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Bio-Inspired DNA Nanoswitches and Nanomachines: Applications in Biosensing and Drug Delivery

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6.1 Introduction

Nature has evolved sophisticated biomolecular switches and nanomachines through billions of years of evolution that can change the shape or activity in response to various stimuli such as temperature, light, pH changes, small molecules, proteins and hormones. One example of such class of natural molecular switches is G protein-coupled receptors (GPCRs), a large protein family composed of more than 1,000 members [1,2]. These proteins can sense and respond to a plethora of extracellular stimuli to activate various biological outputs inside the cell (Figure 6.1). Such bio-nanosystems are of great inspiration to engineers interested in building nanosystems that produce useful output in response to specific chemical signal.

Nanoengineers have explored all types of chemistry in an attempt to recreate switches and nanomachines found in nature (see for example 2016 Nobel Prize [3–6]). While protein chemistry should, in principle, represent a material of choice to do so, it has revealed to be much more complicated than expected: using polypeptides to build useful switches from scratch remains extremely challenging [7–10]. Other types of chemistry have been also exploited with relatively good success (e.g. see 2016 chemistry Nobel Prize), but the switches and nanomachines engineered so far often remain simple proof of concept [3–6]. In recent years, DNA chemistry, with its high programmability and life-compatible features, has risen into a "happy medium" biopolymer that enables to rationally engineer a wide variety of useful nanomachines and molecular switches [11–18]. Our capacity to create specific DNA structures with defined thermodynamics has played a major role in propelling this research field into the spotlight [13,19,20]. Due to its simplicity, DNA chemistry has also enabled to recreate many highly complex signaling mechanism employed by natural switches and nanomachines (e.g. allosteric mechanism, population-shift regulation [21–26] (Figure 6.2)).

Artificial DNA switches are generally programmed and engineered to generate a measurable signal (e.g. fluorescent, electrochemical) or to trigger a precise activity such as drug release in response to a specific input stimulus. They typically alternate between two conformations often referred to "OFF" and "ON". These switches are now used as tools for biosensing (e.g. Kramer et al. 1996 molecular beacon for medical diagnostic [27]), fundamental research and drug delivery applications (see Ref. [12]) for a recent review on this field). In this chapter, we will briefly exemplify why DNA is an ideal polymer to build a wide range of useful nanotechnology. We will explain how we design and engineer DNA switches for useful biological applications such as biosensing and drug delivery. Finally, we will conclude with an outlook on the future of DNA switches and nanomachines in the field of medicine.

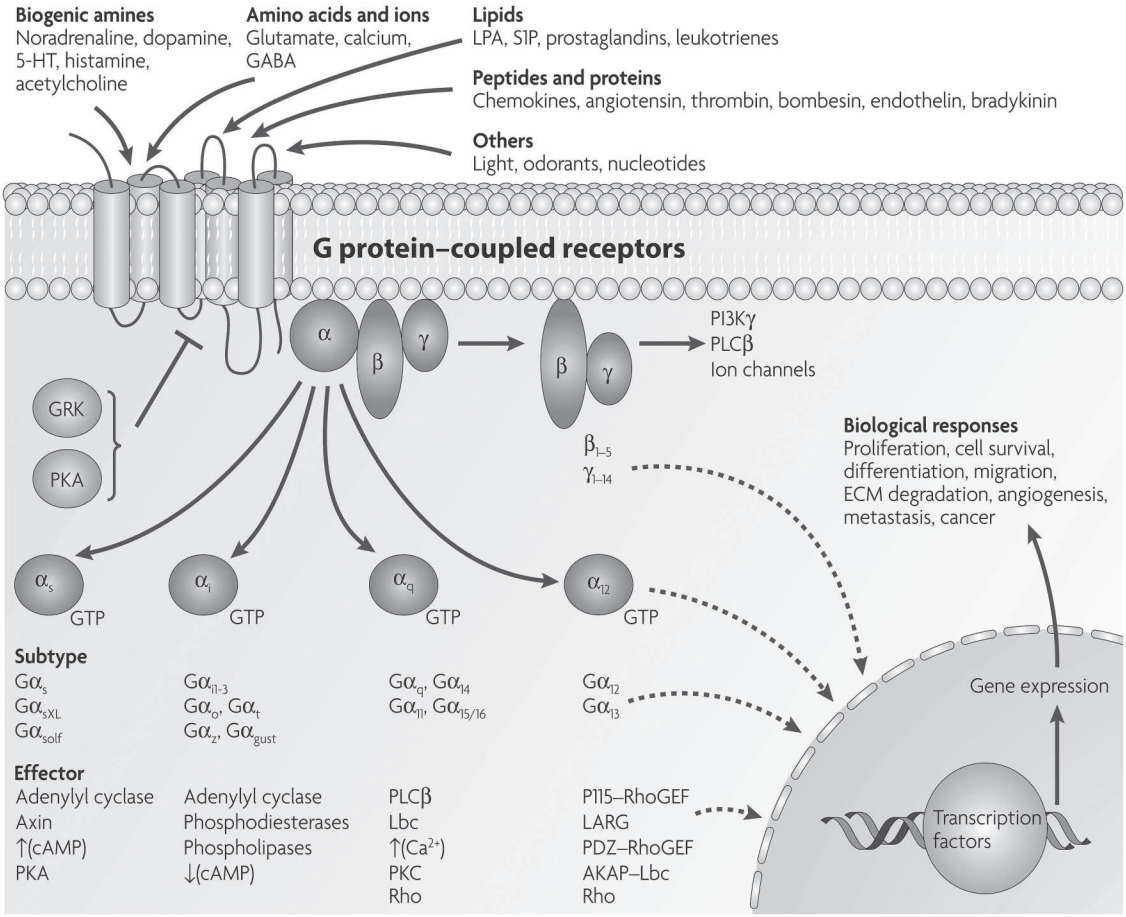

FIGURE 6.1 GPCRs are biomolecular switches that sense a wide variety of input stimuli and transduce this information into a wide variety of signal output. These represent a great inspiration for building nanoswitches or nanomachines for various applications. (Reprinted by permission from Springer customer service center gmbh: Springer Nature, Nature Reviews Cancer. Reference [2]. Copyright 2007.)

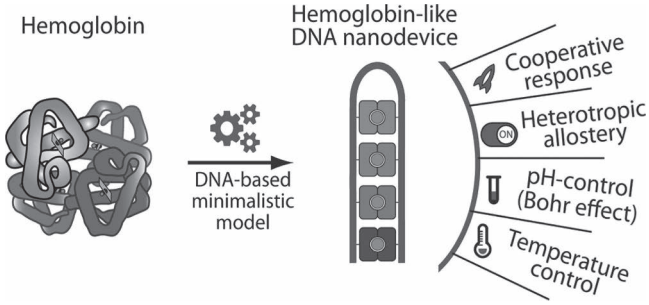

FIGURE 6.2 Bio-inspired DNA switch recreating hemoglobin's response behavior. (Adapted with permission from Ref. [26]. Copyright 2017 American Chemical Society.)

6.2 DNA: A Simple and Versatile Nanomaterial

Even if most natural switches are composed of proteins and to a lower extent of RNA, most recent advances in switch engineering in the field of nanotechnology have been carried out with DNA [13,19,20,28]. Although mainly studied for its genetic carrier role since its discovery by Watson and Crick, the immense potential of DNA programmability has been first applied in the 1980s and early 1990s to build specific 3D structures [29–34] with some displaying catalytic (e.g. DNAzyme) [35] or binding activities (e.g. DNA aptamers) [36,37]. In this section, we will go briefly over the three main reasons that make DNA an ideal programmable biopolymer for engineering nanosystems and will conclude with a brief overview on artificial automated DNA synthesis.

6.2.1 High Programmability

The first reason that makes DNA an ideal polymer is that its simple base pairing code, A-T and C-G, enables to predict its secondary structure and thermodynamics. This simple base pairing code, for example, was well exploited by software prediction tools such as *mfold* and NUPACK [38,39] to predict the folding energy of any DNA sequence into unimolecular or bimolecular structures in different conditions (e.g. salt concentration, temperature). This simple feature has fueled most of the advances in DNA nanotechnology [40–42] since the pioneering work of Seeman in the

1990s [43]. In addition to thermodynamic control, various strategies have been proposed to control DNA association kinetic to create more optimized DNA nanomachines [44,45]. Another feature highlighting the high programmability of DNA chemistry is that DNA sequences can be selected to bind almost any target of interest with high specificity and affinity (e.g. hydrogen ions, metal ions, small organics, proteins and even living cells, bacteria or virus) through natural evolution techniques like SELEX (see Section 6.3.1) [36,37].

6.2.2 Life-Compatible

Second, DNA switches are nanosized and already life-compatible, which allows the engineering of nanotechnological tools that can be directly applied to living systems. Most of the time, DNA requires very little modifications to resist living environment (e.g. mammalian cells or multicellular organisms) while still maintaining biocompatibility properties [46]. They can also be adapted easily to escape immunological response, which could lead to the development of therapeutic agents that could be used in medical settings [47].

6.2.3 Simple Automated Artificial Synthesis

The third and last reason that makes DNA an ideal polymer for nanotechnology is the simplicity of artificial DNA synthesis that produces DNA sequences in high yield and at a low cost (US$0.05–0.15 per nucleotide) [48]. While there are multiple different methods to synthesize oligonucleotides, the phosphoramidite approach [49] is the most often employed. Its adaptation on solid support allowed to build automated systems for faster and more efficient synthesis [50].

The automated method consists of four successive steps repeated for every nucleotide from the starting 3′ to 5′ end (i.e. in the other direction than DNA biosynthesis) (Figure 6.3): (i) detritylation, (ii) coupling, (iii) oxidation and (iv) capping. This synthesis method is usually performed on a column containing a controlled pore glass (CPG) solid support bearing a specific nucleotide load protected with a 5′-DMT ready for the first synthesis step. In the first step, the protective group 5′-DMT (4,4′-dimethoxytrityl) that protects the nascent DNA from unwanted coupling is removed leaving a 5′-OH on the solid support. Then, the next nucleotide phosphoramidite monomer from the DNA sequence is injected allowing its coupling to the DNA on solid support. The coupling step leaves a phosphite triester for the internucleotide linkage that is unstable and needs to be converted to the phosphate triester during the oxidation step. The last step, capping, enables the removal of 5′-OH unreacted DNA that is produced due to the impossibility of reaching a 100% coupling efficiency. If not removed, these sequences could keep reacting and introduce DNA sequences missing some

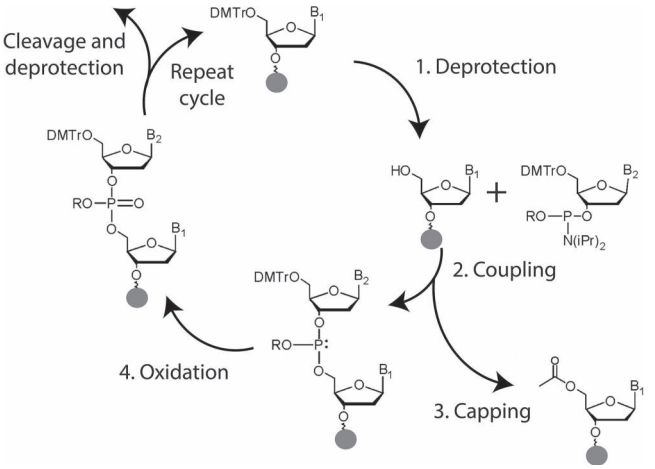

FIGURE 6.3 Automated organic synthesis of DNA oligonucleotide on solid support with the phosphoramidite method [50].

nucleotides. DNA oligonucleotides are then cleaved from the solid support and nucleobases are de-protected. Finally, DNA oligonucleotides are purified by various methods such as reverse-phase cartridge, high-performance liquid chromatography (HPLC) and denaturing electrophoresis.

Many research groups are ordering their specific oligonucleotides (modified or not) from companies (e.g. IDT Biosearch, IBA, Sigma Aldrich) by simply entering their sequence in the webserver. These are generally received within 2 days for unmodified oligonucleotides or in less than a month when special modifications are requested. Many academic laboratories also perform their own DNA synthesis using an automatic synthesizer and commercially available phosphoroamidite monomers. Commercially available phosphoroamidites monomers also include a wide diversity of modified nucleotide (aside normal A, T, C and G) with additional functional groups (e.g. amine, thiol, azide and alkyne), and many other more complex groups (e.g. fluorophore, quencher, cholesterol, tocopherol). It is also possible to work beyond commercially available phosphoroamidite monomers by simply synthesizing either the phosphoroamidites [51–53] or the CPG solid support [54–56] in house, which is routinely performed by various laboratories.

6.3 Engineering DNA Switches

DNA switches engineering is a key task in the field of DNA nanotechnology. Rational design of DNA switches can be summarized into four main steps: (i) a chemical input is first identified based on your research interest (e.g. what do you want to measure; what is the target stimulus you want your DNA switch to respond to); (ii) a DNA scaffold is selected and designed to provide a conformational change mechanism that is triggered by the selected input; (iii) a signal output is then mounted on the DNA switch to translate the input recognition and conformational change into a relevant output signal that will fit your specific application (e.g. fluorescence, current, drug release) and (iv) the DNA

switch must be adapted to work in complex environment with specific chemical modifications (e.g. cellular uptake, nuclease resistance). These four steps will be discussed in detail in the following sections.

6.3.1 DNA Switches Input

DNA chemistry, like protein chemistry, can be adapted to respond specifically to a large variety of chemical inputs (Figure 6.4). Therefore, the first and most important step in DNA switch engineering is to identify the appropriate chemical input that suits the application of your choice. For example, if your application is biosensing, you should find an analyte of interest that can help you answer your scientific question (e.g. protons can be selected if pH measurement is key to your application). Another example, drug delivery DNA switches could involve a disease biomarker as the input signal to trigger the switch to release a specific drug. As such, we can divide the inputs used for DNA switch in two categories: chemical stimuli or physical phenomena. For example, one of the simplest physical change that can impact DNA folding is temperature. DNA unfolding due to temperature increase was one of the first DNA physical property studied extensively and is at the center of a myriad of scientific applications like polymerase chain reaction [57,58].

Melting temperature of DNA (Tm where 50% of DNA strands are in the single-stranded form) was also found to be dependent on the GC content of the DNA duplex since GC base pair is more stable than AT (that is because GC base pair forms three hydrogen bridges compared to two in AT base pair) or to salt concentration (an example of chemical stimuli) [59,60]. This important feature of DNA

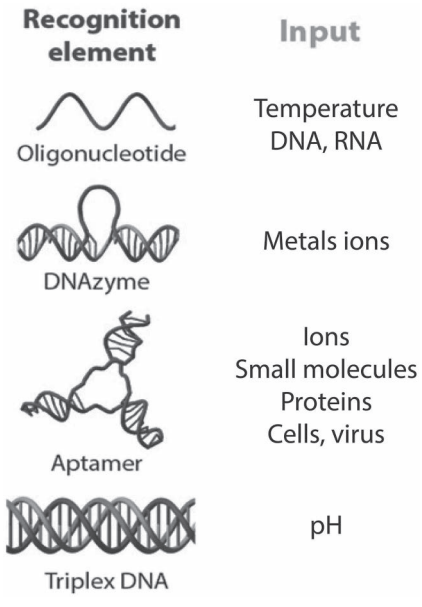

FIGURE 6.4 DNA structure can bind and/or respond to a plethora of input stimuli. (Adapted from Ref. [12] with permission from The Royal Society of Chemistry.)

allowed the engineering of various DNA switches like DNA nanothermometers [61–65].

Like natural GPCR, light can be used as physical stimuli to trigger DNA switches by the specific incorporation of functional groups bearing azo-benzene in the DNA strand that can switch between *cis* and *trans* conformation [66–69]. Another physical property of DNA, its natural conductance [70,71], can be selected as an input signal and successfully used to develop structure-switching mechanism by direct current flow through the DNA switch [72,73]. Electrochemical stimuli is another example of physical phenomena that can be employed to design a structure switching mechanism (e.g. Pb(II) reduction) [74].

On the other hand, chemical stimuli are based on molecules, ions or large biomolecules that bind various DNA structures, which can be used as inputs for DNA switches. The simplest chemical stimuli used to build DNA switch are hydrogen ions which allowed engineering of nanosized pH meter [75]. These DNA switches are based on specific DNA structures such as i-motif [76,77], poly dA helix [78] or parallel triplex [16], which need acidic pH to fold. Hence, a small variation in pH can result in the unfolding of the DNA switch, which allows the precise monitoring of pH variations at the nanoscale. These tools can be used to measure pH inside living cells [79,80] or to engineer drug delivery systems [81]. Similarly, other ions can be used for the folding of specific DNA structures like G-quadruplex that require potassium ion to form [82], which can be adapted into a simple potassium sensors [83] or into sophisticated DNA nanochannels [84]. Other G-quadruplex DNA structures are also of interest for their ability to bind specific metal ions such as Pb(II) [85], Tl(I) [86] and many other monovalent and divalent metal ions [87]. Small organic molecules, like members of the porphyrin family, can also bind various G-quadruplex structures [88,89]. DNA mismatch can also be used to detect metal ions like T:T mismatch for Hg(II) [90] or C:C mismatch for Ag(I) [91]. Nucleic acid sequences made of either DNA or RNA form perhaps one of the simplest class of molecular inputs that can be incorporated and easily programmed within the DNA switch (as it will be described in the next section). An example of DNA switch based on nucleic acid recognition is the molecular beacon, widely used for medical diagnosis since its conception by Tyagi and Kramer [27].

In the event that a specific input target of interest has no known DNA binding sequence, SELEX methods can be employed to identify nucleotide sequences that display high affinity for small organics [92], proteins [93,94] and even viruses [95] or whole bacteria [96] (mostly through surface proteins/sugars). Briefly, SELEX (short for systematic evolution of ligands by exponential enrichment) is a method developed in 1990 [37,97] in which specific nucleotide sequences (referred to as aptamer with typically 15–40 nucleotides) from random libraries are selected to bind specific targets with high affinity through several rounds of selection. This method was first developed for RNA selection but was then rapidly adapted to select DNA

sequences [36]. In this method, one selection round typically consists in a simple binding experiment where DNA sequences bound to the target of interest are amplified and used for the next selection round.

6.3.2 Engineering Switching Mechanisms

Engineering switching mechanism represents, without a doubt, the most important step in the development of DNA switches. This mechanism involves a change in structure from conformation 1 to conformation 2 in the presence of the input selected (also often referred to as OFF and ON states, see output Section 6.3.3). This structural change from conformation 1 to conformation 2 will be adapted to produce a relevant signal output as it will be detailed in the next section. When engineering the switching mechanism, one should always keep in mind the desired signal output mechanism (e.g. fluorescence, electrochemistry, drug delivery). For example, signal output performance (signal-to-noise ratio) is often related to the magnitude of the conformational change that occurs in the presence of the selected input. Some DNA structures intrinsically go through a significant conformational change in the presence of the input. For instance, some G-quadruplex structures will go from a single-stranded DNA to a four-stranded quadruplex structure in the presence of the input (e.g. potassium ions) while other structures will only undergo mild-to-no conformational changes upon binding to their targets. To overcome this limitation and engineer a structure-switching mechanism in a specific DNA structure, one can take advantage of the population-shift model and stabilize an alternative non-binding structure within the original DNA sequence [42,98]. In this strategy, a slightly modified DNA sequence can be made to adopt a distinct conformation from the original one (i.e. a non-competent binding state) (Figure 6.5). This non-binding conformation will switch back and forth with the original conformation through chemical equilibrium in the absence of input molecule. Therefore, one can stabilize this non-binding conformation so that in the absence of input molecule, most of the DNA switches are in the non-binding conformation. Stabilizing the non-binding conformation also means there is now an energetic price required for the DNA switch to adopt its original conformation (to undergo a conformational switching). This energetic price is paid through binding of the input molecule, that form a more stable complex with the DNA switch than the non-binding conformation. This system is conveniently described using a three-state population-shift model: a binding-competent state and an input molecule bound state that both have the same conformation and a non-competent binding state that has a distinct conformation.

Many strategies can be used to engineer a non-binding state within the original DNA structure (Figure 6.6). A first approach consists in adding complementary nucleotides at both extremities of the original DNA sequence to force a stem-loop conformation (Figure 6.6a). This strategy was employed successfully by Kramer et al. in 1996 when they designed now famous molecular beacons that open upon binding to specific complementary nucleotides sequence [27]. Another strategy is to destabilize the original DNA structure by introducing mutations in the binding-competent conformation, which will favor an alternative non-binding conformation (Figure 6.6b) [99]. In the presence of input molecule, this equilibrium toward the non-binding conformation will shift back toward the binding-competent state to form a switch-input complex. It should be noted that mutation should be introduced with care in this strategy to avoid changing the intrinsic K_D and specificity of the DNA sequence with its input molecule. To avoid inserting mutations in the DNA sequence, a simpler strategy is to design a DNA sequence that is complementary to the switch sequence (Figure 6.6c). The addition of a complementary DNA will sequester the switch into a double-stranded DNA whose conformation will be different from the binding-competent

FIGURE 6.5 Population-shift mechanism: a non-binding DNA conformation shifts toward a binding-competent conformation upon binding to a specific chemical input. (Adapted from Ref. [12] with permission from The Royal Society of Chemistry.)

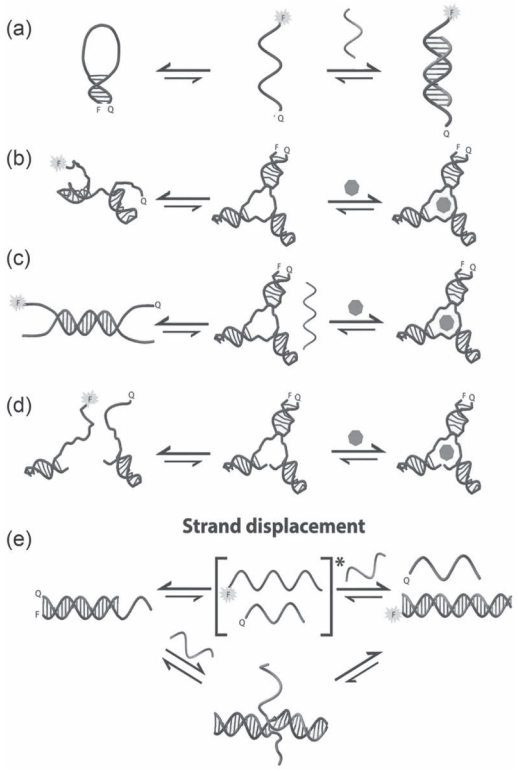

FIGURE 6.6 Engineering strategies to introduce a switching mechanism in DNA recognition elements. F: fluorophore; Q: quencher. (Adapted from Ref. [12] with permission from The Royal Society of Chemistry.)

conformation. Here, the chemical input will compete with the complementary DNA and the switch will undergo a structure switching mechanism upon input binding [100]. This approach is particularly useful when working with DNA aptamers that adopt a unique DNA conformation as it is easy to design a complementary DNA sequence [41,101]. Another way to avoid mutations is to divide the switch sequence in two parts that can dimerize to form the binding-competent state (Figure 6.6d) [98]. This strategy is also largely employed with DNA aptamer sequence whose structures can be roughly modeled using available computational tools like *mfold*. A last strategy, called strand displacement, can also be employed to build DNA switches (Figure 6.6e). In this strategy, the "switch" sequence is sequestered in a non-binding state with a complementary DNA strand like described previously except that there is a single-stranded terminal anchor (toehold domain) that is not bound. This toehold domain is free to hybridize to a part of an input DNA sequence (i.e. the input molecule in this case; see Ref. [100]). This process allows for strand exchange in the presence of the input molecule that results in a significant conformational change [45]. Here the input molecule is an invading DNA strand, but it is possible to adapt the strategy, so the invading input molecule can be replaced by proteins or small molecules. To achieve this, one can employ aptamer-bound toeholds [102,103], G-quadruplex structures [104] or even pH-sensitive DNA sequences [105].

The thermodynamics of the population-shift model controls the performance of the DNA switch. To understand this, let's consider the following two chemical equilibrium: K_S and K_D based on different conformations (C) of the switch:

$$C^{\text{non-binding}} \overset{K_S}{\rightleftharpoons} C^{\text{binding}}$$

$$C^{\text{input}} \overset{K_D}{\rightleftharpoons} C^{\text{binding}} + \text{input}$$

where K_D represents the affinity of the chemical input for the binding-competent conformation. However, the introduction of a non-binding conformation and, thus K_S, modifies the apparent binding affinity of the switch with the following relationship:

$$K_S = \frac{[\text{binding-competent state}]}{[\text{non-binding state}]}$$

$$K_D^{\text{apparent}} = K_D^{\text{instrinsic}} \cdot \left(\frac{1 + K_S}{K_S}\right)$$

This increase in apparent K_D represents the energetic price required to introduce the non-binding conformation (Figure 6.7a). As such, the K_S value should be chosen wisely since the lower its value, the higher the apparent K_D will be (Figure 6.7a). K_S may often be tuned to optimize the activity of the switch at the relevant concentration of the chemical input [21]. When the lowest detection limit of a switch is needed, K_S may be set between 0.1 and 1 as this maintains a low apparent K_D while between 90%–50% of the DNA switch goes from non-binding state to binding-competent and bound state in the presence of the input molecule, respectively (Figure 6.7b and c).

The high programmability nature of DNA is only fully exploited when we couple switching engineering strategies with DNA structure software prediction like freely available webserver *mfold* [38] and NUPACK [39]. These webservers are able to compute the folding and free energy of a DNA sequence at a specific temperature and salt concentration for unimolecular or bimolecular processes. Therefore, these tools are crucial to design non-binding state as they are able to predict the K_S with the Gibbs free energy:

$$\Delta G = -RT \cdot \ln(K_S)$$

For *mfold*, the unimolecular folding prediction is performed with the *mfold* application tool while the bimolecular folding is performed with DINAmelt application [106]. The K_S calculated by *mfold* can then be validated experimentally using urea [107] or temperature [108] denaturation curves.

6.3.3 Engineering Output Functions in DNA Switches

One of the last steps to engineer a functional DNA switch is to translate the conformational change induced by a chemical input into a relevant and measurable output signal.

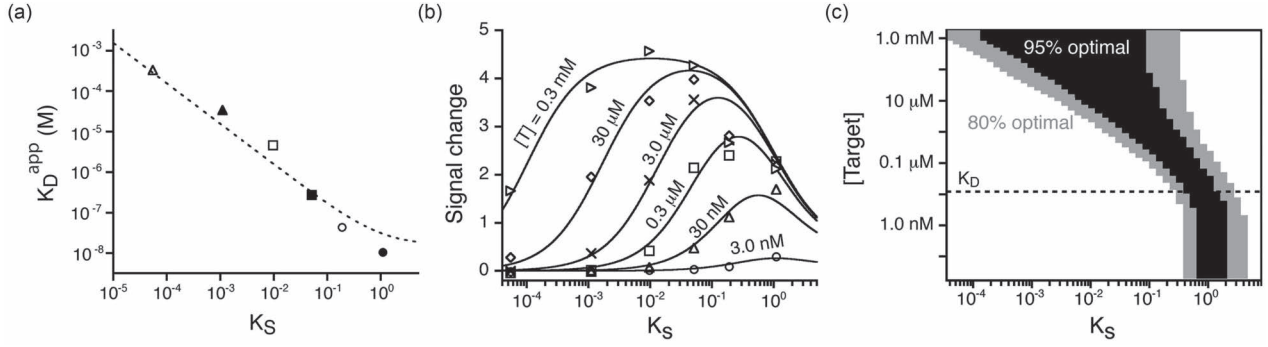

FIGURE 6.7 (a) Stabilizing a non-binding conformation to create a switching molecule with an equilibrium K_S decreases the apparent affinity K_D^{app} of the input molecule for the switch. (b) The amplitude of signal change induced by binding of the input molecule to the DNA recognition element depends on the K_S of the non-binding conformation. (c) A K_S between 0.1 and 1 is ideal to optimize the detection limit of the switch. (Reprinted with permission from Ref. [21]. Copyright 2009 National Academy of Sciences.)

There is a wide variety of output signaling mechanisms that can be discussed, but due to limited space, we will focus on the most commonly used strategies. The simplest and most widely used output signal implemented in DNA switch is fluorescence spectroscopy either by quenching or FRET (Förster resonance energy transfer) [109,110]. For instance, one of the first DNA switch developed, the molecular beacon by Kramer, employed fluorescence quenching as the output signal to monitor binding of a target DNA sequence (Figure 6.8a). To render the DNA switch fluorescent, a fluorophore (e.g. fluorescein, cyanine dyes and many others) is inserted at one of its extremities while a molecular quencher (i.e. a molecule that can absorb the light emitted by the fluorophore) or another fluorophore that can absorb light and re-emit at a higher wavelength is attached at the other extremity. A significant change in fluorescence signal can then be detected when the dyes are separated by a distance larger than their Förster radius (typically ∼50Å) [111]. Since DNA switches alternate between two conformations, one can strategically place the fluorophore/quencher pair so that they are physically close (∼20Å) in one conformation and far from each other (>50Å) in the other conformation. Binding of the input molecule will thus either bring fluorophore/quencher pair close or far from each other. Fluorescent switches can either be qualified as "signal OFF" or "signal ON" switches when the fluorescence decreases or increases upon input molecule addition. Fluorescence is often used for its high sensitivity and simplicity in biosensing applications although it cannot be used in medium that cannot transmit light (e.g. whole blood).

Electrochemistry is also often employed to monitor conformational changes within DNA switches (e.g. cyclic voltammetry (CV) or squarewave voltammetry (SWV)). In this approach, DNA is often covalently attached on an electrode surface via a sulfur linker, for example, if a gold surface is employed [115]. Other linkers may be used for other surface such as glass or glassy carbon [116]. Conformational changes are monitored by adding an electrochemical reporter (e.g. methylene blue or ferrocene) on the switch that undergoes redox reactions when located near the gold surface (Figure 6.8b). As described for fluorescence, this reporter needs to be strategically placed so that it is either brought closer (signal ON) or farther from the surface (signal OFF) in the presence of the input molecule. Classic electro-active molecules employed are methylene blue or ions like ferricyanide [117]). One great advantage of electrochemical output is their ability to signal efficiently directly in whole blood. Electrochemical DNA switches thus show much promises for point-of-care sensing applications in medical diagnostics (Figure 6.8c) [114,118–120].

Alternative signal output approaches like colorimetric changes employ nanoparticles to translate a DNA switch conformational change. In a colorimetric assay, for example, a visible color change can be detected upon nanoparticle aggregation in the presence of a specific input molecule [121,122]. To do so, single-stranded DNA switches are often used to prevent nanoparticles from aggregating. In the presence of their input molecule, these switches fold and decrease their affinity for the nanoparticles surface, which results in their aggregation. This approach can be adapted to a variety of input ranging from pH changes [123] to small organics and proteins [124]. Other approaches involve electrochemical plasmonic sensing systems [125] and surface-enhanced Raman spectroscopy [126,127].

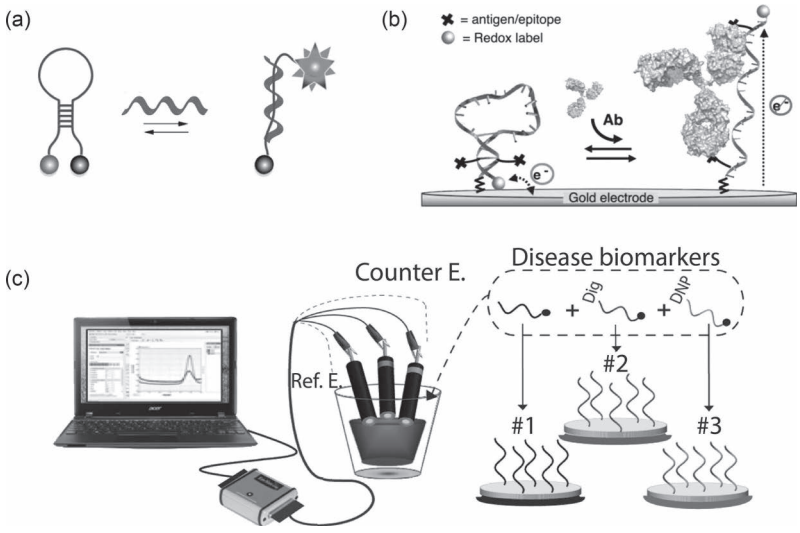

FIGURE 6.8 (a) Fluorescence output to monitor DNA switches requires a conformational change upon binding on the input molecule that brings a fluorophore/quencher pair far from each other. (b) Electrochemical output can be used to monitor DNA switches in which a redox element is brought far from an electrode surface upon binding of the input molecule. (c) Electrochemical output can be adapted to portable systems and multiplexed for point-of-care approaches. (Panel A is adapted with permission from Ref. [112]. Copyright 2010 American Chemical Society. Panel B is adapted with permission from Ref. [113]. Copyright 2012 American Chemical Society. Panel C is adapted with permission from Ref. [114]. Copyright 2015 American Chemical Society.)

6.3.4 Adapting DNA Switches for Complex Environment

An increasing proportion of DNA switches are used for applications related to living organism whether *in vitro* or *in vivo*. Such applications include biosensing inside living cells or drug delivery systems in living animals like mice. As a result, various strategies are required to adapt DNA switches for harsher environment (e.g. nuclease-rich environments) or simply to allow them to reach their final destination (e.g. cellular uptake) [47,128]. These modifications are not intended to impact the switching mechanism but rather to adapt DNA switches to the cellular environment. One of the biggest obstacle when employing DNA switches in living organism is the presence of nucleases that can easily degrade unmodified DNA (e.g. half-life of short unmodified DNA oligonucleotides is in the order of minutes in whole blood) [129]. To overcome this limitation, a myriad of chemical modifications can render DNA resistant to nuclease degradation with often little impact on the DNA thermodynamic and function (although it should be validated experimentally case by case). One common target for chemical modification is the DNA backbone structure in which internucleotide linkage can be modified using phosphorothioate [130], phosphonoacetate [131], morpholino oligomers [132] or methyl phosphonate [133] (Figure 6.9). Other chemical modification will target the nucleotide 2′ position of DNA to reduce nuclease degradation. Examples of 2′ chemical modifications include: 2′-fluoro [134], 2′O-methyl [135] and locked nucleic acid (LNA)-oligo [136] (Figure 6.9).

Some of these chemical modifications have already been approved by the FDA. For example, the phosphorothioate linkage is present in Nusinersen, an antisense oligonucleotides drug [137] used to treat spinal muscular atrophy [138]. Pegaptanib is an example of a 2′ modified oligonucleotide that is now used as a therapeutic agent to treat macular degeneration [139]. Another backbone modification, L-DNA (i.e. the mirror image of the natural form D-DNA) is also resistant to nuclease degradation and was successfully used to build DNA nanothermometers, which were used inside living cells [46].

In order to use DNA switches in living systems, it is also important to ensure that DNA switches are able to reach their final destination. Since cellular uptake of unmodified DNA is inefficient, this typically represents an obstacle for biosensing inside living cells. To overcome this limitation, various chemical modifications have been shown to greatly increase cellular uptake. For example, attaching a folic acid on a DNA switch enables it to target near cancer cells that overexpress folate receptors [140]. Hydrophobic chemical modifications such as cholesterol, uncharged DNA backbone or stearic acid are other examples of modifications that are used to increase cellular uptake of DNA oligonucleotides [141]. Targeting of oligonucleotide to specific cells can be achieved through specific cell surface receptors. One example of such chemical modification, N-acetyl galactosamine (GalNac), has helped to target oligonucleotides to a population of liver cells through binding specific cell surface receptors [141]. Attaching aptamers that target specific receptors (e.g. human transferrin receptor aptamer) is another simple strategy to transport DNA switches inside living cells [142]. Functionalization of DNA switches with protein like transferrin enables to target metabolic pathways (this was used to measure pH along endocytic transferrin pathway) [79]. Attaching specific signal peptide to DNA switches represents another strategy to target them to specific cell and organelle types like the nucleus (e.g. using the nuclear localization signal peptides) [143]. To this aim, researchers have recently developed an *in vivo* phage-display method in mice to select specific peptides that can localize to various organs (similar to homing peptide) [144,145]. Briefly, phage display is a selection method similar to SELEX in which phage bearing surface peptides are selected through binding interaction to a specific target to be then amplified which results in peptides with high affinity for the selected target [146]. Coupling of such method with DNA switches could allow to target them anywhere inside living mammals.

6.4 Applications of DNA Switches

In this section, we will describe two different downstream applications of DNA switches that highlight the high programmability and great potential of DNA nanotechnology. We will start by exploring with more detail the design and engineering of DNA switches as biosensing tools with applications in fundamental research and medical diagnosis (e.g. point-of-care approaches). We will then explore drug delivery systems in greater detail for applications as therapeutic agents in the medical field. For each application, we will integrate the four steps of DNA switches engineering in a single workflow toward downstream applications.

6.4.1 Biosensing Applications

Applications in Fundamental Research

DNA nanothermometers are DNA switches engineered to measure large or small temperature variations within nanosized systems [61]. Temperature variation is the obvious input stimuli selected for this application. A wide variety of DNA structures, however, can be selected since most of them will undergo temperature denaturation at a specific Tm. However, selecting DNA structures whose thermodynamic stability can be easily tuned will offer greater flexibility to build DNA nanothermometers tools that can be optimized for optimal temperature sensing. DNA stem-loop structure (e.g. molecular beacons) is an example of such programmable structure because it is possible to easily tune its folding energy by changing the GC/AT ratio of the stem structure [21]. One can rationally perform this by employing simulation software like *mfold* and change the stem sequence while keeping the loop sequence constant. For this application, a simple fluorescence output

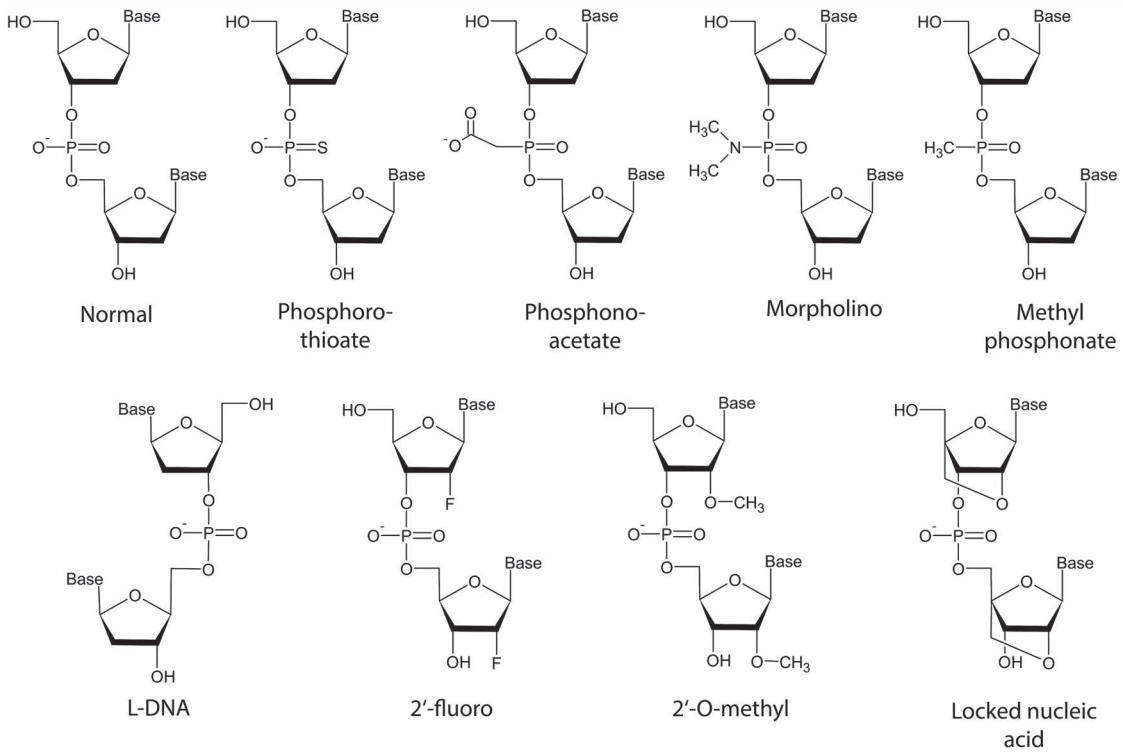

FIGURE 6.9 Chemical modifications of DNA to adapt DNA switches for complex environment (e.g. living organisms). All of these examples of modified nucleotides are commercially available from DNA synthesis companies such as Glen Research, Chemgenes, LINK and many others.

system (fluorophore/quencher pair) was implemented at the extremities of the stem structure whose opening due to increased temperature resulted in a fluorescence increase. To test this design, the GC content of the stem was varied from 0 to 5 GC base pair, which allowed to change the Tm from 40°C to 80°C (Figure 6.10a) [61]. Each nanothermometer has a linear range for a specific temperature interval (∼15°C), but combining all nanothermometers together yielded a single linear range of ∼50°C allowing extended temperature sensing (Figure 6.10b).

These DNA nanothermometers were employed in a real biological setting to monitor temperature inside living cells [46]. To do so, they were chemically modified to L-DNA which limited their binding to cellular proteins and conferred them with increased nuclease resistance. These nanothermometers were used to probe temperature increase in living cells after thermal therapy for cancer consisting in the irradiation of Pd nanosheets. Irradiation caused a temperature increase of up to 12°C that could be monitored by the DNA nanothermometers (Figure 6.11).

More cooperative DNA structures like DNA clamp switch (triple helix) were also adapted into nanothermometers, offering narrower temperature transition to monitor smaller temperature changes (∼7°C) with enhanced precision

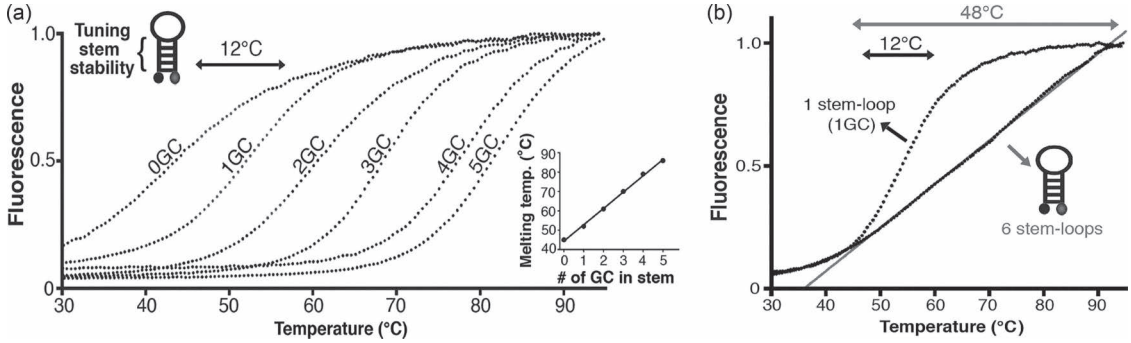

FIGURE 6.10 (a) Molecular beacons can be thermodynamically engineered into DNA nanothermometer tools with various temperature sensitivity. (b) Combining multiple DNA thermometers with different K_S results in an extended linear range to monitor extended temperature variations. (Adapted with permission from Ref. [61]. Copyright 2016 American Chemical Society.)

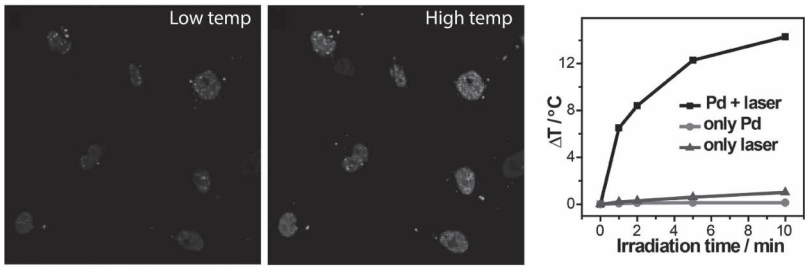

FIGURE 6.11 DNA nanothermometers were adapted through chemical modifications for temperature sensing inside living cells to monitor temperature increase caused by Pd nanosheets in real time. (Adapted with permission from Ref. [46]. Copyright 2012 American Chemical Society.)

(Figure 6.12). These ultrasensitive nanothermometers could be more effective to measure small temperature variations in living cells (e.g. to monitor metabolic activity).

DNA pH-sensitive switches are tools developed to monitor pH change at the nanoscale (i.e. nano pH meter), which resulted in various applications in biosensing and drug delivery. The selected input signal, pH variation, can be monitored, thanks to some DNA structures that are specifically stabilizes by hydrogen ions. As detailed in the section on DNA switch engineering, there is a wide variety of DNA structures whose folding is heavily dependent on specific protonation of nucleobase and hence on pH (e.g. i-motif [76,77], poly dA helix [78] or parallel triplex [16]). In particular, parallel triplex are composed of CGC or TAT triplets that display different pH sensitivities with TAT undergoing transition around pH 10 whereas CGC is around pH 7 (Figure 6.13a) [75]. A universal fluorescent DNA pH meter was therefore designed using a DNA triplex fold. For this application, Alexa Fluor 680 was employed as fluorophore due to their insensitivity to pH variation. This triplex thermometer can be programmed to respond to different pH variation by simply changing its TAT content versus CGC within the sequence (Figure 6.13b).

As described previously, these switches can be combined to extend the pH interval that can be monitored at the same time from 1.8 to 5.5 pH unit by combining three

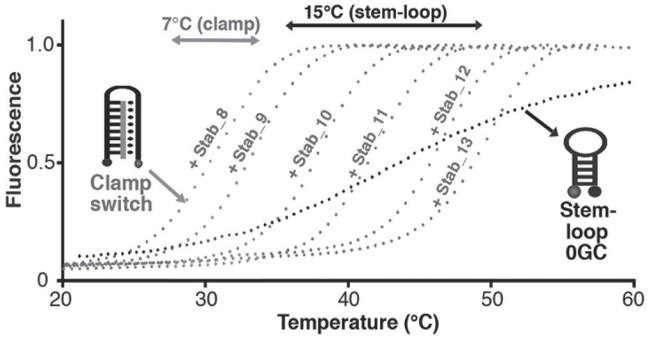

FIGURE 6.12 DNA clamp switch structures can be used to build nanothermometers with increased sensitivity to probe smaller temperature variations with high precision. (Adapted with permission from Ref. [61]. Copyright 2016 American Chemical Society.)

different DNA pH meters (Figure 6.14). Another important feature of these switches is their ability to be completely reversible [75].

DNA pH switches have also garnered interest for *in vitro* real-time pH sensing [79,80]. In this application, a pH-sensitive switch based on an i-motif DNA structure was covalently attached to transferrin, which enabled efficient switch internalization along the endocytic pathway of transferrin. Pulse chase of the DNA switch during time was performed to measure pH change through the endocytic pathway going, for example, from sorting endosome to recycling endosome with significant differences in pH distribution (Figure 6.15). This was also performed on the furin pathway using a specific DNA duplex domain. Interestingly, pH distribution determined by image processing of the DNA switches matched literature values [79]. This represents a unique method to probe pH value of cellular organelles in real time without having to lyse cells for analysis. Interestingly, these tools can be applied to study effects of toxin in real time as shown with Brefeldin A, an antibiotic known to cause extensive tubulation of the trans-Golgi network. These tools were also used on the more complex multicellular organism *Caenorhabditis elegans* to map spatiotemporal pH changes that was associated with endocytosis [147].

Biosensing for Medical Diagnostic Applications

Biosensing with DNA switches can also be used to build efficient diagnostic tools that can be employed in point-of-care setting. One of the earliest example of DNA switch application for diagnostic tools came from Kramer with fluorescent molecular beacons to detect specific DNA sequences (routinely employed in many medical facilities) [148–150]. However, fluorescence sensing is often not suitable for point-of-care approaches since it requires exhaustive sample handling. Another strategy based on electrochemical outputs directly in whole blood was then developed to overcome this limitation as described in the output section [115]. Recently, a DNA-based electrochemical biosensor exploited steric hindrance effect to detect biomarkers (Figure 6.16) [114,119,120]. In this strategy, a single-stranded capturing DNA is functionalized on a gold surface via Au-S chemistry and a complementary signaling DNA sequence bearing a redox element and a recognition element is added to the

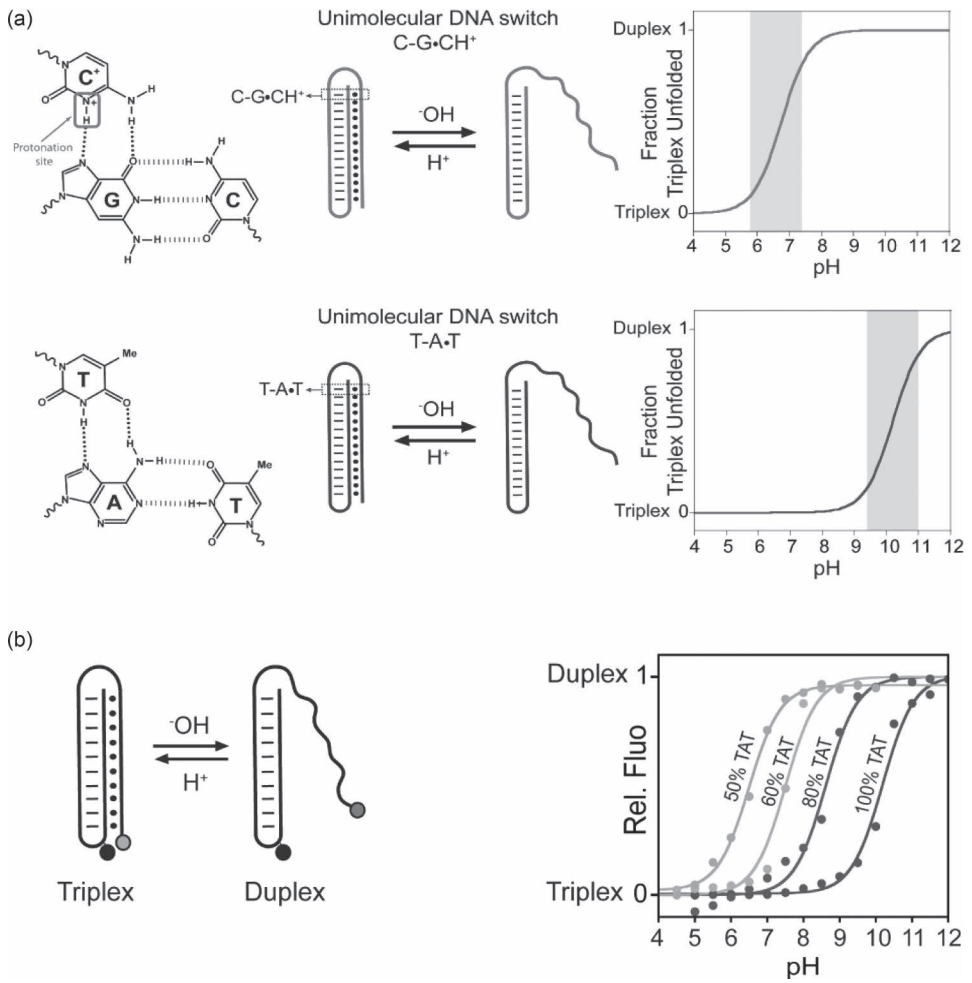

FIGURE 6.13 (a) Parallel triplex DNA structures were used to create pH-sensitive DNA switches to monitor pH variations (nano pH meter). (b) DNA pH meter has pH sensitivity that can be modulated by changing their DNA sequence with parallel triplex TAT or CGC. (Adapted with permission from Ref. [75]. Copyright 2014 American Chemical Society.)

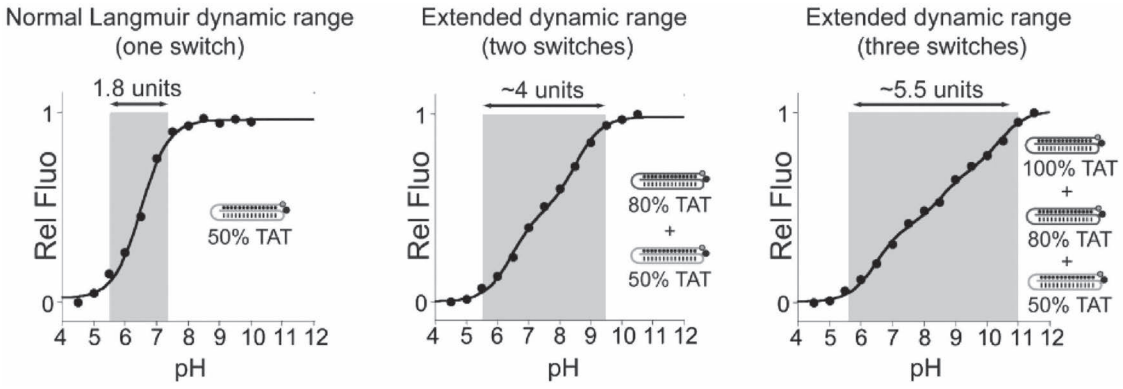

FIGURE 6.14 Combining DNA pH meter of different pH sensitivity can yield extended pH monitoring capacity. (Adapted with permission from Ref. [75]. Copyright 2014 American Chemical Society.)

blood sample. The specific biomarker (e.g. specific antibodies) can bind to the signaling DNA, which reduces the hybridization efficiency of the signaling DNA on the capturing DNA located on the surface of the electrode (>50%) due to steric hindrance. This results in a decrease of the measured current of the redox element (Figure 6.16).

This is an example of a signal-off DNA switch where the amplitude of the signal reduction is correlated with the molecular weight of the biomarker (i.e. steric hindrance effect) (Figure 6.17). This DNA switch can be easily adapted to bind other input molecules given that a small recognition element can be placed on the complementary DNA

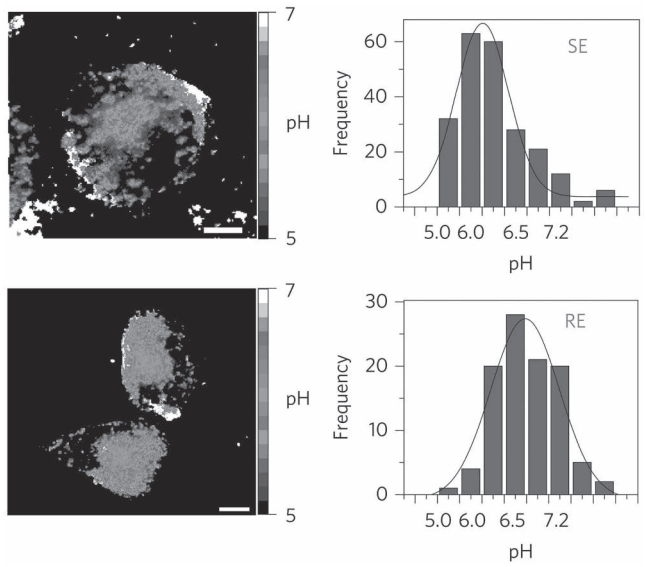

FIGURE 6.15 DNA pH meters were employed to map pH along endocytic pathways. (Adapted by permission from Springer customer service center gmbh: Springer Nature, Nature Nanotechnology. Reference [79]. Copyright 2013.)

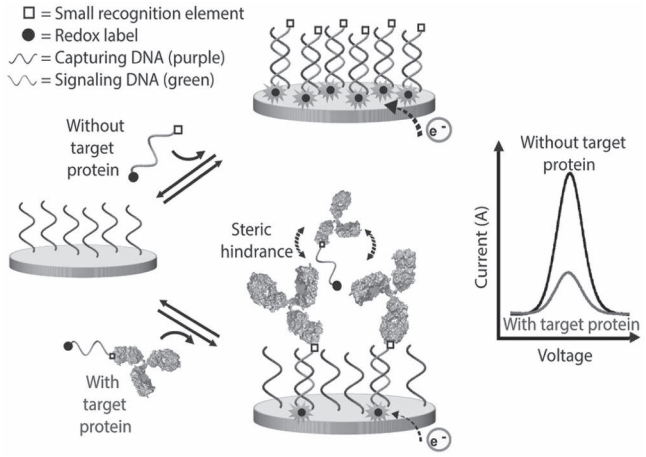

FIGURE 6.16 Steric hindrance was exploited to engineer a DNA switch for the detection of large biomarkers using gold electrodes. (Adapted with permission from Ref. [114]. Copyright 2015 American Chemical Society.)

(e.g. <30 kDa). This approach was tested directly in whole blood in a point-of-care format that employs an inexpensive potentiostat and provided result within 10 min [120].

6.4.2 Drug Delivery System for Medical Therapies

Drug delivery systems can take advantage of the high programmability of DNA. For example, DNA switches can be engineered to transport specific drug cargos that can be released in the presence of specific chemical input such as disease markers. For example, Ricci et al. 2017 have recently developed a DNA switch that can be programmed

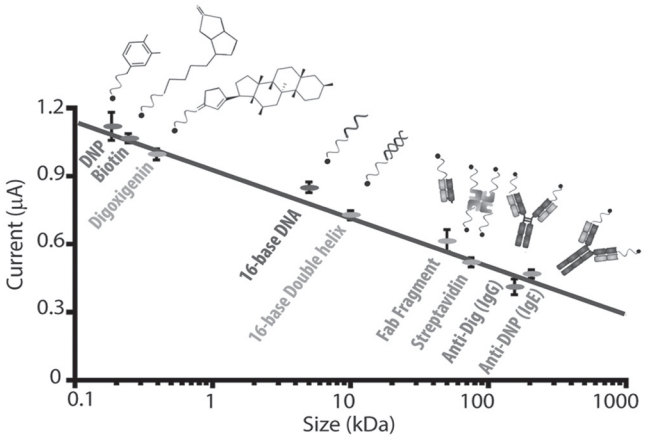

FIGURE 6.17 The steric hindrance effect on the DNA switch response was dependent on the molecular weight of the biomarker detected. (Adapted with permission from Ref. [114]. Copyright 2015 American Chemical Society.)

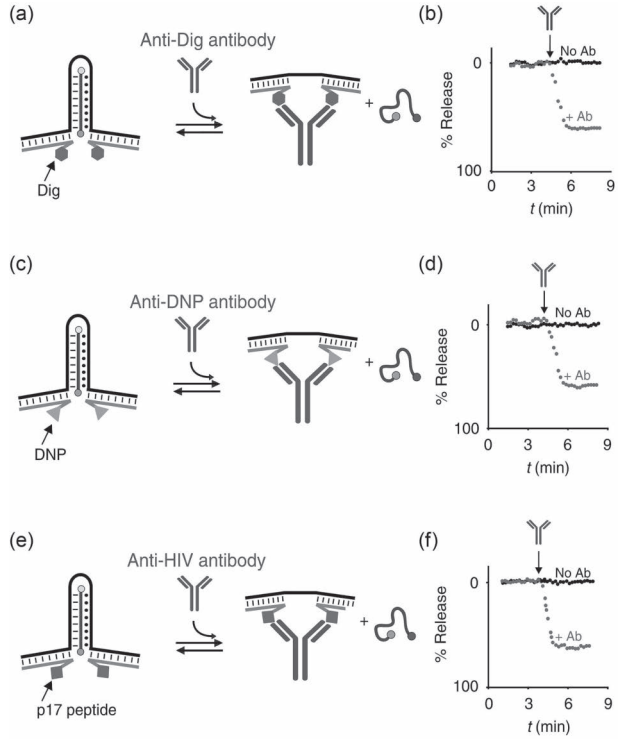

FIGURE 6.18 Antibody-induced drug-releasing nanomachines. DNA-based nanomachines programmed to release a nucleic acid-based cargo in response to: (a and b) anti-digoxigenin antibody; (c and d) anti-dinitrophenol antibody and (e and f) anti-HIV antibody. (Adapted with permission from Ref. [151]. Copyright 2017 American Chemical Society.)

to release a nucleic acid drug cargo in the presence of specific antibodies (Figure 6.18) [151]. The switch–cargo complex consists of a DNA triplex clamp structure. This complex is thermodynamically engineered to be stable in the triplex form but unstable once the triplex unfolds into a DNA duplex, resulting in the release of the drug cargo [151].

The cargo release mechanism is based on the proximity of two recognition elements (i.e. hapten, epitopes) that specifically bind the input antibodies (e.g. digoxigenin binds anti-digoxigenin antibody). Upon binding the two antigens or epitopes on the DNA transporter, one single specific antibody provides the energy to destabilize the triplex structure by stretching the switch (Figure 6.18). This stretching mechanism has already been exploited to build DNA switches for sensing applications [113,152]. This DNA switch architecture is also highly programmable since the input biomarker can be changed to any other antibody by simply changing the hapten/epitope element on the switch scaffold (Figure 6.18c and e). The high programmability of this approach was also employed to build steric hindrance-based logic gates for drug delivery where drug cargo is released only in the presence of two different input biomarkers (i.e. two different antibodies) (Figure 6.19). These DNA switches were tested in blood serum and retained high specificity to its target antibody, which suggests that they could be useful to deliver nucleic acid-based drug in living organism.

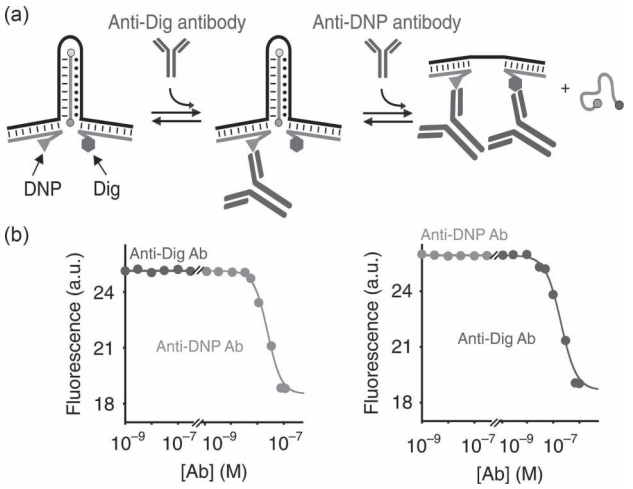

FIGURE 6.19 Antibody-induced drug-releasing nanomachines in response to the presence of two antibodies. The digoxigenin (Dig) and dinitrophenol (DNP) DNA switch were modified into a logic circuit requiring the presence of two different antibodies to induce drug cargo release. (Adapted with permission from Ref. [151]. Copyright 2017 American Chemical Society.)

There is a close proximity between DNA switches designed for biosensing and drug delivery systems since highly specific biosensing switches can be easily adapted to trigger the release of a drug cargo instead of simply providing a fluorescent output signal. Another example of such versatile switch is the adaptation of a DNA pH meter based on parallel triplex into a drug delivery switch triggered by acidic environments [81]. Acidic environments have been the input of a wide variety of drug delivery systems because tumor environment is usually more acidic due to specific tumor metabolism [153–155]. The switching mechanism employed is based on the addition of a pH-sensitive sequence at a distal position on a DNA-based recognition element in order to leave the high affinity and specificity of the DNA switch unchanged. The first design explored consisted of a simple stem-loop DNA that can transport a complementary nucleic acid drug hybridized to its loop (Figure 6.20a and b). When adding a parallel triplex-forming sequence to the DNA transporter (i.e. far from the loop), the triplex structure conformation is stabilized at lower pH (<6.0), therefore reducing the affinity of the transporter for its nucleic acid cargo (Figure 6.20c and d).

This design allowed to load single-stranded nucleic acid drugs on the loop structure of the DNA switch and trigger its release in acidic environment. This strategy was also adapted using more complex drug transporters such as the cocaine-binding aptamer where the same DNA sequence

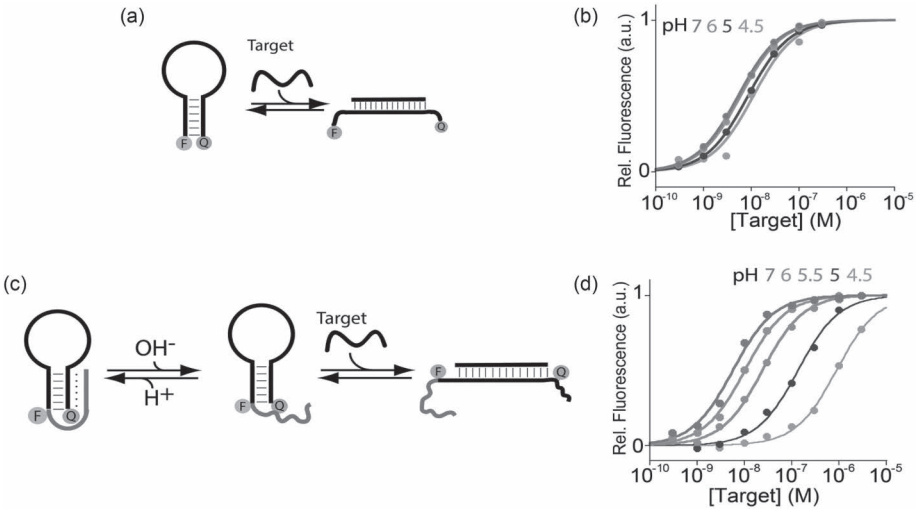

FIGURE 6.20 (a and b) The stem-loop DNA transporter is not sensitive to pH variation. (c and d) The addition of a triplex-forming, pH-sensitive DNA element renders the DNA transporter sensitive to pH variations. F: fluorophore; Q: quencher (Adapted with permission from Ref. [81]. Copyright 2015 American Chemical Society.)

sensitive to acidic environment was added at distal position from the recognition site of the aptamer (Figure 6.21a). The cocaine aptamer served as a mean to load the drug (i.e. cocaine) and the pH-sensitive sequence as the trigger to release cocaine. This cocaine transporter reacted as predicted by releasing cocaine in acidic environment (Figure 6.21b). This strategy is highly modular and could in principle be applied to a wide variety of DNA aptamers to transport and release a variety of therapeutic molecules.

Other smart drug transporters have been developed using DNA aptamers. For example, Krishnan et al. have recently employed a cyclic di-guanosine monophosphate (c-di-GMP)-binding aptamer to deliver fluorescent drug cargo in response to c-di-GMP, a second messenger in bacteria that regulates signal transduction and metabolism [156]. This DNA aptamer was engineered into a DNA switch that undergoes conformational change upon binding to c-di-GMP (Figure 6.22a) [157]. This DNA switch was then employed to build a small DNA cage that can transport fluorescent drugs. The DNA cage was designed to open upon the activation of the DNA switch with increasing concentration of c-di-GMP (Figure 6.22b). This approach is highly modular since it is possible to replace the DNA switch with any other DNA aptamers, and thus, these DNA cages could be in principle activated by any disease biomarker for which we possess a DNA aptamer. This approach also exploits novel properties of DNA cage like their intrinsic resistance to nucleases, which protect the drug cargo from being rapidly metabolized [158]. DNA cage has been successfully used in mammalian cells [158] and in the multicellular organism *C. elegans* [159] for bioimaging, suggesting that they could be eventually applied successfully for drug delivery applications in living organisms. This cage, sometimes referred to as DNA container, retains the programmability of DNA, and thus, it is possible to engineer them within the DNA switching mechanism to make a link between presence of the input and cage opening.

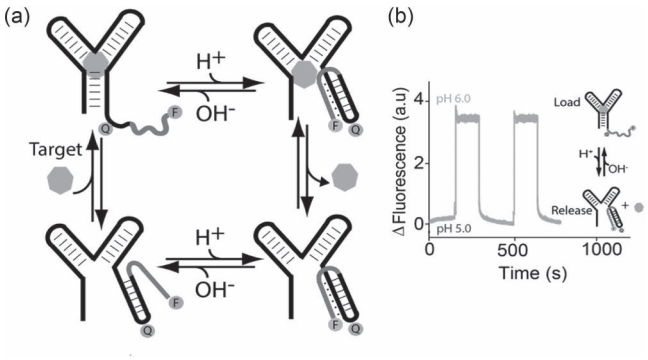

FIGURE 6.21 An aptamer-based pH-induced cocaine transporter that releases cocaine in response to low pH. F: fluorophore; Q: quencher (Adapted with permission from Ref. [81]. Copyright 2015 American Chemical Society.)

6.5 Conclusion and Perspective

DNA is a unique biopolymer that has enabled tremendous research applications in nanotechnology creating a field of its own: DNA nanotechnology. The high versatility of DNA arises from our capacity to predict its structure from the nucleotide sequence. Other biopolymers like proteins, for example, still lack this structure predictability, making it harder to develop them into new functional structures *de novo*. Another unique feature of DNA is its easy organic synthesis using automated synthesizer, which

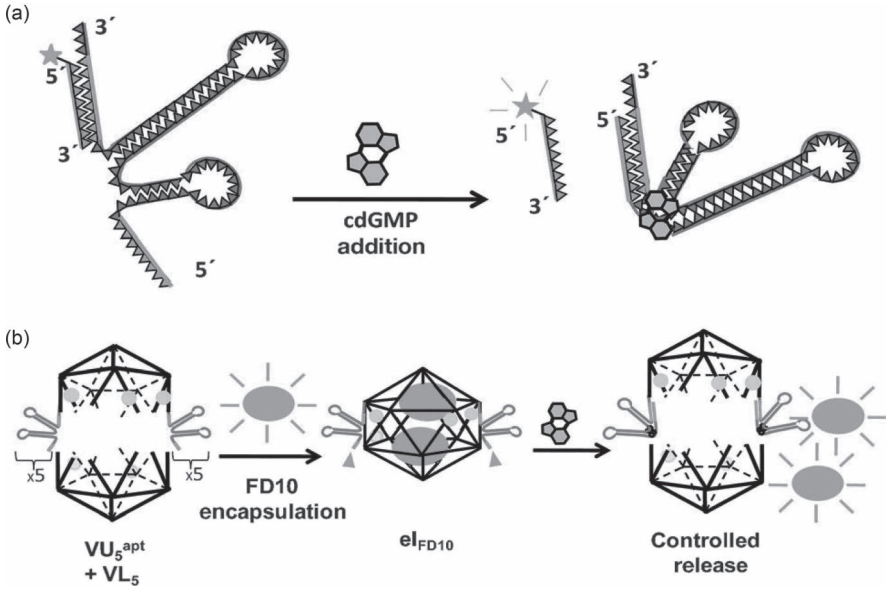

FIGURE 6.22 (a) A DNA aptamer was adapted into a DNA switch that undergoes conformational change upon binding to its input molecule c-di-GMP. (b) This DNA switch was inserted on a DNA cage whose opening, and drug cargo release, is triggered by activation of the DNA switch in the presence of c-di-GMP. (Reprinted with permission from Ref. [157]. Copyright 2013 John Wiley and Sons.)

drastically speeds up the progression in this field. These two features, along with the intrinsic biocompatibility of DNA, are at the center of the development of sophisticated nanotechnological tools that offer stimuli responsive capacity. One such bio-inspired example described in this review are DNA switches employed for biosensing and drug delivery applications. Throughout this chapter, we presented a summary of the step-by-step process to engineer DNA switches through multiple design and their adaptation into various applications. Selection of the input (e.g. disease marker) and the output signals (drug release, signaling mechanism) are the key elements to build DNA switches. The high programmability of DNA allows designing switches that recognize input ranging from light, to small ions, proteins or whole cells and that are able to translate this recognition event into a myriad of output signals from electrical current to fluorescence or drug release, for example. We also presented more than five different strategies to incorporate structure switching mechanisms within DNA switches. All of these strategies are aimed to engineer a non-binding conformation that corresponds to the switch inactive state. This non-binding conformation can switch to the bound-active conformation in the presence of the input through a population-shift model and generate the output signal implemented within the switch.

In addition to applications in biosensing and drug delivery, DNA switches have also found a lot of use in fundamental research and medicine. For example, DNA nanothermometer and nano pH meter have been developed to detect either temperature changes or pH variations at the nanoscale. These tools can be useful to monitor physical change within complex systems like living cells (e.g. mapping the pH of living cells in different conditions) or can also be adapted for drug delivery applications (e.g. pH-dependent drug delivery). Application of DNA switches in molecular diagnostics also represents a promising avenue in the medical field with the development of fast, inexpensive, point-of-care approaches that provide results within less than 10 min. These approaches often rely on DNA switches that provide electrochemical output in the presence of specific disease markers (e.g. HIV antibodies) and that can be employed directly in whole blood.

Although the future of DNA switches looks very promising, several challenges lie ahead before realizing this promise [160]. One example of such challenge is the need to create and develop DNA switches that can achieve sufficient specificity and selectivity. We believe that recent works seeking to expand the DNA code beyond the classic natural A, T, C and G base pairs with artificial nucleotides should greatly contribute to enhance the specificity of nucleic acid-based recognition elements [161,162]. Other challenges faced by DNA nanotechnology to move beyond laboratory-scale systems include improving accuracy (e.g. for DNA switch-based sensors), stability (to enable repeatability in DNA switches) and reproducibility (e.g. over industrial-scale production) [160]. To that end, an improvement and increased understanding of large-scale production of oligonucleotides to decrease cost and increase yield will be useful [163]. Finally, application of DNA switches in various medical fields for therapies will need a better understanding of intracellular uptake and trafficking mechanisms, DNA switches toxicology and their pharmacokinetic properties [164].

References

1. M. C. Lagerström, H. B. Schiöth, Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nature Reviews Drug Discovery* **7**, 339 (2008).
2. R. T. Dorsam, J. S. Gutkind, G-protein-coupled receptors and cancer. *Nature Reviews Cancer* **7**, 79 (2007).
3. D. A. Leigh, Genesis of the nanomachines: The 2016 nobel prize in chemistry. *Angewandte Chemie International Edition* **55**, 14506–14508 (2016).
4. A. E. Friedman, J. C. Chambron, J. P. Sauvage, N. J. Turro, J. K. Barton, A molecular light switch for DNA: Ru (bpy) 2 (dppz) 2+. *Journal of the American Chemical Society* **112**, 4960–4962 (1990).
5. V. Balzani, A. Credi, F. M. Raymo, J. F. Stoddart, Artificial molecular machines. *Angewandte Chemie International Edition* **39**, 3348–3391 (2000).
6. N. Koumura, R. W. Zijlstra, R. A. van Delden, N. Harada, B. L. Feringa, Light-driven monodirectional molecular rotor. *Nature* **401**, 152 (1999).
7. D. Baker, A. Sali, Protein structure prediction and structural genomics. *Science* **294**, 93–96 (2001).
8. P. Bradley, K. M. Misura, D. Baker, Toward high-resolution de novo structure prediction for small proteins. *Science* **309**, 1868–1871 (2005).
9. L. Jiang *et al.*, De novo computational design of retro-aldol enzymes. *Science* **319**, 1387–1391 (2008).
10. B. Kuhlman *et al.*, Design of a novel globular protein fold with atomic-level accuracy. *Science* **302**, 1364–1368 (2003).
11. A. Desrosiers, A. Vallée-Bélisle. Nature-inspired DNA switches: applications in medicine. *Nanomedicine (Lond)* **12**(3), 175–179 (2016).
12. S. G. Harroun *et al.*, Programmable DNA switches and their applications. *Nanoscale* **10**, 4607–4641 (2018).
13. N. C. Seeman, DNA engineering and its application to nanotechnology. *Trends in Biotechnology* **17**, 437–443 (1999).
14. F. Wang, X. Liu, I. Willner, DNA switches: From principles to applications. *Angewandte Chemie International Edition* **54**, 1098–1129 (2015).
15. C.-H. Lu, B. Willner, I. Willner, DNA nanotechnology: From sensing and DNA machines to drug-delivery systems. *ACS Nano* **7**, 8320–8332 (2013).
16. Y. Hu, A. Cecconello, A. Idili, F. Ricci, I. Willner, Triplex DNA nanostructures: From basic properties

to applications. *Angewandte Chemie* **56**, 15210–15233 (2017).
17. L. Qian, E. Winfree, Scaling up digital circuit computation with DNA strand displacement cascades. *Science* **332**, 1196–1201 (2011).
18. C. G. Evans, E. Winfree, Physical principles for DNA tile self-assembly. *Chemical Society Reviews* **46**, 3808–3829 (2017).
19. N. C. Seeman, DNA nanotechnology: Novel DNA constructions. *Annual Review of Biophysics and Biomolecular Structure* **27**, 225–248 (1998).
20. A. V. Pinheiro, D. Han, W. M. Shih, H. Yan, Challenges and opportunities for structural DNA nanotechnology. *Nature Nanotechnology* **6**, 763 (2011).
21. A. Vallée-Bélisle, F. Ricci, K. W. Plaxco, Thermodynamic basis for the optimization of binding-induced biomolecular switches and structure-switching biosensors. *Proceedings of the National Academy of Sciences* **106**, 13802–13807 (2009).
22. F. Ricci, A. Vallée-Bélisle, A. J. Simon, A. Porchetta, K. W. Plaxco, Using nature's "tricks" to rationally tune the binding properties of biomolecular receptors. *Accounts of Chemical Research* **49**, 1884–1892 (2016).
23. F. Ricci, A. Vallée-Bélisle, K. W. Plaxco, High-precision, in vitro validation of the sequestration mechanism for generating ultrasensitive dose-response curves in regulatory networks. *PLoS Computational Biology* **7**, e1002171 (2011).
24. F. Ricci, A. Vallée-Bélisle, A. Porchetta, K. W. Plaxco, Rational design of allosteric inhibitors and activators using the population-shift model: In vitro validation and application to an artificial biosensor. *Journal of the American Chemical Society* **134**, 15177–15180 (2012).
25. A. J. Simon, A. Vallée-Bélisle, F. Ricci, H. M. Watkins, K. W. Plaxco, Using the population-shift mechanism to rationally introduce "Hill-type" cooperativity into a normally non-cooperative receptor. *Angewandte Chemie International Edition* **53**, 9471–9475 (2014).
26. D. Mariottini, A. Idili, A. Vallée-Bélisle, K. W. Plaxco, F. Ricci, A DNA nanodevice that loads and releases a cargo with hemoglobin-like allosteric control and cooperativity. *Nano Letters* **17**, 3225–3230 (2017).
27. S. Tyagi, F. R. Kramer, Molecular beacons: Probes that fluoresce upon hybridization. *Nature Biotechnology* **14**, 303 (1996).
28. N. C. Seeman, DNA in a material world. *Nature* **421**, 427 (2003).
29. P. W. Rothemund, Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 297 (2006).
30. J. Chen, N. C. Seeman, Synthesis from DNA of a molecule with the connectivity of a cube. *Nature* **350**, 631 (1991).
31. N. R. Kallenbach, R.-I. Ma, N. C. Seeman, An immobile nucleic acid junction constructed from oligonucleotides. *Nature* **305**, 829 (1983).
32. N. C. Seeman, Nucleic acid junctions and lattices. *Journal of Theoretical Biology* **99**, 237–247 (1982).
33. M. Churchill, T. D. Tullius, N. R. Kallenbach, N. C. Seeman, A Holliday recombination intermediate is twofold symmetric. *Proceedings of the National Academy of Sciences* **85**, 4653–4656 (1988).
34. R.-I. Ma, N. R. Kallenbach, R. D. Sheardy, M. L. Petrillo, N. C. Seeman, Three-arm nucleic acid junctions are flexible. *Nucleic Acids Research* **14**, 9745–9753 (1986).
35. J. Elbaz *et al.*, DNA computing circuits using libraries of DNAzyme subunits. *Nature Nanotechnology* **5**, 417 (2010).
36. A. D. Ellington, J. W. Szostak, Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. *Nature* **355**, 850 (1992).
37. C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *science* **249**, 505–510 (1990).
38. M. Zuker, Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* **31**, 3406–3415 (2003).
39. J. N. Zadeh *et al.*, NUPACK: Analysis and design of nucleic acid systems. *Journal of Computational Chemistry* **32**, 170–173 (2011).
40. R. Veneziano *et al.*, Designer nanoscale DNA assemblies programmed from the top down. *Science* **352**, 1534–1534 (2016).
41. R. Nutiu, Y. Li, Structure-switching signaling aptamers. *Journal of the American Chemical Society* **125**, 4771–4778 (2003).
42. A. Vallée-Bélisle, K. W. Plaxco, Structure-switching biosensors: Inspired by Nature. *Current Opinion in Structural Biology* **20**, 518–526 (2010).
43. M. R. Jones, N. C. Seeman, C. A. Mirkin, Programmable materials and the nature of the DNA bond. *Science* **347**, 1260901 (2015).
44. D. Y. Zhang, E. Winfree, Control of DNA strand displacement kinetics using toehold exchange. *Journal of the American Chemical Society* **131**, 17303–17314 (2009).
45. D. Y. Zhang, G. Seelig, Dynamic DNA nanotechnology using strand-displacement reactions. *Nature Chemistry* **3**, 103 (2011).
46. G. Ke *et al.*, L-DNA molecular beacon: A safe, stable, and accurate intracellular nano-thermometer for temperature sensing in living cells. *Journal of the American Chemical Society* **134**, 18908–18911 (2012).
47. S. Surana, A. R. Shenoy, Y. Krishnan, Designing DNA nanodevices for compatibility with the immune

system of higher organisms. *Nature Nanotechnology* **10**, 741 (2015).
48. S. Kosuri, G. M. Church, Large-scale de novo DNA synthesis: Technologies and applications. *Nature Methods* **11**, 499 (2014).
49. S. L. Beaucage, R. P. Iyer, Advances in the synthesis of oligonucleotides by the phosphoramidite approach. *Tetrahedron* **48**, 2223–2311 (1992).
50. D. A. Lashkari, S. P. Hunicke-Smith, R. M. Norgren, R. W. Davis, T. Brennan, An automated multiplex oligonucleotide synthesizer: Development of high-throughput, low-cost DNA synthesis. *Proceedings of the National Academy of Sciences* **92**, 7912–7915 (1995).
51. F. A. Aldaye, H. F. Sleiman, Guest-mediated access to a single DNA nanostructure from a library of multiple assemblies. *Journal of the American Chemical Society* **129**, 10070–10071 (2007).
52. Y.-H. M. Chan, B. van Lengerich, S. G. Boxer, Lipid-anchored DNA mediates vesicle fusion as observed by lipid and content mixing. *Biointerphases* **3**, FA17–FA21 (2008).
53. D. Musumeci, D. Montesarchio, Synthesis of a cholesteryl-HEG phosphoramidite derivative and its application to lipid-conjugates of the anti-HIV 5'TGGGAG3'Hotoda's sequence. *Molecules* **17**, 12378–12392 (2012).
54. M. J. Damha, P. A. Giannaris, S. V. Zabarylo, An improved procedure for derivatization of controlled-pore glass beads for solid-phase oligonucleotide synthesis. *Nucleic Acids Research* **18**, 3813–3821 (1990).
55. R. T. Pon, N. Usman, K. Ogilvie, Derivatization of controlled pore glass beads for solid phase oligonucleotide synthesis. *Biotechniques* **6**, 768–775 (1988).
56. S. Padmanabhan, J. E. Coughlin, R. P. Iyer, Microwave-assisted functionalization of solid supports: Application in the rapid loading of nucleosides on controlled-pore-glass (CPG). *Tetrahedron Letters* **46**, 343–347 (2005).
57. K. M. Ririe, R. P. Rasmussen, C. T. Wittwer, Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Analytical Biochemistry* **245**, 154–160 (1997).
58. J. Lee, N. A. Kotov, Thermometer design at the nanoscale. *Nano Today* **2**, 48–51 (2007).
59. M. Frank-Kamenetskii, Simplification of the empirical relationship between melting temperature of DNA, its GC content and concentration of sodium ions in solution. *Biopolymers* **10**, 2623–2624 (1971).
60. C. Schildkraut, S. Lifson, Dependence of the melting temperature of DNA on salt concentration. *Biopolymers* **3**, 195–208 (1965).
61. D. Gareau, A. Desrosiers, A. Vallée-Bélisle, Programmable quantitative DNA nanothermometers. *Nano Letters* **16**, 3976–3981 (2016).
62. A. T. Jonstrup, J. Freds∅e, A. H. Andersen, DNA hairpins as temperature switches, thermometers and ionic detectors. *Sensors* **13**, 5937–5944 (2013).
63. S. Ebrahimi, Y. Akhlaghi, M. Kompany-Zareh, Å. Rinnan, Nucleic acid based fluorescent nanothermometers. *ACS Nano* **8**, 10372–10382 (2014).
64. Y. Wu *et al.*, Novel ratiometric fluorescent nanothermometers based on fluorophores-labeled short single-stranded DNA. *ACS Applied Materials & Interfaces* **9**, 11073–11081 (2017).
65. R. Tashiro, H. Sugiyama, A nanothermometer based on the different π stackings of B-and Z-DNA. *Angewandte Chemie International Edition* **42**, 6018–6020 (2003).
66. H. Asanuma, T. Ito, T. Yoshida, X. Liang, M. Komiyama, Photoregulation of the formation and dissociation of a DNA duplex by using the cis–trans isomerization of azobenzene. *Angewandte Chemie International Edition* **38**, 2393–2395 (1999).
67. C. Dohno, K. Nakatani, Control of DNA hybridization by photoswitchable molecular glue. *Chemical Society Reviews* **40**, 5718–5729 (2011).
68. C. Dohno, S.-n. Uno, K. Nakatani, Photoswitchable molecular glue for DNA. *Journal of the American Chemical Society* **129**, 11898–11899 (2007).
69. H. Kang *et al.*, Single-DNA molecule nanomotor regulated by photons. *Nano Letters* **9**, 2690–2696 (2009).
70. J. Hihath, B. Xu, P. Zhang, N. Tao, Study of single-nucleotide polymorphisms by means of electrical conductance measurements. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 16979–16983 (2005).
71. R. Korol, D. Segal, From exhaustive simulations to key principles in DNA nanoelectronics. *The Journal of Physical Chemistry C* **122**, 4206–4216 (2018).
72. L. Xiang *et al.*, Gate-controlled conductance switching in DNA. *Nature Communications* **8**, 14471 (2017).
73. K. Wang, DNA-based single-molecule electronics: From concept to function. *Journal of Functional Biomaterials* **9**, 8 (2018).
74. B. Y. Won, C. Jung, K. S. Park, H. G. Park, An electrochemically reversible DNA switch. *Electrochemistry Communications* **27**, 100–103 (2013).
75. A. Idili, A. Vallée-Bélisle, F. Ricci, Programmable pH-triggered DNA nanoswitches. *Journal of the American Chemical Society* **136**, 5836–5839 (2014).
76. H. A. Day, P. Pavlou, Z. A. Waller, i-Motif DNA: Structure, stability and targeting with ligands. *Bioorganic & Medicinal Chemistry* **22**, 4407–4418 (2014).
77. Y. Dong, Z. Yang, D. Liu, DNA nanotechnology based on i-motif structures. *Accounts of Chemical Research* **47**, 1853–1860 (2014).
78. S. Chakraborty, S. Sharma, P. K. Maiti, Y. Krishnan, The poly dA helix: A new structural motif for

high performance DNA-based molecular switches. *Nucleic Acids Research* **37**, 2810–2817 (2009).
79. S. Modi, C. Nizak, S. Surana, S. Halder, Y. Krishnan, Two DNA nanomachines map pH changes along intersecting endocytic pathways inside the same cell. *Nature Nanotechnology* **8**, 459 (2013).
80. S. Modi *et al.*, A DNA nanomachine that maps spatial and temporal pH changes inside living cells. *Nature Nanotechnology* **4**, 325 (2009).
81. A. Porchetta, A. Idili, A. Vallée-Bélisle, F. Ricci, General strategy to introduce pH-induced allostery in DNA-based receptors to achieve controlled release of ligands. *Nano Letters* **15**, 4467–4471 (2015).
82. A. Ambrus *et al.*, Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution. *Nucleic Acids Research* **34**, 2723–2735 (2006).
83. Z.-S. Wu, C.-R. Chen, G.-L. Shen, R.-Q. Yu, Reversible electronic nanoswitch based on DNA G-quadruplex conformation: A platform for single-step, reagentless potassium detection. *Biomaterials* **29**, 2689–2696 (2008).
84. X. Hou *et al.*, A biomimetic potassium responsive nanochannel: G-quadruplex DNA conformational switching in a synthetic nanopore. *Journal of the American Chemical Society* **131**, 7800–7805 (2009).
85. Z. Lin, Y. Chen, X. Li, W. Fang, Pb^{2+} induced DNA conformational switch from hairpin to G-quadruplex: Electrochemical detection of Pb^{2+}. *Analyst* **136**, 2367–2372 (2011).
86. M. Hoang, P.-J. J. Huang, J. Liu, G-quadruplex DNA for fluorescent and colorimetric detection of thallium (I). *ACS Sensors* **1**, 137–143 (2015).
87. D. Bhattacharyya, G. Mirihana Arachchilage, S. Basu, Metal cations in G-quadruplex folding and stability. *Frontiers in chemistry* **4**, 38 (2016).
88. D.-L. Ma *et al.*, Recent developments in G-quadruplex probes. *Chemistry & Biology* **22**, 812–828 (2015).
89. D. Zhao, Y. Fan, F. Gao, T.-M. Yang, "Turn-off-on" fluorescent sensor for (N-methyl-4-pyridyl) porphyrin-DNA and G-quadruplex interactions based on ZnCdSe quantum dots. *Analytica Chimica Acta* **888**, 131–137 (2015).
90. Y. Miyake *et al.*, $Mercury^{II}$-mediated formation of thymine$-Hg^{II}-$thymine base pairs in DNA duplexes. *Journal of the American Chemical Society* **128**, 2172–2173 (2006).
91. A. Ono *et al.*, Specific interactions between silver (I) ions and cytosine–cytosine pairs in DNA duplexes. *Chemical Communications* **39**, 4825–4827 (2008).
92. F. Pfeiffer, G. Mayer, Selection and biosensor application of aptamers for small molecules. *Frontiers in Chemistry* **4**, 25 (2016).
93. B. Deng *et al.*, Aptamer binding assays for proteins: The thrombin example—a review. *Analytica Chimica Acta* **837**, 1–15 (2014).
94. N. Hamaguchi, A. Ellington, M. Stanton, Aptamer beacons for the direct detection of proteins. *Analytical Biochemistry* **294**, 126–131 (2001).
95. P. K. Kumar, Monitoring intact viruses using aptamers. *Biosensors* **6**, 40 (2016).
96. A. Davydova *et al.*, Aptamers against pathogenic microorganisms. *Critical Reviews in Microbiology* **42**, 847–865 (2016).
97. A. D. Ellington, J. W. Szostak, In vitro selection of RNA molecules that bind specific ligands. *Nature* **346**, 818 (1990).
98. W. Zhou, P.-J. J. Huang, J. Ding, J. Liu, Aptamer-based biosensors for biomedical diagnostics. *Analyst* **139**, 2627–2640 (2014).
99. M. N. Stojanovic, P. De Prada, D. W. Landry, Aptamer-based folding fluorescent sensor for cocaine. *Journal of the American Chemical Society* **123**, 4928–4931 (2001).
100. Z.-X. Wang, An exact mathematical expression for describing competitive binding of two different ligands to a protein molecule. *FEBS Letters* **360**, 111–114 (1995).
101. Z. Tang *et al.*, Aptamer switch probe based on intramolecular displacement. *Journal of the American Chemical Society* **130**, 11268–11269 (2008).
102. Y. Xing, Z. Yang, D. Liu, A responsive hidden toehold to enable controllable DNA strand displacement reactions. *Angewandte Chemie International Edition* **50**, 11934–11936 (2011).
103. H. Zhang *et al.*, Assembling DNA through affinity binding to achieve ultrasensitive protein detection. *Angewandte Chemie International Edition* **52**, 10698–10705 (2013).
104. W. Tang *et al.*, DNA tetraplexes-based toehold activation for controllable DNA strand displacement reactions. *Journal of the American Chemical Society* **135**, 13628–13631 (2013).
105. A. Amodio *et al.*, Rational design of pH-controlled DNA strand displacement. *Journal of the American Chemical Society* **136**, 16469–16472 (2014).
106. N. R. Markham, M. Zuker, DINAMelt web server for nucleic acid melting prediction. *Nucleic Acids Research* **33**, W577–W581 (2005).
107. A. Idili, F. Ricci, A. Vallée-Bélisle, Determining the folding and binding free energy of DNA-based nanodevices and nanoswitches using urea titration curves. *Nucleic Acids Research* **45**, 7571–7580 (2017).
108. Y. You, A. V. Tataurov, R. Owczarzy, Measuring thermodynamic details of DNA hybridization using fluorescence. *Biopolymers* **95**, 472–486 (2011).
109. T. Schlichthaerle, M. T. Strauss, F. Schueder, J. B. Woehrstein, R. Jungmann, DNA nanotechnology

and fluorescence applications. *Current Opinion in Biotechnology* **39**, 41–47 (2016).
110. C. Feng, S. Dai, L. Wang, Optical aptasensors for quantitative detection of small biomolecules: A review. *Biosensors and Bioelectronics* **59**, 64–74 (2014).
111. R. Roy, S. Hohng, T. Ha, A practical guide to single-molecule FRET. *Nature Methods* **5**, 507 (2008).
112. F. Xia *et al.*, On the binding of cationic, water-soluble conjugated polymers to DNA: Electrostatic and hydrophobic interactions. *Journal of the American Chemical Society* **132**, 1252–1254 (2010).
113. A. Vallée-Bélisle, F. Ricci, T. Uzawa, F. Xia, K. W. Plaxco, Bioelectrochemical switches for the quantitative detection of antibodies directly in whole blood. *Journal of the American Chemical Society* **134**, 15197–15200 (2012).
114. S. S. Mahshid, S. B. Camiré, F. Ricci, A. Vallée-Bélisle, A highly selective electrochemical DNA-based sensor that employs steric hindrance effects to detect proteins directly in whole blood. *Journal of the American Chemical Society* **137**, 15596–15599 (2015).
115. C. Fan, K. W. Plaxco, A. J. Heeger, Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proceedings of the National Academy of Sciences* **100**, 9134–9137 (2003).
116. A. Kowalczyk *et al.*, Construction of DNA biosensor at glassy carbon surface modified with 4-aminoethylbenzenediazonium salt. *Biosensors and Bioelectronics* **26**, 2506–2512 (2011).
117. Y. Tang, B. Ge, D. Sen, H.-Z. Yu, Functional DNA switches: Rational design and electrochemical signaling. *Chemical Society Reviews* **43**, 518–529 (2014).
118. L. Yu, H. Lim, A. Maslova, I. Hsing, Rational design of electrochemical DNA biosensors for point-of-care applications. *ChemElectroChem* **4**, 795–805 (2017).
119. S. S. Mahshid, A. Vallée-Bélisle, S. O. Kelley, Biomolecular steric hindrance effects are enhanced on nanostructured microelectrodes. *Analytical Chemistry* **89**, 9751–9757 (2017).
120. S. S. Mahshid, F. Ricci, S. O. Kelley, A. Vallée-Bélisle, Electrochemical DNA-based immunoassay that employs steric hindrance to detect small molecules directly in whole blood. *ACS Sensors* **2**, 718–723 (2017).
121. C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* **382**, 607 (1996).
122. N. L. Rosi, C. A. Mirkin, Nanostructures in biodiagnostics. *Chemical Reviews* **105**, 1547–1562 (2005).
123. C. Chen, G. Song, J. Ren, X. Qu, A simple and sensitive colorimetric pH meter based on DNA conformational switch and gold nanoparticle aggregation. *Chemical Communications* **2008**, 6149–6151 (2008).
124. F. Xia *et al.*, Colorimetric detection of DNA, small molecules, proteins, and ions using unmodified gold nanoparticles and conjugated polyelectrolytes. *Proceedings of the National Academy of Sciences* **107**, 10837–10841 (2010).
125. A.-M. Dallaire, S. Patskovsky, A. Vallée-Bélisle, M. Meunier, Electrochemical plasmonic sensing system for highly selective multiplexed detection of biomolecules based on redox nanoswitches. *Biosensors and Bioelectronics* **71**, 75–81 (2015).
126. N. H. Kim, S. J. Lee, M. Moskovits, Aptamer-mediated surface-enhanced Raman spectroscopy intensity amplification. *Nano Letters* **10**, 4181–4185 (2010).
127. A. Barhoumi, N. J. Halas, Label-free detection of DNA hybridization using surface enhanced Raman spectroscopy. *Journal of the American Chemical Society* **132**, 12792–12793 (2010).
128. Y.-J. Chen, B. Groves, R. A. Muscat, G. Seelig, DNA nanotechnology from the test tube to the cell. *Nature Nanotechnology* **10**, 748 (2015).
129. T. L. Fisher, T. Terhorst, X. Cao, R. W. Wagner, Intracellular disposition and metabolism of fluorescently-labled unmodified and modified oligouncleotides microijjected into mammalian cells. *Nucleic Acids Research* **21**, 3857–3865 (1993).
130. S. D. Putney, S. J. Benkovic, P. R. Schimmel, A DNA fragment with an alpha-phosphorothioate nucleotide at one end is asymmetrically blocked from digestion by exonuclease III and can be replicated in vivo. *Proceedings of the National Academy of Sciences* **78**, 7350–7354 (1981).
131. D. Sheehan *et al.*, Biochemical properties of phosphonoacetate and thiophosphonoacetate oligodeoxyribonucleotides. *Nucleic Acids Research* **31**, 4109–4118 (2003).
132. R. M. Hudziak *et al.*, Resistance of morpholino phosphorodiamidate oligomers to enzymatic degradation. *Antisense and Nucleic Acid Drug Development* **6**, 267–272 (1996).
133. M. A. Reynolds *et al.*, Synthesis and thermodynamics of oligonucleotides containing chirally pure R P methylphosphonate linkages. *Nucleic Acids Research* **24**, 4584–4591 (1996).
134. T. Ono, M. Scalf, L. M. Smith, 2′-Fluoro modified nucleic acids: Polymerase-directed synthesis, properties and stability to analysis by matrix-assisted laser desorption/ionization mass spectrometry. *Nucleic Acids Research* **25**, 4581–4588 (1997).
135. J. K. Watts, G. F. Deleavey, M. J. Damha, Chemically modified siRNA: Tools and applications. *Drug Discovery Today* **13**, 842–855 (2008).
136. M. Frieden, H. F. Hansen, T. Koch, Nuclease stability of LNA oligonucleotides and LNA-DNA chimeras.

Nucleosides, Nucleotides and Nucleic Acids **22**, 1041–1043 (2003).
137. C. A. Stein, D. Castanotto, FDA-approved oligonucleotide therapies in 2017. *Molecular Therapy* **25**, 1069–1075 (2017).
138. E. W. Ottesen, ISS-N1 makes the first FDA-approved drug for spinal muscular atrophy. *Translational Neuroscience* **8**, 1–6 (2017).
139. E. S. Gragoudas, A. P. Adamis, E. T. Cunningham Jr, M. Feinsod, D. R. Guyer, Pegaptanib for neovascular age-related macular degeneration. *New England Journal of Medicine* **351**, 2805–2816 (2004).
140. S. Ko, H. Liu, Y. Chen, C. Mao, DNA nanotubes as combinatorial vehicles for cellular delivery. *Biomacromolecules* **9**, 3039–3043 (2008).
141. J. Winkler, Oligonucleotide conjugates for therapeutic applications. *Therapeutic Delivery* **4**, 791–809 (2013).
142. S. Saha, V. Prakash, S. Halder, K. Chakraborty, Y. Krishnan, A pH-independent DNA nanodevice for quantifying chloride transport in organelles of living cells. *Nature Nanotechnology* **10**, 645 (2015).
143. L. Liang *et al.*, Single-particle tracking and modulation of cell entry pathways of a tetrahedral DNA nanostructure in live cells. *Angewandte Chemie International Edition* **53**, 7745–7750 (2014).
144. M. G. Kolonin *et al.*, Synchronous selection of homing peptides for multiple tissues by in vivo phage display. *The FASEB Journal* **20**, 979–981 (2006).
145. R. Pasqualini, E. Ruoslahti, Organ targeting in vivo using phage display peptide libraries. *Nature* **380**, 364 (1996).
146. G. P. Smith, Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. *Science* **228**, 1315–1317 (1985).
147. S. Surana, J. M. Bhat, S. P. Koushika, Y. Krishnan, An autonomous DNA nanomachine maps spatiotemporal pH changes in a multicellular living organism. *Nature Communications* **2**, 340 (2011).
148. A. S. Piatek *et al.*, Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis. *Nature Biotechnology* **16**, 359 (1998).
149. S. Tyagi, D. P. Bratu, F. R. Kramer, Multicolor molecular beacons for allele discrimination. *Nature Biotechnology* **16**, 49 (1998).
150. S. A. Marras, F. R. Kramer, S. Tyagi, Multiplex detection of single-nucleotide variations using molecular beacons. *Genetic Analysis: Biomolecular Engineering* **14**, 151–156 (1999).
151. S. Ranallo, C. Prévost-Tremblay, A. Idili, A. Vallée-Bélisle, F. Ricci, Antibody-powered nucleic acid release using a DNA-based nanomachine. *Nature Communications* **8**, 15150 (2017).
152. S. Ranallo, M. Rossetti, K. W. Plaxco, A. Vallée-Bélisle, F. Ricci, A modular, DNA-based beacon for single-step fluorescence detection of antibodies and other proteins. *Angewandte Chemie* **127**, 13412–13416 (2015).
153. R. A. Gatenby, R. J. Gillies, Why do cancers have high aerobic glycolysis? *Nature Reviews Cancer* **4**, 891 (2004).
154. V. Estrella *et al.*, Acidity generated by the tumor microenvironment drives local invasion. *Cancer Research* **73**, 1524–1535 (2013).
155. J. Liu *et al.*, pH-sensitive nano-systems for drug delivery in cancer therapy. *Biotechnology Advances* **32**, 693–710 (2014).
156. R. Hengge, Principles of c-di-GMP signalling in bacteria. *Nature Reviews Microbiology* **7**, 263 (2009).
157. A. Banerjee *et al.*, Controlled release of encapsulated cargo from a DNA icosahedron using a chemical trigger. *Angewandte Chemie International Edition* **52**, 6854–6857 (2013).
158. A. S. Walsh, H. Yin, C. M. Erben, M. J. Wood, A. J. Turberfield, DNA cage delivery to mammalian cells. *ACS Nano* **5**, 5427–5432 (2011).
159. D. Bhatia, S. Surana, S. Chakraborty, S. P. Koushika, Y. Krishnan, A synthetic icosahedral DNA-based host–cargo complex for functional in vivo imaging. *Nature Communications* **2**, 339 (2011).
160. T. R. Fadel *et al.*, Toward the responsible development and commercialization of sensor nanotechnologies. *ACS Sensors* **1**, 207–216 (2016).
161. D. W. Drolet, L. S. Green, L. Gold, N. Janjic, Fit for the eye: Aptamers in ocular disorders. *Nucleic Acid Therapeutics* **26**, 127–146 (2016).
162. S. Gupta *et al.*, Chemically-modified DNA aptamers bind interleukin-6 with high affinity and inhibit signaling by blocking its interaction with interleukin-6 receptor. *Journal of Biological Chemistry* **289**, 8706–8719. doi:10.1074/jbc.M113.532580 (2014).
163. T. Tørring, K. V. Gothelf, DNA nanotechnology: A curiosity or a promising technology? *F1000prime Reports* **5**, 14 (2013).
164. S. Bamrungsap *et al.*, Nanotechnology in therapeutics: A focus on nanoparticles as a drug delivery system. *Nanomedicine* **7**, 1253–1271 (2012).