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REVIEW

Aptamer-Functionalized DNA Nanostructures for Biological Applications

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Abstract

DNA nanostructures hold great promise for various applications due to their remarkable properties, including programmable assembly, nanometric positional precision, and dynamic structural control. The past few decades have seen the development of various kinds of DNA nanostructures that can be employed as useful tools in fields such as chemistry, materials, biology, and medicine. Aptamers are short single-stranded nucleic acids that bind to specific targets with excellent selectivity and high affinity and play critical roles in molecular recognition. Recently, many attempts have been made to integrate aptamers with DNA nanostructures for a range of biological applications. This review starts with an introduction to the features of aptamer-functionalized DNA nanostructures. The discussion then focuses on recent progress (particularly during the last five years) in the applications of these nanostructures in areas such as biosensing, bioimaging, cancer therapy, and biophysics. Finally, challenges involved in the practical application of aptamer-functionalized DNA nanostructures are discussed, and perspectives on future directions for research into and applications of aptamer-functionalized DNA nanostructures are provided.

Keywords Aptamer · DNA nanostructures · DNA origami · Biosensing · Bioimaging · Drug delivery

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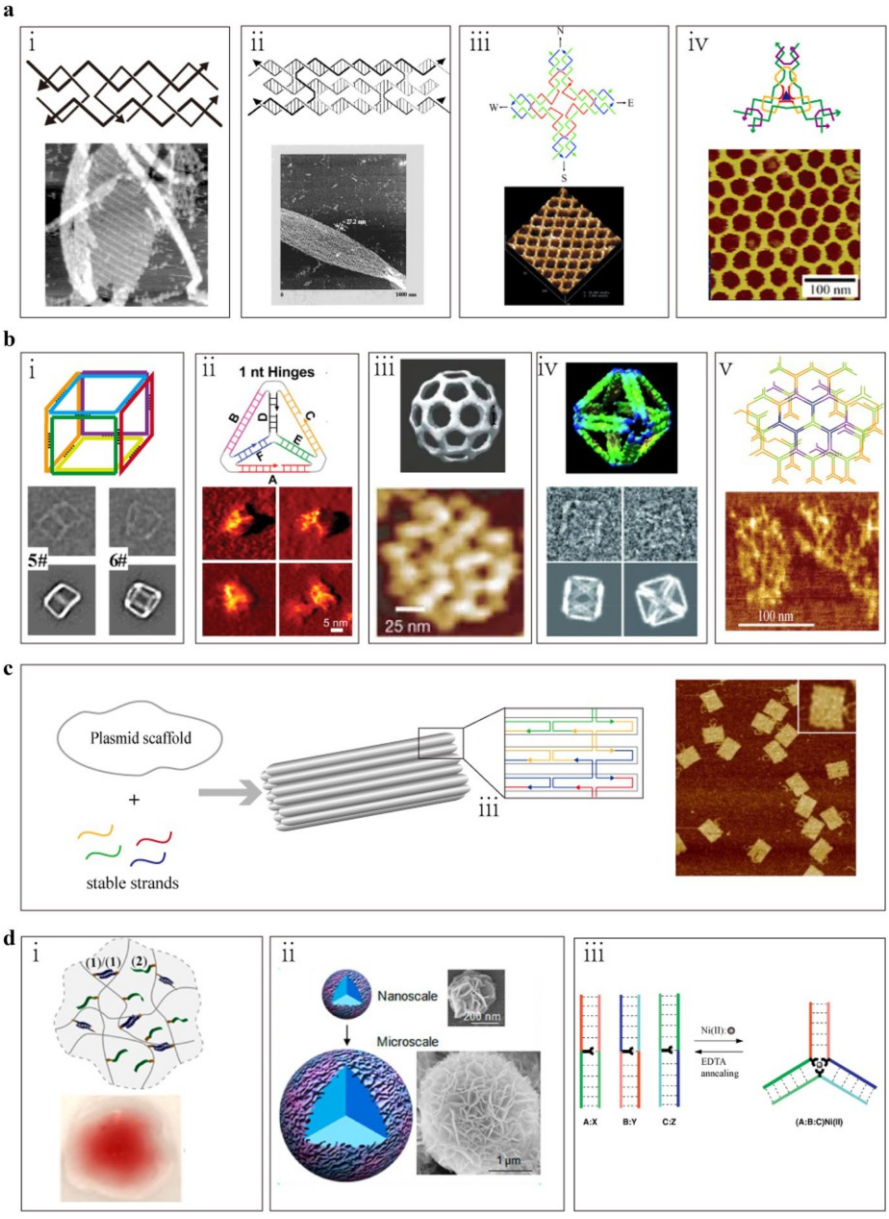
Fig. 1 Examples of representative classes of designed DNA nanostructures. **a** The building blocks of ▶ nanostructures: (*i*) double-crossover (DX) tiles (reproduced with permission from [7], copyright 1998 Springer Nature), (*ii*) triple-crossover (TX) tiles (reproduced with permission from [8], copyright 2000 American Chemical Society), (*iii*) 4×4 tiles (reproduced with permission from [9], copyright 2003 American Association for the Advancement of Science), (*iv*) three-point star tiles (reproduced with permission from [11], copyright 2005 American Chemical Society). **b** Higher-order DNA nanostructures constructed from tiles through bottom-up assembly: (*i*) DNA cube (reproduced with permission from [13], copyright 2009 American Chemical Society), (*ii*) DNA tetrahedron (reproduced with permission from [14], copyright 2005 American Association for the Advancement of Science), (*iii*) DNA polyhedron (reproduced with permission from [10], copyright 2008 Springer Nature), (*iv*) DNA octahedron (reproduced with permission from [16], copyright 2017 Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences), and (*v*) DNA dendrimer (reproduced with permission from [19], copyright 2014 American Chemical Society). **c** DNA origami-based structures (reproduced with permission from [20], copyright 2016 Wiley–VCH Verlag GmbH and Co. KGaA). **d** Other DNA nanostructures, including (*i*) DNA nanohydrogels produced by gelation (reproduced with permission from [21], copyright 2015 American Chemical Society), (*ii*) DNA nanoflowers prepared by rolling circle amplification-templated assembly (reproduced with permission from [22], copyright 2013 American Chemical Society), and (*iii*) metal-induced DNA assembly (reproduced with permission from [23], copyright 2015 Wiley–VCH)

1 Introduction

Nanotechnology, which utilizes various kinds of nanomaterials, has been widely applied in fields such as chemistry [1], biology [2], and medicine [3], among many others [4]. Nucleic acids (DNAs and RNAs), the genetic information carriers in living cells, are regarded as important building blocks for fabricating nanomaterials. Ever since Seeman first proposed the concept of structural DNA nanotechnology in 1982 [5], there has been increasing interest in fabricating DNA structures with controllability and versatility that can be applied in the field of nanobiotechnology. Compared to dsDNA helices, DNA nanostructures possess more complex and rigid structures due to the presence of immobile Holliday junctions. Various kinds of DNA nanostructures have been developed. The most basic DNA nanostructures are simple DNA tiles (Fig. 1a), such as double crossovers (DX) [6, 7], triple crossovers (TX) [8], 4×4 tiles [9], and three-point stars [10, 11].

Higher-order nanostructures can be assembled from the bottom up from DNA tiles (Fig. 1b). Such nanostructures include cubes [12, 13], tetrahedrons [14], polyhedrons [10], octahedrons [15, 16], and DNA dendrimers [17–19]. In 2006, Rothemund [24] reported DNA origami (Fig. 1c), in which DNA self-folds into predesigned shapes. More specifically, hundreds of short synthetic DNA strands termed "staples" are employed to bind at particular sites on a long single-stranded scaffold DNA (such as M13 phage genomic DNA, ~7249 bp), causing the scaffold DNA to fold into the required shape. Other methods of preparing other kinds of DNA nanostructures (Fig. 1d), such as gelation-induced DNA nanohydrogels [25–27], rolling circle amplification-templated DNA nanoflowers [22], and metal-induced DNA assembly [28], have also been reported.

DNA nanostructures have many unique advantages over other nanomaterials. First, the Watson–Crick base pairing of nucleobases (A–T/U and G–C) via hydrogen bonding makes nucleic acids powerful and programmable materials for preparing nanostructures. Second, DNA nanostructures are predictable at the molecular level,



which enables us to fabricate well-defined and uniform DNA nanostructures [29]. Third, DNA binding can occur reversibly in response to various external stimuli such as temperature [30], pH [31], and ionic strength [32], and this response can be tuned by altering the length and sequence of DNA. Fourth, DNA interacts with various chemical and biochemical species such as metal ions [33], tool enzymes [34], and small molecules [35], which can therefore be used to control the construction

or binding abilities of DNA. Fifth, the biocompatibility of DNA nanostructures facilitates their application in vitro and in vivo [36]. Finally, DNA nanostructures can interact with functional nucleic acids (e.g., aptamers [37], DNAzymes [38], and molecular beacons [39]) through simple Watson–Crick base pairing. All of these features mean that DNA nanostructures are attractive tools in fields such as cellular biophysics, medical diagnostics and therapeutics, and biomimetic systems.

Aptamers are short single-stranded nucleic acids that are isolated from a random-sequence library comprising $10^{12–15}$ different oligonucleotides of DNA or RNA through a process termed systematic evolution of ligands by exponential enrichment (SELEX) [40–42]. An aptamer that folds into a unique secondary or tertiary structure can be used to bind to a target of interest with high specificity and affinity. Aptamers have received increasing interest from researchers since they were first reported by the groups of Szastak and Gold in 1990, as they represent a useful alternative to antibodies. While the dissociation constants (K_d) of aptamers are comparable to those of antibodies (they are often regarded as "chemical antibodies"), aptamers possess many advantages over antibodies [43], such as easy synthesis and modification, small size, good stability, a wide variety of possible targets (ranging from small molecules to cells or even pathogens), and good biocompatibility. Therefore, considerable effort has been directed into identifying a variety of aptamers that can be applied in bioanalysis, diagnosis, and therapy.

Given the attraction of combining the unique characteristics of DNA nanostructures with the advantages of aptamers, it is not surprising that a great deal of research has focused on constructing a series of aptamer-functionalized DNA nanostructures for bioanalytical and biomedical applications (Fig. 2). The present review summarizes recent studies (particularly those from the last five years) of aptamer-functionalized DNA nanostructures. It mainly focuses on four representative types of DNA nanostructures: tile-based structures, DNA origami, DNA-based polymers such as dendrimers, micelles, and hydrogels, and DNA nanoflowers. Recent progress in research into these aptamer-functionalized DNA nanostructures is reviewed, as are their applications in the fields of biosensing, bioimaging, and nanomedicine (among others). Finally, the remaining challenges in this area of research and probable future trends in aptamer-functionalized DNA nanostructures are discussed.

2 Aptamer-Functionalized DNA Nanostructures for Biosensing Applications

Biosensors are useful analytical tools that include a biological sensing element. They have been widely applied in the pharmaceutical industry [44], disease diagnosis [45], food safety [46], and environmental monitoring [47]. Because the field of biosensors is undergoing phenomenal growth, an increasing number of novel analytical methods (e.g., nanomaterial-based probes) have been explored in this field in recent years [48]. Aptamers can specifically recognize various targets such as metal ions, proteins, small molecules, and even cells, which makes them promising analytical tools that can be incorporated into various sensing platforms [49]. Because it is possible to attach aptamers at nanometrically precise locations on DNA nanostructures,

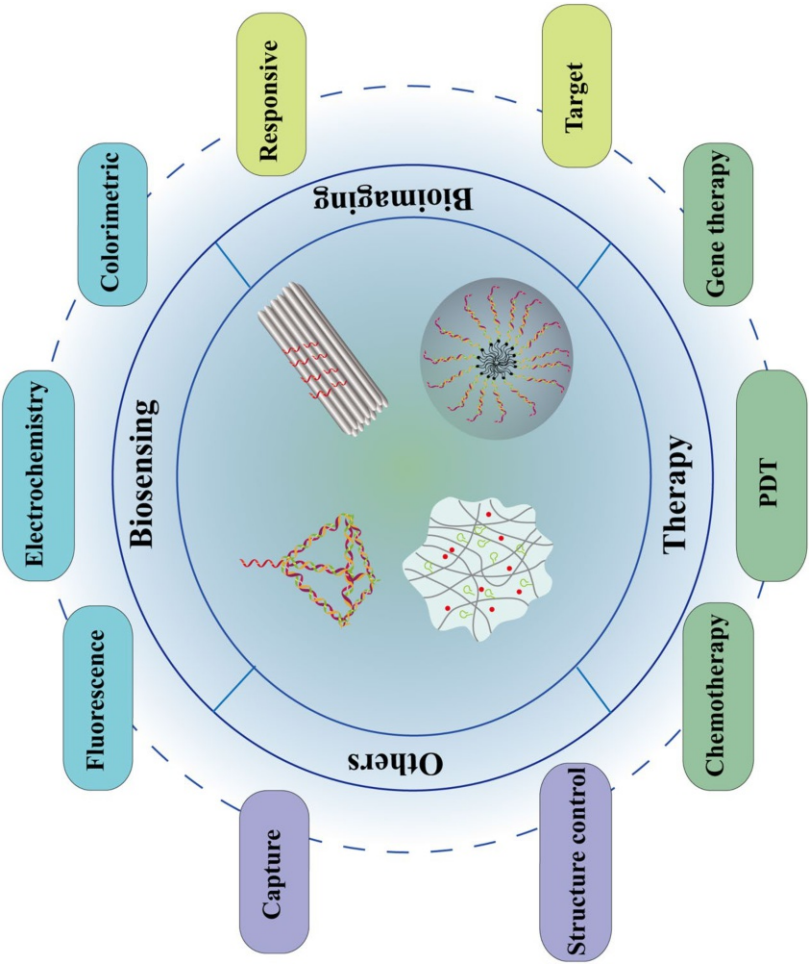


Fig. 2 Summary of the uses of aptamer-functionalized DNA nanostructures for biosensing, bioimaging, therapy, and other applications

aptamer-functionalized DNA nanostructures have great potential to detect targets through various sensing methods [50, 51]. Therefore, an increasing number of electrochemical and optical sensing platforms based on aptamer-functionalized DNA nanostructures have been exploited in recent years [52]. Here, we categorize these sensing methods into fluorescent biosensors, electrochemical biosensors, colorimetric biosensors, and other biosensors, based on the signal transduction mechanism involved.

2.1 Fluorescent Biosensors

Fluorescent biosensors make use of the physicochemical phenomenon known as fluorescence. When the analyte of interest is present, the emission characteristics (e.g., fluorescence intensity, fluorescence lifetime, and fluorescence isotropy) of the fluorophores used in the biosensor change. Below, we review the utilization of aptamer-functionalized DNA nanostructures in fluorescence biosensors employed for extracellular and intracellular biosensing.

2.1.1 Biosensing in a Tube

There are currently two ways to fabricate aptamer-functionalized DNA nanostructures for use as extracellular biosensors. The first approach is based on the rigidity and stability of DNA nanostructures. The fluorescent aptamer-based sensor is directly immobilized on a solid substrate [53, 54], thus expanding its range of biological applications. For example, Li et al. [53] immobilized a micrometer-sized DNA superstructure ("3D DNA") on paper (Fig. 3a). They integrated a fluorescently labeled thrombin (or ATP) aptamer (i.e., an aptamer that binds to thrombin/ATP) into the 3D DNA superstructure using rolling circle amplification, leading to fluorescence enhancement in the presence of thrombin (or ATP). By combining the ability of 3D DNA to adhere to the surface of paper via physisorption with the features of functional DNA (aptamers or DNAzymes), assembled structures of this nature offer a strategy for designing high-density, surface-tethered nucleic acid probe (SNAP)-based paper sensors. Song et al. [54] constructed a DNA-tetrahedron-based fluorescent microarray platform for detecting various target types. By fabricating DNA tetrahedra on a glass substrate via amine–aldehyde interactions, it was possible to position capture probes with a specific alignment and with defined spacing of the probes at the vertices of tetrahedra, which provided the probes with a solution-phase-like environment for them to recognize the target. This strategy enabled the direct application of the fluorescent probe to the solid surface, facilitating high-throughput analysis. Appropriate design of the DNA nanostructure allowed various aptamers for specific biomolecules to be fixed indirectly to other solid surfaces in a specific orientation and with defined probe spacing.

The other way to fabricate aptamer-based sensors is through the rational design of DNA nanostructures that are intended for use in solution [55, 56]. For example, Wagenknecht et al. [55] put forward the concept of a "DNA traffic light" in solution. This design utilizes split aptamers as the recognition unit of a DNA origami tweezer

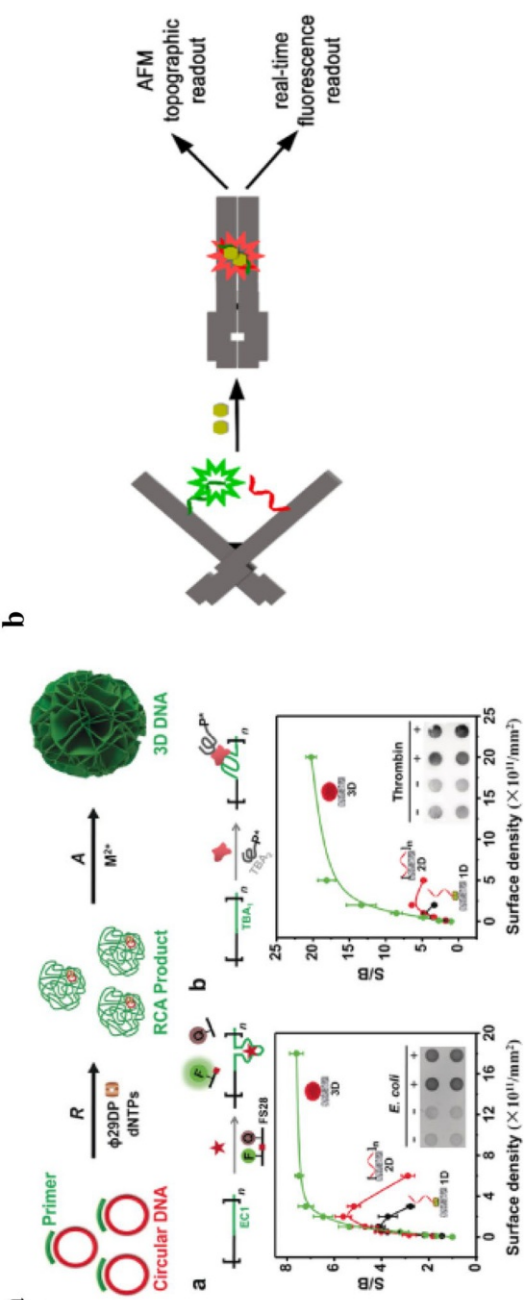


Fig. 3 Aptamer-functionalized DNA nanostructures for biosensing in a tube. **a** Schematic of the sensor based on 3D DNA on paper (reproduced with permission from [54], copyright 2018 Wiley–VCH Verlag GmbH and Co. KGaA). **b** Schematic of a "DNA traffic light" using a DNA origami tweezer with a split ATP aptamers in the presence of ATP (reproduced with permission from [55], copyright 2017 American Chemical Society)

that provides bicolor fluorescence readout (Fig. 3b). The stems of the split aptamers consist of donor and receptor fluorophores, respectively, which exhibit Förster resonance energy transfer (FRET) when the distance between the donor and the receptor is less than 10 nm. In the absence of ATP, the split aptamers are well separated, resulting in green fluorescence (the DNA origami is "open" and the FRET signal is off). When the DNA origami aptamers bind to ATP, the spilt aptamers move closer to each other, causing the fluorescence to change from green to red (the DNA origami is "closed" and the FRET signal is on). However, this assay can provide not only fluorescence readout but also topographic readout upon combining the DNA origami with AFM, representing a new strategy for bimodal sensor platforms. Tanner et al. [57] also reported an aptamer-functionalized DNA nanobox for the detection of the malaria biomarker protein *Plasmodium falciparum* lactate dehydrogenase (PfLDH) based on dual-modality (fluorescence and electron-microscopic) imaging. As well as small biomolecule detection, aptamer-functionalized DNA nanostructures can also be applied to detect whole cells [58].

2.1.2 Intracellular Biosensing

Aptamer-functionalized DNA nanostructures have also been successfully applied to detect biomolecules in cells, again making use of the rigidity and stability of these nanostructures [59]. For example, by modifying split aptamers with a DNA nanoprism, Huan et al. [60] developed an ATP-responsive biosensor for the stable, sensitive, and specific detection of ATP in living cells (Fig. 4a). In their design, the stems of the split aptamers are labeled with Cy3 and Cy5, leading to a FRET signal when the split aptamers bind to two ATP molecules. The use of the nanoprism in this design protects the probe from nuclease degradation.

In addition, some DNA aptamers (e.g., AS1411 and sgc-8) recognize proteins that are overexpressed on the surfaces of cancer cells and are internalized by cells. Thus, aptamers have been widely used as tools for transporting DNA nanoprobes into living cells. For example, Yang et al. [61] reported a DNA octahedron-based fluorescence nanoprobe for mRNA detection in living cells (Fig. 4b). Modification with the aptamer AS1411 allowed the DNA octahedron biosensor to be more efficiently internalized by cancer cells than normal cells, thus permitting the development of biosensors that facilitate specific cancer diagnoses or therapies. In order to enhance the detection sensitivity, Jie et al. [62] prepared a multifunctional DNA nanocage containing a nucleolin aptamer and fluorescent CdTe quantum dots for the fluorescence detection of human 8-oxoguanine DNA glycosylase 1 (hOGG1) with a detection limit of 0.001 U mL^{-1}.

2.2 Electrochemical Biosensors

Electrochemical biosensors have been widely applied in numerous fields, such as biomarker diagnosis [63], cancer cell screening [64], food safety [65], and environmental monitoring [66]. Compared to other aptamer-based biosensors, electrochemical methods possess the advantages of excellent sensitivity, high portability,

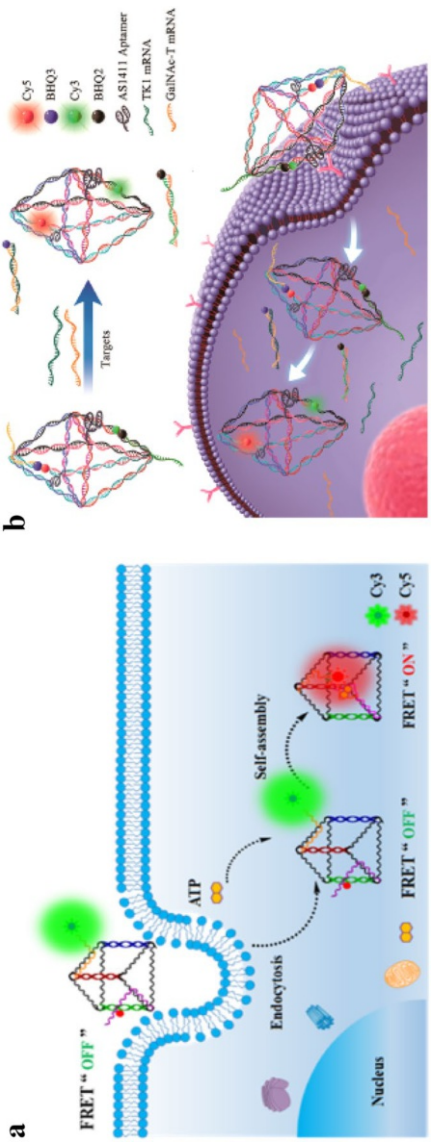


Fig. 4 Aptamer-functionalized DNA nanostructures for intracellular biosensing. **a** Schematic of split aptamers encapsulated in a DNA triangular prism for intracellular ATP sensing (reproduced with permission from [60], copyright 2017 American Chemical Society). **b** Schematic of the DNA octahedron that was integrated with the aptamer AS1411 for tumor-related mRNA detection in cells (reproduced with permission from [61], copyright 2018 American Chemical Society)

and rapid response. In an electrochemical aptasensor, the identification signal is converted to an electrical signal [67–69]. To date, most aptamer-functionalized DNA-nanostructure-based electrochemical biosensors have been based on labeled electrochemical biosensors in which the nucleic acids or aptamers are labeled with an electrochemically active substance or catalytically active inorganic/biological molecules such as ferrocene or peroxidase. Due to the mechanical rigidity and structural stability of DNA nanostructures, an electrochemical aptasensor can be anchored directly to an electrode surface, where it acts as an ultrasensitive and selective detector for a particular target. Electrochemical aptasensors can be divided into two classes according to the mode that causes the change in the electrical signal from the DNA nanostructure.

2.2.1 Immobilization-Based Electrochemical Aptasensors

In a traditional electrochemical aptamer sensor, the flexibility of the single-strand aptamers immobilized on the electrode surface results in a tendency for the aptamers to aggregate or entangle, affecting target recognition by the aptamer [52]. To overcome this limitation, 3D DNA nanostructures can be immobilized on the Au electrode surface to act as a scaffold for the aptamers. Given the mechanical rigidity, stability, and specific orientation of tetrahedral DNA nanostructures (TDNs), Fan et al. [52, 70] constructed a series of efficient electrochemical sensors based on DNA tetrahedra for the detection of nucleic acids, proteins, cell, small molecules, and so on. For example, a DNA tetrahedron structure with a recognition probe at one vertex for thrombin detection and thiol at the other three vertices was constructed on the Au surface through Au–S bonding (Fig. 5a) [70]. The thrombin recognition probe was a 15-nt aptamer that triggered the binding of any thrombin present to a 29-nt aptamer with avidin-HRP (horseradish peroxidase) at another site, resulting in the catalyzed electroreduction of hydrogen peroxide. It was demonstrated that this DNA-tetrahedron-based electrochemical biosensor was easily assembled on the Au surface with a specific orientation, well-controlled tetrahedron spacing, and high stability, leading to enhanced detection performance.

The detection of biological molecules is essential if we are to improve our understanding of their physiological and pathological functions and to diagnose diseases at an early stage. Various groups have recently applied an electrochemical detection strategy based on aptamer-functionalized DNA tetrahedra to detect a range of targets [71–75]. For example, Wei et al. [74] designed an electrochemical biosensor based on tetrahedral DNA nanostructures for detecting 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is a key biomarker of oxidative damage and is widely distributed in various lesion tissues, urine, and exposed cells. The system obtained after immobilizing the TDN on the Au electrode surface and fixing an 8-OHdG aptamer to the vertex of the TDN was found to be almost 300-fold more sensitive to 8-OHdG than other electrochemical methods. Such enhanced sensitivity was also achieved for the detection of cancerous exosomes.

Exosomes [76] transport many macromolecules from their parent cells and are vital for intercellular communication. Dong et al. [77] developed a TDN-assisted aptasensor for the ultrasensitive detection of cancerous hepatocellular exosomes. In

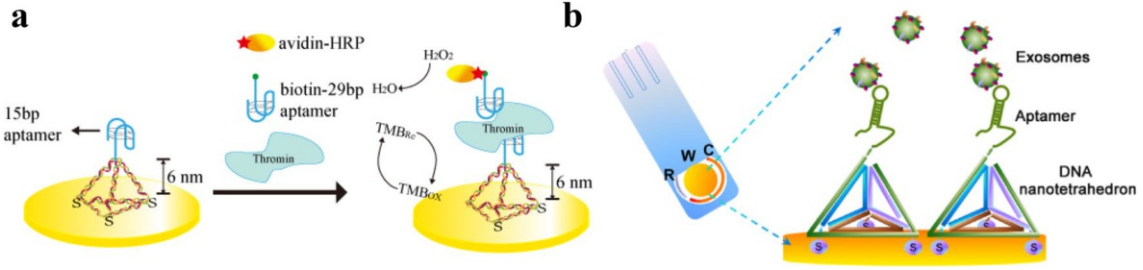


Fig. 5 Electrochemical aptasensors with immobilized DNA nanostructures. **a** Schematic of DNA-tetrahedron-based electrochemical detection of thrombin [70]. **b** Schematic of DNA-tetrahedron-assisted electrochemical aptasensors for the detection of cancerous exosomes (reproduced with permission from [77], copyright 2017 American Chemical Society)

this system, LZH8 aptamers that bind to HepG2 exosomes are affixed to TDNs distributed on a Au electrode surface, where the TDNs are separated by a defined nanometric distance (Fig. 5b). When an aptamer captures a HepG2 exosome, the redox signal would change. This assay made the aptamer strands individually at defined nanoscale distance on the gold electrode, which decreased the hindrance effect and maintained spatial orientation for improving identification of exosomes , leading to 100-fold greater sensitivity to exosomes than achieved with single-stranded aptamer sensors. As well as biomarker detection, electrochemical aptasensors based on DNA nanotetrahedra can be applied to monitor food safety. For instance, Chen et al. [78] constructed a system comprising aptamer-functionalized tetrahedral DNA nanostructures and macroporous MoS_2-AuNPs with three-dimensionally ordered structure for the detection of aflatoxin B1 (AFB1, a common contaminant in food). This detection system presented high linearity from 0.1 fg/mL to 0.1 μg/mL and a detection limit of 0.01 fg/mL.

2.2.2 Electrochemical Aptasensors Based on Conformational Switching

Some electrochemical biosensors utilize the change in electrical signal induced by DNA nanostructure self-assembly or disassembly for detection [79–86]. For example, a DNA-nanoladder-based electrochemical biosensor was recently used by Abnous et al. [80] to detect ampicillin (Fig. 6a). Their sensing strategy utilizes an ampicillin aptamer to form a ladder-shaped DNA and immobilize it on the surface of a gold electrode. The ladder-shaped DNA physically and electrostatically blocks $[Fe(CN)_6]^{3-/4-}$ from reaching the surface of the gold electrode. However, in the presence of ampicillin, the DNA nanoladder dissociates from the electrode surface, allowing the $[Fe(CN)_6]^{3-/4-}$ to access the electrode surface, which in turn increases the electrical signal from the electrode. This analytical approach presented a linear range of 7 pM to 100 nM and a detection limit of 1 pM for ampicillin. In another example, Wu et al. [79] developed a label-free impedimetric aptasensor based on DNA nanoladders. This was coupled with a peroxidase mimic to facilitate the amplified detection of nuclear factor kappa B (NF-kB).

In addition to simple two-dimensional DNA nanostructures, more complicated three-dimensional DNA nanostructures can be employed to create aptamer sensors based on more complex conformational switching. For example, Yin et al. [81] fabricated dendritic DNA structures on magnetic beads as a means to detect *Vibrio alginolyticus*. In this system, when *V. alginolyticus* is present, the dendritic DNA nanostructure disassembles, leading to a change in the electrical signal from the system. In order to enhance the detection sensitivity, Yuan et al. [82] utilized DNA dendrimers as nanocarriers of a novel electrochemiluminescence (ECL) indicator based on luminol. The system yielded a detection limit of 0.18 fg/mL for lipopolysaccharides (LPS) when LPS aptamers were incorporated for recognition purposes. This strategy of monitoring for changes in the electrical signal caused by DNA nanostructure assembly was also applied by Jie et al. [84], who developed an electrochemical/ECL assay based on the self-assembly of a DNA nanotube for Dam methylase (MTase) and aflatoxin B1 (AFB1) detection (Fig. 6b). In this assay, the DNA nanotube acts as the signal amplifier for the biosensor. As shown in Fig. 6b, when Dam MTase

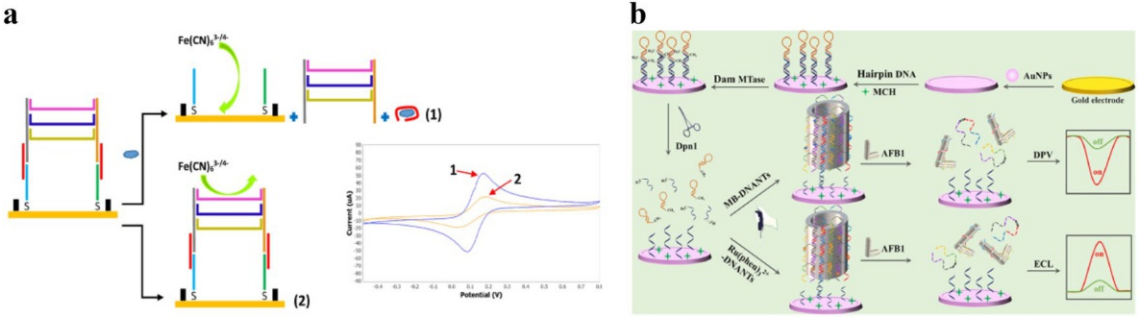


Fig. 6 Electrochemical aptasensors that utilize DNA nanostructures and conformational switching. **a** Schematic of a DNA-nanoladder-assisted electrochemical aptasensor for the detection of ampicillin (reproduced with permission from [80], copyright 2019 Elsevier B.V.). **b** Schematic of a electrochemical system that uses DNA nanotube assembly and disassembly and electrochemiluminescence (for signal amplification) to detect multiple targets (reproduced with permission from [84], copyright 2019 American Chemical Society)

is present, the hairpin DNA on the electrode is methylated and cleaved, inducing the assembly of the DNA nanotube. ECL or EC signal probes (Ru (phen)$^{32+}$ and methylene blue) are then immobilized on the electrode as they hybridize with DNA nanotubes, leading to amplified ECL and EC signals. Moreover, the recognition of AFB1 by the aptamer S2 in DNA nanotubes was observed to induce the dissociation of the DNA nanotube from the electrode, resulting in decreased ECL and EC signals in the presence of AFB1.

2.3 Colorimetric Biosensors

Colorimetric assays possess the advantageous properties of simplicity, robustness, and low cost [87]. In particular, colorimetric methods can be performed without the need for any complicated instrumentation, which is a useful feature for point-of-care cancer diagnostics. The color changes utilized in colorimetric methods are usually due to the presence of colorimetric reagents such as gold nanoparticles (AuNPs) [88], enzymes [89], visible dyes [90], and polymers [91]. Many sensitive methods of converting aptamer–target binding events into color signals have been reported. These strategies are usually based on a DNA-nanostructure-based colorimetric biosensor and employ a DNA hydrogel to control the colorimetric reagent [92], which tend to be either gold nanoparticles or enzymes.

2.3.1 AuNP-Based Colorimetric Detection

Gold nanoparticle (AuNP)-based colorimetric probes have been widely used for colorimetric assays because their extinction coefficients are significantly higher than those of common organic dyes [93]. Two approaches are commonly used in the design of AuNP colorimetric sensors. In the first, the color change is obtained directly through the accumulation or dispersion of AuNPs. In an early example of this type of probe, Lu et al. used DNA-based interactions and aptamer recognition to control the aggregation of AuNPs, thus facilitating the colorimetric sensing of analytes [94]. A system utilizing an aptamer-containing polyacrylamide hydrogel was designed by Tan's group [95] for the colorimetric detection of adenosine. Tan's group also summarized the applications of aptamer-functionalized hydrogel systems in colorimetric detection, drug release, and targeted cancer therapy [96]. These hydrogels have been limited for sol-gel phase transitions because one molecule of specific biomolecular input could only cleave one site at the network, which need a large amount of biomolecular inputs. In order to promote the use of hydrogels, Oishi et al. [97] recently developed novel DNA hydrogels that are capable of responding to biological stimuli via DNA circuit systems. As shown in Fig. 7a, these DNA hydrogels were fabricated by integrating poly(ethylene glycol)-modified gold nanoparticles (PEG-NPs) for use as the colorimetric reagent into the hydrogel. The presence of catalyst DNA triggers the dissociation of crosslinking points and main chains, inducing the transition of the hydrogel from sol to gel. This releases the entrapped PEG-GNPs into a solution that consequently turns from colorless to red. Oishi et al. utilized this DNA hydrogel to realize the enzyme-free amplified

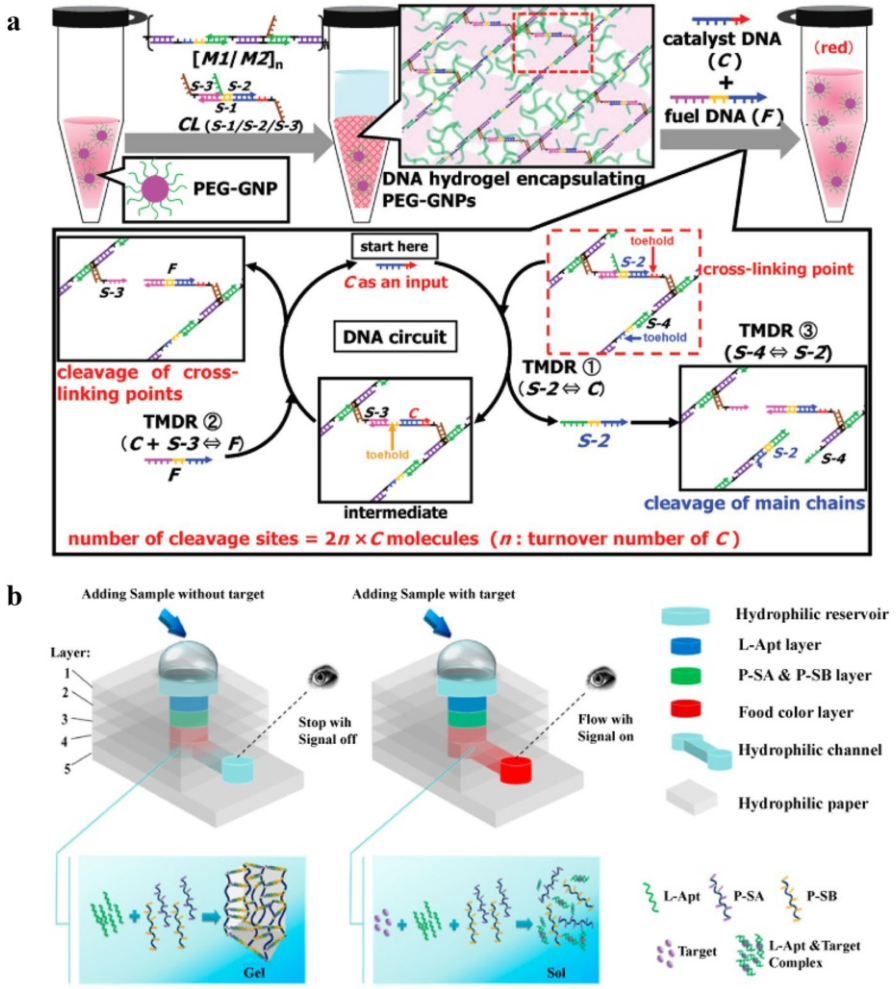


Fig. 7 AuNP-based aptamer-functionalized DNA hydrogels for colorimetric detection. **a** Schematic of a DNA-hydrogel-based colorimetric biosensor containing a DNA circuit system that responds to biological stimuli (reproduced with permission from [97], copyright 2019 Wiley–VCH Verlag GmbH and Co. KGaA). **b** Schematic of a microfluidic paper-based analytical device (μPAD) for multisubstance colorimetric detection (reproduced with permission from [99], copyright 2015 American Chemical Society)

colorimetric detection of ATP by incorporating a structure-switching ATP aptamer. The resulting system was found to be more sensitive (capable of detecting 5.6 μM ATP within 30 min) than other DNA-hydrogel-based ATP-biosensing systems.

The second approach that is used to fabricate AuNP-based colorimetric systems is based on the mobility of the aggregation or dispersion in the presence of the analyte. For example, Lu et al. [98] developed a dipstick test for adenosine that is applicable to serum and is based on AuNP aggregation. Their adenosine-aptamer-based lateral flow device is placed into the solution to be tested. The color of the AuNPs

in the device changes from blue to red in the presence of the target. Yang et al. [99] also used aptamer-functionalized DNA hydrogels and AuNPs to develop another paper-based microdevice, a microfluidic paper-based analytic device (μPAD) for multisubstance detection (Fig. 7b). Their device uses an aptamer as a crosslinker in the target-responsive hydrogel, gold nanoparticles or iodine staining as the colorimetric reagent, and a slipchip as the readout device. In the presence of the target, the aptamer-functionalized hydrogel changes from a solid to a mobile phase, which alters the flow in the μPAD. Yang et al. utilized this platform to realize the simultaneous detection of multiple targets such as cocaine, adenosine, and Pb^{2+}.

2.3.2 Enzyme-Based Colorimetric Detection

Another way to observe a colorimetric reaction is to utilize enzymatic catalysis [100, 101]. Compared to other methods, enzymatic catalytic reactions can provide higher sensitivity when used as signal-amplifying elements. For example, Wang et al. [89] encapsulated HRP as a second signal amplification unit into an aptamer-functionalized DNA hydrogel with a target-switchable response to achieve the colorimetric detection of ochratoxin A (Fig. 8a). The DNA hydrogel was assembled from two kinds of DNA subunits: a Y-scaffold shape and an aptamer domain. In the presence of the target, the DNA hydrogel disassembles and releases the encapsulated HRP, which reacts with H_2O_2 and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) ($ABTS^{2-}$), inducing a color change. Instead of natural enzymes, screened DNA enzymes (e.g., a HRP-mimicking DNAzyme) can be used to develop enzyme-free colorimetric biosensors. For example, Ravan et al. [102] reported an isothermal amplification strategy to detect RNA (Fig. 8b). In their work, they combined a hybridized chain reaction (HCR) assay with a HRP-mimicking DNAzyme to increase target sensitivity. In the presence of the target, the HCR is triggered, leading to DNA concatemers. Upon adding hemin molecules, the inactive HRP-mimicking DNAzyme folds into its active configuration, changing the substrate from colorless to colored. This assay was found to have a sensitivity to the target RNA similar to that of RT-PCR, which has a LOD of 1 pM. Furthermore, Mohammadi et al. [103] used a colorimetric method based on a HRP-mimicking DNAzyme to detect cancer cells.

2.4 Other Biosensors

Recently, other methods have also been used in conjunction with DNA nanostructures to develop novel biosensors. Such methods include atomic force microscopy (AFM) [104], surface plasmon resonance (SPR) [105], surface-enhanced Raman scattering (SERS) [106, 107], circular dichroism (CD) [108, 109], and mechanochemistry [110]. Recent applications of aptamer-functionalized DNA nanostructures in these fields have generally made use of DNA origami. In contrast to inorganic materials, DNA origami permits precise spatial addressability and provides a wide range of sites for probe immobilization. For example, unlike oligonucleotides and proteins, the binding of small molecules with aptamers cannot cause significant

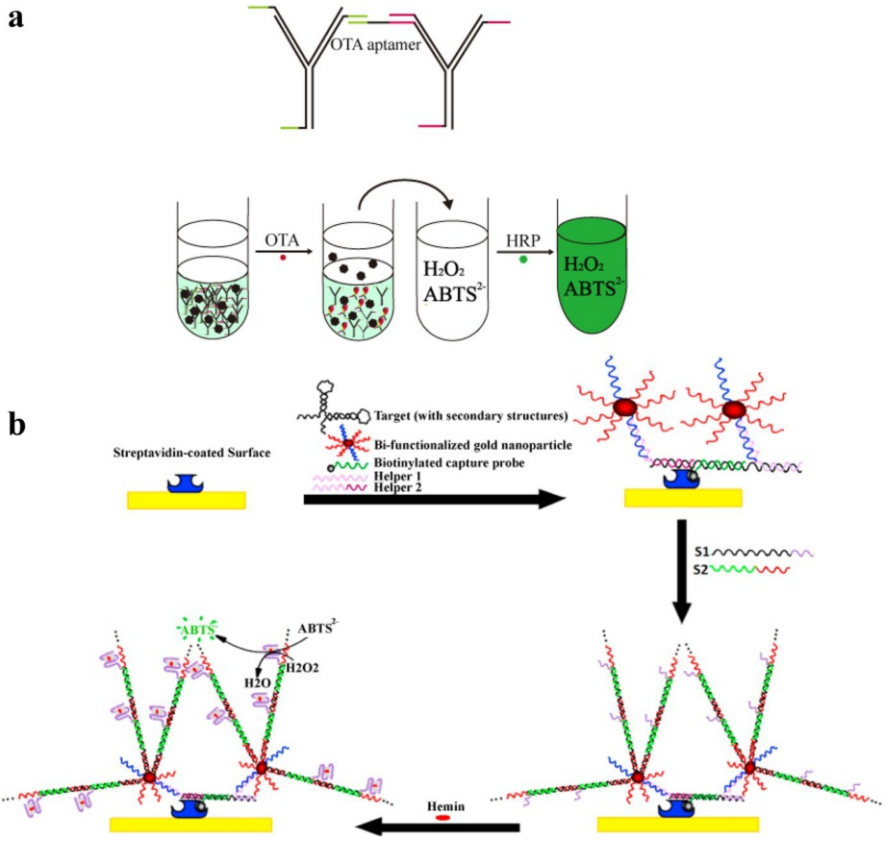


Fig. 8 Enzyme-based aptamer-functionalized DNA hydrogels for colorimetric detection. **a** Schematic of a DNA-hydrogel-based colorimetric biosensor with encapsulated HRP for the detection of ochratoxin A [89]. **b** Schematic of DNA concatemer assembly following HRP-mimicking DNAzyme activation in the isothermal amplified detection of RNA (reproduced with permission from [102], copyright 2016 Elsevier B.V.)

change in their elasticity and height, thus it is extremely hard to directly visualize the binding events of individual aptamer using AFM. To overcome this limitation, Lu et al. [111] constructed an aptamer-functionalized DNA origami biosensor for the AFM detection of small molecules such as aflatoxin B1 (AFB1) (Fig. 9a). In their biosensor, the aptamers attached to the DNA origami are initially bound to ssDNA-modified AuNPs. In the presence of the target (AFB1), the aptamer strands preferentially bind to the target and release the AuNPs, which are detected directly by AFM. In other methods, the DNA origami acts as a signal indicator. For example, Endo et al. [110] utilized the modular design of DNA origami to develop an expanded single-molecule mechanochemical sensor (Fig. 9b). Their design utilizes DNA origami with seven tiles, and the recognition elements are positioned next to the interlocks that connect the contiguous tiles. When present, the target binds to the recognition sites, breaking the interlocks and inducing a change in mechanical

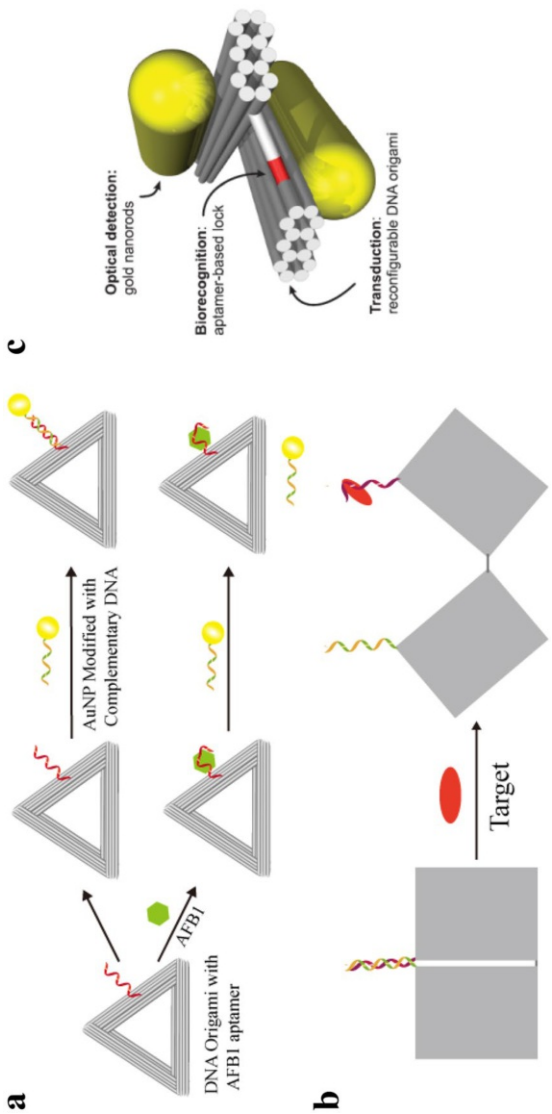


Fig. 9 Other biosensors that employ aptamer-functionalized DNA nanostructures. **a** Schematic of the aptamer-functionalized DNA origami used for target biosensing in conjunction with AFM [111]. **b** Schematic of a DNA-origami-based biosensor that monitors for changes in a mechanical signal [110]. **c** Schematic of a biosensor that employs CD spectroscopy and DNA origami with gold nanorods (reproduced with permission from [108], copyright 2018 American Chemical Society)

signal. The use of multiple recognition elements rather than just one reduces both the detection limit and detection time.

Furthermore, a biosensor that utilizes aptamer-functionalized DNA origami with two gold nanorods attached for optical detection based on CD spectroscopy has been developed (Fig. 9c) [108]. In this design, aptamer biorecognition is used as a molecular lock that controls the spatial configuration of DNA origami with two gold nanorods (AuNRs) attached. Aptamer biorecognition causes the two AuNRs to form a three-dimensional (3D) chiral plasmonic object that exhibits circular dichroism, which is detected via CD spectroscopy.

3 Aptamer-Functionalized DNA Nanostructures for Bioimaging Applications

It is important to monitor the distributions of biomolecules in cells, as this can enhance our understanding of cell function and pathology, improve disease diagnosis, and facilitate drug delivery. Various aptamer-based probes have been applied for bioimaging. In particular, the biocompatibility of aptamer-functionalized DNA nanostructures makes them attractive tools for bioimaging. Aptamer-functionalized DNA nanostructures employed for bioimaging can be divided into two classes depending on whether the aptamer is used as a responsive or a targeting element.

3.1 Aptamers as Responsive Elements

Many emerging intracellular and extracellular bioimaging methods based on DNA materials utilize aptamers as responsive units. For example, Tan et al. [112] proposed the use of switchable aptamer micelle nanostructures for ATP imaging in cells. However, it is important to consider the critical micelle concentration (CMC) of these micelle nanostructures when using them because they could dissociate at low concentrations. Therefore, researchers are interested in designing DNA nanostructures that are stable during cellular applications, especially at low concentrations. For example, Zhang et al. [18] designed an aptamer-functionalized DNA dendrimer for monitoring ATP in situ. Fan et al. [113] designed a DNA tetrahedral nanostructure which can be dynamically regulated by assembling an anti-ATP aptamer in one of the edges. The aptamer was modified with a pair of FRET fluorophores (Cy3 and Cy5), which would be close to each other to generate FRET signal through the conformational change of aptamer after recognizing ATP. This structure can be self-delivered into cells for monitoring the ATP in cells.

Recently, Li et al. [114] developed a series of framework nucleic acid (FNA) nanodevices for subcellular ATP imaging. They initially constructed a FNA nanoplatform based on two tetrahedral nanostructures (TDNs) that was intended for use in lysosomes. As shown in Fig. 10a, the two TDNs have differently branched vertices, but both TDNs have a split ATP aptamer and a biomolecular i-motif at the vertex. When the TDNs enter intracellular acidic lysosomes, the acidic environment induces the TDNs to assemble (via an i-motif unit) into a heterodimeric structure.

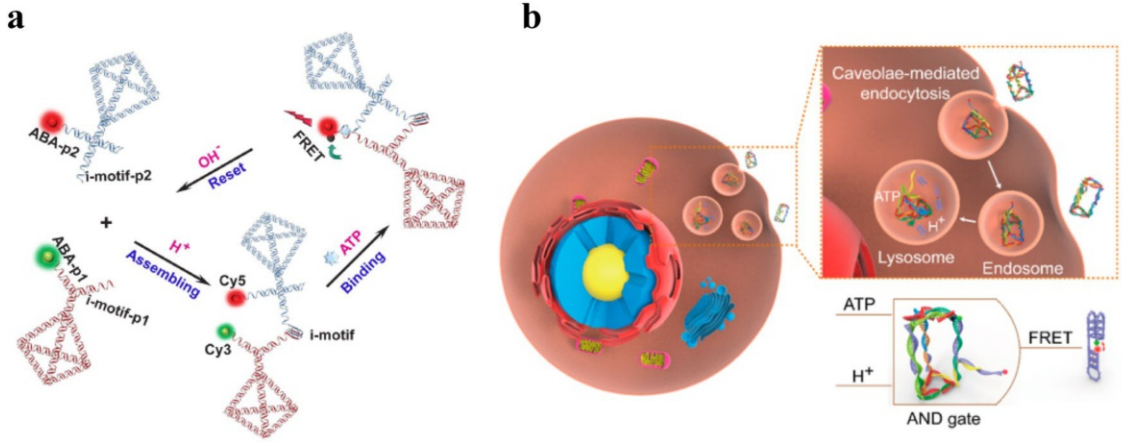


Fig. 10 The utilization of aptamers as responsive probes in bioimaging. **a** Schematic of a TDN-based framework nucleic acid (FNA) nanodevice for ATP imaging in lysosomes (reproduced with permission from [114], copyright 2019 Wiley–VCH Verlag GmbH and Co. KGaA). **b** Schematic of a DNA triangular prism used for the logic-based imaging of protons and ATP (reproduced with permission from [115], copyright 2019 American Chemical Society)

The formation of this large framework brings the split ATP aptamers on the two TDNs into close proximity, permitting ATP imaging within the lysosome via FRET. This i-motif-guided assembly of TDNs mimics the pH-responsive dimerization of natural silk proteins (spidroins). Li et al. subsequently used the same strategy to develop FNA-based logic nanodevices based on DNA triangular prisms for subcellular imaging (Fig. 10b) [115]. In this case, FRET (and therefore fluorescence) only occurs in the FNA-based logic device when both the pH and the ATP level change in the lysosome.

3.2 Aptamers as Targeting Elements

Targeting elements (e.g., aptamers, antibodies, peptides, or small molecules) are able to bind with biomarkers on targeted cells or tissues. Modifying the surfaces of nanomaterials with these targeted elements is an attractive method for realizing sensitive and selective bioimaging in vitro and in vivo. Among the various types of targeting elements, aptamers have attractive features such as easy synthesis and modification, small size, high stability, and good biocompatibility, which make them outstanding candidates for use in targeted imaging schemes [116]. Aptamer-functionalized DNA structures employed for target bioimaging are commonly based on synthetic biomacromolecules such as micelles [112, 117], nanoflowers [118], or dendrimers [119].

Ju et al. [117] developed a multifunctional nanomicelle for targeted imaging and photodynamic therapy in vivo. Although imaging in vivo was achieved using this nanomicelle, this imaging was limited by the instability of the nanomicelle at concentrations lower than the critical micelle concentration. In order to overcome this limitation, Tan et al. [120] recently reported the development of an aptamer-lipid micelle with internal photinduced crosslinking (Fig. 11a). The introduction of aptamers into micelle to give a DNA-lipid micelle (DLM) consisting of hydrophilic aptamer heads and hydrophobic lipid tails led to faster target-cell recognition. In addition, methacrylamide was incorporated as a layer of photoinduced crosslinks between the DNA shell and the lipid core, enhancing the stability of the DLM in the cellular environment. On the other hand, in the absence of crosslinking, it should be possible to modify the lipid core or the aptamer shell of the aptamer-lipid micelle, making such micelles potentially useful in targeted cellular imaging, gene therapy, and drug delivery.

In addition to these large DNA nanostructures, conjugating aptamers with small DNA nanostructures such as a three-dimensional DNA tetrahedron can also improve aptamer targeting efficiency in vitro. Yang et al. [121] utilized a DNA tetrahedron as a phase-transfer agent for hydrophobic nanoparticles and transferred upconversion nanoparticles (UCNPs) for targeted cellular imaging using an aptamer-pendant DNA tetrahedron. Recently, Wu et al. [122] examined the effects of varying the number of MUC1 aptamers attached to a DNA tetrahedron on the efficiency and safety of this functionalized DNA tetrahedron when it was employed for targeted imaging and drug delivery (Fig. 11b). Their results showed that increasing the number of

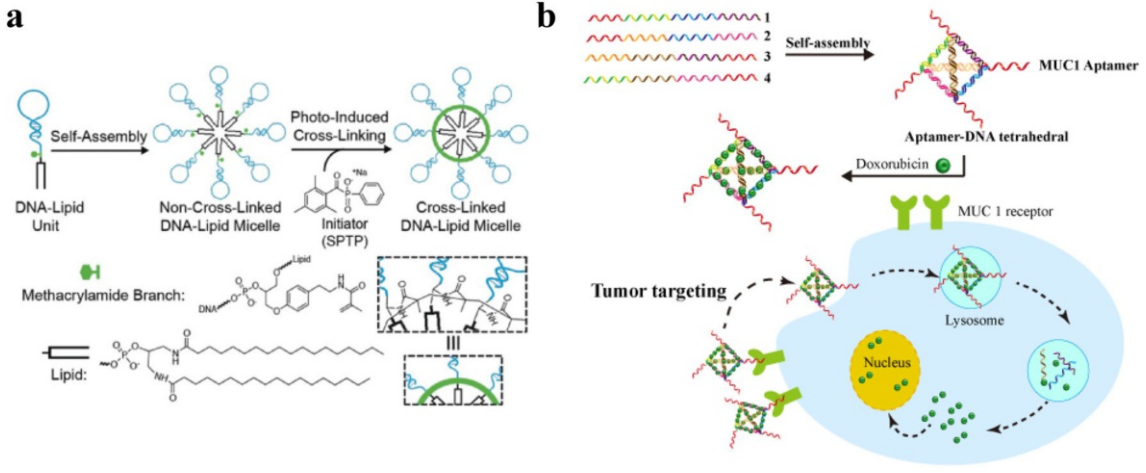


Fig. 11 The utilization of aptamers for targeted bioimaging in live cells and in vivo. **a** Schematic of a crosslinked DNA-methacrylamide-lipid micelle system (reproduced with permission from [120], copyright 2018 Wiley–VCH Verlag GmbH and Co. KGaA). **b** Schematic of a DNA tetrahedron conjugated with different numbers of MUC1 aptamers, allowing targeted drug delivery and in vivo imaging [122]

conjugated aptamers enhanced the specificity of the functionalized DNA tetrahedron for cancer cells.

4 Aptamer-Functionalized DNA Nanostructures for Cancer Therapy

Cancer is one of the most destructive diseases and a leading cause of human mortality. Current cancer treatment methods include surgery, chemotherapy, and radiotherapy. However, one of the major limitations of these therapeutic methods is their poor selectivity for cancer cells, which results in the destruction of normal, healthy cells and can lead to treatment failure and potentially death [123]. Aptamers are considered excellent candidates for use in targeted cancer therapies because they are small, easy to prepare, and show excellent target-specific recognition. Therefore, a wide variety of nanomaterials functionalized with aptamers have been produced for cancer treatments. Among these nanomaterials, DNA nanostructures are particularly popular for many biomedical applications because of their programmability, ease of modification, and high biocompatibility. In this section, we summarize recent progress in the development of aptamer-functionalized DNA nanostructures for use in various cancer treatment methods, including chemotherapy, photodynamic therapy, and gene therapy.

4.1 Chemotherapy

Chemotherapy is a widely used drug treatment for cancers. The drawbacks of using chemotherapeutic drugs are their toxic side effects, poor solubility, nonspecific distribution in the body, and systemic toxicity. However, the advantages of aptamer-functionalized DNA nanostructures—including a capacity for high drug loading, good biocompatibility, and specific recognition—make them attractive tools for bypassing these problems with chemotherapeutic drugs. Since chemotherapeutic agents can be conjugated with DNA through covalent [124] and noncovalent [125] bonding, DNA nanostructures are considered ideal candidates for drug delivery. Most aptamer-functionalized DNA nanostructures used in drug delivery are noncovalently conjugated with chemotherapeutic agents (e.g., doxorubicin, DOX). The aptamers most commonly used in this context are the mucin 1 protein (MUC1) aptamer, the nucleolin aptamer AS1411, and the aptamer sgc-8c.

MUC1 is a cell-surface glucoprotein that is overexpressed in many kinds of adenocarcinomas, making it an attractive target in anticancer drug delivery schemes [126]. Early in 2011, Huang et al. [127] attached a MUC1 aptamer to a DNA icosahedron and used the resulting system to efficiently and specifically deliver DOX to epithelial cancer cells for cancer therapy. Following that work, many groups utilized the MUC1 aptamer in TDN-based drug delivery systems [128, 129]. In addition to these small DNA nanostructures, the MUC1 aptamer can also guide relatively large DNA nanostructures into cells. For example, Ding et al. [130] attached the MUC1 aptamer to a triangular DNA origami–AuNR complex that permitted increased internalization of DOX and AuNRs (Fig. 12a). In their scheme, the first step is to

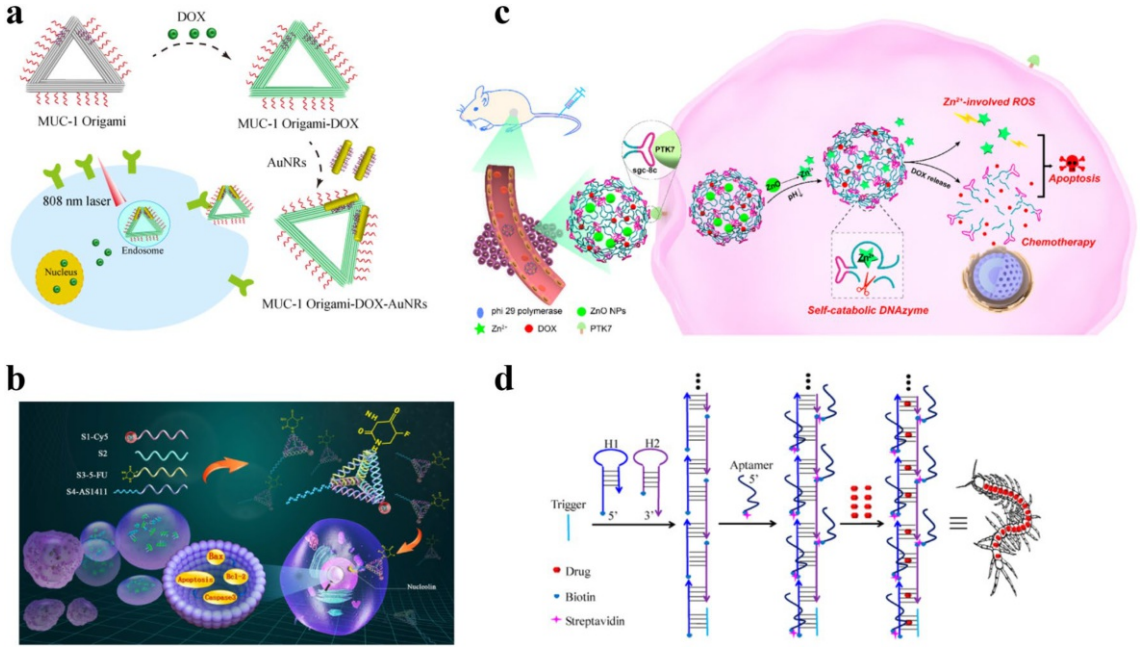


Fig. 12 Aptamer-functionalized DNA nanostructures used for chemotherapy. **a** Schematic of triangular DNA origami functionalized with the MUC-1 aptamer for drug delivery [130]. **b** Schematic of a DNA tetrahedron conjugated with the aptamer AS1411, which can be used for 5-fluorouracil (5-FU) delivery (reproduced with permission from [135], copyright 2019 American Chemical Society). **c** Schematic of yarn-like DNA nanosponges (NSs) used for drug delivery. These NSs encapsulate ZnO and the aptamer sgc-8c and incorporate DNAzyme (reproduced with permission from [137], copyright 2019 American Chemical Society). **d** Schematic of the nanocentipede that is assembled via a HCR and has aptamer "legs" for cell-targeted drug delivery (reproduced with permission from [140], copyright 2016 American Chemical Society)

prepare the triangular DNA origami functionalized with the MUC1 aptamer. The DOX is then loaded into the DNA origami via an interaction between the DOX and GC-rich regions in DNA pairs. After that, two AuNRs are assembled on the origami template at a predesignated location. Guided by the MUC1 aptamer, the MUC1-DNA origami-DOX-AuNR (MODA) nanostructure is effectively internalized by cancer cells. This approach inhibits P-glycoprotein (multidrug resistance pump) expression, thus avoiding P-gycoprotein-mediated drug efflux and, in turn, increasing the sensitivity of multidrug-resistant breast cells to DOX. MCF-7/ADR cells can then be killed by a combination of DOX chemotherapy and hyperthermia induced by near-infrared (NIR) laser irradiation. Jiang et al. [131] developed a protein-scaffolded DNA nanohydrogel based on three types of streptavidin (SA)-based DNA tetrads and functionalized this nanohydrogel with the MUC1 aptamer. The resulting nanohydrogel, which can incorporate therapeutic agents, was found to facilitate activatable target imaging and therapy.

The aptamer AS1411 is a 26-mer DNA sequence with a G-rich region that can bind to nucleolin, a cell-surface protein that is overexpressed in tumor cells [132]. When the G-quadruplex structure of AS1411 is bound to nucleolin, the nucleolin can transfer the aptamer from cytomembrane to cytoplasm and nucleus. Furthermore, AS1411 possesses anticancer activity when it is present at high concentrations [133, 134]. There is therefore increasing interest in combining AS1411 with DNA nanostructures for targeted drug delivery. For example, Lin et al. [135] developed a DNA-based nanomedicine based on a AS1411-modified DNA tetrahedron for the targeted therapy of breast cancer cells (Fig. 12b). In their scheme, the anticancer drug 5-fluorouracil (5-FU) was inserted at the vertex of the DNA tetrahedron. Lin et al. demonstrated the importance of including AS1411 to facilitate targeted cell therapy through comparison with therapy based on free 5-FU. To further enhance the targeting of cancer cells, Chen et al. [129] simultaneously modified a DNA tetrahedron with the MUC1 aptamer and AS1411 in order to fabricate a system that targets both cancer cells and nucleolin.

Sgc-8c is another aptamer that is commonly used in anticancer drug delivery schemes. It has 41 oligonucleotides and specifically binds to the cell membrane protein tyrosine kinase 7 (PTK-7), which is overexpressed in CCRF-CEM (human T-cell acute lymphoblastic leukemia) [136] and other tumors (such as colon and gastric cancers). Therefore, sgc8 is commonly combined with DNA nanostructures when developing drug delivery systems that selectively target lymphoblastic leukemia. For example, Yang et al. [119] designed an sgc-8c-modified, DOX-loading DNA dendrimer that can selectively distinguish target cancer cells from normal cells and is internalized into CCRF-CEM and cervical cancer HeLa cells. Recently, Wang et al. [137] developed a drug delivery system based on a DNA hydrogel for smart targeted drug delivery (Fig. 12c). Their design involves the fabrication, through a method known as isothermal rolling circle amplification (RCA), of sgc-8c-functionalized, monodisperse, and sophisticated, yarn-like DNA nanosponges (NSs) that encapsulate ZnO and are bound to a DNAzyme. The assembled DNA/ZnO NSs are designed to act as carriers for DOX. The sgc-8c guides the assembly into cancer cells, where intracellular acids trigger the conversion of the ZnO into Zn^{2+} ions, stimulating reactive oxygen species (ROS) generation. The Zn^{2+} also acts as

a cofactor in DNAzyme digestion, leading to the digestion of the NSs and therefore the release of DOX.

There are also some DNA-nanostructure-based drug delivery systems in which other novel aptamers are applied. For example, Zhao et al. [138] site-specifically attached the novel aptamer C2NP [139], which is highly specific for the tumor cell marker CD30, to DNA origami for the purposes of targeted drug delivery. Also, Yang et al. [140] constructed a centipede-like DNA structure via a HCR to use for drug delivery in targeted therapy (Fig. 12d). In this scheme, the trunk of the nanocentipede is the DNA scaffold and the legs are aptamers that bind to SMMC-7721 cells (human hepatocellular carcinoma cells).

The anti-HER2 aptamer HApt specifically recognizes and binds to HER2, an epidermal growth factor receptor [28]. HApt can translocate HER2 to lysosomes for degradation, stimulating cell death and inhibiting cell growth. Lin et al. [141] recently reported that they had improved the stability and increased the blood circulation time of HApt by combining it with a tetrahedral nucleic acid framework.

4.2 Photodynamic Therapy

Photodynamic therapy (PDT) is a photochemistry-based method that utilizes light to activate chemicals that are cytotoxic in their activated state. PDT utilizes three components: a light source, a photosensitizer, and tissue oxygen [142]. When all three components are present, reactive oxygen species (ROS) are generated through an interaction between the photosensitizer and the tissue oxygen in the presence of radiation, and these ROS are toxic to cells. PDT is a highly efficient and noninvasive therapeutic method and is therefore an attractive treatment for malignant diseases [143]. PDT circumvents the problems of systemic therapies because the therapy is only applied at irradiated locations. Also, the photosensitizer used in PDT is selectively accumulated in cancer cells. Moreover, this method is inexpensive.

Aptamer-functionalized DNA nanostructures have also been used in PDT. There are three main ways to load the photosensitizer onto a DNA nanostructure: direct covalent modification, noncovalent binding, and encapsulation. Various strategies have been used to directly attach the photosensitizer to a DNA strand. One of the main advantages of this approach is that the resulting photosensitizer-modified DNA nanostructure can be integrated with a carrier. For example, Wang et al. [144] labeled a DNA tetrahedron (TDN) with the photosensitizer pyropheophorbide a, which activates the toxic species $^1O_2$ when it is irradiated with light. This system was then combined with a chemotherapeutic agent, thus facilitating the localized destruction of circulating tumor cells (CTCs) via simultaneous PDT and chemotherapy (Fig. 13a). In Wang et al.'s approach, the TDN-based system is first immobilized on a microchannel supporter by a hairpin aptamer switch at the vertex of the TDN, and the chemotherapeutic agent DOX is loaded into the dsDNA of the TDN. When CTCs enter the microchannel, the hairpin aptamer specifically recognizes and binds to them, releasing the TNA, which is internalized into the CTCs. DOX is therefore delivered into the target cells by the TDN, and PDT is realized by irradiating the photosensitizer with light.

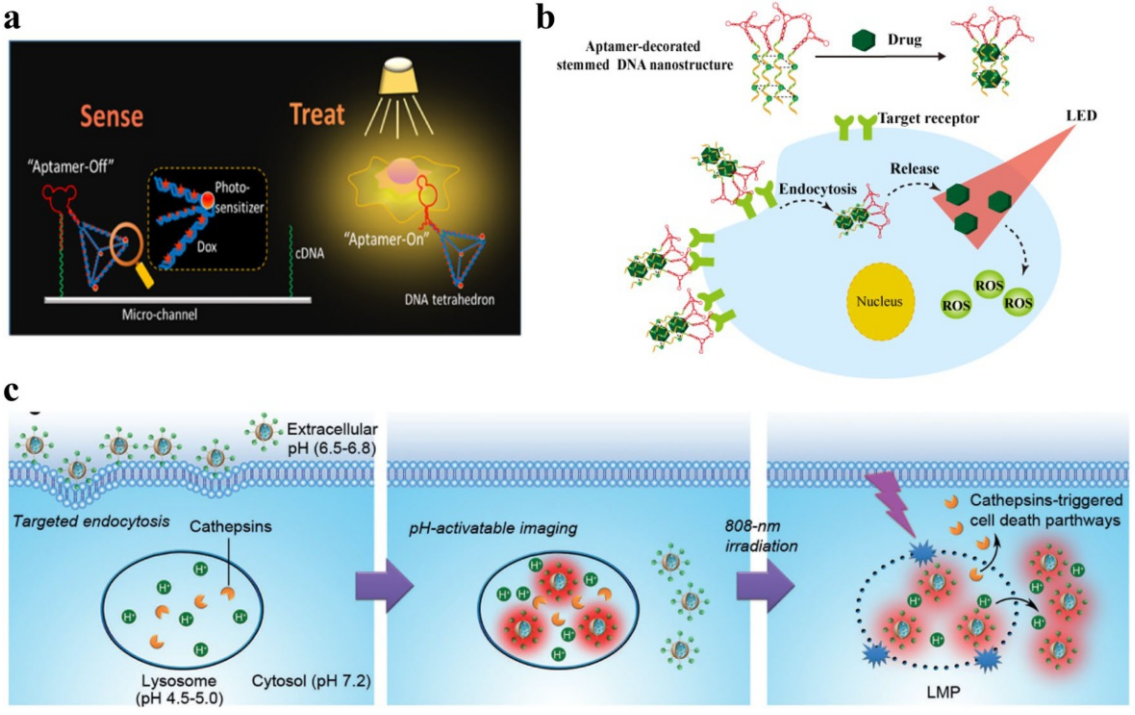


Fig. 13 Aptamer-functionalized DNA nanostructures for photodynamic therapy. **a** Schematic of a DNA tetrahedron labeled with a photosensitizer for photodynamic CTC therapy (reproduced with permission from [144], copyright 2016 American Chemical Society). **b** Schematic of an aptamer-based stemmed DNA nanostructure that loads a photosensitizer used for cell-specific therapy [145]. **c** Schematic of the utilization of a multifunctional nanomicelle encapsulating the fluorescent probe BDP-688 and a new photosensitizer for cell-targeted lysosome-based imaging and near-infrared PDT (reproduced with permission from [117], copyright 2014 Wiley–VCH Verlag GmbH and Co. KGaA)

Noncovalent binding strategies generally utilize the ability of the G-quadruplex of a DNA strand to load a photosensitizer such as methylene blue (MB) or 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin (TMPyP4). MB, a phenothiazine, is commonly used for cell staining, to treat methemoglobinemia, and to delineate the edge of a tumor during surgery. MB has also been widely used as a photosensitizer in PDT because it generates $^1O_2$ when irradiated at wavelengths between 620–670 nm. MB can also be loaded onto an aptamer-functionalized DNA nanostructure carrier as a means to improve the cell selectivity of PDT. For example, Shim et al. [145] developed a stemmed DNA nanostructure integrated with an aptamer (an aptamer-modified oligoguanine quadruplex) to load MB for PDT therapy with enhanced cell selectivity (Fig. 13b). In a similar way, TMPyP4, a porphyrin derivative, can be combined with a G-quadruplex-forming aptamer to improve cell selectivity. For example, Shieh et al. [146] utilized the guanine-rich aptamer AS1411 to selectively deliver TMPyP4 to breast cancer cells.

The third method of delivering a photosensitizer using a DNA nanostructure is encapsulation. For example, Yang et al. [117] developed a nanomicelle based on DNA that can simultaneously encapsulate the pH-activatable fluorescence probe BDP-688 and the NIR photosensitizer R16FPm, making it useful for cancer therapy (Fig. 13c). This system also uses the aptamer Apt S1 to specifically recognize and bind to human breast cancer cells (cell line MDA-MB-231). Results indicated that, after the nanomicelle had been internalized into the cells and transferred to lysosomes, the BDP-688 produced a fast and reversible fluorescence response to pH, allowing the pH in lysosomes to be monitored in real time. Irradiation with NIR caused the photosensitizer R16FP to generate ROS, inducing cell death. This strategy could allow cancer treatments to be visualized in vivo.

4.3 Gene Therapy

Gene therapy is an attractive approach for treating heritable or acquired diseases such as cancers, viral infections, and thalassemia. Among these, cancer is the most common gene therapy target, as it often has a genetic basis that is difficult to cure. DNA nanostructures are regarded as ideal nonviral vectors for nucleic acid delivery due to their high loading capacities and biocompatibilities. Generally speaking, these DNA-nanostructure-based therapeutic systems can be categorized into antisense, RNA interference (RNAi), and gene delivery approaches.

Antisense strands are single-stranded DNA or RNA sequences that are complementary to their target genes and knock them down, whereas RNAi is a method in which small interfering RNA (siRNA) or short hairpin DNA (shRNA) is used to recognize and cleave target messenger RNA (mRNA) [147]. However, it is difficult for siRNA, shRNA, and antisense strands to internalize into cells, so there is considerable interest in developing tools for delivering them into their targets. DNA nanostructures are outstanding candidates. For example, Tan et al. [148] developed an aptamer-based DNA nanohydrogel for delivering antisense oligonucleotides into A549 cells (Fig. 14a). Their design uses three building blocks for the hydrogel: a Y-shaped monomer (A) containing a DNAzyme, another Y-shaped monomer (B)

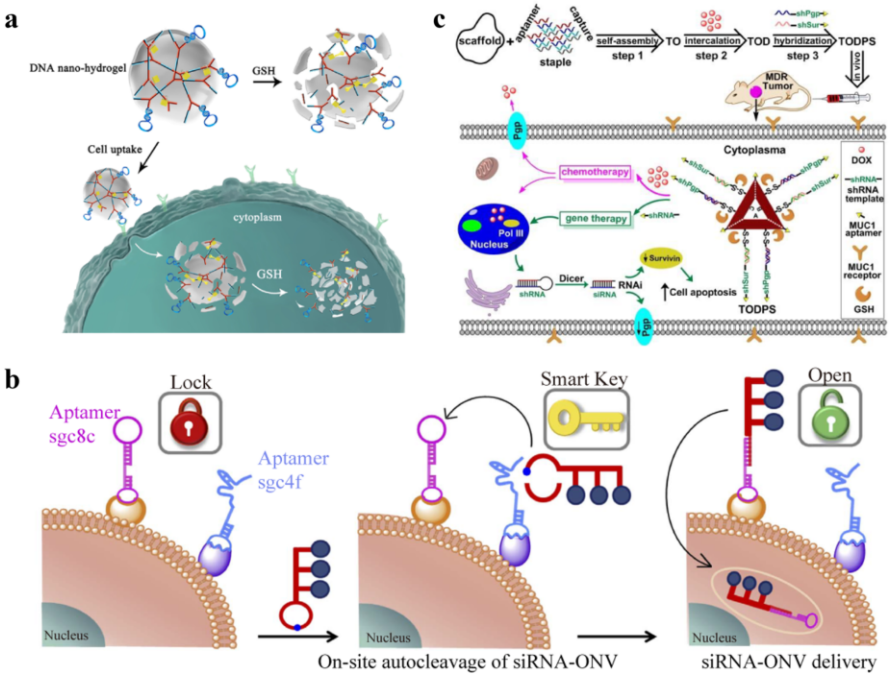


Fig. 14 Use of aptamer-functionalized DNA nanostructures in gene therapy. **a** Schematic of the application of a stimuli-responsive DNA nanohydrogel to deliver antisense DNA into target cells for gene therapy (reproduced with permission from [148], copyright 2015 American Chemical Society). **b** Schematic of a DNA dual lock and key strategy for cell-subtype-specific binding and siRNA delivery (reproduced with permission from [149], copyright 2016 Springer Nature). **c** Schematic of a platform based on triangular DNA origami for delivering shRNA transcription templates and chemodrugs in vivo (reproduced with permission from [150], copyright 2018 Wiley–VCH Verlag GmbH and Co. KGaA)

containing an aptamer that is used as a cell-targeting unit, and a linker containing the antisense DNA. Disulfide linkages are incorporated into each block, which causes the DNA hydrogel to disassemble in the presence of the reducing agents found in cells. This strategy of incorporating various functional units into a DNA hydrogel was found to be highly effective from a therapeutic perspective.

Ju et al. [149] developed DNA logic nanotubes for the precise delivery of siRNA (Fig. 14b). These dual lock and key DNA nanotubes, which were modified with a self-cleavable hairpin structure, were considered to act as a "key" for two "locks," which were two different aptamers (sgc-8c and sgc-4f) that specifically bind to the target cell membrane. This "dual lock and key" strategy avoids nonspecific siRNA adsorption and off-target toxicity. Qian et al. [128] recently investigated the utilization of a siRNA-loaded DNA nanoprism for gene therapy. They decorated the DNA nanoprism with two functional units: the siRNA Rab26 (the gene drug) and the aptamer MUC1 (the targeting unit). Targeting and drug loading was regulated by tuning the number of aptamers on the nanoprism. However, approaches such as these have the disadvantage that siRNA is easily degraded in complex biological

systems, reducing treatment efficacy. Therefore, strategies that enhance the stability of RNAi technology are of considerable interest. Ding et al. [150] recently proposed a method of maintaining the stability of shRNA transcription templates during their delivery to the target through the use of triangular DNA origami, thus allowing effective RNAi chemotherapy in vitro and in vivo (Fig. 14c). In their strategy, they attached two different shRNA transcription templates along with an aptamer used as a targeting unit to the triangular DNA origami through a stimuli-responsive and cleavable disulfide linkage. The chemotherapeutic drug DOX was then loaded through intercalation onto the DNA origami. This multifunctional DNA nanoplatform was found to permit both RNAi and chemotherapy at the same time, leading to enhanced therapeutic effects on antagonistic tumors in vitro and in vivo. They also applied this strategy to efficiently and simultaneously deliver the gene p53 and chemotherapeutic drugs to multidrug-resistant tumors [151].

4.4 Other Therapies

As well as the more common therapeutic methods discussed above, there are other promising strategies for treating diseases that make use of aptamer-functionalized DNA nanostructures. For instance, some proteins and enzymes are considered potential therapeutic agents [152–154]. Due to their molecular recognition and cell internalization abilities, aptamer-functionalized DNA nanostructures have been utilized to achieve the targeted delivery of these therapeutic agents [155–157]. For example, Zhao, Ding, Yan, and Nie et al. [155] recently constructed a DNA nanorobot that uses DNA origami as a carrier to transport thrombin to a tumor, where it coagulates (Fig. 15a). In their design, four thrombin molecules are attached to the inner surface of hollow tube-shaped DNA origami, which was then closed with predesigned fastener DNA strands containing the aptamer AS1411. The AS1411 serves as both a targeting unit and a molecular switch that controls whether the DNA robot is open. The therapeutic activity of the thrombin molecules is activated when the aptamer interacts with nucleolin (a tumor vessel marker), meaning that the release of the thrombin leads to vascular infarction of the tumor (i.e., cancer treatment). In another application, Wang et al. [156] constructed aptamer-modified DNA supersandwich assemblies that transport a catalase into cells. Once inside a cell, this catalase scavenges reactive oxygen species.

Another route to achieving a therapeutic effect is through the anticancer abilities of aptamers. Aptamers can combine directly and specifically with various proteins, some of which are potential therapeutic targets [158]. The anticancer aptamers that are most commonly combined with DNA nanostructures are AS1411 and thrombin aptamer. AS1411 is a G-rich oligonucleotide that inhibits NF-kB signaling and reduces the expression of Bcl-2, so it has been explored as an anticancer agent in a phase 2 clinical trial [159]. By attaching this aptamer to a DNA nanostructure with an enhanced cell internalization ability, AS1411 can be efficiently delivered into cancer cells, inhibiting their growth [48]. For example, Lin et al. [48] attached AS1411 and drug molecules to TDN nanostructures (Fig. 15b), thus permitting the efficient delivery of both the aptamer and drugs to the target cells in a synergetic

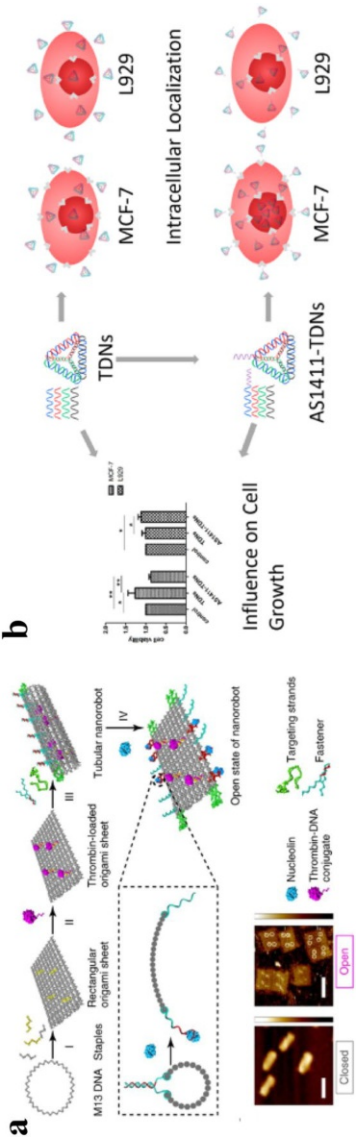


Fig. 15 Use of aptamer-functionalized DNA nanostructures in other therapeutic schemes. **a** Schematic of a DNA nanorobot that uses DNA origami as a carrier to transport thrombin for coagulation at the tumor site (reproduced with permission from [155], copyright 2018 Springer Nature). **b** Schematic of AS1411-modified TDN nanostructures for suppressing tumor cell growth (reproduced with permission from [48], copyright 2017 American Chemical Society)

cancer therapy scheme. Thrombin aptamer is reported to exhibit similar antithrombotic activity to anticoagulation drugs, and has therefore also been applied therapeutically [160]. For instance, Gianneschi et al. [161] recently developed polymeric micelles with thrombin aptamer for use as a nanoscale anticoagulant.

5 Aptamer-Functionalized DNA Nanostructures for Other Applications

Aside from biological applications, aptamer-functionalized DNA nanostructures have also been applied in fields such as protein or molecular immobilization and analysis (due to the addressable and programmable nature of DNA nanostructures) and structural control (due to the specific recognition abilities of aptamers). Because DNA nanostructures feature addressable decorations, allow high spatial resolution, and can be directly imaged using AFM, they are excellent candidates for biophysical research into aptamers. For example, the immobilization of a protein on a nanostructure can facilitate investigations of spatially precise biological interactions because biomolecular recognition events commonly happen at the nanoscale. In this context, in 2008, Yan et al. [162] performed the first investigation of the distance dependence of multivalent binding effects at the single-molecule level by immobilizing an aptamer at a precisely controlled position on a DNA nanostructure.

The application of an aptamer and a single-chain antibody against that aptamer has been shown to be an effective strategy for assembling proteins in a highly specific manner. Fabrega et al. [163] utilized DNA origami as an addressable support to investigate proteins at the single-molecule level (Fig. 16a). Using the ability of a thrombin aptamer to recognize and bind to α-thrombin, they were able to visualize via AFM, for the first time, the enzymatic activity of human O^6-alkylguanine-DNA alkyltransferase (hAGT) while it was supported by the DNA origami platform. Recently, Seminario et al. [164] investigated the molecular dynamics of the interactions of the thrombin aptamers TBA26 and TBA29 with thrombin. In addition to protein immobilization, aptamer-functionalized DNA nanostructures have been used to investigate the biological functions of RNA [165] and cells [166].

Furthermore, an aptamer can be integrated into a DNA nanostructure as a building block that controls nanostructure formation. This method can be utilized to construct stimuli-responsive materials. For example, Mao et al. [167] constructed multilayered DNA nanocages where the formation of each layer was controlled by the binding of an aptamer to its target (Fig. 16b). ATP aptamers (blue strands) were embedded into the linkers between two tetrahedra by hybridization with a single strand (red strand) on the smaller tetrahedron. In the presence of ATP, the ATP aptamer bound to that ATP and separated from the red strand, triggering the dissociation of the two tetrahedra. Also, the macrostructure can be tuned by using a dynamic DNA structure containing an aptamer. Plaxco et al. [168] engineered a series of adenosine-responsive DNA hydrogels based on an aptamer. They investigated the response kinetics of these aptamer-based hydrogels at different length scales from nanometers to hundreds of microns (Fig. 16c). In their work, an adenosine-binding aptamer was incorporated into Y-shaped DNA hydrogel monomers

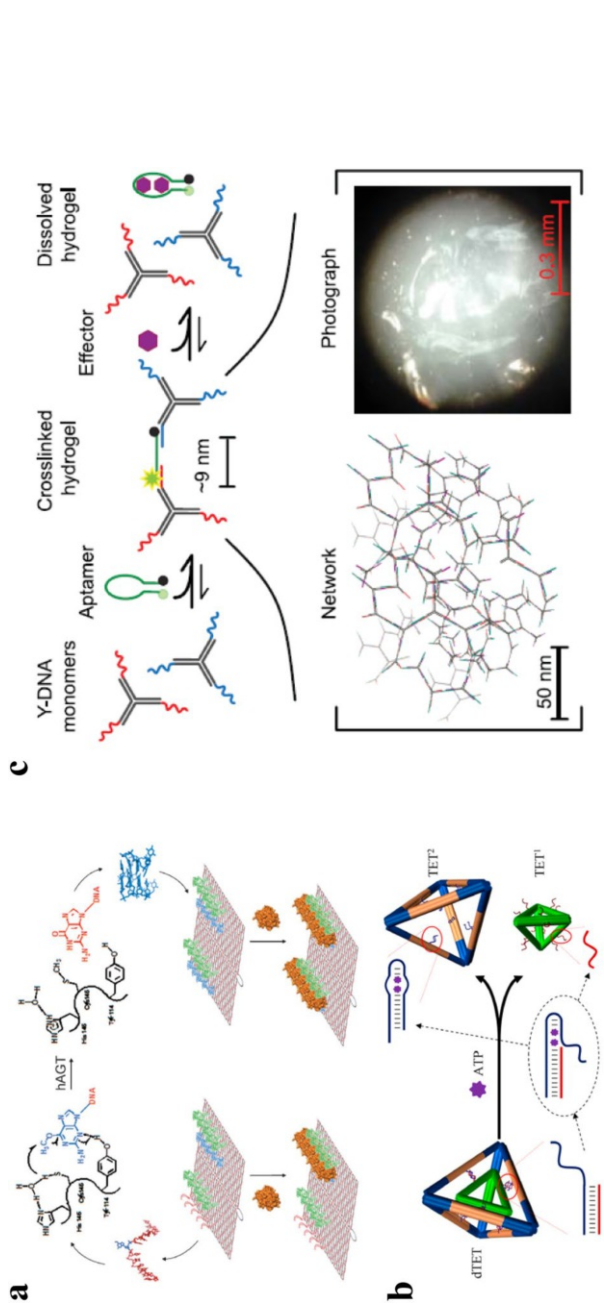


Fig. 16 Use of aptamer-functionalized DNA nanostructures for other applications. **a** Scheme in which α-thrombin is immobilized on DNA origami by binding the thrombin to a thrombin aptamer and the DNA repair activity is monitored by AFM (reproduced with permission from [163], copyright 2013 Wiley–VCH Verlag GmbH and Co. KGaA). **b** Schematic of the ATP-activated separation of multilayered DNA nanocages (reproduced with permission from [167], copyright 2015 American Chemical Society). **c** Schematic of the ATP-activated dissolution of a hydrogels at scales ranging from nanometers to hundreds of microns (reproduced with permission from [168], copyright 2017 American Chemical Society)

that were disrupted when the aptamer recognized adenosine. This dissolution of the DNA hydrogel could be monitored because the aptamer was fluorescently labeled. They then investigated the effect of modulating the aptamer-based crosslink stability on the DNA hydrogel. DNA hydrogel characteristics such as volume can also be controlled through schemes that incorporate DNA strand displacement. For example, Schulman et al. [169] incorporated a HCR into a macroscopic polyacrylamide-based hydrogel using DNA as a responsive crosslinker. The HCR caused the volume of the hydrogel to expand 100-fold. Schulman et al. later demonstrated that this change in the DNA hydrogel was induced by various molecular triggers, including ATP. When triggered by ATP, there was a change of fluorescence as well as DNA hydrogel swelling.

6 Conclusions and Perspectives

Over the past few decades, aptamer-functionalized DNA nanostructures have facilitated impressive advances in various fields. A range of strategies have been used to construct aptamer-functionalized DNA nanostructures, and these nanostructures have been widely used for biological applications due to their superior performance. In this review, we have summarized the most recent applications of aptamer-functionalized DNA nanostructures in biosensing, bioimaging, cancer therapy, and other promising fields. Despite the considerable achievements that such nanostructures have made possible, there are still issues with the use of aptamer-functionalized DNA nanostructures that need to be addressed.

The first of these issues is that some important targets don't have respective aptamers so far. Thus, it is necessary to screen more aptamers for these targets for broadening respective research (e.g., in studies of the interactions of aptamers with nonmammalian cells). Second, it is important to find a way to enhance the stability of aptamers in complex biological environments (e.g., serum), although some recent strategies for achieving this based on the electrostatic steric hindrance of nanoparticles [170] and the design of a circular aptamer [171] appear promising. Third, the preparation of DNA nanostructures via scalable methods [172] is crucial to facilitating their widespread application. To achieve this goal, Yan et al. [173] proposed a framework for designing and assembling a single DNA or RNA strand that self-folds into a predesigned complex unknotted structure and can be amplified by polymerases in vitro or by clonal production in vivo. Also, Dietz et al. [174] recently utilized the self-replicating ability of bacteriophages to produce macroscopic amounts of DNA origami. Furthermore, it is important to improve the purity of DNA nanostructures before applying them in any subsequent application [175]. Fourth, most bioanalytical schemes that use aptamer-functionalized DNA nanostructures are currently based on one-photon fluorescent imaging, which has a low penetration depth and has a problem with tissue autofluorescence. Using near-infrared (NIR) fluorescent imaging, photoacoustic imaging, and nuclear magnetic resonance imaging (NMRI) would further improve the accuracy and sensitivity of bioanalysis based on aptamer-functionalized DNA nanostructures. Fifth, in addition to the traditional biological applications mentioned above, aptamer-functionalized DNA nanostructures

present considerable potential for use in computing. For example, Bachelet et al. [176] developed DNA origami robots for computing in animals. Also, Tan et al. [177] engineered a 3D DNA nanostructure on a target cell membrane for logic computing. This appears to be a promising route to the creation of intelligent systems for molecular medicine and sensing. Lastly, the operation of aptamer-functionalized DNA nanostructures in vivo remains challenging due to the presence of numerous biological barriers during delivery. Although there are a few reports of attempts to apply aptamer-functionalized DNA nanostructures in vivo, the complex physiological environment makes such studies very difficult for a myriad of reasons: nuclease degradation, nonspecific adsorption of proteins, immunological rejection, and so on. Therefore, further mechanistic investigations of the fate of DNA nanostructures in vivo and the development of efficient methods to minimize interference from the physiological environment would drive the practical application of aptamer-functionalized DNA nanostructures in vivo.

Current developments in the construction and application of aptamer-functionalized DNA nanostructures indicate that there is great potential to improve their design, which should lead to important uses for these nanostructures in materials science and healthcare.

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