DNAZYME BIOSENSORS: A REVIEW

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ABSTRACT

The most commendable objective that challenges science and technology is the upkeep of health. The prerequisite for the prevention and cure of a disease is diagnosis. Biosensors are indispensable in-patient analysis and disease diagnosis. Also, utilization of biosensors for the analysis of food quality and environmental assessment can be seen as strive towards the central objective of maintaining good health. The requirement for vigorous diagnostics is an exigency for the advancing world. This article gives an overview of DNAzyme biosensing and reviews various sensing applications, underlining important advances and breakthrough contributions in the realm of portable biosensing tools and point-of-care diagnostics. In particular, the field of functional DNAzymes is discussed. Subsequently, the incorporation of DNA zymes into bioassays will be described.

Keywords

Biosensors, dnazymes, nanoparticles, detection, diagnosis

INTRODUCTION

Biosensors are basically analytical devices comprising different biological sensing elements and transducer which sense the presence or absence of the particular analyte in question. Every biosensor count on recognising biological interplay on a surface. The manipulation of subtlety of the biological element is the impetus of a biosensor. Preferably, biosensor is a device proficient at responding incessantly and reversibly without disturbing the sample. Home pregnancy kits and glucose biosensors are the most popular biosensors used today.

As per the IUPAC norms 1999, "a biosensor is an autonomously amalgamated receptor-transducer analytical device with the ability of delivering precise quantitative or semi-quantitative data based on a biorecognition event" (Thevenot et al., 1999).

Ever since the introduction of the Leyland Clark blood glucose biosensor, the realm of biosensing technology has been consistently striving towards improved selectivity, sensitivity and multiplexing capability of the biosensors. Biosensing technology is an emerging dynamic tool for advancements in the field of healthcare research, environment, food industry, diagnostics and military. Scientists across the world are working diligently to upgrade biosensors through build out of better cohering technologies, introduction of new materials and incorporation of new and advanced transducers(Perumal and Hashim, 2014).

TIMELINES IN THE HISTORY OF BIOSENSORS

1916	First immobilization of a protein was reported by Nelson and Griffin
1922	Discovery of glass pH electrode
1956	Clark developed the oxygen electrode
1962	Description of enzyme electrode (Clark) based on the principle of amperometry
1969	First potentiometric biosensor to identify urea
1970	Ion Selective Field Effect Transistor (ISFET) was invented by Bergveld
1972-75	Yellow Springs Instruments glucose biosensor- the first mercantile biosensor

1975	First microbial biosensor First immunosensor
1976	First bedside artificial pancreas
1980	First pH sensor based on fibre optics for detecting in vivo blood gases
1982	First fibre optic biosensor for glucose monitoring
1983	First surface plasmon resonance (SPR) immunosensor
1984	First mediated amperometric biosensor: recognition of glucose using GOx with ferrocene
1987	Establishment of MediSense ExacTech™ blood glucose detector
1990	Inauguration of the Pharmacia BIACore SPR-based biosensor system
1992	Handheld blood analyser launched by i-STAT
1996	Launch of glucocard
1998	Development of LifeScan FastTake blood glucose biosensor Roche Diagnostics: collaboration of Roche and Boehringer Mannhelm
2001	Inverness medical's glucose detecting business was purchased by LifeScan
2003	i-STAT bought by Abbott for $39.2 million
2004	Abbott gains Therasense for $1.2 billion
2012	Biosensor based on acetylcholinesterase inhibition
2013	Invention of hydrogel and quartz crystal biosensor
2014	Discovery of HbA1C biosensor, piezoelectric biosensor, silicon biosensor
2015	Microbial fuel cell-based biosensor
2011-15	Nanoparticle based biosensor
2013-15	Uric acid biosensors
current	Semiconductor dots, nanotubes, nanowire, carbon tubes etc

DNAZYMES

DNAzymes are basically enzymes based on DNA that have catalytic activity(Devon, Rothenbroker and Li, 2018). These enzymes are usually single stranded oligonucleotides with high specificity and binding affinity(Hollenstein, 2015). These are man-made enzymes selected from a pool of randomly sequenced synthetic deoxy-ribonucleotides(Rothenbroker et al., 2019). DNAzymes can be modified easily to facilitate signal amplification(Gong et al., 2014). Ronald breaker and George Joyce in 1994 discovered the first type of DNA zyme which can catalyse transesterification reaction in presence of metal cations like lead by a method known as in vitro selection. They also demonstrated that single stranded DNA can act as catalyst, which is similar to the activity of ribozymes. Over the recent past years the scope of DNAzymes have increased rapidly in various fields such as logic gate operations, therapeutic uses, diagnostic tools and biosensing technology(Hollenstein, 2015). This has mainly been possible due to the appreciable folding properties and chirality of the DNA structure(Hollenstein, 2015). DNAzymes are said to be potential gene silencing agents as they can selectively cleave mRNA stretches(Hollenstein, 2015). G-quadruplex is a type of DNA zyme which has G-rich sequence and can show peroxidase activity in presence of hydrogen peroxide and luminol. In presence of cations like ammonia, lead, potassium these G-rich sequence fold into a quadruplex due to which they are used as biosensors for detecting lead or potassium ions in colorimetry(Gong et al., 2014). DNAzymes are found to act as potential biocatalysts in many chemical reactions(Hollenstein, 2015). They act as bond cleaving catalysts. DNAzymes are capable of cleaving various bonds, they are capable of hydrolyzing ether bonds and phosphodiester bonds(Hollenstein, 2015).

Based on bond cleaving DNAzymes can be classified as RNA-cleaving DNAzymes, DNA-cleaving DNAzymes and other bond cleaving DNAzymes.

- **RNA-Cleaving DNAzymes**.

  The first ever DNAzyme to be discovered in the year 1994 by Beaker and Joyce by and in-vitro selection method(Hollenstein, 2015). RNA-cleaving DNAzymes are the most studied group of DNAzmes that catalyze the cleavage of single stranded RNA linkage embedded within the DNA strand(Zhang, 2018).

These DNAzymes consists of substrate and enzyme strands, which are further divided into enzymatic region, active site and two binding arms(Zhang, 2018). Here, the substrate strands consists of the cleavage site of RNA and the enzyme strands with the cofactors form the second structure with the catalytic properties to cleave the RNA linkage(Zhang, 2018). The catalytic property is said to be so specific that a single misplace in the antisense arms decreases the cleavage efficiency(Zhang, 2018).

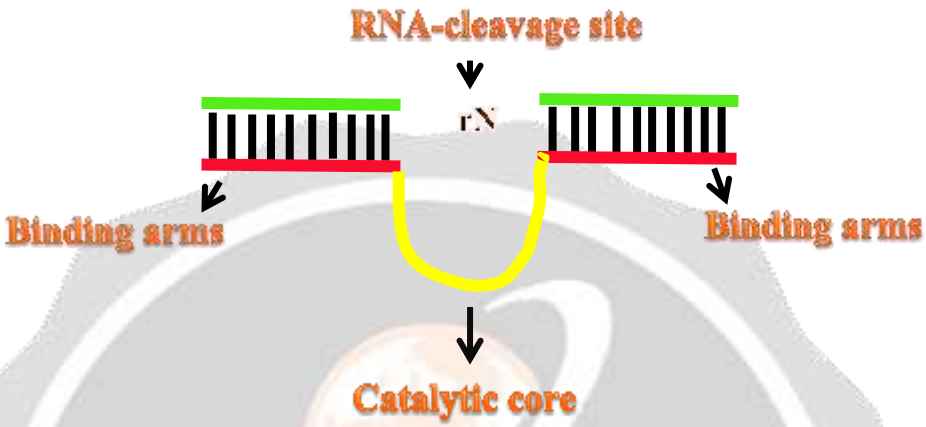


Figure 1. A representative of a RNA-Cleaving DNAzyme(Zhang, 2018)**.**

In recent years many researchers have tried to obtain the exact structure of the RNA-cleaving DNAzymes, Nowakowski et al have reported the crystal structure of the10-23 RNA-cleaving DNAzyme(Zhang, 2018). The first crystal structure of the 8-17 RNA-cleaving DNAzyme has been recently obtained as reported by Liu et al at a resolution of 2.55A^{o} (Zhang, 2018). In the 10-23 RNA-cleaving DNAzymes the catalytic core is made of 15 nucleotides of which almost all are conserved(Schlosser and Li, 2009). Whereas, in the 8-17 RNA-cleaving DNAzymes the catalytic core is made of only 4 highly conserved nucleotides(Schlosser and Li, 2009). They interact with substrates with the help of 2 binding arms through Watson and crick methods of pairing(Liu, Chang and Li, 2017). RNA-cleaving DNAzymes are also said to be actively functioning at a pH of 3-5 which is an acidic condition(Liu, Chang and Li, 2017).

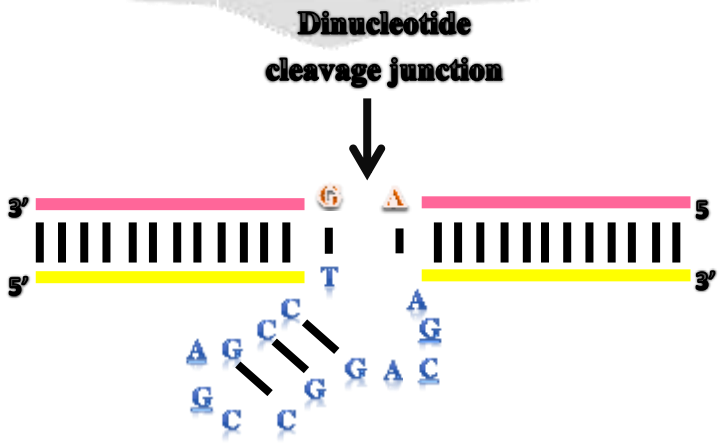


Figure 2. Secondary structure and sequence of 8-17 RNA-Cleaving DNAzymes(Schlosser and Li, 2009)
The nucleotides of only the catalytic core are shown here. The catalytic core in the 8-17 RNA-Cleaving DNAzymes have only 4 (underlined) highly conserved nucleotides. The sequence of 8-17 RNA-Cleaving DNAzyme is shown as depicted by Santaro and Joyce in 1997(Schlosser and Li, 2009)**.**

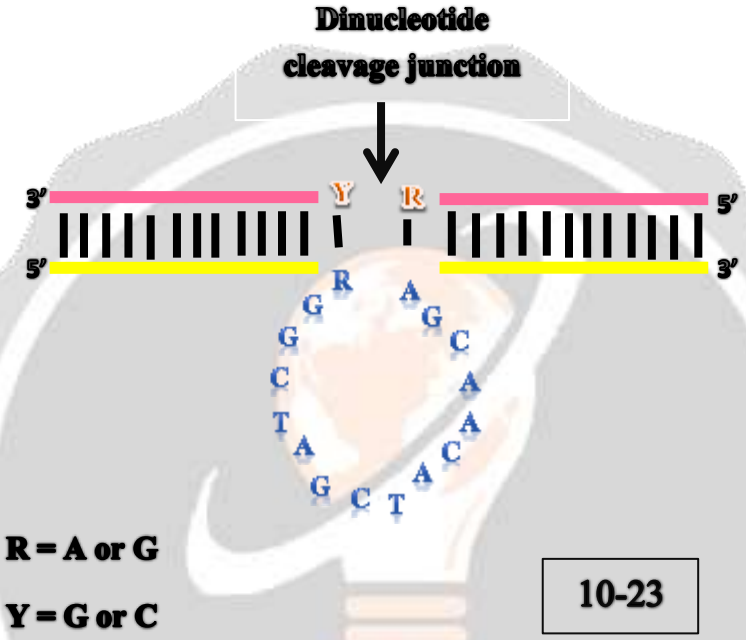


Figure 3. Secondary structure and sequence of 10-23 RNA-Cleaving DNAzymes(Schlosser and Li, 2009)
The nucleotides of only the catalytic core are shown here. The catalytic core in the 10-23 RNA-Cleaving DNAzymes have 15 nucleotides of which many nucleotides are highly conserved(Schlosser and Li, 2009)**.**

- **DNA-cleaving DNAzymes**

  The first ever DNAzyme with DNAse activity was discovered by Silverman et al(Hollenstein, 2015). This discovery lead to identifying the possibility of DNA-mediated peptide bond hydrolysis(Hollenstein, 2015). These type of DNAzymes have very low pH tolerance and show poor substrate tolerance(Hollenstein, 2015). The DNA-cleaving DNAzymes are known to cleave the DNA at any random site without damaging any genetic information(Hollenstein, 2015). These DNAzymes consists of substrate and enzyme strands, which are further divided into enzymatic region, active site and two binding arms(Zhang, 2018)

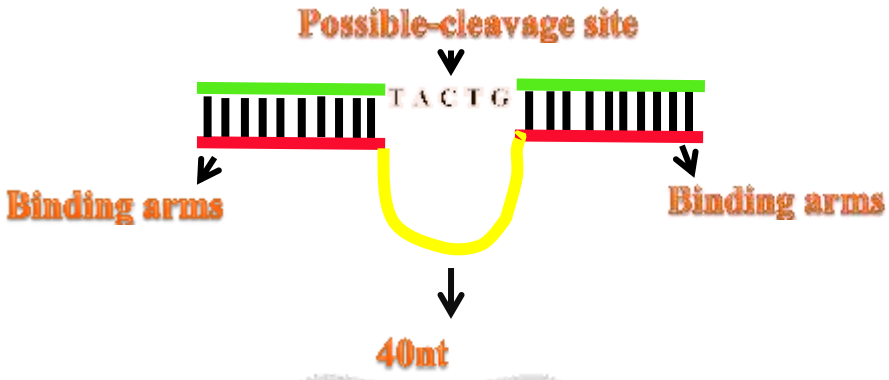
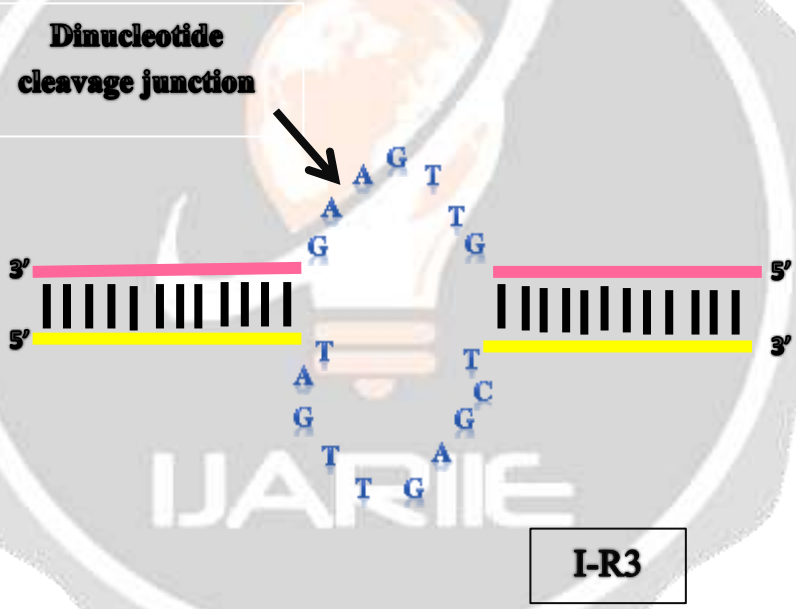


Figure 4. A representative of a DNA-Cleaving DNAzyme(Wang et al., 2014)

The structures of some of the DNA-cleaving DNAzymes are shown below.



5. Secondary structure and sequence of I-R3 DNA-Cleaving DNAzymes(Hollenstein, 2015)

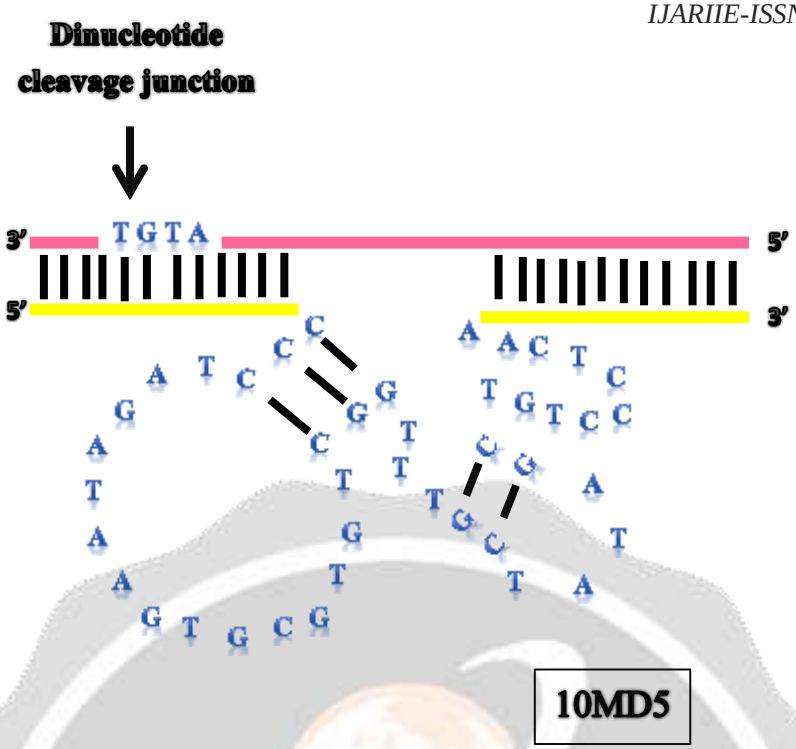


Figure 6. Secondary structure and sequence of 10MD5 DNA-Cleaving DNAzymes(Hollenstein, 2015)

- **Other bond cleaving DNAzymes**

  P-O bond cleaving and C-O bond cleaving DNAzymes are the most common bond cleaving DNAzymes(Hollenstein, 2015). Lesions in the DNA caused due to UV radiations can be repaired by the DNA photolyases(Hollenstein, 2015). Hydrolysis of the phosphomonoesters on side chains of amino acids is catalyzed by the 14WM9 DNAzymes(Hollenstein, 2015). Elimination of the phosphate on serine side chain is catalyzed by DNAzyme DhaDz1(Hollenstein, 2015). These elimination reaction takes place in presence of the metal co factors such as Mn^{2+}, Zn^{2+} and Mg^{2+}(Hollenstein, 2015).

Ce13d DNAzyme is the most active lanthanide dependent DNAzyme(Zhou et al., 2016). They are most active with trivalent lanthanides(Zhou et al., 2016). Ce13d DNAzyme has a catalytic loop of 16 nucleotides that are conserved(Zhou et al., 2016). These DNAzymes are also active with many other metal ions other than Cr^{3+}, they are longer sequences and highly conserved(Zhou et al., 2016). These DNAzymes are also found to be active with Cd^{2+} when the substrate used is a PS-modified(Huang and Liu, 2015).Here the PS refers to phosphorothioate substrate(Huang, Jimmy and Liu, 2014). here it is said that the thymine bases are not important for the activity of the enzymes(Zhou et al., 2016). The secondary structure is shown below.

Biosensors	Detection limit	Applications
Electrochemical biosensor for Pb2+	0.05 -400,000.0nM	Simple, enclose good responsiveness in actual Sample detection, applicable for Field detection of Pb2+ in water
Electrochemical biosensor using gold nanoparticles for uranyl ion	8.1 pM(S/N=3) Detection limit and logarithm of electrochemical response intensity and the Concentration (UO2)2+ajnabi resulted in wide linear range of 10~100 pM.	Successful in establishment of uranyl ion in water sample.
Electrochemical biosensor using silica nanoparticle for uranyl ion	low limit as 0.15pM, linear response with 20pM to 0.1nM concentration of uranyl ion.	Highly Selective, specific and not hindered by the presence of other metal ion.
Electrochemical biosensor using gold nanoparticles with electroactive hexaammineruthenium (lll) to intensify electrochemical signal.	5 pM for concentration of uranyl ion that has linear calibration ranging from 13pM to 0.15nM	The existence of other metal ions had no effect on the detection of UO2 2+, indicating the high specificity of the biosensor.
Scanometric biosensor using gold nanoparticles for lead	2 to 1000nmol/L	Simple, sensitive, portable tool for the on-the-spot detection of Lead(ll) in nanomolar level
Surface plasmon resonance biosensor using gold nanoparticles for lead ion	80pM for 1uM dnazyme	Simple, highly sensitive to pb2+, successful in groundwater detection of Pb2+ ions.
Fiber surface plasmon resonance biosensor using gold nanoparticles for lead ion	Low detection limit ~8.56pM and showed huge response ranges of 10^-11M to 10^-6M	Highly stable, excellent selectivity with regard to lead ion compared to other metal ions, Successful Detection in clinical human serum samples.
Fluorescence biosensor using gold nanoparticles for lead ion	250pM with a Linear range of 1-100nM	Exhibited satisfying results in detection of pb2+ in Lake water sample, know to show possible results in environmental and biomedical fields.
Fluorescence biosensor based on label-free gold nanoparticles for lead ion	Detected Pb2+ across 10nM to2500nM and detection limit of 1.7nM	Simple, cost effective, sensitive, rapid detection tool for Pb2+.
Fluorescence biosensor using gold nanoparticles for uranyl ion	The linear relationship between fluorescence intensity and	Ultra – sensitive, Have potential application in future for real water sample.

	concentration of Uranyl ion ranges from 30pM to 5nM with detection limit of 13pM	
Colorimetric biosensor using gold nanoparticles for lead ion	At 17E enzyme strand Detection range from 100nM to 4uM and 100nM to 200uM at the ratio of 1:20.	Highly selective and sensitive, detect even at 0.5% lead Content.
Colorimetric biosensor using labeled and label – free gold nanoparticles for uranyl ion	Labeled Nanoparticle – 50nM After 30mins. Label- free Nanoparticle – 1nM and liner fitting range from 1nM to 100nM after 6mins.	Both sensors exhibited satisfying selectivity over various metal ions. They have shown detection limit for uranyl ion that is below the maximum contamination level of 130nM in drinking water.

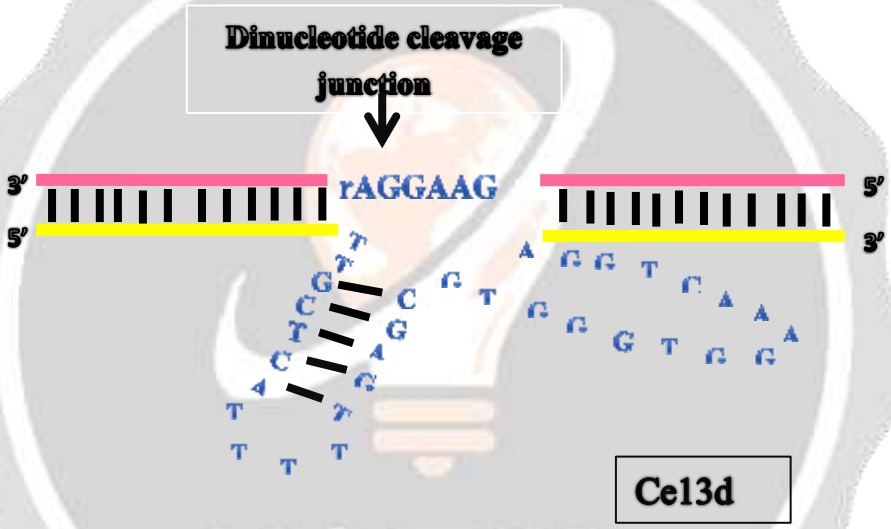


Figure 7. Secondary structure and sequence of Ce13d RNA-Cleaving DNAzymes(Zhou et al., 2016)

BN-Cd16 DNAzyme is an RNA-cleaving DNAzyme. BN-Cd16 have high selectivity, specificity and also show optimal activity for Cd^{2+}(Huang and Liu, 2015). Here the nucleotides are absolutely conserved in BN-Cd16 Enzyme(Huang and Liu, 2015). There are many examples from this abundant family of DNAzymes such as BN-Cd13, BN-Cd04, BN-Cd18, BN-Cd40(Huang and Liu, 2015). The BN-Cd13 DNAzymes show high activity but lack selectivity process whereas the DNAzyme BN-Cd04 show very poor activity(Huang and Liu, 2015). The DNAzyme BN-Cd18 show poor selectivity on the other had DNAzyme BN-Cd40 have high selectivity but act very slow(Huang and Liu, 2015). Therefor the dnazymeBN-Cd16 is most popular among the group. The secondary structure of the DNAzyme is shown below.

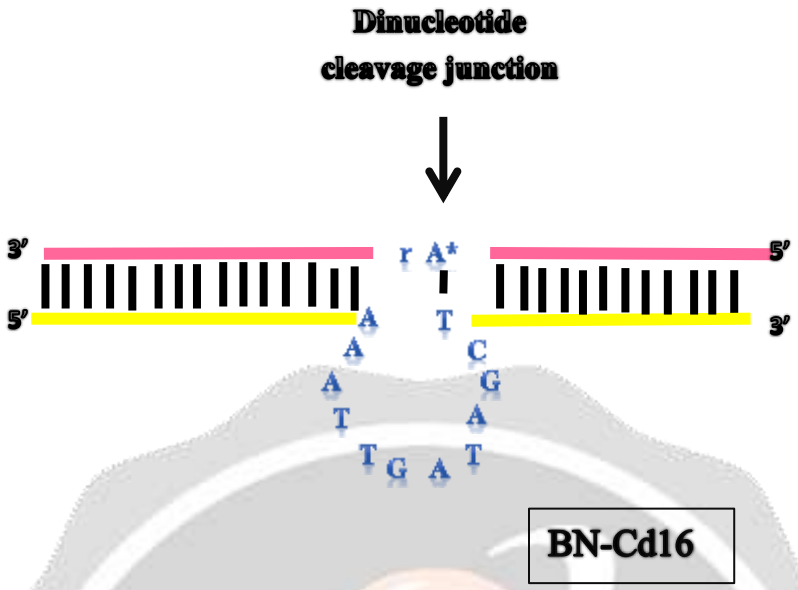


Figure 8. Secondary structure and sequence of BN-Cd16 RNA-Cleaving DNAzymes

Many other different DNAzymes are also available that are being used for different strain detection used as biosensors(Liu, Chang and Li, n.d.). The DNAzyme VAE-2 is an RNA-cleaving DNAzyme used for biosensors in detection of aquatic bacteria *Vibrio anguillarum* (Gu et al., 2019). These DNAzymes have been said to show high activity and specificity towards different strains of the bacteria(Gu et al., 2019). They require polyvalent metal ions for their activity(Gu et al., 2019).

The 17E DNAzyme is also a RNA-cleaving DNAzyme that show activity with the Pb^{2+} and other divalent metals(Ren et al., 2020). Here the nucleotides of the substrate strands are similar and have highly conserved(Ren et al., 2020).

DNAZYME BIOSENSOR FOR DETECTION OF METAL IONS:

DNAzyme coupled nanoparticles biosensors in Disease Diagnosis

- Silver nanoparticles

Detection of pathogenic bacteria such as Escherichia coli can be done using an ultrasensitive fluorescent biosensor using bacteria specific RNA cleaving DNAzyme. The sensing system was based on DNA – templated fluorescent silver nanoclusters (AgNCs) that was integrated along magnetic nanoparticles (MNP) - DNAzyme – AchE complex. The ultrasensitivity of the sensor is due to the DNAzyme recognition element and DNA – AgNCs associated enzyme fluorescent siganl element.S. Typhimuricum bacteria were also detected through fluorescent biosensor with the help of G – Quadruplex DNAzyme and DNA labeled with silver nanoclusters. Using silver nanoparticles

(AgNPs) and hemi/ G Quadruplex DNAzyme an chemiluminescence (CL) based assay has developed for determination of the cardiac troponin T (cTnT), a cardiovascular disease marker was developed. The disposal immunosensor was constructed by the process of immobilization of capture antibody on corresponding sensing site nof the glass chip. The detection strategy was produced by chemiluminescene signals. Ultrasensitivity Metal enhanced nanosenors using silver nanoparticles, functional DNA dendrimer (FDD) and proximity dependent DNAzyme in the determination of cardiac troponin T and carcinoma antigen 125. The introduction of silver nanoclusters as a versatile electrochemiluminescene(ECL) and electrochemical signal probe, a sensitive biosensor in thrombin detection are established. The DNAzyme – assisted target recycling and hybridization chain reaction (HCR) are used in multiple amplification strategy. A detection method for antibiotics such as chloramphenicol (CAP) has been reported using the interaction of DNA/ metal ion that is coupled with silver – DNAzyme cleavage-mediated signal amplification. The analysis dependent on the recognition between the antibiotic CAM and its aptamer. The increase in presence of CAM increases the release of more Ag+, due to this activation of more Ag – DNAzyme resulting in excessive electrochemical signals for sensitive identification of CAM.

- Gold nanoparticles

Colorimetric biosensor based on G – Quadruplex DNAzyme functionalized with gold nanoparticles are associated in myoglobin detection that has a Major role in acute myocardial infraction (AMI) diagnosis. The DNA2 probe that has been modified on AuNPs would gold into G- Quadruplex structure, further bind to hemin resulted in the catalyze to oxidize colorless (ABTS)2- to green (ABTS)2- by H2O2. The detected myoglobin concentration was as low as 2.5nM. Split – type photo electrochemical (PEC) immunosensor has been constructed with designed p-type semiconductor material in alpha – fetoprotein detection which is assisted with hemin for cathode photocurrent enhancement. The PEC is assembled by *p*- CuBi2O4 on the layer of gold nanoparticles. On the presence of AFP, sandwich type immunoreaction takes place in the capture antibody coated microplate with detection antibody and the hemin-based G – Quadruplex DNAzyme in producing signal probe on - site detection of pathogens that can lower the risk of transmission of disease infection. On that case, rapid detection of pathogens such as Salmonella choleraesis can be done with plasmonic biosensor that utilize DNAzyme- immobilized gold nanoparticles. Electrochemiluminescene(ECL) aptasensors were designed based in biobar- coded gold nanoparticles and DNAzyme to detect protein such as thrombin with a strategy of dual amplification signal using CdSeTe@Zns quantum dots (QD). Highly sensitive method for Protein kinase activity and inhibition Assay that was based on the double-quenching of graphene quantum dots (GQDs) was constructed using electro chemiluminescence (ECL)strategy, with G- Quadruplex hemin DNAzyme and gold nanoparticles. The method includes graphene quantum dots that has been modified upon indium-tin oxide (ITO) electrode further fabricated of the substrate peptide of protein kinase over covalent coupling to result in stable ECL signals. As an essential biomarker of cancer and some of the ischemic diseases, high speed detection of microRNAs results in early diagnosis and treatment. Utilizing this, a rapid AuNPs - DNAzyme molecular motor biosensor was initiated using optimum gold nanoparticles in the ideal detection of miRNA -155. Activated DNAzyme cleave substrate strands release of fluorescence fragments from AuNPs. The subsequent hybridization of catalytic strand with other substrate strand achieve the movement of AuNPs-DNAzyme molecular motor that can be monitored using fluorescence signals. Detection of invA gene of salmonella was established through colorimetric sensor integration DNAzyme probe self-assembled gold nanoparticles with PCR method and 3,3′,5,5′-tetramethylbenzidine dihydrochloride hydrate (TMB) that is oxidized to cause colour change.

- Carbon nanosheets, graphene, Zirconia nanoparticle

The homogenous chemiluminescence aptasensors (CL) are developed as a sensitive biomarker detector like carcinoembryonic antigen (CEA) with graphitic carbon nitride nanosheet (g-C_3N_4 NS) in quenching CL luminol-hydrogen peroxide (H_2O_2) system. In the presence of a specific target DNA hybridization reaction leads to DNA – DNA duplex, inhibits the adsorption on the surface of g-C_3N_4 NS due to which electron transfer will be blocked and enhance CL emission. This method also has potential in detection of adenosine triphosphate (ATP). Pathogenic bacteria such as Escherichia coli determination was developed by a real time highly selective, sensitive fluorescent

biosensor based on Escherichia coli – activated RNA – cleaving DNAzyme/ graphene hybrid nanomaterial. Detection of oligonucleotide associated with hepatitis B virus DNA segment was proposed using label free DNAzyme biosensor, based on electrochemical reduction of graphene – oxide carboxly multi – walled carbon nanotube composite (GO – CMWCNTs) and the gold nanoparticles electrodeposition on a glassy carbon electrode. In the presence of hemin DNAzyme and k+, the hemin/G-quadruplex DNAzyme starts generating on electrode surface that trigger the process of electrochemical H2O2-mediated oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB). Highly effective DNAzyme cycling process of DNA and zirconia nanoparticles were used in detection of target proteins. The DNAzyme substrate was previously modified on the surface of electrode which expose 5'phosphate end for conjugation with zirconia nanoparticles (ZrNPs), that has been associated with numerous amounts of signal molecular peptide (GHK) produce amplify electrochemical signals for the Assay.

Sensor platform	Analyte	Linear range	Limit of detection
Fluorescence biosensor/ MNP – RNA cleaving DNAzyme- AChE complex/ DNA-AgNCs/ATCH	Pathogenic Bacteria E- coli	NA	60 cfu mL^{-1}
Flouresecence biosensor/silver nanocluster-labeled DNA/G-Quadruplex DNAzyme/PET	Pathogenic bacteria *S. Typhimurium*	NA	8cfu·mL-
Chemiluminescence array/AgNPs/G-quadruplex DNAzyme/	Cardiac troponin	0.003 to 270 ng L^{-1}	84 fg L^{-1}
EL/ECL/HCR/AuNPs modified electrode/DNAzyme- assisted target recycling/hairpin DNA	Thrombin	NA	NA
MEC nanosenors/FDD/AgNPs/G-quadruplex DNAzyme	Protein Markers	$5*10^{-5}$ ng L^{-1}	$1.8*10^{-4}$ UmL^{-1}
EC signal/DNA/metal ion interaction/ Ag – DNAzyme	Chloramphenicol	NA	NA
AuNPs/ G-quadruplex DNAzyme/polystyrene microplate	Myoglobin	NA	NA
Plasmon biosensor/ DNAzyme/AuNPs/	*Salmonella choleraesis*	NA	NA
ECL aptasensors/biobar-coded/ AuNPs/CdSeTe@ZnS QDs/	Thrombin	NA	0.28 fM
ECL /GQDs/G-quadruplex–hemin DNAzyme/AuNPs/	Protein kinase activity	0.05 to 5 U mL^{-1}	0.04 U mL^{-1}
AuNPs -DNAzyme/ fluorescence fragments	miRNA - 155	NA	low as 50 fM
COLORIMETRIC BIOSENSOR/PCR/TMB/AuNPs/DNAzyme	*InvA* gene Salmonella	0.5 to 50 nM	0.44 nM Salmonella detection in Water- $3*10^3$ to $3*10^6$ CFU mL^{-1}
CL aptasensor/g-C3N4 NS/hemin/G-quadruplex DNAzyme/CL quenching	Carcinoembryonic antigen	NA	63.0 pg/mL
Fluorescent biosensor/RNA-cleaving DNAzyme/graphene/	Pathogenic bacteria *Escherichia coli*	NA	10^5 CFU/mL
Label- free DNAzyme/GO – CMWCNTs/	Oligonucleotide	10 pM	0.5 pM

AuNPs/TMB	Hepatitis b DNA segment	to 10 nM	
Electrochemical sensor/ZrNPs/GHK/ DNAzyme	Proteins		

Advantages and Disadvantages of DNAzymes in Biosensors

Advantages

- Since DNAzymes show combinatorial selection hence are commonly used in biosensors for theranostics (Wenhu *et al.*, 2017).
- The advantage of in vitro selection of DNAzymes is that the DNAzymes can be isolated under any physiological conditions as extremely high temperature or very low pH range (Wenhu *et al.*, 2017).
- DNAzymes also show high specificity for certain metal ions as they interact with the phosphates of DNA (Huang and Liu, 2015).
- DNA is more stable than the RNA and proteins hence DNAzymes are the most popular class of enzymes for biosensors (Schlosser and Li, 2009).
- DNAzymes are capable of using and recognizing small molecules and non-nucleosidic substrates (Hollenstein, 2015b).
- DNAzymes show high flexibility (Zhang, 2018).
- They can be modified to increase their stability (Baum and Silverman, 2008).
- They have high target selectivity when compared to antisense oligonucleotides (Baum and Silverman, 2008).
- They are easier to synthesize when compared to other ribozymes (Baum and Silverman, 2008).
- They show high catalytic activity when compared to proteins (Gong *et al.*, 2014).
- They tend to have small molecular weight (ZhiJie *et al.*, 2012).
- The DNAzymes which are based on metal ions can be easily stored (Liang *et al.*, 2016).
- They show more resistance to nuclease activity when compared to ribozymes (Benson, Khachigian and Lowe, 2008).

Disadvantages

- The heat sterilization technique cannot be implemented as this may damage the DNAzyme (Bagde and Borkar, 2013).
- This application is found to be very costly (Bagde and Borkar, 2013).
- These DNAzymes are mostly not reusable and cannot be retrieved once used (Bagde and Borkar, 2013).
- Some of the DNAzyme based biosensors have also shown cell intoxication (Bagde and Borkar, 2013).
- Use of DNAzymes in biosensors for therapeutic use have shown to have problems of delivery and cellular uptake (Benson, Khachigian and Lowe, 2008).
- They show lower divalent metal ion concentrations when compared to RNA interference (Baum and Silverman, 2008).

- DNAzymes have been found to show off-target effects (ZhiJie *et al.*, 2012).
- The DNAzymes may lose their activity during immobilization on the transducer (K, 2017).

Comparison of DNAzyme based biosensor with optical and electrochemical biosensors

	Dnazyme based biosensor	**Optical biosensor**	**Electrochemical biosensor**
Working principle	Catalytic reaction and binding capabilities for required specific analyte detection	Generate signals propotional to analyte concentration for real time detection	electrochemical properties of the transducer and analyte
Specificity	High	high	high
storage	High care required	Optimum conditions not required	Does not require optimum conditions
sensitivity	high	high	high
Limit of detection	high	high	low
cost	low	low	low

CONCLUSION

In spite of tremendous progress in the field, only few of the aforementioned biosensors have been commercialized like the other kinds of sensors like enzyme- and antibody-based sensors. To advance the field and fully realize the potential of DNAzymes, we must overcome several challenges. Better DNAzymes that can detect a wider range of targets must be developed. To achieve this goal, we need to develop DNAzymes with higher activity and specificity toward their targets to meet the requirements of high sensitivity, selectivity, and rapid assay speed for real samples. The development of portable and miniaturized devices that use DNAzyme as MREs is a growing area. By incorporating additional functions, such as sample collection and pretreatment, amplification, and multiplex readout, production of commercially viable sample-to-readout devices may be possible, that can be used in resource-limited regions. With the increasing research on DNAzyme-based devices, we expect commercially viable PON sensors to come to market in the recent future.

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