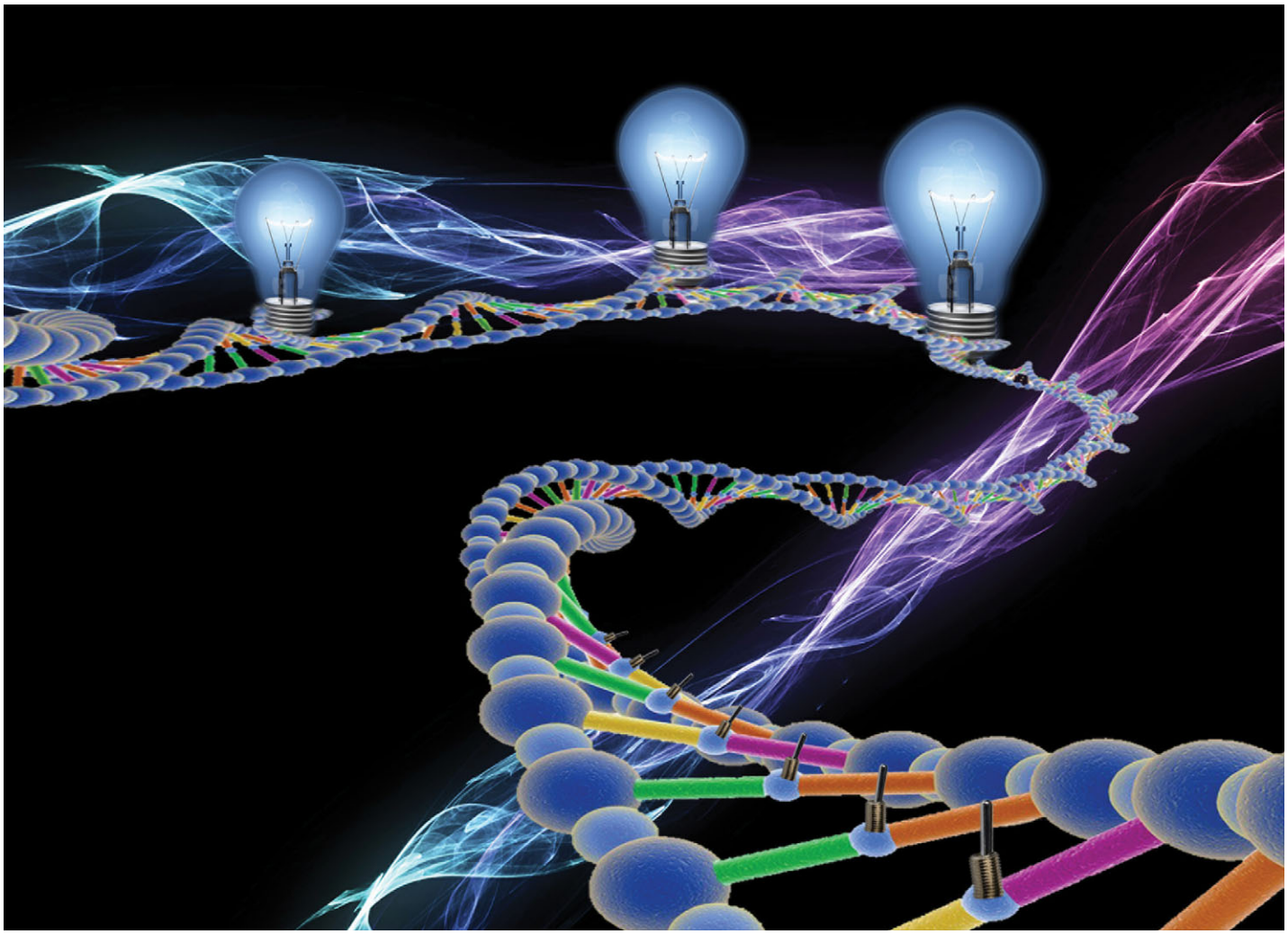

Featuring work from the research groups of Dr Hogan Yu and Dr Dipankar Sen at Simon Fraser University, Vancouver, Canada.

Functional DNA switches: rational design and electrochemical signaling

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Functional DNA switches: rational design and electrochemical signaling

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Recent developments in nanoscience research have demonstrated that DNA switches (rationally designed DNA nanostructures) constitute a class of versatile building blocks for the fabrication and assembly of electronic devices and sensors at the nanoscale. Functional DNA sequences and structures such as aptamers, DNAzymes, G-quadruplexes, and i-motifs can be readily prepared *in vitro*, and subsequently adapted to an electrochemical platform by coupling with redox reporters. The conformational or conduction switching of such electrode-bound DNA modules in response to an external stimulus can then be monitored by conventional voltammetric measurements. In this review, we describe how we are able to design and examine functional DNA switches, particularly those systems that utilize electrochemical signaling. We also discuss different available options for labeling functional DNA with redox reporters, and comment on the function-oriented signaling pathways.

Key learning points

(1) Recent progress in DNA nanoscience/nanotechnology has led to the discovery of DNA switches
(2) DNA switches are rationally designed dynamic nanostructures that are synthesized and assembled *in vitro*
(3) Aptamers, DNAzymes, G-quadruplexes, and i-motifs are the key modules to create DNA switches
(4) The conformational and conductivity variation of DNA switches can be transduced electrochemically upon redox-labeling
(5) DNA switches are governed by different function-oriented signaling pathways

1. Introduction

DNA, as the biological polymer for the storage and propagation of genetic information, has remarkable structural and functional properties; its highly specific base-pairing interactions allow for replication and transcription.[1] In the last few decades, however, DNA has gained increasing recognition as a powerful and versatile building block for the fabrication of self-assembled nanostructures.[2] A variety of DNA-based nanodevices have been designed and synthesized chemically. Among the first generation DNA nanodevices was one that exploited the B–Z transition (right-handed helix to left-handed helix) of DNA.[3] In this device, the relative positions of two double-crossover structures connected by a poly-CG sequence could be reversed by adding $[Co(NH_3)_6]^{3+}$. However, while DNA nanotechnology has made great strides in the fabrication of two- and three-dimensional static nanostructures, it still offers notable challenges in the design of dynamic nanostructures.[4]

DNA switches are "responsive" DNA constructs which can undergo structural or other physical property changes in the presence of certain external stimuli. A number of DNA nanodevices have been shown to respond to a variety of stimuli, including ions, proteins, and oligonucleotides. These devices are often constructed by way of incorporation of functional DNA modules, such as aptamers, DNAzymes, G-quadruplexes, and i-motifs. This review starts with a description of the structure and properties of such functional DNA modules that are distinct from conventional DNA double helices. We define herein DNA switches as specific sequences of DNA which adopt tertiary structures capable of various activities, including ligand recognition or chemical catalysis. When DNA switches are self-assembled on electrode surfaces, electrochemical methods can be used to monitor their switching behavior. In the following, we will first review various designs of functional DNA switches, from both DNA mechanical and biochemical perspectives. The redox labeling methods for carrying out electrochemical characterization will also be briefly discussed.

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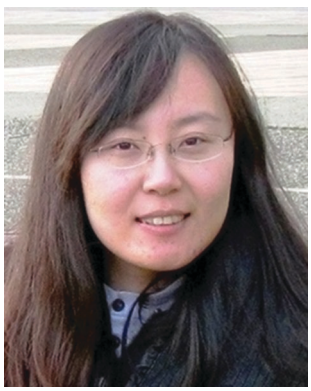
2. Functional DNA used for creating DNA switches

The majority of DNA nanodevices in the early years relied on hybridization-induced mechanical motions, where the role of DNA was to provide a structural frame and the chosen base sequence determines the movements concomitant with conformational changes. Thus, the DNA construct in most cases was relatively passive.[5] To progress to the next level of complexity, DNA nanodevices should be able to perform functional tasks that require the incorporation of active functional units (non-standard B-DNA) within these devices. DNA aptamers and DNAzymes are among the most widely known functional nucleic acids. Two other unique DNA structures that can be adapted as active components in DNA switches are G-quadruplexes and i-motifs. Below, we will elucidate the structural and functional properties of these "functional" DNAs, which will then enable us to understand the design principles of DNA switches (*vide infra*).

2.1. Aptamers

Aptamers are oligonucleotides which bind specifically to target molecules with high affinity. The targets can be organic dyes, amino acids, metal ions, proteins, and even whole cells.[6,7] The affinity range is from micromolar to picomolar in terms of the dissociation constant of the aptamer–ligand complex (K_d). Aptamers were first reported independently by Szostak and by Gold in the 1990s.[8,9] Since then, they have been widely recognized as highly promising building elements for a variety of important applications.

DNA aptamers for given targets are obtained by a methodology called *in vitro* selection or SELEX (systematic evolution of ligands by exponential enrichment).[10] As shown in Fig. 1, a very large



Yiting Tang

Yiting Tang graduated from Tongji University (Shanghai, P. R. China) with a BSc in Applied Chemistry in 2006 and an MSc in Physical Chemistry in 2009. After one-year study at Stony Brook University, she came to Simon Fraser University (Vancouver, Canada) and obtained her MSc degree in Analytical Chemistry in 2013 under the supervision of Dr Hua-Zhong "Hogan" Yu. Yiting has been working as a research associate for Vancouver Biotech Ltd. since 2012. Her research is focused on the development of DNA-based electrochemical biosensors/switches.

Bixia Ge

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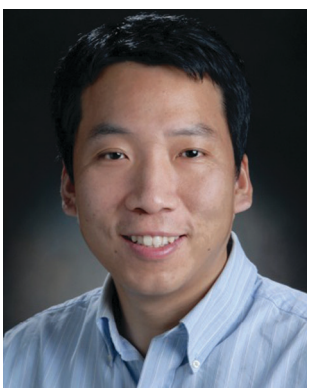
Dipankar Sen

Dipankar Sen is a professor in the Department of Molecular Biology & Biochemistry, jointly appointed in the Department of Chemistry at Simon Fraser University (British Columbia, Canada). Dr Sen did his undergraduate studies at the University of Cambridge, UK, and obtained his PhD from Yale University in 1985. From 1985 to 1991 he was a postdoctoral associate at Harvard University with Nobel Laureate Walter Gilbert. Dr Sen's research interests focus on DNA and RNA biochemistry, with special regard to novel structural and functional properties of these nucleic acids. More recently, Dr Sen has been a Fellow of the Canadian Institute for Advanced Research.



Hua-Zhong Yu

Hua-Zhong "Hogan" Yu is a professor in the Department of Chemistry at Simon Fraser University, Canada; he also holds joint appointments with 4D Labs and the Department of Molecular Biology and Biochemistry. Dr Yu received his PhD from Peking University (Beijing) in 1997, and carried out his postdoctoral research at the California Institute of Technology with Ahmed Zewail (1999 Nobel Laureate in Chemistry) and Fred Anson. Dr Yu's research spans a broad range of topics in physical, analytical, and materials chemistry; he is the recipient of a number of national and international awards in analytical chemistry and electrochemistry.

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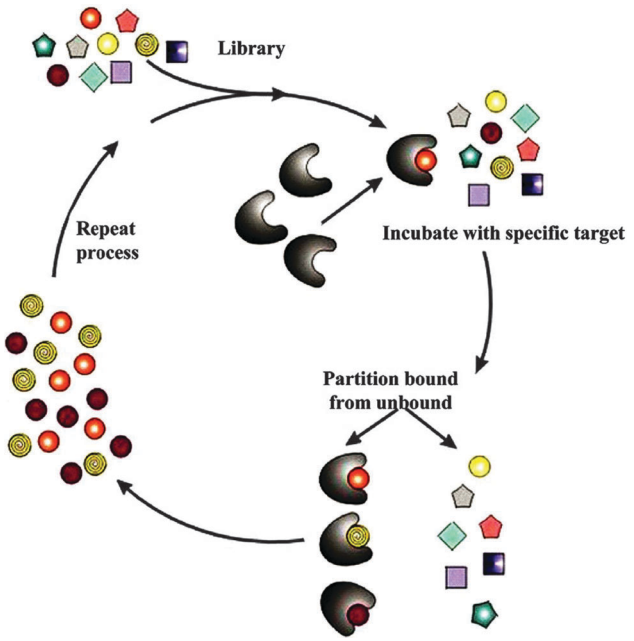

Fig. 1 The systematic evolution of ligands by exponential enrichment (SELEX) process to isolate nucleic acid aptamers from a large random sequence pool. Reproduced with permission from ref. 10. Copyright (2006), Elsevier.

(10^{13}–10^{15} individuals) random-sequence DNA library, in which the individual single-stranded DNA molecules have been allowed to fold into their secondary and tertiary structures, is typically allowed to flow through a column in which the target molecules are immobilized. Some DNA molecules bind to the target; those that do not bind are washed off. The bound DNA molecules are eluted from the column and amplified by PCR (polymerase chain reaction), and the duplex library thus produced is used to generate the single-stranded DNA library afresh. This process is repeated, iteratively, to yield highly specific target-binding DNA aptamers, which can be cloned and sequenced. To obtain high target-specificity, sometimes counter-selection is performed against a secondary target with similar structure and properties to the target, to ensure that only aptamers with very high affinity and specificity for the desired target are obtained. In counter-selection, alternating selection steps against target and the target analogue are carried out, such that those sequences that bind indiscriminately to the two can be eliminated from the selection pool. Once an aptamer has been identified by cloning and sequencing, it can be synthesized *de novo* by automated chemical synthesis. Its binding activity to the target and to target analogues can then be confirmed using different methods to validate its affinity and specificity.

Aptamers have many advantages compared to antibodies, which are biologically generated proteins capable of binding target molecules (antigens).[11] Aptamers do not require an immune response in host animals to obtain them, as they are produced chemically, in large quantities, by automated nucleic acid synthesis. Antibodies cannot be easily obtained for targets that are too small (*e.g.*, metal ions) or for molecules with poor immunogenicity or high toxicity. Although aptamers are selected for target binding *in vitro*, they are also capable of binding to their targets *in vivo* with good affinity. Besides, aptamers are readily chemically modified, which allows for their immobilization onto substrates with ease. For example, in the electrochemical detection, one of the popular electrode materials is gold; modification with a thiol group enables the formation of robust self-assembled DNA monolayers on gold. As a result, the electrochemical properties of immobilized DNA switches on gold electrodes can be directly characterized.[12]

The secondary and tertiary folding structures of aptamers are essential to their binding affinity and specificity. DNA aptamers typically undergo a conformational change upon target–ligand binding: they can fold to form internal loops, three-way junctions, or G-quartet structures, depending on their sequences and the structures of the target molecules they are binding. The capability of folding upon binding ensures the high affinity of aptamers for their targets. Small target molecules can be enfolded in the folded DNA structure. For larger target molecules such as proteins,[13] the folded DNA aptamer may bind to a particular epitope.

The functionality of aptamers has been used in the construction of DNA switches, which can controllably bind and release a target molecule. In principle, such a nanodevice can be constructed to respond to any protein or ligand for which an aptamer exists. They are widely regarded as ideal recognition elements for biosensing applications, including environmental analysis, food inspection, and medical diagnostics.

2.2. DNAzymes

DNAzymes are DNA-based enzymatic molecules that catalyze chemical and biological reactions including nucleic acid cleavage, ligation, and peptide bond formation. Although natural DNAzymes can be found, the majority of them have been obtained by *in vitro* selection techniques (SELEX), like aptamers. Since the early 1990s, many different DNAzymes have been screened, such as metal-ion-dependent DNA/RNA-cleaving DNAzyme,[14] histidine-dependent DNAzyme,[15] and DNA ligase DNAzyme.[16] We have also obtained a peroxidase-mimicking DNAzyme,[17] which has been extensively explored for analytical applications.[18] Versatile DNAzyme-based biosensing platforms for the detection of nucleic acid, protein and enzyme activity have recently been reported, which showed the true enzymatic multiple turnovers.[19,20] Even though DNAzymes have proved their usefulness in many applications, the detailed structure of DNAzymes and the catalytic reaction mechanisms are still largely unclear. Based on previous biochemical studies, it is believed that the reaction mechanism of most RNA-cleaving DNAzymes is metal-assisted general acid–base catalysis.[14]

These DNAzymes became new members of the enzyme family, joining protein enzymes and ribozymes, more importantly, they exhibit many intrinsic advantages over conventional protein enzymes, including cost-effective synthesis, high chemical stability, and easy molecular modification.[16,17] Most DNAzymes can be denatured and regenerated many times without losing their binding ability or activity toward substrates. All these features have made DNAzymes particularly attractive in diverse areas for versatile

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applications such as biosensing and nanoelectronics (*e.g.*, logic gate applications).

2.3. G-quadruplexes and i-motifs

G-quadruplexes are guanine-rich DNA sequences which involve the enthalpically favorable reorganization of guanines to form planar structures through hydrogen bonding.[21] The quadruplex structure is further stabilized by the presence of a metal cation, particularly K^+, which sits in the central channel between pairs of guanine tetrads. The G-quartet secondary structure exists in many aptamers, *e.g.*, the thrombin-binding aptamer selected by Bock *et al.*[22] has been shown using both NMR and X-ray crystallography to fold into a G-quartet structure in the presence of stabilizing salts and upon target binding.[23]

In a similar fashion, the i-motif structure was discovered by Guéron and co-workers in 1993,[24] in which four DNA strands are held together by an unconventional base pair between protonated and unprotonated cytosine bases. The difference compared to a G-quadruplex is that i-motif formation is "fuelled" by protons rather than by metal ions. It is based on the fact that cytosine can be protonated at the N3 position at low pH values (pK_a = 4.2). These protonated bases can form non-WC base pairs, for example, $C–C^+$. The incorporation of i-motifs into DNA nanomachines, in which they switch structure in response to pH changes, was initially demonstrated by Liu and Balasubramanian.[25] Below pH 6.5, the C-rich DNA strand forms an intramolecular quadruplex conformation in which $C–C^+$ base pairs occur in a staggered arrangement. At higher pH values, the C-rich DNA strand hybridizes with its complementary strand. Therefore, the DNA conformation can be cycled between an extended duplex state and a closed quadruplex state by subsequent addition of acid and base.

3. Electrochemical signaling of DNA constructs

For DNA switches, transduction of the switching event to a measurable signal is usually obtained in an optical form (typically, fluorescence). Traditional fluorescence-based readout methods of DNA switches not only require highly precise and expensive instrumentation but also involve sophisticated numerical algorithms to interpret the data. Recently, a number of innovative designs of electrochemical DNA switches have been reported. These types of devices combine DNA switches with electrochemical transducers to generate an electrical signal, and therefore provide a simple, accurate and inexpensive platform for applications such as medical diagnosis and environmental monitoring.

3.1. Advantages of electrochemical methods

A typical electrochemical device monitors electrical current variations produced by redox reactions occurring on the electrode surface. This approach has many advantages, including simplicity, rapidity, low cost and high sensitivity. In particular, electrochemical reactions yield an electronic signal directly; therefore, expensive signal transduction equipment is not needed. As functional DNA constructs can be immobilized readily on electrode surfaces, chip-based, miniaturized and portable systems can be developed accordingly.

For electrochemical DNA switches, one of the most frequently used electrode materials is gold, on whose surface thiolated DNA constructs can be tethered *via* strong sulfur–gold linkages.[12] The modification of DNA with a terminal thiol can be easily achieved using simple phosphoramidite chemistry, during the chemical synthesis of the oligos. DNA immobilization on a gold surface can be readily accomplished by immersing a cleaned gold slide in a thiolated DNA solution to form a self-assembled monolayer (SAM). Gold electrodes modified with mixed monolayers formed from thiolated DNA and alkanethiol alcohols produce significantly better quality (uniform packing and less nonspecific adsorption) than those comprised purely of thiolated DNA. Gold electrode surfaces passivated by longer alkanethiols (*e.g.*, $HS(CH_2)_{11}OH$) have been found to be more stable during long-term dry storage,[26] although shorter alkanethiol (*e.g.*, $HS(CH_2)_6OH$)–DNA mixed monolayers have been generally adopted.[26,27]

3.2. Redox labeling methods for DNA

Sensitive electrochemical signaling is usually based on the redox reactions of reporter molecules confined to the electrode surface. Different redox labeling methods for DNA have been developed in the past two decades. A DNA construct can be labeled with a redox reporter through a covalent linker or intercalation. Besides, the solution-diffused redox reporters can also be used to indirectly examine the property of DNA modified surfaces. The change in the electrochemical response of the redox reporter therefore signals the switching event occurring in a DNA nanostructure.

3.2.1. Covalently tethered redox systems. Various reporters, including ferrocene (Fc) and methylene blue (MB), have been covalently tethered to the distal terminus of DNA through flexible alkyl linkages. Because the mobility of the redox reporters is restricted, their position in the DNA film can be controlled by changing the DNA orientation or surface density. When the length of alkyl linkages is limited, the redox reaction of reporters can only be a surface-controlled process without the consideration of diffusive processes, which simplifies the interpretation of electrochemical data. More importantly, there is usually only one redox reporter on each DNA construct; therefore, it can be used to quantify the amount of DNA (in a 1 : 1 ratio with the reporter) on the electrode surface.

The reaction protocol for covalent tethering of Fc and MB is well established.[28,29] It involves the preparation of an-amino-modified DNA oligonucleotide and subsequent attachment of the redox reporter, *via* the amino functionality, to the terminal phosphodiester. For Fc, the utilization of *N*-hydroxysuccinimide (NHS) ester of ferrocenecarboxylic acid has been the gold-standard route. For MB, the NHS-ester of *N*-(carboxypropyl)methylene blue is typically prepared. Since the activated ester is unstable, it must be freshly prepared right before tethering to the amino-modified DNA.

3.2.2. Intercalation-based systems. Intercalation is the insertion of a ligand between the base pairs of a DNA duplex by a non-covalent interaction. The ligands, called intercalators,

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are mostly polycyclic, aromatic, and planar. Intensively studied DNA intercalators include daunomycin (DM) and methylene blue (MB). In a typical assay, thiolated DNA duplexes with matched or mismatched sequences are first self-assembled on a gold surface, and then treated with micromolar (μM) concentrations of a redox-active intercalator. Upon intercalation, the reporter can be electrochemically addressable.

Intercalated redox reporters are widely used for studying DNA-mediated charge transport. The intercalator can be used not only to report the amount of DNA on the surface, but also to distinguish between double- and single-stranded DNA. In addition, the coupling of intercalators into the π-stack of DNA critically affects the DNA-mediated electron transfer at long range. It has been demonstrated that the electrochemical signals of redox reporters are sensitive to DNA sequences and subtle perturbations in the base stacking;[30] structural damages, mismatches, and protein binding all result in less efficient charge flow within DNA.

It has been reported that DNA intercalators like DM or MB bind predominantly near the solvent-exposed terminus of the double-stranded DNA monolayer.[31] The diffusion of intercalators into the monolayer is inhibited by the tight packing of the DNA duplexes at high surface density. Thus, intercalators appear to be constrained to the top of closely packed DNA monolayers. However, at lower surface density, a larger signal is obtained for a redox-active DNA intercalator, which is likely due to increased access to the interior of the monolayer.

3.2.3. Solution-diffused systems. Negatively charged redox reporters, which are freely diffusing in solution, cannot get into close proximity with the DNA-modified electrode surface owing to electrostatic interactions. For example, $[Fe(CN)_6]^{3-/4-}$ ions in solution are electrostatically repelled by the negatively charged DNA phosphate backbone, and cannot reach the electrode surface. This leads to a high electron transfer resistance, which can be detected by the method of electrochemical impedance spectroscopy (EIS). However, upon incubation or binding to positively charged proteins, the diffusion of $[Fe(CN)_6]^{3-/4-}$ ions towards the electrode surface is facilitated which results in a decrease in the electron transfer resistance.[32]

Another solution-based redox system, $[Ru(NH_3)_6]^{3+/2+}$, is often used as a reliable measure to report the amount of DNA on the electrode surface.[33] The $[Ru(NH_3)_6]^{3+}$ cations diffuse to the electrode surface and bind electrostatically to the DNA backbone. Their characteristic surface redox reaction can be adapted to discriminate the contribution of solution-diffused redox centers: DNA dehybridization can be monitored by a decreased signal from the electrostatically bound $[Ru(NH_3)_6]^{3+}$ (Fig. 2). Besides, the surface densities of both single- and double-stranded oligonucleotides can be determined by integration of the reduction peak of $[Ru(NH_3)_6]^{3+}$ to $[Ru(NH_3)_6]^{2+}$. Following incubation in $[Ru(NH_3)_6]^{3+}$ with a concentration of 5.0 μM or lower at low ionic strength, the surface concentration of $[Ru(NH_3)_6]^{3+}$, Γ_{Ru}, can be calculated using the following equation:

$$\Gamma_{Ru} = \frac{Q}{nFA} \quad (1)$$

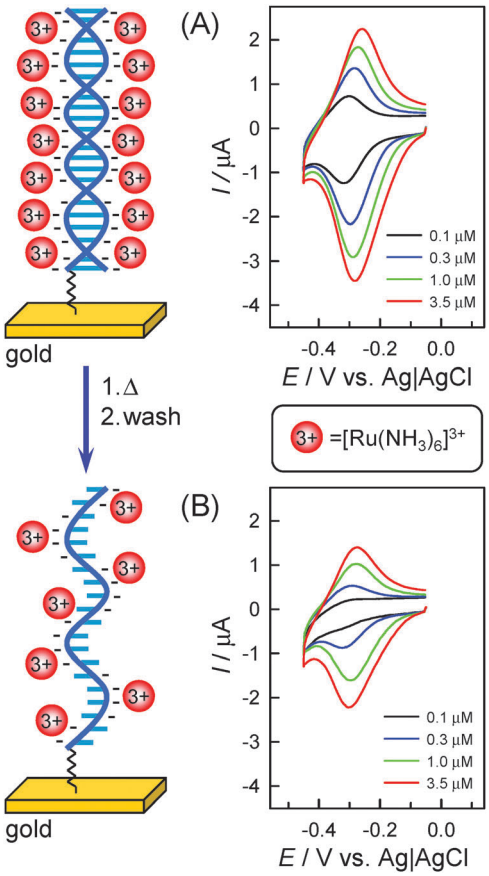

Fig. 2 Schematic representation and voltammetric response of $[Ru(NH_3)_6]^{3+}$ at different concentrations electrostatically bound (A) to dsDNA- and (B) to ssDNA-modified gold electrodes in 10 mM Tris buffer (pH 7.4). Scan rate = 50 mV s^{-1}. Reproduced with permission from ref. 33. Copyright (2003), American Chemical Society.

where Q is the charge obtained by integrating the reduction peak area of surface-bound $[Ru(NH_3)_6]^{3+}$, n is the number of electrons involved in the redox reaction (which is 1), F is Faraday's constant, and A is the electrode area. This equation assumes the following:[34] the interaction between $[Ru(NH_3)_6]^{3+}$ is purely electrostatic; there is complete exchange of $[Ru(NH_3)_6]^{3+}$ with compensation by Na^+ ions; full saturation of the DNA-modified surface with $[Ru(NH_3)_6]^{3+}$ occurs; and, every phosphate molecule is accessible for electrostatic binding to the redox cations.

Under saturation conditions (*i.e.*, where the highest possible concentration of surface-bound $[Ru(NH_3)_6]^{3+}$, Γ_{Ru}, was achieved), the measured Γ_{Ru} value can be converted to the surface density of DNA, Γ_{DNA}, using the following equation:

$$\Gamma_{DNA} = \Gamma_{Ru}\left(\frac{z}{m}\right)N_A \quad (2)$$

where z is the valence of the redox cation (+3) and m is the number of nucleotides in the DNA.

3.2.4. Comparison between the redox labeling methods. In comparison, all three redox labeling methods have their own advantages and disadvantages. From the preparation point of view, either solution-diffused redox ions or intercalated redox ligands can

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be simply added to the electrolyte before testing, without any additional activation and coupling reactions. The solution diffused systems as discussed above, in particular, are solely dependent on the electrostatic interactions between the redox ions in solution and the surface charges (the phosphate backbone), and they cannot distinguish different sequences or conformations.[32,33] Nevertheless, useful information about electrode-bound DNA switches can be readily obtained with this method including the determination of the interfacial electron transfer resistance with redox anions and the estimation of surface packing densities using redox cations. The intercalated redox ligands typically bind to double-stranded DNA only at particular sites (with preferential base-pairs),[31] which can be used to differentiate between single-stranded and double-stranded DNA targets. The covalently tethered redox labeling, on the other hand, involves additional modification and coupling steps. It is the best option to study structural-dependent electronic properties of DNA switches; the definite position and quantity of the redox labels on each DNA switch are critical in the evaluation of different charge transfer pathways.

4. Rational design of electrochemical DNA switches

In functional DNA-based electrochemical switches, DNA constructs undergo various structural or conformational changes in the presence of external stimuli leading to electrochemical signal changes. In the following section, electrochemical DNA switches are classified into three categories based on the type of functional DNA and its property: aptamer–ligand binding, DNAzyme substrate cleavage, and quadruplex formation. To facilitate a general understanding of their respective function and signaling mechanism, in Table 1 we have summarized the various DNA switches by listing the targets, recognition elements, and testing methods.

4.1. Aptamer–ligand binding

When aptamer-containing DNA constructs are immobilized on electrode surfaces, the binding of a ligand to the aptamer may induce structural changes. With redox-active reporters, the signal switching can be monitored by conventional electrochemical methods. The DNA constructs are either double-stranded or single-stranded, in which ligand binding induces the dissociation of duplexes or the folding of single strands.

4.1.1. Dissociation of double-stranded DNA constructs. DNA aptamers selected by *in vitro* selection experiments (SELEX) typically have a high affinity for their respective binding ligands. Thus, single-stranded DNA aptamers may participate in two different types of molecular recognition events: (i) binding to their ligand and (ii) binding to their Watson–Crick complementary strand. This competition can be used to

Table 1 Classification of electrochemical DNA switches

Category	Target/ligand	Recognition element	Redox reporter	Method	Signal (+/−)	Ref.
Aptamer–ligand binding	ATP	Adenosine aptamer in duplex	Fc	SWV	(+)	37 Fig. 4A
	Adenosine	Adenosine aptamer in partial duplex	Fc	CV	(−)	38 Fig. 4B
	Adenosine	Adenosine aptamer in partial duplex	$[Ru(NH_3)_6]^{3+}$	CC CV	(−)	39 and 40
	Adenosine	Adenosine aptamer in partial duplex	$[Fe(CN)_6]^{3-/4-}$	EIS	(−)	41
	Adenosine or thrombin	Adenosine or thrombin aptamer in duplex	Fc		(+)	42 Fig. 5
	Thrombin	Thrombin aptamer in duplex	Fc	DPV CV	(−)	43
	Thrombin	Single-stranded thrombin aptamer	MB	ACV	(−)	44 Fig. 6A
	Cocaine	Single-stranded cocaine aptamer	MB	ACV	(+)	45 Fig. 6B
	Thrombin	Single-stranded thrombin aptamer	Fc	CV, DPV EIS	(+)	46
DNAzyme substrate cleavage	Pb^{2+}	DNAzyme and substrate strands	MB	ACV	(+)	47 Fig. 7A
	L-Histidine	DNAzyme and substrate strands	Fc	SWV	(+)	48
	Mg^{2+}	DNAzyme and substrate strands	Fc	CV, EIS DPV	(−)	50
	Pb^{2+}	GNP-attached substrate and DNAzyme strand	$[Ru(NH_3)_6]^{3+}$	CV	(−)	51 Fig. 7B
Quadruplex formation	Pb^{2+}	G-rich hairpin DNA	$[Fe(CN)_6]^{3-/4-}$	EIS	(+)	52
	H^+	i-Motif DNA attached to SWCNTs	C^+:C(C-rich i-motif)	CV	(+)/(−)	53
	K^+	Contractile dsDNA/G4-DNA	Fc	SWV	(+)	55

Abbreviations: (+), electrochemical signal increased upon target binding; (−), electrochemical signal decreased upon target binding; ACV, alternating current voltammetry; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CC, chronocoulometry; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; Fc, ferrocene; GCE, glassy carbon electrode; GNPs–GNSs, gold nanoparticles–graphene nanosheets; MB, methylene blue; SWCNTs, single-walled carbon nanotubes; SWV, square wave voltammetry.

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construct aptamer-based DNA switches. This type of switch starts from a fully or partially double-stranded DNA construct with one strand incorporating an aptamer sequence. Upon incubation with the ligand, the aptamer strand selectively binds the ligand, concomitant with the dissociation of the DNA duplex. Afterwards, the DNA switch can be regenerated by hybridization with the leaving strand in the absence of the ligand.

The adenosine aptamer selected by Huizenga and Szostak in 1995 has been studied extensively in the context of this type of switch.[35] This aptamer binds adenosine, adenosine monophosphate (AMP) and adenosine triphosphate (ATP), with comparable affinities. The binding of two adenosine molecules results in the folding of the aptamer strand and formation of a partial double-stranded region (Fig. 3).[36] Researchers have taken advantage of this unique tertiary structure to develop aptamer-based sensors or nano-switches. Zuo *et al.* tethered ferrocene to the surface-bound aptamer strand which was hybridized with its fully complementary strand.[37] In its initial state, ferrocene was far from the electrode surface. Upon binding ATP, the aptamer strand dissociated from the double helix and folded back, bringing the ferrocene closer to the electrode (Fig. 4A). A remarkable increase in the current was monitored by square wave voltammetry (SWV) upon increasing the concentration of ATP. With an aptamer strand modified with ferrocene close to the electrode surface, Liu *et al.*[38] discovered that upon binding adenosine, the aptamer strand dissociated from the surface-bound partially complementary strand, and the electrochemical signal of ferrocene decreased, as characterized by cyclic voltammetry (CV) (Fig. 4B).

Redox labeling methods other than covalent tethering have also been used in this type of DNA switches, including solution-diffused and intercalated redox reporters. The dissociation of a partial double-stranded DNA construct results in the decrease of integrated charge of the surface-bound $[Ru(NH_3)_6]^{3+}$ cations,

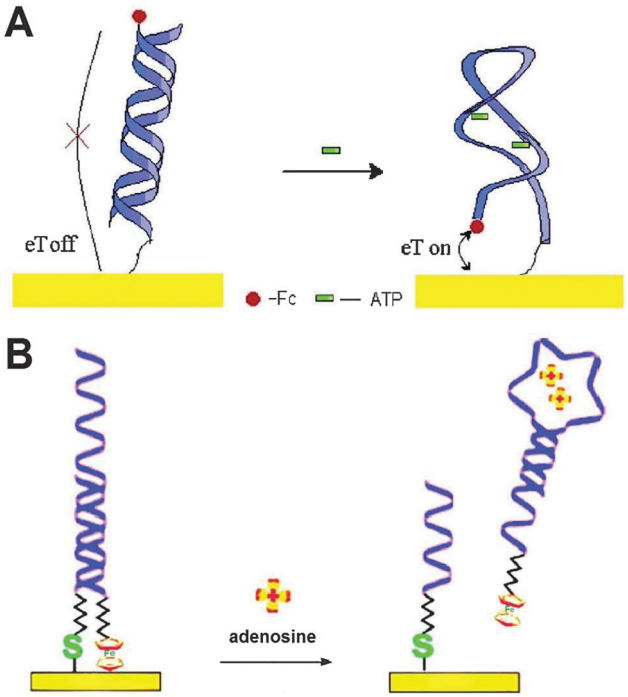

Fig. 4 Double-stranded aptamer-based electrochemical switches with covalently tethered ferrocene based on duplex dissociation upon adenosine–ATP binding. Ferrocene is modified at the (A) distal and (B) proximal end of the aptamer strand. (A) Reproduced with permission from ref. 37. Copyright (2007), American Chemical Society. (B) Reproduced with permission from ref. 33. Copyright (2009), Elsevier.

which can be monitored by chronocoulometry (CC)[39,40] and cyclic voltammetry (CV).[40] And the decrease of electron transfer resistance can be monitored by electrochemical impedance spectroscopy (EIS).[41]

Another popularly investigated system, the anti-thrombin aptamer, is known to fold into a G-quadruplex upon thrombin binding.[23] Lu *et al.*[42] immobilized double-stranded DNA constructs in which the aptamer strand was not labeled, while the complementary strand was labeled with a ferrocene at the distal end and with thiol at the proximal end. Upon binding thrombin, the aptamer strand dissociated from the surface; the complementary strand then folded into a hairpin in the presence of Mg^{2+}, and the ferrocene moved much closer to the electrode surface resulting in a higher electrochemical signal (Fig. 5). When the anchored strand on the surface was the aptamer strand, the complementary strand was released, along with its tethered ferrocene. A decrease in the current was observed in the presence of thrombin.[43] These surfaces are also reusable with regeneration by repeating the on-chip hybridization of the leaving DNA strand.

4.1.2. Folding of single-stranded DNA constructs. The advantages of aptamer-based DNA switches consisting of single strands, over double-stranded DNA, are (1) the DNA conformational changes upon ligand binding are simple and do not involve dissociation of the duplex; (2) regeneration of the surface is easier and does not require rehybridization of the dissociated strand.

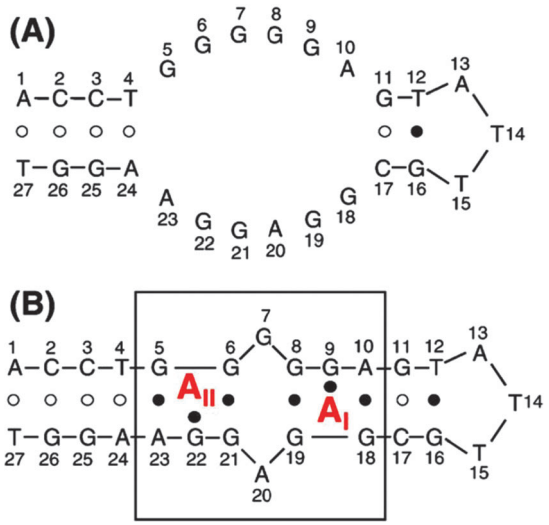

Fig. 3 Secondary structure of the adenosine aptamer (A) before and (B) after binding to adenosine at the two non-equivalent sites (A_I and A_{II}). Reproduced with permission from ref. 36. Copyright (1997), Elsevier.

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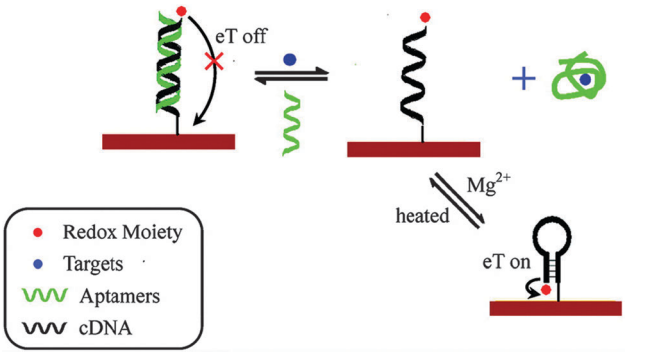

Fig. 5 Aptamer-based electrochemical switch based on the formation of a hairpin structure with ferrocene attached at the distal end of the complementary strand. Reproduced with permission from ref. 42. Copyright (2008), American Chemical Society.

A single-stranded aptamer immobilized on a gold electrode surface typically folds into a unique tertiary structure in the presence of its ligand. Plaxco and co-workers designed two aptamer-based electrochemical switches against the protein thrombin and the small molecule cocaine,[44,45] respectively (Fig. 6). Each is comprised of a specific DNA aptamer which is modified at its 5′-terminus with a thiol group and at its 3′-terminus with a MB molecule. In the absence of the ligand, the aptamers are thought to be entirely (thrombin) or partially (cocaine) unfolded. Upon ligand binding, the aptamer folds into a G-quadruplex (thrombin) or three-way junction (cocaine),

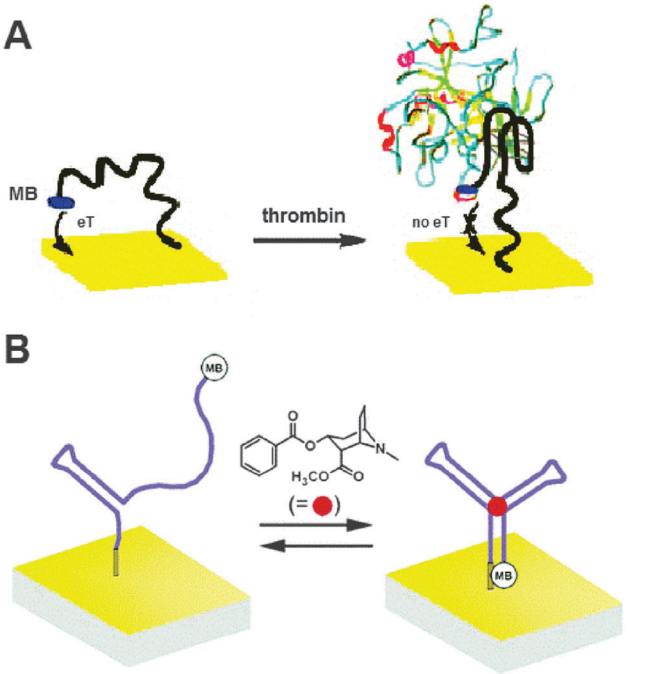

Fig. 6 Single-stranded aptamer-based electrochemical switches with covalently tethered methylene blue based on folding of the aptamer strand upon ligand binding: (A) thrombin; (B) cocaine. (A) Reproduced with permission from ref. 44. Copyright (2005), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Reproduced with permission from ref. 45. Copyright (2006), American Chemical Society.

and relocates the position of the redox reporter relative to the electrode surface. When MB moves farther away from the gold surface upon thrombin binding, a decrease in the electrochemical current can be measured by alternating current voltammetry (ACV); when MB moves closer to the gold surface upon cocaine binding, the current increases. Regeneration of these systems can be easily realized by simply washing the surface using 6 M guanidine hydrochloride (in the case of thrombin), or immersion in ligand-free buffer (in the case of cocaine).

In contrast to the thrombin aptamer-based electrochemical switch of Xiao *et al.*,[44] Radi *et al.*[46] designed an electrochemical switch using a shorter 15-nucleotide thrombin aptamer. As a result of this modification, the electrochemical signal does not decrease but rather increases with increasing concentrations of thrombin. The aptamer was immobilized *via* the 5′-thiol group onto a gold electrode, which was then passivated with 2-mercaptoethanol, forming a mixed monolayer. Because of the short length of the aptamer, folding of the aptamer upon thrombin binding brought the 3′-ferrocene moiety closer to the electrode surface, thus increasing the electrochemical signal. The switch was characterized using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS). In addition, the switch could be fully regenerated by simply unfolding the aptamer in 1.0 M HCl, and there was no loss in electrochemical signal upon subsequent thrombin binding, even after being regenerated up to 25 times.

4.2. DNAzyme substrate cleavage

Electrochemical DNA switches based on DNA-cleaving DNAzymes usually involve the dissociation of double-stranded DNA constructs comprised of a DNAzyme strand and its substrate strand.[47] Xiao *et al.* reported a DNAzyme-based electrochemical switch for Pb^{2+} detection (Fig. 7A).[48] The DNAzyme strand was labeled with a redox-active reporter (MB) and immobilized on a gold electrode *via* a thiol–gold linkage. The DNAzyme was then hybridized to its substrate strand, prohibiting any "contact" between MB and the electrode. In the presence of Pb^{2+}, the substrate was cleaved and the fragments were released. Such release made the enzyme strand more flexible and shortened the distance between the redox label and the electrode, leading to a higher current signal proportional to the concentration of Pb^{2+} present.

A novel design for the detection of L-histidine was reported by Liang *et al.*[49] based on a switching structure of DNAzyme and the gold nanoparticle–graphene nanosheet (GNP–GNS) composite. Before immobilization of the thiolated DNA duplexes, GNPs were deposited on the surface of GNSs through chemical reduction of chloroauric acid by sodium citrate; a glassy carbon electrode (GCE) was then modified with the GNPs–GNSs. The introduction of L-histidine induces self-cleavage of the DNAzyme on the GNPs–GNSs/GCE, and the redox reporter ferrocene approaches the electrode surface, subsequently. Because of the excellent conductivity and high surface area of graphene, the distribution of GNPs on GNSs

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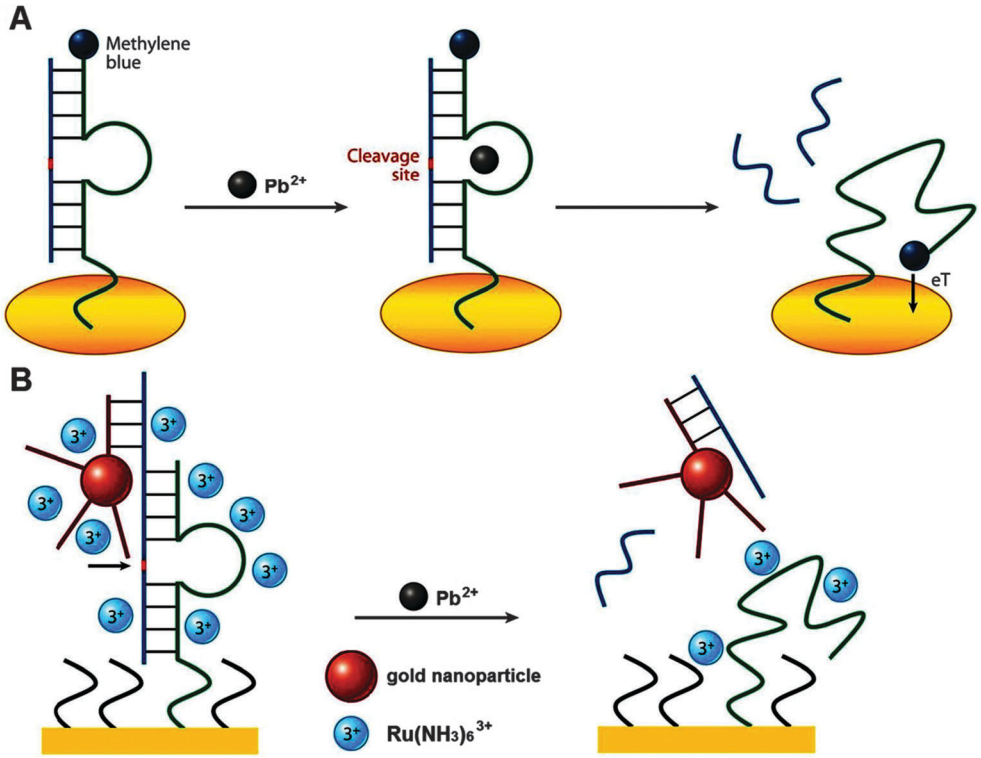

Fig. 7 DNAzyme-based electrochemical switches for the detection of Pb^{2+} with (A) covalently tethered methylene blue and (B) solution-diffused $[Ru(NH_3)_6]^{3+}$. (A) Reproduced with permission from ref. 48. Copyright (2007), American Chemical Society. (B) Reproduced with permission from ref. 51. Copyright (2008), American Chemical Society.

made the fabricated switch exhibit an expanded linear range and excellent sensitivity for the detection of L-histidine.

The redox reporter at the end of the substrate strand can also be modified. Gao *et al.*[50] developed a DNAzyme-based switch for magnesium ions (Mg^{2+}). Their system consisted of Mg^{2+}-dependent DNAzyme and a ferrocene-modified DNA substrate strand. In the absence of Mg^{2+}, the obvious faradaic current was obtained. In the presence of Mg^{2+}, the substrate was cleaved and removed resulting in a reduction of the faradaic current. The current decreased with increasing Mg^{2+} concentration, permitting a quantitative determination of the Mg^{2+} concentration.

Shen and co-workers[51] employed short oligonucleotides functionalized with gold nanoparticles to improve the sensitivity of the DNAzyme-based switch for the detection of Pb^{2+} (Fig. 7B). The DNAzyme hybridizes to the partially complementary substrate strand which also hybridizes to a short DNA strand with a gold nanoparticle. Because each nanoparticle carries a large number of short DNA strands which can bind to the redox reporter $[Ru(NH_3)_6]^{3+}$, more $[Ru(NH_3)_6]^{3+}$ cations bind to the DNA construct on the surface, and a higher electrochemical signal is obtained before Pb^{2+} is added. Upon binding Pb^{2+}, the DNAzyme catalyzes the hydrolytic cleavage of the substrate strand, resulting in its removal (along with the short DNA strands) from the electrode surface. As a result, fewer $[Ru(NH_3)_6]^{3+}$ cations are confined on the electrode surface, and a lower electrochemical signal is expected. The surface may be regenerated by hybridization after the substrate cleavage, allowing multiple switching cycles.

4.3. Quadruplex formation

The formation of unique G-quadruplex or i-motif quadruplex structures for G-rich or C-rich sequences has also been employed in the design of DNA-based conformational switches. Switches containing the anti-thrombin aptamer are not included in this section. Lin and co-workers[52] developed a DNA conformational switch from G-rich hairpin DNA induced by Pb^{2+}. Upon incubation with Pb^{2+}, the G-rich hairpin DNA opens the stem-loop and forms a G-quadruplex structure, which gives rise to a sharp increase in the electron transfer resistance determined by electrochemical impedance spectroscopy (EIS) using $[Fe(CN)_6]^{3-/4-}$ as the redox reporter. Shin *et al.*[53] reported pH induced conformational changes of an i-motif DNA nanomachine attached to single-walled carbon nanotubes (SWCNTs). With increasing pH, the protonated cytosines of the i-motif are deprotonated and the DNA strand adopts a random coil structure. Cyclic voltammetry also indicated that the electrochemical signal decreases at the SWCNT–DNA hybrid-modified glassy carbon electrode. Varying the pH between 5 and 8 resulted in reversible switching demonstrating its potential application as switchable pH-controlled electrode with switchable redox activity.

A G-quadruplex can be stabilized by coordination with specific metal ions, such as K^+ or Sr^{2+} ions, which bind between

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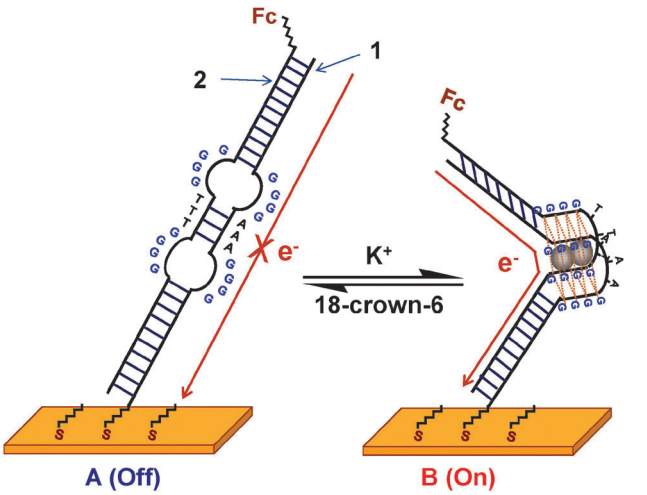

Fig. 8 A contractile DNA nanoswitch between a structurally extended "off" state (a duplex) and a contracted "on" state (a G-quadruplex) on gold controlled by the addition and removal of K^+ ions. Reproduced with permission from ref. 55. Copyright (2010), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

successive G-quartets. We have constructed a contractile DNA nanoswitch which switched repeatedly between "off" (an extended duplex) and "on" (a G-quadruplex) without strand dissociation or duplex regeneration.[54,55] The transition between duplex and G-quadruplex was controlled by the addition and removal of K^+ ions (Fig. 8). The electrochemical properties of a ferrocene-labeled DNA nanoswitch immobilized on gold were investigated by square wave voltammetry (SWV). After treatment with K^+ ions, G-quadruplex formation gave rise to a much higher oxidation current. The subsequent addition of a potassium chelator ([18]crown-6) reversed the contraction induced by the K^+ ions and restored the signal to its original low level. Reversible extension and contraction of the contractile DNA switch can therefore be repeatedly realized at the solid–liquid interface, and monitored by electronic measurements.[55]

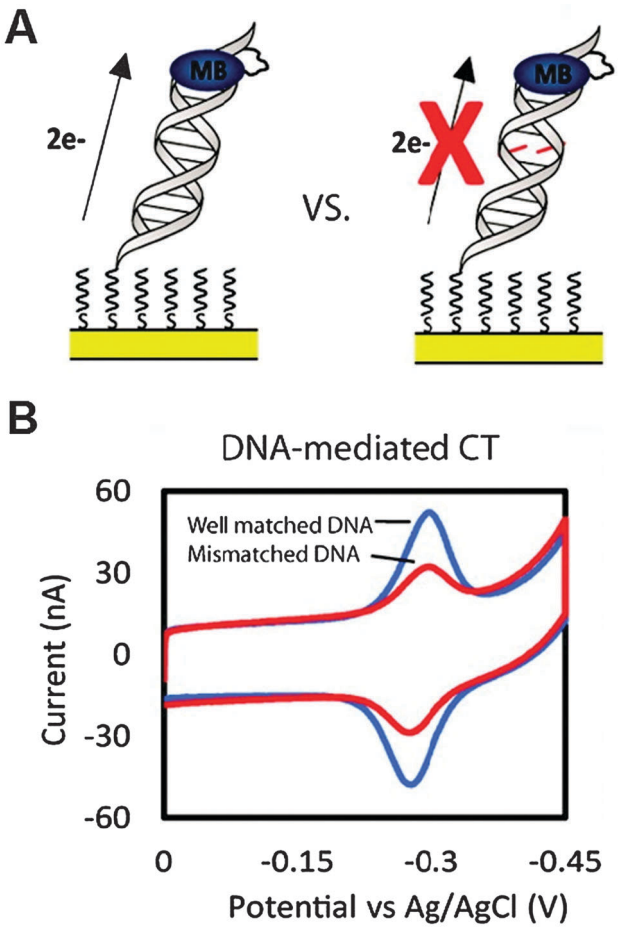

Fig. 9 (A) Schematic representations and (B) cyclic voltammograms acquired at 100 mV s^{-1} for a well-matched DNA sequence and a sequence containing a single mismatch on the gold surface. Reproduced with permission from ref. 29. Copyright (2012), American Chemical Society.

5. Signal-switching mechanisms of electrochemical DNA switches

The observed signal switching induced by functional DNA is believed to be due to possible changes of (1) the DNA-mediated electron transfer rate, (2) the distance between the redox reporter and the electrode surface, and (3) electrostatic interactions.

5.1. Changes in the DNA-mediated electron transfer rate

Barton and co-workers[29,30] have reported that electrons transfer through continuous base stacks of DNA, and that any perturbations such as mismatches will decrease the overall charge passed. Therefore, the introduction or removal of perturbations is one of the causes of signal switching. The ability of DNA to mediate electron transfer is taken advantage of to detect even a single-base mismatch. When the base pair stack is intact, current can flow between the redox reporter and the electrode surface. A single-base mismatch decreases the current significantly (Fig. 9).

5.2. Changes in the redox-to-surface distance

In most functional DNA-based electrochemical switches, the distance between the redox reporter (attached at the end of the DNA probe) and the electrode surface is of key importance for signal transduction. Plaxco and co-workers[44,45,48] have reported that when this distance is short, the redox reporter can be reduced directly at the surface and electron transfer is facilitated, leading to a high rate constant and electrochemical signal. Anne and Demaille have shown that DNA flexibility is related to the redox-to-surface distance and the electron transfer rate.[56,57] Since single-stranded DNA is more flexible than double-stranded DNA, double-stranded DNA-based switches often dissociate first to the more flexible single strand, leading to a short redox-to-surface distance. Pheeney *et al.*[29] recently reported direct reduction of a redox reporter modified at the distal end of double-stranded DNA on gold at low DNA surface density.

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5.3. Changes in electrostatic interactions

It is known that the DNA phosphate backbone has negative charges, which interact electrostatically with solution-diffused ions. The negative charges on the electrode surface can be diminished by binding to positively charged proteins[32,34] or by dissociation of DNA strands.[39–41] The electrostatic repulsion between DNA and redox anions like $[Fe(CN)_6]^{3-/4-}$ ions is lowered owing to less negative charge on the surface, resulting in a lower electron transfer resistance as measured by electrochemical impedance. Electrostatic attraction binds cations like $[Ru(NH_3)_6]^{3+}$ to the DNA on the surface.[28] Thus, the amount of $[Ru(NH_3)_6]^{3+}$ cations measured by cyclic voltammetry (CV) reflects the amount of negative charges on DNA.[33,34]

6. Challenges and future directions

In evaluating the relative benefits and challenges of the various DNA switching paradigms reviewed above, whereby aptamers or DNAzymes are adapted to generate ligand-dependent electrochemical signals, each of the designs (with the corresponding mechanism) described above brings with its certain strengths as well as shortcomings. For instance, some of the switches provide a positive signal output in response to a cognate ligand's binding, whereas others report a decrease of signal in response to such recognition event. Some generate signal in a straightforward manner, requiring only addition of the ligand to the DNA switch in question, while others require the addition, removal, or replenishment of accessory components, such as complementary DNA strands. A further issue of importance is versatility. Aptamers, in general, come in many sizes and shapes, which undergo different degrees of conformational change upon binding their cognate ligand. Some of these changes are of global scale, while others show relatively little overall structural variation. To be widely applicable, a switching methodology should ideally be maximally versatile, *i.e.*, is able to work using a broad spectrum of aptamers. In our view, the following features are particularly desirable in a ligand-sensing DNA switch: (a) a "signal-on" output is generally more valuable than a "signal-off" output; (b) a simpler system, involving fewer components, is likely to prove more robust than a more complex one; and, (c) a switch whose design features afford the greatest versatility, in terms of both aptamer choice and ligand range, is also likely to be the most useful.

As for the G-quadruplex and i-motif-based DNA switches described above, they represent, in themselves, excellent demonstrations of mechano-electronic nano-devices. In principle, they can also be adapted for vital sensing purposes. Small molecular ligands that specifically bind to and stabilize G-quadruplexes have been shown to inhibit the activity of telomerase enzymes within living cells. These ligands therefore represent a new class of potential anti-cancer pharmaceuticals, and there is a broad research effort worldwide to find the best of them.[58] The G-quadruplex forming contractile DNA switches, described above, should be adaptable for the large-scale electronic screening of G-quadruplex-binding ligands. i-Motifs, too, have recently been implicated in the control of expression of certain genes.[59] Therefore, the i-motif forming DNA switches may find eventual utility towards the screening of i-motif stabilizing ligands.

The future of the methodologies of constructing and testing DNA switches reviewed in this paper is likely to be multi-faceted. One immediate field of application, described above, lies in their evident utility as versatile biomedical and environmental sensors. In fact, many of the switches described here may find a longer-term utility in the construction of mechano-electronic devices on a nano-scale. They may also function in the creation of a class of DNA-based logic gates capable of providing electronic outputs. These latter applications might prove particularly useful in computational systems in which DNA has been found to be a powerful raw material.[60]

7. Conclusion

To date, functional DNA has been used extensively as building block for the synthesis and assembly of a variety of nanoscale devices. To progress to the next stage of complexity, DNA devices should be able to perform diverse tasks, including electrochemical signal switching. Redox reporters for electrochemical signaling can be covalently tethered, intercalated or solution diffused. Functional DNA-based electrochemical switches are dynamic devices which reversibly undergo structural changes in the absence and presence of certain external stimuli. The designs of these various switches are categorized in this review, based on the types of functional DNA. The binding of the ligand to the aptamer induces either the dissociation of double-stranded DNA constructs or the folding of single-stranded DNA constructs. The DNAzyme-based electrochemical switches involve the cleavage and dissociation of the substrate strand in response to ligand binding. Quadruplexes (G-quadruplex and the i-motif) can be formed in G-rich or C-rich DNA sequences, respectively, and their formation can also be switchable. The mechanisms of electrochemical signal changes due to structural switching include DNA-mediated electron transfer, changes in the redox-to-surface distance, and electrostatic interaction changes.

Acknowledgements

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