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DNA G-Quadruplex as a Reporter System for Sensor Development

Qiuting Loh and Theam Soon Lim

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Abstract

The versatile DNA G-quadruplex structure has emerged as an interesting alternative reporter system applied in different biosensor platforms. In comparison to the conventional reporter systems like enzymatic or fluorescent, DNA G-quadruplex has some distinct advantages, as it is thermostable, easy to produce, low cost and most importantly able to be amplified. Such remarkable advantages have led many researchers to exploit DNA G-quadruplex as the reporter system in colorimetric, fluorescence and luminescence sensors. There has also been integration of DNA G-quadruplex with electrochemical methods and quantum dot for sensing applications. Therefore, this chapter highlights some recent examples of different biosensor platforms that use DNA G-quadruplex as a reporter system with different detection methods.

Keywords: G-quadruplex, Biosensor, Protein Detection, DNA Detection, Metal Detection

1. Introduction

All genetic information with regards to every living organism is stored in the deoxyribonucleic acid, DNA. This is the fundamental application of DNA that makes it the basic building block of life. However, DNA is a very dynamic molecule whereby its function is not confined only to information storage and delivery. DNA also has the ability to form a number of spatial arrangements such as single-stranded hairpins, homoduplexes, triplexes and quadruplexes with high-order complexity. In nature, the formation of these structures has been found to be involved in many cellular mechanisms such as DNA recombination, regulation of gene expression and possibly the proliferation of tumor cells [1, 2].

One of these DNA structures that are well studied is the G-quadruplex (G-quad) structure. The G-quad structure is made up of a stack of nucleic acid sequence that is rich in guanine (G)

[3]. Besides, G-quad structure is found to be polymorphic as it forms many different structural arrangements depending on the variation in DNA composition and environment. Different formation of G-quad structure has different yet specific functions in nature. Thus, the study of G-quad has driven the structure into many applications, especially in the field of medicine, biology and material sciences.

The biological functions of the G-quad structure are well documented and the principles of it can be exploited for use as biosensors and therapeutics. One of the functions of the structure is the formation of DNA enzyme or catalytic enzymes, in short, DNAzyme, that can exhibit catalytic capabilities such as exhibiting peroxidase-like activity. G-quad can also serve as internal fluorescent probes in which its nucleobases are modified or attached with fluorescent dyes for sensing. In addition, G-quad can be combined with some electrochemical methods to produce signal readout. The discovery of such remarkable advantages of G-quad have given rise to the development of assays exploiting the G-quad structure such as DNA detection assay, protein detection assay and even the detection of molecules and ions [4-7].

Taken together, the advantages and flexibilities accorded to DNA G-quad have made G-quad very useful for the development of a variety of reporter systems for sensing applications. Consequently, DNA-based assay has now become a potential alternative to the conventional diagnostic platforms that use enzymes. In this chapter, the focus of our discussion would be on the structural features and application of DNA G-quad structures for the development of various sensing platforms.

2. Basic structures of DNA G-quad

DNA is often described as a double helix structure based on the typical Watson-Crick base pairing, where hydrogen bonds are formed between guanine and cytosine or adenine with thymine [8]. However, it was later discovered that a different bonding interaction based on hydrogen bonding could contribute to base pairing, called the Hoogsteen bonding. The basic structure of G-quad involves four G bases forming a square planar array called the G-quartet that is stabilized by eight Hoogsteen and Watson-Crick hydrogen bonds. G-quad are formed by stacking up square planar arrays that is joined by the phosphodiester backbone and stabilized by the π - π stacking interactions of the stacked G-quartets and specific cations such as K^+ , Na^+ , Li^+ , NH_4^+ , Pb^{2+} and Sr^{2+} that gives rise to the strong electrostatic interaction between G and the cations [9-10].

NMR and crystallography studies have shown G-quad structures to be highly polymorphic [11]. They can form many different structures depending on the length of the DNA, orientation of the chains, positions of the loops and nature of the cations. G-quad can be found in many different forms ranging from one, two or four separate chains that give rise to unimolecular, bimolecular and tetramolecular structures [12]. There are also a variety of topologies of G-quad due to different possible combinations of the stretches of G-rich sequences, loop formation and also sequences [13-14]. Generally, the stretches of G can fold into different forms that include the parallel, basket, hairpin and chair conformation. Four strands of G can fold to form a

parallel four-stranded structure while two chains of G will fold into dimeric structures by dimerization of a pair of hairpin that results in a bimolecular G-quad with two loops [15]. Two structures of different loop orientations can be formed which are 'edgewise' loops that connect adjacent anti-parallel chains and the 'diagonal' loops that connect cross-over anti-parallel chains. As reported, the use of different cations in these two structures will form different loop conformations. The NMR solution of these two structures showed that with K^+ ions, the G-quad structure gave 'edgewise' loops, and, on the other hand, the G-quad structure in Na^+ ions produced 'diagonal' loops [16, 17]. A single-stranded G-rich sequence with four G repeats will form a unimolecular G-quad structure. This single stretch of G will fold and form an intramolecular G-quad with three loops in the presence of cations. Due to the steric hindrance and electrostatic repulsion caused by the loops, the orientation of the three loops is not entirely anti-parallel [13].

The polymorphism in the G-quad is the result of a balance between several stabilizing factors. The G-quad structures are mainly determined by monovalent cations as these structures are cation-dependent and require it for stability. Besides, other factors such as hydrogen bonding, base-stacking forces and hydrophobic effects also affect the formation of different topologies of the G-quad structures. However, this remarkable polymorphism has driven the increasing influence of G-quad in various functions and applications, especially in the field of medicine, biology and material sciences [18]. The main attractive application of G-quad revolves around the potential diagnostic application of G-quad as a reporter system where signal readouts are easily amplified by standard DNA amplification processes to yield a sensitive sensing system.

3. G-quad mimicking peroxidase activity in colorimetric-based sensors

There are several forms of readouts that G-quad structures are capable of producing. A major readout format is by absorbance value where G-quad structures are able to form DNAzymes that exhibit catalytic activity [19]. In nature, DNAzymes are initially known as catalytic enzymes or DNA enzymes because of its ability to catalyze many reactions such as ligation, DNA modification [20, 21], cleavage of DNA or RNA [21, 22] and also methylation of porphyrin rings [9, 23]. One of the most important features of G-quad DNAzyme activity is the peroxidase mimicking activity when hemin is bound to the G-quad structure. The hemin-quadruplex complex will catalyze the peroxide-mediated oxidation of the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) to generate a coloured product [24-26]. This colorimetric change has allowed the development of assays detecting metal ions, aptamer-substrate complexes and even proteins. The conventional method to generate such change in colour is based on enzymatic reactions involving enzymes such as horseradish peroxidase and alkaline phosphatase. These enzymes are usually active in a narrow temperature range and denatures at high temperatures. DNAzymes are stable in a broad temperature range and even at very high temperatures, making it an interesting alternative to enzymes. Another advantage of DNAzymes is the ease in preparation by chemical synthesis or by PCR, whereas protein enzymes require tedious preparation and purification processes. A key advantage of G-quad DNAzyme is the ability to carry out signal amplification by conventional DNA amplification

methods, which is impossible with normal protein set-ups. The attractiveness of G-quad DNAzyme to mimic peroxidase-like activity has allowed it to be exploited for the development of biosensors [27-30].

An assay to detect silver ions (Ag^+) was developed using G-quad as the reporter system. Ag^+ ions are able to stabilize cytosine–cytosine (C–C) mismatches by forming the C– Ag^+ –C base pairs. Therefore, in the absence of Ag^+ , the G-rich sequence will form an intramolecular duplex. The addition of Ag^+ in the mixture will allow the G-rich sequence to fold into a quadruplex structure and readily bind to hemin to form a DNAzyme. This will allow the G-quad to exhibit the peroxidase-like activity resulting in a change of the colourless ABTS to a coloured product in the presence of Ag^+ ions. The application of G-quad is useful to detect other molecules other than metal ions [31].

Our group has shown the application of G-quad as a sensitive reporter system for the detection of antibody–antigen interaction. The system was based on a pre-formed reporter system whereby a probe was pre-formed by conjugating streptavidin gold nanoparticles with biotinylated antigen and biotinylated daunomycin aptamer to exhibit the hemin-dependent peroxidase-like activity (Figure 1). Thus, the pre-assay generation of such reporter probes allows for rapid one-step incubation in a one-pot synthesis by exploiting the simple yet strong streptavidin–biotin interaction. This helps to eliminate multiple tedious steps of incubation and wash of conventional immunoassay systems. In this direct antibody–antigen assay, antibodies against the target antigen was coated to the microtiter plates and incubated with the probe. The wells were then developed with ABTS solution. The assay was able to generate sensitive readouts for both competitive and direct assays [32].

Figure 1. Schematic diagram of the immunoassay that is based on the preformed-reporter system.

Commonly, the peroxidase-like activity of DNAzymes is based on the oxidation of ABTS solution mediated by hydrogen peroxide (H_2O_2). This mechanism can be manipulated to initiate different sensing outcomes based on the same principles. This was evident in a detection assay for cholesterol by substituting the H_2O_2 with the catalysed cholesterol oxidase reaction of cholesterol and oxygen to produce the required H_2O_2 (Figure 2). Therefore, the G-quad DNAzyme will be able to exhibit the peroxidase-like activity after the binding of hemin to catalyze the oxidation of colourless ABTS mediated by the cholesterol oxidase reacted production of H_2O_2 . Therefore, only with the presence of cholesterol, the changes in colour of ABTS will occur. Amplification of the DNA sequences to produce more DNAzyme was carried out to further improve the signal readout [33].

Figure 2. Schematic diagram of the G-quad-based colorimetric sensor for cholesterol.

A new strategy was reported by Tang et al. for nucleic acid detection of the viral load of Hepatitis B virus (HBV) that utilizes G-quad DNAzyme as the probe and involves polymerase chain reaction (PCR). The DNA probe was designed to form a hairpin structure at room temperature. During the denaturation step of PCR, the dsDNA HBV templates will denature, and the DNA probe will be linearized and allowed to hybridize with the target HBV ssDNA. The loop of the DNA probes will anneal to the conserved region of the HBV genome. The DNA probes can be amplified and cleaved so that they can form G-quad DNAzyme. The stem part of the probe is used to prohibit DNAzyme sequence to fold into G-quad at room temperature in order to reduce the background reading. With the involvement of PCR, the sensitivity and specificity of the HBV DNA assay was improved [34].

For further improvement of the DNA probe amplification, single temperature amplification (isothermal amplification) was carried out instead of going through the conventional temperature cycling with the PCR. As reported by Liu et al., an aptamer-modified microchip that combines rolling circle amplification with the peroxidase mimicking activity by G-quad DNAzyme was developed for thrombin detection (Figure 3). The assay involved an aptamer-modified microchip and a reporter aptamer consisting of the thrombin aptamer sequence and a primer with G-quad circular template. When the sample was introduced into the microchip, thrombin was captured by the chip immobilized thrombin aptamer and the reporter aptamer will act as primer for the RCA amplification process. This will allow the amplification process to generate increased amounts of G-quad to form DNAzyme upon binding of hemin. The

generated hemin–DNAzyme complex will catalyse the oxidation of ABTS to produce a coloured product [35]. Isothermal DNA amplification strategies can be employed easily for DNAzyme sensor detection.

Figure 3. Ultrasensitive detection of thrombin by RCA and G-quad DNAzyme.

Besides RCA, G-quad structures can also be amplified using other isothermal strategies like quadruplex priming amplification (QPA) as reported by Kankia et al. QPA allows for efficient generation of G-quad structures by isothermal amplification with the additional ability to self-dissociate for continuous amplification. During the QPA elongation process, the 5' end of the product will fold into intramolecular quadruplex and self-dissociates from the primer-binding site of the template, allowing the template to be accessible to the incoming primer for the next priming cycle. Thus, more quadruplexes were formed and eventually leading to increased signal readout. Our group incorporated the QPA system for the development of a sandwich immunoassay, called immuno-QPA (IQPA). The system exploits the peroxidase mimicking function of G-quad DNAzyme and the quadruplex amplification by QPA. A recombinant scFv was coated on the well and the biotinylated antigen was added and left to bind with the antibody. Streptavidin was introduced as a bridge between the biotinylated antigen and biotinylated QPA template. QPA was then carried out to amplify the QPA template with its specific primer to generate self-dissociating G-quad structures. These self-dissociating G-quad structures will bind to hemin to generate the similar colour change readout with ABTS as mentioned earlier [36].

4. G-quad as fluorescence probes

The photophysical properties of G-quad structures are highly dependent on the bound metal ions at the location of nucleobase electrons of the structure due to the coordination of ions in

the center of the quadruplex. Thus, G-quad is able to exhibit two- to ten-fold higher quantum fluorescence yields. This is due to the structure serving as energy donors to energy acceptors in close proximity to yield fluorescence resonance energy transfer (FRET) systems. G-quad can be utilized as a fluorescent probe in which the nucleobases in the structure are labelled or attached with fluorescent dyes. Besides, the enhancement of the fluorescence of a small dye can be exhibited based on the interaction of the G-quad with the fluorescence ligand [7,37,38]. Some of the ligands such as thiazole orange were reported to yield higher fluorescence yield with the aid of the G-quad structure [39]. On the other hand, the G-quad could also cause fluorescence quenching phenomena. For instance, the classic intercalating agent, ethidium bromide (EtBr), binds to double-stranded DNA and exhibit fluorescence intensity up to 30-fold. However, in the presence of G-quad, the fluorescence intensity of the EtBr can be quenched upon binding to the structure [7].

One of the label-free G-quad DNA-based fluorescence biosensors reported was designed to detect cisplatin, which is an anticancer drug widely used in chemotherapy. However, cisplatin overdose can cause neurotic cell death that makes it dangerous for administration without proper monitoring. Therefore, Zhou et al. developed a simple label-free fluorescence biosensor by utilizing the interaction of G-quad and cisplatin as cisplatin was found to bind to G-quad naturally (Figure 4). In the context of the assay, the absence of cisplatin will allow the formed G-quad to bind to N-methyl mesoporphyrin IX (NMM), resulting in an increase in fluorescence intensity. However, when cisplatin was added into the assay, it will bind to the G-quad structure and disintegrate the structure. This will then cause a drastic decrease in fluorescence intensity due to the collapse of the G-quad structure [40].

Figure 4. Label-free turn on and turn off fluorescence DNA G-quad-based sensor for the detection of cisplatin.

Wang et al. had proposed a label-free fluorescence biosensor based on G-quad formation in order to detect the lead (II) ion (Pb^{2+}). This biosensor consists of a G-rich DNA strand with its partially complementary strand. The heme-oxygenase-1 inhibitor, zinc protoporphyrin (ZnPPiX) has been utilized as a fluorescence probe, whereby it can interact with the Pb^{2+} /G-quad complex, producing fluorescence readout. In the absence of the Pb^{2+} ions, both DNA strands will form a DNA duplex. The addition of Pb^{2+} will unwind the DNA duplex to allow the G-rich strand to fold into the G-quad structure. The Pb^{2+} /G-quad complex will then interact with ZnPPiX to enhance its fluorescence intensity, which is not possible with the DNA duplex. This biosensor could overcome the cumbersome step in which most of the fluorescence-based biosensors would require fluorescence dyes to be probed on the DNA. In addition, this biosensor can be reset easily to the original state by dissociating the G-quad structure. In order

to dissociate the G-quad structure, the strong Pb^{2+} chelator DOTA (1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid) was used as it has high binding capacity against Pb^{2+} to remove Pb^{2+} from the G-quad structure, leaving the G-quad structure to dissociate and form a DNA duplex with the complementary strand again [41].

As reported by Kankia and co-workers, a further improvement to their original QPA method was reported [42]. The improved method involves the use of two linear processes that are the QPA and linear nicking amplification, which is based on the study done by Galas and co-workers. The probe DNA was hybridized with the target DNA and polymerase was used to extend the target DNA strand and form QPA-PBS (primer-binding site). Then, the nicking enzyme Nt.BSTNBI was introduced to nick the target strand and release the QPA-primer-binding strand. The QPA-primer-binding strand will bind to the QPA primer and the polymerase will function to extend the strand further. The primer will then dissociate to emit fluorescence signals. The following priming step of QPA will be initiated again when the next primer binds with the QPA-primer binding strand. This method was reported to be able to reduce the background activity to allow sufficient sensitivity of the assay (Figure 5).

Figure 5. Principle of the QPA integrating nicking enzyme for diagnostics.

5. G-quad in luminescence-based sensors

Although applied widely, fluorescent labelling for sensor development has some limitations. Labelling the oligonucleotide covalently can reduce the binding affinity or selectivity of the oligonucleotide, which in turn hampers the efficiency of the assay. Besides, fluorescent labelling is costly and time consuming. Therefore, the use of luminescent probes in sensor development was an attractive solution. Unlike fluorescent probes, luminescent probes are not covalently attached to the nucleic acid; instead, it can bind with DNA through end stacking or electrostatic interactions, intercalation and groove binding. Such interactions will not affect the functionality of the DNA [43].

A label-free G-quad assay for metal detection was developed by Ma et al. This assay was based on the transition of a DNA structure, which involved specially designed oligonucleotide that is rich in guanine and cytosine. The transition of DNA structure would be from a quadruplex to duplex conformation induced by silver (Ag^+) ions. In this assay, platinum (II) metallo intercalator was used for the switch on/ switch off response towards the Ag^+ ions. Initially, a low luminescence background was generated due to the weak interaction of platinum (II) metallo intercalator and the G-quad structure without the Ag^+ ions. However, after adding the Ag^+ ions, cytosine- Ag^+ -cytosine mismatched base pairing occurred, causing the G-quad structure to revert to a duplex structure. This allowed the intercalation of the platinum(II) metallo intercalator to the duplex DNA structure and generates a luminescent signal [44].

Besides metal detection assays, the same group also developed an assay for the detection of gene deletion (Figure 6). They designed a split G-quad assay whereby it consists of two short oligonucleotides, namely P1 and P2. Both of these strands have complementary regions that can recognize the deletion site of the chemokine receptor gene CCR5 and also contain guanine-rich overhangs. These guanine-rich overhangs of both strands can form a split G-quad in close proximity. CCR5 was used as the target because it was reported to be a co-receptor for macrophage-tropic human immunodeficiency virus type 1 (HIV-1) strain and it allows HIV-1 to enter the CD4^+ T cells. Mutation on CCR5 gene caused no expression of functional protein, which gives rise to the resistance against HIV infection. In this assay, P1 and P2 strands were able to hybridize with the wild-type CCR5 DNA strand. Due to the large spatial separation between both G-rich overhangs of P1 and P2, a split G-quad structure was not formed, causing the generation of a lower luminescent signal of the iridium(III) complex. However, mutant of the CCR5 DNA sequence that is shorter in length can cause the two G-quad-forming sequences of P1 and P2 to close proximity upon hybridizing with the shorter mutant sequence. This resulted in the formation of the split G-quad, generating a switch-on luminescence effect of the iridium (III) complex [45].

Figure 6. Split G-quad luminescent turn-on detection for the detection of gene deletion of HIV DNA sequence.

6. G-quad integrates with electrochemistry

Apart from the applications that exploit the properties of the G-quad structures, new strategies were established by integrating G-quad with electrochemistry. Recent studies developed different electrochemical G-quad sensors based on magnetic particles, nanoparticles labelled

with metal tags, nanotubes and other nanomaterials. Wang et al. developed a thrombin detection assay using amplified electrochemical signal. This assay consists of an aptamer modified gold electrode and another aptamer modified with Cds hollow nanosphere. These two aptamers are complementary in most part except for the middle bases that are not complementary. This design will allow the aptamer to form a hairpin. Thus, in the presence of thrombin, the conformation of the aptamer will change from a hairpin to a G-quad structure. The nanospheres will allow the electron transfer between the gold electrode and $K_3[Fe(CN)_6]$, producing an electrocatalytic response [46].

Besides the protein detection sensor, electrochemical methods have also been exploited in the development of biosensor for cancer monitoring or impedimetric biosensor that measures the swelling behaviour of different cancer cells. An electrochemical-based G-quad sensor to detect cancer cells was developed by Qu et al. The biosensor consists of a graphene-modified electrode where aptamer, AS1411 and its complementary strand were used. In the presence of the cations, the aptamer, AS1411 forms G-quad and binds specifically to nucleolins that are expressed on the cancer cell surface with high binding affinity. Thus, only the cancer cells can be captured and it is capable to differentiate cancer cells from normal cells. The cyclic voltammetry (CV) signal of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ decreased over time as the anchored aptamer folded to form a G-quad. However, after the binding of the cancer cell to the G-quad, there was little to no observable CV signal of the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The sensor is then regenerated using the AS1411 complementary strand to allow it to be reused for the next round of cancer cell detection [47].

Many studies also incorporated electrochemical methods in the DNA detection sensors. Recently, Yao and co-workers integrated the isothermal exponential amplification (EXPAR) with hybridization chain reaction (HCR) of DNAzyme in addition to the merits of electrochemical method for the development of an ultrasensitive DNA sensor for avian flu strain H7N9 (Figure 7). A single-stranded DNA derived from the hemagglutinin (HA)-encoding sequences from avian influenza A (H7N9) was used as the target gene in their sensor development. The molecular beacons (MBs) contained the G-quad that cannot be opened up or assembled together without the target. A duplex probe was anchored to the surface of the electrode. Once the target DNA hybridized with one of the sequence of the probe, the amplification through toe-hole-mediated strand displacement (TMSDR) was initiated. The duplex with the target gene was released to the solution and initiated the EXPAR with a primer. On the other hand, the other bound strand of the probe on the electrode was then hybridized with the MBs. The hairpin structure of the MBs was opened through TMSDR and resulted in the formation of G-quad nanowires. Hemin was bound to the G-quad nanowires and formed DNAzyme that catalysed the oxidation of TMB, generating an increase in electrochemical signal in reduction current to be measured. This DNA sensor for avian flu (H7N9) was ultrasensitive with the limit of detection at femtomolar levels [48].

Applying electrochemical methods, the detection limits of biosensors can be improved to allow remarkably low levels to be detected. In addition to the reduced cost of the biosensor with rapid response, the ability to miniaturize the assay allows it to be considered for point-of-care applications. Such remarkable advantages have drawn many researchers to the development

Figure 7. Schematic diagram of the DNA detection of H7N9 integrating EXPAR and HCR for electrochemical assay.

of biosensor systems based on electrochemistry for the detection of protein, DNA and other analytes.

7. G-quad integrates with quantum dots

The emergence of quantum dots (QD) in molecular sensing methods has attracted a lot of attentions from many researchers. QD have been extensively used as optical labels, probes for FRET and also as energy acceptors from metal complexes or from energy generated by luminescence or photoelectrochemical for the detection of DNA or formation of aptamer–substrate complexes [49]. Wilner and co-workers utilized the G-quad forming DNAzyme, which is conjugated to semiconductor QDs. It functions as a light source that promotes chemiluminescence resonance energy transfer (CRET) to QDs.

In this assay, anti-thrombin aptamer was used where it was also previously used in a colorimetric sensor for thrombin whereby hemin/G-quad forming DNAzyme was used to catalyse the oxidation of ABTS in the presence of H_2O_2 . Meanwhile, in this assay, the hemin/G-quad forming DNAzyme aptamer–thrombin complex was used to generate chemiluminescence in the presence of H_2O_2 /luminol. Then, in close proximity, this complex was able to excite the CdSe/ZnS QDs, resulting in CRET to the QDs triggering the luminescence of QDs. The glutathione-modified CdSe/ZnS QDs were attached with the anti-thrombin aptamer. A low CRET and chemiluminescence signal was observed even if it was without thrombin due to the diffusional hemin. Upon adding thrombin into the assay, the chemiluminescence signal and the CRET-stimulated luminescence of the QDs were greatly increased. The increase in CRET signals was proportional to the concentration of thrombin detected [50].

Besides the detection of aptamer–substrate complex, the detection of metal ions can also be carried out by exploiting the hemin/G-quad forming DNAzyme complex. The same group also developed a G-quad-based sensor to detect mercury ions (Hg^{2+}) (Figure 8). This assay

comprised two strands of DNA whereby each of the strand has G-rich sequence forming DNAzyme (1) and (2) and a T-containing site that functions as the recognition site of Hg^{2+} , (3) and (4). As the T-containing sites of (3) and (4) were partially complementary, (1) and (2) could not assemble to form a G-quadruplex. However, in the presence of Hg^{2+} , the T- Hg^{2+} -T complexes formed between the T-containing sites of (3) and (4) and formed a duplex structure. Therefore, this led to the formation of the hemin/G-quad complex between (1) and (2). The hemin/G-quad complex catalyzed the oxidation of luminol in the presence of H_2O_2 , generating chemiluminescence signal. Then, this signal acted as an internal light source that can excite CdSe/ZnS QDs, resulting in CRET to the QDs. Then, this stimulated the luminescence signal from QDs [51].

Figure 8. Detection of Hg^{2+} through CRET from luminol that is oxidized by DNAzyme to QD.

8. Conclusion

In comparison with conventional reporter systems, G-quad-based systems provide many advantages in terms of cost, thermostability and ease of synthesis. With all these advantages, it makes them very useful for the development of sensing probes for diagnosis, applicable to DNA, protein and metal detection. The vast application of G-quad structures to generate various forms of readouts ranging from colorimetric to electrochemical-based readouts puts it at the forefront of sensing reporters. G-quad-based assays are flexible as they can be adapted to many different types of diagnostic platforms. This makes G-quad an attractive alternative for the development of sensitive reporter systems for the sensing of various samples ranging from small drug molecules, chemical compounds to large biomolecules.

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Author details

Qiuting Loh and Theam Soon Lim*

*Address all correspondence to: theamsoon@usm.my

Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden, Penang, Malaysia

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