ELSEVIER

Development of the DNA-based biosensors for high performance in detection of molecular biomarkers: More rapid, sensitive, and universal

Qiong Wang [a,1], Jing Wang [b,c,1], Yan Huang [a], Yichen Du [b,d], Yi Zhang [a], Yunxi Cui [a,c,*], De-ming Kong [b,c,**]

[a] College of Life Sciences, Nankai University, Tianjin, 300071, PR China
[b] Tianjin Key Laboratory of Biosensing and Molecular Recognition, Research Centre for Analytical Sciences, College of Chemistry, Nankai University, Tianjin, 300071, PR China
[c] State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin, 300071, PR China
[d] Life and Health Intelligent Research Institute, Tianjin University of Technology, Tianjin, China, 300384, PR China

ARTICLE INFO

ABSTRACT

The molecular biomarkers are molecules that are closely related to specific physiological states. Numerous molecular biomarkers have been identified as targets for disease diagnosis and biological research. To date, developing highly efficient probes for the precise detection of biomarkers has become an attractive research field which is very important for biological and biochemical studies. During the past decades, not only the small chemical probe molecules but also the biomacromolecules such as enzymes, antibodies, and nucleic acids have been introduced to construct of biosensor platform to achieve the detection of biomarkers in a highly specific and highly efficient way. Nevertheless, improving the performance of the biosensors, especially in clinical applications, is still in urgent demand in this field. A noteworthy example is the Corona Virus Disease 2019 (COVID-19) that breaks out globally in a short time in 2020. The COVID-19 was caused by the virus called SARS-CoV-2. Early diagnosis is very important to block the infection of the virus. Therefore, during these months scientists have developed dozens of methods to achieve rapid and sensitive detection of the virus. Nowadays some of these new methods have been applied for producing the commercial detection kit and help people against the disease worldwide. DNA-based biosensors are useful tools that have been widely applied in the detection of molecular biomarkers. The good stability, high specificity, and excellent biocompatibility make the DNA-based biosensors versatile in application both *in vitro* and *in vivo*. In this paper, we will review the major methods that emerged in recent years on the design of DNA-based biosensors and their applications. Moreover, we will also briefly discuss the possible future direction of DNA-based biosensors design. We believe this is helpful for people interested in not only the biosensor field but also in the field of analytical chemistry, DNA nanotechnology, biology, and disease diagnosis.

1. Introduction

1.1. Molecular biomarkers

Biomarkers are a series of indicators that may provide measurable or evaluable information for specific physiological states. Traditional biomarkers of a particular biological object include many kinds of characteristics of it(Rifai et al., 2006; Sawyers, 2008; Srinivas et al., 2001). For example, the biomarkers of a particular disease may involve the temperature of the patients' body, the number or shape of a kind of cell in unique tissues, the particular mutation(s) on the genome, the concentrations of a series of molecules in blood or urine, the existence of a kind of bacteria or virus, and so on. Molecular biomarkers including chemical and biological molecular characters have attracted intense attention from the new century. Depending on the development of molecular biology and chemical biology, it is more convenient to identify the

---

special molecules as the biomarkers of particular biological objects now. Comparing with other characteristics, a representative molecule is always endowing with some advantages such as better recognizability and a more straightforward signal readout. Usually, the molecules collected from body fluid systems, cells, and tissues cultured *in vitro* can be still applied as accurate markers, making the detection process easier to operation and lower risk. Nowadays, numerous molecular biomarkers have been identified as targets for disease diagnosis, molecularly targeted therapy(Hood et al., 2004), biological research(Czapla and McPhail, 2015; Dimri et al., 1995), and even in environmental monitoring(Abdel-Halim et al., 2006; Marin and Matozzo, 2004). Bio-related molecules, such as nucleic acid, protein, lipid, and small ions which may participate in the bioprocess are the most prominent molecular biomarkers. For example, the level of microRNA is interrelated with many kinds of cancers(Lu et al., 2005; Qiu et al., 2018) and neurological diseases(Christensen and Schratt, 2009); abnormal DNA methylation is considered as a biomarker of neck squamous cell carcinoma(Herman et al., 1995), cardiovascular disease risk(Zhang and Zeng, 2016), and other disease(Jones, 2012); the prostate-specific antigen is now a general clinical biomarker for prostate cancer(Catalona et al., 2011); the activity of multitudinous enzymes such as telomerase(Wang et al., 2017b), glycosylase(Zhang et al., 2018b), metalloproteinase(Wang et al., 2016), and others are also found to involve in cancers; the intracellular concentration of the trace elements such as Fe^{2+}, Cu^{2+}, Zn^{2+} are important for human health(Halliwell, 1996; Stohs and Bagchi, 1995). These molecular biomarkers exist not only in bioactive systems including cells and tissues, but also across a wide spectrum of body fluids including serum, lymph, and urine(Mitchell et al., 2008). Due to the convenience of biofluid extraction, these molecules are suitable candidates for liquid biopsy which is quite popular in diagnostics and pathology in recent(Diaz and Bardelli, 2014). One of the research pursuits in this field is to detect molecular biomarkers in a fast and accurate way. A particular example is the Corona Virus Disease 2019 (COVID-19) that outbreak globally in a short time in 2020(Wu and McGoogan, 2020; Zhou et al., 2020a). Early diagnosis is critical to block the infection. However, only the real-time quantitative PCR (RT-qPCR) could provide reliable results in the first months(Liu et al., 2020b; Pfefferle et al., 2020). The RT-qPCR process costs several hours for an experienced person in the laboratory to verify the existence of the virus (named SARS-CoV-2) in each sample. As a result, few people are diagnosed and quarantined in the first place. The low efficiency limited the whole world to restrict the spread of the plague, as well as the deal with the virus. To date, the COVID-19 has spread toward more than 200 nations (Bettinelli et al., 2020; Lone and Ahmad, 2020). In total, over 200 million people were infected and more than 4 million of them died (data from Johns Hopkins University, https://www.arcgis.com/apps/dashboards/bda7594740fd40299423467b48e9ecf6). Although vaccines have been developed and promoted globally, the number of the infected keeps increasing because of the unexpected mutation of the virus(Callaway, 2021). So that efficient clinical diagnosis and quarantine are still necessary to eradicate the virus. The good news is, during these months, scientists have introduced dozens of methods for rapid and sensitive detection of the virus(Han et al., 2021; Koh and Cunningham, 2020; Pang et al., 2020; Qiu et al., 2020; Vandenberg et al., 2021; Xiong et al., 2020). Obviously, better strategies that are more convenient to use should be well prepared to keep waiting for the summoning of the world. People can probably adapt to the plague today, but we must be prepared well for tomorrow.

1.2. DNA-based biosensors

In general, the biosensor is a platform that can convert the invisible properties of a special target into a readable digital signal(Wang, 2008). To date, the electronic, fluorescent, chemiluminescent, and many other kinds of signals have been applied as the readouts of the biosensors (Chen et al., 2003; Fan et al., 2008). Lots of biosensors have been widely

introduced in the fields of analytical chemistry, bioanalysis, food chemistry, and clinical diagnosis(Arya et al., 2006). It is a promising direction that using the biosensors for detection of molecular biomarkers. To date, some biosensors such as the famous glucometer have already performed well in their detection tasks(Wang, 2001). However, as we mentioned above, more biosensors are still urgent demands for the analysis of the newly generated biomarkers rapidly and sensitively. It is also challenging to make the biosensors working in living cell systems or even in living bodies.

DNA is a well-known biomacromolecule that is famous for its function of gene regulation in the biological field. In recent decades, DNA has also been identified as a kind of nanomaterial by scientists(Seeman, 2010). As a material, DNA is believed to be endowed with the following properties: i) Biocompatibility: The biomacromolecule such as DNA is better to be accepted by the biosystem; ii) Stability: The DNA molecule can maintain its original structure in relatively acid or basic conditions, and can also recover from even 95 °C denaturing process after cooling down; iii) Specificity: Not only because of the principles of Watson-Crick base pairing but also because of the special recognition between the DNA aptamers and the targets, which was newly developed in recent years; iv) Programmability: The sequence of a DNA strand is highly free in design, and the construction of different structures can be achieved through artificial control. Taking these great advantages, people have fabricated many biosensors using DNA as the substrate. DNA-based biosensors have been used extensively for chemical, biological and medical applications (see Scheme 1). In this review, we will introduce the recent design of DNA-based biosensors for high-performance molecular biomarker analysis. In addition, we will also discuss the possible future direction of DNA-based biosensors design.
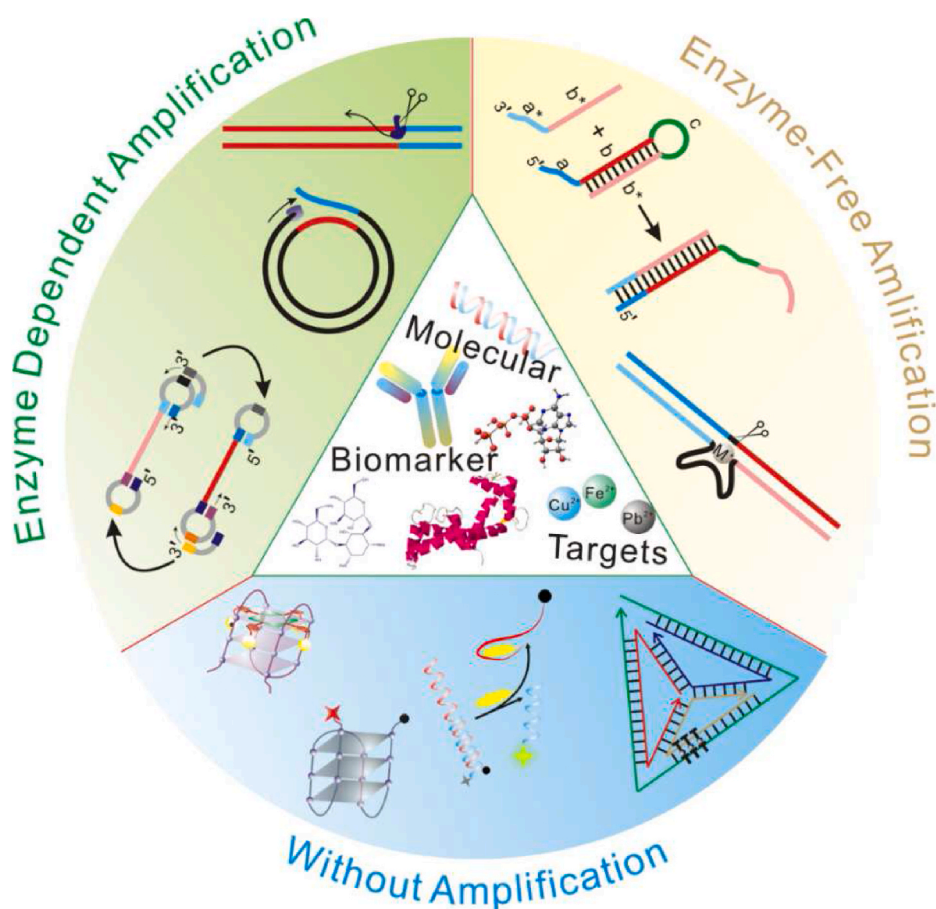
2. Design of DNA-Based biosensors

2.1. DNA-amplification related methods

When we talk about "high performance", higher sensitivity is probably a general character. So finding some pathway to amplify the signal to improve the sensitivity of the sensors will definitely provide high performance of the systems. For the DNA-based biosensors, the DNA substrate is the key that is usually applied for amplification. Starting from the polymerase chain reaction (PCR), which won the Nobel Prize in 1993(Mullis et al., 1986), scientists have brought a wealth of strategies for DNA amplification. In this chapter, we will discuss how people design the sensing platforms utilizing different DNA-amplification methods, including enzyme-dependent and enzyme-free methods respectively. We will talk about how people keep the sensitivity as well as ensuring the stability of the whole platform.

2.1.1. Enzyme-dependent strategies

It is no doubt that the PCR is a powerful and widely applied technique. Products as high as even 10^6-fold of the targets can be obtained within a 2h reaction. So that a signal response to a target with a concentration low to nanomolar (nM) level can be observed through the PCR detection method. As a development of the regular PCR, real-time quantitative PCR (RT-qPCR) has also been established to achieve a more convenient way for biomarkers detection(Livak and Schmittgen, 2001). Linking with the advanced instrument, RT-qPCR technique allows people to obtain the step-by-step readout during the PCR process, thus making the analysis more rapid and precise. As we mentioned above, the RT-qPCR is the earliest method that was applied for the clinical diagnosis of SARS-COV-2(Bustin and Nolan, 2020; Corman et al., 2020; Wang et al., 2020e). However, the biggest limitation of PCR in practical applications is the complicated temperature alternate processes. Temperature as high as 95 °C is necessary for a complete PCR, and many of the molecular biomarkers may be denatured under such an extreme environment. In the late 1990s, more enzymes were discovered and they worked in a friendlier environment. Since then, numerous

Scheme 1. Basic concern of DNA-based biosensor. DNA is a material of target probe, signal transducer, and reporter carrier.
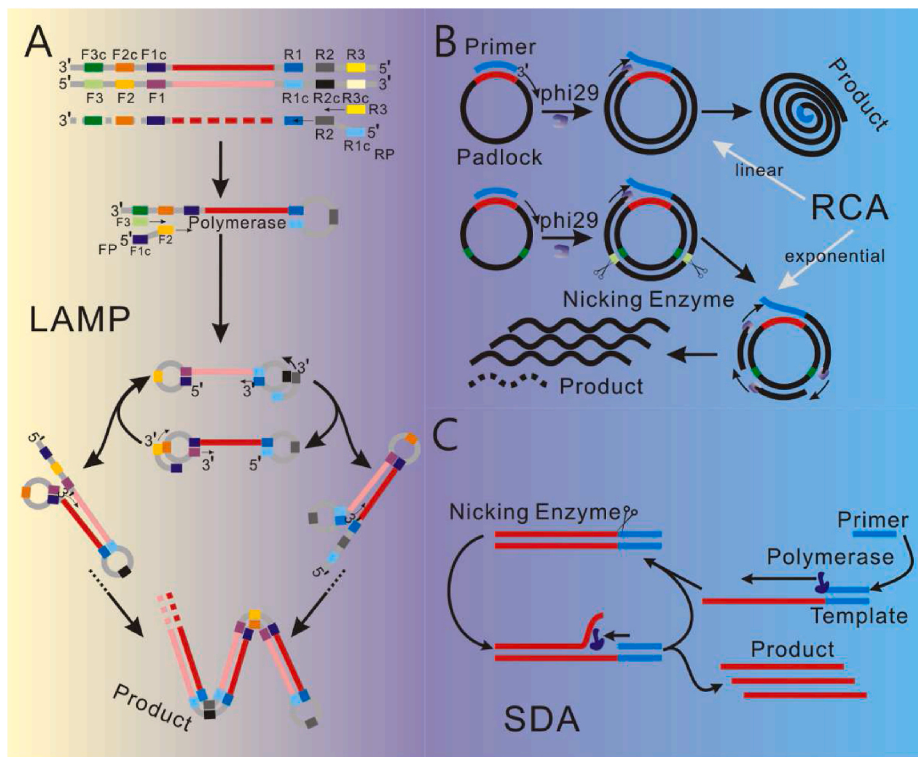

Fig. 1. Basic principle of the enzyme assisted DNA amplification reaction (A) loop-mediated isothermal amplification (LAMP) (B) rolling circle amplification (RCA) and (C) strand displacement amplification (SDA).

methods with simple temperature-controlled processes (isothermal, as so-called) have been introduced to build DNA-based biosensors(Zhao et al., 2015). For example, the loop-mediated isothermal amplification (LAMP, Fig. 1A) that works under a constant temperature (63–65 °C) performed good amplification efficiency(Notomi et al., 2000). Furthermore, some methods that work under gentle temperature (close to room temperature) were achieved. The rolling circle amplification (RCA, Fig. 1B) processes under a constant 30 °C(Dean et al., 2001), and the nicking-enzyme dependent strand displacement amplification (SDA, Fig. 1C) worked at around 25 °C(Walker et al., 1992). Compare to PCR, the isothermal methods are more convenient so that they have been applied for biosensors design widely(Liang et al., 2020; Tröger et al., 2015). In most recent, Yan et al. introduced a reverse transcription LAMP assay for the rapid detection of SARS-CoV-2(Yan et al., 2020). They designed the primers specific to the orf1ab and S genes of the virus and used a commercially purchased Loopamp RNA amplification kit to carry the detection process. As a result, they achieved a 100% specific detection within 30 min, showing that the LAMP assay can be a powerful tool for the detection of the SARS-CoV-2.

Nowadays, scientists do not satisfy with the μM/nM level in the limit of detection (LOD) of the DNA-based biosensors, so more sophisticated designs were proposed. Generally, people combined several amplification cycles, where the product(s) of the first cycle may initiate the next cycle(s), to realize the extension of the amplification process. Such a process was always called "exponential amplification" or "cascade amplification"(Li et al. 2020a, 2020b; Qiu et al., 2018; Zhao et al., 2019b). The name "exponential amplification reaction" was first proposed and abbreviated as EXPAR by Galas and coworkers in 2003(Van Ness et al., 2003). Now the EXPAR idea has been widely developed in the detection of different targets(Huang et al., 2018b; Reid et al., 2018). There is a basic idea on the design of such a biosensor: First of all, you need a probe that can recognize and react with the target, and the reaction may generate **product1**. Then the new assistant probe is added into the system to recognize and react with the **product1**, triggering the new amplification cycles. The final amplification product will be used for the output such as fluorescent or electric signals.

The key of such a design is how to combine different cycles efficiently. Different amplification reactions should be compatible so that they won't interrupt each other. Huang et al. reported a multiple primers-like RCA platform for the detection of DNA methyltransferase (MTase) (Fig. 2A) (Huang et al., 2017). They used a dumbbell-like template with special DNA sites for MTase recognition. The RCA process could keep going only in the presence of the target because the methylation may protect the intact template from digestion. To increase the sensitivity of this platform, they introduced several nicking sites for recognition of the nicking enzyme Nb.BbvcI, during the proceeding of RCA, the long-chain product would be cleaved by Nb.BbvcI, generating multiple short-stranded DNA which could work as the new primers to initiate new RCA cycles. As a result, 0.001 U ml^{-1} of LOD was achieved. The Zhang group reported a couple of designs of the DNA biosensing platforms that contained multiple amplification cycles with high sensitivity(Wang et al., 2018; Zhang et al. 2017, 2018b, 2019b). One of the highlights of Zhang's work is the new enzymes they adopted, such as Endonuclease IV and RNase HII (Fig. 2B) (Wang et al., 2018). These kinds of enzymes were applied as a highly specific substitute of the nicking enzyme, thus making the designed sensing platform selectively respond to the target only(Li et al., 2020d). During the development of enzyme engineering, more and more enzymes with unique functions have been introduced and employed in the construction of DNA-based biosensors to improve the performance of the systems(Sun et al., 2018). In most recent, an impressive system-CRISPR/Cas (Cluster Regularly Interspaced Short Palindromic Repeats) (Cong et al., 2013) has been introduced in the design of biosensors(Aman et al., 2020; Huang et al., 2018b; Li et al., 2019b). As the most compelling gene-editing tool, CRISPR/Cas has been demonstrated to perform efficient and accurate DNA detection, so the introduction of this system into the DNA-based sensing platform will definitely attract intense interest from researchers. Luo's group reported an RCA-assisted CRISPR/Cas9 platform for the detection of extracellular vesicle microRNA (EV-miRNA) (Wang et al., 2020d). In this platform, the RCA product was applied to initiate the subsequential Cas9-based cleavage. Using this platform, they identified multiple EV-miRNAs simultaneously in single-base mismatch resolution. Further, Sun et al. reported a sensing platform that combined SDA, RCA, and CRISPR/Cas9, such a system was applied to detect a pathogenic bacteria-*E. coli* O157:H7(Sun et al., 2020). Zhang et al. introduced a method using CRISPR/Cas9-mediated proximity ligation assay to achieve the imaging of single-nucleotide variation in mitochondrial DNA *in situ(Zhang* et al. *2018a)*. In their method, the CRISPR/Cas9 system was applied for accurate recognition of the target site. Two DNA strands linked with the system would be brought proximity to assist the forming of a template. This template could be further applied for an RCA process to realize efficient signal amplification. In recent, a member of the CRISPR-associate protein family called Cas12a has been demonstrated shown unique collateral DNase activities(Chen et al., 2018; Li et al., 2018). People have linked Cas12a with other DNA-amplification methods to achieve high specific detection of targets include nucleic acids(Dai et al., 2019; Wang et al., 2019a), enzymes(Du et al., 2021; Wang et al., 2020a), and small molecules(Liang et al., 2019). Just recently, Broughton e*t al* presented a SARS-COV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) which combined CRISPR/Cas12 system with the LAMP method (Fig. 2C) (Broughton et al., 2020). Using this DETECTR strategy, they achieved a rapid and accurate detection of SARS-COV-2 with a visualized lateral flow paper-strip assay, gave a 95% positive predictive agreement within 40 min.

One challenge of such a multiple-cycle design is the complicated assistant materials. People usually insert more than two amplification cycles to achieve a satisfactory sensitivity. The more cycles included, the more materials such as enzymes or oligonucleotides would be added into the whole system. Obviously, a complicated sensing platform is not good enough for a task in the situation which is also complicated because too many elements may interrupt the expected procedure through the non-specific reactions. To overcome this drawback, the Kong group proposed an integrated probe design strategy to build a sensing platform of high performance in recent. They designed a hairpin-like DNA template that could react with targeted MTase (Fig. 2D) (Cui et al., 2019b). The product would initiate an SDA process, which generated new primers that retroacting on the hairpin template and triggering another SDA. Therefore, the original DNA template played triple roles as a probe of the target, a template of signal amplification, and a reporter of fluorescence. The integrated design avoided the requirement of the superfluous auxiliary materials, thus simplifying the whole sensing system. In recent, the same group proposed a more integrated probe constructed with only one DNA strand, which was termed as probe-amplifier-reporter trifunctional integrated platform (PARTIP) (Fig. 2E) (Cui et al., 2019a). Using terminal deoxynucleotidyl transferase (TdT)-a template-independent polymerase, they applied a single probe DNA strand as the substrate to be elongated in the presence of the target. Then the elongated product could also play as the substrate to react with the probe thus initiating the signal amplification itself. Moreover, scientists demonstrated that the TdT assisted DNA-amplification method could be applied for sensitive detection of different molecular biomarkers in a one-pot way with only simple modification, thus offering a promising strategy for simplified biosensor design. In most recent, Jiang et al. have improved the PARTIP design to construct a sensing platform to detect Pb^{2+} in food and water samples(Jiang et al., 2021). Combining the TdT assisted amplification and CRISPR/Cas12 method, Zhang et al. have introduced a high-performance sensing system to detect polynucleotide kinase(Zhang et al., 2021).

During the development of DNA nanotechnology, DNA-based nanodevices have been introduced to construct the DNA-based biosensors. Combining with the DNA-amplification process, DNA
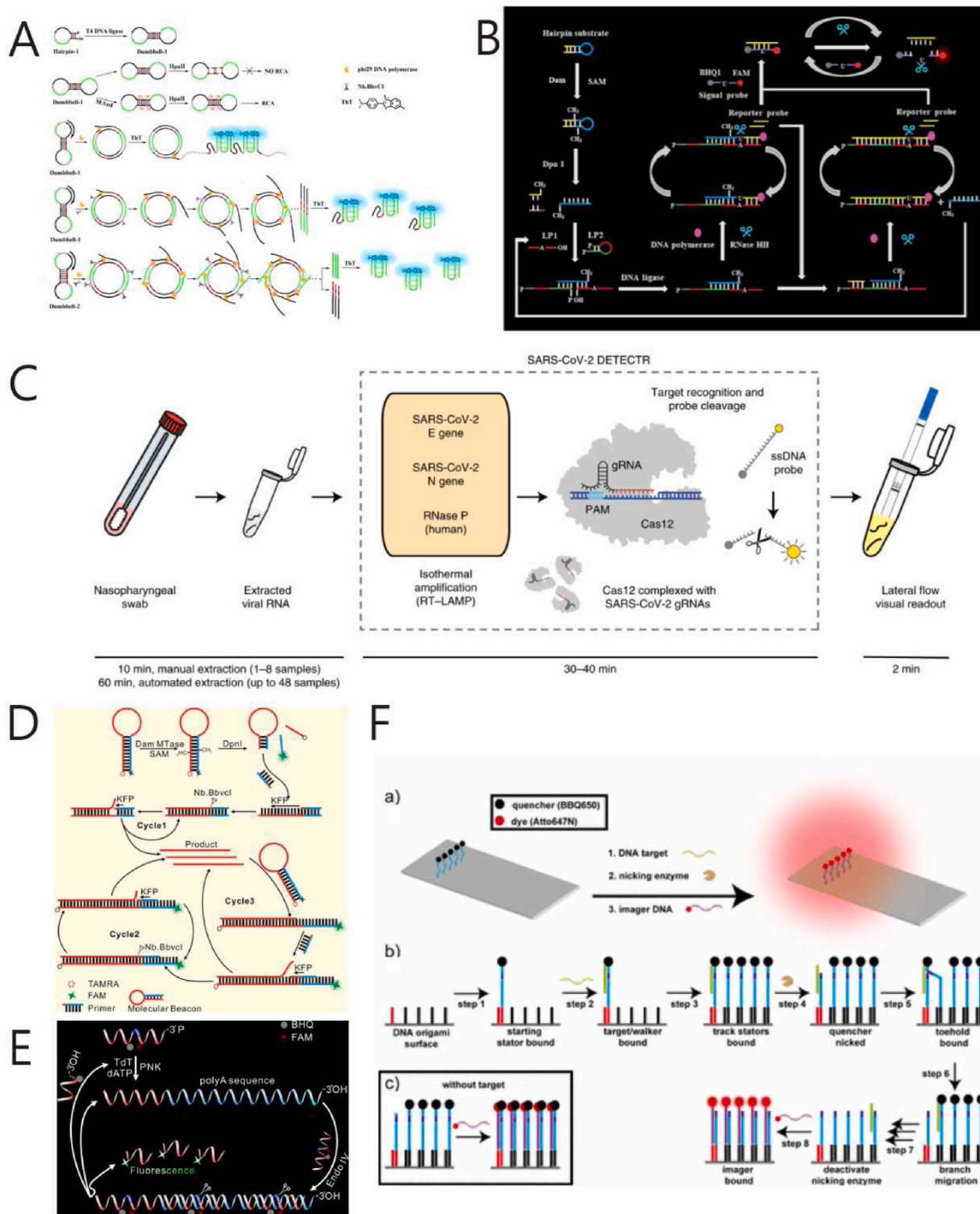
Fig. 2. Design of the DNA-based biosensors depended on enzyme-assisted DNA amplification processes. (A) The nicking-enzyme-dependent exponential RCA method. Reproduce from ref. (Huang et al., 2017) with permission from the Elsevier, copyright 2017 (B) High specific RNase HII assisted multiple amplification. Reproduce from ref. (Wang et al., 2018) with permission from the Royal Society of Chemistry, copyright 2018 (C) Cas12 related amplification system for detection of SARS-CoV-2. Reproduce from ref. (Broughton et al., 2020) with permission from the Nature Publishing Group, copyright 2020 (D) SDA-based multi-cycle amplification using the integrated probe. Reproduce from ref. (Cui et al., 2019b) with permission from the Royal Society of Chemistry, copyright 2019 (E) Design of the probe-amplifier-reporter trifunctional integrated platform (PARTIP). Reproduce from ref. (Cui et al., 2019a) with permission from the Royal Society of Chemistry, copyright 2019 (F) Nicking-enzyme assisted DNA nanowalker platform using target ssDNA as the fuel. Reproduce from ref. (Wang et al., 2017a) with permission from the American Chemical Society, copyright 2017.

nanostructures have been proved that can highly improve the detection efficiency for DNA-based biosensor construction. One typical instance is the DNA walker, which is well known in the field of DNA nanorobot and DNA computing(Jung et al., 2016; Omabegho et al., 2009; Qian and Winfree, 2011). Wang et al. reported a DNA origami-based nanowalker device for sensitive detection of target nucleic acids (Fig. 2F) (Wang et al., 2017a). They built a rectangular DNA origami substrate with extended short DNA tails. Then quencher labeled stator stands were hybridize with the short tails. When the target DNA was added in, it would hybridize with the stator strand to form a double-stranded structure. The stator stand part of this double-stranded structure could be cleaved into two pieces by a unique nicking enzyme. The structure would become unstable because of the cleavage, so the quencher labeled part could be released and the target strand could walk to hybridize with another stator stand. Finally, the fluorescent signal could be observed because the quenchers had been released. In this DNA walker platform, one target DNA strand can walk dozens of steps, triggering the cleavage of the stator stands thus achieving the signal amplification. Besides the flat-surface substrates, spheres such as gold nanoparticles (AuNPs) are also popular for DNA nanowalker construction now. The stator stand can be easily anchored on the surface of AuNPs and targets including nucleic acids and proteins can be detected(Qu et al., 2017; Yang et al., 2016; Zhang et al., 2015; Zhao et al., 2021).

2.1.2. Enzyme-free strategies

The enzyme-based DNA amplification strategies have shown good potential in the design of high-performance DNA-based sensing platforms. However, it sometimes failed when working in a complicated environment because the efficiency of the enzymes cannot be well controlled. In addition, it is a big challenge for the exogenous enzymes to cross the cellular barrier to work the living system. Some methods such as nanoparticle carrier and transfection strategy have been introduced to facilitate the enzyme-assisted DNA amplification biosensors entering living cells to perform intracellular detections(Deng et al., 2014; Zhang et al., 2019a; Zhou et al., 2019). However, it remains drawbacks. For example, the enzymes may be digested by the intracellular proteases, and the exogenous enzymes may trigger unexpected immune responses. Some scientists took a detour through the enzyme-free DNA amplification pathway, such as hybridization chain reaction (HCR) (Dirks and Pierce, 2004) and catalytic hairpins assembly (CHA) (Yin et al., 2008), both of which were first proposed by Pierce's group. The driving force of both CHA and HCR is toehold-mediated DNA hybridization(Zhang et al., 2012). As shown in Fig. 3A&B, two hairpin-like DNA templates can automatically be assembled in the presence of a single-stranded DNA initiator.

These enzyme-free methods provide new directions for biosensor design. Over the past decade, dozens of biosensing platforms based on CHA or HCR were constructed and showed outstanding performance in the analysis of different molecular biomarkers both extracellularly and intracellularly(Choi et al., 2010; Huang et al., 2011; Kitamura et al., 2020; Li et al., 2019a; Xing et al., 2020). Similar to the enzymatic strategies, people designed biosensors with branched HCR, or combined CHA/HCR method, to achieve the high sensitivity through multiple amplification cycles(Bi et al., 2017; Jiang et al., 2019; Shang et al., 2020; Wang et al., 2020c). Although the circumvention of enzyme utilization made the constructed sensing platform simpler and more convenient, the efficiency of the amplification reactions was limited without the help of the enzymes. Sophisticated sequence design through precise entropy
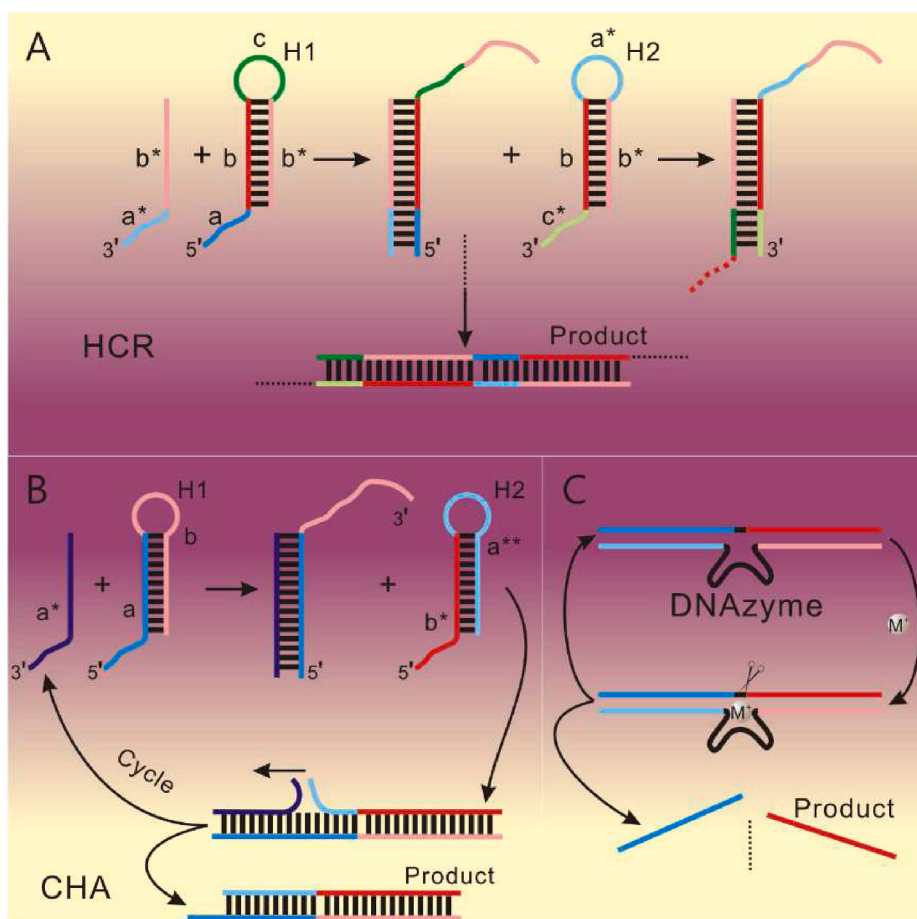

Fig. 3. Basic principle of the enzyme-free DNA amplification reaction (A) hybridization chain reaction (HCR) (B) catalytic hairpins assembly (CHA) and (C) DNAzyme assisted amplification.

calculation may maximize the efficiency(Figg et al., 2020; Haley et al., 2020; Zhang et al., 2012). However, people are still looking for some better strategies to improve it. Recently, a model that confining re-actants together in a limited space was shown to accelerate the reaction rate of the DNA stands displacement reactions through a locally concentrated mechanism(Bui et al., 2018; Chatterjee et al., 2017; Engelen et al., 2018; Ren et al., 2018). Inspiring by this model, scientists have developed some sensing platforms based on DNA nanotechnology for highly efficient intracellular biomarker detections. Huang et al. built a protein-scaffold DNA tetrad for intracellular miRNA detection (Fig. 4A) (Huang et al., 2018a). One streptavidin molecule was used as a core and four biotinylated hairpin DNA probes were conjugated on the core to construct a four-arm DNA nanostructure. The existed miRNA

would trigger a crosslink HCR between two DNA tetrads. They achieved ultrasensitive and accurate miRNA imaging in living cells using this biosensor. Wei et al. reported a localized CHA (LCHA) platform constructed by interval hybridization of DNA hairpin probe pairs to a DNA nanowire generated by alternating chain hybridization (Fig. 4B) (Wei et al., 2018). Comparing to the conventional CHA, they found that the LCHA performed a significantly more rapid response time and higher sensitivity toward miRNA in living cells. Just recently, Wang et al. proposed a quadrivalent tetrahedral DNA nanostructure (qTDN)-mediated hyperbranched HCR (Fig. 4C) (Wang et al., 2019c). By assembling DNA hairpins at the vertexes of qTDNs, they discovered that the HCR between the qTDN-assembled hairpins had greatly accelerated reaction kinetics. People supposed this was because of the synergetic
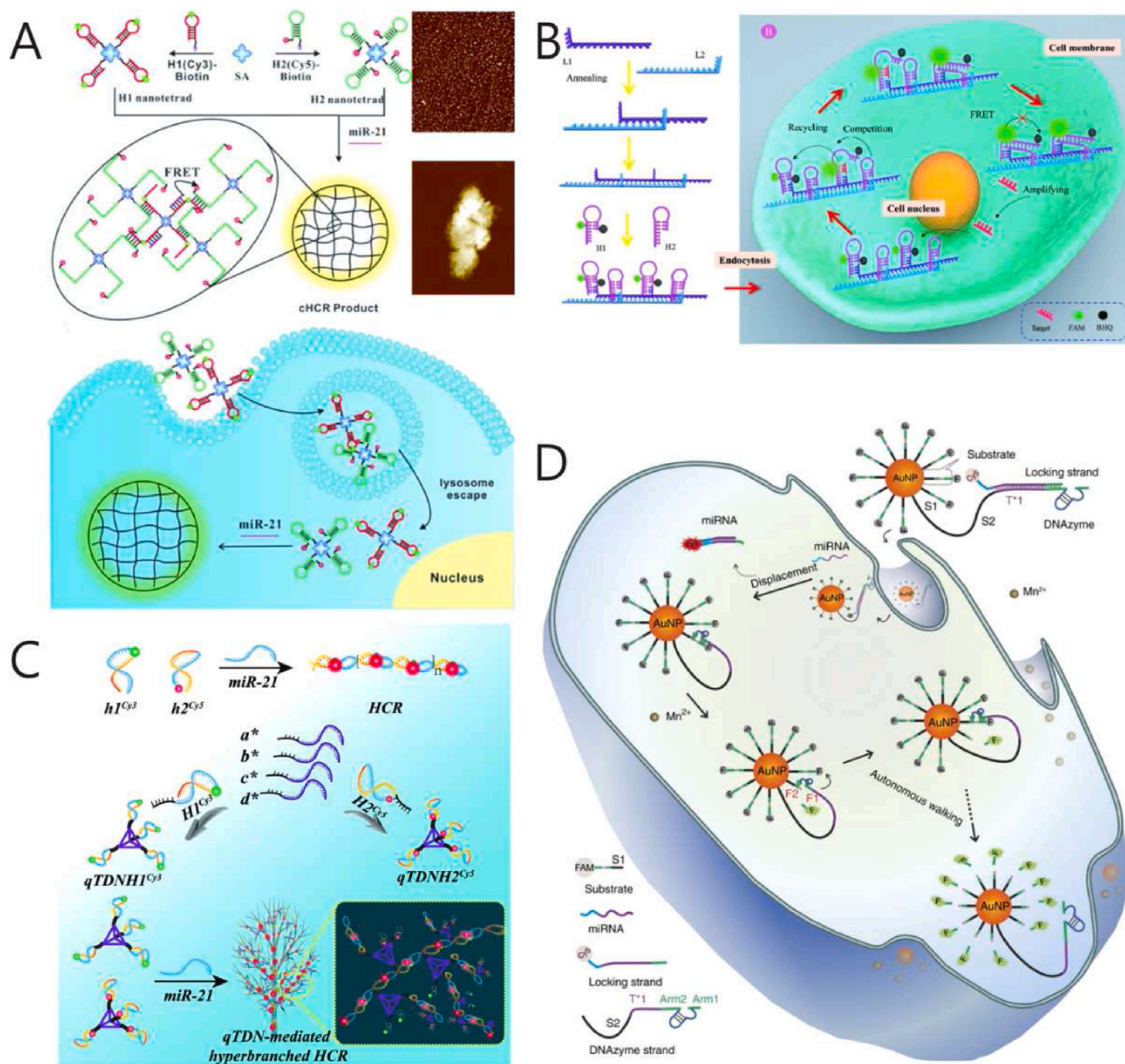
Fig. 4. Designs of the DNA-based biosensors dependent on the enzyme-free DNA amplification methods. (A) HCR between DNA tetrads based on the streptavidin-biotin scaffold. Reproduce from ref. (Huang et al., 2018a) with permission from the Royal Society of Chemistry, copyright 2018. (B) Localized CHA based on a DNA nanowire template. Reproduce from ref. (Wei et al., 2018) with permission from the Royal Society of Chemistry, copyright 2018. (C) Using qTDN structure to accelerate the efficiency of HCR. Reproduce from ref. (Wang et al., 2019c) with permission from the Royal Society of Chemistry, copyright 2019. (D) AuNP supported DNA nanowalker based on catalytic DNA cleavage of the DNAzyme. Reproduce from ref. (Peng et al., 2017) with permission from the Nature Publishing Group, copyright 2017.

contributions of multiple reaction orientations, increased collision probability, and enhanced local concentrations mediated by the tetrahedral structure. Such a local-concentration acceleration can be achieved through not only the tetrahedral DNA nanostructure(Ji et al., 2021) but also other kinds of compact structures such as DNA Y-shape scaffold(Wu et al., 2020) and DNA nanolantern(Wang et al. 2019b, 2021a).

Besides this detour through the strand hybridization-related strategies, another pathway using cleavage catalytic DNAzyme as the key component is also popular in biosensor design. DNAzyme is a series of DNA that are endowing with catalytic activation(Brown et al., 2003; Liu and Lu, 2003; Wang et al., 2017c). A typical cleavage DNAzyme includes three components: a catalytic DNA strand with a unique sequence; a cleavable substrate strand, which usually contains one ribonucleotide site; and a special ion that may be selectively recognized by the catalytic strand thus initiating the cleavage (Fig. 3C). The catalytic strand and the substrate strand are partially complementary and hybridize with each other. In the presence of the special metal ion, the cleavage activation of the catalytic strand will be initiated, resulting in a truncation of the substrate strand at the ribonucleotide position. Through the DNAzyme system, DNA cleavage can be realized without any help from exogenous proteinaceous enzymes. Therefore, a sensing system constructed based on the DNAzyme can achieve an efficient signal amplification without the protein substrates. To date, DNAzymes responding to Mg^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Na^+ and many other different ions have been proposed and widely applied for biosensor construction(Cao et al., 2020; Chen et al., 2020; Huang et al., 2020; Nakama et al., 2020; Ren et al. 2020a, 2020b; Zhang et al., 2020c). The DNAzyme can work with the strand hybridization methods synergistically to create a sensing platform based on enzyme-free exponential amplification(Liu et al., 2017b; Wang et al., 2019d; Wu et al., 2017; Zhou et al., 2020b). Furthermore, combining DNAzyme and DNA nanotechnology is also a promising way for the design of DNA-based biosensors(Chen et al., 2017a; Meng et al., 2018). Le's group reported a DNA walker system using DNAzyme as the trigger force for miRNA detection in living cells (Fig. 4D) (Peng et al., 2017). The structure was constructed by an Au nanoparticle decorated with lots of locked DNAzyme templates. The locked DNAzyme template included hundreds of substrate DNA strands and dozens of catalytic DNA strands. In the presence of the target miRNA, the catalytic strands will be unlocked. The catalytic strand could work as a "walker" to cleave multiple substrates one by one, thus achieving signal amplification without the addition of external materials. Motivated by this model, a few self-powered DNAzyme motors have also been introduced for the detection of different molecular biomarkers *in vitro* and *in vivo* in recent (*Chen* et al. *2017b*; *Liang* et al. *2017*; *Liu* et al. *2017a*; *Ma* et al. *2018*).

2.2. DNA amplification-free strategies

DNA amplification strategies are interesting and powerful to provide a sensitive signal to improve the performance of the DNA-based biosensors, but some scientists insist that amplification-free is the better access. Some people claimed that the nucleic acid amplification designs are overelaborate, time-consuming, costly, and (importantly) sometimes lose their specificity. By removing complex steps, DNA amplification-free detection can perform direct and rapid detection of target molecules. In fact, amplification-free is the style of DNA-based sensing strategy which existed much earlier. Such as the Southern blot and Northern blot from 1970s(Engler-Blum et al., 1993), they are important techniques for the detection of DNA and RNA, but how to improve the sensitivity is the problem since their birth.

During the development of molecular biology and biochemistry, people can not only synthesize DNA strands with programmed sequences but also make various particular modifications on the unique site of the DNA strands. In the past decades, attempts have been made to modify DNA with unique signal transducers to improve the performance of the sensors. The high selectivity and sensitivity of the novel transducer molecules make the signal amplification by the DNA-amplification process can be refrained(Dan et al., 2019; Kim and Winssinger, 2020; Thekkan et al., 2019; Tyagi and Kramer, 1996; Xu et al., 2017; Yang et al., 2019; Yue et al., 2020). Motivated by enzyme-linked immunosorbent assay (ELISA), some DNA-based sensing platforms have been proposed using modified DNA as substitutes for the conventional antibody. Li et al. proposed a DNA-based electronic sensor (Fig. 5A) (Li et al., 2016). They immobilized the hairpin-like DNA probes on the surface of the gold electrode. This probe can form a stable Y-shape structure with the target DNA and a reporter DNA strand. The reporter DNA contained a biotin modification so it can bind with the streptavidin (SA) linked horseradish peroxidase (HRP). The next steps would be quite similar to ELISA: HRP could catalyze the oxidation of TMB in the presence of H_2O_2, resulting in the change of electronic signal. Some similar designs have been reported in recent, in which modified DNA probes were anchored on the surface of different kinds of electrodes, and the targets might bring some materials which acted as electronic transduction mediators to the electrodes through interaction with the DNA probes(Gong et al., 2017; Tripathy et al., 2017). Besides the design based on the hybridization between DNA strands, another approach using allosteric DNA is also a promising design for amplification-free biosensor construction. DNA with special sequences can fold into some unique three-dimensional structures such as G-quadruplex (Gq) and i-Motif (iM) (Simonsson and Sjoback, 1999). The conformational change will be utilized to generate different signals. As an early example, Nagatoishi et al. reported a sensing platform constructed by a guanine-rich sequence that can fold into stable Gq in the presence of K^+(Nagatoishi et al. 2005, 2006). It is not difficult to imagine that the forming of Gq will bring the 5′ and 3' ends of the single-stranded DNA to a proximal distance. So if there are some particular labels on these two ends, the change in distance may cause a readable signal. Nagatoishi used fluorophore-quencher pairs in their example, while other people using FRET or electronic transducers can also construct efficient sensors (Liu et al., 2020a). In recent Zhang et al. designed a special DNA strand with inserted azobenzene as the probe of the thrombin (Fig. 5B) (Zhang et al., 2020b). The probe was anchored on the electrode with one end, and modified with ferrocene on the other end. In the presence of targeted thrombin, the probe would fold into a Gq structure, thus bringing the ferrocene close to the electrode to generate the electric signal. In addition, the inserted azobenzene could perform a *trans-cis* structure change thus opening the folded Gq under UV-light irradiation. This *cis*-azobenzene might reverse to *trans*-structure under visible light, making the sensing platform regenerate. Nowadays, more and more unique DNA structures have been employed in the design of amplification-free biosensors by their allosteric characters. In recent, the Ricci group proposed a DNA-based nanomachine that can respond to the target antibodies and realize controllable DNA releasing for signal produce (Fig. 5C) (Ranallo et al., 2017). In their design, the DNA probe was constructed by a hairpin DNA labeled with both ends and a signal DNA that can form a stable triplex structure with the hairpin. In the presence of the target, the two ends of the hairpin would be held on two separated sites of the target. The distance between the two separated sites was bigger than the end-to-end distance of the hairpin, so the triplex would be unfolded after this holding. Then the signal stand will be released from the unfolded triplex thus generating the signal.

Another interesting DNA material that performs allosteric binding toward a particular target molecule is aptamers. In 1990, Tuerk and Gold introduced a series of short DNA oligos, which were selected through a technic called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) (Tuerk and Gold, 1990). Similar to antibodies, the aptamers can recognize and bind to special target molecules, forming stable complexes with particular configurations. Nowadays, many DNA-based biosensors with aptamers as major components have been fabricated(Debiais et al., 2020; Tan et al., 2020; Zhao et al., 2019a). In most of the designs, aptamers worked as probes of the targets as well as signal reporters due to the allosteric recognition. Modifying with unique
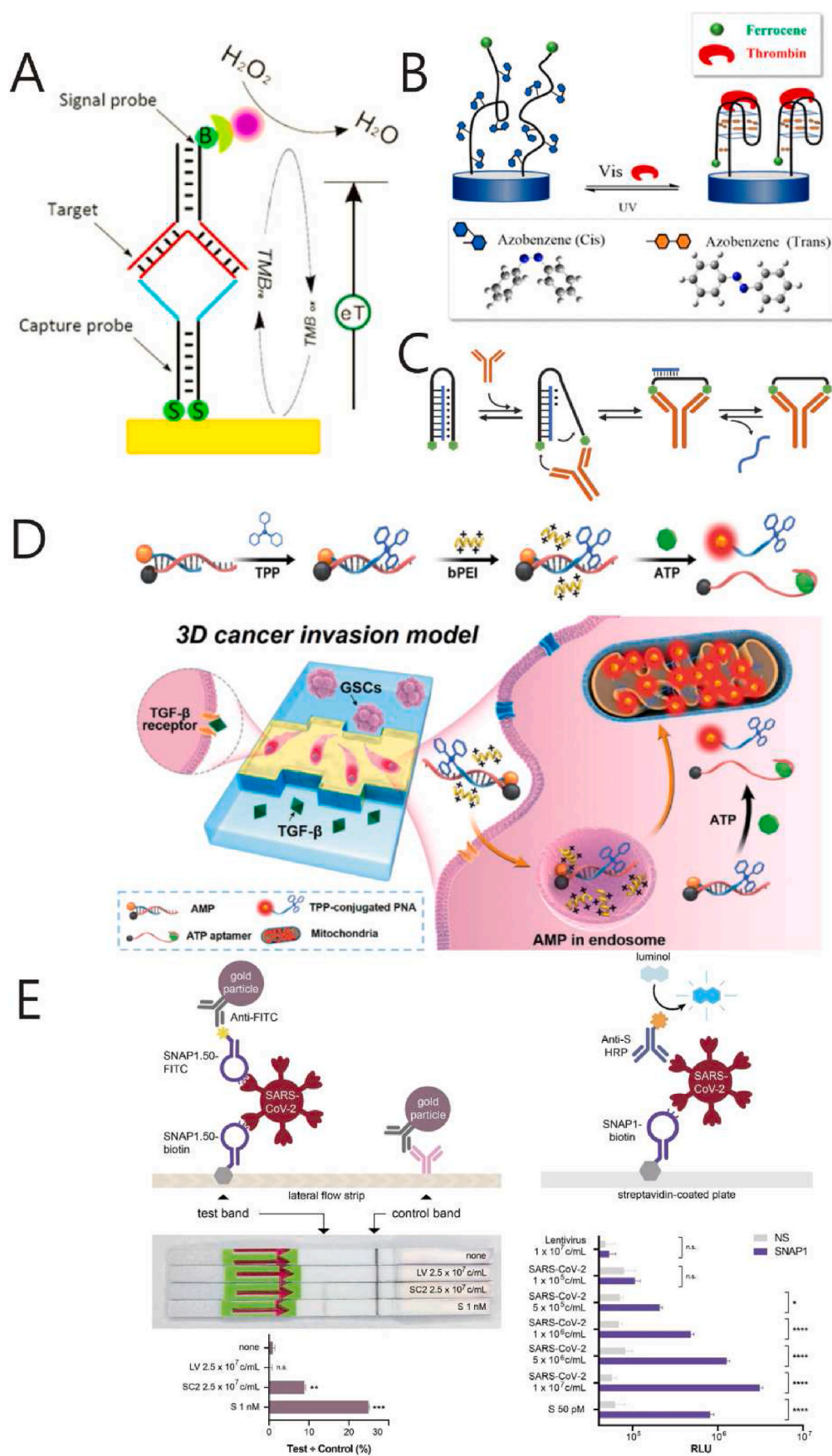
Fig. 5. Designs of the DNA-based biosensors without the DNA amplification process. (A) The modified target nucleic acid strands can form a Y-shape structure with the probes anchored on the electrode for further electric signal transduce. Reproduce from ref. (Li et al., 2016) with permission from the American Chemical Society, copyright 2016. (B) Special DNA strands can form a G-quadruplex structure and bring the ferrocene molecules close to the electrode. Reproduce from ref. (Zhang et al., 2020b) with permission from the American Chemical Society, copyright 2020 (C) Allosteric regulation of a DNA triplex structure to control the release of the signal strand by the targeted antibody. Reproduce from ref. (Ranallo et al., 2017) with permission from the Nature Publishing Group, copyright 2017 (D) An ATP-responsive mitochondrial probe constructed based on hybridized PNA and DNA aptamer. Reproduce from ref. (Lin et al., 2020) with permission from the Royal Society of Chemistry, copyright 2020 (E) Using the DNA aptamers as the probe of SARS-COV-19 for detection of the virus by a lateral flow assay. Reproduce from ref. (Kacherovsky et al., 2021) with permission from the Wiley-VCH, copyright 2020.

signal transducer molecules, the aptamer-based sensing platform can perform highly efficient detection without any DNA amplification processes. For example, Lin et al. have reported an ATP-responsive mitochondrial probe that can monitor the ATP level in living cells (Fig. 5D) (Lin et al., 2020). In their design, they hybridized a fluorophore and triphenylphosphonium (TPP) modified peptide nucleic acid (PNA) strand and a quencher modified ATP aptamer DNA strand. The recognition and binding of the ATP with the aptamer strand will release the PNA strand. The fluorescent signal can be observed because of the separation between the fluorophore and the quencher. During the development of SELEX, aptamers targeting toward more kinds of molecules with higher efficiency have been screened, promoting the aptamer-related biosensors in the applications of a wide range of biomarkers(Iliuk et al., 2011; Meng et al., 2016). To date, people have successfully screened several DNA aptamers toward SARS-COV-19 (Fig. 5E) (Kacherovsky et al., 2021; Zhang et al., 2020a). Since the discovery of these aptamers, the biosensors based on the interaction between the aptamer and the target have been proposed for detection of the SARS-COV-19(Amouzadeh Tabrizi et al., 2021; Chen et al., 2021; Deng et al., 2021; Idili et al., 2021). It has been demonstrated that the constructed biosensors can perform rapid and sensitive result without any DNA-amplification process.

The amplification-free platforms require not only the sophisticated designs but also the high-resolution equipment. Thanks to the development of advanced technics and instruments, people can now observe the tiny signals more straightforward without any amplification. For example, the CRISPR/Cas9 system was integrated into a microfluidic chip in combination with a graphene-based field-effect transistor. Hajian et al. used this sensing platform for ultrasensitive detection of target DNA with a concentration as low as 1.7 fM without amplification methods(Hajian et al., 2019). Moreover, total internal reflection fluorescence microscopies (TIRF) (Lee et al., 2019; Peng et al., 2019), optical tweezers(Mitra and Ha, 2019; Shrestha et al., 2018), and nanopore technology(Wang et al., 2020b) are all great technologies that provide single-molecule level resolution. To date, more and more attempts using advanced high-resolution technologies to detect the signals carried by DNA have been reported(Hu et al., 2019; Li et al., 2020c; Mandal et al. 2016, 2019; Wang et al., 2021b). Although the major concept is still based on the engineering development of the instruments themselves, we believe that scientists will focus on the design of DNA biosensors more to better link the instruments with large-scale molecular biomarkers in living systems in the future.

3. Conclusions and outlook

DNA-based biosensors have developed rapidly over the past two decades. Many designs of DNA sensing platforms have been proposed for response to different molecular biomarkers including nucleic acids, proteins, lipids, small ions, and many other molecules involved in the bioprocesses. Exquisite designs based on sophisticated sequence programs, accurate base modifications, novel enzyme engineering, and modern nanotechnology, promoted the application of DNA-based biosensors both *in vitro* and *in vivo*. Nowadays, a variety of well-established DNA-based sensing platforms have been reported and widely applied in different fields including environmental monitoring, food safety, clinical diagnosis, disease prognosis, etc. The journeys ahead of these biosensors maintain two directions: making it more general, or more professional.

i) A more general design means a biosensor more suitable for general people to use even at home. Usually, people need a final result in a simple and fast way. Time-consuming, complicated operation, high cost, and instrument dependency are all limitations to introduce a biosensor globally. For example, when people trying to confirm the existence of the SARS-COV-19, the lateral flow assay is definitely more achievable for a global analysis compared to the RT-qPCR.

Maybe the striving of the engineers in handy equipment such as a household fluoroscope also encourages a biosensor to a wider audience. However, scientists in the chemical and biological fields also keep on going to make the biosensors more compact. DNA is a powerful tool, then how to reduce the required components, how to make the materials longer stable on a portable chip, how to transmit the signal of DNA to readable household equipment such as smartphone or glucometer, are all obstacles still waiting for perfect curvets. Some attempts have been proposed and we believe the mature designs will emerge sooner or later.

ii) It is probably biologists rather than chemists who desire a more professional biosensor. An ideal biosensor may provide accurate information to monitor the existence of a bioprocess or understand the mechanism. Therefore, it is very important to develop biosensors of high performance in living cells or even in living bodies. How to improve the permeability of the biosensor to cross the biological barrier, how to improve the anti-interference performance of the biosensors, how to maintain the high sensitivity, are the major challenges that have to be overcome. Obviously, DNA nanotechnology provides more interesting ideas for the design of biosensors with higher sensitivity and better performance in living biosystems. Moreover, the development of advanced instruments has offered a more straightforward way for signal detection. Therefore, we believe the cutting-edge biosensors that combine DNA nanotechnology and nice apparatus will be raised as an encouraging direction.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

References

Abdel-Halim, K.Y., Salama, A.K., El-Khateeb, E.N., Bakry, N.M., 2006. Organophosphorus pollutants (OPP) in aquatic environment at Damietta Governorate, Egypt: implications for monitoring and biomarker responses. Chemosphere 63 (9), 1491–1498.

Aman, R., Mahas, A., Mahfouz, M., 2020. Nucleic acid detection using CRISPR/Cas biosensing technologies. ACS Synth. Biol. 9 (6), 8.

Amouzadeh Tabrizi, M., Nazari, L., Acedo, P., 2021. A photo-electrochemical aptasensor for the determination of severe acute respiratory syndrome coronavirus 2 receptor-binding domain by using graphitic carbon nitride-cadmium sulfide quantum dots nanocomposite. Sensor. Actuator. B Chem. 345, 130377-130377.

Arya, S.K., Chaubey, A., Malhotra, B.D., 2006. Fundamentals and applications of biosensors. Proceedings of the Indian National Science Academy 72 (4), 249–266.

Bettinelli, G., Delmastro, E., Salvato, D., Salini, V., Placella, G., 2020. Orthopaedic patient workflow in CoViD-19 pandemic in Italy. J. Orthop. 22, 158–159.

Bi, S., Yue, S., Zhang, S., 2017. Hybridization chain reaction: a versatile molecular tool for biosensing, bioimaging, and biomedicine. Chem. Soc. Rev. 46 (14), 4281–4298.

Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J., Miao, X., Streithorst, J.A., Granados, A., Sotomayor-Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C.-Y., Guevara, H., Wadford, D.A., Chen, J.S., Chiu, C.Y., 2020. CRISPR-Cas12-based detection of SARS-CoV-2. Nat. Biotechnol. 38, 870–874.

Brown, A.K., Li, J., Pavot, C.M.B., Lu, Y., 2003. A lead-dependent DNAzyme with a two-step mechanism. Biochemistry 42 (23), 7152–7161.

Bui, H., Shah, S., Mokhtar, R., Song, T., Garg, S., Reif, J., 2018. Localized DNA hybridization chain reactions on DNA origami. ACS Nano 12 (2), 1146–1155.

Bustin, S.A., Nolan, T., 2020. RT-qPCR testing of SARS-CoV-2: a primer. Int. J. Mol. Sci. 21 (8).

Callaway, E., 2021. Remember Beta? New data reveal variant's deadly powers. Nature.

Cao, C., Liu, J.Q., Tang, S.Y., Dai, Z.R., Xiao, F.B., Rang, W.Q., Liu, L., Chen, T., Yuan, Y. L., Li, L., 2020. Amplified electrochemical determination of UO22+ based on the cleavage of the DNAzyme and DNA-modified gold nanoparticle network structure. Microchim. Acta 187 (5).

Catalona, W.J., Partin, A.W., Sanda, M.G., Wei, J.T., Klee, G.G., Bangma, C.H., Slawin, K. M., Marks, L.S., Loeb, S.S., Broyles, D.L., Shin, S.S., Cruz, A.B., Chan, D.W., Sokoll, L.J., Roberts, W.L., van Schaik, R.H.N., Mizrahi, I.A., 2011. A multicenter study of -2 pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. J. Urol. 185 (5), 1650–1655.

Chatterjee, G., Dalchau, N., Muscat, R.A., Phillips, A., Seelig, G., 2017. A spatially localized architecture for fast and modular DNA computing. Nat. Nanotechnol. 12 (9), 920–927.

Chen, F., Bai, M., Cao, K., Zhao, Y., Cao, X.W., Wei, J., Wu, N., Li, J., Wang, L.H., Fan, C. H., Zhao, Y.X., 2017a. Programming enzyme-initiated autonomous DNAzyme nanodevices in living cells. ACS Nano 11 (12), 11908–11914.

Chen, H., Park, S.-G., Choi, N., Kwon, H.-J., Kang, T., Lee, M.-K., Choo, J., 2021. Sensitive detection of SARS-CoV-2 using a SERS-based aptasensor. ACS Sens. 6 (6), 2378–2385.

Chen, J.B., Zuehlke, A., Deng, B., Peng, H.Y., Hou, X.D., Zhang, H.Q., 2017b. A target-triggered DNAzyme motor enabling homogeneous, amplified detection of proteins. Anal. Chem. 89 (23), 12888–12895.

Chen, J.H., Pan, J.F., Liu, C.S., 2020. Versatile sensing platform for Cd2+ detection in rice samples and its applications in logic gate computation. Anal. Chem. 92 (8), 6173–6180.

Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M., Doudna, J.A., 2018. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science 360 (6387), 436–439.

Chen, R.J., Bangsaruntip, S., Drouvalakis, K.A., Kam, N.W.S., Shim, M., Li, Y.M., Kim, W., Utz, P.J., Dai, H.J., 2003. Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. Proc. Natl. Acad. Sci. U. S A 100 (9), 4984–4989.

Choi, H.M.T., Chang, J.Y., Trinh, L.A., Padilla, J.E., Fraser, S.E., Pierce, N.A., 2010. Programmable in situ amplification for multiplexed imaging of mRNA expression. Nat. Biotechnol. 28 (11), 1208-U1103.

Christensen, M., Schratt, G.M., 2009. microRNA involvement in developmental and functional aspects of the nervous system and in neurological diseases. Neurosci. Lett. 466 (2), 55–62.

Cong, L., Ran, F.A., Cox, D., Lin, S.L., Barretto, R., Habib, N., Hsu, P.D., Wu, X.B., Jiang, W.Y., Marraffini, L.A., Zhang, F., 2013. Multiplex genome engineering using CRISPR/Cas systems. Science 339 (6121), 819–823.

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K.W., Bleicker, T., Bruenink, S., Schneider, J., Schmidt, M.L., Mulders, D.G.J.C., Haagmans, B.L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J.-L., Ellis, J., Zambon, M., Peiris, M., Goossens, H., Reusken, C., Koopmans, M.P.G., Drosten, C., 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 25 (3), 23–30.

Cui, Y.-X., Feng, X.-N., Li, X.-Y., Zhang, Y.-P., Tang, A.-N., Kong, D.-M., 2019a. Trifunctional integrated DNA-based universal sensing platform for detection of diverse biomolecules in one-pot isothermal exponential amplification mode. Chem. Commun. 55 (53), 7603–7606.

Cui, Y.X., Feng, X.N., Wang, Y.X., Pan, H.Y., Pan, H., Kong, D.M., 2019b. An integrated-molecular-beacon based multiple exponential strand displacement amplification strategy for ultrasensitive detection of DNA methyltransferase activity. Chem. Sci. 10 (8), 2290–2297.

Czapla, C., McPhail, S.M., 2015. Electrophoretic mobility of cell nuclei (EMN index) as a biomarker of the biological aging process: considering the association between EMN index and age. Homo 66 (6), 549–560.

Dai, Y., Somoza, R.A., Wang, L., Welter, J.F., Li, Y., Caplan, A.I., Liu, C.C., 2019. Exploring the trans-cleavage activity of CRISPR-cas9 (cpf1) for the development of a universal electrochemical biosensor. Angew. Chem. Int. Ed. 58 (48), 17399–17405.

Dan, K., Veetil, A.T., Chakraborty, K., Krishnan, Y., 2019. DNA nanodevices map enzymatic activity in organelles. Nat. Nanotechnol. 14 (3), 252–259.

Dean, F.B., Nelson, J.R., Giesler, T.L., Lasken, R.S., 2001. Rapid amplification of plasmid and phage DNA using phi29 DNA polymerase and multiply-primed rolling circle amplification. Genome Res. 11 (6), 1095–1099.

Debiais, M., Lelievre, A., Smietana, M., Mueller, S., 2020. Splitting aptamers and nucleic acid enzymes for the development of advanced biosensors. Nucleic Acids Res. 48 (7), 3400–3422.

Deng, J., Tian, F., Liu, C., Liu, Y., Zhao, S., Fu, T., Sun, J., Tan, W., 2021. Rapid one-step detection of viral particles using an aptamer-based thermophoretic assay. J. Am. Chem. Soc. 143 (19), 7261–7266.

Deng, R.J., Tang, L.H., Tian, Q.Q., Wang, Y., Lin, L., Li, J.H., 2014. Toehold-initiated rolling circle amplification for visualizing individual MicroRNAs in situ in single cells. Angew. Chem. Int. Ed. 53 (9), 2389–2393.

Diaz, L.A., Bardelli, A., 2014. Liquid biopsies: genotyping circulating tumor DNA. J. Clin. Oncol. 32 (6), 579–586.

Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S A 92 (20), 9363–9367.

Dirks, R.M., Pierce, N.A., 2004. Triggered amplification by hybridization chain reaction. Proc. Natl. Acad. Sci. U. S A 101 (43), 15275–15278.

Du, Y.C., Wang, S.Y., Wang, Y.X., Ma, J.Y., Wang, D.X., Tang, A.N., Kong, D.M., 2021. Terminal deoxynucleotidyl transferase combined CRISPR-Cas12a amplification strategy for ultrasensitive detection of uracil-DNA glycosylase with zero background. Biosens. Bioelectron. 171.

Engelen, W., Wijnands, S.P.W., Merkx, M., 2018. Accelerating DNA-based computing on a supramolecular polymer. J. Am. Chem. Soc. 140 (30), 9758–9767.

Engler-Blum, G., Meier, M., Frank, J., Muller, G.A., 1993. Reduction of background problems in nonradioactive northern and Southern blot analyses enables higher sensitivity than 32P-based hybridizations. Anal. Biochem. 210 (2), 235–244.

Fan, X.D., White, I.M., Shopova, S.I., Zhu, H.Y., Suter, J.D., Sun, Y.Z., 2008. Sensitive optical biosensors for unlabeled targets: a review. Anal. Chim. Acta 620 (1–2), 8–26.

Figg, C.A., Winegar, P.H., Hayes, O.G., Mirkin, C.A., 2020. Controlling the DNA hybridization chain reaction. J. Am. Chem. Soc. 142 (19), 8596–8601.

Gong, Q., Wang, Y., Yang, H., 2017. A sensitive impedimetric DNA biosensor for the determination of the HIV gene based on graphene-Nafion composite film. Biosens. Bioelectron. 89, 565–569.

Hajian, R., Balderston, S., Tran, T., DeBoer, T., Etienne, J., Sandhu, M., Wauford, N.A., Chung, J.-Y., Nokes, J., Athaiya, M., Paredes, J., Peytavi, R., Goldsmith, B., Murthy, N., Conboy, I.M., Aran, K., 2019. Detection of unamplified target genes via CRISPR-Cas9 immobilized on a graphene field-effect transistor. Nature Biomedical Engineering 3 (6), 427–437.

Haley, N.E.C., Ouldridge, T.E., Ruiz, I.M., Geraldini, A., Louis, A.A., Bath, J., Turberfield, A.J., 2020. Design of hidden thermodynamic driving for non-equilibrium systems via mismatch elimination during DNA strand displacement. Nat. Commun. 11 (1).

Halliwell, B., 1996. Antioxidants in human health and disease. Annu. Rev. Nutr. 16, 33–50.

Han, T., Cong, H., Shen, Y., Yu, B., 2021. Recent Advances in Detection Technologies for COVID-19. Talanta 233.

Herman, J.G., Merlo, A., Mao, L., Lapidus, R.G., Issa, J.P., Davidson, N.E., Sidransky, D., Baylin, S.B., 1995. Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res. 55 (20), 4525–4530.

Hood, L., Heath, J.R., Phelps, M.E., Lin, B.Y., 2004. Systems biology and new technologies enable predictive and preventative medicine. Science 306 (5696), 640–643.

Hu, Z.-L., Li, M.-Y., Liu, S.-C., Ying, Y.-L., Long, Y.-T., 2019. A lithium-ion-active aerolysin nanopore for effectively trapping long single-stranded DNA. Chem. Sci. 10 (2), 354–358.

Huang, D.-J., Huang, Z.-M., Xiao, H.-Y., Wu, Z.-K., Tang, L.-J., Jiang, J.-H., 2018a. Protein scaffolded DNA tetrads enable efficient delivery and ultrasensitive imaging of miRNA through crosslinking hybridization chain reaction. Chem. Sci. 9 (21), 4892–4897.

Huang, J., Li, X.-Y., Du, Y.-C., Zhang, L.-M., Liu, K.-K., Zhu, L.-N., Kong, D.-M., 2017. Sensitive fluorescent detection of DNA methyltransferase using nicking endonuclease-mediated multiple primers-like rolling circle amplification. Biosens. Bioelectron. 91, 417–423.

Huang, J., Wu, Y., Chen, Y., Zhu, Z., Yang, X., Yang, C.J., Wang, K., Tan, W., 2011. Pyrene-excimer probes based on the hybridization chain reaction for the detection of nucleic acids in complex biological fluids. Angew. Chem. Int. Ed. 50 (2), 401–404.

Huang, M., Zhou, X., Wang, H., Xing, D., 2018b. Clustered regularly interspaced short palindromic repeats/cas9 triggered isothermal amplification for site-specific nucleic acid detection. Anal. Chem. 90 (3), 2193–2200.

Huang, P.J.J., de Rochambeau, D., Sleiman, H.F., Liu, J., 2020. Target self-enhanced selectivity in metal-specific DNAzymes. Angew. Chem. Int. Ed. 59 (9), 3573–3577.

Idili, A., Parolo, C., Alvarez-Diduk, R., Merkoci, A., 2021. Rapid and efficient detection of the SARS-CoV-2 spike protein using an electrochemical aptamer-based sensor. ACS Sens. 6 (8), 3093–3101.

Iliuk, A.B., Hu, L.H., Tao, W.A., 2011. Aptamer in bioanalytical applications. Anal. Chem. 83 (12), 4440–4452.

Ji, P., Han, G., Huang, Y., Jiang, H., Zhou, Q., Liu, X., Kong, D., 2021. Ultrasensitive ratiometric detection of Pb2+ using DNA tetrahedron-mediated hyperbranched hybridization chain reaction. Anal. Chim. Acta 1147, 170–177.

Jiang, H., Ji, P., Xu, Y., Liu, X., Kong, D., 2021. Self-paired dumbbell DNA -assisted simple preparation of stable circular DNAzyme and its application in Pb2+sensor. Anal. Chim. Acta 1175.

Jiang, X., Wang, H., Chai, Y., Li, H., Shi, W., Yuan, R., 2019. DNA cascade reaction with high-efficiency target conversion for ultrasensitive electrochemiluminescence microRNA detection. Anal. Chem. 91 (15), 10258–10265.

Jones, P., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat. Rev. Genet. 13 (7), 484–492.

Jung, C., Allen, P.B., Ellington, A.D., 2016. A stochastic DNA walker that traverses a microparticle surface. Nat. Nanotechnol. 11 (2), 157–163.

Kacherovsky, N., Yang, L.F., Dang, H.V., Cheng, E.L., Cardle, I.I., Walls, A.C., McCallum, M., Sellers, D.L., DiMaio, F., Salipante, S.J., Corti, D., Veesler, D., Pun, S. H., 2021. Discovery and characterization of spike N-terminal domain-binding aptamers for rapid SARS-CoV-2 detection. Angew. Chem. Int. Ed. 60.

Kim, K.T., Winssinger, N., 2020. Enhanced SNP-sensing using DNA-templated reactions through confined hybridization of minimal substrates (CHOMS). Chem. Sci. 11 (16), 4150–4157.

Kitamura, Y., Azuma, Y., Katsuda, Y., Ihara, T., 2020. Catalytic formation of luminescent lanthanide complexes using an entropy-driven DNA circuit. Chem. Commun. 56 (27), 3863–3866.

Koh, D., Cunningham, A.C., 2020. Counting coronavirus disease-2019 (COVID-19) cases: case definitions, screened populations and testing techniques matter. Ann. Acad. Med. Singapore 49 (3), 161–165.

Lee, C.-Y., McNerney, C., Myong, S., 2019. G-quadruplex and protein binding by single-molecule FRET microscopy. In: Yang, D., Lin, C. (Eds.), G-quadruplex Nucleic Acids: Methods and Protocols. Humana Press Inc, 999 Riverview Dr, Ste 208, Totowa, Nj 07512-1165 USA, pp. 309–322.

Li, C.-c., Chen, H.-y., Hu, J., Zhang, C.-y., 2020a. Rolling circle amplification-driven encoding of different fluorescent molecules for simultaneous detection of multiple DNA repair enzymes at the single-molecule level. Chem. Sci. 11 (22), 5724–5734.

Li, C., Wu, D., Hu, X.L., Xiang, Y., Shu, Y.Q., Li, G.X., 2016. One-step modification of electrode surface for ultrasensitive and highly selective detection of nucleic acids with practical applications. Anal. Chem. 88 (15), 7583–7590.

Li, D., Zhang, T., Yang, F., Yuan, R., Xiang, Y., 2020b. Efficient and exponential rolling circle amplification molecular network leads to ultrasensitive and label-free detection of MicroRNA. Anal. Chem. 92 (2), 2074–2079.

Li, F., Mao, X., Li, F., Li, M., Shen, J., Ge, Z., Fan, C., Zuo, X., 2020c. Ultrafast DNA sensors with DNA framework-bridged hybridization reactions. J. Am. Chem. Soc. 142 (22), 9975–9981.

Li, N., Liu, L., Xiang, M.-H., Liu, J.-W., Yu, R.-Q., Jiang, J.-H., 2019a. Proximity- induced hybridization chain assembly with small- molecule linked DNA for single- step amplified detection of antibodies. Chem. Commun. 55 (30), 4387–4390.

Li, S.-Y., Cheng, Q.-X., Liu, J.-K., Nie, X.-Q., Zhao, G.-P., Wang, J., 2018. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. Cell Res. 28 (4), 491–493.

Li, X.Y., Cui, Y.X., Du, Y.C., Tang, A.N., Kong, D.M., 2020d. Isothermal cross-boosting extension-nicking reaction mediated exponential signal amplification for ultrasensitive detection of polynucleotide kinase. Analyst 145 (10), 3742–3748.

Li, Y., Li, S., Wang, J., Liu, G., 2019b. CRISPR/Cas systems towards next-generation biosensing. Trends Biotechnol. 37 (7), 730–743.

Liang, C.P., Ma, P.Q., Liu, H., Guo, X.G., Yin, B.C., Ye, B.C., 2017. Rational engineering of a dynamic, entropy-driven DNA nanomachine for intracellular MicroRNA imaging. Angew. Chem. Int. Ed. 56 (31), 9077–9081.

Liang, K., Wang, H., Li, P., Zhu, Y., Liu, J., Tang, B., 2020. Detection of microRNAs using toehold-initiated rolling circle amplification and fluorescence resonance energy transfer. Talanta 207.

Liang, M., Li, Z., Wang, W., Liu, J., Liu, L., Zhu, G., Karthik, L., Wang, M., Wang, K.-F., Wang, Z., Yu, J., Shuai, Y., Yu, J., Zhang, L., Yang, Z., Li, C., Zhang, Q., Shi, T., Zhou, L., Xie, F., Dai, H., Liu, X., Zhang, J., Liu, G., Zhuo, Y., Zhang, B., Liu, C., Li, S., Xia, X., Tong, Y., Liu, Y., Alterovitz, G., Tan, G.-Y., Zhang, L.-X., 2019. A CRISPR- Cas12a-derived biosensing platform for the highly sensitive detection of diverse small molecules. Nat. Commun. 10 (1), 3672.

Lin, L., Yi, L., Zhao, F., Wu, Z., Zheng, Y., Li, N., Lin, J.-M., Sun, J., 2020. ATP-responsive mitochondrial probes for monitoring metabolic processes of glioma stem cells in a 3D model. Chem. Sci. 11 (10), 2744–2749.

Liu, F., Yang, M., Song, W., Luo, X., Tang, R., Duan, Z., Kang, W., Xie, S., Liu, Q., Lei, C., Huang, Y., Nie, Z., Yao, S., 2020a. Target-activated transcription for the amplified sensing of protease biomarkers. Chem. Sci. 11 (11), 2993–2998.

Liu, J., Cui, M.R., Zhou, H., Yang, W.R., 2017a. DNAzyme based nanomachine for in situ detection of MicroRNA in living cells. ACS Sens. 2 (12), 1847–1853.

Liu, J.W., Lu, Y., 2003. A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles. J. Am. Chem. Soc. 125 (22), 6642–6643.

Liu, M., Zhang, Q., Chang, D., Gu, J., Brennan, J.D., Li, Y., 2017b. A DNAzyme feedback amplification strategy for biosensing. Angew. Chem. Int. Ed. 56 (22), 6142–6146.

Liu, R., Han, H., Liu, F., Lv, Z.H., Wu, K.L., Liu, Y.L., Feng, Y., Zhu, C.L., 2020b. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. Clin. Chim. Acta 505, 172–175.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real- time quantitative PCR and the 2(T)(-Delta Delta C) method. Methods 25 (4), 402–408.

Lone, S.A., Ahmad, A., 2020. COVID-19 pandemic - an African perspective. Emerg. Microb. Infect. 9 (1), 1300–1308.

Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebet, B.L., Mak, R.H., Ferrando, A.A., Downing, J.R., Jacks, T., Horvitz, H.R., Golub, T.R., 2005. MicroRNA expression profiles classify human cancers. Nature 435 (7043), 834–838.

Ma, P.Q., Liang, C.P., Zhang, H.H., Yin, B.C., Ye, B.C., 2018. A highly integrated DNA nanomachine operating in living cells powered by an endogenous stimulus. Chem. Sci. 9 (13), 3299–3304.

Mandal, S., Selvam, S., Shrestha, P., Mao, H.B., 2016. Mechanochemical sensing of single and few Hg(II) ions using polyvalent principles. Anal. Chem. 88 (19), 9479–9485.

Mandal, S., Zhang, X.Q., Pandey, S., Mao, H.B., 2019. Single-molecule topochemical analyses for large-scale multiplexing tasks. Anal. Chem. 91 (21), 13485–13493.

Marin, M.G., Matozzo, V., 2004. Vitellogenin induction as a biomarker of exposure to estrogenic compounds in aquatic environments. Mar. Pollut. Bull. 48 (9–10), 835–839.

Meng, H.M., Liu, H., Kuai, H.L., Peng, R.Z., Mo, L.T., Zhang, X.B., 2016. Aptamer- integrated DNA nanostructures for biosensing, bioimaging and cancer therapy. Chem. Soc. Rev. 45 (9), 2583–2602.

Meng, X.D., Zhang, K., Dai, W.H., Cao, Y., Yang, F., Dong, H.F., Zhang, X.J., 2018. Multiplex microRNA imaging in living cells using DNA-capped-Au assembled hydrogels. Chem. Sci. 9 (37), 7419–7425.

Mitchell, P.S., Parkin, R.K., Kroh, E.M., Fritz, B.R., Wyman, S.K., Pogosova- Agadjanyan, E.L., Peterson, A., Noteboom, J., O'Briant, K.C., Allen, A., Lin, D.W., Urban, N., Drescher, C.W., Knudsen, B.S., Stirewalt, D.L., Gentleman, R., Vessella, R. L., Nelson, P.S., Martin, D.B., Tewari, M., 2008. Circulating microRNAs as stable blood-based markers for cancer detection. Proc. Natl. Acad. Sci. U. S. A 105 (30), 10513–10518.

Mitra, J., Ha, T., 2019. Nanomechanics and co-transcriptional folding of spinach and mango. Nat. Commun. 10.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., Erlich, H., 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol. 51 (Pt 1), 263–273.

Nagatoishi, S., Nojima, T., Galezowska, E., Juskowiak, B., Takenaka, S., 2006. G quadruplex-based FRET probes with the thrombin-binding aptamer (TBA) sequence designed for the efficient fluorometric detection of the potassium ion. Chembiochem 7 (11), 1730–1737.

Nagatoishi, S., Nojima, T., Juskowiak, B., Takenaka, S., 2005. A pyrene-labeled G- quadruplex oligonucleotide as a fluorescent probe for potassium ion detection in biological applications. Angew. Chem. Int. Ed. 44 (32), 5067–5070.

Nakama, T., Takezawa, Y., Sasaki, D., Shionoya, M., 2020. Allosteric regulation of DNAzyme activities through intrastrand transformation induced by Cu(II)-Mediated artificial base pairing. J. Am. Chem. Soc. 142 (22), 10153–10162.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28 (12).

Omabegho, T., Sha, R., Seeman, N.C., 2009. A bipedal DNA brownian motor with coordinated legs. Science 324 (5923), 67–71.

Pang, J., Wang, M.X., Ang, I.Y.H., Tan, S.H.X., Lewis, R.F., Chen, J.I.P., Gutierrez, R.A., Gwee, S.X.W., Chua, P.E.Y., Yang, Q., Ng, X.Y., Yap, R.K.S., Tan, H.Y., Teo, Y.Y., Tan, C.C., Cook, A.R., Yap, J.C.-H., Hsu, L.Y., 2020. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): a systematic review. J. Clin. Med. 9 (3).

Peng, H., Li, X.-F., Zhang, H., Le, X.C., 2017. A microRNA-initiated DNAzyme motor operating in living cells. Nat. Commun. 8.

Peng, M.S., Fang, Z.Y., Na, N., Ouyang, J., 2019. A versatile single-molecule counting- based platform by generation of fluorescent silver nanoclusters for sensitive detection of multiple nucleic acids. Nanoscale 11 (35), 16606–16613.

Pfefferle, S., Reucher, S., Norz, D., Lutgehetmann, M., 2020. Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. Euro Surveill. 25 (9), 18–22.

Qian, L., Winfree, E., 2011. Scaling up digital circuit computation with DNA strand displacement cascades. Science 332 (6034), 1196–1201.

Qiu, G., Gai, Z., Tao, Y., Schmitt, J., Kullak-Ublick, G.A., Wang, J., 2020. Dual-Functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection. ACS Nano 14 (5), 5268–5277.

Qiu, X., Xu, J.Y., Guo, J.J., Yahia-Ammar, A., Kapetanakis, N.I., Duroux-Richard, I., Unterluggauer, J.J., Golob-Schwarzl, N., Regeard, C., Uzan, C., Gouy, S., Dubow, M., Haybaeck, J., Apparailly, F., Busson, P., Hildebrandt, N., 2018. Advanced microRNA-based cancer diagnostics using amplified time-gated FRET. Chem. Sci. 9 (42), 8046–8055.

Qu, X., Zhu, D., Yao, G., Su, S., Chao, J., Liu, H., Zuo, X., Wang, L., Shi, J., Wang, L., Huang, W., Pei, H., Fan, C., 2017. An exonuclease III-powered, on-particle stochastic DNA walker. Angew. Chem. Int. Ed. 56 (7), 1855–1858.

Ranallo, S., Prevost-Tremblay, C., Idili, A., Vallee-Belisle, A., Ricci, F., 2017. Antibody- powered nucleic acid release using a DNA-based nanomachine. Nat. Commun. 8, 1–9.

Reid, M.S., Le, X.C., Zhang, H., 2018. Exponential isothermal amplification of nucleic acids and assays for proteins, cells, small molecules, and enzyme activities: an EXPAR example. Angew. Chem. Int. Ed. 57 (37), 11856–11866.

Ren, K., Xu, Y., Liu, Y., Yang, M., Ju, H., 2018. A responsive "nano string light" for highly efficient mRNA imaging in living cells via accelerated DNA cascade reaction. ACS Nano 12 (1), 263–271.

Ren, W., Huang, P.J.J., He, M.L., Lyu, M.S., Wang, C.H., Wang, S.J., Liu, J.W., 2020a. Sensitivity of a classic DNAzyme for Pb2+ modulated by cations, anions and buffers. Analyst 145 (4), 1384–1388.

Ren, W., Jimmy Huang, P.-J., de Rochambeau, D., Moon, W.J., Zhang, J., Lyu, M., Wang, S., Sleiman, H., Liu, J., 2020b. Selection of a metal ligand modified DNAzyme for detecting. Ni2. Biosens. Bioelectron. 165, 112285.

Rifai, N., Gillette, M.A., Carr, S.A., 2006. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. Nat. Biotechnol. 24 (8), 971–983.

Sawyers, C.L., 2008. The cancer biomarker problem. Nature 452 (7187), 548–552.

Seeman, N.C., 2010. Nanomaterials based on DNA. In: Kornberg, R.D., Raetz, C.R.H., Rothman, J.E., Thorner, J.W. (Eds.), Annual Review of Biochemistry, vol. 79. Annual Reviews, Palo Alto, pp. 65–87.

Shang, J., Wei, J., Wang, Q., Wang, J., Zhou, Y., Yu, S., Liu, X., Wang, F., 2020. Adaption of an autonomously cascade DNA circuit for amplified detection and intracellular imaging of polynucleotide kinase with ultralow background. Biosens. Bioelectron. 152.

Shrestha, P., Cui, Y.X., Wei, J., Jonchhe, S., Mao, H.B., 2018. Single-molecule mechanochemical pH sensing revealing the proximity effect of hydroniums generated by an alkaline phosphatase. Anal. Chem. 90 (3), 1718–1724.

Simonsson, T., Sjoback, R., 1999. DNA tetraplex formation studied with fluorescence resonance energy transfer. J. Biol. Chem. 274 (24), 17379–17383.

Srinivas, P.R., Kramer, B.S., Srivastava, S., 2001. Trends in biomarker research for cancer detection. Lancet Oncol. 2 (11), 698–704.

Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med. 18 (2), 321–336.

Sun, X., Wang, Y., Zhang, L., Liu, S., Zhang, M., Wang, J., Ning, B.A., Peng, Y., He, J., Hu, Y.G., Gao, Z.X., 2020. CRISPR-Cas9 triggered two-step isothermal amplification method for E. coli O157:H7 detection based on a metal-organic framework platform. Anal. Chem. 92 (4), 3032–3041.

Sun, Y., Sun, Y., Tian, W., Liu, C., Gao, K., Li, Z., 2018. A novel restriction endonuclease GlaI for rapid and highly sensitive detection of DNA methylation coupled with isothermal exponential amplification reaction. Chem. Sci. 9 (5), 1344–1351.

Tan, W., Li, L., Xu, S., Yan, H., Li, X., Yazd, H.S., Li, X., Huang, T., Cui, C., Jiang, J., 2020. Nucleic acid aptamers for molecular diagnostics and therapeutics: advances and perspectives. Angew. Chem. 60 (5), 2221–2231.

Thekkan, S., Jani, M.S., Cui, C., Dan, K., Zhou, G., Becker, L., Krishnan, Y., 2019. A DNA-based fluorescent reporter maps HOCl production in the maturing phagosome. Nat. Chem. Biol. 15 (12), 1165–1172.

Tröger, V., Niemann, K., Gärtig, C., D, K., 2015. Isothermal amplification and quantification of nucleic acids and its use in microsystems. J. Nanomed. Nanotechnol. 6 (3), 19.

Tripathy, S., Vanjari, S.R.K., Singh, V., Swaminathan, S., Singh, S.G., 2017. Electrospun manganese (III) oxide nanofiber based electrochemical DNA-nanobiosensor for zeptomolar detection of dengue consensus primer. Biosens. Bioelectron. 90, 378–387.

Tuerk, C., Gold, L., 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science (New York, N.Y.) 249 (4968), 505–510.

Tyagi, S., Kramer, F.R., 1996. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. 14 (3), 303–308.

Van Ness, J., Van Ness, L.K., Galas, D.J., 2003. Isothermal reactions for the amplification of oligonucleotides. Proc. Natl. Acad. Sci. U. S. A 100 (8), 4504–4509.

Vandenberg, O., Martiny, D., Rochas, O., van Belkum, A., Kozlakidis, Z., 2021. Considerations for diagnostic COVID-19 tests. Nat. Rev. Microbiol. 19 (3), 171–183.

Walker, G.T., Fraiser, M.S., Schram, J.L., Little, M.C., Nadeau, J.G., Malinowski, D.P., 1992. Strand displacement amplification–an isothermal, in vitro DNA amplification technique. Nucleic Acids Res. 20 (7), 1691–1696.

Wang, B., Wang, R., Wang, D., Wu, J., Li, J., Wang, J., Liu, H., Wang, Y., 2019a. Cas12aVDet: a CRISPR/cas12a-based platform for rapid and visual nucleic acid detection. Anal. Chem. 91 (19), 12156–12161.

Wang, D.-X., Wang, J., Cui, Y.-X., Wang, Y.-X., Tang, A.-N., Kong, D.-M., 2019b. Nanolantern-based DNA probe and signal amplifier for tumor-related biomarker detection in living cells. Anal. Chem. 91 (20), 13165–13173.

Wang, D., Vietz, C., Schroeder, T., Acuna, G., Lalkens, B., Tinnefeld, P., 2017a. A DNA walker as a fluorescence signal amplifier. Nano Lett. 17 (9), 5368–5374.

Wang, D., Yuan, Y.L., Zheng, Y.N., Chai, Y.Q., Yuan, R., 2016. An electrochemical peptide cleavage-based biosensor for matrix metalloproteinase-2 detection with exonuclease III-assisted cycling signal amplification. Chem. Commun. 52 (35), 5943–5945.

Wang, D.X., Wang, J., Du, Y.C., Ma, J.Y., Wang, S.Y., Tang, A.N., Kong, D.M., 2020a. CRISPR/Cas12a-based dual amplified biosensing system for sensitive and rapid detection of polynucleotide kinase/phosphatase. Biosens. Bioelectron. 168.

Wang, J., 2001. Glucose biosensors: 40 years of advances and challenges. Electroanalysis 13 (12), 983–988.

Wang, J., 2008. Electrochemical glucose biosensors. Chem. Rev. 108 (2), 814–825.

Wang, J., Li, M.-Y., Yang, J., Wang, Y.-Q., Wu, X.-Y., Huang, J., Ying, Y.-L., Long, Y.-T., 2020b. Direct quantification of damaged nucleotides in oligonucleotides using an aerolysin single molecule interface. ACS Cent. Sci. 6 (1), 76–82.

Wang, J., Wang, D.-X., Ma, J.-Y., Wang, Y.-X., Kong, D.-M., 2019c. Three-dimensional DNA nanostructures to improve the hyperbranched hybridization chain reaction. Chem. Sci. 10 (42), 9758–9767.

Wang, J., Wang, H., Wang, H., He, S., Li, R., Deng, Z., Liu, X., Wang, F., 2019d. Nonviolent self-catabolic DNAzyme nanosponges for smart anticancer drug delivery. ACS Nano 13 (5), 5852–5863.

Wang, J.R., Xia, C., Yang, L., Li, Y.F., Li, C.M., Huang, C.Z., 2020c. DNA nanofirecrackers assembled through hybridization chain reaction for ultrasensitive SERS immunoassay of prostate specific antigen. Anal. Chem. 92 (5), 4046–4052.

Wang, L.J., Han, X., Li, C.C., Zhang, C.Y., 2018. Single-ribonucleotide repair-mediated ligation-dependent cycling signal amplification for sensitive and specific detection of DNA methyltransferase. Chem. Sci. 9 (28), 6053–6061.

Wang, L.J., Ma, F., Tang, B., Zhang, C.Y., 2016. Sensing telomerase: from in vitro detection to in vivo imaging. Chem. Sci. 8 (4), 2495–2502.

Wang, R., Zhao, X., Chen, X., Qiu, X., Qing, G., Zhang, H., Zhang, L., Hu, X., He, Z., Zhong, D., Wang, Y., Luo, Y., 2020d. Rolling circular amplification (RCA)-Assisted CRISPR/Cas9 cleavage (RACE) for highly specific detection of multiple extracellular vesicle MicroRNAs. Anal. Chem. 92 (2), 2176–2185.

Wang, S., Yue, L., Shpilt, Z., Cecconello, A., Kahn, J.S., Lehn, J.-M., Willner, I., 2017c. Controlling the catalytic functions of DNAzymes within constitutional dynamic networks of DNA nanostructures. J. Am. Chem. Soc. 139 (28), 9662–9671.

Wang, Y.-X., Wang, D.-X., Ma, J.-Y., Wang, J., Du, Y.-C., Kong, D.-M., 2021a. DNA nanolantern-based split aptamer probes for in situ ATP imaging in living cells and lighting up mitochondria. Analyst 146 (8), 2600–2608.

Wang, Y., Zhang, H., Cui, Y., Duan, S., Lin, W., Liu, B., 2021b. A complementary-DNA-enhanced fiber-optic sensor based on microfiber-assisted Mach-Zehnder interferometry for biocompatible pH sensing. Sensor. Actuator. B Chem. 332.

Wang, Y.S., Kang, H.Y.J., Liu, X.F., Tong, Z.H., 2020e. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. J. Med. Virol. 92 (6), 538–539.

Wei, Q., Huang, J., Li, J., Wang, J., Yang, X., Liu, J., Wang, K., 2018. A DNA nanowire based localized catalytic hairpin assembly reaction for microRNA imaging in live cells. Chem. Sci. 9 (40), 7802–7808.

Wu, H., Chen, T.-T., Wang, X.-N., Ke, Y., Jiang, J.-H., 2020. RNA imaging in living mice enabled by an in vivo hybridization chain reaction circuit with a tripartite DNA probe. Chem. Sci. 11 (1), 62–69.

Wu, Z., Fan, H., Satyavolu, N.S.R., Wang, W., Lake, R., Jiang, J.-H., Lu, Y., 2017. Imaging endogenous metal ions in living cells using a DNAzyme-catalytic hairpin assembly probe. Angew. Chem. Int. Ed. 56 (30), 8721–8725.

Wu, Z.Y., McGoogan, J.M., 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China summary of a report of 72 314 cases from the Chinese center for disease control and prevention. JAMA, J. Am. Med. Assoc. 323 (13), 1239–1242.

Xing, X., Li, J., Qiu, L., Tan, W., 2020. A molecular recognition-activatable DNA nanofirecracker enables signal-enhanced imaging in living cells. Chem. Commun. 56 (21), 3131–3134.

Xiong, Z., Fu, L., Zhou, H., Liu, J.K., Wang, A.M., Huang, Y., Huang, X., Yi, B., Wu, J., Li, C.H., Quan, J., Li, M., Leng, Y.S., Luo, W.J., Hu, C.P., Liao, W.H., 2020. Construction and evaluation of a novel diagnosis pathway for 2019-Corona Virus Disease. Zhonghua Yixue Zazhi 100 (16), 1223–1229.

Xu, Q., Ma, F., Huang, S.-q., Tang, B., Zhang, C.-y., 2017. Nucleic acid amplification-free bioluminescent detection of MicroRNAs with high sensitivity and accuracy based on controlled target degradation. Anal. Chem. 89 (13), 7077–7083.

Yan, C., Cui, J., Huang, L., Du, B., Chen, L., Xue, G., Li, S., Zhang, W., Zhao, L., Sun, Y., Yao, H., Li, N., Zhao, H., Feng, Y., Liu, S., Zhang, Q., Liu, D., Yuan, J., 2020. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. Clin. Microbiol. Infect. 26 (6), 773–779.

Yang, X., Tang, Y., Mason, S.D., Chen, J., Li, F., 2016. Enzyme-powered three-dimensional DNA nanomachine for DNA walking, payload release, and biosensing. ACS Nano 10 (2), 2324–2330.

Yang, Z., Wang, N., Wen, H., Cui, R., Yu, J., Yang, S., Qu, T., Wang, X., He, S., Qi, J., Wang, J., Ye, Q., Liu, Y., 2019. An amplification-free detection method of nucleic acids by a molecular beacon probe based on endonuclease activity. Sensor. Actuator. B Chem. 298.

Yin, P., Choi, H.M.T., Calvert, C.R., Pierce, N.A., 2008. Programming biomolecular self-assembly pathways. Nature 451 (7176), 318–U314.

Yue, X., Qiao, Y., Gu, D., Wu, Z., Zhao, W., Li, X., Yin, Y., Zhao, W., Kong, D., Xi, R., Meng, M., 2020. Reliable FRET-ON imaging of telomerase in living cells by a tetrahedral DNA nanoprobe integrated with structure-switchable molecular beacon. Sensor. Actuator. B Chem. 312.

Zhang, D.Y., Chen, S.X., Yin, P., 2012. Optimizing the specificity of nucleic acid hybridization. Nat. Chem. 4 (3), 208–214.

Zhang, H., Lai, M., Zuehlke, A., Peng, H., Li, X.-F., Le, X.C., 2015. Binding-Induced DNA nanomachines triggered by proteins and nucleic acids. Angew. Chem. Int. Ed. 54 (48), 14326–14330.

Zhang, J., He, M., Nie, C., He, M., Pan, Q., Liu, C., Hu, Y., Yi, J., Chen, T., Chu, X., 2019a. Biomineralized metal-organic framework nanoparticles enable enzymatic rolling circle amplification in living cells for ultrasensitive MicroRNA imaging. Anal. Chem. 91 (14), 9049–9057.

Zhang, K., Deng, R., Teng, X., Li, Y., Sun, Y., Ren, X., Li, J., 2018a. Direct visualization of single-nucleotide variation in mtDNA using a CRISPR/Cas9-Mediated proximity ligation assay. J. Am. Chem. Soc. 140 (36), 11293–11301.

Zhang, L., Fang, X., Liu, X., Ou, H., Zhang, H., Wang, J., Li, Q., Cheng, H., Zhang, W., Luo, Z., 2020a. Discovery of sandwich type COVID-19 nucleocapsid protein DNA aptamers. Chem. Commun. 56 (70), 10235–10238.

Zhang, L., Zhang, X., Feng, P., Han, Q., Liu, W., Lu, Y., Song, C., Li, F., 2020b. Photodriven regeneration of G-quadruplex aptasensor for sensitively detecting thrombin. Anal. Chem. 92 (11), 7419–7424.

Zhang, Q., Wang, W., Huang, S., Yu, S., Tan, T., Zhang, J.-R., Zhu, J.-J., 2020c. Capture and selective release of multiple types of circulating tumor cells using smart DNAzyme probes. Chem. Sci. 11 (7), 1948–1956.

Zhang, X., Zheng, C., Ding, L., Wu, Y., Xu, H., Sun, Y., Zeng, Y., Liu, X., Liu, J., 2021. CRISPR-Cas12a coupled with terminal deoxynucleotidyl transferase mediated isothermal amplification for sensitive detection of polynucleotide kinase activity. Sensor. Actuator. B Chem. 330.

Zhang, Y., Hua, R.-n., Zhang, C.-y., 2019b. Analysis of the isolated and the clustered DNA damages by single-molecule counting. Anal. Chem. 91 (16), 10381–10385.

Zhang, Y., Li, Q.N., Li, C.C., Zhang, C.Y., 2018b. Label-free and high-throughput bioluminescence detection of uracil-DNA glycosylase in cancer cells through tricyclic cascade signal amplification. Chem. Commun. 54 (51), 6991–6994.

Zhang, Y., Wang, X.-y., Zhang, Q., Zhang, C.-y., 2017. Label-free sensitive detection of DNA methyltransferase by target-induced hyperbranched amplification with zero background signal. Anal. Chem. 89 (22), 12408–12415.

Zhang, Y., Zeng, C.Y., 2016. Role of DNA methylation in cardiovascular diseases. Clin. Exp. Hypertens. 38 (3), 261–267.

Zhao, L., Qi, X., Yan, X., Huang, Y., Liang, X., Zhang, L., Wang, S., Tan, W., 2019a. Engineering aptamer with enhanced affinity by triple helix-based terminal fixation. J. Am. Chem. Soc. 141 (44), 17493–17497.

Zhao, Y., Fang, X., Chen, F., Bai, M., Fan, C., Zhao, Y., 2019b. Locus-patterned sequence oriented enrichment for multi-dimensional gene analysis. Chem. Sci. 10 (36), 8421–8427.

Zhao, Y., Wang, Y., Yang, R., Zhang, H., Zhao, Y., Miao, X., Lu, J., 2021. A zero-background fluorescent aptasensor for ultrasensitive detection of pesticides based on magnetic three-dimensional DNA walker and poly(T)-templated copper nanoparticles. Sensor. Actuator. B Chem. 343.

Zhao, Y.X., Chen, F., Li, Q., Wang, L.H., Fan, C.H., 2015. Isothermal amplification of nucleic acids. Chem. Rev. 115 (22), 12491–12545.

Zhou, F., Yu, T., Du, R.H., Fan, G.H., Liu, Y., Liu, Z.B., Xiang, J., Wang, Y.M., Song, B., Gu, X.Y., Guan, L.L., Wei, Y., Li, H., Wu, X.D., Xu, J.Y., Tu, S.J., Zhang, Y., Chen, H., Cao, B., 2020a. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395, 1054–1062, 0229.

Zhou, W., Li, D., Yuan, R., Xiang, Y., 2019. Programmable DNA ring/hairpin-constrained structure enables ligation-free rolling circle amplification for imaging mRNAs in single cells. Anal. Chem. 91 (5), 3628–3635.

Zhou, Z., Brennan, J.D., Li, Y., 2020b. A multi-component all-DNA biosensing system controlled by a DNAzyme. Angew. Chem. Int. Ed. 59 (26), 10401–10405.