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Review Article

SARS-CoV-2 Detection by Functional Nucleic Acids – A Review

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Abstract:

Nowadays, rapid, and accurate detection of SARS-CoV-2 is a challenging task due to its mutations. Therefore, scientists are developing novel methods to detect and prevent the spread of SARS-CoV-2 more effectively using different biomolecules. The current review mainly focuses on the functional nucleic acids (FNA), *in-vitro* selection of FNA, and development of FNA-based biosensors and assays to detect SARS-CoV-2 infection and a summary of how these FNA are used to detect different targets effectively. Even though gold standard analytical methods are available to detect virus infections, and microorganisms, more specifically, those methods involve time-consuming, complicated sample pre-treatment and sophisticated instruments. Functional nucleic acid-based sensors have emerged as an alternative due to the benefits of non-destructive rapid analysis with *in situ* and real-time detection with high sensitivity and selectivity.
Keywords: Functional nucleic acid, *In-vitro* selection, Aptamer, DNAzyme, Theranostic systems, SARS-CoV-2.

1 Introduction

The emergence of the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), known as the Corona Virus Disease 2019 (COVID-19) outbreak in December 2019, caused the global pandemic [1]. Coronavirus is an RNA-type virus usually associated with pneumonia and the common cold. However, the first human coronavirus was identified in 1960 [2], and later, an outbreak of severe acute respiratory syndrome (SARS) was reported in Guangdong province, southern China, in 2002 and was etiologically identified as a novel coronavirus (CoV). The CoV belongs to the genus *b-coronavirus* and is designated SARS-CoV [1]. Due to the SARS-CoV, nearly 8422 people were infected, which caused 916 deaths in 30 countries on five continents in 2002 – 2003 [2,3]. SARS is due to an airborne RNA virus that spreads directly through the droplets of saliva and indirectly via surfaces exposed to a SARS-infected person [4].

Furthermore, SARS-CoV is known to have a zoonotic origin and is transmitted from an animal reservoir traced back to the Chinese horseshoe bat (*Rhinolophus sinicus*) [5]. SARS-CoV was identified as a highly lethal viral disease, and it disappeared with time due to intense health mitigation plans. SARS-CoV and SARS-CoV-2 are genetically similar, and the basic reproductive rate (R_0) for SARS-CoV-2 has been identified as 2.5 and 2.4 for SARS-CoV [6]. COVID-19 threatens human health around the globe due to its spreading over the globe with unimaginable rate and scale; therefore, World Health Organization (WHO) declared COVID-19 as a global pandemic on 11th March 2020 [7]. COVID-19 has infected 442 million and caused the deaths of 5.98 million people worldwide [8]. According to the latest statistics, the pandemic affects the world economy and public health infrastructure, severely stagnating the global economy.

SARS-CoV-2 is a relatively large, spherical-shaped, linear single-stranded positive-sense RNA ((+) ssRNA) genome encapsulated by a membrane envelope. The RNA genome is responsible for the encoding of essential proteins of viruses such as a spike glycoprotein (S), nucleocapsid protein (N), membrane protein (M), and a small envelope protein (E), as shown in Fig.1. Spike proteins consist of three similar monomers and two distinct subunits known as S1 and S2. The S1 proteins on the viral membrane are responsible for the crown-like appearance of the coronavirus. The S1 subunits also consist of the receptor binding domain (RBD) and the N-terminal domain (NTD). The RBD uses human angiotensin-converting enzyme-2 (ACE-2) as a receptor on the host cell for attachment and plays a significant role in the infection process of human respiratory epithelial cells [1,9]. Whereas the subunit S2 immobilizes the spike protein on the host cell membrane and facilitates the fusion of the viral envelope within the host cell membrane. The N protein encloses the viral genome, and the other S, E, and M proteins integrate to form the virus's outer shell, which functions as a protective barrier.

Physical and clinical indications of COVID-19 vary among the infected people, and the reports revealed the existence of symptomatic and asymptomatic patients around the globe. Therefore, early diagnosis and treatment of suspected infected patients are required to control and confine the spread of COVID-19. However, viral proteins (e.g.: Spike

protein) and RNA are diagnostic molecules for detecting SARS-CoV-2.

Detecting viral infection based on nucleic acids attracted scientists since it provides rapid and reliable information. Currently, the viral diagnosis is carried out by the polymerase chain reaction (PCR), which can amplify the fragments of the desired DNA genome. Over the years, with the advancement of technology, various PCR-based methods such as real-time PCR (RT-PCR) and reverse transcription polymerase chain reaction (RT-PCR), have been developed to detect nucleic acid efficiently and rapidly. PCR and reverse transcriptase-polymerase chain reaction (RT-PCR) tests have been identified as the gold standards for diagnosing COVID-19-infected patients. Nevertheless, attempts to suppress the spread of COVID-19 have failed due to limitations associated with these diagnosis methods, such as time consumption, expensiveness, pre-treatment, sample preparations, extensive labor, and lack of PCR and RT-PCR instruments. Also, reports revealed a high rate of false-negative results during the COVID-19 diagnosis. A possible explanation for this might be that the serum samples from the nasal cavity and throat may not contain enough genetic material to conduct respective tests. Since the genetic materials of the coronavirus are based on ribonucleic acid (RNA), it is challenging to carry out a PCR directly. To conduct the PCR, it requires converting the RNA sequence into the complementary DNA (cDNA) by reverse transcriptase enzyme. Therefore, PCR methods are ineffective in detecting coronavirus-infected people due to the higher infectious rate of the COVID-19 virus.

Recently, scientists developed an immunoassay-based detection method known as a rapid test to identify SARS-CoV-2 infected patients. The human immune system produces immunoglobulin G (IgG) and immunoglobulin M (IgM) due to the infection of SARS-CoV-2, and these antibodies protect against COVID-19. The new immunoassay-based detection test is based on an antibody's specific and rapid fusion to its respective antigen**.** In addition, the new detection method provides qualitative detection of IgG and IgM available in human serum, whole blood, or plasma within 10-15 minutes. However, these antibody tests can be performed only after the post-infection of COVID-19, not for the early detection of COVID-19 infections.

To overcome limitations associated with the PCR diagnosis methods, different alternative approaches, including isothermal methods such as rolling circle amplification (RCA), recombinase polymerase amplification (RPA), loop-mediated amplification (LAMP)[10], nucleic acid sequences-based amplification (NASBA)[11], strand-displacement amplification (SDA)[12], and isothermal exponential amplification reaction (EXPAR)[13] have been developed to replace the PCR diagnose methods. Due to the isothermal nature of these methods, sophisticated laboratory equipment for thermocycling is avoided and efficiently facilitates the cost-effective diagnosis of nucleic acid. Detection of COVID-19 based on isothermal techniques is yet to be developed. Therefore, the detection of COVID-19 is limited for the RT-PCR, PCR, and antigen tests. However, access to RT-PCR equipment is still limited to countries such as low-and-middle-income countries with poor resources.

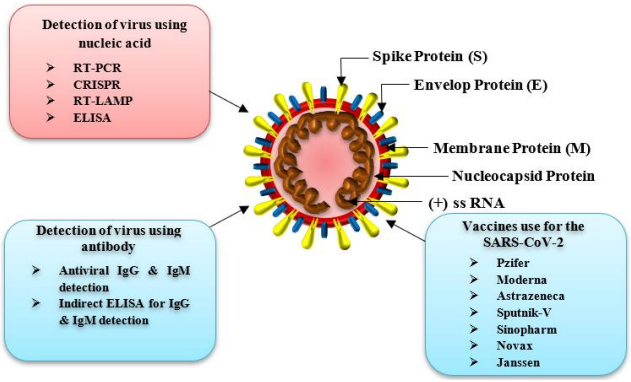

Fig. 1. SARS-CoV-2 diagnosis methods and vaccines for SARS-CoV-2 and schematic illustration of the SARS-CoV-2 virus

Therefore, it is imperative to develop an alternative detection method to achieve effective and rapid detection of the COVID-19 infection, which is cost-effective and reachable to developing countries to prevent further spread.

Recently, scientists utilized an interesting molecule known as functional nucleic acids (FNA) to develop biosensors to effectively diagnose COVID-19 since it provided the platform to create novel sensors.

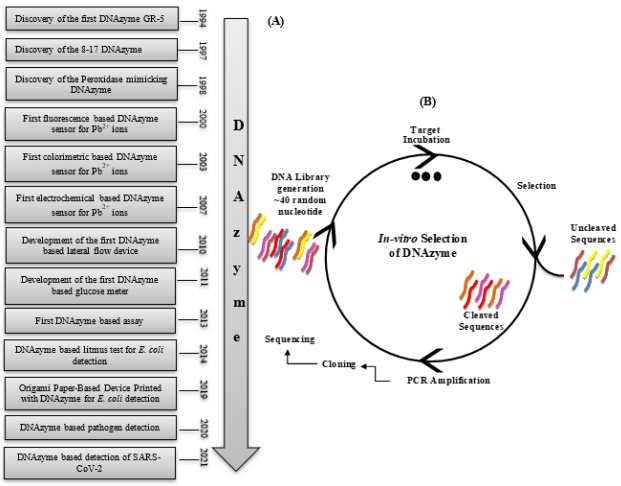

Fig. 2. (A) Evolution of the DNAzyme from metal detection to SARS-CoV-2 detection, (B) In-vitro selection of the DNAzyme

The current review mainly focuses on recent trends in developing effective diagnosis methods and assays based on FNA to detect SARS-CoV-2 infection and a summary of FNA-based biosensors developed so far to detect different targets effectively.

2 Functional nucleic acid

The advancement in biology over the past few decades proved that nucleic acids are not confined to storing and transmitting genetic information to the next generation.

Nucleic acids can be used as a versatile biomolecule, which functions beyond the genetic role. The functional nucleic acids consist of aptamers (single-stranded DNA/RNA), catalytically active RNA molecules (ribozymes), catalytic DNA (DNAzymes), and aptazymes (a combination of both aptamer and DNAzyme). Mainly aptamers and DNAzymes have been isolated *via* an *in-vitro* selection process as shown in Fig.2 (A), also known as the systematic evolution of ligands by exponential enrichment (SELEX). FNAs have been used to develop different biosensors to detect metal and pathogen contaminations due to their distinctive properties, such as precise and sensitive target recognition and catalysis. These FNAs can be produced cost-effectively in large quantities and are easy to modify compared to the other biomolecules. These FNAs can be integrated with diverse signaling methods such as fluorescent, colorimetric, and electrochemical to diagnose the analyte. FNAs are currently employed in various fields such as detecting metal contaminations, detecting therapeutic agents for cancer, and detecting diseases caused by pathogens. Therefore, FNAs are ideal for diagnosing COVID-19 due to their remarkable activity.

2.1 In-vitro selection process/SELEX of DNAzymes

The *in-vitro* selection process of DNAzymes starts with a DNA library consisting of approximately 50 nucleotides (N_{50}) randomized regions flanked by the unique binding arms. The initial library contains ~10^{15} random DNA sequences in the catalytic core of the catalytic strand. The substrate strand embedded with the single ribonucleotide (rA) linkage [1,2] is kept in close proximity to the catalytic strand by the unique binding arms. Even though DNA and RNA contain the same functional groups, DNA differs from RNA due to the lack of a 2' hydroxyl group (2'–OH) in the pentose sugar. Therefore, the RNA molecule is more susceptible to hydrolysis than DNA[3] due to the availability of 2'–OH in the pentose sugar.

In DNAzyme cleavage, metal ions are directly involved and affect the catalysis process differently [4,5]. In an RNA cleavage reaction, a cofactor (e.g.: metal ion) assists the 2'–OH deprotonation to flank the covalent bond, making the 2'–OH a strong nucleophile [6]. The nucleophilic attack (S_N-2) by the deprotonated 2'–OH on the phosphorous centre causes the formation of the pentacoordinate intermediate, which split into 2', 3' – cyclic phosphate, and 5'–hydroxyl product. The cyclic phosphate group can be further hydrolysed with specific co-factors [6–9]. During the initial selection step, parameters such as concentrations of the metal ions, temperature, pH value, and incubation time are optimized to obtain a highly selective and sensitive DNAzyme.

2.2 Aptamers: Brief Introduction

An aptamer is a short oligonucleotide capable of binding to a specific target. Aptamers are excellent functional molecules selected *via in-vitro* selection / systematic evolution of ligands by exponential enrichment (SELEX). In 1990, the isolation of aptamers was first reported by three research groups independently [10–13]. These groups developed *in-vitro* selection and amplification methods to isolate RNA sequences, which could specifically bind to specific targets with a higher degree of specificity [11,14]. These artificial single-strand RNA oligonucleotides were named aptamers and could fold into different structures. Due to the different structures, these aptamers exhibit high specificity for the target binding [11].

The aptamer technology has attracted many research interests in different fields due to the identification of aptamers as unique molecules that are capable of forming strong interactions with a broad range of target molecules (e.g.: Protein, peptides, even whole cells, and metal ions such as K^+, Hg^{2+}, Pb^{2+}).[15] Due to their folding ability, aptamers exhibit a higher affinity for the target molecules with a remarkable dissociation constant (K_d), ranging from picomolar to nanomolar [11,16]. These aptamers react in different ways with the target molecule, such as the incorporation of small target molecules (e.g.: metal ions) to the nucleic acid structure integration with the target molecule (e.g.: proteins) [17]. Aptamers are also described as "chemical antibodies" due to their *in-vitro* selection/ SELEX. The SELEX enables the synthesis and modification of aptamers for non-immunogenic and toxic molecules that are unable to obtain by the immune system [18].

This process allows for the synthesis of aptamers for specific target molecules' particular regions, which is not easy for antibodies. Aptamers have become an essential molecular biological tool for diagnostics, biosensing, and therapeutics. Aptamer-based biosensors also possess advantages over sensors based on natural receptors, such as antibodies and enzymes. An aptamer with high specificity and affinity can be isolated *via in-vitro* selection using a synthetic library for a broad range of target molecules (small molecules to large proteins) and make it possible to develop a wide range of aptamer-based sensors [11,14]. The selection and isolation of aptamers can undergo amplification *via* polymerase chain reaction (PCR) to achieve higher reproducibility and purity than other sources. The DNA aptamers are also highly stable (able to tolerate harsh conditions such as high pH and high temperatures) compared to the proteins and antibodies [11,14]. Aptamers undergo conformational changes in target molecules' presence, offering great flexibility to design biosensors with higher sensitivity and selectivity [11,14].

2.2.1 SARS-CoV-2 detection based on the Aptamer

Aptamers, or the "chemical antibodies," are recognized as versatile molecules due to their high specificity, and they provide advantages over the antibodies such as small size, rapid testing ability, higher chemical and thermal stability, ability to integrate with other compounds for the detection and amplification of signals and rapid and accurate modification. Therefore, aptamers have been identified as a potential tool to develop novel assays to diagnose COVID-19 [19].

In 2020, Song's group[20] isolated two novel aptamers that have been denoted as CoV2-RBD-1C and CoV2-RBD-4C using the human ACE-2 in the SELEX process. The selected aptamers were ideal candidates for diagnosing SARS-CoV-2 RBD in patients' samples due to their higher binding affinity and smaller size. The dissociation constant (K_d) values against the RBD been have recorded as 5.8 nM for CoV2-RBD-1C and 19.9 nM for CoV2-RBD-4C, respectively [20]. This discovery leads to the development of novel aptamer-based SARS-CoV-2 virus diagnosis methods.

During the SARS-CoV outbreak in 2002, scientists identified the SARS-CoV nucleocapsid (N) protein as a good biomarker due to its higher detection sensitivity than the viral nucleic acid or antibodies in patients. Earlier, in 2011, Cho's group[21] has used a single-stranded DNA aptamer (Aptamer 1) attached explicitly to SARS-CoV N protein. Later, Chen's group[22] modified the previously identified DNA aptamer to detect the SARS-CoV-2 N proteins since both SARS-CoV N and SARS-CoV-2 N proteins share ~ 91% sequence homology. They utilized the modified aptamers for the Enzyme-Linked Aptamer Binding Assay (ELAA) to investigate the detection of SARS-CoV-the 2 N protein. It revealed that the modified aptamers could detect SARS-CoV-2 N proteins with a low concentration of 10 ng/mL [22].

Recently, Pramanik's group[23] discovered that the SARS-CoV-2 S protein-specific DNA aptamer attached gold nanostars (GNS) could detect and prevent the infection of the virus. They utilized a distance-dependent nanoparticle surface energy transfer (NSET) spectroscopy to develop this novel assay to detect the SARS-CoV-2 virus infection in patients. In their strategy, DNA aptamer discovered by the song's group has modified with a Rhodamine 6G (Rh-6G) dye, and it was fused with the GNS *via* thiol-gold linkage. Rh-6G dye fluorescence emission was drastically quenched *via* NSET due to the proximity of GNS. Upon incubating with spike antigen or virus particles, the DNA aptamer binds and facilitates the increase of the distance between GNS and RH-6G. This enables the sudden enhancement of the fluorescence in the sample. They reported that this novel assay could effectively detect SARS-CoV-2 S antigen at a concentration of 130 fg mL^{-1} and virus particles at a concentration level of 8 particles mL^{-1}[23].

In another case, Chen's group[24] had developed a highly sensitive assay utilizing the surface-enhanced Raman scattering (SERS)-based aptamer to detect SARS-CoV-2 from the patients' sample lysates. To develop this novel sensor a previously discovered aptamer by Song's group was used [20]. The S protein-specific DNA aptamer was modified by attaching Raman reporter molecules (Cy3) to the aptamer's terminal. It was immobilized onto the auto-grown gold nano popcorn by DNA hybridization. Upon the incubation of the SARS-CoV-2 lysate, DNA aptamer binds with the S proteins in the lysate. It separates from the gold nano popcorn substrate due to the strong bond between DNA aptamer and S protein. The peak intensity of the SERS upon the new binding is monitored. Data indicate that the peak intensity of the Raman reporter molecule decreases with the increase in virus concentration. The research group reported that the sensor is capable of detecting SARS-CoV-2 virus particles with a limit of detection (LOD) of less than 10 PFU mL^{-1} within 15 minutes.

Subsequently, Zhang's group[25] developed a high-affinity dimeric aptamer DSA1N5 based on the earlier identified aptamer to recognize the S protein of the SARS-CoV-2 wild type, Alpha variant, and Delta variant. The dimeric aptamer's polythymidine (polyp) linkage has significantly enhanced the binding affinity towards the S protein. This dimeric aptamer was capable of recognizing SARS-CoV-2 wildtype virus (K_d = 120 pM) and its B.1.1.7 Alpha variant (K_d = 290 pM) and B.1.617.2 Delta variant (K_d = 480 pM) and also the pseudo-typed lentiviruses (PL), which are expressing the SARS-CoV-2 wildtype and alpha trimeric S proteins with the affinity constants of 2.1 pM and 2.3 pM. The research group immobilized the DSA1N5 onto a gold electrode to develop an electrochemical impedance sensor to produce a susceptible diagnosis test for SARS-CoV-2. This sensor could detect viral particles within less than 10 minutes from a patient's saliva samples. The clinical evaluation data indicate 80.5 % sensitivity and 100 % selectivity for the SARS-CoV-2 virus detection, which is on par with the rapid antigen tests to date. However, spike-binding neutralizing antibodies in the saliva prevents the virus detection by aptamers and lead to the insufficient detection sensitivity of the virus [25].

Recently, Zhang's group[26] reported a unique DNA aptamer (MSA52) that exhibits higher binding affinity towards the Alpha, Beta, Gamma, Epsilon, Kappa, Delta, and Omicron variants of the SARS-CoV-22 virus. The research group reported that MSA52 aptamer exhibits a higher binding affinity for all the variant S proteins, with K_d values ranging from 2 – 10 nM. This aptamer can also detect different types of S protein of SARS-CoV-2 expressed by the pseudo typed lentiviruses (PL) with a K_d value ranging from 20 – 50 pM [26].

2.3 DNAzyme: Brief Introduction

The discovery of catalytic DNA (DNAzymes) in 1994 by Ronald R. Breaker and Gerald F. Joyce revolutionized biosensor development in analytical chemistry. These pioneer researchers isolated the first DNAzyme named GR-5 by the in-vitro selection using Pb^{2+} ions as the specific cofactor, as shown in Fig.2(B). According to the literature, GR-5 exhibited the detection of Pb^{2+} ions with a rate of ~1 min^{-1} [1]. DNAzyme is a double-stranded molecule comprised of a catalytic and substrate strand. An RNA molecule was embedded into the substrate strand to achieve an RNA/DNA chimera. This ribonucleotide linkage acts as the cleavage site, while the catalytic strand acts as the target recognition site. However, DNAzyme exhibits remarkable specificity for the sequence of the substrate strand. Even a single mismatch in the DNA sequence affects the DNAzyme activity. Therefore, DNAzymes are recognized as exceptional molecules due to their higher sensitivity, selectivity, signal amplification ability, catalytic activity,

thermal stability, and cost-effectiveness attracting the attention of the researchers. To date, scientists have isolated various DNAzymes to catalyse different types of reactions, as shown in Table 1 in the presence of specific metal ions and metal oxides such as Pb^{2+}, Cu^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Hg^{2+}, and UO_2^{2+}ions [27].

Table 1: Reactions catalysed by the different DNAzymes

Reaction	Cofactor	Ref
RNA cleavage	Pb^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, UO_2^{2+}	[28–36]
RNA ligation	Mg^{2+}, Zn^{2+}, Mn^{2+}	[37–41]
DNA cleavage	Cu^{2+}	[42,43]
DNA hydrolysis	Mn^{2+}, Zn^{2+}	[44]
DNA ligation	Mn^{2+}, Cu^{2+}, Zn^{2+}	[45,46]
DNA adenylation	Cu^{2+}	[47]
DNA phosphorylation	Ca^{2+}	[48]
DNA depurination	-	[49]
Phosphonamidite bond cleavage	Mg^{2+}	[50]
Thymine dimer cleavage	-	[51]
Carbon-carbon bond formation	Ca^{2+}	[52]
N-glycosylation	Ca^{2+}	[53]
Porphyrin metalation	Cu^{2+} /Zn^{2+}	[54,55]

2.3.1 SARS-CoV-2 detection based on the DNAzyme

Generally, DNAzymes carry two main activities: catalytic activity and peroxidase mimicking activity. DNAzymes with catalytic activity exhibit the ribonucleotide linkage's cleavage upon the specific analyte incubation. This cleavage facilitates the release of the DNA fragment from the DNAzyme substrate strand, which is utilized as the signalling molecule to develop the biosensors. These catalytic DNAzymes were already employed in virus detection and therapeutic application, as shown in Table 2. The peroxidase mimicking the activity of the DNAzyme has been incorporated to develop colorimetric biosensors. Formation of the G-quadruplex (G4) structure by guanine-rich DNA sequence and upon the binding to the hemin, the G4/hemin complex exhibit the peroxidase activity by catalysing the oxidation of H_2O_2/2, 2'-azino-bis diammonium salts ($ABTS^{2-}$) to ($ABTS^{\cdot+}$) and H_2O_2/3,3',5,5'-tetramethylbenzidine sulfate (TMB) to oxTMB to generate a color change which the naked eye can observe [56]. The functional nucleic acids are not confined to storing and transferring genetic information. It can be used as a versatile biomolecule, which operates beyond nucleic acids' genetic role. G-quadruplex was extensively explored as molecular tool for various applications to develop recognition elements and catalytic units for bioanalysis and nanotechnology.

Table 2: 10-23 DNAzyme-based gene down-regulation of the virus

Virus Type	DNAzyme target	Ref
Human immunodeficiency virus (HIV-1)	HIV-1 tar,	[57]
	HIV-1 gag,	[58]
	tat/rev RNA,	[59–62]
	CCR5	[63]
	HIV-1 integrase	[64]
	CXCR-4	[65]
	ENV	[66–68]
	NEF	[58]
	Vpr	[69]
	env	[70]
Dengue virus	5'cyclization sequence (5' CS)	[71]
Human papilloma virus type 16 (HPV16)	E6, /E7	[72]
	c-myc	[73]
Huntington's disease (HD)	Huntingtin mRNA	[74]
Human rhinovirus type 14 (HRV14)	5′-non-translated region (NTR)	[75,76]
Epstein-Barr virus (EBV)	Latent membrane protein (LMP1), cis-acting replication element	[77–82]
Respiratory syncytial virus (RSV)	NS2	[83]
	F	[84]
Japanese encephalitis	3'-Non-coding regions	[85]
Influenza A	PB2	[86,87]
	M1	[88]
Influenza B	BM2	[89]
Hepatitis B	DR1 and PA	[90]
	HBsAg, HBeAg	[91,92]
	S	[92,93]
	C	[90,94]
	X	[95,96]
	e	[97]
Hepatitis C	Core protein	[98,99]
	NS5B	[100]
	NS3	[101,102]
	Internal ribosome entry site (IRES)	[103]
Autographa californica nucleopolyhedrovirus (*Ac*NPV)	pk1 mRNA	[104]
Enterovirus	EV71 and CVB3	[105]

In 2007, Wu's[106] group reported using DNAzyme as a therapeutic agent and an effective screening method for detecting SARS-CoV infection. A DNAzyme, Dz-104, was developed with 9+9 arm sequences with conserved 15 nucleotides catalytic motif of the 10-23 DNAzyme targeting the 5′ untranslated region (5′ UTR) of the SARS virus. The 5′ UTR is mainly responsible for viral gene expression. The 9+9 arm sequences were designed in a complementary manner to the 5′ UTR sequences to promote the effective binding of the DNAzyme with the viral RNA sequence. Wu's group identified a highly conserved RNA sequence in the other SARS-CoV strains. The targeting of the 5′ UTR provides the opportunity to prevent viral gene expression and avoid the emergence of the mutant strains [106]. According to the results, the modified DNAzyme was capable of cleaving the 5′ UTR of the SARS-CoV effectively. A similar approach is possible to down-regulate the proliferation and develop effective vaccines against SARS-CoV-2.

Anantharaj's[107] research group recently utilized the DNAzymes peroxidase-like activity to develop a colorimetric sensor to detect SARS-CoV-2. The development of colorimetric-based sensors omits the requirement of highly sophisticated instruments such as fluorescence spectrophotometers for SARS-CoV-2 detection. The research team designed the forward primer (P1A) and reverse primer (P1B), specifically corresponding to the SARS-CoV-2 genome. Both primers were modified to have a reverse complementary sequence of the DNAzyme

with the peroxidase activity. Samples isolated from the COVID-19-positive or negative patients were subjected to amplifying the SARS-CoV-2 genome via RT-PCR. The resultant amplified genome contains the peroxidase-like DNAzyme sequence due to the amplification of the primer sequences. In the presence of hemin/KCl and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS)/ 3,3′,5,5′-tetramethylbenzidine (TMB) solution mixtures, the color change was observed from colorless to greenish-blue color by the naked eye within 5 minutes. The LOD of this assay ranged from 10^1–10^7 copies, and the developed DNAzyme sensor was able to detect the SARS-CoV-2 with a threshold cycle (Ct) value of 35 with a better correlation with a Ct value of fluorescence-based real-time PCR [107]. This DNAzyme-based sensor provides the rapid, efficient, cost-effective, and accurate diagnosis of COVID-19 by eliminating the need for expensive instruments. This DNAzyme based assay can be easily modified to detect the variants of the SARS-CoV-2 compared to the other existing methods.

Later, Brink's[108] research group developed a novel method designated "Rapidemic," a label-free DNAzyme based assay to detect nucleic acid. This assay consists of three major components such as recombinase polymerase amplification (RPA), linear strand-displacement amplification (LSDA), and DNAzyme with peroxidase activity (G-quadruplex). Both RPA and LSDA are isothermal amplification methods used as an alternative amplification method to avoid the thermal cycling steps of the PCR. The research team combined the RPA and LSDA to amplify the specific nucleic acid sequence and DNAzyme with peroxidase mimicking activity as the signaling molecule. At the RPA stage, the target sequence was amplified and extended, and the primer was designed to have an overhang to introduce a short DNA sequence. This short DNA strand contains the reverse complementary sequence responsible for the recognition site for the nicking endonuclease enzyme and G-quadruplex sequence. The nicking endonuclease enzyme promotes the single strand cleavage at the recognition site of the amplified double-strand DNA sequence. It facilitates the elongation of the 3′ ends of the nicked DNA strand by displacing the G-quadruplex (G4) sequence. This G4 forms a 3-dimensional (3D) structure that consists of the peroxidase activity in the presence of the KCl and hemin. In the presence of hydrogen peroxide (H_2O_2), this 3D complex oxidizes the TMB and promotes the color change from colorless to greenish-blue color [108]. The research team already validated the performance of this method using *Saccharomyces cerevisiae*, and with further optimization, this method can be utilized as an effective diagnostic tool to detect viral infections due to its simplicity, easy modification, and cost-effectiveness. Compared to the previous PCR-based diagnosis methods, this method provides accurate, time-efficient diagnoses due to avoiding thermal cycling steps. Changing the primer sequences can be used to diagnose. However, this method still requires the conversion of the viral RNA genome to its cDNA by reverse transcriptase to amplify the sequences. With further modification, this obstacle can be overcome.

The efficiency of SARS-CoV-2 detection by RT-PCR and rapid antigen tests is becoming limited because of its time consumption, the requirement of sophisticated laboratories, and, most importantly, the higher mutation rate of the virus. Therefore, at the current moment, detection methods with reliable, rapid, cost-effective, and ability to detect different variants are required to contain the further spread of COVID-19. Zhang's[109] research group designed an ultrasensitive chemiluminenace strategy to address this issue to diagnose the SARS-CoV-2 virus. The research group combined the proximity hybridization and rolling circle amplification (RCA) technique to amplify the signal response in this design. RPA is also an isothermal amplification technique that utilizes circular DNA and phi29 DNA polymerase (PolΦ29) to extend the DNA primer around the circular DNA to generate a long single-stranded sequence. This diagnosis method consists of the proximity ligand complex (Ab-1/SARS-CoV-2/Ab-2), block/primer complex, and cytosine-rich circular DNA. The research group designed two DNA-antibody conjugates (Ab-1, Ab-2) to identify the SARS-CoV-2 proteins to achieve higher sensitivity. This recognition of the SARS-CoV-2 proteins *via* Ab-1 and Ab-2 promotes the formation of the proximity ligand complex and facilitates the primer's displacement from the block/primer complex. The released primer binds to the circular DNA template and triggers the RCA reaction to synthesize guanine (G) rich long DNA sequence. This G-rich DNA sequence assumes the G4, which exhibits the horseradish peroxidase activity in the presence of hemin. The resulting G4 catalyses the luminol/H_2O_2 releasing a strong chemiluminescence signal. The research group claimed that this assay was capable of detecting SARS-CoV-2 with a detection limit of 6.46 fg mL^{-1} [109]. This assay provides higher sensitivity, selectivity, and an accurate diagnosis method to detect SARS-Cov-2 compared to the other peroxidase-like DNAzyme based methods. However, this method's major drawback is the cost-effectiveness due to the use of the antibodies.

Pan's[110] research group recently developed a novel strategy by utilizing an Mg^{2+} specific DNAzyme to develop a logical DNA circuit to diagnose COVID-19. The exonuclease III and DNAzyme activities were combined to promote the synergetic signal amplification. The major components of the DNA logic circuit were the input signals (SARS-CoV-2 specific DNA sequence and homologs sequence), exonuclease III, substrate strand, and three hairpin structures designated as H1, H2, and H3. In the presence of the specific sequence, both H1 and H2 synergistically hybridized with the specific sequence *via* the 3′ end overhangs and formed a partial duplex complex. This complex promotes the binding of exonuclease III, and upon binding of exonuclease III catalyzes the hydrolysis of the nucleotides from the 3′ ends of the H1 and H2 hairpin structures. Once the partial duplex is hydrolyzed, it releases the DNA1 and DNA2 strands required to construct the Mg^{2+} specific DNAzyme. Similarly, H3 interacts with the

homologous sequence. Upon the completion of the hydrolysis by exonuclease III, the catalytic enzyme strand is released from the H3/Homologous sequence complex. The synergetic hybridization of the enzyme strand, substrate strand, DNA1, and DNA2 strands promotes the formation of Mg^{2+} specific DNAzyme in the shape of the "H" junction. In the presence of the Mg^{2+} ion, the substrate strand cleaved into two DNA fragments due to the catalytic activity of the DNAzyme. The release of the FAM-tagged fragment from the substrate strand emits an intense fluorescence signal. The research group reported that the assay was capable of detecting COVID-19 in the range of femtometre (fM) [110]. Compared to the other DNAzyme based COVID-19 diagnosis methods, this method contains dual recognition sites to identify COVID-19 and facilitate highly sensitive detection. However, this strategy consists of drawbacks such as the requirement of the fluorescence spectrometer to detect the fluorescence signal and conversion of the SARS-CoV-2 genome into cDNA to facilitate the formation of the partial duplex complexes.

Isothermal amplification techniques attract scientists to develop novel COVID-19 detection methods due to their simplicity, inexpensiveness, and rapidness over the RT-PCR and antigen tests. Reverse-transcriptase loop-mediated isothermal amplification (RT-LAMP)[111] and reverse-transcriptase recombinase polymerase amplification (RT-RPA)[112] methods were generally used to develop novel detection methods for the COVID -19 detection. However, the non-specific DNA amplification affects the detection process, and researchers incorporated a preamplification step to overcome this obstacle. This type of preamplification step was utilized in specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) and DNA endonuclease-targeted CRISPR *trans* reporter (DETECTR) systems for the detection of COVID-19.[113] Both SHERLOCK and DETECTR systems[113–115] are based on the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) protein. Yang's[116] research group recently developed a novel platform to detect nucleic acid called RNA-Encoded Viral Nucleic Acid Analyte Reporter (REVEALR) [116]. The research group designed the REVEALR by utilizing novel Xeno nucleic acid (XNA) derived XNAzyme 10-23 (X 10-23), a synthetic analog of the previously discovered 10-23 DNAzyme. The splitting of the X 10-23 into two parts facilitates the development of the multicomponent sensor to detect SARS-CoV-2. The research group modified the X 10-23 split halves with a reporter arm and a trigger arm, synthesized using Xeno nucleic acid derivative 2′-fluoroarabino nucleic acid. Reporter's arms contain the complementary sequence for hybridizing the ribonucleotide embedded reporter sequence, consisting of a fluorophore and quencher molecules at the end of the sequence. The trigger arm includes the complementary sequence for the S-gene of the SARS-CoV-2 genome. In the viral RNA target sequence, two catalytic cores of the X 10-23 self-assembled to generate an active DNAzyme. After the incubation in a pH 8.5 buffer containing $MgCl_2$, the cleavage of the DNAzyme facilitates the release of the fluorophore from the DNAzyme complex. The released fluorophore emits an intense fluorescence signal. To achieve the atamolar (aM) level detection of SARS-CoV-2, the research group incorporated a preamplification by *in vitro* transcription (IVT) of viral RNA and RT-RPA. The research group reported that the developed sensor is capable of detecting COVID-19 with a limit of detection ≤ 20 aM (~10 copies/µL)[116] within 1 hour. This assay provides the rapid and accurate detection of COVID-19. This can be utilized for the early detection of many samples and prevent the spread of COVID-19 effectively compared to other COVID-19 detection methods due to its unique characteristics. Apart from the diagnosis of the SARS-CoV-2 virus, FNA has been employed in detecting metal contaminations and pathogen contaminations. The following sections will discuss the utilization of DNAzymes to detect metal contaminations.

3 DNAzyme based biosensors for the detection of various analytes.

Human activities such as discharging industrial effluent, mining, coral & fossil fuel burning, waste dumping, pesticides, and herbicides consumption in irrigation release metals into the environment, increasing the exposure and causing severe damage to the environment and health [117] Therefore, detecting metals in the environment is vital in many aspects. Many bio-analytical techniques are being used to detect metal ion distribution and fluctuations in bio-organisms. Various sensitive and highly accurate techniques are available to detect metals in the environment, such as atomic absorption/emission spectroscopy (AAS/AES) [118], Inductively coupled plasma mass spectrometry (ICP-MS), Inductively coupled plasma-optical emission spectrometry (ICP-OES)/Inductively coupled plasma atomic emission spectroscopy (ICP-AES) which can detect trace amounts of metal ions. However, these techniques are costly and require laboratories, and expertise in sample preparation equipment handling, and prevents on-site or real-time monitoring of the samples [119]. Therefore, efforts have been taken to overcome the limitations to develop sensors capable of detecting metal ions in the in-situ environment. However, different sensors were developed over time, and research was focused on incorporating biological molecules such as proteins and peptides into sensors to develop biosensors.

Biosensors have the advantage of fast analysis and higher sensitivity due to their characteristic features. The main components of the biosensors are the target recognition site and signal transduction. The target recognition site can be a protein, peptide, carbohydrate, or nucleic acid. These elements usually have a high metal-binding affinity, high selectivity for specific metal ions, fast reaction time, and a wide dynamic range for detecting ions [120]. Signal transduction is mainly responsible for converting the target recognition element reaction with the specific ions into a physically detectable signal. Depending on the signal transduction site, the signals can be colorimetric [65, 66], fluorescence [67, 68], or electrochemical [121–124]. Since DNAzyme consists of the target recognition site due to the

catalytic core and exhibits signal transduction due to the cleavage of the ribonucleotide linkage in the presence of specific cofactors intrigue the scientist to develop novel biosensors. The release of the DNA fragment of the DNAzyme substrate strand upon the incubation of the cofactor is utilized as the platform to develop various biosensors to detect different cofactors such as metal ions, *etc*. Hence, signal transduction methods such as fluorescent, colorimetric, and electrochemical have been incorporated into the DNAzyme to develop novel biosensors [125–127].

Fluorescence sensors are developed based on fluorescence-labelling organic chelators. Many biomolecules such as proteins and peptides emerged as potent compounds to achieve the fluorescence biosensor. Some of these attempts make remarkable progress in developing fluorescence biosensors to detect metal ions such as calcium (Ca^{2+}) and zinc (Zn^{2+}) ions. But the designing and synthesis of such sensors is a challenging process. Fluorescent detection attracted the attention of many researchers due to their unique properties since these fluorescence detection methods give an immediate response and can use for real-time detection of the analyte. Due to the non-radioactive behaviour of the fluorescence labels, disposal and waste management is effectively achieved [128]. Since nucleic acids are non-fluorescent, all the fluorescence-based DNAzyme sensors are composed of an external fluorophore to achieve the fluorescence [125]. Therefore, the conversion of DNAzyme into sensor was achieved by incorporating a fluorophore (F) at the 5′ end of the substrate strand and incorporating the 3′ end of the catalytic strand of the DNAzyme with a quencher molecule (Q).

Researchers have been using fluorescence based DNAzymes to develop novel biosensors. Many of them incorporated both fluorophore and quenchers to improve the detection of biosensors by reducing the background signal. The Li's[77] group initially carried out this pioneering work, and afterward, many scientists followed the same path to develop the fluorescent-based DNAzyme biosensors. In the presence of the specific cofactor, the substrate strand is cleaved due to the catalytic activity of the DNAzyme. The release of the fluorophore attached DNA fragment of the substrate strand results in the sudden enhancement of the fluorescence signal. Both fluorophore and quencher molecules are arranged in close proximity to reduce the background signal. These F/Q pairs can be positioned differently to optimize the fluorescent signals, as illustrated in Fig. 3. It was reported that this F/Q pair hinders the metal ion binding to the catalytic core of the DNAzyme and facilitates the selectivity against other metal ions.

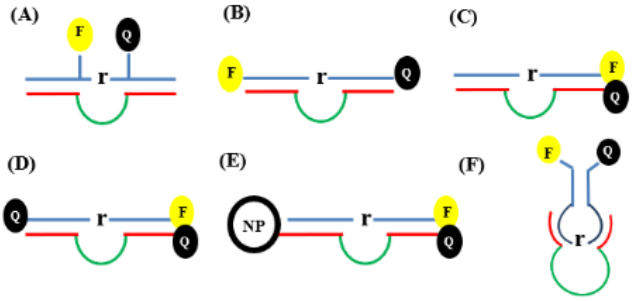

Fig. 3. Schematic illustration of the different fluorophore and quencher (F/Q) arrangements on the DNAzyme (A) F and Q were placed next to the cleavage site flanking the cleavage site. (B) F and Q were placed at the two ends of the substrate strands. (C) F and Q were placed on the same side but with different strands. (D) A second Q was introduced on the other end of the substrate. (E) Nanoparticles as a quencher. (F) F and Q were placed at the two ends of a hairpin-shaped catalytic and molecular beacon (CAMB)

However, the fluorescence-based DNAzyme biosensors require sophisticated, expensive instruments such as fluorescence spectrometers for fluorescence detection. To overcome this drawback, colorimetric-based DNAzyme sensors were developed [129]. These colorimetric biosensors exhibit a color change in the presence of the target molecule, which can be observed in the visible range. The development of colorimetric sensors provides easy analyte detection compared to fluorescence-based sensors. Usually, nucleic acid absorbs light at the ultra-violet (UV) range, and it is impossible to detect those signals in the visible range. Therefore DNAzyme based colorimetric sensors required color reporting groups; small organic dyes, conjugated polymers, and metallic nanoparticles [125]. Even though colorimetric biosensors provide the easy detection of the analyte, compared to the fluorescence-based biosensors, the sensitivity of the biosensor is relatively low. Apart from fluorescence and colorimetric-based DNAzyme sensors, electrochemical-based detection has been incorporated into the DNAzyme based biosensors. These electrochemical-based DNAzyme sensors contains the advantages in practical applications such as; low instrumentation cost, higher sensitivity, reproducibility and rapid response compared to fluorescence and colorimetric DNAzyme based detection, high sensitivity provided by electronic devices. Plaxco's[130] group first reported the development of the electrochemical-based DNAzyme biosensor in 2007. They reported that the biosensor was highly sensitive and capable of detecting the Pb^{2+} ions with the limit of detection (LoD) of 300 nM. They also claimed that the sensor could detect Pb^{2+} contamination in soil samples [130]. An overview of the use of DNAzymes with different signal transduction to detect various metals and metal molecules is summarized in Table 3.

Table 3: Different types of sensors developed using DNAzymes for the detection of different analyte.

DNAzyme	Signalling method	LOD	Linear range	Ref
		Na^+		
NaA43	FLR	135 µM	0.135–50 mM	[131]

EtNa	FLR	-	-	[132]
		Mg^{2+}		
E5	FLR	10 mM	-	[29]
Bipartite I	FLR	10 mM	-	[133]
17EV1	FLR	1.1 mM	-	[134]
Mg^{2+} specific DNAzyme	FLR	200 nM	-	[135]
E6	FLR	300 pM	0–20 nM	[136]
		Ca^{2+}		
Mg5	FLR	-	-	[137]
17EV1	FLR	2.5 mM	-	[134]
EtNa	FLR	17 µM	-	[127]
		UO_2^{2+}		
39E	FLR	45 pM	Up - 20 nM	[36]
39E	FLR	2.4 pM	Up -100 nM	[138]
UO_2^{2+} specific DNAzyme	FLR	0.6 nM	1.0 nM - 3.0 µM	[139]
“	FLR	13 pM	30 pM – 5 nM	[140]
E-DNA	FLR	100 fM	0.2 - 1000 pM	[141]
39E	FLR	190 pM	-	[142]
E-DNA	CLR	20 pM	50 pM - 2 nM	[141]
UO_2^{2+} specific DNAzyme	CLR	330 pM	0.5 - 15.0 ppb	[143]
“	CLR	0.08mg L^{-1}	2.9 – 5.0 nM	[144]
		Pb^{2+}		
GR-5	FLR	3.7 nM	-	[28], [145]
17E	FLR	7.8 nM	-	[145]
8-17	FLR	600 pM	Up - 200 nM	[146]
Pb^{2+}-Specific DNAzyme	FLR	5 nM	-	[147]
8-17	FLR	61.8 pM	Up - 100 nM	[148]
GR-5 DNAzyme	FLR	300 pM	Up -100 nM	[149]
Pb^{2+}-Specific DNAzyme	FLR	0.5 nM	-	[150]
“	FLR	0.2 nM	1.0 – 50 nM	[151]
“	FLR	1 nM	-	[152]
GR-5 DNAzyme	FLR	0.1 nM	200 pM - 100 nM	[153]
8-17	FLR	3.9 nM	5.0 - 3.0 µM	[139]
17E	FLR	1.7 nM	5.0 - 3.0 µM	[154]
17E	FLR	10 nM	10 – 4.0 µM	[155]
GR-5 L-DNAzyme	FLR	3 nM	5 - 100 nM	[156]
GR-5	CLR	59.39 pM	-	[157]
“	CLR	0.05 nM	0.01 - 100 µM	[158]
“	CLR	32 pM	10^2 - 10^8 pM	[159]
8-17	FLR	50 pM	100 pM - 10 µM	[160]
“	FLR	500 pM	500 pM - 100 nM	[161]
GR-5	FLR	3 nM	-	[162]
GR-5	FLR	0.16 nM	0.5 – 75 nM	[163]
GR-5	ELC	290 fM	1 pM - 0.1 µM	[164]
GR-5	ELC	0.048 pM	0.2 pM - 100 nM	[165]
17E	ELC	17.4 fM	10^{-13} - 10^{-7} $molL^{-1}$	[166]
Pb^{2+}-Specific DNAzyme	FLR	0.3 nM	1.0 – 500 nM	[135]
“	CLR	~100 nM	-	[125,167]
“	CLR	3 nM	-	[168]
GR-5	CLR	0.05 nM	0.01 - 100 µM	[169]
“	ELC	300 nM	-	[130]
“	ELC	0.028 nM	-	[170]
8-17	ELC	1 nM	-	[171]
custom Pb^{2+} DNAzyme	ELC	96 pM	100 pM - 5 µM	[172]
8-17	ELC	0.07 pM	5.0pmol/L-2.0µmol/L	[173]
15−12 DNAzyme	Plasmon	8.0 nM	-	[174]
		Hg^{2+}		
Modified 39E	FLR	2.4 nM	Up to 200 nM	[175]
E6	ELC	4.2 fM	0.1 pM - 10 nM	[176]
MZ	ELC	23 fM	0.1 pM - 200 nM	[177]
E6	FLR	30 pM	0.1 - 5 nM	[178]
E6	CLR	33 pM	50 - 1200 pM	[179]
-	CLR	5 pM	10 pM - 100 nM	[180]
		Cu^{2+}		
Cu^{2+}-Specific DNAzyme	FLR	35 nM	up to 20 µM	[181]
“	FLR	0.5 nM	1.0 – 50 nM	[151]

“	FLR	1.0 nM	2.0 – 500 nM	[135]
“	FLR	500 pM	1–100 nmol L^{-}	[182]
PSCu10	FLR	1.6 nM	Up to 100 nM	[183]
		Zn^{2+}		
9NL27	FLR	1 nM	-	[184]
I-R3	FLR	1 nM	-	[185]
ALP – PPi - Regulated DNAzyme	FLR	20 pM	5 - 800 µM	[186]
		Cd^{2+}		
BN-Cd16	FLR	1.1 nM	Up to 100 nM	[187]

Abbreviations: CLR: colorimetric, ELC: electrochemical, FLR: fluorometric

Furthermore, the development of biosensors using DNAzyme further expanded with the advancement of the technology and intrigued the scientists to utilize this remarkable molecule to develop novel sensors. This shows that the DNAzymes are not confined to a particular field, but there is still room for improvement.

4 Conclusion

This review comprehensively analyses the use of functional nucleic acids known as aptamers, and DNAzymes as a versatile tool in developing effective diagnosis methods of SARS-CoV-2 virus detection. The discovery of FNA revolutionized the designing of biosensors and assays with higher sensitivity and selectivity for the different analyte. The detection of SARS-CoV-2 *via* aptamers and DNAzyme is a novel discovery. It might provide the platform to develop sensors to detect micro-orgasms more effectively than traditional methods and treat SARS-CoV-2 virus infection effectively. We conclude that, even though FNA-based sensors and assays have extensive potential applications in various fields, more studies are vital to improving the selectivity, stability, and sensitivity of this fascinating and promising biosensor technology.

Conflicts of Interest

There are no conflicts to declare.

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References

[1] Breaker R R, and Joyce G F, 1994 *Chem. Biol.* 1 (4) 223–229.

[2] Ihms H E, and Lu Y, 2012 *Methods Mol. Biol.* 848 297–316.

[3] Li Y, and Breaker R R, 1999 *J. Am. Chem. Soc.* 121 (23) 5364–5372.

[4] Takagi Y, Warashina M, Stec W J, Yoshinari K, and Taira K, 2001 *Nucleic Acids Res.* 29 (9) 1815–1834.

[5] NAGRAJ N, 2010 *DEVELOPMENT OF FUNCTIONAL DNA-BASED SENSORS AND INVESTIGATIONS INTO THEIR MECHANISM* .

[6] Schlosser K, and Li Y, 2010 *ChemBioChem* 11 (7) 866–879.

[7] Brown A K, Li J, Pavot C M B, and Lu Y, 2003 *Biochemistry* 42 (23) 7152–7161.

[8] Sigel R K O, and Pyle A M, 2007 *Chem. Rev.* 107 (1) 97–113.

[9] Ward W L, Plakos K, and Derose V J, 2014 *Chem. Rev.* 114 (8) 4318–4342.

[10] Robertson D L, and Joyce G F, 1990 *Nature* 344 (6265) 467–468.

[11] Iliuk A B, Hu L, and Tao W A, 2011 *Anal. Chem.* 83 (12) 4440–4452.

[12] Tuerk C, and Gold L, 1990 *Science (80-.).* 249 (4968) 505–510.

[13] Ellington A D, and Szostak J W, 1990 *Nature* 346 (6287) 818–822.

[14] Song S, Wang L, Li J, Fan C, and Zhao J, 2008 *TrAC - Trends Anal. Chem.* 27 (2) 108–117.

[15] Tombelli S, Minunni M, and Mascini M, 2007 *Biomol. Eng.* 24 (2) 191–200.

[16] Jenison R D, Gill S C, Pardi A, and Polisky B, 1994 *Science (80).* 263 (5152) 1425–1429.

[17] Hermann T, and Patel D J, 2000 *Science (80-.).* 287 (5454) 820–825.

[18] Jayasena S D, 1999 *Clin. Chem.* 45 (9) 1628–1650.

[19] Liu R, He L, Hu Y, Luo Z, and Zhang J, 2020 *Chem. Sci.* 11 (44) 12157–12164.

[20] Song Y, Song J, Wei X, Huang M, Sun M, Zhu L, Lin B, Shen H, Zhu Z, and Yang C, 2020 *Anal. Chem.* 92 (14) 9895–9900.

[21] Cho S J, Woo H M, Kim K S, Oh J W, and Jeong Y J, 2011 *J. Biosci. Bioeng.* 112 (6) 535–540.

[22] Chen Z, Wu Q, Chen J, Ni X, and Dai J, 2020 *Virol. Sin.* 35 (3) 351–354.

[23] Pramanik A, Gao Y, Patibandla S, Mitra D, McCandless

M G, Fassero L A, Gates K, Tandon R, and Ray P C, 2021 *J. Phys. Chem. Lett.* 12 (8) 2166–2171.

[24] Chen H, Park S G, Choi N, Kwon H J, Kang T, Lee M K, and Choo J, 2021 *ACS Sensors* 6 (6) 2378–2385.

[25] Zhang Z, Pandey R, Li J, Gu J, White D, Stacey H D, Ang J C, Steinberg C J, Capretta A, Filipe C D M, Mossman K, Balion C, Miller M S, Salena B J, Yamamura D, Soleymani L, Brennan J D, and Li Y, 2021 *Angew. Chemie - Int. Ed.* 60 (45) 24266–24274.

[26] Zhang Z, Li J, Gu J, Amini R, Stacey H D, Ang J C, White D, Filipe C D M, Mossman K, Miller M S, Salena B J, Yamamura D, Sen P, Soleymani L, Brennan J D, and Li Y, 2022 *Chem. - A Eur. J.* e202200078.

[27] Zhang X-B, Kong R-M, and Lu Y, 2011 *Annu. Rev. Anal. Chem.* 4 (1) 105–128.

[28] Breaker R R, and Joyce G F, 1994 *Chem. Biol.* 1 (4) 223–229.

[29] Breaker R R, and Joyce G F, 1995 *Chem. Biol.* 2 (10) 655–660.

[30] Faulhammer D, and Famulok M, 1996 *Angew. Chemie Int. Ed. English* 35 (2324) 2837–2841.

[31] Santoro S W, and Joyce G F, 1997 *Proc. Natl. Acad. Sci. U. S. A.* 94 (9) 4262–4266.

[32] Li J, 2000 *Nucleic Acids Res.* 28 (2) 481–488.

[33] Feldman A R, and Sen D, 2001 *J. Mol. Biol.* 313 (2) 283–294.

[34] Mei S H J, Liu Z, Brennan J D, and Li Y, 2003 *J. Am. Chem. Soc.* 125 (2) 412–420.

[35] Liu Z, Mei S H J, Brennan J D, and Li Y, 2003 *J. Am. Chem. Soc.* 125 (25) 7539–7545.

[36] Liu J, Brown A K, Meng X, Cropek D M, Istok J D, Watson D B, and Lu Y, 2007 *Proc. Natl. Acad. Sci. U. S. A.* 104 (7) 2056–2061.

[37] Coppins R L, and Silverman S K, 2004 *Nat. Struct. Mol. Biol.* 11 (3) 270–274.

[38] Flynn-Charlebois A, Wang Y, Prior T K, Rashid I, Hoadley K A, Coppins R L, Wolf A C, and Silverman S K, 2003 *J. Am. Chem. Soc.* 125 (9) 2444–2454.

[39] Flynn-Charlebois A, Prior T K, Hoadley K A, and Silverman S K, 2003 *J. Am. Chem. Soc.* 125 (18) 5346–5350.

[40] Hoadley K A, Purtha W E, Wolf A C, Flynn-Charlebois A, and Silverman S K, 2005 *Biochemistry* 44 (25) 9217–9231.

[41] Wang Y, and Silverman S K, 2003 *J. Am. Chem. Soc.* 125 (23) 6880–6881.

[42] Carmi N, Shultz L A, and Breaker R R, 1996 *Chem. Biol.* 3 (12) 1039–1046.

[43] Carmi N, Balkhi S R, and Breaker R R, 1998 95 .

[44] Chandra M, Sachdeva A, and Silverman S K, 2009 *Nat. Chem. Biol.* 5 (10) 718–720.

[45] Cuenoud B, and Szostak J W, 1995 *Nature* 375 (6532) 611–614.

[46] Sreedhara A, Li Y, and Breaker R R, 2004 *J. Am. Chem. Soc.* 126 (11) 3454–3460.

[47] Li Y, Liu Y, Biochemistry R B-, and 2000 undefined, 2000 *ACS Publ.* 39 (11) 3106–3114.

[48] Li Y, Sciences R B the N A of, and 1999 undefined, 1999 *Natl. Acad Sci.* 96 2746–2751.

[49] Höbartner C, Pradeepkumar P I, and Silverman S K, 2007 *Chem. Commun.* (22) 2255–2257.

[50] Burmeister J, Von Kiedrowski G, and Ellington A D, 1997 *Angew. Chemie (International Ed. English)* 36 (12) 1321–1324.

[51] Chinnapen D J F, and Sen D, 2004 *Proc. Natl. Acad. Sci. U. S. A.* 101 (1) 65–69.

[52] Chandra M, and Silverman S K, 2008 *J. Am. Chem. Soc.* 130 (10) 2936–2937.

[53] Sheppard T L, Ordoukhanian P, and Joyce G F, 2000 *Proc. Natl. Acad. Sci. U. S. A.* 97 (14) 7802–7807.

[54] Li Y, and Sen D, 1996 *Nat. Struct. Biol.* 3 (9) 743–747.

[55] Li Y, and Sen D, 1997 *Biochemistry* 36 (18) 5589–5599.

[56] Li B, Du Y, Li T, and Dong S, 2009 *Anal. Chim. Acta* 651 (2) 234–240.

[57] Chakraborti S, and Banerjea A C, 2003 *Mol. Ther.* 7 (6) 817–826.

[58] Dash B C, and Banerjea A C, 2004 *Oligonucleotides* 14 (1) 41–47.

[59] Sood V, Unwalla H, Gupta N, Chakraborti S, and Banerjea A C, 2007 *AIDS* 21 (1) 31–40.

[60] Unwalla H, Chakraborti S, Sood V, Gupta N, and Banerjea A C, 2006 *Antiviral Res.* 72 (2) 134–144.

[61] Unwalla H, and Banerjea A C, 2001 *Antiviral Res.* 51 (2) 127–139.

[62] UNWALLA H, and BANERJEA A C, 2001 *Biochem. J.* 357 (1) 147–155.

[63] Goila R, and Banerjea A C, 1998 *FEBS Lett.* 436 (2) 233–238.

[64] Singh N, Ranjan A, Sur S, Chandra R, and Tandon V, 2012 Inhibition of HIV-1 Integrase Gene Expression by

10-23 DNAzyme, in Journal of Biosciences, pp. 493–502.

[65] Basu S, Sriram B, Goila R, and Banerjea A C, 2000 *Antiviral Res.* 46 (2) 125–134.

[66] Sugiyama R, Hayafune M, Habu Y, Yamamoto N, and Takaku H, 2011 *Nucleic Acids Res.* 39 (2) 589–598.

[67] Zhang X, Xu Y, Ling H, and Hattori T, 1999 *FEBS Lett.* 458 (2) 151–156.

[68] Dash B C, Harikrishnan T A, Goila R, Shahi S, Unwalla H, Husain S, and Banerjea A C, 1998 *FEBS Lett.* 431 (3) 395–399.

[69] Bano A S, Gupta N, Sharma Y, Sood V, and Banerjea A C, 2007 *Oligonucleotides* 17 (4) 465–472.

[70] Dash B C, Harikrishnan T A, Goila R, Shahi S, Unwalla H, Husain S, and Banerjea A C, 1998 *FEBS Lett.* 431 (3) 395–399.

[71] Carter J R, Balaraman V, Kucharski C A, Fraser T S, and Fraser M J, 2013 *Virol. J.* 10 1–15.

[72] Reyes-Gutiérrez P, and Alvarez-Salas L M, 2009 *Oligonucleotides* 19 (3) 233–242.

[73] Sun L Q, Cairns M J, Gerlach W L, Witherington C, Wang L, and King A, 1999 *J. Biol. Chem.* 274 (24) 17236–17241.

[74] Yen L, Strittmatter S M, and Kalb R G, 1999 *Ann. Neurol.* 46 (3) 366–373.

[75] Schubert S, Gül D C, Grunert H P, Zeichhardt H, Erdmann V A, and Kurreck J, 2003 *Nucleic Acids Res.* 31 (20) 5982–5992.

[76] Potaczek D P, Unger S D, Zhang N, Taka S, Michel S, Akdağ N, Lan F, Helfer M, Hudemann C, Eickmann M, Skevaki C, Megremis S, Sadewasser A, Alashkar Alhamwe B, Alhamdan F, Akdis M, Edwards M R, Johnston S L, Akdis C A, Becker S, Bachert C, Papadopoulos N G, Garn H, and Renz H, 2019 *J. Allergy Clin. Immunol.* 143 (4) 1403–1415.

[77] Ke X, Yang Y C, and Hong S L, 2011 *Med. Oncol.* 28 (SUPPL. 1) 336–332.

[78] Chen Y, Yang L, Huang S, Li Z, Zhan L, He J, Xu Z, Liu L, Cao Y, and Sun L, 2013 *Int. J. Nanomedicine* 8 3107–3118.

[79] Ma X, Xu Z, Yang L, Xiao L, Tang M, Lu J, Xu S, Tang Y, Wen X, Deng X, Sun L, and Cao Y, 2013 *Int. J. Oncol.* 43 (5) 1541–1548.

[80] Lu Z X, Ma X Q, Yang L F, Wang Z L, Zeng L, Li Z J, Li X N, Tang M, Yi W, Gong J P, Sun L Q, and Cao Y, 2008 *Cancer Lett.* 265 (2) 226–238.

[81] Lu Z X, Ye M, Yan G R, Li Q, Tang M, Lee L M, Sun L Q, and Cao Y, 2005 *Cancer Gene Ther.* 12 (7) 647–654.

[82] Yang L, Lu Z, Ma X, Cao Y, and Sun L Q, 2010 *Molecules* 15 (9) 6127–6139.

[83] Xie Y Y, Zhao X D, Jiang L P, Liu H L, Wang L J, Fang P, Shen K L, Xie Z De, Wu Y P, and Yang X Q, 2006 *Antiviral Res.* 71 (1) 31–41.

[84] Zhou J, Yang X Q, Xie Y Y, Zhao X D, Jiang L P, Wang L J, and Cui Y X, 2007 *Virus Res.* 130 (1–2) 241–248.

[85] Appaiahgari M B, and Vrati S, 2007 *Mol. Ther.* 15 (9) 1593–1599.

[86] Takahashi H, Hamazaki H, Habu Y, Hayashi M, Abe T, Miyano-Kurosaki N, and Takaku H, 2004 *FEBS Lett.* 560 (1–3) 69–74.

[87] Toyoda T, Imamura Y, Takaku H, Kashiwagi T, Hara K, Iwahashi J, Ohtsu Y, Tsumura N, Kato H, and Hamada N, 2000 *FEBS Lett.* 481 (2) 113–116.

[88] Kumar B, Khanna M, Kumar P, Sood V, Vyas R, and Banerjea A C, 2012 *Mol. Biotechnol.* 51 (1) 27–36.

[89] Kumar B, Kumar P, Rajput R, Saxena L, Daga M K, and Khanna M, 2013 *Nucleic Acid Ther.* 23 (5) 355–362.

[90] Asahina Y, Ito Y, Wu C H, and Wu G Y, 1998 *Hepatology* 28 (2) 547–554.

[91] Miao J, Yang X, Shang X, Gao Z, Li Q, Hong Y, Wu J, Meng T, Yuan H, and Hu F, 2021 *Mol. Ther. - Nucleic Acids* 24 (June) 127–139.

[92] Hong Y, Mao D, Wu R, Gao Z, Meng T, Wang R, Liu L, and Miao J, 2019 *RSC Adv.* 9 (27) 15196–15204.

[93] Wo J E, Wu X L, Zhou L F, Yao H P, Chen L W, and Dennin R H, 2005 *World J. Gastroenterol.* 11 (23) 3504–3507.

[94] He W, Wo J er, and Liu K zhou, 2006 *Zhejiang Da Xue Xue Bao. Yi Xue Ban* 35 (5) 507–511.

[95] GOILA R, and BANERJEA A C, 2001 *Biochem. J.* 353 (3) 701–708.

[96] Hou W, Ni Q, Wo J, Li M, Liu K, Chen L, Hu Z, research R L-A, and 2006 undefined, *Elsevier*.

[97] Miao J, Zhang X G, Hong Y, Rao Y F, Li Q, Xie X J, Wo J E, and Li M W, 2012 *Carbohydr. Polym.* 87 (2) 1342–1347.

[98] Trepanier J, Tanner J E, Momparler R L, Le O N L, Alvarez F, and Alfieri C, 2006 *J. Viral Hepat.* 13 (2) 131–138.

[99] Oketani M, Asahina Y, Wu C H, and Wu G Y, 1999 *J. Hepatol.* 31 (4) 628–634.

[100] Kumar D, Chaudhury I, Kar P, and Das R H, 2009 *J. Gastroenterol. Hepatol.* 24 (5) 872–878.

[101] Lee B, Bo Kim K, Oh S, Sig Choi J, Park J S, Min D H,

and Kim D E, 2010 *Oligonucleotides* 20 (6) 285–296.

[102] Ryoo S R, Jang H, Kim K S, Lee B, Kim K B, Kim Y K, Yeo W S, Lee Y, Kim D E, and Min D H, 2012 *Biomaterials* 33 (9) 2754–2761.

[103] Roy S, Gupta N, Subramanian N, Mondal T, Banerjea A C, and Das S, 2008 *J. Gen. Virol.* 89 (7) 1579–1586.

[104] Mishra G, Chadha P, Chaudhury I, and Das R H, 2008 *Virus Res.* 135 (1) 197–201.

[105] Du M, Zheng J, Tian S, Liu Y, Zheng Z, Wang H, Xia J, Ji X, and He Z, 2021 *Anal. Chem.* 93 (13) 5606–5611.

[106] Wu S, Xu J, Liu J, Yan X, Zhu X, Xiao G, Sun L, and Tien P, 2007 *J. Gene Med.* 9 (12) 1080–1086.

[107] Anantharaj A, Das S J, Sharanabasava P, Lodha R, Kabra S K, Sharma T K, and Medigeshi G R, 2020 *Front. Mol. Biosci.* 7 444.

[108] van den Brink M, Tandar S T, P van den Akker T A, Jovikj S, Defourt V, B Langelaar T G, Delzenne T O, van Strien K, Schonk A W, A M Beers A J, Golov E, Chong L J, Özer Bergman G, W D Meijdam J J, Crooijmans M E, Claessen D, and de Winde J H, 2020 *bioRxiv* 2020.10.14.337808.

[109] Zhang R, Wu J, Ao H, Fu J, Qiao B, Wu Q, and Ju H, 2021 *Anal. Chem.* 93 (28) 9933–9938.

[110] Pan J, He Y, Liu Z, and Chen J, 2021 *Chem. Commun.* 57 (9) 1125–1128.

[111] T N, H O, H M, T Y, K W, N A, and T H, 2000 *Nucleic Acids Res.* 28 (12) .

[112] O P, CH W, DL S, and NA A, 2006 *PLoS Biol.* 4 (7) 1115–1121.

[113] Mustafa M I, and Makhawi A M, 2021 *J. Clin. Microbiol.* 59 (3) .

[114] Bhattacharyya R P, Thakku S G, and Hung D T, 2018 *ACS Infect. Dis.* 4 (9) 1278–1282.

[115] Gootenberg J S, Abudayyeh O O, Lee J W, Essletzbichler P, Dy A J, Joung J, Verdine V, Donghia N, Daringer N M, Freije C A, Myhrvold C, Bhattacharyya R P, Livny J, Regev A, Koonin E V., Hung D T, Sabeti P C, Collins J J, and Zhang F, 2017 *Science (80-.).* 356 (6336) 438–442.

[116] Yang K, and Chaput J C, 2021 *J. Am. Chem. Soc.* 143 (24) 8957–8961.

[117] Järup L, 2003 *Br. Med. Bull.* 68 (September) 167–182.

[118] Ozbek N, Baysal A, and Akman S, 2015 Heavy Metal Determination in Environmental Samples, in Environmental Applications of Instrumental Chemical Analysis, pp. 441–470.

[119] Reardan D T, Meares C F, Goodwin D A, McTigue M, David G S, Stone M R, Leung J P, Bartholomew R M, and Frincke J M, 1985 *Nature* 316 (6025) 265–268.

[120] Jung Y, Jeong J Y, and Chung B H, 2008 *Analyst* 133 (6) 697–701.

[121] Wang J, Xu D, Kawde A N, and Polsky R, 2001 *Anal. Chem.* 73 (22) 5576–5581.

[122] Katz E, and Willner I, 2003 *Electroanalysis* 15 (11) 913–947.

[123] Gerard M, Chaubey A, and Malhotra B D, 2002 *Biosens. Bioelectron.* 17 (5) 345–359.

[124] Que E L, Domaille D W, and Chang C J, 2008 *Chem. Rev.* 108 (5) 1517–1549.

[125] Liu J, Cao Z, and Lu Y, 2009 *Chem. Rev.* 109 (5) 1948–1998.

[126] Gong L, Zhao Z, Lv Y F, Huan S Y, Fu T, Zhang X B, Shen G L, and Yu R Q, 2015 *Chem. Commun.* 51 (6) 979–995.

[127] Zhou W, Saran R, and Liu J, 2017 *Chem. Rev.* 117 (12) 8272–8325.

[128] Proudnikov D, and Mirzabekov A, 1996 *Nucleic Acids Res.* 24 (22) 4535–4542.

[129] Li, Yingfu; Lu Y, 2009 *Functional Nucleic Acids for Analytical Applications*, Springer New York.

[130] Xiao Y, Rowe A A, and Plaxco K W, 2007 *J. Am. Chem. Soc.* 129 (2) 262–263.

[131] Torabi S F, Wu P, McGhee C E, Chen L, Hwang K, Zheng N, Cheng J, and Lu Y, 2015 *Proc. Natl. Acad. Sci. U. S. A.* 112 (19) 5903–5908.

[132] Zhou W, Saran R, Chen Q, Ding J, and Liu J, 2016 *ChemBioChem* 17 (2) 159–163.

[133] Feldman A R, and Sen D, 2001 *J. Mol. Biol.* 313 (2) 283–294.

[134] Zhou W, Zhang Y, Ding J, and Liu J, 2016 *ACS Sensors* 1 (5) 600–606.

[135] Yun W, Wu H, Liu X, Fu M, Jiang J, Du Y, Yang L, and Huang Y, 2017 *Anal. Chim. Acta* 986 115–121.

[136] Wang J, Wang X, Tang H, He S, Gao Z, Niu R, Zheng Y, and Han S, 2018 *Sensors Actuators, B Chem.* 272 146–150.

[137] Peracchi A, 2000 *J. Biol. Chem.* 275 (16) 11693–11697.

[138] Cao J X, Wang Y S, Xue J H, Huang Y Q, Li M H, Chen S H, Zhou B, Tang X, Wang X F, and Zhu Y F, 2016 *RSC Adv.* 6 (110) 108662–108667.

[139] Zhou W, Liang W, Li D, Yuan R, and Xiang Y, 2016 *Biosens. Bioelectron.* 85 573–579.

[140] Yun W, Wu H, Yang Z, Wang R, Wang C, Yang L, and Tang Y, 2019 *Anal. Chim. Acta* 1068 104–110.

[141] Yun W, Wu H, Liu X, Zhong H, Fu M, Yang L, and Huang Y, 2018 *Sensors Actuators, B Chem.* 255 1920–1926.

[142] Feng M, Gu C, Sun Y, Zhang S, Tong A, and Xiang Y, 2019 *Anal. Chem.* 91 (10) 6608–6615.

[143] Zhang H, Cheng X, Chen L, Mo F, Xu L J, and Fu F F, 2017 *Anal. Chim. Acta* 956 63–69.

[144] Huang C, Fan X, Yuan Q, Zhang X, Hou X, and Wu P, 2018 *Talanta* 185 258–263.

[145] Lan T, Furuya K, and Lu Y, 2010 *Chem. Commun.* 46 (22) 3896–3898.

[146] Zhang X B, Wang Z, Xing H, Xiang Y, and Lu Y, 2010 *Anal. Chem.* 82 (12) 5005–5011.

[147] Kim J H, Han S H, and Chung B H, 2011 *Biosens. Bioelectron.* 26 (5) 2125–2129.

[148] Wang L, Jin Y, Deng J, and Chen G, 2011 *Analyst* 136 (24) 5169–5174.

[149] Zhao X H, Kong R M, Zhang X B, Meng H M, Liu W N, Tan W, Shen G L, and Yu R Q, 2011 *Anal. Chem.* 83 (13) 5062–5066.

[150] Wen Y, Peng C, Li D, Zhuo L, He S, Wang L, Huang Q, Xu Q H, and Fan C, 2011 *Chem. Commun.* 47 (22) 6278–6280.

[151] Wu C S, Khaing Oo M K, and Fan X, 2010 *ACS Nano* 4 (10) 5897–5904.

[152] Yin B C, Zuo P, Huo H, Zhong X, and Ye B C, 2010 *Anal. Biochem.* 401 (1) 47–52.

[153] Zhang D, Fu R, Zhao Q, Rong H, and Wang H, 2015 *Anal. Chem.* 87 (9) 4903–4909.

[154] Wang X Y, Niu C G, Guo L J, Hu L Y, Wu S Q, Zeng G M, and Li F, 2017 *J. Fluoresc.* 27 (2) 643–649.

[155] Li J, and Lu Y, 2000 *J. Am. Chem. Soc.* 122 (42) 10466–10467.

[156] Liang H, Xie S, Cui L, Wu C, and Zhang X, 2016 *Anal. Methods* 8 (39) 7260–7264.

[157] Huang Z, Chen J, Luo Z, Wang X, and Duan Y, 2019 *Anal. Chem.* 91 (7) 4806–4813.

[158] Wang H B, Ma L H, Fang B Y, Zhao Y Di, and Hu X Bin, 2018 *Colloids Surfaces B Biointerfaces* 169 305–312.

[159] Rong M, Li J, Hu J, Chen A, Wu W, and Lyu J, 2018 *J. Chem. Technol. Biotechnol.* 93 (11) 3254–3263.

[160] Pan J, Li Q, Zhou D, and Chen J, 2019 *New J. Chem.* 43 (15) 5857–5862.

[161] Zhang T, Liu C, Zhou W, Jiang K, Yin C, Zhang Z, and Li H, 2019 *J. Anal. Methods Chem.* 2019 .

[162] Fu T, Ren S, Gong L, Meng H, Cui L, Kong R M, Zhang X B, and Tan W, 2016 *Talanta* 147 302–306.

[163] Wu J, Lu Y, Ren N, Jia M, Wang R, and Zhang J, 2019 *Sensors (Switzerland)* 19 (12) 2732.

[164] Liao X, Luo J, Wu J, Fan T, Yao Y, Gao F, and Qian Y, 2018 *J. Electroanal. Chem.* 829 129–137.

[165] Cai W, Xie S, Zhang J, Tang D, and Tang Y, 2018 *Biosens. Bioelectron.* 117 312–318.

[166] Yu Z, Li N, Hu X, Dong Y, Lin Y, Cai H, Xie Z, Qu D, and Li X, 2019 *Synth. Met.* 254 164–171.

[167] Liu J, and Lu Y, 2003 *J. Am. Chem. Soc.* 125 (22) 6642–6643.

[168] Wang Z, Lee J H, and Lu Y, 2008 *Adv. Mater.* 20 (17) 3263–3267.

[169] Wang H B, Ma L H, Fang B Y, Zhao Y Di, and Hu X Bin, 2018 *Colloids Surfaces B Biointerfaces* 169 305–312.

[170] Yang X, Xu J, Tang X, Liu H, and Tian D, 2010 *Chem. Commun.* 46 (18) 3107–3109.

[171] Shen L, Chen Z, Li Y, He S, Xie S, Xu X, Liang Z, Meng X, Li Q, Zhu Z, Li M, Le X C, and Shao Y, 2008 *Anal. Chem.* 80 (16) 6323–6328.

[172] Song X, Wang Y, Liu S, Zhang X, Wang J, Wang H, Zhang F, Yu J, and Huang J, 2019 *Microchim. Acta* 186 (8) 1–8.

[173] Wang Y, Zhao G, Zhang G, Zhang Y, Wang H, Cao W, Li T, and Wei Q, 2020 *Sensors Actuators, B Chem.* 319 128313.

[174] Diao W, Wang G, Wang L, Zhang L, Ding S, Takarada T, Maeda M, and Liang X, 2020 *ACS Appl. Bio Mater.* 3 (10) 7003–7010.

[175] Lu Y, 2009 (February 2009) .

[176] Cai W, Xie S, Zhang J, Tang D, and Tang Y, 2017 *Biosens. Bioelectron.* 98 466–472.

[177] Li Y, Chang Y, Ma J, Wu Z, Yuan R, and Chai Y, 2019 *Anal. Chem.* 91 (9) 6127–6133.

[178] Yun W, Zhong H, Zheng S, Wang R, and Yang L, 2018 *Sensors Actuators, B Chem.* 277 456–461.

[179] Deng P, Zheng S, Yun W, Zhang W, and Yang L, 2019 *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* 210 335–340.

[180] Chen J, Pan J, and Chen S, 2017 *Chem. Commun.* 53 (73) 10224–10227.

[181] Liu J, and Lu Y, 2007 *J. Am. Chem. Soc.* 129 (32) 9838–9839.

[182] Chen Y, Chen L, Ou Y, Wang Z, Fu F, and Guo L, 2016

Talanta 155 245–249.

[183] Huang P J J, and Liu J, 2016 *Anal. Chem.* 88 (6) 3341–3347.

[184] Xu J, Sun Y, Sheng Y, Fei Y, Zhang J, and Jiang D, 2014 *Anal. Bioanal. Chem.* 406 (13) 3025–3029.

[185] Liu N, Hou R, Gao P, Lou X, and Xia F, 2016 *Analyst* 141 (12) 3626–3629.

[186] Kong R M, Fu T, Sun N N, Qu F L, Zhang S F, and Zhang X B, 2013 *Biosens. Bioelectron.* 50 351–355.

[187] Huang P J J, and Liu J, 2015 *Nucleic Acids Res.* 43 (12) 6125–6133.