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Review article

New insight into G-quadruplexes; diagnosis application in cancer

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ABSTRACT

Biochemical properties and flexibility of nitrogenous bases allow DNA to fold into higher-order structures. Among different DNA secondary structure, G-quadruplexes (tetrapelexes-G4) - which are formed in guanine rich sequences – have gained more attention because of their biological significance, therapeutic intervention, and application in molecular device and biosensor. G4-quadruplex studies categorize into three main fields, in vivo, in vitro, and in silico. The in vitro field includes G4 synthetic oligonucleotides. This review focuses on the G-quadruplex synthetic aptamers structure features and considers the applicability of G4-aptamers for cancer biomarkers detection. Various biosensing methods will be reviewed based on G-quadruplex aptamers for cancer detection.

1. Introduction

1.1. Nucleic acid aptamer (Nu-Apt)

Nucleic acid molecules are involved in many crucial biological processes such as expression, transport [1], and genetic material storage [2]. They can function as an enzyme (deoxyribozimes and ribozymes) and as receptors (aptamers). Nucleic acids are also one of the most promising materials in nano-biotechnology because of their programmable and predictable assembly [3].

Nucleic acid aptamers are single-stranded DNA or RNA molecules, usually between 30 and 70 nucleotides in length. Nu-Apts can recognize and bind with high affinity to a wide range of target molecules, with a dissociation constant from the micromolar range to lower values, because of their unique three-dimensional folding [4,5]. Compared to their protein counterparts (antibodies), Nu-Apts have many advantages [6,7]: 1. Smaller size can cause efficient uptake of the aptamer to biological targets 2. production in animal-free systems by a chemical process which is low cost and readily scalable 3. Lower immunogenicity 4. Astonishing stability (thermal and pH stability) and can be reversibly denatured.

Due to these features, Nu-Apts are a valid alternative to antibodies. They can be used in various applications, including diagnostic, imaging, and therapeutic (as drug, drug delivery system and targeting moieties) applications [5,7–9].

The most effective method to produce Nu-apt with high-affinity for a certain target is a process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) (Fig. 1) [10,11].

1.2. G- quadruplex aptamer; structure and feature

It is necessary for the nucleic acid to adopt a variety of three-dimensional structures to do their functional roles. For years, DNA was regarded as a passive and rigid molecule with a canonical B-form structure, which was responsible for storing genetic information. However, according to experimental data, it is evident that this macromolecule can adopt an assortment of structures based on its distinctive sequence motif and interaction with a different protein, such as slipped structures, bent DNA and sticky DNA, Z-DNA, triplexes (H-DNA), tetraplexes, cruciform, and hairpins [12–14]. One of these interesting noncanonical structures is G-quadruplexes or DNA-tetraplexes. In 1910, Ivar Bang's paper revealed that guanylic acid forms a gel at a high millimolar concentration [15]. The unusual physical property resulting from Bang's experience suggests that G-rich sequences in DNA have the potential to form higher-order structures. Fifty years later, X-ray diffraction data on guanylic acid demonstrated that guanylic acids can

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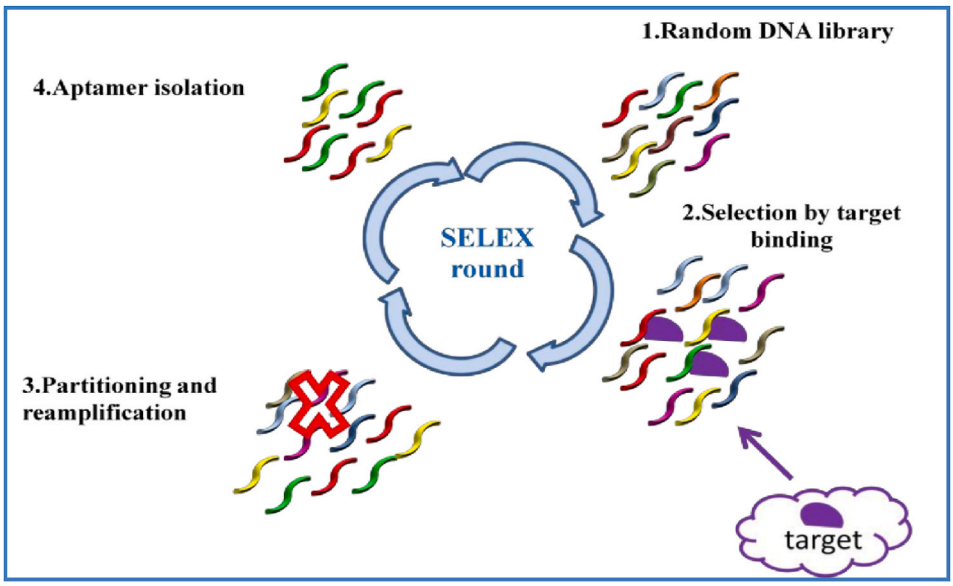


Fig. 1. Schematic presentation for SELEX process.

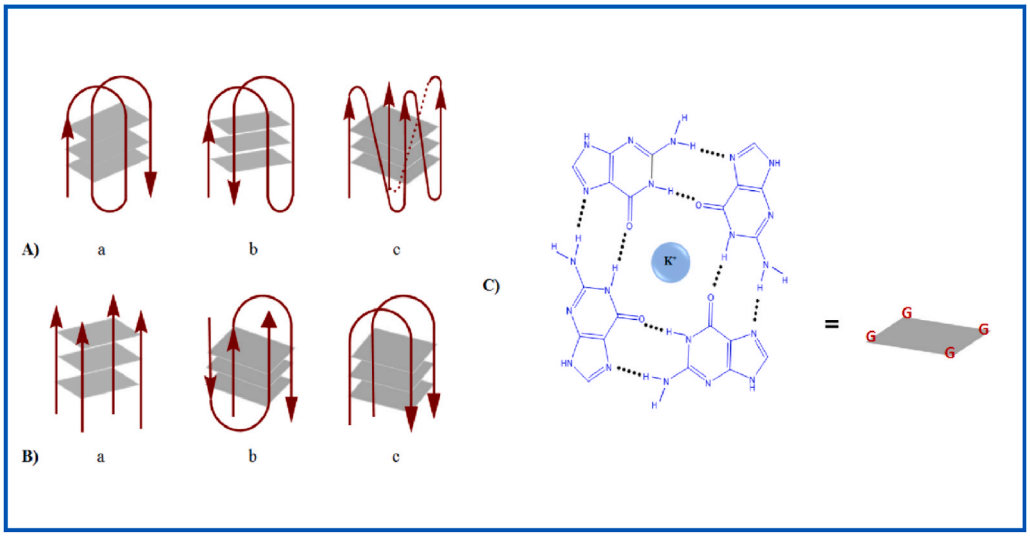


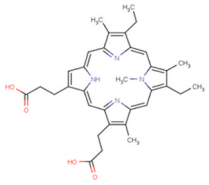
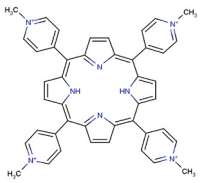
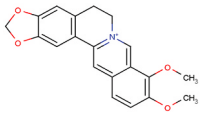
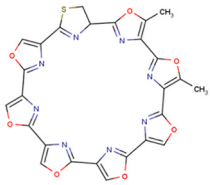
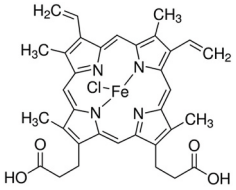
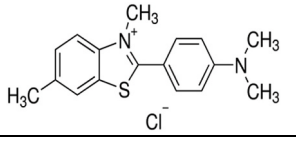
Fig. 2. Structural polymorphism of G-quadruplex. A) intramolecular structure: a,b)antiparallel c) parallel. B) Intermolecular structure including a) tetramolecular parallel structure and b,c) Bimolecular G-quadruplex. C) G-quartet structure.

assemble into tetrameric structures. These tetrameric units account for a gel-like property in the aqueous solution [16]. In the late 1980s, it was shown that a particular oligonucleotide sequence containing runs of three or four adjacent guanines (G-tract, G-quartet), formed tetra-stranded DNA structures called G-quadruplexes or G4 [17,18]. G-quartets _ the building blocks of G-quadruplex_ are a square co-planar arrangement of guanine in which each atom bind to the adjacent guanine by Hoogsteen-type hydrogen bonds. Two or more G-quartets stack on top of each other, giving rise to a G4 structure and the interfering sequences are extruded as a loop from the structure (also G4 structure may do not have loops). There is a difference in the sequence and size of the loop regions, but they are usually small in length (1–7 nucleotide), and smaller loops lead to a more stable structures [19]. The stability and formation of the structures is dependent on monovalent cations such as K^+ and Na^+, which become occupied in the central cavities between the stacked quartet and neutralized the electrostatic repulsion of guanine oxygens [20]. G-quadruplex DNA structures show extremely high polymorphism, which can be divided into several subclasses based on the number of participant strand, strand orientation (parallel, antiparallel, or hybrid) and connecting loop structure (Fig. 2). According to strand stochiometry, G-quadruplex forms intramolecular (one strand) and intermolecular folding (two or four strands) [21].

It is worth mentioning that G-quadruplexes have twice the negative charge density per unit length compared to duplex DNA. Due to this high electrostatic potential, G-quadruplexes are prone to bind strongly to positively charged proteins or small molecules, although some of these ligands are neither neutral or negatively charged, such as hemin or *N*-methyl mesoporphyrin IX (NMM) [22]. Also, the relationship between different quadruplex structures (parallel, antiparallel) and different binding affinities for hemin has been confirmed. More precisely, various G4 structures indicate different peroxidase-like activities [23].

Apart from the possibility of DNA quadruplex formation in vitro, numerous studies have demonstrated the existence of G-quadruplexes in vivo. It is worth mentioning that the distribution of G-quadruplexes in the genome is not random, and these structures are particularly found in gene promoters and telomeres. Also, researchers have studied the

Table 1
Some example of G-quadruplex binding ligands.

Name	More detail	Structure
NMM (*N*-Methyl Mesoporphyrin IX)	Anionic Porphyrin, water soluble, increase in fluorescence in the presences of G-quadruplex, preferentially recognize parallel strand G4 rather than antiparallel	
TMPyP4	Cationic porphyrin, human telomerase inhibitor, Fluoresces highly in the presence of quadruplex DNA	
Berberin	An Isoquinoline alkaloids with antibacterial activity, bind with G-quadruplex and inhibit telomere elongation	
Telomestatin	Natural macrocyclic molecule telomestatin, Soluble in DMSO, A potent telomerase inhibitor	
Hemin	Natural porphyrin, soluble in ammonium hydroxide and DMSO, stacks externally the G-quartet and show enhanced peroxidase-like activity	
Thioflavin T (ThT)	A benzothiazole dye that increases in fluorescence upon binding to G4-quadruplex	

functional role of quadruplex structures; here are some of the most important functions: regulating telomere maintenance, DNA replication, transcription, and epigenetic regulation. Due to its potential biological relevance such as a functional role in telomere maintaining process, it becomes an ideal target for drug discovery in cancer treatment. Today's knowledge about DNA quadruplex structure provides a way to design new specific g-quadruplex ligands using in silico drug screening approaches. Since this review study intended to examine the application of DNA quadruplex-aptamers in cancer disease diagnosis, hence the aforementioned brief explanation about in vivo and in silico G-quadruplex studies would be sufficed [12,24].

1.3. Specific ligands and proteins for G-quadruplex aptamer

There are more than a thousand ligands that bind to G4-aptamers and stabilize them. A group of researchers established a database to record and categorize these ligands [25]. (See http://g4ldb.org/). Although ligands have high-affinity binding to the structure, usually, they are not specific. Nonetheless, a few examples of good G-quadruplex binding elements with more detail are mentioned in Table 1.

There is also a wide variety of proteins that have been reported to bind to G4-aptamers like proteins that stabilize G-quadruplexes, helicases, G-quadruplex-specific nucleases and so on [26]. Furthermore, many aptamers which recognize proteins adopt G4 –folding, the best-known example being the thrombin-binding aptamer [26]. Among the variety of ligands introduced for G4-aptamers, we were focused on two well-known ligands, hemin and thioflavin T, and reviewed their application in biodiagnostics, also analyzing the G4-aptamer folded potential in cancer therapy.

1.4. Cancer disease overview

Cancer is a group of diseases characterized by abnormal cell growth without control. The cancer cells can spread and invade the other part of the body by blood and lymph systems [27]. Cancer demonstrates heterogeneity in its biological features. However, cancer usually shows hallmarks such as resistance to cell death and senescence, continuous proliferation even with the contrary signal, aberrant metabolism, promoted blood vessel construction, self-renewal, metastasis and so on [28]. Different substances, known as cancer biomarkers, such as protein, DNA, RNA, etc. [29], can be measured to indicate either the presence of disease or the stages of the disease. According to the WHO report, cancer is one of the top causes of death worldwide. Namely, it is of great importance to diagnose the disease in the early stages and find effective ways to cure it. Nowadays, there has been a large growing body of research on cancer biology, detection, and therapy. So many researchers have worked on cancer biomarkers to detect the diseases in the pre-cancerous phase, and also many have studied the most effective molecular target to cure it [30–34]. In this review, we are going to focus on the G-quadruplex based aptamers and its diagnostic applications in cancer.

2. G-quadruplex in sensing platform

Accurate and early detection of disease is a crucial factor in the effective treatment of any disease. In the case of cancer due to its long latency, screening and early detection of cancers in the preliminary stage has great importance and can improve the patient's survival. Aptamers have been used widely in the sensory field as bio-recognition elements due to their advantages (small size, low cost, stability, sensitivity), and they can detect a wide range of biomarkers like small molecules, ions, biomarkers, and even whole cells [35,36]. Many articles research the application of aptamers in biosensors as biorecognition elements, but here we aimed to review the biosensors which used quadruplex aptamers as signal transduction probe in cooperating with different kinds of biorecognition elements.

As mentioned above, there are several ligands for quadruplex aptamer. One of the most interesting ligands is [Fe (III)-protoporphyrin IX] Hemin, which binds specifically to the G-quadruplex and forms a Hemin/G-quadruplex complex, often referred to as a peroxidase-mimicking DNAzyme. Many researchers studied the relation between the different G-quadruplex structures and peroxidase-like activity, affected by the ability to bind hemin. The results showed that all-parallel or mixed strand polarity has the highest enzymatic activity. Precisely hemin attaches to the external guanine, so the parallel form is more favorable for ligand binding through end-stacking [37–39]. In 1998, Sen and co-workers reported the DNA aptamer-hemin complex with peroxidase activity for the first time [40]. G-quadruplex DNAzyme with peroxidase activity can catalyze different chromogenic substrates (2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid or 3, 3′, 5, 5′-tetramethylbenzidine sulfate (TMB)), fluorogenic substrates (such as Amplex red) and chemiluminescence substrates (luminal) to produce a signal [41].

Thioflavin T (4-(3,6-dimethyl-1,3-benzothiazol-3-ium-2-yl)-N,Ndimethylaniline, ThT) is reported as a selective fluorescence probe for human telomeric quadruplexes [42]. There are so many quadruplex binding dyes like *N*-methyl mesoporphyrin IX (NMM) or berberine, but their interaction is non-specific. Indeed these dyes interact with other

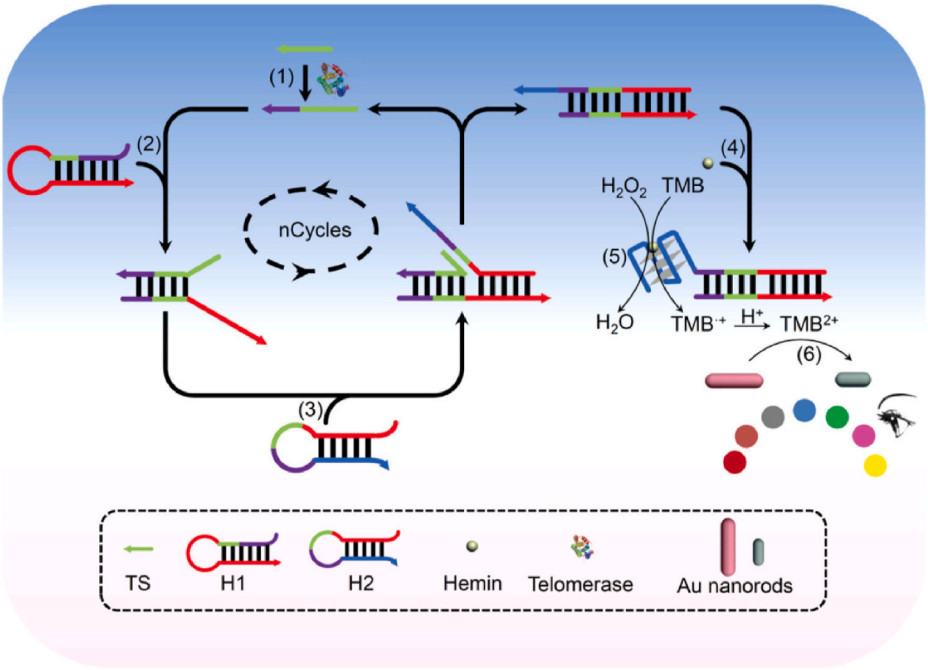


Fig. 3. Colorimetric method for determination of telomerase activity by employing etching of Au nanorods and catalytic hairpin assembly.

forms of DNA (like double-strand DNA) and cannot differentiate quadruplexes from other structures [43–45]. The free ThT has a weak fluorescence signal, whereas bound ThT to G-quadruplex leads to an enhancement in the fluorescence signal. Therefore, several detection systems have been introduced based on ThT as a light-up fluorescence probe [46–49].

2.1. Optical sensors

Biosensors can be classified into different groups depending on the method of signal transduction: optical, electrochemical, thermometric, piezoelectric, or magnetic. The optical and electrochemical sensing platforms are the most common among biosensors. Optical biosensors work by measuring the absorbed and emitted light followed by a specific reaction. Broadly speaking, optical biosensors are based on general modes divided into label-free and label-based. Optical phenomena such as fluorescence, absorption, luminescence, surface plasmon resonance (SPR), reflection produce the output transducer signals in optical biosensors [50–52].

2.1.1. Colorimetric approach

Colorimetric detection has been widely used in biosensing applications due to its inherent advantages, including simple operation, low cost, and adaptable sensitivity [53–56].

MicroRNAs are single-stranded non-coding RNA molecules which lengths between 18 and 23 nucleotides that function as a post-transcriptional regulation factor in gene expression. These small RNA molecules have emerged as a novel cancer biomarker, which provides the new area for numerous research based on microRNA assay for cancer detection [57–59]. Wu et al. have developed a colorimetric platform for amplified detection of let-7 microRNA [60]. Catalyzed assembly and hybridization chain reaction between four unlabeled hairpins produced numerous G-quadruplex structures, which are able to show peroxidase-like activity upon association with hemin. The proposed sensing strategy was shown highly sensitive for miRNA detection with a detection limit of 7.4 fM and a linear range between 10 fM to 10 nM. Recently Hosseinzadeh and co-workers reported an ultrasensitive biosensor based on triple amplification strategy for miR-21 detection. The G-rich sequences are responsible for the third step in the signal amplification process, which form G-quadruplex DNAzymes in the presence of hemin produced the colorimetric signal [61].

Human telomerase is a ribonucleoprotein complex that catalyzes the reverse transcription of telomeric repeat $(TTAGGG)_n$ at the end of chromosomes. Many studies had been proven that telomerase is over-expressed in human tumor cells (over 85%) and causes unlimited proliferation, tumorigenesis, and immortalization [62–64]. Thus the assessment of the telomerase activity will be a valuable biomarker in cancer diagnosis. Wang and co-workers designed a new visual technique to detect telomerase activity. This colorimetric method is based on the etching of gold nanorods (Au NRs) induced by the oxidation state of TMB. Also, catalytic hairpin assembly (CHA) was operated as a signal amplification strategy to enable naked-eye detection. As it is shown in Fig. 3, two hairpin DNA probes were involved in the CHA. The presence of telomerase could start the extension of the telomerase substrate and subsequent hybridization chain reaction. Finally, cyclic CHA produced G-quadruplex DNAzyme in the presence of hemin, and this DNAzyme with peroxidase-like activity catalyzed the oxidation between the H_2O_2 and TMB. The resulted TMB^{2+} could etch Au NRS and decrease their aspect ratio and led to blue-shifted of longitudinal localized surface plasmon resonance (LSPR), and correspondingly multiple obvious colors [65].

Also, many researchers have worked on cancer-related genes as a biomarker [66–69]. Shen and co-workers utilized a multifunctional molecular beacon with palindromic tail for colorimetric detection of the p53 gene. The presence of the target gene led to the opening of the palindromic molecular beacon and the beginning of strand displacement amplification (SDA). The enhanced colorimetric signal was achieved by the intervening of nickase and generation of G-quadruplex DNAzymes. This sensitive proposed method has a linear response range from 10 pM to 200 nM [70].

2.1.2. Fluorometric approach

Another optical biosensor subclass is fluorescence-based detection. This technique has been widely used in biomedical applications due to its easy operation, high sensitivity, and high spatial and temporal resolution [71–75]. Here we mention some biosensors based on quadruplex fluorescence probes (including ThT and NMM) which were used in cancer biomarkers detection, including microRNA, telomerase assay,

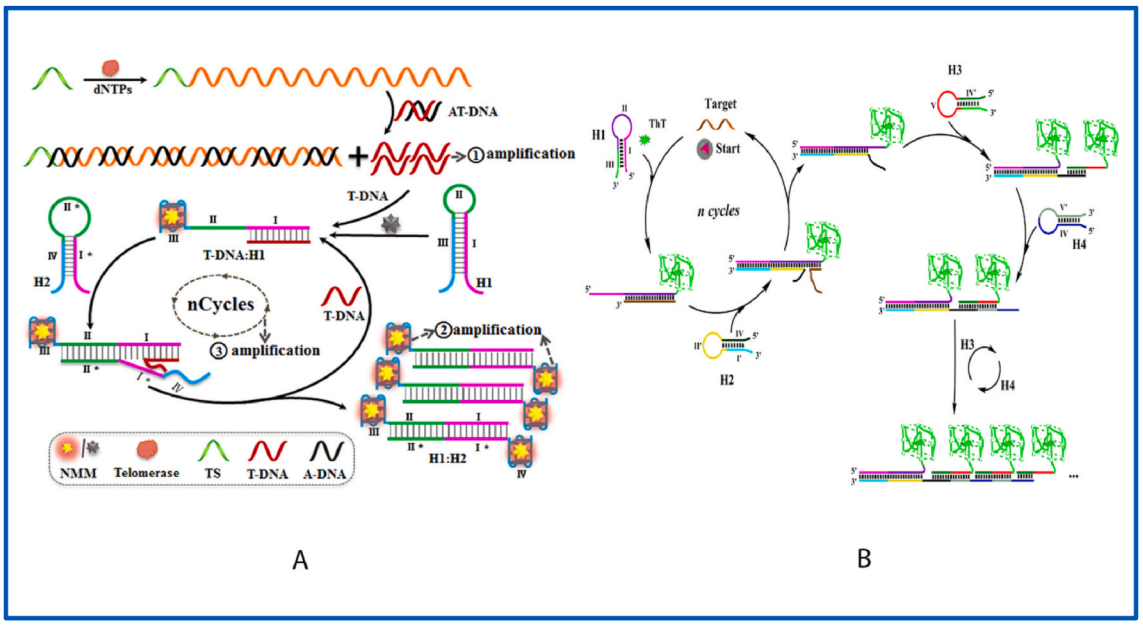


Fig. 4. Schematic illustration of Fluorescence detection for A) Telomerase activity in HeLa cell B) microRNA based on catalyzed hairpin assembly amplification and hybridization chain reaction.

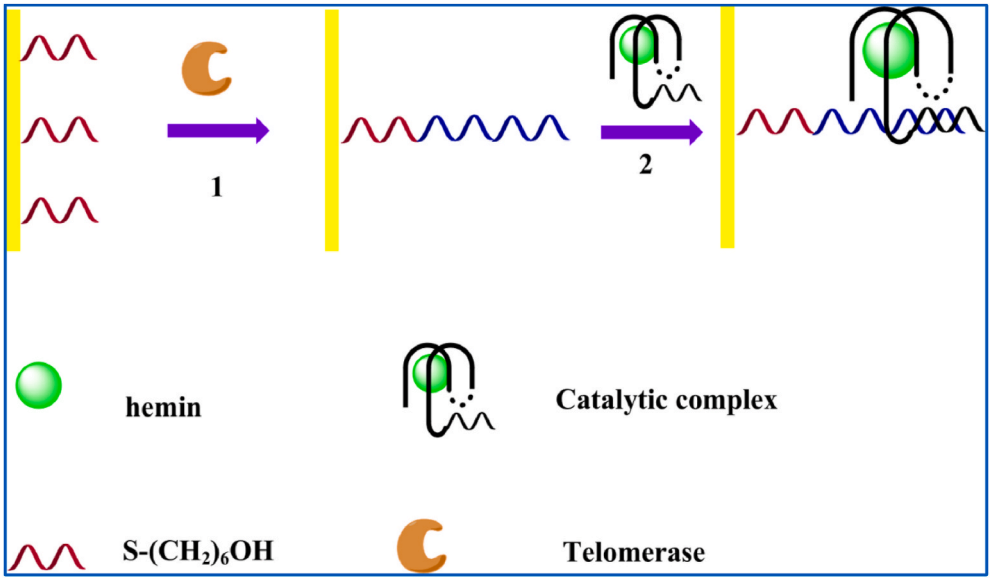


Fig. 5. Amplified Chemiluminescence Surface Detection of Telomerase Activity Using G-quaruplex structure,1) Primer strand (red) elongation via telomerase 2) hybridization between complementary domain from catalytic complex and telomere repeat units which generate chemiluminescence signal. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

DNA methyltransferase assay, protein biomarkers, and so on.

A label-free fluorescent strategy based on triple amplification was developed using a quadruplex fluorescence probe for telomerase activity detection. Telomerase activity in HeLa cells has been detected in the range of from 1 to 3000 cells in a homogeneous solution [76] (Fig. 4A). Li et al. proposed a novel fluorescent biosensor for telomerase activity detection. Two oligonucleotides had been used in this work, a telomerase primer and a padlock probe. Telomerase can extend the primer into a short telomerase extension product (GGGTT), in the presence of dGTP and dTTP. Hybridization between telomerase extension product and padlock probe and also T4 DNA ligase involvement led to the conversion of linear padlock probe into a ligated, circularized molecule. The obtained circular probe serves as a template and telomerase product acts as a primer to initiate circular rolling circle amplification (RCA). A nicking enzyme-mediated exponential RCA produced numerous G-rich sequence which could fold into G-quadruplex structures. Specific binding between thioflavin T and G-quadruplex structure could bring fluorescence signal to telomerase activity detection [77].

It is worth mentioning that there is a comprehensive review article in the context of detection and analysis of telomerase activity, based on a novel series of fluorescence probes (gold and mesoporous silica nanoparticles, quantum dot, and labels such as aggregation-induced emission luminogens and conjugated polymer) other than quadruplex fluorescence probes [78].

Li and colleagues have reported a dual-amplified approach for fluorescence detection of microRNAs. Hybridization chain reaction

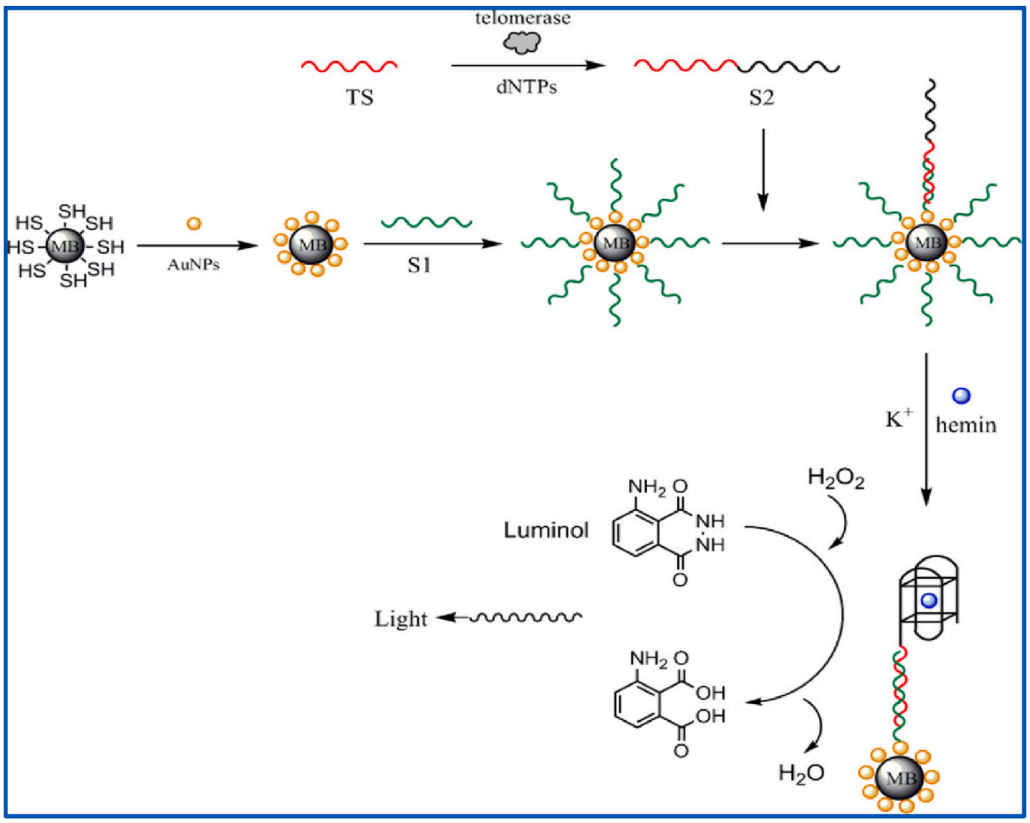


Fig. 6. Schematic illustration of chemiluminescence detection of telomerase activity based on telomere elongation and formation of G-quadruplex DNAzyme. The chemiluminescence signal was generated in the presence of luminol and H2O2.

(HCR) circuits and catalyzed hairpin assembly (CHA) amplification methods were used in this platform to enhance the signal and improve the sensitivity of the sensor. These two non-enzymatic amplification methods utilized four hairpin probes, H1, H2, H3, and H4. The presence of the target strand (miRNA-141) leads to the interaction between H1 and target and also a subsequent set of reactions which finally cause the formation of numerous G-quadruplex structures for fluorescence detection in cooperation with ThT. This sensor achieves a wide concentration range for microRNA detection and a detection limit of 36 fM [79]. The manner of hairpin probes involvement in CHA cycle and HCR circuit is illustrated in Fig. 4B.

A group of enzymes known as the DNA methyltransferases (DNMT) is responsible for the DNA methylation process, which includes the covalent addition of the methyl group from *S*-adenosylmethionine (SAM) to the carbon 5 position of the cytosine ring. Abnormal DNA methylation and its related carcinogenesis role make it a reliable cancer biomarker [80,81]. In this regard, our group has designed the fluorimetric sensors based on DNA-templated silver nanoclusters and graphene quantum dots for activity assay of DNA methyltransferase [82,83]. Cui et al. have used strand displacement amplification (SDA) and exponential rolling circle amplification (ERCA) technique for sensitive fluorescence detection of DNA MTase activity. First, they designed a hairpin probe with a long stem which contains a methylation site for DNA MTase recognition. The next hairpin probe was methylated by DNA MTase and specifically cleaved by DnpI endonuclease, respectively. The resulting product was an unstable hairpin probe and underwent structural changes and ultimately became a single-strand DNA (ssDNA). This ssDNA hybridized with the overhang of the hairpin probe and serve as a trigger strand to begin SDA. Many primers were produced during the SDA process in the presence of polymerase and nicking enzyme. The produced primer from SDA went into RCA platform, which finally led to synthesize large numbers of G-rich sequences. Upon the addition of K^+, the G-rich sequence folds into the quadruplex structure and interact with *N*-methylmesoporphyrin IX (NMM) and showed an enhanced fluorescent signal [84]. Also, a nicking endonuclease-mediated multiple primers-like rolling circle amplification (RCA) strategy was developed for sensitive and label-free for DNA MTase activity detection. They used a dumbbell probe as an RCA template which was constructed through blunt-end ligation of two hairpin DNA probes. This dumbbell shape template has two pivotal parts in the loop, the primer binding sequence and the c-rich sequence, and also includes specific sequences in the stem region as a recognition site for both M.SssI MTase and restriction endonuclease *Hpa*II. The next step was the interaction between dumbbell and restriction endonuclease. In the presence of M.SssI MTase, the methyl group was added to the cytosine and the recognition site was blocked for endonuclease cleavage, so the dumbbell structure stayed complete and integrated. This complete dumbbell can be used for RCA. On the other side, endonuclease can be recognized and cleaved the specific recognition site in the absence of MTase, due to the lack of methyl group. The destroyed dumbbell was useless for RCA. RCA amplification will be initiated with hybridization between phi29 DNA polymerase and primer, resulting in numerous copies of G-rich sequences. The G-rich sequence can be formed into G-quadruplex structure and specifically interacted with ThT, and provide an enhanced fluorescent signal for M.SssI MTase activity detection [85].

2.1.3. *Chemiluminescence approach*

Chemiluminescence, which is the generation of light from a chemical reaction, takes its place among other spectroscopic techniques owing to its sensitivity, cost-effectiveness, selectivity, simple instrument and no need for external excitation light sources and monochromator filter [86,87]. Hemin/G-Quadruplex DNAzyme can catalyze the oxidation reaction of luminal-H_2O_2 and use for producing the chemiluminescence output signal [88].

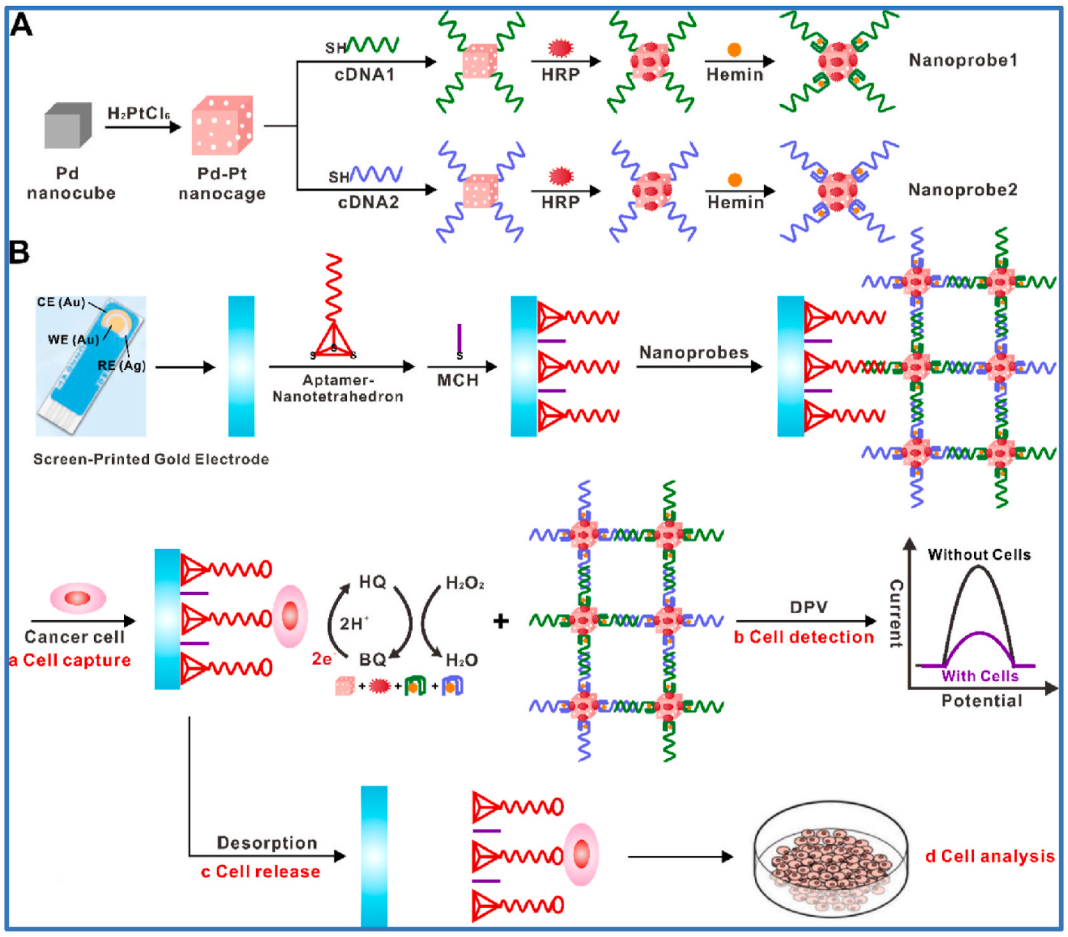


Fig. 7. Schematic diagram of electrochemical biosensor for the monitoring of HepG2 tumor cells with self-assembled DNA nanostructure. A) General procedure for the fabrication of Pd–Pt nanocage–HRP–cDNA/hemin/G-quadruplex hybrid nanoprobes. B) The proposed aptasensor for the efficient capturing and detection and release of circulating tumor cells (CTCs).

The amplified chemiluminescence based strategy was introduced for the analysis of DNA and telomerase activity. Hemin/G-quadruplex DNAzyme had been used as a label and catalyzed the luminal-H_2O_2 reaction. Fig. 5 shows a schematic illustration of the above biosensor [89]. In the other study of evaluation of telomerase activity, Li et al. proposed a chemiluminescence method based on G-quadruplex formation. The sensor consists of magnetic microbeads (MBs) which decorated by gold nanoparticles (AuNPs). The MBs had thiol groups on their surface, and AuNPs were placed on MBs surface through Au–S bond. Two DNA strands were used in the sensing system, the capture DNA which is immobilized on MB-AuNPs and the telomerase primer. The elongation process had happened in the presence of telomerase and dNTPs, and a series of TTAGGG sequences were added to the primer. Then the primer strand hybridized to the capture DNA. Finally, by providing the right condition, the G-quadruplex structure was formed and produced chemiluminescence signal [90] (Fig. 6). Furthermore, Wang and co-workers introduced a simple and sensitive chemiluminescence sensing strategy for telomerase activity detection based on a two-stage isothermal amplification reaction. An elaborate probe consisting of several different parts was designed and served as a telomerase substrate primer. The primer was elongated during telomerization via telomerase and dNTPs. The new extended primer acted as a template for strand displacement amplification (SDA). The output products of SDA were DNAzymes (C), G-rich telomeric repeat units (R), and short oligonucleotides (A′). Then short oligonucleotide functioned as a trigger for exponential amplification reaction (EXPAR). The final result was the production of a large number of DNAzymes and telomeric repeats which were capable of forming G-quadruplex nanostructures. Enhanced chemiluminescence signal was produced by the catalytic activity of the hemin/G-quadruplex complex in the presence of luminal-H_2O_2 [91].

Carcinoembryonic antigen (CEA), a glycoprotein involved in cell adhesion, is a significant tumor marker for cancer detection. CEA is commonly found at a very low level in the blood of healthy adults while it had been shown that CEA blood level might be increased in certain types of cancer such as colon cancer, lung cancer, gastric cancer, breast cancer, and ovarian cancer [92]. To evaluate the CEA as a biomarker in cancer diagnosis, Khang and co-workers have proposed an all-in-one dual-aptasensor with 1, 1′-oxalyldiimidazole (ODI) chemiluminescence detection. The dual aptamer consisted of three parts, hemin aptamer, linker sequence (AAAAA), and CEA aptamer. G-quadruplex DNAzyme was formed through the interaction between hemin and its specific aptamer. The HRP mimicking DNAzyme catalyzed the amplex red and H_2O_2 reaction and produced resorufin. Addition of ODI and H_2O_2 in the following step led to the generation of a chemiluminescence signal. Because of the competitive condition between hemin and CEA to bind dual aptamer, the signal output was lessened in the presence of CEA [93]. The limit of detection for the above biosensor was as low as 0.58 ng/ml.

Recently, professor Li's group developed a novel chemiluminescence sensing strategy for miRNA detection which involved an acute myocardial infarction (AMI). The above-mentioned projects utilized spherical nucleic acid enzyme (SNAzyme) to developed sensitive methodologies and the presence of a dense layer of G-quadruplex on gold nanoparticles responsible for amplified chemiluminescence signal.

Table 2
A summary of developments of quadruplex-based biosensors in cancer biomarker detection.

Strategy	Biomarker	Limit of detection	Linear rang	Ref
Colorimetric detection	Let-7a microRNA	7.4 fM	10 fM to10 nM	[60]
	miR-21	1 aM	1 aM to 5 pM	[61]
	Telomerase activity	15 cells	20-500 cells	[65]
	PIK3CA gene mutation	0.2%	0.2%–50.0%	[66]
	K-ras gene	10 pM	0.01–150 nM	[67]
	p53 gene	1 fM		[68]
	p53 gene	25 fM		[69]
	target p53 gene	Down to 10 pM	10 pM to 200 nM	[70]
Fluorescent Detection	Telomerase activity		1-3000 cells	[76]
	Telomerase activity	1 cell	1 to 100 HeLa cells	[77]
	miRNA-141	36 fM		[79]
	DNA methyltransferase activity	8.1×10^{-5}U/mL	4.0×10^{-4} to1.0×10^{-2}U/mL	[84]
	DNA methyltransferase activity	0.0011 U/mL	0.008–50 U/mL	[85]
chemiluminescence	Telomerase Activity	1000 HeLa cells		[89]
	Telomerase Activity		100 to 1000	[90]
	Telomerase Activity			[91]
	carcinoembryonic antigen (CEA)	0.58 ng/ml	0–200 ng/ml	[93]
Electrochemical	DNA species related to oral cancer	0.02 fM	0.08–8.0 fM	[98]
	miRNA-21	1.6 fM	5.0 fM to 1.0 pM	[99]
	Cancer cell (HepG2)	30 cells per mL	1×10^2 to 1×10^7 cells/mL	[100]
	Cancer cell (HepG2)	15 cells per mL	1×10^2–1×10^7 cells/mL	[101]
	Cancer cell (HepG2)	5 cells per mL	10 to1×10^6 cells/mL	[102]

The interesting and admirable results of the above researches can be generalized to AMI point-of-care diagnosis [94,95].

2.2. Electrochemical detection

Electrochemical biosensors analyze the biological sample based on redox reactions and electron transfer between electroactive species. According to the type of generated signal, these devices have three categories: amperometric, conductometric, and potentiometric. Various kinds of biomolecules can be used as biological recognition elements such as nucleic acids, enzymes, proteins, antibodies, etc. [96]. Among them, DNA-electrochemical biosensors are formed by the immobilization of a DNA layer (the biological recognition element) on the electrode surface (the electrochemical transducer). The interaction between target analytes and DNA causes the structural, morphological, and electrochemical changes in the DNA layer and produces an electrical signal used for an accurate diagnosis. Some advantages of DNA electrochemical biosensors are high sensitivity, easy modification, and requirement of small volumes of the analyte [97]. Here, the results from the more specific studies recently reported on G-quadruplex-based electrochemical biosensor will be discussed in the field of cancer detection.

A electrochemical biosensor was developed for detection of DNA species related to oral cancer based on nuclease-assisted target recycling and G-quadruplex/hemin DNAzyme. The strategy showed high sensitivity toward the target DNA owing to the target recycling mechanism. The proposed sensor showed a good linear range for the target and could detect amounts as low as 0.02 fM of the target DNA [98]. Miao and colleagues have designed an electrochemical biosensor for miRNA-21 detection by employing iridium (III) complexes as the catalyst. Two probes were used in this sensor, a capture probe immobilized on the Au electrode surface and a detection probe, which was a G-rich sequence, labeled with methylene blue (MB) and modified onto gold nanoparticles. Both probes were immobilized on the gold surface based on Au–S chemistry. Target strand (miRNA-21) could act as a linker to connect the nanoparticles and the electrode surface forming a sandwich DNA complex. The detection probe then formed a quadruplex structure in the presence of K^+ and selectively interacted with the iridium (III) complex. The immobilized iridium (III) complex catalyzed the reduction of H_2O_2, followed by an electrochemical signal change using MB as an electron mediator [99].

Accurate detection of cancer cells in the early stage of the disease is important for clinical diagnostic and cancer therapy. Sun et al. have designed an electrochemical biosensor for the detection of human liver hepatocellular carcinoma cells (HepG2) based on dual recognition and enzymatic signal amplification. The above sensory system was based on the formation of the aptamer-cell-nanoprobe sandwich-like superstructure on a gold electrode surface in the presence of the target cell. The specific HepG2 aptamer was immobilized on the electrode surface via Au–S chemistry, which could recognize the HepG2 cells. Nanoprobes were gold nanoparticles modified with horseradish peroxidase (HRP) and G-quadruplex/hemin/aptamer. The HRP and DNAzyme catalyzed the oxidation of the hydroquinone (HQ) and benzoquinone (BQ) in the presence of H_2O_2 and produced an electrochemical signal by measuring the increase in the reduction current [100]. They later proposed an elaborate electrochemical cytosensor for HepG2 detection using hybrid nanoelectrocatalysts and enzymes for signal amplification. The principle of this sensor is similar to the former one, which is based on the formation of a sandwich-like architecture but has some characteristic differences. In this cytosensing platform, a modified glassy carbon electrode was used instead of the gold electrode and the new nanoprobe introduced. Au@Pd core-shell nanoparticles and modified magnetic Fe_3O_4/MnO_2 beads were prepared separately. The nanoprobe includes the immobilized G-quadruplex aptamer and HRP on the Fe_3O_4/MnO_2/Au@Pd nanoelectrocatalysts surface. An enhanced electrochemical signal is the result of the catalytic activity of hybrid nanoelectrocatalysts and G-quadruplex/hemin DNAzyme and the natural HRP enzyme [101]. Recently they have developed an ultrasensitive label-free electrochemical biosensor for HepG2 cell detection. Disposable screen-printed gold electrodes (SPGEs), the capture probe and hybrid nanoprobes were used to fabricate this cytosensor. Capture probe and DNA nanotetrahedron-based (NTH-based) TLS11a aptamers were immobilize on the SPGE surface. Nanoprobes consisted of Pd–Pt nanocages labeled with complementary DNA (cDNA1, cDNA2), G-quadruplex DNAzyme and HRP. These nanoprobes were placed on the SPGE surface via DNA hybridization, which led to the formation of dendritic structure (DS) nanoprobes. HepG2 cells and DS nanoprobes competed with each other to bind with NTH-based aptamer probes. Presence of target cells resulted in the denaturation of double-stranded DNA and the release of the DS nanoprobe from the electrode surface (Fig. 7) [102]. Comparing the limit of detection (LOD) of these sensors, successful improvement is achieved through their designed platform (30, 15, 5 cells per ml respectively).

Moreover, previous research has introduced tumor-derived exosomes and micro-vesicles as cancer biomarkers. Exosomes are a class of extracellular vesicles and play a significant role in intercellular communications and disease physiology. Several attempts have been made to achieve sensitive and accurate cancer-derived exosomes detection; for example, researchers developed new strategies based on G-quadruplex

units as recognition elements [103–105]. Huang et al. developed an electrochemical sensing system for gastric cancer exosome detection using rolling circle amplification and G-quadruplex units. It was reported that the above aptasensor response was linear from 4.8×10^3 to 4.8×10^6 exosomes per milliliter with a detection limit of 9.54×10^2 ml^{-1} [106].

3. Discuss the future of the field

It has been shown that there is a relationship between the rise in mortality rates and delayed cancer diagnosis. Until now, many biosensors based on nanoparticles and nanostructures have developed to achieve early cancer detection. In this review, we surveyed the recent biosensors based on G-quadruplex structures in this field. First, the features and structure of G-quadruplex were discussed briefly. Later application facets of quadruplex structure in cancer biomarker detection and various detection methods were summarized (Table 2). Regarding research development in the "specific G-quadruplex ligand" field, quadruplex based biosensors are still at the beginning and have a long way to go. In most cases, G-rich sequence functions are studied to find a relationship between cancer biomechanism and the presence of quadruplex in the genome. Next G-rich sequences are used to designed specific ligand to stabilize the structure and control the disease. The other application of quadruplex aptamers is the usage in developing drug delivery systems. But using quadruplex as a recognition element in the bioanalytical methods is a new way forward in cancer diagnosis. There are many nanostructures and nanoparticles which are used for this purposes but this article focused on the quadruplex based sensing platforms developed for the first time.

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