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Recent progresses in DNA nanostructure-based biosensors for detection of tumor markers

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ABSTRACT

DNA has emerged as a promising biomaterial for assembling a variety of nanostructures based on its programmable base pairing. It also has other remarkable properties including stability, prominent biocompatibility, and can easily be modified with functional groups for further applications. In the past few decades, researchers have established various design rules and assembly technologies to improve the stability and complexity of DNA nanostructures. The detection of cancer-associated biomarkers has significant importance in identifying patients with different clinical stages and also in developing adaptive therapeutic strategies. Due to their unique advantages, DNA nanostructures can be designed to serve as universal units to form biosensors for the detection of tumor biomarkers. In this review, we first present a brief introduction of the development of structural DNA nanotechnology. Then we summarize recent strategies for DNA nanostructure-based optical, electrochemical and mass sensitive biosensors in cancer detection. Finally, we discuss the challenges and opportunities these technologies provide.

1. Introduction

It is well known that DNA plays a significantly important role in storing and transmitting genetic information (Crick, 1970; Watson and Francis, 1953). The Watson-Crick base pairing rules guide single strand DNA (ssDNA) assembling into double strand helical structures. DNAs have been considered as novel building blocks for bottom-up fabricating construction at the nanoscale since Seeman first performed pioneering research in the early 1980s (Seeman, 1982). Self-assembled DNA structures began with crossovers and other topological structures such as branched junction (Kallenbach et al., 1983) and knots (Wang et al., 1993), progressing to higher-order periodic and aperiodic structures *via* DNA crossover tiles (LaBean et al., 2000; Li et al., 1996; Mathieu et al., 2005; Winfree et al., 1998). The introduction of DNA origami which used long-stranded DNA chain to fold into a desired shape with the help of a set of designed 'staple strands' brought structural DNA nanotechnology to a new level in 2006 (Rothemund, 2006). This technique led to well-formed 2D structures in near-quantitative yields, even when unpurified staple strands are used (Zhang et al., 2014a, 2014b). The scalability and complexity of DNA nanostructures are also markedly improved (Hong et al., 2017). Substantial efforts have more recently been taken to extend the DNA origami fabrication to the third dimension (Andersen et al., 2009; Hendrik Dietz and William, 2009). Through different strategies, multifarious nanostructures have been created, such as wireframe (Benson et al., 2015; Zhang et al., 2015) and supracolloidal fibrils (Tigges et al., 2016). Nowadays, the rapid growth of technology makes it possible to design one-, two- and three-dimensional DNA nanomaterials for a broad range of applications in biochemistry, materials science and medical science.

Cancer is a leading cause of premature death and disability worldwide. Cancer-associated biomarkers including DNA, RNA, proteins, lipids, metabolites, etc, are quantifiable indicators of specific cancer states and their detection has significant importance in identifying patients with different clinical stages and also in developing adaptive therapeutic strategies (Huang et al., 2017a, 2017b). These biomarkers can be classified into three categories: predictive biomarkers, prognostic biomarkers and diagnostic biomarkers. However, due to the structural characteristics of nucleic acids, specific protein signatures

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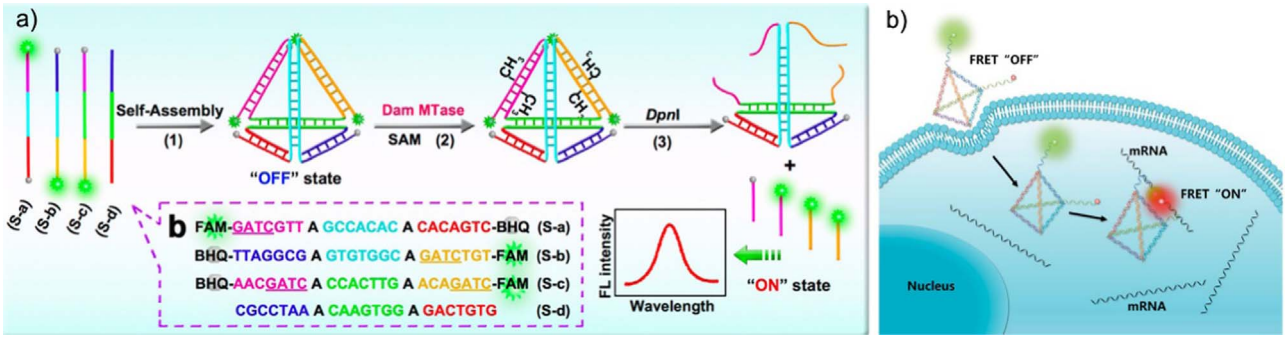

Fig. 1. DNA nanostructure based fluorescence sensing. (a) Schematic illustration of DNA tetrahedron nanostructure to detect DNA methyltransferase activity. With the aid of SAM, the sequences used to assemble DNA tetrahedron was methylated and cleaved. Which led to the recovering of the fluorescence signal. (b) Schematic illustration of DNA tetrahedron nanotweezer to detect intracellular mRNA. The structure of DNA tetrahedron altered in the presence of target mRNA and led to the change of fluorescence signal. Adapted with permission from Ref. (Zhou et al., 2017a, 2017b) and (He et al., 2017).

caused by tumor heterogeneity and difficulty to achieve sample concentration, sensitive detection of cancer biomarkers represents a challenge. DNA nanostructures provide effective platforms in the bioanalytical field. Much attention has been paid to DNA nanostructures for cancer detection in the last three decades. First, the strict base-pairing principle (A-T and G-C) endows the DNA with precise predictability and reproducibility. Second, DNA nanostructures exhibit strong nuclease resistance compared with ssDNA and dsDNA (Song et al., 2017a, 2017b). Third, DNA nanostructures are natural biopolymers with prominent biocompatibility and biodegradability, indicating a great potential for their *in vivo* application (Kumar et al., 2016; Li et al., 2017a; b, c, d, e, f, g; Zhang et al., 2014a, 2014b). Forth, DNA nanostructures can be further modified with quantum dots (Wen et al., 2018), fluorescent dyes (Domljanovic et al., 2017; Schlichthaerle et al., 2016), antibodies (Wang et al., 2017a, b, c, d, e), and cancer-targeting organic compounds including peptides and aptamers (Jiang et al., 2016; Li et al., 2017a; b, c, d, e, f, g; Song et al., 2017a, 2017b) to achieve versatile applications. Thus, DNA nanostructures can be designed and modified to serve as universal units to form biosensors for detection of cancer related biomarkers (Xie et al., 2017). This review therefore focuses on recent progress on application of DNA nanostructure-based biosensors in different classifications for the detection of cancer. We also attempt to discuss the challenges and potential applications regarding these biosensors.

2. DNA nanostructure-based biosensors

Biosensors possess several preponderances including strong specificity to their targets, high sensitivity, simple operation, transportable and able to perform real-time detection compared with traditional analytical approaches like enzyme-linked immunosorbent assay (ELISA) and chromatography (Salek-Maghsoudi et al., 2018). A classical biosensor is formed by a biological recognition unit which specifically recognizes target and transducer which converts the biologic interaction into physical signals to determine the quantities of the target (Chao et al., 2016; Meng et al., 2016). Based on the transduction mechanism, biosensors can be categorized based on the transduction mechanism: optical, electrochemical, thermal, resonant and ion-sensitive biosensors (Chaubey and Malhotra, 2002). Among them, optical and electrochemical biosensors are the most widely used types in the field of tumor biomarker detection and will be discussed in details in the next sections.

The main components of an optical biosensor are illumination supply, optical transducer and detecting system (Patel et al., 2010). The output transducer signals of optical biosensors are based on fluorescence, absorption, luminescence, surface plasmon resonance (SPR), reflection and other optical phenomena (Jayanthi et al., 2017).

2.1. Fluorescence detection

Fluorescence-based device measuring the fluorescence emission of the fluorescent tags labeled target is one of the most sensitive strategies in biosensing. Among the various DNA nanostructures fabricated in the recent years, a classical 3D framework named DNA tetrahedron holds prominent mechanical properties, including simplicity, flexibility, structural stability, and ability to modify with functional decorations or to load drugs and other molecules (Goodman et al., 2005; Xie et al., 2017; Li et al., 2014). Goodman and co-workers first reported a single-step synthesis of tetrahedron (Goodman et al., 2004). In their experiment, four designed oligonucleotides were mixed in salt-containing buffer followed by an annealing process. Since then, DNA tetrahedron has been utilized in the study of cancer mechanisms, tumor biomarker detection and cellular imaging. Zhou and co-workers described an 'off-on' fluorescence system based on DNA tetrahedron to monitor DNA methyltransferase (MTase) activity which plays a crucial role in cancer suppressor gene expression, cancer growth and therapeutics (Hossain et al., 2017). In their experiment, three of the oligonucleotides used to form the DNA tetrahedron were modified with fluorescein and quencher at their 5′ and 3′ ends (Zhou et al., 2017a, 2017b). In the presence of MTase, methyl groups were transferred from S-adenosyl methionine (SAM) to induce methylation of three labeled strands. The strands were then removed from the DNA tetrahedron with the help of *Dpn*I to provide measurable fluorescence signal (Fig. 1a). The detection limit for the fluorescence system reached as low as 0.045 U mL^{-1}.

Furthermore, reports showed that the DNA tetrahedron is able to penetrate cellular membrane without a transfection reagent (Walsh et al., 2011) so that it can be used for cellular detections. He and co-workers reported a DNA tetrahedron nanotweezer for the detection of cancer-related mRNA in living cells (He et al., 2017). The presence of target mRNA caused a structural change for the nanoprobe which led to a high fluorescence resonance energy transfer (FRET) (Fig. 1b). The method they described had the potential to be applied in biomedical research and clinical field. Other than linking the vertexes of tetrahedron with functional groups (Feng et al., 2016; Wang et al., 2017a, b, c, d, e), modificators such as molecular beacons and selective DNAzyme can also be integrated into DNA tetrahedron (Xie et al., 2016; Zhou et al., 2016a, 2016b). Xie and co-workers encoded a molecular beacon into the DNA tetrahedron to detect cancer-associated mRNA in living cells. The target mRNA would induce a configuration change of the DNA tetrahedron, which resulted in the rise of fluorescence intensity (Xie et al., 2016).

In addition to DNA tetrahedron, other DNA nanostructures such as DNA nanohydrogel and DNA origami scaffolds are also applied for bioanalytical application (Fu et al., 2016; Wang et al., 2017a, b, c, d, e; Wang et al., 2015; Wang et al., 2014). These materials are not only utilized to immobilize functional molecules but also participate in the