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DNA molecules site-specific immobilization and their applications

Review Article

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Abstract: In addition to its role as a carrier of genetic information, DNA has been recognized as a construction material for the assembly of different objects and structural arrangements with nanoscale features. As a result of DNA's self-recognition properties (based on the specific base-pairing of G-C and T-A), monolayer films of nucleic acids on solid supports have attracted an escalating attentions. Recently, numerous novel materials based on two-dimensional (2D) and three-dimensional (3D) DNA structures have been reported, which extends their utility to a large number of appliations. This review paper intends to be a new and comprehensive overview of recent strategies to site-specifically immobilized DNA on various materials, including carbonaceous substances, gold, and silica substrate, emphasizing the applications of site-specific DNA nanostructure-based devices for diagnostic, bioanalytical, food safety and environmental monitoring. Additionally, an up-to-date perspective is proposed at the end of this review.

Keywords: *DNA • Site-specific immobilization • Application • Sensors*

1. Introduction

As the foundation of modern microarray and biosensing technologies, molecular interaction at solid surface is widely used in applied science and engineering [1,2]. DNA, a carrier of genetic information, comprises only four bases: adenine (A), thymine (T), cytosine (C), and guanine (G), has been recognized as a construction material for the assembly of different objects and structural arrangements with nanoscale features [3-5]. Taking advantage of the self-recognition properties of DNA (based on the specific base-pairing of G-C and T-A) [6] , monolayer films of nucleic acids on solid supports are utilized in diagnostic and bioanalytical applications [7-11], environmental monitoring [12] and food safety testing [13] for detection and quantification of chemical and biological molecules [14]. All of above applications will be discussed in a later section of this review paper. Due to their wide-spread applications, immobilization of DNA molecules on solid supports has attracted an escalating attention, both in the research and commercial fields [15,16]. Many novel materials based on two-dimensional (2D) and three-dimensional (3D) DNA structures have been reported recently [17]. Additionally, numerous immobilization strategies have also been published, describing how to precisely control and organize these functional materials.

One other important feature of DNA-based devices is that they allow easier, faster and cheaper results than traditional assays, while keeping high sensitivity and specificity of detection [16]. In general, DNA-based devices are composed of probes, supports, and targets. A probe is the immobilized or fixed DNA with a known sequence. Supports are the biocompatible materials, which allow probes to be attached to them. The target is the free DNA or other molecules that can interact with the probe specifically [18]. It is important to minimize the non-specific linkages between DNA and surface for a specific, selective and reliable analysis. With this in mind, fundamental studies of the experimental conditions and optimization become necessary. Significant efforts have been made and a variety of studies for the improvement

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of DNA site-specific immobilization have been reported [19-25]. Based on these investigations, ionic strength, temperature, even washing procedures are believed to effect the probe immobilization, then inpact the performance of DNA based devices. In this article, we aim to summarize the recent advances in DNA molecules site-specific immobilization, particularly with regards to DNA probe immobilization on various supports, as well as the application of these probes in developing DNA-based devices.

2. Routs for the DNA surface immobilization

In most cases, the methodology employed for DNA immobilization ranges from simple physiosorption and covalent chemisorption to biospecific interaction. Whereas physisorption relies on weak van der Waals forces between DNA and surfaces [26], chemisorption indicates covalent coupling formation with high affinity. Biospecific interaction relates to the adsorption of biomolecules to their complementary constituents, such as the biotin-streptavidin motif [27]. Although a number of strategies have been published, the basic rules for methods of immobilizing DNA remain simple, robust, and cost-effective [28].

Obviously, fast adsorption/desorption kinetics are one of the advantages of the physisorption approach. For example, Yousef Elahi and co-workers published a report describing how double stranded DNA was physisorbed onto a polypyrrole (PPy) modified Pt electrode. The electrode was over-oxidized prior to immobilization of the DNA. The binding resulted from the electrostatic interaction between the negatively charged DNA phosphate groups and the positively charged oxidized PPy backbone. Compared with the couple of hours normally required for the chemisorption approach [29], 30 mins incubation time were all that were required in this study for the DNA immobilization, which clearly shortened the process of sample preparation significantly. After removing the weakly absorbed DNA with PBS solution, the interaction of DNA with salicylic acid (SA) and acetylsalicylic acid (ASA) was studied on the electrode surface using differential pulse voltammetry [30].

Although physical adsorption of DNA on the substrate is simple and straightforward, loss of DNA bioactivity may occur due to its random orientation with respect to the surface [31]. The introduction of the covalent immobilization of DNA can help largely avoid this issue. It is very likely that covalent attachment will yield better results, which is of great interest for many medical and bioanalytical applications [18]. Covalent attachment of DNA is typically achieved by chemical derivatization of the phosphate group at either the 5' or 3' terminus to form a moiety, which can covalently bond with the substrate surface [32]. For example, in Seefeld's work, an amino linker was added to the DNA (see Fig. 1). The 5'-amino-modified DNA was covalently attached to the poly-L-glutamic acid (pGlu) monolayer coated gold substrate via NHS-EDC coupling chemistry (a three-step reaction for attaching amine-terminated ssDNA onto surfaces) [33]. Based on the resulting DNA microarrays, a simultaneous detection of protein system was built up for the first time. After PCR reaction and the creation of mRNA, the expressed His-tagged protein was specifically adsorped onto an adjacent Cu(II)-NTA modified gold surface and simultaneously detected via surface plasmon resonance imaging (SPRI). The significant advantage of this on-chip synthesis process is that it greatly reduces the risk of protein degradation and realizes protein detection in a timely manner [34].

On the other hand, the internucleotide can also be modified with phosphorothioate function to attach the DNA to the corresponding surface. A case involving the chemisorption of oligodeoxynucleotide phosphorothioate (s-oligo) was reported by Yoshinaga and co-workers, in which the internucleotide phosphodiester linkage was developed to attach the entire molecule to gold surfaces. Its corresponding complementary DNA sequences would bind on either side of this oligodeoxynucleotide phosphorothioate (s-oligo) DNA sequence (Fig. 2). The consequent electrochemical characterization indicated that the hybridization event had occurred between the probe DNA and its complementary sequence. This method made the immobilization of the DNA native sequences (rather than terminal modified ones) possible. Furthermore, this DNA sequence with an interior phosphodiester link offered a bimodal hybridization capability [32].

Another method of stabilizing DNA on the surface is the use of a bioaffinity interaction. A biofunctionalization strategy is aimed at achieving a superior surface bioactivity and tighter, higher specific binding than that provided by physical adsorption [31]. This improved functioning is mainly attributed to the presence of site-oriented biomolecules on the surface [35]. Escorihuela and co-workers reported the successful DNA patterning on a silicon surface through bioaffinity interaction combined with thiol-ene chemistry. Thiol-ene coupling is a methodology for covalently attaching thiol with alkenes under UV irradiation [36]. Biotin derivatives were introduced into the thiol-functionalized silicon slides for the thiol-ene coupling. Followed by the specific adsorption of streptavidin, 3'-Cy5, 5'-biotin oligonucleotide was

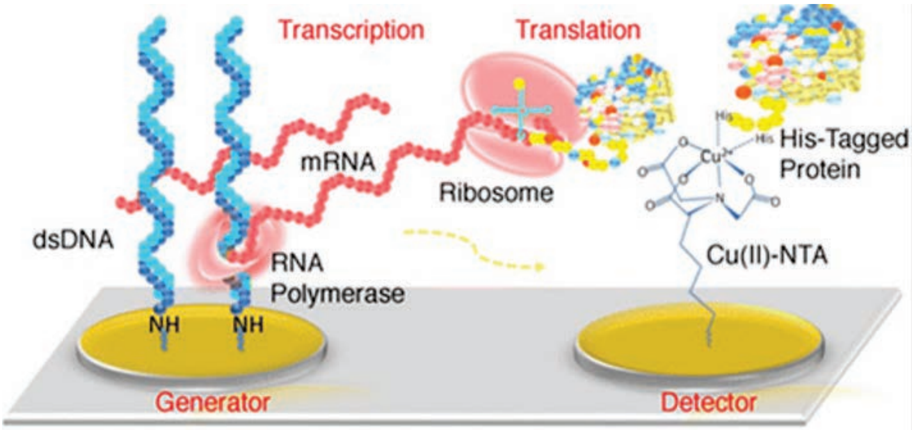

Figure 1. Schematic diagram of the on-chip synthesis of protein microarray from DNA microarray via surface *in vitro* transcription-translation. Reprinted with permission from [34]. Copyright 2012 American Chemical Society [34].

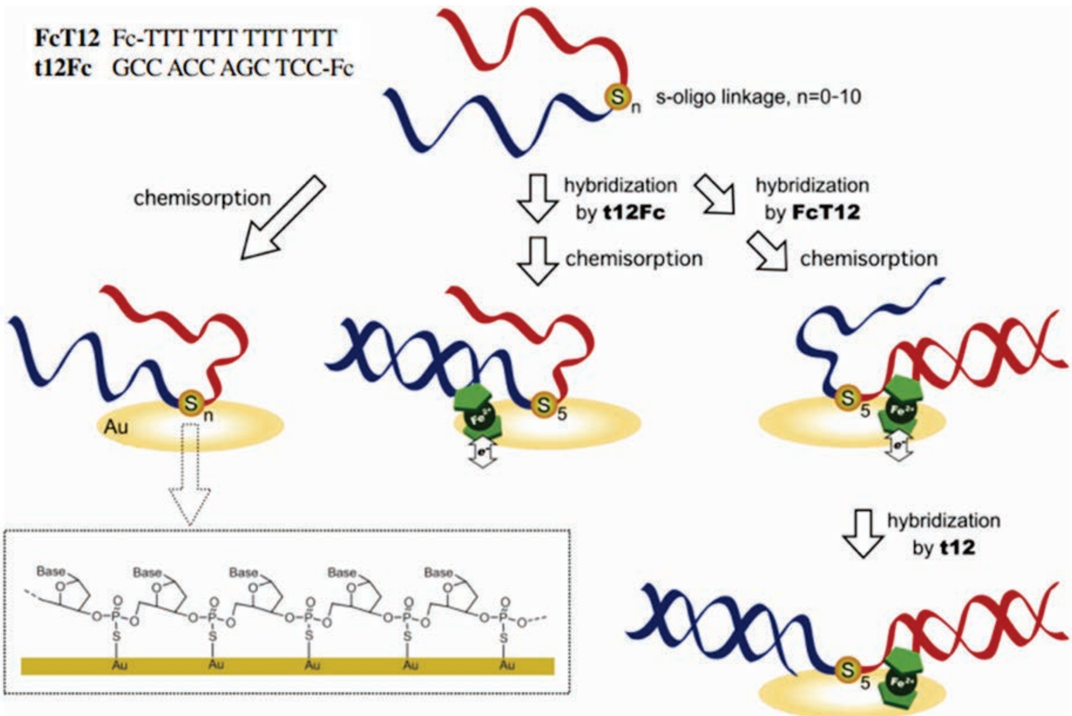

Figure 2. Schematic illustration of the covalent attachment of the model capture probe DNA molecule, oligodeoxynucleotide phosphorothioate (s-oligo), or its corresponding half-hybridized species formed with a ferrocene-modified complementary DNA. In the box is shown the expected surface structure of the s-oligo surface-adlayer. Reprinted with permission from [32]. Copyright 2012 Analytical Sciences [32].

successfully immobilized on the biotinylated surface (Fig. 3). The fluorescence intensity increase indicated that the biotin terminated DNA was selectively attached to the streptavidin through active sites [37].

Another attempt to position biotin-modified ssDNA wrapped single-walled carbon nanotubes (SWCNTs) on DNA self-assembled structures based on the use of streptavidin-biotin interaction was reported by Eskelinen and co-workers. In the study, the researchers assembled streptavidin onto the rectangular origami structures with biotin modified staple strands. Then, biotion-modified ssDNA was aligned on the origami structure through streptavidin-biotin interaction (Fig. 4). Since the SCNTs were wrapped with the ssDNA, this technique can facilitate the accurate positioning and alignment of SWCNTs. This simple and cost efficient method provided a potential possibility for assembling devices and circuits accurately on the nanoscale [38].

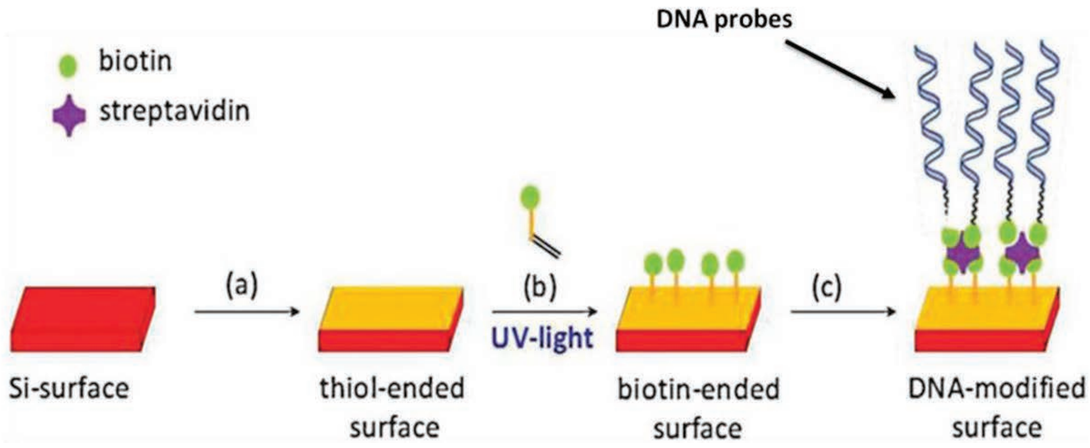

Figure 3. Functionalization of Si/SiO_2 surface through thiol-ene chemistry. Reprinted with permission from [37]. Copyright 2012 Royal Society of Chemistry [37].

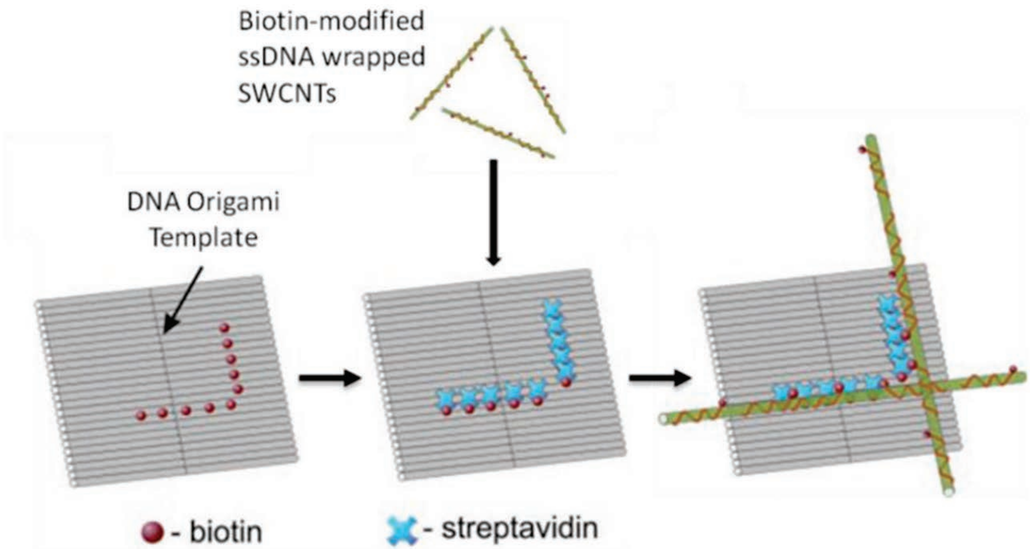

Figure 4. Schematic diagram of SWCNT assembly on DNA origami templates using streptavidin-biotin interaction. Reprinted with permission from [38]. Copyright 2011 WILEY-VCH [38].

3. DNA immobilization on different surfaces

The immobilization of DNA on different solid supports is an interesting research topic, as it can strongly influence the later quality of detection for a DNA based analytical device. Therefore, it is crucial to the development of DNA-based biosensors and other diagnostic techniques. Among the different materials, carbonaceous materials, gold, and silica are the most popular choices.

3.1. Carbonaceous materials

Carbonaceous materials are widely used for DNA immobilization due to their extraordinary electrochemical, physical and mechanical properties. Their commercial availability and compatibility with microchip fabrication technology also make them a strong candidate for DNA attachment. Moreover, their diversity of structural forms, such as that seen in graphite, graphene, and carbon nanotubes, provide more choices for DNA immobilization [18].

3.1.1. Graphite

As a layered material, graphite has very promising electrical properties at low cost, which give it strong potential in playing a vital role in electroanalytical applications. Therefore, the investigation of DNA-functionalized graphite has become an interesting research topic in materials science and engineering [39]. Ensafi's research group very recently reported a design

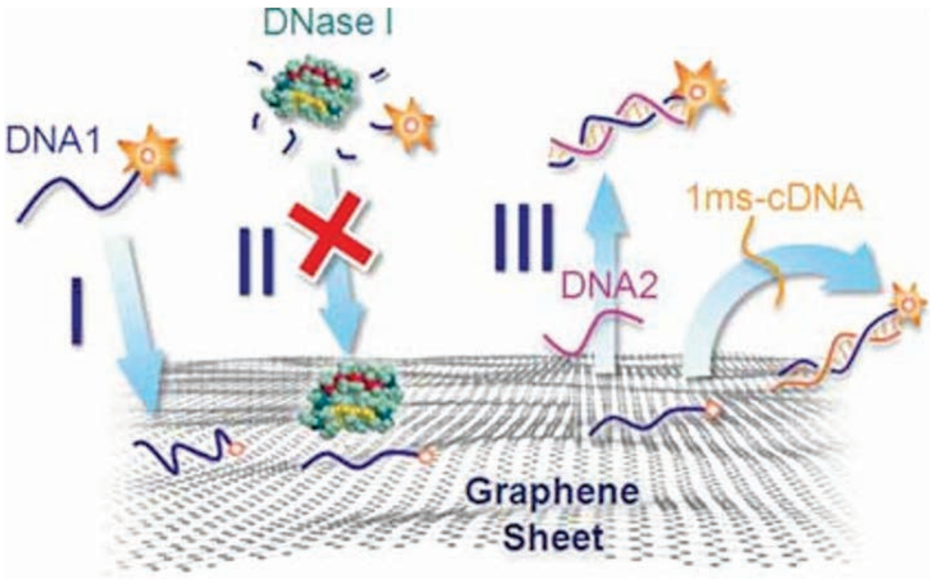

Figure 5. Schematic illustration of the constraint of DNA molecules on functionalized graphene and its effects. I) The single-stranded DNA can be effectively constrained on the surface of graphene via adsorption. II) DNAse I can digest free DNA but not graphene-bound DNA. III) The constrained DNA shows improved specificity response towards target sequences that can distinguish the complementary and single-mismatch targets. Reprinted with permission from [43]. Copyright 2010 WILEY-VCH [43].

strategy for the construction of a DNA-functionalized biosensor for riboflavin detection on pretreated graphite electrodes (a +1.4V potential was applied on a graphite electrode for 60s in a quiescent solution). In this study, the salmon sperm ds-DNA was firstly electrostatically adsorbed on the graphite surface through the negatively charged sugar-phosphate backbone. Then, electrochemical detection based on the interaction of riboflavin with ds-DNA resulting in the decrease in the intensity of the guanine and adenine oxidation signals. These intensity changes were used as indicator signals for the determination of riboflavin [40].

Another important advantage of graphite is its flat and oriented surface, which is suitable for performing studies using of high resolution techniques, such as atomic force microscopy (AFM) [18]. For example, in Boulanouar's study, DNA plasmids and 1,3-diaminopropane complex were precipitated onto the hydrophobic HOPG surface, forming a thin film suitable for quantitative low energy electron (LEE) irradiation studies. The thickness of this film was investigated and characterized by AFM [41].

3.1.2. Graphene

Graphene is a single-layer carbon crystal. Because of its exceptional electron/hole carrier mobility, remarkable mechanical strength, and biocompatibility, it recently became an important substrate for DNA immobilization and hybridization. A number of reports describing the incorporation of DNA and graphene in new hybrid materials have been published. One of the first studies exploring the interactions between DNA and graphene was reported by Tang and co-workers. They used a pre-treated graphene, sonicated with 25% nitric acid and 75% sulphuric acid for a better level of dispersibility in an aqueous solution, to interact with single-stranded DNA. In the solution, graphene was able to strongly adsorb single-stranded DNA (ssDNA) *via* a π-stacking interaction between the ring structures in the nucleobases and the hexagonal cells of grapheme [42]. The subsequent fluorescence, anisotropy, nuclear magnetic resonance (NMR), and circular dichroism (CD) characterization indicated that the molecular interactions between the functionalized graphene and single-stranded DNA effectively prevented enzymes from digesting the constrained DNA. Their further study indicated that the adsorbed single-stranded DNA can desorb from the graphene surface with its complementary sequence, leading to an enhancement of the fluorescence signal (Fig. 5). This feature showed its promising potential in biomedical and bioassay applications [43].

Compared to the physisorption of the single-stranded DNA, the formation of a covalent bond between probe DNA and graphene has also been recently developed. An approach was demonstrated in Dubuisson's work. The epitaxial graphene (EG) was anodized to form carboxyl groups, which acted as tethering groups for the covalent grafting of an amino group of modified ssDNA on its top. Then, methylene bule, an aromatic heterocycle, was used as an electrochemical intercalator to monitor the DNA hybridization reaction. It was noted that the anchoring of the DNA probe with covalent grafting can serve a larger dynamic range and a more sensitive response than the π-π stacked DNA probe for DNA detection. The direct voltammetric sensing of single-nucleotide mismatch also becomes possible on these DNA-modified anodized EG surfaces [44].

Despite a cascading expansion in the quantity of articles about DNA-graphene interaction, technical improvements are still required. One of the biggest challenges concerns the reaction mechanism between DNA and graphene, which should be addressed more clearly so that the hybrid materials can be used appropriately. Another challenge is to establish powerful procedures for these hybrid materials to be synthesized with reproducible and scalable properties [45].

3.1.3. Carbon nanotubes

Carbon nanotubes (CNTs) have recently emerged as one of the most extensively studied nanomaterials due to their excellent optical, electronic, thermal, and mechanical properties [46]. It has been noted that the attachment of DNA oligomers to single-walled carbon nanotubes (SWCNTs) can greatly enhance their structural functionality, making the CNT-DNA complexes strong candidates for biomedical applications. Their large surface areas, combined with their excellent charge-transport characteristics greatly promote electron transfer reactions, which dramatically improve electrochemical performance [18]. Molecular dynamics simulations have clearly illustrated that a single DNA molecule with sufficient length (>14 nucleotides) can wrap around a SWCNT within 20 ns spontaneously [46] and will be stabilized by significant numbers of non-Watson-Crick hydrogen bonds in addition to π-π stacking between DNA bases and nanotube surfaces and Watson-Crick pairs [47].

Following the same principle, an electrically conductive polyethylene oxide nanofiber web incorporating DNA-wrapped double-walled carbon nanotubes (DWNTs) was built. This work was described by Kim *et al*. High-purity DWNTs were prepared by catalytic CVD and then dispersed in an aqueous solution of DNA. DNA molecules were able to wrap around the sidewalls of the carbon nanotube. After a polyethylene oxide (PEO) solution was added to a DNA-dispersed DWNT solution, the blend was electrospun into nanofiber webs. The following electrical conductivity measurement by the four-probe method indicated that PEO/DNA/DWNT nanofiber web had a relatively high electrical conductivity. This result suggested that the DNA-wrapped nanotubes acted as electrical conductors within the PEO nanofiber web. Because PEO is highly biocompatible, this nanofiber web could be potentially useful for tissue engineering [48,49].

A more sophisticated way of covalently attaching a single-stranded DNA sequence to a carbon nanotube was demonstrated by Sorgenfrei and co-workers, in which the carboxyl defects on the carbon nanotubes were generated by applying a 30 mV bias in sulphuric acid. Then, an amine terminated probe DNA was covalently attached to the carboxyl defect on the nanotube. By recording the fluctuations in conductance of nanotube in the presence of a complementary DNA target (the conductance of a device with duplex DNA is lower than that of a device with unbounded probe DNA), the molecular kinetics of this single-molecule label-free bioanalytical system was studied at microsecond timescales (Fig. 6). In contrast to previously reported methods, this system is capable of probing single molecule behavior (*e.g*. binding kinetics) with fast time resolution. Moreover, the single molecule study for more intricate objects, such as protein folding and enzymatic activity can be obtained through this methodology [50].

Obviously, a carbon substrate can be considered as a promising alternative material for DNA sensors and chip construction. However, it also should be noted that the interaction between DNA and carbonaceous surfaces must be thoroughly understood before taking full advantage of the capabilities of immobilized DNA. To this end, base-base interaction of DNA molecules with highly ordered pyrolytic graphite (HOPG) was investigated via single molecule force spectroscopy (SMFS) for four homopolymer sequences (5'-poly(dT_{50}), 5'-poly(dT_{100}), 3'-poly(dA_{50}), and 5'-poly(dG_{100})). In the experiment, a gold-coated AFM probe was functionalized with thiol group modified ssDNA, and the final force jump in the retraction region of the force-distance curve originated from a single DNA molecule detaching from the graphite surface (Fig. 7). The forces required to detach four sequences from the graphite surface were measured, and the rank of the effective average binding energy per nucleotide for four homopolymer sequences to graphite was T ≥ A > G ≥ C. This experimental study helped to develop insights into interaction between DNA oligomers and SWCNTs (the graphite substrate was assumed to serve as an appropriate analogue for SWCNTs) [51]. However, based on Lee's work, the van der Waals (vdW) interactions between the DNA nucleobases and graphene followed an order of G > A > T > C. The binding energy strengths were obtained via vdW energy-corrected DFT calculation [26].

3.2. Gold surfaces

Gold is another promising substrate for DNA immobilization, because although gold is chemically

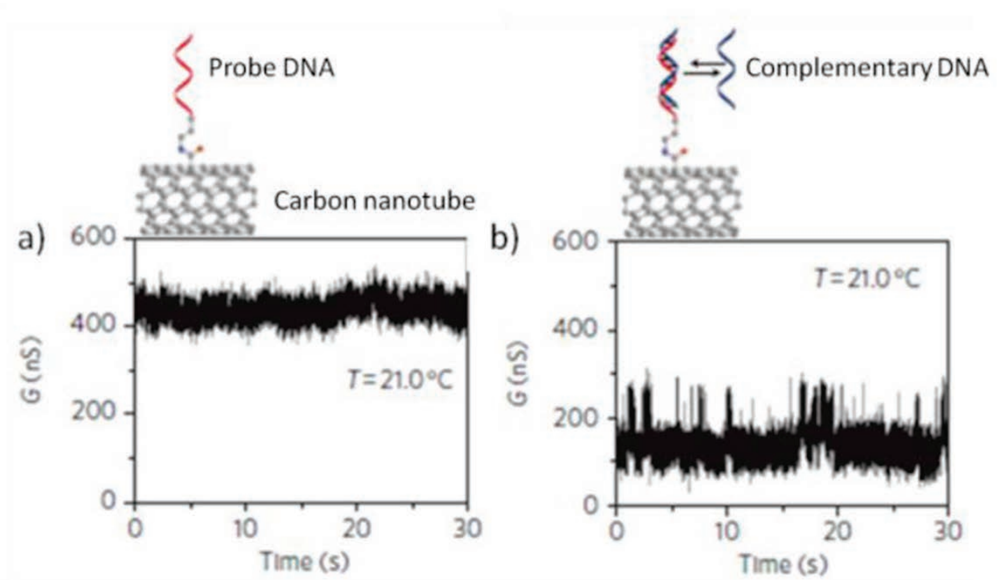

Figure 6. Conductance recordings of device over one 30s interval with DNA oligonucleotide probe NH_2-5'-GGAAAAAAGG-3'(A6) without and after exposure to the complementary DNA target. Reprinted with permission from [50]. Copyright 2011 Nature Publishing Group [50].

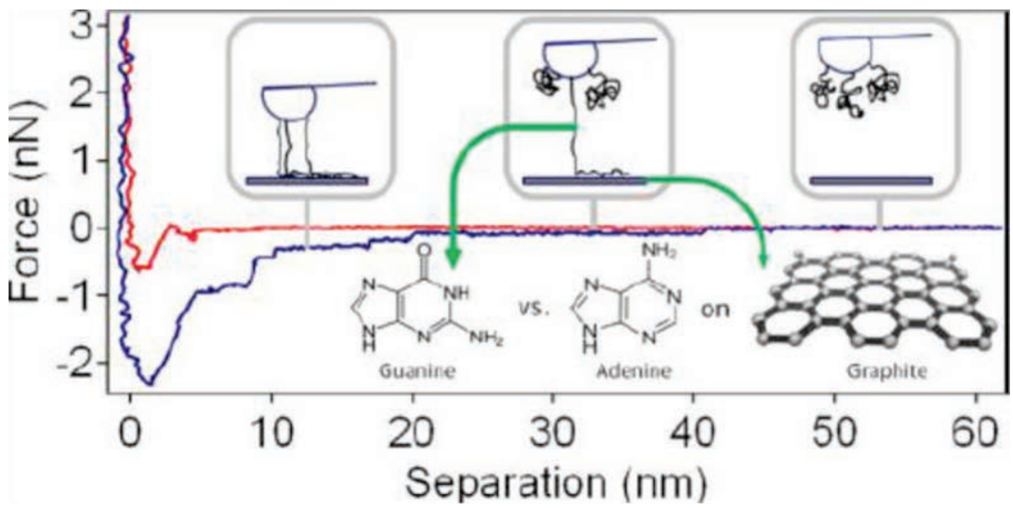

Figure 7. Cartoon (not drawn to scale) of frictionless peeling of an ssDNA homopolymer attached to a gold-coated force probe, from a graphite surface, with a typical force-distance curve for peeling ssDNA from the surface of graphite obtained at a tip velocity of 200 nm/s. Red curve is approach, blue is retraction. Reprinted with permission from [26]. Copyright 2013 American Chemical Society [26].

inert, DNA can be conveniently bonded to it *via* thiol group [52]. Moreover, DNA arrays on the gold substrate are compatible with a wide variety of detection techniques, including fluorescence, surface plasmon resonance, electrochemical methods, and matrix-assisted laser desorption/ionization mass spectrometry (MALDIMS). This means that gold substrates can provide more choices for the signal monitoring and analysis [53].

Recently, several very interesting applications of DNA gold hybrid structures have been published. For example, a functionalization of DNA tweezer with Au nanoparticles was presented by Shimron *et al.* This can be programmed and controlled, based upon binding and unbinding with fuels. In particular, the researchers demonstrated that fluorophores associated with this DNA tweezer exhibit interesting and unique photophysical properties—the relative position of fluorophore to the gold nanoparticle varies the fluorescence intensity. Although the reversible fluorescence quenching and enhancement could be observed upon the closure and opening of the tweezers (Fig. 8), this technique has its limitations in synthesizing and purifying single nucleic acid modified Au NPs with certain difficulties. Additionally, the excitation enhancement or scattering

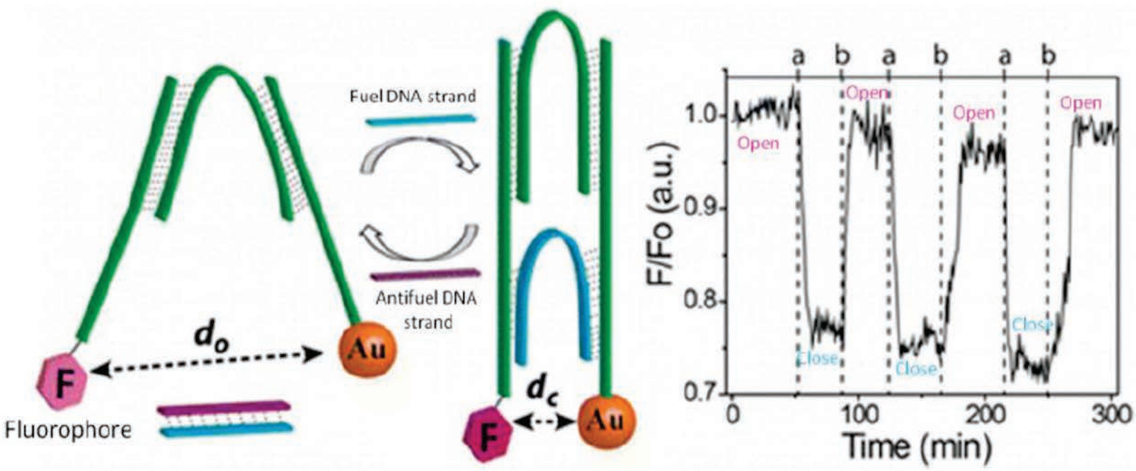

Figure 8. Mechanical control of the fluorescence properties of different fluorophore/Au NP-functionalized tweezers through the cyclic opening and closure of the tweezers by means of fuel/antifuel DNA strands. Reprinted with permission from [54]. Copyright 2013 American Chemical Society [54].

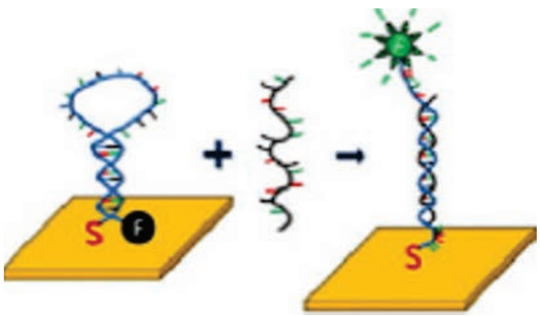

Figure 9. Schematic diagram of DNA hairpin immobilization and hybridization. Reprinted with permission from [55]. Copyright 2011 Elsevier [55].

phenomena can also contribute to the fluorescence enhancement [54].

In Huang's work, DNA hairpins with stem and loop structures were immobilized on the gold surface via thiol-Au bonds (see Fig. 9). Their study suggested that this stem-loop structure provides an increase in specificity of probe hybridization. Upon probe-target hybridization, the conformation of the probe was altered, resulting in an extension of the folded hairpin into a linear duplex. In response to the extension, the fluorophore was detached from the Au surface, which led to an increase of the fluorescent signal. Compared to the conventional hairpin probes with chemical quenchers, the fluorescence enhancement after hybridization in this hairpin immobilization technique was much higher. This was because gold was an active quencher, which may provide a more efficient quenching—the energy was transferred to the gold surface directly. The effect of the distance of the fluorophor to the gold surface was thoroughly investigated in this study [55].

Although gold is widely used as a substrate for the site-specific immobilization of DNA molecules, it has been proven that DNA can be adsorbed onto the Au via not only the Au-S bond, but also through Au-N interaction nonspecifically. Therefore, the critical challenges are preventing interstrand entanglement of DNA molecules on the gold surface and increasing the orderliness of DNA layers [17]. The physical and chemical properties of DNA microarrays are highly dependent on the roughness of the substrate. However, to achieve an atomically smooth Au substrate remains a challenge [56].

3.3. Silica and silicon surfaces

Silicon is also widely used as an alternative substrate in DNA immobilization, because of the ease and speed of fabrication, its excellent optical and morphological properties, and its versatile surface chemistries. An additional advantage of using silicon as a substrate for DNA immobilization is that it allows researchers to take full advantage of existing microelectronics technologies associated with the silicon semiconductor industry [57]. Generally, the routine for linking DNA onto silica surfaces involves fuctionalizing silica substrates with silane molecules followed by further covalent coupling of DNA molecules. An example of a Si substrate used in the DNA immobilization is shown in Fig. 10. DNA arrays were patterned in silicon-based self-assembled polymer brush layers. This flexible spacer provided a biologically simulated environment for the bioactivity maintenance of the attached biomolecules. In this report, poly (acrylic acid) (PAA) patterns were first generated by photolithography. Then, the amine-modified

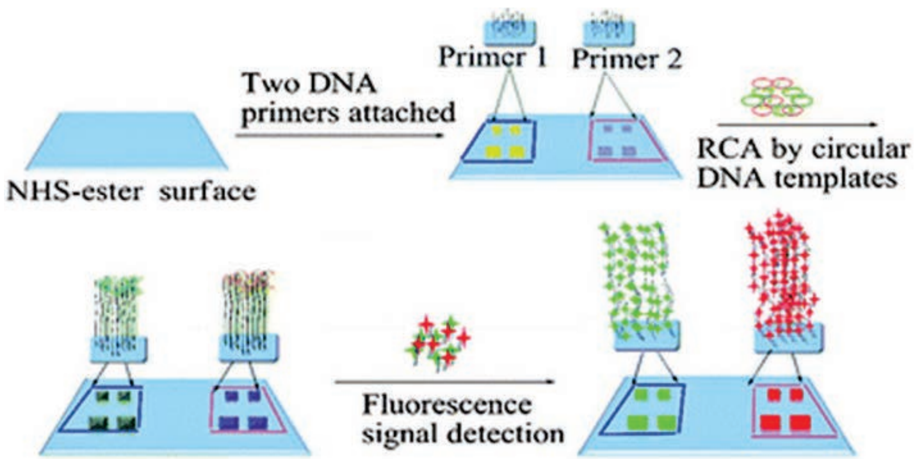

Figure 10. Preparation of a two-color DNA microarray. Reprinted with permission from [57]. Copyright 2012 Royal Society of Chemistry [57].

oligonucleotide sequences were linked to the polymer brushes for the synthesis of long ssDNA through rolling circle amplification (RCA). These DNA microarrays complemented the Cy3-labelled oligonucleotide probes, generating a fluorescence signal. Furthermore, by activating the PAA brushes via EDC-NHS to NHS-ester surface, a two-color microarray was fabricated [57].

As a parallel and low-cost process, Lv and co-workers developed a novel porous silicon (PSi) polybasic structure, following the different refractive index PSi photonic crystal layers with a symmetrical structure for antifreeze protein gene detection. The optical properties of this PSi photonic crystal structure exhibit a sharp resonance in the reflectance spectrum, giving a greater sensitivity for sensing performance under the reflectance spectrum. The symmetrical PSi photonic crystal layers were formed using electrochemical etching by alternating the anodization current with different etching times. The freshly etched surface was oxidized by H_2O_2 and followed with silanization by aminopropyltriethoxysilane (APTES) and glutaraldehyde treatment for the probe DNA immobilization. The reflectance spectra shift with complementary DNA demonstrates the effectiveness for DNA hybridization detection of these PSi multilayered films with polybasic symmetrical structure [58].

Although silicon is an attractive substrate for DNA immobilization, it can not always generate enough sum frequency generation (SFG) signal to allow for the observation of hybridization and dehybridization in situ, which limits its application to a certain degree [59]. In summary, the selection of the substrate for DNA immobilization depends on the intended application. For instance, silica surfaces are the primary choice for optical sensors, while carboneous materials are preferred for electrochemical detection due to their excellent electrical properties.

4. Super-DNA molecules (DNA-origami) surface immobilization

Since it was first proposed by Seeman in 1982 [3], DNA origami (DO) has became a very powerful approach for the design and construction of nanometer-sized DNA objects. Since the DO structure can be a scaffold for the subsequent construction of functional materials, the ability to precisely position DO on functionalized substrates has emerged as a challenge.

Progress has been reported with the site-specific attachment of DO structures onto substrate. In 2011, Yun *et al*. used graphene oxide (GO) and nitrogen-doped reduced graphene oxide (NrGO) for the selective nanopatterning of DO structures. In their strategy, spin-coated GO films are lithographically patterned, followed by further reduction or N doping modification. Their work demonstrated that DO structures were preferably adsorbed onto GO and NrGO with a high yield, while they were barely adsorbed onto the reduced graphene oxide (rGO) or graphene surface (Fig. 11). The explanation is that the negatively charged DNA strands were attracted by Mg^{2+} cations, which more easily interact with GO and NrGO surfaces due to either the negatively charged carboxylates group or the attraction from the lone-pair electrons of the nitrogen atoms [60].

In another example, Ding and coworkers integrated the bottom-up self-assembly of DO with the top-down lithographic methods for the surface patterning. To achieve site-specific attachment of DO, surface patterned gold islands (tens of nanometers in diameter) were first fabricated using electron beam lithography (EBL). Then, the fixed-length DO nanotubes with modified multiple thiol groups located at both ends were interconnected with the discreted gold islands on the substrate when the interisland distance matched the

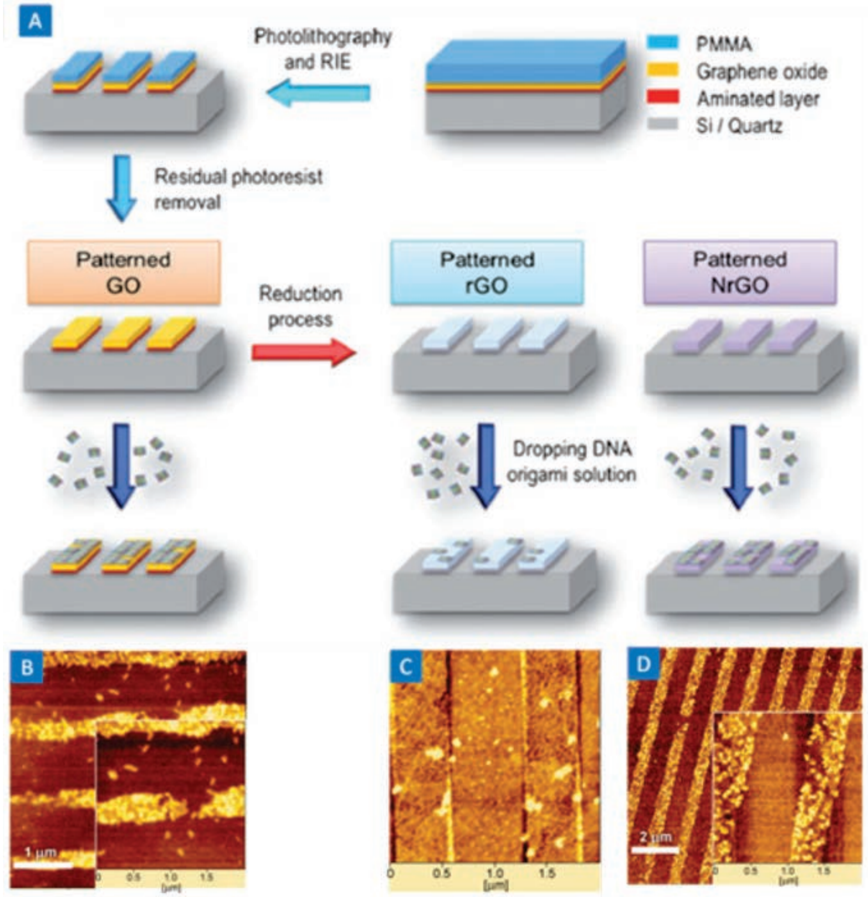

Figure 11. (A) Patterning of DNA origami structures on graphene-based substrates. Spin-cast GO films are lithographically patterned and chemically modified by reduction or N doping. DNA origami structures were assembled on patterned graphene-based films from buffer solution (RIE=reactive ion etching). AFM images of DNA origami structures assembled on the patterned B) GO, C) rGO, and D) NrGO, respectively. Reprinted with permission from [60]. Copyright 2011 WILEY-VCH [60].

length of the thiolated tube structure (Fig. 12). Their further studies indicated that the binding efficiency of DO nanotubes could be affected by incubation time, the number of thiol groups, the size of the gold islands, and the DO nanotube concentration. This interconnection strategy may lead to novel methods for fabrication of electronic devices [61].

The Tinnefeld group also studied the specific immobilization of DO on surfaces. A pillar-shaped DO was folded from an 8634-nucleotides-long scaffold strand and 199 short staple strands. By changing the quantity and position of the biotin-modified staple strands, the DNA origami nanopillars were able to specifically bind, vertically or horizontally, onto the BSA-biotin-coated coverslip through the mediation of neutravidin (Fig. 13). Since the fluorescent dye was also attached to either the top or bottom of the DNA origami nanopillars, the structure and orientation of these DNA origami pillars can be determined and visualized by three-dimensional fluorescence super-resolution microscopy [62].

The overall size, as large as several hundred nanometers, allows DO to be an ideal module for the higher-level functional structure construction. The majority of recent reported research focused on the decoration of DO with various types of functional molecules, while a lesser amount dealt with the methods for the attachment of DO with site-specific and covalent association on the surface. Since nanometer precision is significant for the industrial production of functional materials and devices, the study of the site-specific attachment of DO on surfaces would be an excellent direction for future research.

5. From immobilization to functionalisation

DNA immobilization provides the opportunity to construct DNA biosensors for human health and safety [63]. The basis for various DNA probe techniques is that single strand DNA is immobilized by attachment

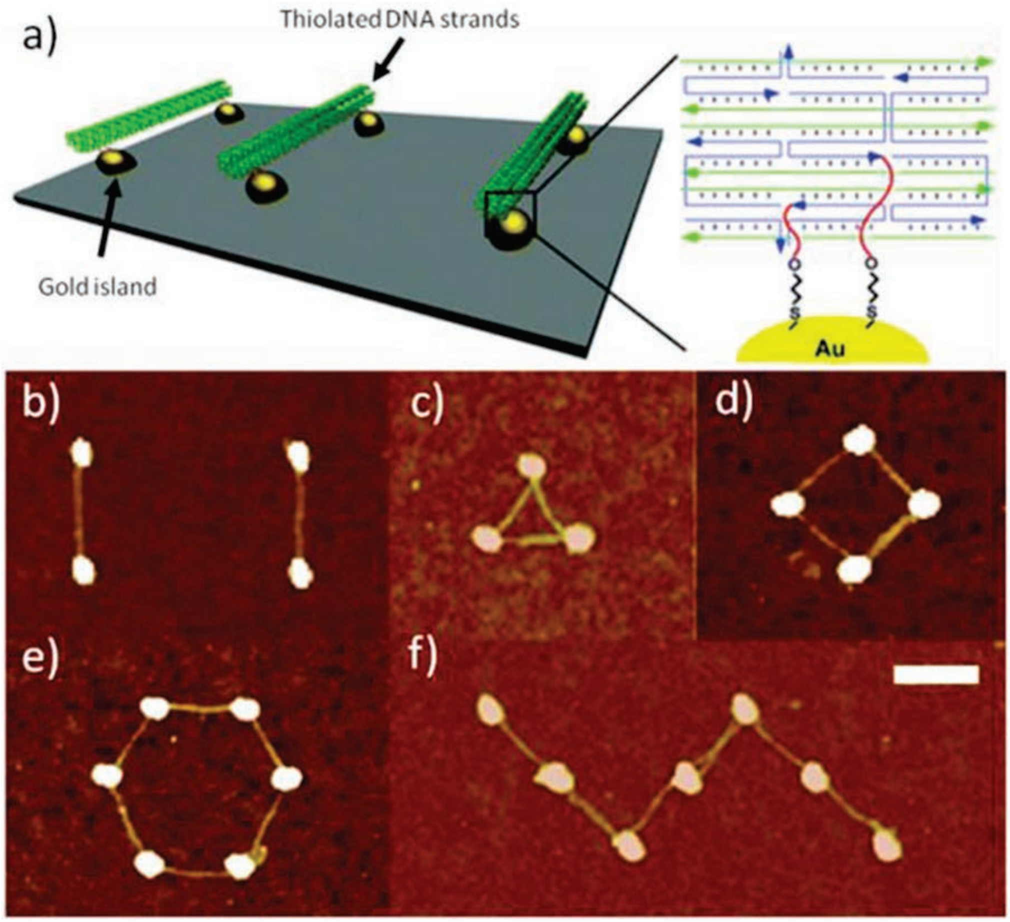

Figure 12. (a) Schematic drawing of gold islands connected by DNA origami tubes on the substrate surface. Various structures were formed by connecting gold islands with DNA origami tubes: (b) straight line, (c) triangle, (d) square, (e) hexagon, (f) “z” shape. The scale bars is 300 nm. Reprinted with permission from [61]. Copyright 2010 American Chemical Society [61].

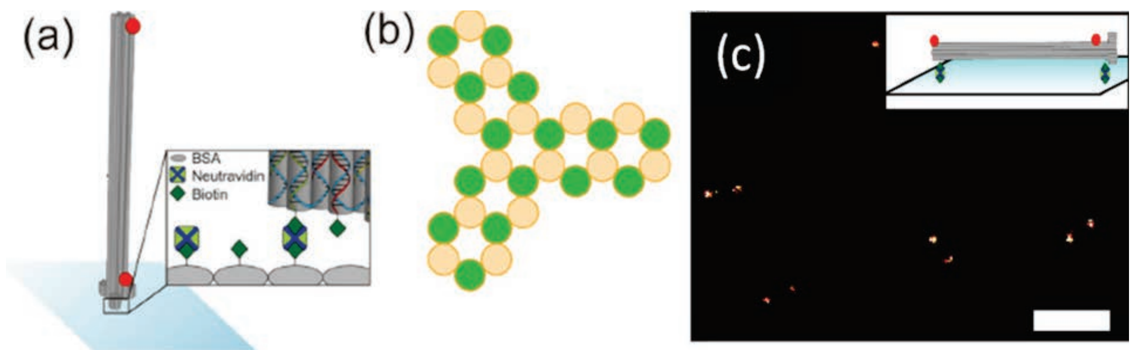

Figure 13. (a) Sketch of the DNA origami nanopillar with Alexa647 dye (shown in red). (b) Cross section of helices for the base of the nanopillar. Biotin-labeled helices are shown in green. Reprinted with permission from [62]. Copyright 2013 American Chemical Society [62].

to a solid support; its complementary strand will be hybridized and retained by the immobilized DNA [64]. The traditional method of DNA detection is *via* the fluorescence technique, in which single-stranded (ss) DNA probes are first stabilized on the transducer surface with a defined orientation. The following association of an appropriate complementary sequence with labeled fluorophore is detected by a fluorescence detector. Although labeling can enhance the sensor’s sensitivity, it increases the time, complexity, and cost of the measurement. Moreover, it might affect the bioaffinity of the probe DNA [65]. Therefore, a direct detection assay,

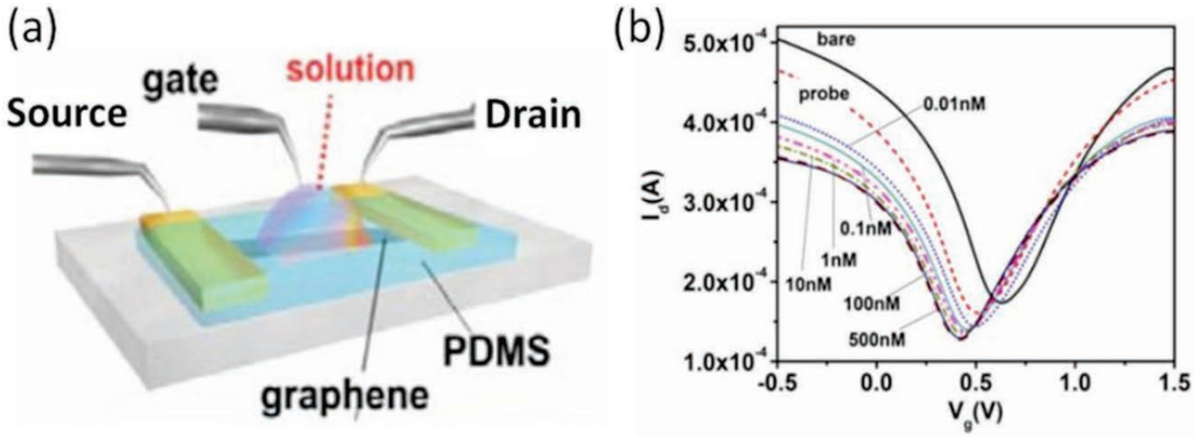

Figure 14. (a) Schematic illustration of the graphene device operated by liquid gating. (b) Transfer curves for the graphene transistors before adding DNA, after immobilization with probe DNA, and after reaction with complementary DNA molecules with the concentration ranging from 0.01 to 500 nM. Reprinted with permission from [71]. Copyright 2010 WILEY-VCH [71].

a label-free DNA biosensor array, is currently attracting much more interest [66]. Generally, the signal in a label-free approach mainly comes from the changes in the electrical properties of unlabelled DNA sequences [67]. Since the first electrochemical DNA sensor was reported by Millan and Mikkelsen [68], DNA electrical detection has received a great deal of attention, as it allows label-free, sensitive, and rapid measurement. The 2010 paper by Dong and co-workers reported an approach for DNA hybridization detection using a graphene-based Field-effect transistor (FET). FET is a transistor in which the drain and source current can be selectively and controllably varied by controlling the gate voltage[69]. Because the drain current I_d is sensitive to the interfacial potential, it can be used in DNA detection (the adsorbed DNAs carry highly negative charges, which come from each phosphate group, at neutral pH) [70]. In their design, probe DNAs first saturated the graphene surface (Fig. 14). Because the limited space restricts the complementary DNAs interaction with graphene directly during hybridization, the shift of gate voltage (Vg) that gives the minimum graphene conductance from the transfer curve becomes a good indicator for DNA hybridization and detection of single-base mutation. In their further experiment, AuNPs, which covalently bind with thiolated DNA molecules, were decorated on the graphene surface for DNA hybridization detection. It was found that this improvement is capable of extending the upper limit of DNA detection from 10 to 500 nM, with detection sensitivity of 0.01 nM target DNA and capability to distinguish single-base mismatch [71].

Following the construction of a DNA sensor based on a two-dimensional graphene sheet, Kergoat and co-workers published a report of a DNA sensor based on a water-gated organic FET. Compared to the inorganic FET, the organic FET is more easily produced at a low cost. In order to address the issue of ions affecting the sensitivity of the transistors for DNA detection, the deionized water was used as a gate dielectric in the experiment. In the study, Poly [3-(5-carboxypentyl) thiophene-2,5-diyl] (P3PT-COOH) was first coated onto the SiO_2 substrate through spin coating, which acted as the transistor channel material [72]. Then, DNA probes were covalently grafted on the substrate *via* NHS-EDC coupling chemistry. The horizontal shift of the transfer curve after DNA probe immobilization and complementary DNA target hybridization in deionized water indicates a successful application of organic FET in DNA detection [73].

Lin and co-workers described a more compact design for label-free DNA sensing. An organic electrochemical transistor (OECT) was integrated in a flexible microfluidic system for the first time. In the study, ssDNA probes were immobilized on the surface of an Au gate electrode through a thiol group. The modulation of the surface potential of the gate electrode caused by the immobilization and the hybridization of DNA molecules is attributed to the mechanism of this DNA sensor. By applying an electric field pulse to the gate electrode, the detection limit of the DNA sensor was extended from 1 nM to 10 pM. It is believed that the electric field reduces steric barriers through a reorientation of DNA molecules, which speeds the hybridization dramatically. A schematic representation of the system is shown in Fig. 15 [74]. Following the same design principle, a single-walled carbon nanotube (SWNT) network was used as a channel of FET for the detection of DNA molecules. The study illustrated that the sensitivity of this SWNT network-based FET depends on the ratio of metallic (M-SWNTs) to semiconducting (S-SWNTs)

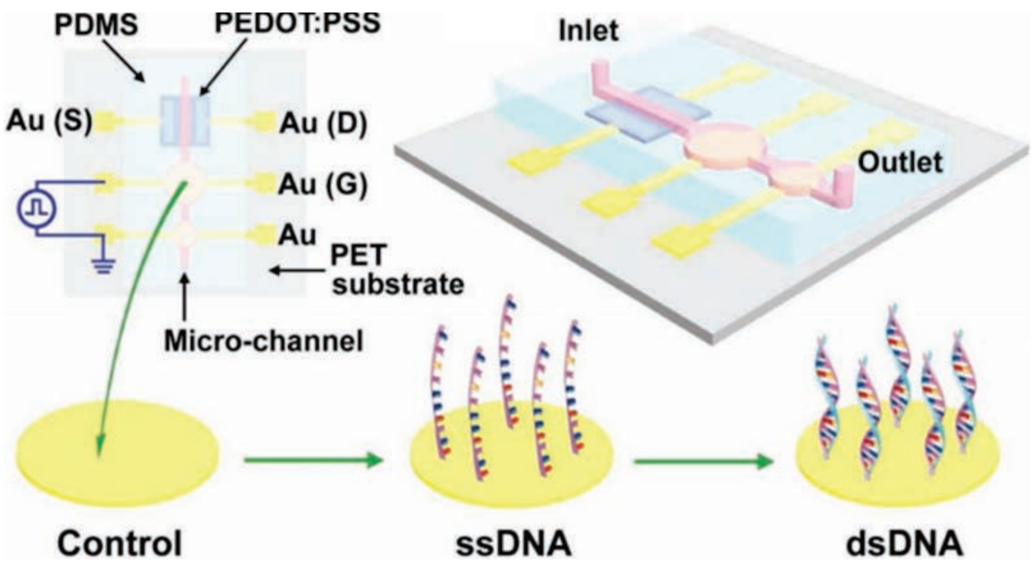

Figure 15. Schematic diagram of an OECT integrated in a flexible microfluidic system. Reprinted with permission from [74]. Copyright 2011 WILEY-VCH [74].

nanotubes and the tube density in the networks (a high content of S-SWNTs or low network density would enhance the device's sensitivity). The highest level of the detection capability of this SWNT network-based FET can reach down to 0.1 fM (~100 DNA molecules) [75].

Obviously, the target molecules for the DNA hybrid materials are not simply limited to its complementary DNA; other large molecules, like proteins, can also specifically bind with probe DNA on the substrate, because the commonly used nucleic acids can be randomly coded for binding to the protein target specificity. Lately, the study of DNA-protein interaction has attracted a great deal of attention [76,77].

One example of a protein-DNA recognition system was reported by Furukawa and coworkers. In their design strategy, the graphene oxide (GO) surface was modified by pyrene, which acted as a link to the sp^2 domain existing on the GO surface. Later, an amino group of terminated oligonucleotides with a thrombin aptamer sequence, which is a single strand DNA that can bind to specific target molecules, was immobilized on the pyrene-modified GO surface. Since GO is known as an effective fluorescence quencher, the dye probe fluorescence, which initially connected with oligonucleotides, was quenched by GO. After thrombin, which is a serine protease and an important protein for blood clotting, was added into system, the protein adsorption and recognition by thrombin aptamer results in recovery of the fluorescence intensity (Fig. 16). A confocal laser scanning microscope and an atomic force microscope were used for the observation, and it was claimed that 10 μg mL^{-1} of thrombin in the solution can be detected successfully upon using this GO system. Since the corresponding aptamer sequence is versatile, based on variable biological molecules, this approach is promising for multiple biosensing applications [78]. In a label-free approach, Pillet and co-workers functionalized an amino-gold surface with phosphorus dendrimers. This sensing surface was then covalently bonded with amino-modified oligonucleotides, which contain a single *sop*C binding site. Followed by SopB protein deposition, the SopB-*sop*C interaction was visualized through surface plasmon resonance imaging (SPRi) under optimized conditions, which can not be discriminated on an untreated-gold surface [79].

Besides biomolecule analysis, DNA sensors can also allow the detection of other biological objects, because molecules and ions can interact with DNA, causing changes in the structure of DNA and the base sequence. Electrostatic, groove-binding, and intercalation are the three significant types of interaction between DNA and molecules/ions [80]. The response of DNA-carbon nanotube sensor arrays to vapor compounds was recently demonstrated by Kybert and coworkers, who functionalized CNTs-FET arrays with ssDNA by incubation of the devices in droplets of DNA solution for 30 min. These DNA physisorbed CNT sensors are able to clearly discriminate analytes with highly similar molecular structures (see Fig. 17). Since individual DNA sequences have differential responses to various analytes, this technique could potentially be incorporated into an electronic nose system [81].

The application of DNA biosensors in food safety examination has also been developed. Of great significance, Ensafi and co-worker first reported the

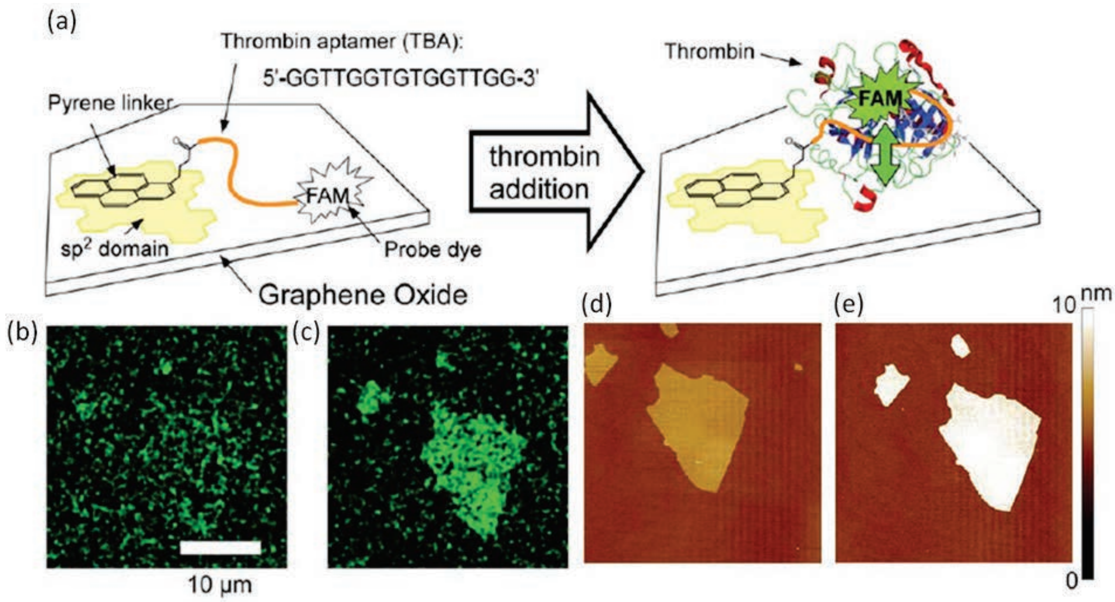

Figure 16. (a) Schematic illustration of the modified GO surface and the principle of thrombin detection. (b) before thrombin addition. (c) 80 s after thrombin addition. (d) and (e) are the AFM topographies of the GO piece in (b) and (c), respectively. Reprinted with permission from [78]. Copyright 2013 Royal Society of Chemistry [78].

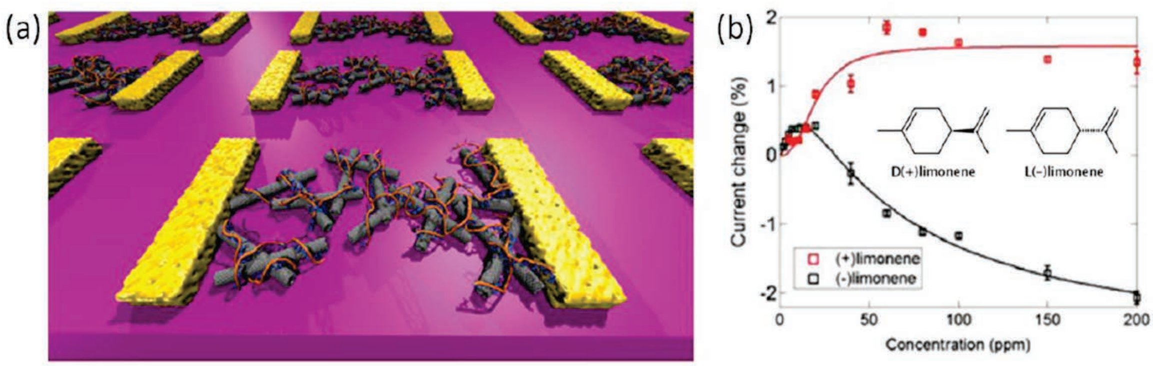

Figure 17. (a) Device schematic. (b) DNA-CNT devices clearly distinguish limonene enantiomers. Reprinted with permission from [81]. Copyright 2013 American Chemical Society [81].

approach for electrochemical determination of Sudan II, which has been assessed as a group 3 geologic carcinogen by the World Health Organization [82], based on its interactions with ds-DNA-modified pencil graphite electrode (PGE). The Sudan II concentration was quantitatively recorded through differential pulse voltammetry (DPV) based upon the differences of guanine and adenine oxidation signals. This approach was further applied to analysis of real samples (chili and ketchup sauce). Compared with other analytical methods, such as HPLC/MS and HPLC/UV, this DNA biosensor is simple and inexpensive. Moreover, it has an extremely low limit of detection (0.4 ug mL^{-1}) for Sudan II determination [83].

6. Conclusion and perspective

Despite the fact that a vast number of novel materials and applications related to DNA immobilization are available, and DNA nanostructure-based electronics can allow analysis to be reliably performed [84,85], offering advantages compared to conventional identification procedures that are tedious, expensive, and time consuming, there remains a need for technical improvement. One of the challenges in using the nucleic acid films on solid supports is the loss of stability under aggressive conditions, such as high temperatures or the requirement for long-term storage [7], Some studies are focusing on addressing these issues. Civit and co-workers reported that diazonium salts with one and two diazo-groups were thermally stable up to significantly higher temperatures (95°C) than alkanethiol SAMs on gold surfaces [86], Phares and co-workers [87] demonstrated that a flexible trihexylthiol anchor was able to significantly improve the solution-phase storage stability and thermostability, due to the formation of the three thiol-gold bonds.

It must be emphasized that most of the reported DNA sensors use synthetic short oligonucleotides as the model targets. Problems might arise when dealing with real samples as a result of the huge steric hindrance encountered by very large targets (a thousand to several hundred thousand base pairs).

Additional approaches remain to be developed, including multiplex analysis of the aptamer and the amplified detection of the DNA targets [88]; further understanding of the structure and dynamics of DNA immobilized on the surfaces, as well as the processes and interactions involved [89]; and optimization of the approaches/parameters for DNA immobilization to provide an "industry standard" for the future (*e.g.* precisely controlling the DNA density on a chip) [18,90].

References

[1] D.J. Graves, Trends Biotechnol. 17, 127 (1999)
[2] M. Schena, R.A. Heller, T.P. Theriault, K. Konrad, E. Lachenmeier, R.W. Davis, Trends Biotechnol. 16, 301 (1998)
[3] N.C. Seeman, J. Theor. Biol. 99, 237 (1982)
[4] N.C. Seeman, Nature 421, 427 (2003)
[5] V.Z. Singh, M. Zharnikov, A. Gulinoc, T. Gupta, J. Mater. Chem. 21, 10602 (2011)
[6] B. Sacca, C.M. Niemeyer, Angew Chem. Int. Ed. Engl. 51, 58 (2012)
[7] D. Ge, X. Wang, K. Williams, R. Levicky, Langmuir 28, 8446 (2012)
[8] A.H. Wu, J.J. Sun, R.J. Zheng, H.H. Yang, G.N. Chen, Talanta 81, 934 (2010)
[9] A. Bonanni, M. del Valle, Anal. Chim. Acta 678, 7 (2010)
[10] F. Yan, H. Tang, Expert review of molecular diagnostics 10, 547 (2010)
[11] X.C. Dong, Y.M. Shi, W. Huang, P. Chen, L.J. Li, Adv. Mater. 22, 1649 (2010)
[12] N.J. Kybert, M.B. Lerner, J.S. Yodh, G. Preti, A.T.C. Johnson, Acs Nano 7, 2800 (2013)
[13] A.A. Ensafi, B. Rezaei, M. Amini, E. Heydari-Bafrooei, Talanta 88, 244 (2012)
[14] F. Lagarde, N. Jaffrezic-Renault, In: V. Somerse (Ed.), Environmental Biosensors (InTech, China, 2011)
[15] W. Wang, Y. Wang, L. Tu, T. Klein, Y.L. Feng, J.P. Wang, IEEE Transactions Magn. 49, 296 (2013)
[16] F.R.R. Teles, L.R. Fonseca, Talanta 77, 606 (2008).
[17] H. Pei, X.L. Zuo, D. Pan, J.Y. Shi, Q. Huang, C.H. Fan, NPG Asia Materials 5, 1 (2013)
[18] C. Wittmann, S. Alegret, Immobilisation of DNA on chips (Springer, Berlin, New York, 2005)
[19] D.S. Dandy, P. Wu, D.W. Grainger, Proc. Natl. Acad. Sci. USA 104, 8223 (2007)
[20] M. Glazer, J.A. Fidanza, G.H. McGall, M.O. Trulson, J.E. Forman, A. Suseno, C.W. Frank, Anal. Biochem. 358, 225 (2006)
[21] J.B. Fiche, A. Buhot, R. Calemczuk, T. Livache, Biophys. J. 92, 935 (2007)
[22] J.H. Watterson, P.A.E. Piunno, C.C. Wust, U.J. Krull, Langmuir 16, 4984 (2000)
[23] M.S. Shchepinov, S.C. CaseGreen, E.M. Southern, Nucleic Acids Res. 25, 1155 (1997)
[24] E.L.S. Wong, E. Chow, J.J. Gooding, Langmuir 21, 6957 (2005)
[25] P. Gong, C.Y. Lee, L.J. Gamble, D.G. Castner, D.W. Grainger, Anal. Chem. 78, 3326 (2006)
[26] J.H.C. Lee, Y.K. Choi, H.J. Kim, R.H. Scheicher, J.H. Cho, J. Phys.Chem. C 117, 13435 (2013)
[27] M. Malmqvist, Nature 361, 186 (1993)
[28] H.O. Ham, Z. Liu, K.H. Lau, H. Lee, P.B. Messersmith, Angew Chem. Int. Ed. Engl. 50, 732 (2011)
[29] H.T. Ngo, H.N. Wang, A.M. Fales, T. Vo-Dinh, Anal. Chem. 85, 6378 (2013)
[30] M. Yousef Elahi, S.Z. Bathaie, S.H. Kazemi, M.F. Mousavi, Anal. Biochem. 411, 176 (2011)
[31] H.K. Tran, K.L. Killops, L.M. Campos, Soft Matter. 9, 6578 (2013)
[32] H. Yoshinaga, K. Nakano, N. Soh, R. Ishimatsu, T. Imato, Anal. Sci. 28, 1059 (2012)
[33] L.K. Gifford, I.E. Sendroiu, R.M. Corn, A. Luptak, J. Am. Chem. Soc. 132, 9265 (2010)
[34] T.H. Seefeld, A.R. Halpern, R.M. Corn, J. Am. Chem. Soc. 134, 12358 (2012)
[35] L.E. Valenti, E. Herrera, M.F. Stragliotto, V.L. Martins, R.M. Torresi, C.E. Giacomelli, ACS Symposium Series (Oxford University Press, American Chemical Society, Washington, DC, 2012)
[36] C.E. Hoyle, C.N. Bowman, Angew Chem. Int. Ed. Engl. 49, 1540 (2010)
[37] J. Escorihuela, M.J. Banuls, R. Puchades, A. Maquieira, Chem. Commun. (Camb) 48, 2116

(2012)
[38] A.P. Eskelinen, A. Kuzyk, T.K. Kaltiaisenaho, M.Y. Timmermans, A.G. Nasibulin, E.I. Kauppinen, P. Torma, Small 7, 746 (2011)
[39] S. Manohar, A.R. Mantz, K.E. Bancroft, C.Y. Hui, A. Jagota, D.V. Vezenov, Nano Lett. 8, 4365 (2008)
[40] A.A. Ensafi, E. Heydari-Bafrooei, M. Amini, Biosens. Bioelectron. 31, 376 (2012)
[41] O. Boulanouar, M. Fromm, A.D. Bass, P. Cloutier, L. Sanche, J. Chem. Phys. 139, 055104 (2013)
[42] X. Tan, T. Chen, X. Xiong, Y. Mao, G. Zhu, E. Yasun, C. Li, Z. Zhu, W. Tan, Anal. Chem. 84, 8622 (2012)
[43] Z. Tang, H. Wu, J.R. Cort, G.W. Buchko, Y. Zhang, Y. Shao, I.A. Aksay, J. Liu, Y. Lin, Small 6, 1205 (2010)
[44] E. Dubuisson, Z. Yang, K.P. Loh, Anal. Chem. 83, 2452 (2011)
[45] T.G. Premkumara, K.E. Geckeler, Progress in Polymer Science 37, 515 (2012)
[46] D. Roxbury, J. Mittal, A. Jagota, Nano Lett. 12, 1464 (2012)
[47] D. Roxbury, A. Jagota, J. Mittal, J. Phys. Chem. B 117, 132 (2013)
[48] J.H. Kim, M. Kataoka, Y.C. Jung, Y.I. Ko, K. Fujisawa, T. Hayashi, Y.A. Kim, M. Endo, ACS Appl. Mater. Interfaces 5, 4150 (2013)
[49] D.P. Kai, M.P. Prabhakaran, G. Jin, S. Ramakrishna, J. Mater. Chem. B 1, 2305 (2013)
[50] S. Sorgenfrei, C.Y. Chiu, R.L. Gonzalez, Jr., Y.J. Yu, P. Kim, C. Nuckolls, K.L. Shepard, Nat. Nanotechnol. 6, 126 (2011)
[51] K.W. Sara Iliafar, S. Manohar, A. Jagota, D. Vezenov, J. Phys. Chem. C 116, 13896 (2012)
[52] M. Hegner, P. Wagner, G. Semenza, Febs. Lett. 336, 452 (1993)
[53] M.R. Lockett, M.F. Phillips, J.L. Jarecki, D. Peelen, L.M. Smith, Langmuir 24, 69 (2008)
[54] S. Shimron, A. Cecconello, C.H. Lu, I. Willner, Nano Lett. 13, 3791 (2013)
[55] C. Huang, T. Stakenborg, Y. Cheng, F. Colle, T. Steylaerts, K. Jans, P. Van Dorpe, L. Lagae, Biosens. Bioelectron. 26, 3121 (2011)
[56] L.T.R. Banner, A. Richter, E. Pinkhassik, Surf. Interface Anal. 41, 49 (2009)
[57] C. Wang, X.M. Jia, C. Jiang, G.N. Zhuang, Q. Yan, S.J. Xiao, Analyst 137, 4539 (2012)
[58] X. Lv, L. Chen, H. Zhang, J. Mo, F. Zhong, C. Lv, J. Ma, Z. Jia, Biosens. Bioelectron. 39, 329 (2013)
[59] A.V. Ladik, F.M. Geiger, S.R. Walter, Nanoscape 7, 19 (2010)
[60] J.M. Yun, K.N. Kim, J.Y. Kim, D.O. Shin, W.J. Lee, S.H. Lee, M. Lieberman, S.O. Kim, Angew Chem. Int. Ed. Engl. 51, 912 (2012)
[61] B. Ding, H. Wu, W. Xu, Z. Zhao, Y. Liu, H. Yu, H. Yan, Nano Lett. (2010)
[62] J.J. Schmied, C. Forthmann, E. Pibiri, B. Lalkens, P. Nickels, T. Liedl, P. Tinnefeld, Nano Lett. 13, 781 (2013)
[63] S.J. Kim, B. Kim, J. Jung, D.H. Yoon, J. Lee, S.H. Park, H.J. Kim, Appl. Phys. Lett. 100 (2012).
[64] T.M. Lee, Sensors 8, 5535 (2008)
[65] E. Ozkumur, S. Ahn, A. Yalcin, C.A. Lopez, E. Cevik, R.J. Irani, C. DeLisi, M. Chiari, M.S. Unlu, Biosens. Bioelectron. 25, 1789 (2010)
[66] A.H. Wu, J.J. Sun, R.J. Zheng, H.H. Yang, G.N. Chen, Talanta 81, 934 (2010)
[67] A.V. Bonannia, M.D. Valle, Anal. Chim. Acta 678, 7 (2010)
[68] K.M. Millan, S.R. Mikkelsen, Anal. Chem. 65, 2317 (1993)
[69] J.T. Wallmark, Field-effect transistor, U.S. Patent No. 2,900,531, 1959)
[70] F. Yan, H. Tang, Expert review of molecular diagnostics 10, 547 (2010)
[71] X. Dong, Y. Shi, W. Huang, P. Chen, L.J. Li, Adv. Mater. 22, 1649 (2010)
[72] L. Kergoat, L. Herlogsson, D. Braga, B. Piro, M.C. Pham, X. Crispin, M. Berggren, G. Horowitz, Adv Mater 22, 2565 (2010)
[73] B.P. Loig Kergoat, M. Berggren, M.-Ch. Pham, A. Yassar, G. Horowitz, Org. Electron 13, 1
[74] P. Lin, X. Luo, I.M. Hsing, F. Yan, Adv Mater 23, 4035 (2011)
[75] D. Fu, H. Okimoto, C.W. Lee, T. Takenobu, Y. Iwasa, H. Kataura, L.J. Li, Adv. Mater. 22, 4867 (2010)
[76] Y. Cao, S. Zhu, J. Yu, X. Zhu, Y. Yin, G. Li, Anal. Chem. 84, 4314 (2012)
[77] B.R. Coad, T. Scholz, K. Vasilev, J.D. Hayball, R.D. Short, H.J. Griesser, ACS Appl. Mater. Interfaces 4, 2455 (2012)
[78] K. Furukawa, Y. Ueno, E. Tamechika, H. Hibino, J. Mater. Chem. B 1, 1119 (2013)
[79] F. Pillet, A. Sanchez, C. Formosa, M. Severac, E. Trevisiol, J.Y. Bouet, V. Anton Leberre, Biosens. Bioelectron. 43, 148 (2013)
[80] P.N. Bartlett, Bioelectrochemistry : fundamentals, experimental techniques and applications (John Wiley & Sons, Chichester, England, Hoboken, NJ, 2008)
[81] N.J. Kybert, M.B. Lerner, J.S. Yodh, G. Preti, A.T. Johnson, ACS Nano 7, 2800 (2013)
[82] European Commission, Notification of new chemical substances in accordance with directive 67/548/EEC on the classification, packaging, and labelling of dangerous substances, EINECS corrections, (European Commission, Bernan Associates

distributor, Luxembourg Lanham, MD, 1997)
[83] A.A. Ensafi, B. Rezaei, M. Amini, E. Heydari-Bafrooei, Talanta 88, 244 (2012)
[84] T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechnol. 21, 1192 (2003)
[85] M.J. Heller, Annual Review of Biomedical Engineering 4, 129 (2002)
[86] L. Civit, A. Fragoso, C.K. O'Sullivan, Electrochem. Commun. 12, 1045 (2010)
[87] N. Phares, R.J. White, K.W. Plaxco, Anal. Chem. 81, 1095 (2009)
[88] X. Liu, R. Aizen, R. Freeman, O. Yehezkeli, I. Willner, ACS Nano 6, 3553 (2012)
[89] A.E. Singh, H. Eksiri, Y.G. Yingling, Polymer Physics 49, 1563 (2011)
[90] S. Nakano, T. Kanzaki, M. Nakano, D. Miyoshi, N. Sugimoto, Anal. Chem. 83, 6368 (2011)