REVIEW

# Scaffolded biosensors with designed DNA nanostructures

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In addition to its fundamental function as a genetic code carrier, the utilization of DNA in various material applications has been actively explored over the past several decades. DNA is intrinsically an excellent type of self-assembly nanomaterial owing to its predictable base-pairing, high chemical stability and the convenience it possesses for synthesis and modification. Because of these unparalleled properties, DNA is widely used as excellent recognition elements in biosensors and as unique building blocks in nanodevices. A critical challenge in surface-based DNA biosensors lies in the reduced accessibility of target molecules to the DNA probes arranged on heterogeneous surfaces, especially when compared to probe–target recognition in homogeneous solutions. To improve the recognition abilities of these heterogeneous surface-confined DNA probes, much effort has been devoted to controlling the surface chemistry, conformation and packing density of the probe molecules, as well as the size and geometry of the surface. In this review, we aim to summarize the recent progress on the improvement of the probe–target recognition properties by introducing DNA nanostructure scaffolds. A range of new strategies have proven to provide a significantly enhanced range in the spatial positioning and the accessibility of the probes to the surface over previously reported linear structures. We will also describe the applications of DNA nanostructure scaffold-based biosensors.
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## INTRODUCTION

Detection of nucleic acids (DNA or RNA) is an integral step in many applications, including in clinical diagnosis, environmental monitoring and antibioterrorism.[1] With these applications in mind, significant efforts have been made to develop sensitive, selective, rapid and cost-effective DNA (or RNA) biosensors. In parallel, DNA-based devices have also attracted a significant, recent interest owing to their promising applications in nanoelectronics,[2,3] biomolecular computations,[4–8] cellular imaging[9] and drug delivery.[10–12] In a typical design of DNA biosensors and nanodevices, oligonucleotides are often used as the molecular recognition layer. Because DNA is comprised of only four bases with A–T and G–C pairing, DNA hybridization processes are highly predictable and can be finely tuned with a rational design of the sequence. In addition, DNA oligonucleotides are usually easy to design, chemically stable and cost-effective.

Understanding the physical structure of a DNA probe immobilized on a surface is critical in applications using DNA as a molecular recognition element. The properties of the DNA molecular recognition layer (such as selectivity, sensitivity and reproducibility) highly depend on the structure of the self-assembled DNA film. Modeling studies with thiolated DNA have demonstrated that efficient hybridization occurs in the well-controlled condition of surface-attached probes with large interprobe distances and upright conformations.[13–24]

However, constructing a reproducible DNA recognition layer with both of these properties is a critical challenge owing to unexpected surface adsorptions, disordered conformations, inconsistencies in grafting density and the flexibility of probe molecules. It is thus imperative to obtain an in-depth understanding of the factors that influence the structure of immobilized DNA layers. In this article, we aim to summarize the recent advances in this field, particularly in the evolution of using DNA probes from one-dimensional (1D) to two-dimensional (2D) and ultimately to three-dimensional (3D) probes (Figure 1), as well as the application of these probes in developing DNA biosensors.

## 1D DNA PROBES

Single-stranded (ss) oligonucleotides (1D DNA probes, typically constructed of 15–50 bases) are widely used in developing most DNA sensors and chips owing to their lack of secondary structure. 1D DNA probes modified with thiol groups at either the 3′ or 5′ terminus can readily self-assemble on gold surfaces; this self-assembly has become a paradigm for the design of DNA recognition layers. Thiolated DNA is expected to form self-assembled monolayers at Au surfaces via a single-point attachment, causing the probe to possess an upright orientation.

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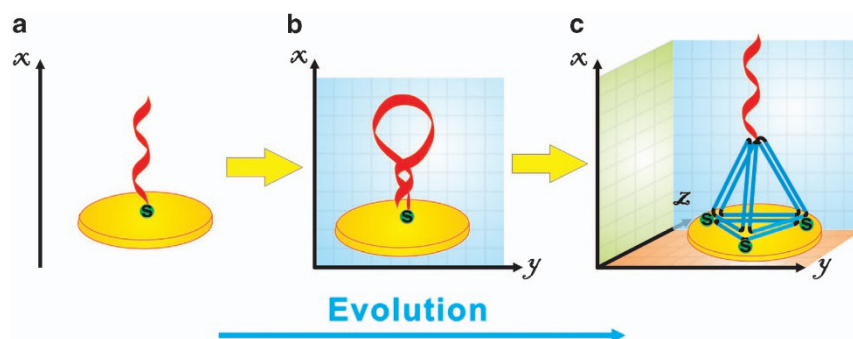E-mail: fchh@sinap.ac.cn

**Figure 1** The evolution of surface-confined DNA probes from one-dimensional (1D) probes to two-dimensional (2D) and then three-dimensional (3D). Gold surfaces are anchored with thiolated DNA probes with (**a**) 1D single-stranded oligonucleotides (usually between 15 and 50 bases) without any secondary structure; (**b**) 2D DNA structure; and (**c**) 3D scaffolded DNA nanostructure.

However, because significant interactions exist between the DNA bases and the Au surface, in reality, DNA may lie down and adsorb to Au via multiple nitrogen atoms,[25] which largely restricts the accessibility of the target DNA molecules (Figures 2a–c). In 1997, Herne and Tarlov[17] provided evidence that thiolated single-stranded DNA (SH-ssDNA) is not only 'specifically' adsorbed onto the Au via the Au–S bond but also 'nonspecifically' via Au–N interactions.[17] Moreover, X-ray photoelectron spectroscopy data revealed that such nonspecifically adsorbed ssDNA could not be removed, even with extensive rinsing or heating to 75 °C. An Au surface assembled with 25-base SH-ssDNA only has a film thickness of $3.3 \pm 0.2$ nm in air, which is approximately 20% of the expected maximum thickness (16 nm), suggesting that the SH-ssDNA monolayer is not tightly packed and that the DNA chains are not oriented perpendicularly to the surface.

It was suggested that the attached DNA could extend further into the solution when either repulsive electrostatic fields[26] were applied onto the surface or with a high surface density of DNA.[27] By using high-resolution X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy, Petrovykh *et al.*[20] characterized the structure of SH-ssDNA films on Au and showed that 1D DNA probes lay nearly flat on the substrate with multiple anchoring points at low densities. At high grafting densities, randomly coiled ssDNA probes were predominantly anchored via thiols and possibly one or two other bases. Further studies using near-edge X-ray absorption fine structure spectroscopy[28] suggested that DNA bases in short probes tended to orient parallel to the surface. In contrast, long DNA probes formed disordered films similar to polyelectrolyte brushes. Clearly, short DNA probes may take upright conformations in densely packed films, which nevertheless prohibit efficient target accessibility.

Ideally, surfaces with a low density of DNA probes that possess the upright orientation should favor target hybridization. Tarlov and co-workers[18] introduced a clever strategy that uses a coassembling 'helper' molecule, mercaptohexanol (MCH), to solve this problem (Figure 2d). Treatment of the DNA-modified Au surface with MCH not only largely removes nonspecifically adsorbed DNA but also further projects the DNA probes out into the solution owing to the repulsion between the net negative dipole of the alcohol terminus and the negatively charged DNA backbones. These mixed ssDNA/alkylthiol monolayers have been extensively characterized with neutron scattering, X-ray photoelectron spectroscopy, surface plasmon resonance and electrochemistry, which have all confirmed the favorable upright orientation of the DNA probes.[29] The two-step sequential adsorption protocol has been extensively utilized in
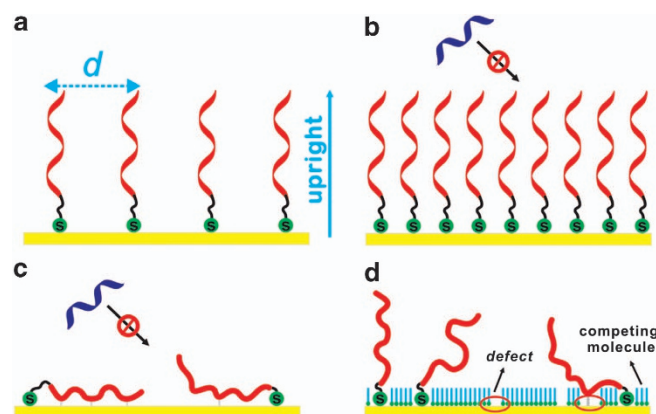


**Figure 2** Impact of probe layer structure. (**a**) Schematic illustration of an ideal DNA recognition layer with large interprobe distances and upright conformation. (**b**) Densely packed DNA probes with an upright conformation, but resulting in poor hybridization efficiency that is due to steric hindrance. (**c**) Probes tend to lie flat on the surface owing to nonspecific adsorption in the sparse film. (**d**) Mixed single-stranded (ss)DNA/alkylthiol monolayers.

developing various types of DNA sensors and DNA-based devices.[30] When coupled with signal amplification with enzymes or nanomaterials, high-sensitivity DNA sensors with detection limits down to the pM–fM scale have been realized, which allows for the detection of a range of pathogenic or tumor targets. The use of MCH was later expanded with other diluent thiols.[31,32] An example of this was a mixed ssDNA/oligo-ethylene glycol monolayer that was used to improve the protein resistance of the surface, which makes it broadly applicable in biological medium.[33]

The DNA probe density is a controlling factor for the efficiency of the target capture, as well as for the kinetics of the target/probe hybridization.[14,15] Gong and Levicky[34] studied the hybridization behavior as a function of the ionic strength ($C_B$) and probe coverage ($S_P$) in the form of a diagram of hybridization regimens[34] (Figure 3). They predicted that DNA hybridization is most favorable in the 'Langmuir' (L) regimen, which exists in the limits of sparse films where probes are so far apart that they do not interact. However, because significant lateral interactions exist in DNA films, even at the lowest densities, this L regimen was hardly reachable. Soft DNA polymers usually adopt disordered structures in the films, particularly at long base lengths.[22,29,35] Recently, Josephs and Ye[36] characterized the conformation of surface-tethered double-stranded DNA in a

single-molecule view using electrochemical atomic force microscopy. They provided evidence that the conformation and dynamics of the tethered double-stranded DNA were far more complicated than expected from the view of ensemble measurements. The presence of aggregation patches significantly reduces target accessibility, which, nevertheless, could not be easily removed using MCH as the dilution molecule.

## 2D STRUCTURED DNA PROBES

ssDNA strands with internal complementary sequences can form secondary structures (2D or pseudo-2D), such as hairpin, quadruplex and pseudoknot structures. Such relatively rigid and ordered structures may, in principle, prevent interstrand entanglement at the surface and/or increase the orderness of DNA layers.

In 2003, by attaching 2D DNA probes with a hairpin (stem-loop) structure at Au electrodes, Fan et al.[37] designed a conceptually new electrochemical DNA (E-DNA) sensor strategy that exploits target-induced conformational changes of 2D DNA structures (Figure 4a). The 3′ end and 5′ end of the stem are labeled with a thiol and an electroactive ferrocene, respectively. The hairpin probe was then self-assembled on Au via the 3′-alkanethiol. Target hybridization



**Figure 3** Diagram of surface hybridization regimes. $C_B$: ionic strength; $S_P$: probe coverage; non-hybridizing (NH) regimen; suppressed hybridizing (SH) regimen; pseudo-Langmuir (PL) regimen; and a true 'Langmuir' (L) regimen (adapted from Gong and Levicky[34]).

alters the conformation of the probe, resulting in the opening of the stem and the extension of the loop into a linear duplex. In response to that extension, the distance between ferrocene and Au is increased, which leads to large variations in electron transfer and in turn produces measurable changes in electrochemical signals. This type of sensor can readily detect picomolar concentrations of target DNA without the need for exogenous reagents. This E-DNA sensor is rapid, portable and reusable, which is particularly useful in medical and military applications. By using this strategy, Lubin et al.[43] successfully demonstrated an E-DNA sensor-based approach for detecting DNA authentication tags that are associated with paper or drugs. Lai et al.[44] achieved the sequence-specific detection of unpurified amplification products of the gyrB gene of Salmonella typhimurium, which may open the path toward effective, field-portable sample-to-answer pathogen identification.

While the initial E-DNA sensor design has proven successful for the highly selective detection of target DNA in a wide range of mediums, the sensitivity is limited to picomolar concentrations, partially because one ferrocene label can only exchange one electron with the Au electrode. In 2005, Bockisch et al.[45] reported a colorimetric method with stem-loop structured probes that relied on horseradish peroxidase (HRP) to transduce the conformation-associated signal. Given that one HRP can convert ∼10 000 reactions of hydrogen peroxide to water, the target binding-induced signal change could be greatly amplified. Liu et al.[38] then developed a highly sensitive enzyme-based E-DNA sensor that pushed the detection limit down to low femtomolar concentrations, to nearly three orders of magnitude lower than the initial E-DNA setup[38] (Figure 4b). Wei et al.[46] developed a similar sensor for electrochemical detection of salivary mRNA targets with 0.4 fM sensitivity.

In an alternative approach, Du et al.[47,48] proposed a fluorescent DNA sensor using DNA hairpins immobilized on the planar Au surface. Their study suggested that the hybridization efficiency is sensitive to the secondary structure of the hairpin, as well as to the distribution of the DNA hairpins on the substrate. Along this line, a range of fluorescent DNA sensors was developed using nanoscale metal surfaces.[49–51] For example, we constructed multicolor nanobeacons by immobilizing three stem-loop probes labeled with different fluorophores on the surface of 15-nm gold nanoparticles (AuNPs). Three probes labeled with FAM, Cy5 and Rox were designed to target three types of tumor-suppressor genes (exon segments of
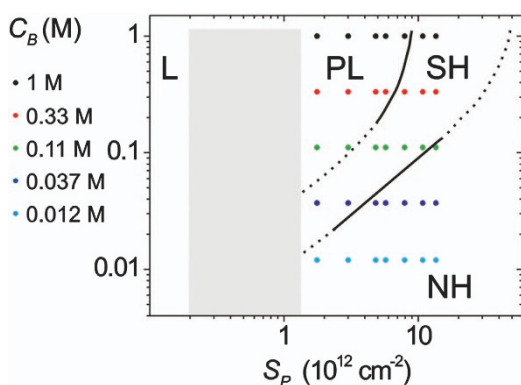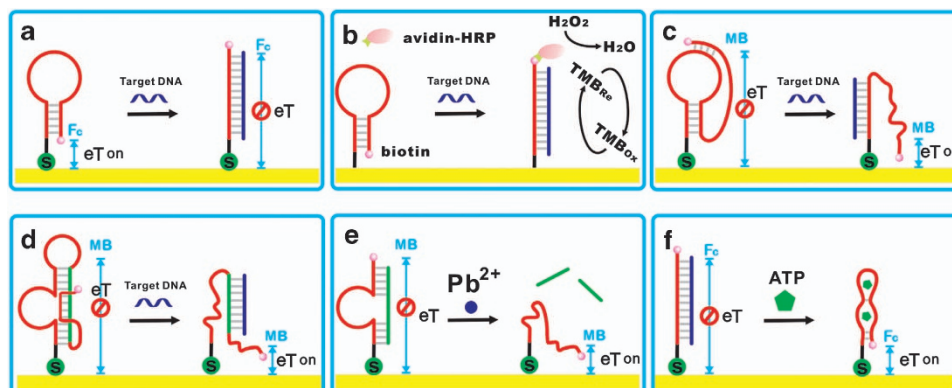


**Figure 4** Two-dimensional (2D) DNA probe. (a) The stem-loop-structured probe design for an electrochemical DNA (E-DNA) sensor (adapted from Fan et al.[37]). (b) A stem-loop-structured probe-based E-DNA sensor with enzyme amplification (adapted from Liu et al.[41]). (c) DNA probe with two stem-loop structures (adapted from Qu et al.[51]). (d) DNA probe with three stem-loop structures (adapted from Xiao et al.[52]). (e) DNAzyme-based 2D probe as an ion ($Pb^{2+}$) sensor (adapted from Plaxco et al.[53]). (f) DNA aptamer-based 2D probe as an ATP sensor (adapted from Zuo et al.[54]). HRP, horse radish peroxidase; MB, multicolor beacons.

*p16, p21* and *p53*), and the nanobeacons could respond to the specific target with negligible crosstalk.[52] It is interesting that AuNP–DNA conjugates can be efficiently taken up by cells. Jayagopal *et al.*[53] presented a strategy for live cell imaging of messenger RNA using DNA hairpin functionalized AuNPs, which shows high promise for real-time analysis of mRNA transport and processing in live cells.

In addition to stem-loop probes, it is also possible to design 2D probes with various different secondary structures. Xiao *et al.*[54] designed a discontinuous duplex DNA probe for constructing a signal-on, label-free electronic DNA sensor, which utilized a strand-displacement mechanism and achieved a sub-picomolar concentration detection limit. This improved detection limit is believed to be attributed to the enhanced rigidity of the sensing probe. Qu *et al.*[39] designed another 2D probe using the pseudoknot structure that consists of two stem-loop structures in which a portion of each loop forms one strand of the stem of the other stem-loop (Figure 4c). They demonstrated that this probe was sufficiently stable for deployment directly in complex matrices, including in blood serum. Furthermore, Xiao *et al.*[40] also used a triple-stem structure that could realize effective single-nucleotide polymorphism (SNP) detection at room temperature (Figure 4d). By comparing the thermodynamic analysis among single-, double- and triple-stem probes, they suggested that this probe took advantage of the large thermodynamic changes in enthalpy and entropy resulting from major conformational rearrangements in response to binding with the complementary target, which gave rise to exquisite sensitivity to single-base mismatches even in the complex medium of blood serum.[40]

It is worth mentioning that functional nucleic acids, such as DNAzymes or aptamers (RNA or DNA), can also be regarded as pseudo-2D probes. In 2007, Plaxco *et al.*[41] used a $Pb^{2+}$-requiring DNAzyme, the '8–17' DNAzyme, as a probe for electrochemical detection of $Pb^{2+}$ (Figure 4e). The probe consisted of a methylene blue-modified catalytic DNA strand hybridized to its complementary substrate oligonucleotide. The presence of $Pb^{2+}$ induced cleavage of the complex and then disturbed the rigid structure of the complexes, which produced specific electrochemical signals. Zuo *et al.*[42] designed an adenosine triphosphate (ATP) sensor using an anti-ATP aptamer. Their study verified that ATP could stabilize the tertiary aptamer structure at the surface and could responsively denature the initial aptamer duplex (Figure 4f). By incorporating a DNA aptamer into the three-way junction elements, Huang *et al.*[55] reported a rationally designed probe architecture for the detection and quantification of thrombin, which is a plasma protein. With the same design principle, Ge *et al.*[56] designed a contractile DNA duplex probe that incorporated two short, separated motifs of G/G mismatches that were flanked by Watson–Crick base-paired DNA. The presence of $K^+$ induced the transition of the duplex DNA to G-quadruplex and changed the charge-conducting properties of the DNA structure. Again, these 2D probes can be assembled on AuNPs and can be used to image molecular targets in live cells owing to the cellular uptake ability of AuNPs. Mirkin and co-workers[57] attached an aptamer probe to AuNPs to develop an intracellular 'nanoflare' probe for quantification of small molecules or proteins in living systems.

Compared with 1D probes, 2D probes have shown superior properties as sensing elements in several aspects. First, the enhanced rigidity over the linear probes offers better ordered orientation of DNA probes on metal surfaces. Second, because of their internal hybridized nature, these 2D probes are inherently resistant to intraprobe interactions at the surface. Third, thermodynamic studies with 2D probes (single- or triple-stem structure) have demonstrated their capability to sensitively discriminate single-base mismatched DNA.

However, it should be noted that 2D probes are not sufficiently rigid to survive on highly crowded surfaces; hence, appropriate modulation of the surface density is still critically important for high-performance sensors. In addition, MCH is still required to help 2D structures stand upright at the surface, which makes the surface inevitably a heterogeneous one.

## 3D-STRUCTURED DNA PROBES

It is critically important to control precisely the density and orientation of grafted DNA probes on the surface to minimize the lateral interactions between probes and to maximize the target accessibility. To this end, Pei *et al.*[58] proposed a new concept to improve the probe–target recognition performance by introducing a 3D-structured DNA probe. As an example, a tetrahedron-shaped DNA nanostructure with a pendant DNA probe at one vertex and three thiol groups at the other three vertices was designed and used for the development of DNA sensors. This probe is expected to attach to the Au surface with the three-point attachment via the thiol–Au bond and to leave a free-standing probe at the top vertex. This tetrahedron-structured probe (TSP) is self-assembled with a probe-containing, 80-base oligonucleotide and three thiolated 55-base oligonucleotides. The formation of the TSP is essentially conducted in solution and is completed within only 2 min with a high yield of ~85%, as confirmed by non-denatured electrophoresis analysis. TSP is rapidly and firmly adsorbed at the surface of gold owing to the presence of the three thiol legs, a process that was monitored with a combination of surface-sensitive technologies, including quartz crystal microbalance, surface plasmon resonance and microcantilever. The probe-to-probe spacing of TSP is approximately 4 nm, as estimated from the measured surface density of TSP ($4.8 \times 10^{12}$ TSP cm$^{-2}$). The pendant probe can selectively capture its complementary sequence, with high discrimination ability toward non-cognate sequences.

By using TSP-decorated Au electrodes, Pei *et al.*[58] constructed an E-DNA sensor with a conventional sandwich detection strategy (Figure 5a). The electrochemical signal is produced via the substrate catalysis of HRP. Of significance, this non-optimized, proof-of-concept sensor exhibits a 250-fold improvement in sensitivity (~1 pM) compared with that of 1D probes with the similar HRP-based signal-transduction approach. To further improve the sensitivity, Wen *et al.*[61] increased the interprobe distance using interfacial engineering and multienzyme amplification, which results in an extremely low detection limit of 10 aM (~600 molecules in 100 μl).

The presence of bulky tetrahedral structures not only avoids interprobe entanglement via spatially segregating pendant probes but also reduces the surface effects and places the probes in a solution-phase-like environment. The high mechanical rigidity of TSP allows it to stay at the Au surface with a highly ordered, upright orientation. Hence, the nanostructured surface has superior molecular accessibility to conventional ones. For example, Howorka and co-workers[62] reported that molecular recognition of receptors on such a surface was improved by an order of magnitude. Squires *et al.*[63] also found that the TSP-based DNA sensor possesses remarkable selectivity toward single-base mismatches. In addition, diffusion and convection at the nanostructure interfaces are also expected to be higher than those at macroscopic ones. This 3D-structured platform also demonstrates a combination of other advantages. First, the presence of the three thiol legs greatly increases the stability of the surface-confined probes by approximately 5000 times compared with the mono-thiolated DNA, which is beneficial for constructing stable and robust biosensors.[64] In addition, the 'helper' molecule MCH is not necessary in this case. Second, this TSP-modified surface is fully
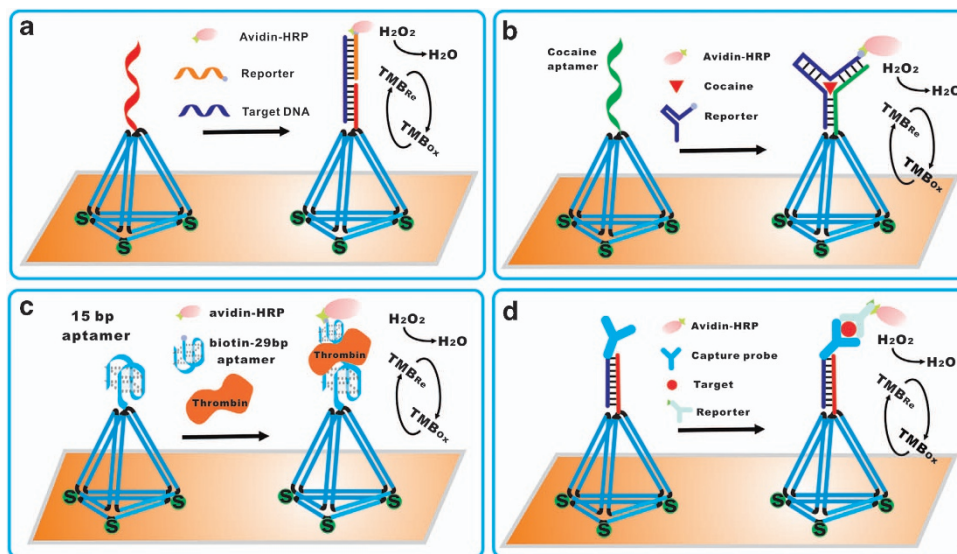
**Figure 5** The tetrahedron-structured probe (TSP)-based biosensing platform for (**a**) DNA targets (adapted from Pei et al.[58]); (**b**) a small molecule, cocaine, with the split anticocaine aptamer (adapted from Wen et al.[63]); (**c**) a protein, thrombin, with a pair of antithrombin aptamers (adapted from Pei et al.[58]); and (**d**) immunological assays (adapted from Pei et al.[66]). HRP, horse radish peroxidase.

compatible with electrochemical interrogations. While the TSP layer is relatively thick ($\sim$6 nm), the hollow structure of the TSP allows facile communications of electroactive species with the electrode. Third, the TSP-decorated surfaces can prevent nonspecific adsorption of proteins, which makes it possible to use directly TSP-based sensors in the serum for practical applications.

The TSP-based surface has proven to be a versatile platform for the detection of a broad range of biomolecules. The pedant probe can be replaced with various functional nucleic acids for biodetection. As a proof of concept, a split aptamer for a small molecule, cocaine, was incorporated to develop an electrochemical sensor for cocaine[59] (Figure 5b). This sensor could detect a concentration of 33 nM cocaine in a complex media, exceeding the sensitivity of previously reported aptamer-based sensors by more than two orders of magnitude. In another approach, Pei et al.[58] replaced the DNA probe with an antithrombin aptamer at the top of the tetrahedron, which formed a thrombin sensor that could detect a concentration as low as 100 pM thrombin (Figure 5c). In addition, Ge et al.[65] developed an electrochemical sensor for the genotyping of SNPs, and Bu et al.[66] constructed a sensor for mercury ions using a mercury-specific oligonucleotide. To make this nanostructured surface more generally usable, Pei et al.[60] introduced a DNA-directed antibody immobilization to convert the DNA recognition layer into a protein recognition one (Figure 5d). Antibodies can be easily and reversibly anchored onto this nanostructured surface via a DNA bridge. These immunosensors demonstrated superior sensitivity and selectivity over conventional ones.

Because surface-confined tetrahedra are highly rigid scaffolds, it is possible to place different dyes or redox molecules on the different bases of the structure to measure the distance-dependent energy transfer or charge transport. When a tetramethylrhodamine dye is placed at the top vertex, the fluorescence is intense; the fluorescence is observed to be weaker when the dye is attached at one of the bottom vertices that are close to the Au surface. This change in the strength of fluorescence is typical of Au-quenched fluorescence, which is due to the energy transfer distance. Similarly, Lu et al.[67] conjugated either

methylene blue or ferrocene redox molecules to different positions of the surface-confined DNA tetrahedron and studied the through-duplex and through-space DNA-mediated charge transport. Such interesting properties are particularly useful for the design of molecular sensors for external stimuli. For example, Pei et al.[68] incorporated different functional nucleic acids into one or two edges of the tetrahedra, which made them dynamic structures that were responsive to different molecules. Because DNA tetrahedra can effectively enter mammalian cells without the help of a transfection reagent,[9,10] this tetrahedron-based sensor can be used to image intracellular ATP in live cells.

## NANOSTRUCTURED DNA SUBSTRATES

Self-assembled DNA nanostructures can also serve as the substrate for biosensing and bioassays (Figure 6). Compared with solid-state substrates (for example, gold or glass), DNA-based nanostructured substrates are essentially in solution and fully compatible with addressable DNA immobilization. This places the biological reactions in the homogeneous solution-like environment, which allows the reactions to occur faster than those that occur at heterogeneous interfaces.

Instead of using simple self-assembled DNA nanostructures, Ke et al.[69] used a DNA origami[70]-based rectangular structure of $70 \times 100$ nm$^2$, which was appended with 20-base probes at predefined positions. This system could detect microRNAs (200 pM) in a high background of total cellular RNA. Because the probes are placed on the rectangular substrate with nanometer-scale precision, the site-specific effects of the probes could be explored with molecular resolution. However, the hybridization signal was only detectable with atomic force microscopy. Given that this tiny DNA origami chip might be easily scaled down to a sample volume comparable to that of single cells, it shows high promise for single-cell gene expression analysis.

This origami substrate of a rectangular shape is readable only when built-in 'index' oligonucleotides are present to break the symmetry. To overcome this problem, Zhang et al.[71] reported the design of an
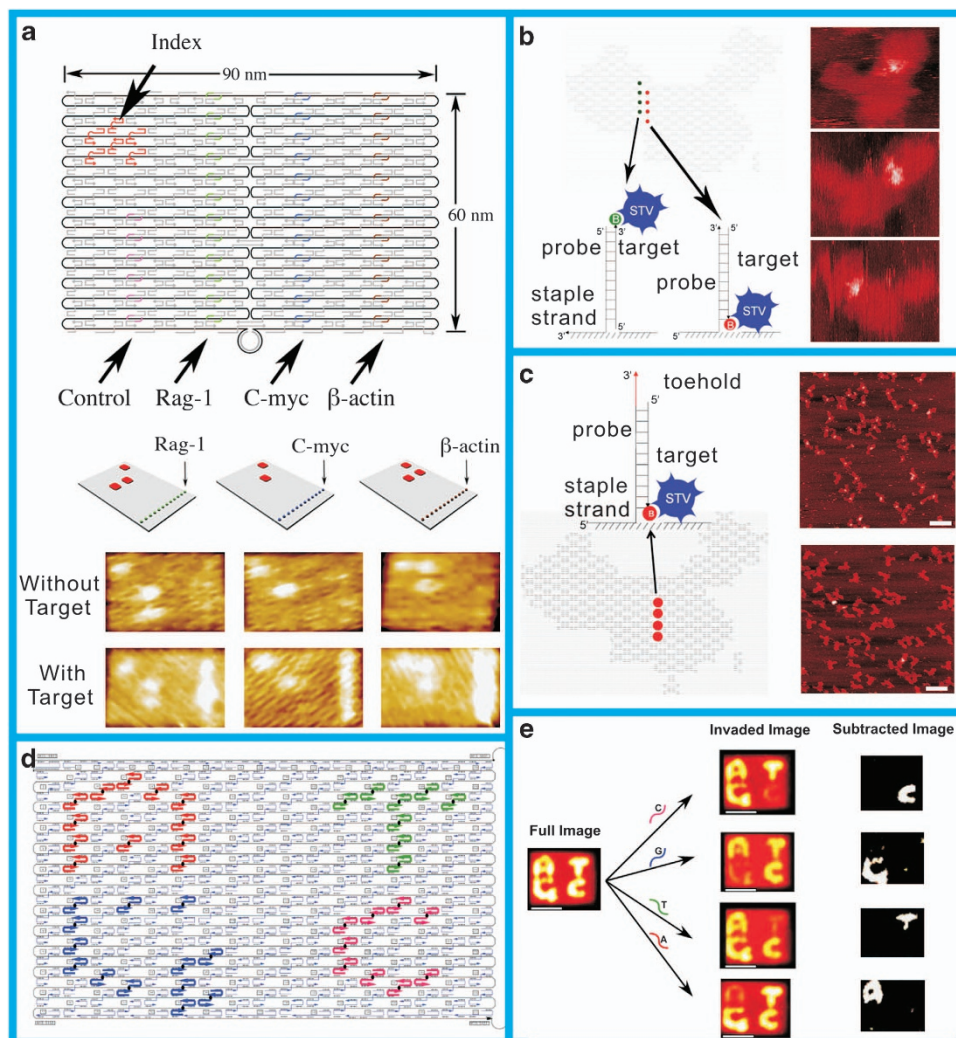
**Figure 6** (**a**) A rectangular DNA origami chip for label-free detection of microRNAs (adapted from Ke *et al*.[70]). A map-shaped asymmetric DNA origami chip for the detection of (**b**) DNA hybridization and (**c**) single-nucleotide polymorphism (SNP) genotyping (adapted from Zhang *et al*.[71,72]); and (**d** and **e**) visual SNP genotyping on DNA origami with a symbolic display (adapted from Subramanian *et al*.[73]).

index-free nanoscale DNA chip by using an asymmetric DNA origami substrate with the shape of a Chinese map. The asymmetric nature of the map makes the position of each DNA probe fully spatially addressable under atomic force microscopy imaging. Zhang *et al*.[72] also developed a chip for SNP genotyping on the DNA origami chip by introducing a toehold strand-displacement reaction that could effectively differentiate single-base mismatches at ambient temperature. Seeman and co-workers[73] developed a visual DNA origami chip for SNP genotyping with a symbolic display. This DNA origami chip contains graphical representations of the four nucleotide alphabetic characters, A, T, G and C, and these patterns can be imaged with atomic force microscopy.

## CONCLUSION

Along with the rapid emergence and development of biotechnology and nanotechnology, various DNA nanostructure scaffolds have been designed, characterized and exploited for a range of applications.[74] In particular, we have observed the evolution of surface-confined DNA probes with rational design from 1D to 2D and then to 3D, which greatly improves our ability to control the density, orientation and

passivation of the surface. On the basis of these studies, a variety of electrochemical and optical biosensors have been constructed for the detection of DNA/RNA hybridization, SNP genotyping and microRNA biomarkers. Other types of 3D-structured DNA probes with different shapes and sizes should be explored in the future, which may provide additional advantages. Nevertheless, DNA sequence synthesis and modification form the major barrier for the design of 3D-structured DNA probes. Ideally, the availability of DNA sequences modified with functional biomolecules, for example, functional nucleic acids that can be readily folded into the desired DNA nanostructure, is necessary for the establishment of a generic platform for biosensing using 3D DNA probes. However, the synthesis of long DNA sequences ($>100$ nucleotides) is not readily commercially available. In addition, extra functional biomolecules may influence the assembly of the DNA nanostructure. The purity of the assembled DNA nanostructure is another concern and relies on the improvement of amenable separation techniques, for example, electrophoresis or high-performance liquid chromatography.

More recently, emerging DNA nanotechnology offers the unprecedented ability to control the assembly of large and well-defined DNA

nanostructures that may serve as substrates for site-specific immobilization of biomolecules. This control will provide new opportunities to perform chip-based multianalyte bioassays in homogeneous solutions. Parallel to these advances in biosensing, DNA nanostructure-based nanodevices have also attracted intense interests that have led to the development of nanorobots, nanocarriers for drug delivery and nanoscale computing devices. All of these advances rely on the active interdisciplinary cooperation among chemistry, physics, material science, biology and electronics, which will clearly have a bright future regarding diagnosis and therapy of cancers and other important diseases.

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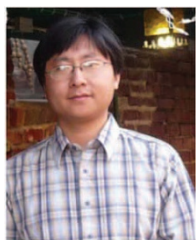
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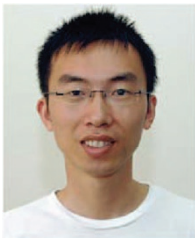
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