
[115] Qi L., Xiao M., Wang X., Wang C., Wang L., Song S., Qu X., Li L., Shi J., Pei H., DNA-encoded raman-active anisotropic nanoparticles for microRNA detection, Anal. Chem., 2017, 89, 9850-9856.
[116] Shen J., Tang Q., Li L., Li J., Zuo X., Qu X., Pei H., Wang L., Fan C., Valence-engineering of quantum dots using programmable DNA scaffolds, Angew. Chem. Int. Ed., 2017, 56, 16077.
[117] Lo P.K., Karam P., Aldaye F.A., McLaughlin C.K., Hamblin G.D., Cosa G., Sleiman H.F., Loading and selective release of cargo in DNA nanotubes with longitudinal variation, Nat. Chem., 2010, 2, 319-328.
[118] Zhao Z., Jacovetty E.L., Liu Y., Yan H., Encapsulation of gold nanoparticles in a DNA origami cage, Angew. Chem. Int. Edit., 2011, 50, 2041-2044.
[119] Walsh A.S., Yin H., Erben C.M., Wood M.J.A., Turberfield A.J., DNA cage delivery to mammalian cells, ACS Nano, 2011, 5, 5427-5432.
[120] Li J., Pei H., Zhu B., Liang L., Wei M., He Y., Chen N., Li D., Huang Q., Fan C., Self-assembled multivalent DNA nanostructures for noninvasive intracellular delivery of immunostimulatory CpG oligonucleotides, ACS Nano, 2011, 5, 8783-8789.
[121] Lee H., Lytton-Jean A.K.R., Chen Y., Love K.T., Park A.I., Karagiannis E.D., Sehgal A., Querbes W., Zurenko C.S., Jayaraman M., Peng C.G., Charisse K., Borodovsky A., Manoharan M., Donahoe J.S., Truelove J., Nahrendorf M., Langer R., Anderson D.G., Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery, Nat. Nanotechnol., 2012, 7, 389-393.
[122] Modi S., Swetha M.G., Goswami D., Gupta G.D., Mayor S., Krishnan Y., A DNA nanomachine that maps spatial and temporal pH changes inside living cells, Nat. Nanotechnol., 2009, 4, 325-330.
[123] Surana S., Bhat J.M., Koushika S.P., Krishnan Y., An autonomous DNA nanomachine maps spatiotemporal pH changes in a multicellular living organism, Nat. Commun., 2011, 2.
[124] Stevens M.M., George J.H., Exploring and engineering the cell surface interface, Science, 2005, 310, 1135-1138.
[125] Chandra R.A., Douglas E.S., Mathies R.A., Bertozzi C.R., Francis M.B., Programmable cell adhesion encoded by DNA hybridization, Angew. Chem. Int. Edit., 2006, 45, 896-901.
[126] Bokel C., Brown N.H., Integrins in development: Moving on, responding to, and sticking to the extracellular matrix, Dev. Cell, 2002, 3, 311-321.
[127] Qu X., Wang S., Ge Z., Wang J., Yao G., Li J., Zuo X., Shi J., Song S., Wang L., Li L., Pei H., Fan C., Programming cell adhesion for on-chip sequential boolean logic functions, J. Am. Chem. Soc., 2017, 139, 10176-10179.
[128] Douglas E.S., Chandra R.A., Bertozzi C.R., Mathies R.A., Francis M.B., Self-assembled cellular microarrays patterned using DNA barcodes, Lab Chip, 2007, 7, 1442-1448.
[129] Toriello N.M., Douglas E.S., Thaitrong N., Hsiao S.C., Francis M.B., Bertozzi C.R., Mathies R.A., Integrated microfluidic bioprocessor for single-cell gene expression analysis, Proc. Natl. Acad. Sci. U.S.A., 2008, 105, 20173-20178.

[130] Hsiao S.C., Crow A.K., Lam W.A., Bertozzi C.R., Fletcher D.A., Francis M.B., DNA-coated AFM cantilevers for the investigation of cell adhesion and the patterning of live cells, Angew. Chem. Int. Edit., 2008, 47, 8473-8477.
[131] Hsiao S.C., Shum B.J., Onoe H., Douglas E.S., Gartner Z.J., Mathies R.A., Bertozzi C.R., Francis M.B., Direct cell surface modification with DNA for the capture of primary cells and the investigation of myotube formation on defined patterns, Langmuir, 2009, 25, 6985-6991.
[132] Gartner Z.J., Bertozzi C.R., Programmed assembly of 3-dimensional microtissues with defined cellular connectivity, Proc. Natl. Acad. Sci. U.S.A., 2009, 106, 4606-4610.
[133] Coyle M.P., Xu Q., Chiang S., Francis M.B., Groves J.T., DNA-mediated assembly of protein heterodimers on membrane surfaces, J. Am. Chem. Soc., 2013, 135, 5012-5016.
[134] Langecker M., Arnaut V., Martin T.G., List J., Renner S., Mayer M., Dietz H., Simmel F.C., Synthetic lipid membrane channels formed by designed DNA nanostructures, Science, 2012, 338, 932-936.