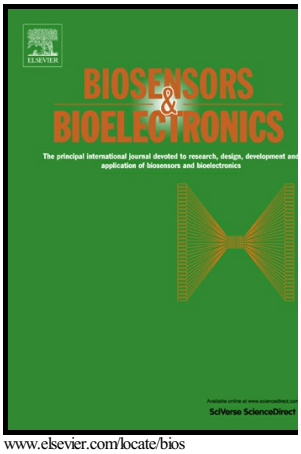

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PII: S0956-5663(15)30257-8
DOI: http://dx.doi.org/10.1016/j.bios.2015.07.007
Reference: BIOS7824

To appear in: *Biosensors and Bioelectronic*

Received date: 3 May 2015
Revised date: 3 July 2015
Accepted date: 5 July 2015

Cite this article as: Jie Chao, Dan Zhu, Yinan Zhang, Lianhui Wang and Chunha Fan, DNA nanotechnology-enabled biosensors, *Biosensors and Bioelectronic* http://dx.doi.org/10.1016/j.bios.2015.07.007

DNA nanotechnology-enabled biosensors

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Abstract

Biosensors employ biological molecules to recognize the target and utilize output elements which can translate the biorecognition event into electrical, optical or mass-sensitive signals to determine the quantities of the target. DNA-based biosensors, as a sub-field to biosensor, utilize DNA strands with short oligonucleotides as probes for target recognition. Although DNA-based biosensors have offered a promising alternative for fast, simple and cheap detection of target molecules, there still exist key challenges including poor stability and reproducibility that hinder their competition with the current gold standard for DNA assays. By exploiting the self-recognition properties of DNA molecules, researchers have dedicated to make versatile DNA nanostructures in a highly rigid, controllable and functionalized manner, which offers unprecedented opportunities for developing DNA-based biosensors. In this review, we will briefly introduce the recent advances on design and fabrication of static and dynamic DNA nanostructures, and summarize their applications for fabrication and functionalization of DNA-based biosensors.

Keywords: DNA-enabled biosensors; DNA nanostructures; DNA dynamic devices

1. Introduction

Sensors have witnessed an everlasting interest because of their wide applications in different areas including machinery manufacturing, medicine, innovation and environmental monitoring. In between, biosensors are one type of small devices for detecting target analytes that are usually biomoleculers such as protein, peptide, DNA and RNA et al. Biosensors employ biological molecules to recognize the target and utilize output elements which can translate the biorecognition event into electrical, optical or mass-sensitive signals to determine the quantities of the target (Borisov and Wolfbeis 2008; Suginta et al. 2013). As a sub-field of biosensors, DNA-based biosensors utilize DNA strands as probes for sensing targets and the processes of target recognition are always in two ways. 1) Hybridization of probes with DNA or RNA targets, based on which a wide range of sensors have been designed, including probes for *in vitro* DNA damage detection, 2) Association of probes with targets or subunits related to specific properties of targets (Teles and Fonseca 2008; Zhao et al. 2014). For example, various sensors have been developed based on the association of aptamers with proteins or small molecules and the intercalation of molecules to DNA double helix. Unlike common biosensors based on enzyme or antibodies, DNA-based biosensors with high sensitivity can be prepared with low cost and high assembly efficiency.

The utilization of new coming nanomaterials has promoted the development of DNA biosensors towards the goal of smart, simple and inexpensive detection for targets analyses. While these currently available DNA biosensors have shown high promise, to our knowledge, there still remain three directions for improvement: 1) Enhancement of the assembly production of DNA probes onto recognition layers to increase the sensitivity, 2) Utilization of new magnification method s to decrease the limit of detection, 3) New output signals to fulfill the detection style. DNA nanotechnology can provide new opportunities in these directions. By exploiting the unique properties of nucleic acids including double helix structure, Watson-Crick base pairing interactions and programmable sequences, thousands of DNA nanostructures with well-defined size, shape and geometry have been created in nanoscale precision. In this concept, we will give a brief introduction to the history of DNA nanotechnology, summarize the recent progress in DNA nanotechnology based biosensors with their mechanisms, and discuss the prospective development

of DNA nanotechnology for design and fabrication of advanced biosensors with their underlying applications.

2. History and current status of DNA nanotechnology

DNA nanostructures subvert the traditional understanding of nucleic acids as genetic information carriers in living cells by using them as building blocks for bottom-up self-assembly. Based on the unique DNA characters including unparalleled base pairing, double helix with diameter precision and programmable sequences, various DNA static nanostructures with well-defined size, shape and geometry and dynamic devices with precisely controlled motions and functions have been created by using DNA nanotechnology. A brief introduction of the history and current status of DNA nanotechnology is provided as follows.

Ever since the pioneer work of immobile four-arm junction in 1983, DNA nanotechnology experiences three periods, in which epoch-making milestones were marked by an immobile four-arm junction was born from its prototype holiday junction which is a mobile junction representing in genetic recombination (Figure 1a) (Kallenbach et al. 1983). Later, this family was expanded by three-arm, five-arm, six-arm, eight-arm and even twelve-arm junctions (Ma et al. 1986; Wang and Seeman 2007; Wang et al. 1991). Based on these arm junctions, DNA tiles were designed by program and fabricated by annealing. Concerning the difficulty of observing topology of these DNA nanostructures under microscope, DNA tiles were used as building blocks for the fabrication of large one-dimensional (1D) nanotubes and ribbons (Kuzuya et al. 2007), two-dimensional (2D) arrays (Ding et al. 2004; Winfree et al. 1998; Zheng et al. 2006) and even three-dimensional (3D) crystals (Figure 1b) (Wang et al. 2010; Zheng et al. 2009). DNA origami technology broke through the traditional design concept of DNA tiles by exploiting virus DNA (M13mp18), which is single-stranded and 7249-nucleotide long, with hundreds of staple strands to form 2D and even 3D DNA nanostrcutures with well-defined shapes. Elegant examples were square-, triangle-, dolphin-, China map- and box-shaped DNA origamis (Figure 1c) (Andersen et al. 2008; Andersen et al. 2009; Qian et al. 2006; Rothemund 2006). Later, more intricate 3D structures, such as monolith and railed bridges, spherical shells and nanoflasks, and gridiron-like balls and tubes were created by uisng ‘six-helix honeycomb lattice’, ‘concentric-ring’ and ‘four-arm-junction vertex’ strategies (Figure 1d) (Dietz et al. 2009; Douglas et al. 2009; Han et al.

2011; Han et al. 2013; Liedl et al. 2010). Recently, single-stranded tiles/bricks with concatenated sticky ends were employed to fabricate 2D/3D DNA canvas that generated complex 2D and 3D nanostructures, whose strategy combined the advantages of tile- and origami-assembly (Figure 1e) (Ke et al. 2012; Wei et al. 2012). Also, DNA nanostructures with larger or smaller sizes were investigated for their applications in drug delivery and plasmonics (Ma et al. 2013; Ouyang et al. 2013; Yang et al. 2012; Zhang et al. 2012).

Dynamic DNA devices play vital roles in the research field because they present the ability of human beings to control the world in nanoscale. Since this review is focused on DNA nanotechnology-enabled biosensors, we will group the dynamic DNA devices with "triggers", which are primarily target analytes for sensing and can be mainly divided into three types as shown below. Other types of dynamic DNA devices can be found in several excellent reviews (Krishnan and Simmel 2011; Wang et al. 2014). 1) DNA or RNA strands. Overhangs with special designed sequences in DNA or RNA strands are widely used as "triggers" because they could initiate branch migration to realize the transformation of the devices. Elegant examples were DNA tweezers, PX-JX_2 based devices and DNA walkers (Figure 1f) (Gu et al. 2010a; Gu et al. 2009; Omabegho et al. 2009; Sherman and Seeman 2004; Shin and Pierce 2004; Yan et al. 2002; Yurke et al. 2000). 2) Enzymes or deoxyribozymes (DNAzymes). The first walking device that triggered by enzymes was a DNA walker which utilized DNA ligase to combine two DNA strands together and DNA restriction enzymes to cleave their connections (Figure 1g) (Tian et al. 2005). Similar concepts were later demonstrated by DNA nicking enzyme. DNAzymes or ribozymes, which catalyze certain biochemical reactions such as phophodiester cleavage or ligation can also drive the DNA motors to walk on double-stranded tracks. This concept was also used to trigger "molecular spiders" to walk on the tracks within 100 nm range which were set on a origami platform (Figure 1h) (Lund et al. 2010; Wickham et al. 2012). 3) Ions. Certain cations such as hexamminecobalt (III) ($Co(NH_3)_6^{3+}$) can drive the double helix of DNA transition from the B (right-handed) to the Z structure (left handed). This concept were applied to construct the first rotary nanomechanical "B-Z device" (Figure 1i) (Mao et al. 1999b). Also, magnesium ions could induce a simple form of nanoscale motion by DNA supercoiling effect and a conformation switch of the Holliday junction from compact to flip state as a nanoscale "metronome". More recently, the change of magnesium ions concentration would finely induce the transformation of

3D DNA nanodevices including an actuator, a switchable gear, an unfoldable nanobook and a nanorobot, which were specifically self-assembled in solution on the basis of shape-complementarity without base pairing (Figure 1j) (Gerling et al. 2015). Because ions have unique affinity to specific bases or sequences such as thymine-Hg^{2+}-thymine complex and the i-motif structure, Hg^{2+}/cysteine together with H^{+}/OH^{-} were employed to trigger a "bipedal walker" and a "bipedal stepper" on a circular DNA template consisting of four tethered footholds (Figure 1k) (Wang et al. 2011). Almost all the dynamic DNA devices mentioned above were monitored optically by appropriate labeling of the components with fluorophores/quenchers units which were readily preparedfor sensing.

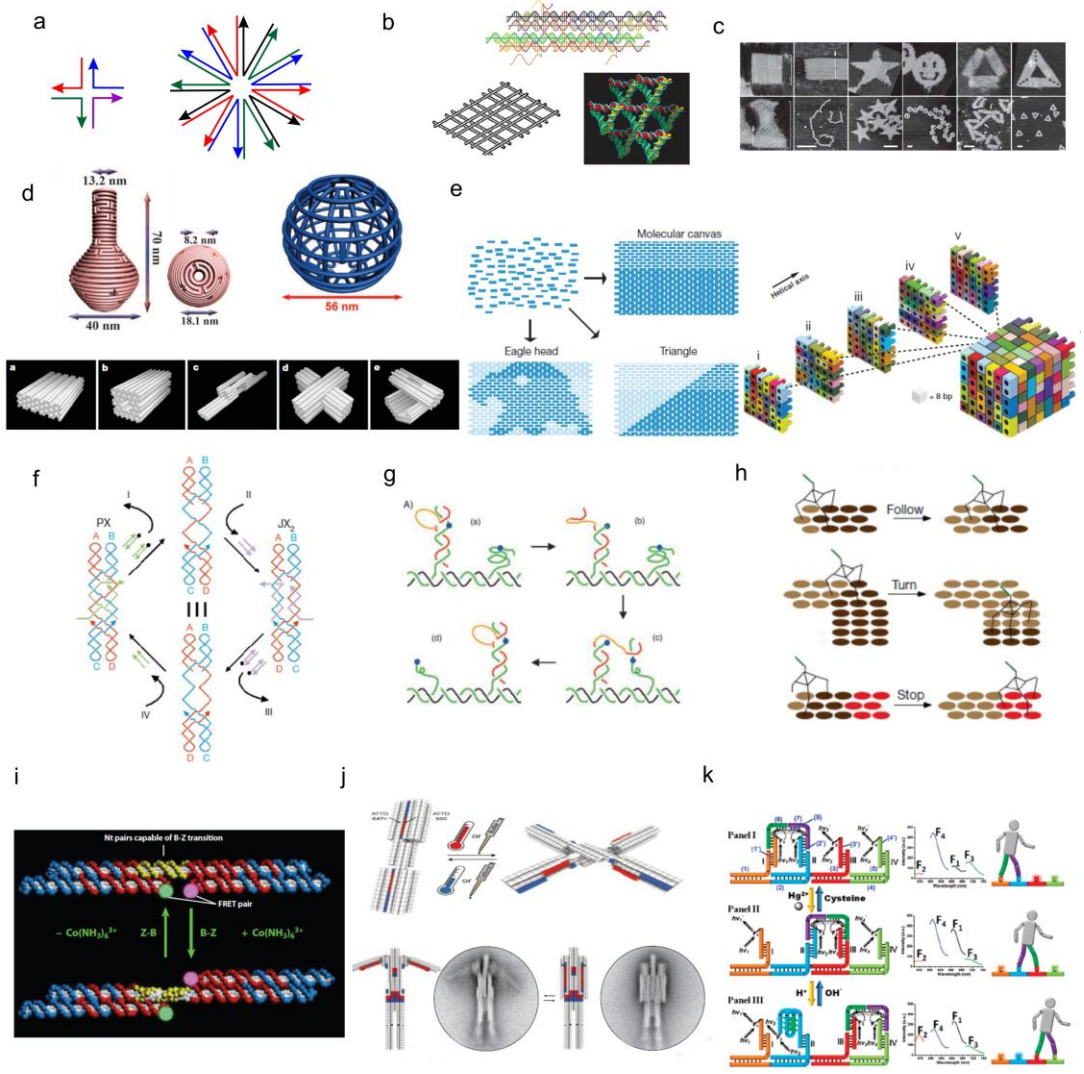

Figure 1. (a) Left: the four-arm junction originated form Holiday junction (Kallenbach et al. 1983). Right: the twelve-arm junction (Kuzuya et al. 2007). (b) The self-assembled DNA nanotube, (Kuzuya et al. 2007) 2D arrays (Mao et al. 1999a) and 3D crystals (Zheng et al. 2009). (c) Different shapes of DNA origamis (Rothemund 2006). (d) 3D DNA structures made by "concentric-ring" (top left), (Han et al. 2011) "four-arm-junction vertex" (top right), (Han et al.

2013) and "six-helix honeycomb lattice" (bottom) strategies . (e) 2D and 3D DNA structures made by DNA canvas (left) (Wei et al. 2012) and DNA bricks (right) (Ke et al. 2012). (f) A PX-JX2 device (Yan et al. 2002). (g) A walker based on deoxyribozymes (Tian et al. 2005). (h) A "nanospider" walking along a track on a DNA origami (Lund et al. 2010). (i) The transition of the DNA double helix from the B (right-handed) to the Z structure (left handed) driven by $Co(NH_3)_6^{3+}$ (Mao et al. 1999b). (j) A dynamic DNA device formed by shape-complementary, non–base pairing 3D components (Gerling et al. 2015). (k) A bipedal walker activiated by Hg^{2+}/cysteine and H^+/OH^- inputs (Wang et al. 2011).

3. Biosensors based on DNA nanostructures

The mechanisms of DNA biosensors are all based on the affinity of DNA nanostructures with target analytes which may cause the transformation of DNA nanostrutures. Elegant examples are G-quadruplexes formed by K^+ with G-rich sequences (Gellert et al. 1962), ion-bridged complexes formed between bases, such as T-Hg^{2+}-T or C-Ag^+-C bridges (Ono et al. 2008; Yamaguchi et al. 2014), DNA three-arm junction with cocaine and DNA aptamers with ATP _ENREF_54and so on (Mohaghegh and Hickson 2001). In the following sections, we will group the DNA sensors based on the dimension of DNA nanostructures.

3.1. One dimensional DNA nanostructures

3.1.1. DNA "stem-loop" structure

Two single-stranded DNA (ssDNA) with complementary sequences can hybrid together by hydrogen bonding, which is a noncovalent bases interaction of adenosine/thymine (A/T) and cytosine/guanine (C/G). Based on this, DNA hybridization biosensor utilize probe DNA with known sequence as the biorecognition element to detect the target DNA in the sample with various signaling mechanisms. Fan et. al. firstly demonstrated a DNA biosensor by sensing the electrochemical properties (E-DNA) for the conformational change of DNA (Figure 2a) (Fan et al. 2003). They self-assembled ferrocene-tagged DNA stem-loop structure onto a gold electrode by gold-thiol interaction. Target DNA would initiate the hybridization making the single-stranded loop to double-stranded helix. Hence, the electron-transfer tunneling distance between the electrode and the redoxable label was altered which induced the change of electron transfer efficiency measurable by cyclic voltammetry. Using this approach, target DNA could be detected at the concentrations as low as 10 pM. Later, this E-DNA sensor was improved by the introduction

of enzyme. They anchored the biotin and digoxigenin (DIG) labeled "stem-loop" onto an avidin-modified electrode surface as DNA probes (Figure 2b) (Liu et al. 2008). Before addition of target DNA, the stem-loop structure could hinder the interaction of a bulky horseradish peroxidaselinked-anti-DIG antibody (anti-DIG-HRP) with DIG by steric effect. The target DNA broke the stem-loop structure into a double helix that forced DIG away from the electrode. Then, anti-DIG-HRP could interact with DIG, which sensitively transduced the hybridization into an amplified electrochemical current signal. This enzyme-based E-DNA sensor would be able to detect as low as femtomolar DNA targets with excellent differentiation ability for even single mismatches. Other materials with new strategies were also studied to create new DNA hybridization biosensors. For example, Zhao et. al employed quantum dots and nanoparticles for detection of target DNA molecules in photoelectrochemical system (Figure 2c) (Zhao et al. 2012). Through the layer-by-layer self-assembly of oppositely charged cationic polyelectrolytes poly(diallyldimethylammonium chloride), CdS QDs were firstly immobilized onto the indium tin oxide (ITO) electrode. Then the capture DNA was connected to the electrode via the classic 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) coupling reaction subsequently blocked by monoethanolamine (MEA). Considering the overlap in absorption spectra, AgNPs was selected to label the target DNA. Upon DNA hybridization, the distances between AgNPs and CdS QDs were small enough for exciton-plasmon interactions which generated changes of photocurrent.

3.1.2 DNA aptamers

Aptamers, usually single-stranded oligonucleotides, are generated by an *in vitro* selection process called SELEX (systematic evolution of ligands by exponential enrichment). They can bind with high affinity and specificity to a wide range of target molecules including ions (e.g., K^+, Hg^{2+} and Pb^{2+}), small organic molecules (e.g., ATP, amino acids, cocaine, vitamins and antibiotics), organic dyes, peptides, proteins (e.g., thrombin and growth factors) and even whole cells or microorganisms (e.g., bacteria) (Song et al. 2008; Tombelli et al. 2005).

Upon the strong coordinating interaction to thymine (binding constant higher than A-T), Hg^{2+} is often detected by aptamers which are a series of T-rich single-stranded DNA to form stable mismatched DNA duplexes. To construct a sensitive biosensor, the question is how to translate the

T-Hg^{2+}-T contained DNA duplex into output signals. A typical method is gold nanoparticle (AuNP)-based colorimetric assay. For example, Xue et al. functionalized AuNPs with two thiol modified DNA as probes to recognize the appropriate DNA linker (Xue et al. 2008). In the presence of Hg^{2+}, these DNA linkers would tie up the two AuNP probes to form the stable DNA duplexes with AuNP conjugates, leading to a clear red-to-purple/pinkish colorimetric response. Under naked eye, the limit of detection for target linkers was about 3 μM of Hg^{2+} in the 14 nm NP system while 1 μM of Hg^{2+} in the 30 nm NP system. Mercury-specific oligonucleotides were also modified on the Au electrodes to capture free Hg^{2+} in aqueous media. The electrochemically reduction of Hg^{2+} to Hg^{+} provided a readout signal for its quantitative detection. They also employed AuNPs to amplify the electrochemical signals, whose linking probe was complementary to the mercury-specific oligonucleotides. This method made the limit of detection for Hg^{2+} improved even to 3 orders of magnitude by electrochemical devices (Zhu et al. 2009). These are example methods for the detection of other ions such as K^{+} and Pb^{2+}.

For the detection of cocaine, a multi-detection aptasensor was created by Golub et al. using supramolecular aptamer complex and nanopaticles (NPs) (Figure 2d) (Golub et al. 2009). As a typical assay, anticocaine aptamer, a three-arm DNA junction, was departed into two subunits. One was thiol functionalized to be assembled on an Au transducer, the other was modified by NP as signal reporter, which would become one supramolecular complexes upon the addition of cocaine. AuNPs were brought much closer to the electrode surface, PtNPs allowed the detection of cocaine by the electrocatalyzed reduction of H_2O_2, CdS NPs by photocurrent transfer of triethanol amine and AuNPs by the reflectance changes stimulated through the electronic coupling between the localized plasmon of the AuNPs and the surface plasmon wave. Lack of background interfering signals was the major advantage of this aptasensing platform that enabled the analysis of cocaine with a detection limit of 10^{-6} to 10^{-5} M. Similar approach was recently employed to detect cocaine in the finger print using a dark-field microscopy. (Li et al. 2013) This approach was a typical method for detecting small organic molecules.

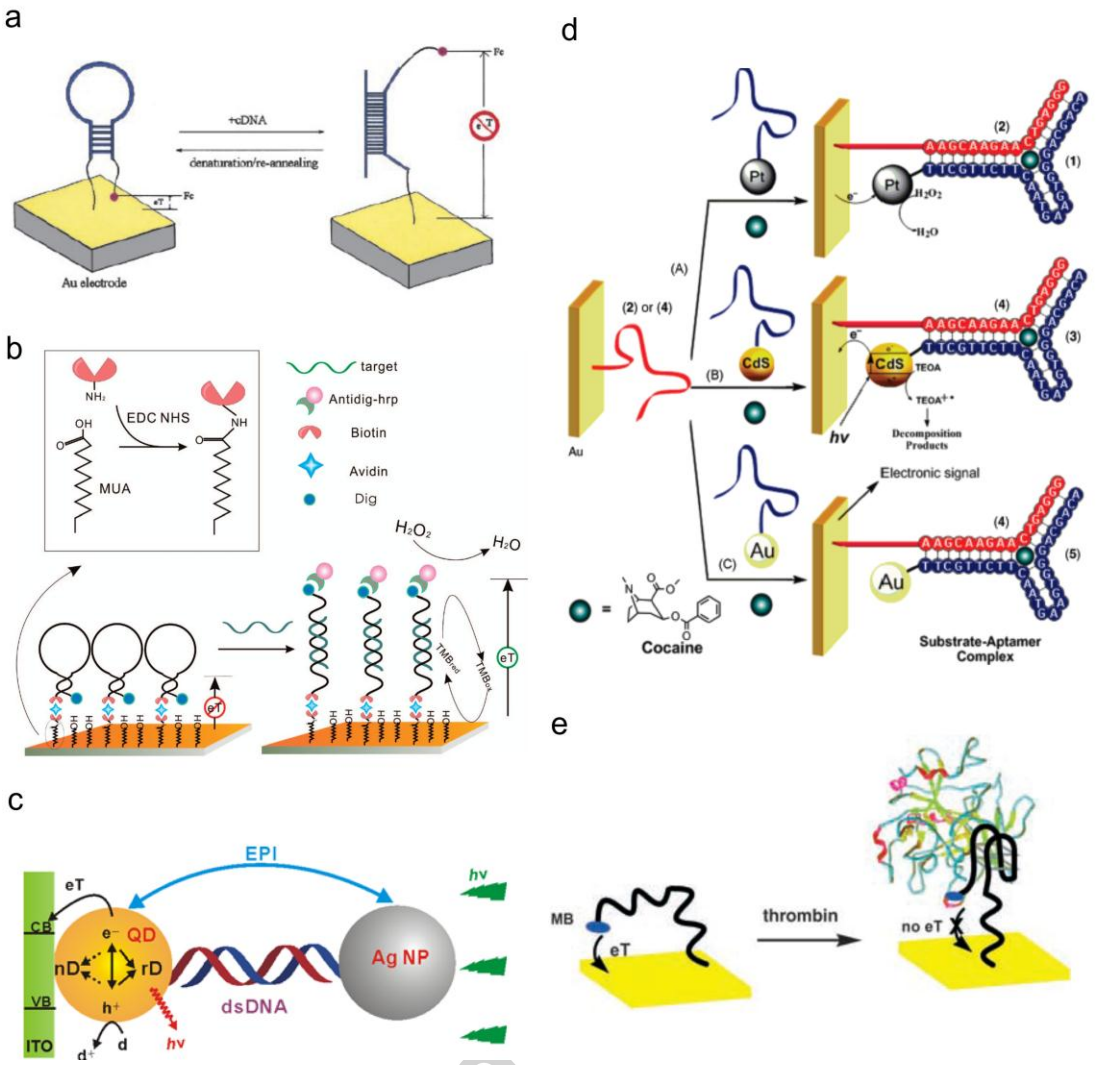

Figure 2. (a) The electrochemical DNA biosensor (E-DNA sensor) based on the conformational change of DNA (Fan et al. 2003). (b) The combination of enzymes with the E-DNA sensor (Liu et al. 2008). (c) The employment of quantum dots and nanoparticles for detection of target DNA molecules in a photoelectrochemical system (Zhao et al. 2012). (d) The multi-detection aptasensor using supramolecular aptamer complex and nanopaticles (NPs) for the detection of cocaine (Golub et al. 2009). (e) The schematic of the electrochemical aptamer-based (E-AB) sensor (Xiao et al. 2005).

Aptamers are suitable for the detection of proteins because compared to the monoclonal antibodies, the dissociation constants of aptamers to target molecules are typically from the micromolar to picomolar range, which demonstrates their high affinity. An elegant example is electronic aptamer-based (E-AB) sensors for thrombin. Xiao et. al. firstly immobilized an

anti-thrombin aptamer with a redox-active methylene blue (MB) labeling on an electrode, the flexible conformation of which enabled the electrical communication of MB with the electrode (Figure 2e) (Xiao et al. 2005). After the addition of thrombin, the conformation of the aptamer transformed from a loose state to a compacted G quadruplex structure which shielded MB from electron transfer communication with the electrode. As a result of the association of target thrombin with aptamer, the negative signal caused by the decrease of amperometric response was an obvious disadvantage for this sensing format. Therefore, several signal-on aptosensors were later developed. One typical approach utilized an anti-thrombin aptamer bifunctionalized with an electroactive group as the redox label and a thiol group anchored on Au electrode (Radi et al. 2006). The signals were in off-state when the aptamer chain was flexible because it prevented electrical contact of the ferrocene label with the electrode. The addition of thrombin made the aptamers form the condensed state of G-quadruplex configuration that facilitated the electron-transfer between the electro-active ferrocene units and the electrode and thus generated the signal-on state.

3.1.3 DNA single-stranded structure for signal amplification

Rolling circle amplification (RCA) is an isothermal nucleic acids amplification strategy which generates long ssDNA from a circle template DNA in the presence of a special phi29 DNA polymerase, a short DNA primer and deoxynucleotide triphosphates (dNTPs) (Shi 2001; Zhao et al. 2008). Based on hundreds and even thousands of times circular replication of the DNA template, the micrometer-long ssDNA own tandem repeat units with translated sequence to its template that leading to extensively applications for amplified biosensing. RCA process can be activated by aptamer functionalized DNAzyme to construct various amplified aptasensors. Ellington and coworkers designed a DNA aptazyme by appending an anti-ATP DNA aptamer to a selected deoxyribozyme ligase which was immobilized on a streptavidin-coated glass slide (Figure 3a) (Cho et al. 2005). Without ATP, this DNA aptazyme was on an “inert” state that resisted the RCA process. When ATP was introduced to the system to bind with the aptamer domain, the tertiary structure of the ligation DNAzyme was cooperatively stabilized and the intramolecular ligation of the 3′- and 5′-ends of the DNAzyme substrate was induced, which generated a circle template for RCA. With the addition of Phi29 DNA polymerase and dNTPs mixture, the long

ssDNA with constant repeat units replicating the circular DNA template was obtained. After the hybridization of this ssDNA with fluorophore labeled probe nucleic acid, the amplified signal enabled the fluorescence detection of ATP, with a detection limit corresponding to 1 μM. DNA/peptide nucleic acid (PNA) duplex that specifically bind the 3, 3′-diethylthiadicarbocyanine dye (DiSC2) were affected by ATP to result in a blue-to-purple color change. RCA process demonstrated its facilitation to the analysis of ATP with a detection limit of 50 μM (Ali and Li 2009). To avoid the susceptible to background signals, the analyte was employed as the substrate for a deoxyribozyme catalyzed self-phosphorylation reaction, whose products could be circularized and subjected to massive signal amplification by rolling circle amplification. This approach obtained better detect limitation to 25 nM GTP even in the presence of 1 μM ATP as a potential contaminant (McManus and Li 2013). Improved strategy with RCA process would reach a lower detection limit of femtomol even attomol. Tang et. al. employed a circle template-half aptamer hybrid and a biotin labeled half-aptamer anchored on to avidin modified magnetic beads together to recognize protein (Tang et al. 2012). Their recognition would initiate the RCA process with the help of dNTP, BSA and phi29 polymerase. Because of the production contained G-quadruplex moieties that could mimic the functions of peroxidase opon combination with hemin, 2, 2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was oxidized to generate a blue-green colorimetric signal. After employing the platelet-derived growth factor B-chain (PDGFBB) as a model system, they demonstrated the detection of a protein marker specifically in a serum-containing medium at a concentration as low as 0.2 pg/mL in ~2 h. Our group also utilized a RCA and DNA enzyme system to detect the microRNA. Here, microRNA (miRNA) was initiators that triggered the RCA process (Figure 3b) (Wen et al. 2012). The resulting RCA product then autonomously replicated a multiple machinery cutter cycle and generated accumulated amount of products which contained G-quadruplex moieties that catalyze a colorimetric change. With these cascade amplifications, 2 aM miRNA could be detected.

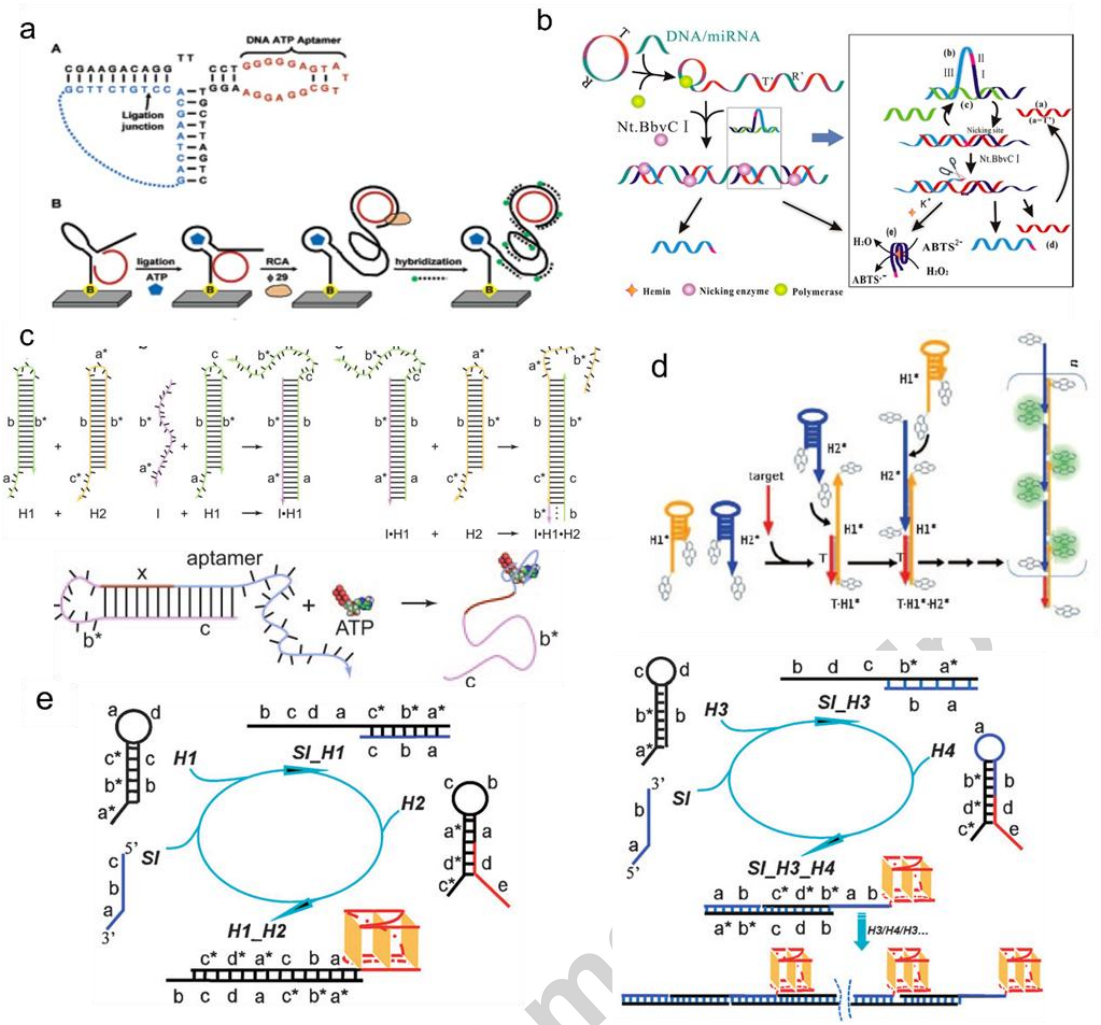

Figure 3. (a) The DNA sensor involving RCA and deoxyribozyme ligase for detection of ATP (Cho et al. 2005). (b) The RCA-based DNA machine for miRNA detection (Wen et al. 2012). (c) The principle of hybridization chain reaction (HCR) and detection for ATP (Pierce 2004). (d) The detection a target DNA by pyrene residues modied H1 and H2 in the HCR process with enhanced excimer emission (Huang et al. 2011). (e) The detection a target DNA by autonomous HCR-triggered cross-opening of two hairpins H1 and H2 that yield hemin/G-quadruplex DNAzyme-functionalized supramolecular nanowires (Ren et al. 2011).

Hybridization chain reaction (HCR) was another method for DNA based signal amplification. A typical HCR system contains two hairpins, H_1 and H_2 and a initiator (I) (Figure 3c) (Pierce 2004). Although H1 and H2 have the same sequences at the stem part, they do not interact with each other unless the initiators were added. Firstly, the initiator breaks the stem-loop state of H1 by hybridization with its toehold. Then the I·H1 hybrid would interact with the toehold of H_2 to generate a I·H1·H2 hybrid which would open a new H1 hairpin. The process cannot stop until H1 or H2 runs up which can be monitored by the fluorescence. A third hairpin (H3) containing ATP

aptamer and the initiator were introduced into the DNA mixture consisting of the functional hairpins H1 and H2 to generate the long-range hybridization. In the presence of the ATP analyte, formation of the ATP/aptamer complex is energetically favored, leading to release of the single-stranded DNA chain comprising domain which initiated the HCR process for signal amplification. In another design, pyrene residues modified H1 and H2 were employed to detect initiator DNA. The polymeric nanowires of the production of HCR process brought the two pyrene units much closer, resulting in the decrease of the monomer emission and the enhancement of excimer emission. Based on this, the detection limit of the target DNA was up to 256 fM (Figure 3d) (Huang et al. 2011). HCR process was also applied into a G-quadruplex system (Ren et al. 2011). Ren et al. designed two HCR process (Figure 3e left and right) to kinetically graft G-quadruplex onto the end of duplex DNA, respectively. In the left system, G-quadruplex was formed at the end of one HCR process and the initiator SI was released which could be reused in the next HCR process. In the right system, different design of H1, H2 and initiator made the formation of G-quadruplex with 1D DNA nanoribbon that amplify the signals of fluorescence, colorimetric or chemiluminescence for target DNA detection.

3.1.4 DNA double helix

DNA double helix has special affinities to small molecules such as drugs and chemicals which is of importance in disease diagnosis and drug discovery. Typically, their noncovalent interaction are in three main types, i.e., intercalation, electrostatic interaction and groove binding. The intercalators are a type of small organic molecules with an appropriate size and chemical nature including a planar structure with polycyclic aromatic rings.(Tokudome et al. 2005) They usually fit into dsDNA base pairs through unwinding DNA and the formed structures are stabilized by ð-stacking. The local structural changes of dsDNA caused by some intercalators can not only inhibit the transcription and replication in DNA repair processes, making intercalators potential application in chemotherapeutic treatment, but also induce photonic or electronic changes of the duplex that can be used as effective photoactive intercalators in DNA hybridization, DNA damage and aptasensing. Because the stability of DNA duplex depends strongly on the compatibility between the two strands, one-base mismatch would make the duplex less energetically favorable. Therefore, as an sensitive reporter, photoactive intercalators can aid detect gene mutation. A

typical example was photoelectrochemical detection of target nucleic acids with a nucleic acid/photo reporter adduct layer on an ITO electrode. After the hybridization of target nucleic acids with capture probes, the subsequent binding of a photo reporter which was a photoactive threading bis-intercalator consisting of two N,N'-bis(3-propyl-imidazole)-1,4,5,8-naphthalene diimides (PIND) linked by a $Ru(bpy)_2^{2+}$ (bpy=2, 2'-bipyridine) complex (PIND-Ru-PIND) would generate the photoelectrochemical signals for target DNA detection(Baugh et al. 2001). Because the adducts owned an extremely low dissociation rate and the photoelectrochemical response was highly reversible under visible light illumination (490 nm), this biosensor was capable of one-base-mismatch discrimination with the enhanced sensitivity to four orders of magnitude over voltammetry. Likewise, a TiO_2 electrode was used to anchor the DNA modified AuNPs as probes and signal transducers (Lu et al. 2006). Target DNA hybridized with the probe was detected by the decreased photocurrent of the TiO_2 electrode. Compared to label-free probe, AuNPs enhanced the photocurrent shifts after the hybridization. Their specificity experiment showed that target DNA with one or more base mismatches could be all discriminated.

Due to the negative charge of phosphate backbones of DNA duplex, positive charged guest molecules may have the opportunities for electrostatic interaction. Recently, the interaction of o-aminophenol (OAP) with DNA duplex was examined for OAP detection (Yan et al. 2014b). They employed CdSe QDs and DNA modified TiO_2 (DNA-CdSe/TiO_2) film electrode to create a photoelectrochemical biosensor. The interaction of OAP with dsDNA led to an effectively enhanced visible light absorption of TiO_2 film. Under optimized conditions, the detection limit of OAP was 8.0×10^{-8} mol L^{-1}. Proteins can also bind to DNA nonspecifically or in a sequence-dependent manner. A typical example was the novel PEC biosensor for DNA methyltransferase (MTase) activity assay (Zhou et al. 2014b). In this work, M. SssI MTase was used as methylation reagent and methyl binding domain protein (MBD1 protein) as methylation recognition element. When the cytosine methylation event occurred at the site of 5'-CG-3', MBD1 in the solution recognized and bound tightly to dsDNA at the methylated site, which generated a decreased photocurrent due to the hindrance on electron donor transferring. The decreased photocurrent was caught with the detection limit of 0.035 unit/mL (S/N=3).

3.2. Two dimensional DNA nanostructures

Although 2D DNA nanostructures inherit most characters form 1D DNA nanostructures, they still own two obvious advantages: 1) higher dimensionality which enlarges their effective space for molecules immobilization; 2) controllable motion which facilitates the study of molecules interactions.

3.2.1. DNA tiles

Dynamic devices based on DNA tiles are usually employed to study the interaction between proteins and DNA or two proteins. In 2004, Shen et al. developed a protein-activated device which allowed the estimation of the amount of remaining energy of protein after binding to DNA by measuring the distortion of DNA aroused by the protein (Shen et al. 2004). In their design, Triple crossover motifs were joined by a domain containing the binding site for the integration host factor (IHF, E.coil was chosen in this paper). The device endured a distortion upon binding of the protein. They also designed a TX-based device in which the bottom domains were connected by a sticky end. By varying the length of the sticky end, the ability of IHF to arouse distortion could be tuned. They considered this device sensitive to the relatively small spatial displacements associated with rotations. Using same strategy, Gu and coworkers created a scissors-like biosensor which utilized two DX molecules connected by a flexible Holliday junction (Gu et al. 2010b). The biorecognition part was a binding site on a double helix that connected two ends of the DX molecules. The output part was another joint of the two DX molecules on an edge adjacent to the binding site whose sticky ends were flanked by a pair of dyes. When the target molecule MutS was introduced into the system, it would bind to the double helix at the binding site, which generated a force to bend the double helix. This force was also transferred by the main body of the scissors to disrupt the sticky ends and separate the dyes in the output part, which was monitored by fluorescence resonance. By altering the length of the sticky ends, the interaction of MutS with double helix would be quantified. Recently, Liu and coworkers constructed a similar device by Triple crossover (TX) molecules(Liu et al. 2012). They chose a different protein, SoxR, to examine the distortion behavior of the device and monitored the process using fluorescence resonance energy transfer (FRET) methods. The nanomechanical device made of DNA could be used for investigation of the interaction of DNA regulatory proteins with DNA. Liu et al. involved the enzymes/cofactor pair in the dynamic DNA device and demonstrated a tweezer-like nanoreactor

(Liu et al. 2013). Through introduction of the fuel strand or the set strand, the tweezer underwent a conformational change between the open and the closed state. In the open state the G6pDH/NAD+ enzyme/cofactor pair was inactive for a relative long distance while in the closed state the activity of the pair was actuated. This nanoreactor was expected to act as specific chemical amplifiers for medical or other purposes in the future.

3.2.2. DNA origami

DNA sensing in a two-dimensional platform becomes available readily with the emerging of DNA-origami method. DNA origami can act as an ideal base for sensing based on its intrinsic addressability and shape controllability. In 2008, Ke et al. developed a type of rectangular DNA origami for simultaneous detection of three different RNA targets (Figure 4a) (Ke et al. 2008). The nucleic acid probes could hybridize to corresponding RNA targets in solution, which resulted in stiffer DNA-RNA duplex readily detectable with the AFM cantilever. Hence, the result could be read out directly under the AFM view.

This read-out scheme was then extended for single-nucleotide polymorphisms (SNPs) detection. As the most common genetic variation in the human genome, SNP has been attracting extensive attention and a number of detection methods have been developed. Zhang et. al. developed a DNA origami chip for SNP detection. In an asymmetric China map-shaped DNA origami, a streptavidin (STV) label was specifically absorbed on a biotin-modified reporter probe (Zhang et al. 2010). In presence of DNA targets, a branch migration was started to release the STV-decorated reporter probe. AFM iamges before and after the strand displacement could confirm the process (Figure 4b). This approach was improved by toehold-mediated strand displacement. As a kinetic dependent process, the branch migration can be inhibited by a mispair, which terminated the displacement process. Hari et al. designed a rectangular DNA origami carrying a pattern of alphabetic characters of the four nucleotide, A, T, G and C (Subramanian et al. 2011). In the presence of probes, only the corresponding branch migration was conducted while other migrations were inhibited by a single base mispair (Figure 4c). After the migration, the duplexes that formed the character disappeared, which in turn led to vanishing of the character under the AFM view.

Besides SNP detection, enzymes were involved as well to extend the range of sensing. In

2012, Fu et al. (Figure 4d) and Fan et al. (Figure 4e) utilized the excellent addressability of DNA origami to study behaviors of an enzyme cascade, respectively (Fu et al. 2012; Fu et al. 2013). They both anchored glucose oxidase (GOx) and horseradish peroxidase (HRP) on the surface of DNA origami platform. The former studied the distance between two enzymes that effected their interaction and claimed the nearer distance would facilitate the enzyme cascade. While the latter obtained the conclusion that enzyme cascade efficiency was promoted by rolling the rectangular DNA origami into a nanotube. Recently, Yan et al. combined RCA with DNA origami method to produce a DNA belt for highly sensitive and specific detection of prostate-specific antigen (PSA) (Figure 4f) (Yan et al. 2014a). Prostate cancer is among the primary causes of cancer lethality for male in developed countries. As an androgen-regulated serine protease, PSA is considered as a paramount serum biomarker which can be used for early diagnostics. With their strategy it's possible to detect specifically as low as 50 aM of PSA.

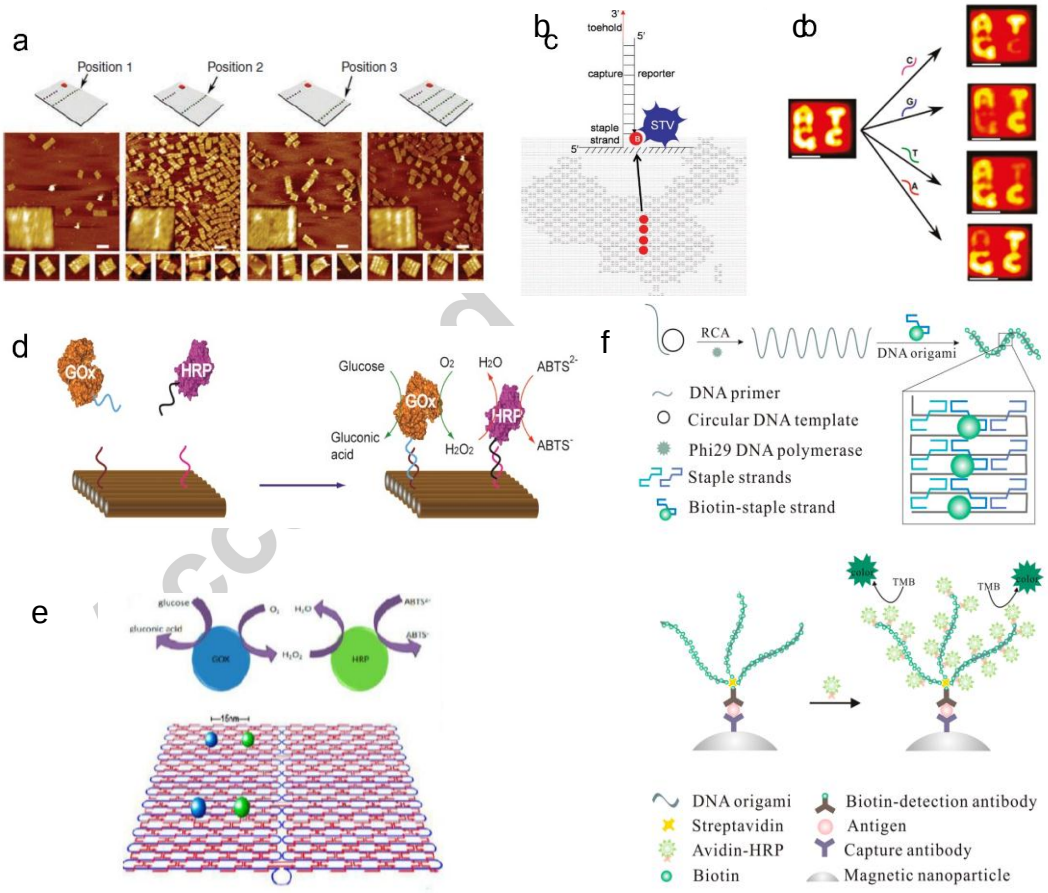

Figure 4. (a) The first read-out sensor based on DNA origami (Ke et al. 2008). (b) An asymmetric DNA origami platform used for SNP detection (Zhang et al. 2010). (c) SNP detection with the read-out sensor (Subramanian et al. 2011). (d) The introduction of enzymes in the two dimensional platform (Fu et al. 2012). (e) Another example of enzymes cascade system on this platform (Fu et

al. 2013). (f) A two dimensional sensor for PSA involving RCA nanoribbons (Yan et al. 2014a).

Chen et al. further confirmed that this kind of 2D platform was able to act as a siRNA delivery vehicle in human cancer cells other than an intracellular pH sensor (Chen et al. 2015). They constructed periodic DNA nanoribbons (DNRs) in which the scaffold was produced with RCA technique as well. The endocytosis process proved efficient for DNRs. By conjugation to a pH-sensitive dye, the intracellular DNRs turned in to a pH sensor and by delivering siRNA they could lead to controllable gene silencing.

3.3. Three dimensional DNA nanostructures

3.3.1. DNA tetrahedron nanostructures

3D DNA nanostructures play vital roles in DNA nanotechnogy. They present the most advanced design concepts and relatively difficult fabrication approaches. They own versatile structures and 3D precise orientations in nanoscale which make them the most promising materials for multidisciplinary applications.

DNA tetrahedron nanostructures seem to be among the mostly used DNA materials for biosensensors and drug delivery. In 2010, Pei et al. constructed a three dimensional DNA nanostructure as a sensor platform (Figure 5a) (Pei et al. 2010). The robust DNA tetrahedron platform is superior to the linear or stem-loop probes due to the greatly enhanced spatial positioning range and accessibility of the probes on the surface. Besides, they confirmed the three dimensional sensors were endowed with high production yields, high protein resistance ability and excellent compatibility.

Based on this design, several further studies were conducted. Lin et al. utilized the platform for microRNA analysis (Figure 5b) (Lin et al. 2014). They designed an E-DNA sensor which involved enzymatic amplification in the tetrahedron platform. By rational design and structure optimization, they achieved a detection limit as low as 1 fM. Ge and coworkers introduced hybridization chain reaction (HCR) amplification to the tetrahedral platform and improved the detection limits by 3 orders of magnitude to reach 100 aM for DNA and 10 aM for microRNA (Ge et al. 2014). Chen et al. constructed an ultrasensitive electrochemical sensor for prostate-specific antigen detection (Figure 5c) (Chen et al. 2014). By controlled assembly of immobilized

antibodies and usage of AuNPs as carriers of HRP modified PSA antibody, they succeeded in getting extremely high sensitivity with a low detection limit of 1pg/mL.

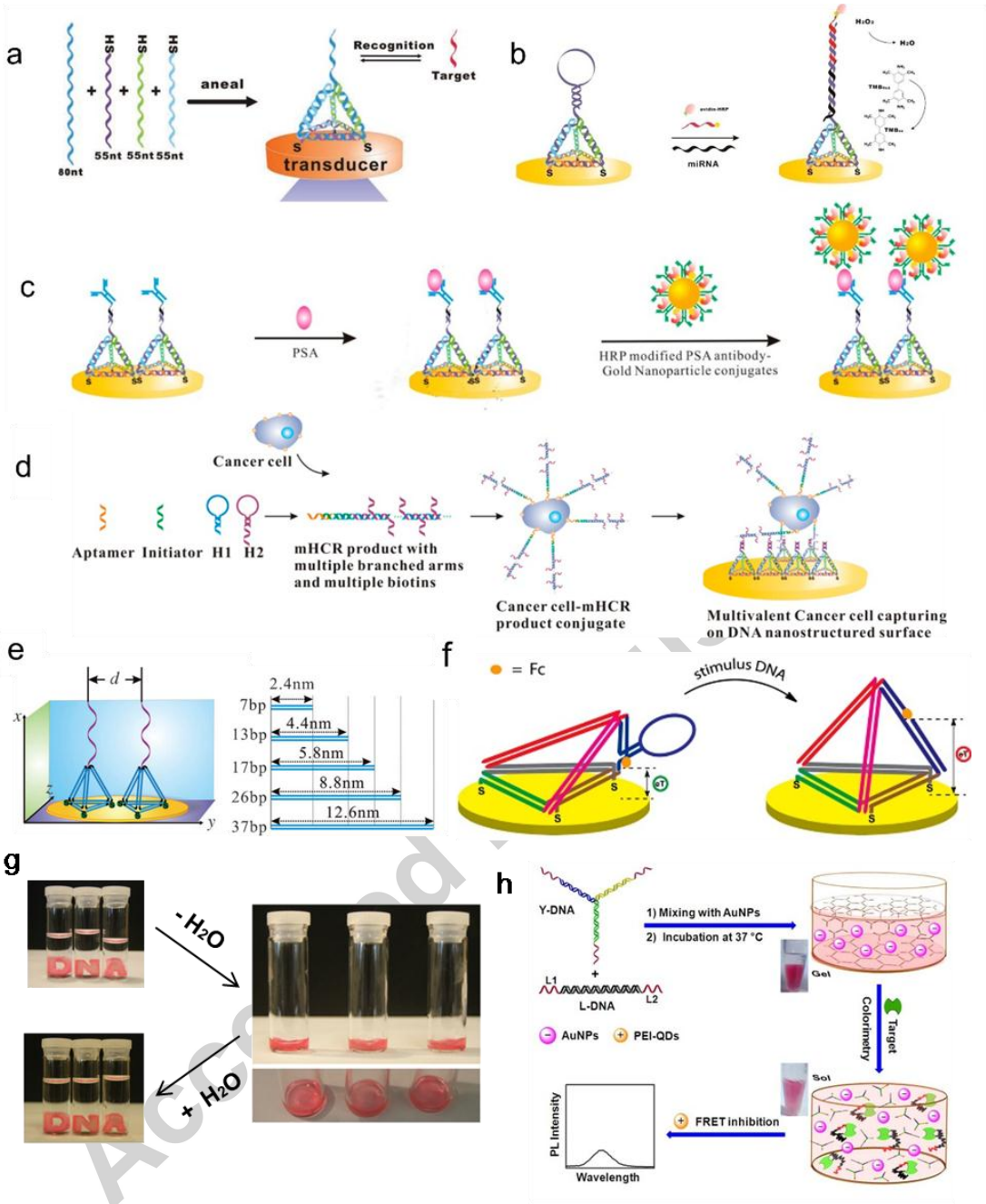

Figure 5. (a) The three dimensional DNA nanostructure as a sensor platform (Pei et al. 2010). (b) A microRNA analysis based on the platform (Lin et al. 2014). (c) An ultrasensitive electrochemical sensor for prostate-specific antigen detection performed on this platform (Chen et al. 2014). (d) The combination of hybridization chain reaction (HCR) amplification with the tetrahedral platform that was capable of cancer cell detection (Zhou et al. 2014a). (e) "soft lithography" to the modification of electrochemical sensors with tetrahedral DNA nanostructures (Lin et al. 2015). (f) A switchable three dimensional platform (Abi et al. 2014). (g) The transformation of the D-, N- and A-shaped hydrogel was tested by removing and replacing water (Lee et al. 2012). (h) A DNA hydrogel based sensor (Zhang et al. 2013).

Zhou et al. extended the detection range to involve cancer cells (Figure 5d) (Zhou et al. 2014a). By producing a long helix with multiple biotins for enzyme binding and multiple branched arms for multivalent anchoring on the electrode gold surface using multibranched HCR (mHCR), they demonstrated a simple and sensitive electrochemical sensor capable of detecting four cancer cells. More recently, Lin and coworkers introduced a concept of "soft lithography" to the modification of electrochemical sensors with tetrahedral DNA nanostructures (TDNs) (Figure 5e). (Lin et al. 2015) The change on the size of the TDNs could improve the efficiency of the sensors to even four orders of magnitudes. Involvement of polymeric enzyme amplification made attomolar sensitivity realizable. Abi et al. endowed the sensor platform with dynamic switching properties (Figure 5f) (Abi et al. 2014). A stimulus DNA was added to open the stem-loop hairpin and the reconfiguration was detected by a non-intercalative ferrocene (Fc) redox label anchored to the tetrahedron edge near the electrode surface.

3.3.2. DNA hydrogels

As a special member in DNA nanostructures, DNA hydrogels are unlimited three-dimensional structures that are synthesized by DNA ligases and polymerase with inter-unit i-motif structures. Luo et. al. created a novel DNA meta-hydrogel through RCA and multi-primed chain amplification (MCA) (Figure 5g) (Lee et al. 2012). The resulting material flowed freely in the containers in absence of water and deformed into a solid when in water. The character can be taken to detect electric current using water as a switch. For providing electric conductivity, the DNA meta-hydrogel was doped with 10 nm AuNPs. When it was surrounded by water, the gel moved away from the electrode in 15 seconds and the electric circuit was shut off. When removing water from the hydrogel, it bacame 'liquid-like' and the two electrodes were linked together. As a result, the circuit was turned on.

Ju and his coworkers designed a DNA hydrogel which was assembled by Y-shaped DNA and linker DNA. L-DNA was a duplex, consisting of an aptamer sequence (L1) with two different recognition sites for thrombin and a complementary sequence (L2) (Fugire 5h) (Zhang et al. 2013). To indicate the gelling formation and transition, AuNPs modified with bovine serum albumin (BSA) can be encapsulated in the rigid space of the DNA hydrogel as a visualizing agent. When

the hydrogel is surrounded by water, two layers would be observed in the absence of the model analyte. Considering the strong affinity of the aptamer to thrombin, the DNA hydrogel would quickly collapse and dissolve upon adding thrombin due to its competitively bind with the L-DNA aptamer. A red lower layer of AuNP was released from the gel to the colorless upper solution. Furthermore, the negatively charged AuNPs approached the positively charged QDs because of the electrostatic interaction. Based on the excellent FRET inhibition between PEI-QDs and AuNPs, DNA hydrogel can be used to detect protein in a sensitive way. In another way, Liu and coworkers employed polyacrylamide hydrogel to covalently interact with acrydite-modified DNA during gel formation (Baeissa et al. 2010). They have obvious advantages such as easy handling and low optical background as a biosensor. Using DNA-functionalized AuNPs as colorimetric agents, even amplified with Ag^{+}, the detection limit of target DNA could reach 1 pM.

4. Perspectives

DNA nanostructures have shown great potential in fabricating biosensors. However, as a newcomer in this area, their widespread and practical applications seem to be premature. There are several key challenges remaining to be addressed. 1) Most currently available DNA nanostructures have their own functions. Hence, it is possible to design and fabricate suitable DNA nanostructures for special use in a fixed biosensor. 2) Since DNA origami nanostructures contain hundreds of ssDNA, their high cost would prevent large-scale applications of DNA-based biosensors. 3) DNA is a type of biomolecules with limited conductivity which in some conditions inhibits their applications in electron relative biosenors. More recent advances in this area have brought new hopes. Several recent studies broke the size limit of Rothemund's origami by using RCA-based scaffolds and several ssDNA (Iinuma et al. 2014; Marchi et al. 2014). Excitingly, lithography technique together with DNA triangular origami have generated large-area spatially ordered arrays of AuNPs, which may help to homogenize their binding to the surfaces such as electrode (Hung et al. 2010). DNA nanostructures have shown unexpected properties such as permeability across the cellular membranes, leading to several exciting applications including targeted drug delivery (Jiang et al. 2012; Li et al. 2011; Schuller et al. 2011), nanoreactors and bioimaging (Le Liang 2014). One would envisage that specifically functionalized DNA biosensors are delivered into cells to detect the diseases *in vivo* and transfer the information out of the cells,

which should have a bright future for theranostic applications in biomedicine.

Acknowledgement

This work was supported by NUPTSF (Grant No.214175), the Ministry of Science and Technology of China (2012CB825805, 2012CB932603, 2013CB933802) and the National Science Foundation of China (21390414, 21227804, 61378062).

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