# Biosensors for Nucleic Acid Detection

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**Abstract**— The treatment and the early stage detection of diseases can be done based on the identification of biomarkers. The biomarkers can be detected with the application of selective and sensitive analytical devices called biosensors. The units of nucleic acid recognition are highly specific while the electrochemical signal transduction is highly sensitive. The combination of both these properties leads to the development and improvement of nucleic acid biosensors. There has been a great development of the experimental techniques in the construction of nucleic acid based biosensors for the detection of biological molecules. The electrochemically active DNA and RNA molecules are detected at the working electrode by using voltammetry. The electromechanical devices are more modified and are capable of identifying nucleic acids at femtogram level with the help of polymerase chain reaction.

The nucleic acid recognition method is the main basis for designing DNA biosensors. The identification of infectious as well as genetic diseases can be conducted in a simple, economic and rapid manner by the biosensors. The identification of the specific DNA sequence finds application in the arena of clinical, food and environmental analysis. The DNA biosensors use advanced technologies like SAM and SELEX for specific and selective detection. The synthesis of the nucleic acid recognition layers is carried out easily and can also be regenerated and reused which is an asset over the antibodies or enzymes. The sequence specific information can be gathered smoothly and cheaply by using gene chips and DNA biosensors. The sensing properties of the nucleic acid biosensors can be enhanced by the use of nano materials. The immobilisation of the biomolecules can be made highly stable by using gold nano particles along with retention of their bioactive properties. However, the strategy of electrode improvement by the use of nano material is still very complex. Thus, design of nano structured modified electrodes for biosensors is a significant field of research.



## INTRODUCTION

A small device having the capacity of recognising biological properties for a selective bio analysis is termed as biosensor (Prummond, 2003). These devices have been used extensively due to its ability for on-site rapid analytical applications which can be conducted feasibly at low cost (Sharma et al, 2003). The basic components of a biosensor are receptor, transducer and a processor. The antibodies, whole cells, nucleic acids or enzymes are used as the sensing elements which forms a layer of recognition. The transducer is integrated with the recognition layer by different techniques like covalent binding, adsorption or cross linking. The measurement parameters dictate the type of transducers. In the case of a potentiometric transducer (Wang et al, 2001), the potential is analysed keeping the current constant while for an amperometric transducer (Ho et al, 2004), the current is measured at constant potential. Additionally, the optical transducer (Ivniski et al, 1999) detects the alteration in the light transmission (Mebravar et al, 2000), and piezoelectric transducer (Bunde et al, 1998) analyses the change in the mass whereas the thermal transducer calculates the temperature change. The techniques associated with biosensors are modified over the general analytical techniques.

The biosensor whose probe is made of RNA, DNA or an analogue of nucleic acid is termed as nucleic acid biosensor. The overall performance of the biosensor depends on the immobilisation of the probe on the biosensor. The reaction of the probe with a target nucleic acid generates a signal (Sassolas et al, 2008). This class of biosensors are used in various areas like drug discovery (Debouck et al, 1999), studies regarding gene expression and genotyping (Xie et al, 2004), diagnosis of different diseases (Callewaert et al, 2004), etc. They have also been used extensively in bioterrorism (Burkle, 2003) and food analysis (Eden-Firstenberg et al, 1988). Recent trends reports the use of nucleic acid analogues as they have certain advantages over natural nucleic acids. The deoxyribonucleic acid is a molecule present in each cell of almost all living organisms. Each protein present in the living organisms functions in a specific manner according to the specific DNA code. The oligonucleotides with a fragment of DNA or RNA or a known base sequence are used as sensing elements in these types of biosensors. The basis of the nucleic acid biosensors are to either act as a specific receptor of a chemical or biochemical species or specific hybridisation between the complementary strands of DNA-RNA molecules (Campbell et al, 2002). The generation of the nucleic acid recognition layer can be conducted easily and can also be regenerated for multiple applica-

tions. The specificity and sensitivity of the nucleic acid biosensors can be highly enhanced by combination with polymerase chain reaction (Arora et al, 2007a). The main asset with nucleotides is that they can retain their activity at high temperature over a broad range of pH values. The treatment can be fabricated as The DNA sensors have the capability of genotyping a virus (Lereau et al, 2013).

## 2.1. The role of biosensors

The biosensors based on nucleic acid operate in a very complex background but has the ability for detection of the targets within a very short span of time. The cells at very low concentrations were detected within two hours devoid of sample treatment (Rijal et al, 2013). The sensitivity of these biosensors can be increased by making ribosomal RNA as the target (rRNA). The rRNA is present extensively in the target organisms. Among the techniques used in the biosensors, the commonly used techniques are electromechanical, optical and electrochemical techniques (Figure 1).

The surface enhanced Raman spectroscopy (SERS) and the surface plasmon resonance (SPR) are widely used in optical sensors (Sipova et al, 2013). The intensity of the Raman spectroscopy peaks are enhanced by using gold, silver or nano surfaces of the surface enhanced Raman spectroscopy. The use of colloidal silver surface increases the intensity of methylene violet peak by a factor of 1014 (Kneipp et al, 1997). The surface plasmon resonance sensor is made of highly refractive prism which is coated with metal. The electromagnetic modes termed as surface

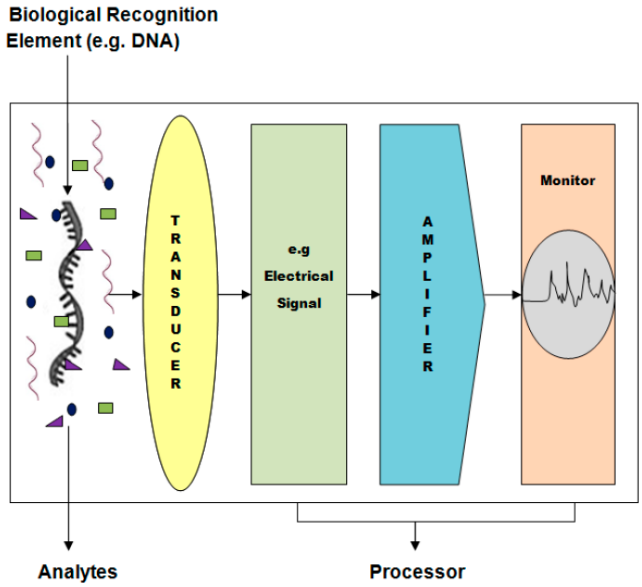


Figure 1: Schematic representation of a biosensor device (Abu-Salah *et al*, 2015)

plasmons are excited by an incident laser light ay the interface of the metal and dielectric which finally decays into both media. The alteration of the resulting refractive index due to the binding of the analyte to the metal surface will bring an alteration in the phase, coupling angle and coupling wavelength of the incident light. The nucleic acids of the viruses, pathogens present in food and parasites present in water can be detected with the aid of surface enhanced Raman spectroscopy and the surface plasmon resonance sensors.

## 2.2. Conventional techniques

The enzyme linked immunosorbent assay (ELISA) is a conventional biological technique for detection of pathogens in selective medium. The types of metabolic activity which are specific to the target organism are shown by the presence of chromogens present in the special media. The enrichment phases are between 48 to 52 hours for the plating methods based on the type of bacteria (Hitchins et al, 2011). The biological entities were detected by ELISA which was the oldest and common commercial methods. The antibodies are stationed on a solid substrate and are revealed to the target organisms. The pathogens are targeted by the antibodies which bind to the antigen and form a sandwich type structure of antibody-antigen-antibody. A change in colour is detected after the addition of a substrate to the enzyme which can be identified due to fluorescence. However, the detection limit of ELISA is of the order 103 cfu/ml of sample which makes it a need for enhancement of the concentration of the pathogens in the food sample. Moreover, the plating process is not compatible to waterborne pathogens. Thus, a positive verification method is required to discard the false negativities. Thus, the conventional methods require manual endeavour which always have the probability of contamination and error.
Another conventional effective method is polymerase chain reaction (PCR) which is widely used for the monitoring as well as the detection of the efficiency of antiviral treatment of infection caused due to hepatitis (Ghany et al, 2009). The polymerase enzymes are repressed by the food proteins and the serum proteins thereby degrading the efficiency of the quantitative polymerase chain reaction (qPCR) (Karmekchiev et al, 2009). Thus, the sensitivity as well as the amplification is hindered leading to the occurrence of false negatives. Additionally, the process requires a large number of steps for the preparation of sample. The primer design and the cycle temperature are some experimental conditions required during polymerase chain reactions which can be obtained by using thermodynamics and kinetics of the nucleic acids in solution.

## 2.3. Methods of immobilisation of probe and hybridisation scheme for biosensors

On the basis of thermodynamics and kinetics the process of hybridisation of DNA in solution is diverse from that on a surface (Springer et al, 2010). A single stranded DNA (ss DNA) probe is immobilised on a surface. The single stranded DNA probe is complementary to the target chains of nucleic acid having either single strand DNA or single strand RNA. The signal for hybridisation which is obtained after the probe detects the target molecule is transduced by different techniques like optical, electromechanical or electrochemical methods. The process of hybridisation to a solution probe is

faster as compared to that of a surface probe. The large extent of electrostatic repulsion between the target and the phosphate backbone present in the probe and large concentration of the nucleic acid charge decreases the rate of hybridisation for surface probes. The density of the probe should be properly controlled for hybridisation of the probes to the target having high ionic strengths. The efficacy of hybridisation is 25 – 40% of probe density is more than 2 x $10^{12}$ chains per $cm^2$. The effect of the density of probe on hybridisation is shown in figure 2. The probe deposition can be inhibited by reducing the incubation time of the probe and using less concentration of salt in their immobilisation buffer.

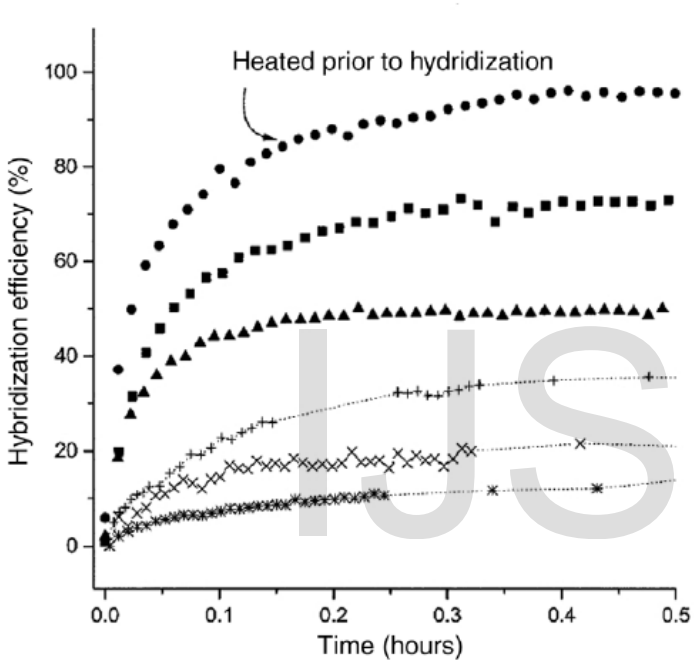


Figure 2: The effect of hybridisation on probe density (Peterson et al, 2001)

The concentration of the surface charge which helps to balance the cation also retards the efficiency and speed of hybridisation. The concentration of the cations locally provides a barrier thermodynamically to the hybridisation of DNA probe. The charge present on the phosphate is balanced by the cations from the solution. The binding of the target single strand DNA as well as single strand RNA is inhibited is hindered by a strong thermodynamic barrier when the concentration of the bulk cation is much lower than that of the concentration of the local cation. This phenomenon leads to a condition where the cations move against the concentration gradient to the surface for balancing the negative charges (Gong et al, 2008). The three various rules which dictate the condition of hybridisation are pseudo-Langmuir, non hybridizing and suppressed hybridisation condition which are classified according to the following equation:

$$\pi = \frac{C_{c,s}}{C_{c,b}}$$

$$C_{c,s} = \frac{S_p N}{h N_a}$$

Where □ = parameter which classifies the condition of hybridisation

$C_{cs}$ = Local cation concentration
$C_{cb}$ = Bulk cation concentration
$S_p$ = Probe surface density
N = Number of phosphates per chain
h = Height of the probe layer
$N_A$ = Avogadro's number

The boundaries of hybridisation system of 18-mer DNA as a function of □ is shown in figure 3. The above formula depicts the thermodynamics of hybridisation at a surface which is generally used in experiments associated with biosensors. When □ is greater than 1, $C_{cs}$ is more than that of $C_{cb}$ and the hybridisation is not favoured by the concentration gradient. When the value of surface probe density is low, the extent of hybridisation attains a constant value it gives rise to pseudo Langmuir behaviour. The intermediate condition under which the hybridisation of the nucleic acid takes place to certain point still there exists strong interactions between the probe sites is described as the suppressed hybridisation regime.

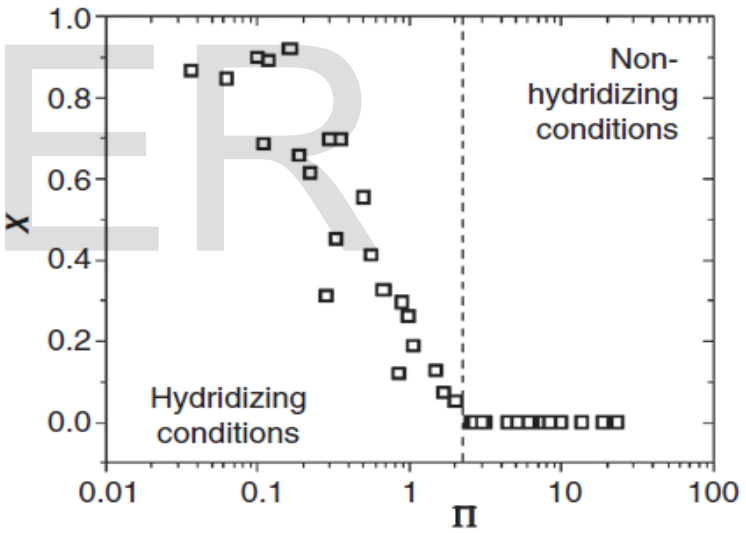


Figure 3: The extent of hybridisation (x) as a function of p (Gong et al, 2008)

The kinetics of the hybridisation of the DNA strands in solution is of second order. The time of complete hybridisation of the complementary strands at the micromolar concentration is 3 seconds. The reaction time should be longer at lower concentration according to the second order rate law. The presence of a hairpin in an oligonucleotide decreases the reaction rate by more than an order of magnitude. The pseudo- Langmuir behaviour is exhibited by the rate constant for the hybridisation of the DNA on the surface which is expressed by the following equation:

$$\frac{dC_{PT}}{dt} = k_\alpha C_T(\sigma - C_{PT}) - k_d C_{PT}$$

$$k_s = k_\alpha C_T + k_d$$

The integration of the above equation when $C_T$ is in excess

gives the following equation:

$$C_{pT}(t) = C_{pT}^{eq}[1 - exp(-k_s t)]$$

Where $C_{PT}$ = Target concentration of surface hybridisation

$C_T$ = Bulk target concentration of surface hybridisation

□□ = Concentration of the probe of immobilised layer

$k_a$ = Association rate constant

$k_d$ = Disassociation rate constant

$k_s$= Effective rate constant

$C_{pT}^{eq}$ = Equilibrium surface concentration of the hybridised probes

For the experiments with nearly 20 mer oligonucleotides, the accurate rate constant is within the range of 0.5 – 5.10x $10^4$ $m^{-1}s^{-1}$. Thus, the order is lower than an order of magnitude of the rate constant of the second order of solution (Gao et al, 2006). As compared to the homogeneous reaction, the local concentration of the surface probe is much towards the higher side which brings the equilibrium state of the hybridisation within 10 – 15 minutes (Li et al, 2013).

## 2.4. SENSING PRINCIPLE OF ELECTROMECHANICAL BIOSENSORS

The two most popular electromechanical biosensing devices made of piezoelectric materials are resonating cantilevers and quartz crystal microbalance (QCM). The shape of the material is altered in a cyclic manner which is induced by an applied AC potential. The matching of the inherent resonant frequency with that of the excited potential frequency alters the electrical properties of the piezoelectric material to a large extent mainly the electrical impedance. The alteration is calculated by observing the current obtained in the circuit. The geometry of the resonating cantilevers and quartz crystal microbalance devices dictate the resonant frequency. A reduction in the resonant frequency indicates the transduction of the additional mass at their surface. The hybridisation of the target nucleotides to the surface of the immobilised complementary capture probes reduces the value of the resonant frequency. The non targets fail to hybridise with the capture probes. The polymerase chain reaction is used for pre-sensing amplication of the target while the secondary hybridisation is used for the post-sensing amplification.

The most commonly used sensing mode by cantilever based sensors is the flexural resonant modes among the other modes which respond to the alteration of mass. The frequency shifts are detected by moving an impedance analyser in the area of a resonant mode having a range of excitation frequencies. The deformation of the crystal lattice of the piezoelectric material induces the charge accumulation which is the basis of the impedance based resonance detection. The nearing of the resonant frequency of the device towards the exciting frequency of the AC potential will enhance the deflection of the cantilever. The condition at which both the frequency resonates there is an enhancement of the amount of accumulation of charge and rate of change with respect to time. The inverse of impedance is termed as admittance. The relationship between accumulation of charge and admittance is given by the following equation (Lee et al, 1999):

$$Y = \frac{1}{V} = \frac{I_p + I_C}{V} = \frac{1}{V}\frac{dq_p}{dt} + \left[\frac{d(CV)}{Vdt} + \frac{1}{R}\right]$$

Where Y = Admittance

V= Applied voltage

□□ = Total output of current

$I_p$ = Piezoelectric component

$I_c$ = Capacitive component

The time derivative of the accumulated charge is a $I_P$ component and is given by the following equation:

$$q_p = -d_{31} E Z_p w \int_0^1 \frac{d^2 v(x)}{dx^2} dx$$

Where $d_{31}$ = Piezoelectric constant

E= Young's modulus

$Z_p$□= Distance between the neutral plane and the plane of zero strain of the piezoelectric layer

w= Width of the cantilever

l= Length of the cantilever

dx = Displacement of the cantilever with respect to x

The current generated by the piezoelectric device as well as the phase angle of the output current is at maximum when the resonance occurs between the frequencies.

### 2.4.1. THE DESIGN OF RESONATING CANTILEVER DEVICES

Electrodes are coated on both sides of a ceramic piezoelectric material like barium titanate, zirconate titanate, aluminium nitride and zinc oxide to constitute a piezoelectric cantilever (Shrout et al, 2007). There are four resonant mode shapes of a three dimensional cantilever. The modes are namely torsional, lateral, flexural and extension modes (Figure 4). The different types of modes like lateral, bending and torsional modes are exhibited by dynamic cantilevers among which the most commonly used mode is the bending mode for biosensing. The resonant frequency and the displacement can be depicted by a set of partial differential equations for a distributed mass system (Sader, 1998). A dynamic cantilever is considered as a spring mass system which acts as a single harmonic oscillator showing a single mode.

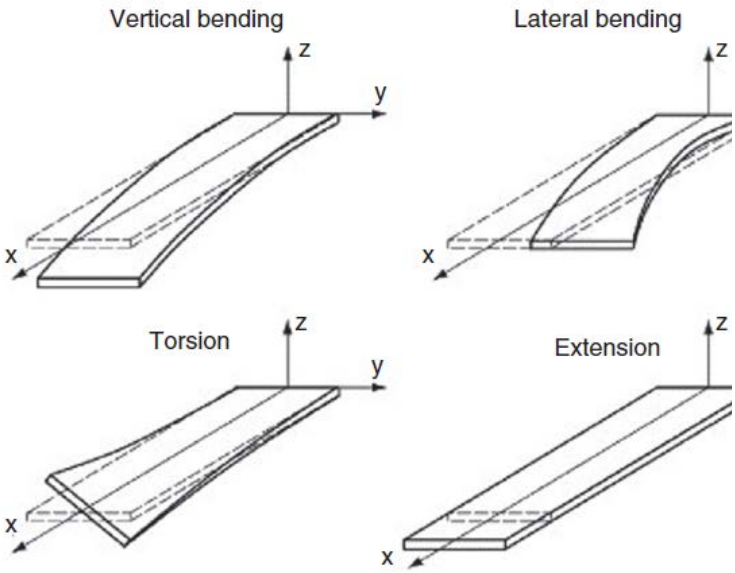


Figure 4: Schematic representation of shapes of resonant modes of cantilever (Song et al, 2006)

The relationship between resonant frequency and added mass is given by the following equation (Fritz, 2008):

$$\Delta m = \frac{k}{4\pi^2}\left(\frac{1}{f_m^2} - \frac{1}{f_o^2}\right)$$

Where k = spring constant

$f_m$= Resonant frequency after the loading of mass

$f_o$= Resonant frequency before the loading of mass

The combination of length of the cantilever, Young's modulus and the mass distribution is called the spring constant. The bending mode of a resonating cantilever is shown in figure 5. The respond of the dynamic cantilevers to the motion of the surrounding fluid is similar to that of the quartz crystal microbalance devices. There is the presence of an inertial component as well as viscous forces which induces the cantilever to operate in out of plane fashion. The cantilever pushes the fluid which leads to the generation of a viscous force. The viscous and the inertial force is given by the following equation:

$$R_s = \frac{\rho\omega b^2}{4\eta}$$

Where □□ = resonant frequency of the cantilever

b= Width of the cantilever

□= Density of fluid

□= Viscosity

$R_e$ = Reynolds number

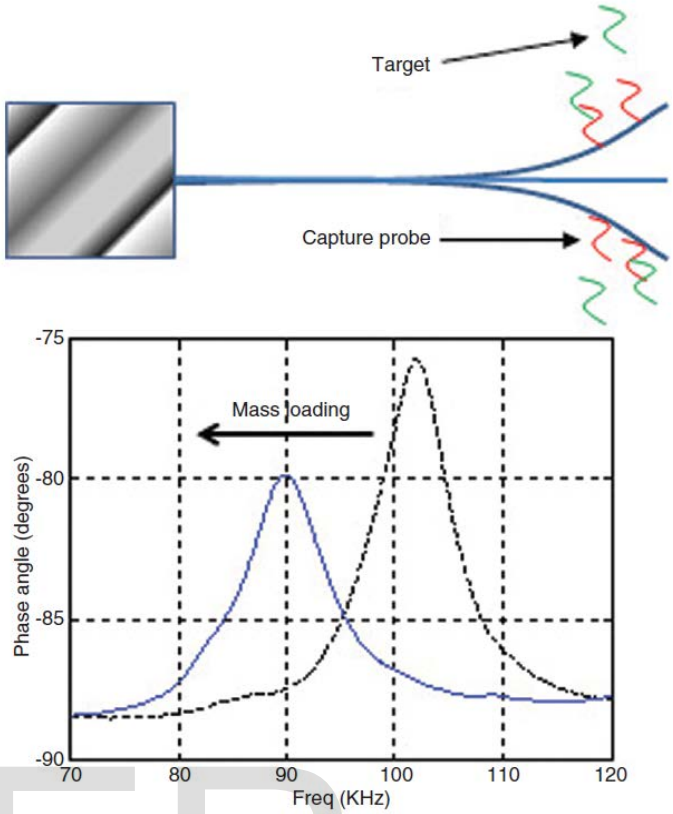


Figure 5: Schematic diagram of a resonating cantilever (Song et al, 2006)

It can be observed from the above equation that the increase in the width of the cantilever or resonant frequency leads to the dominance of the inertial forces.

The resonant frequency of a cantilever in an inviscid medium having a density [□) is expressed by the following equation (Sader, 1998):

$$\frac{f_{fluid}}{f_{vacuum}} = \left(1 + \frac{\pi\rho b}{4\rho_c h}\right)^{-\frac{1}{2}}$$

Where $f_{fluid}$ = Resonant frequency in fluid

$F_{vac}$ = Resonant frequency in vacuum

□= Density of cantilever

h= Thickness of cantilever

The contribution of viscosity gets enhanced if the Reynolds number decreases due to the increase in viscosity or decrease in width of the cantilever. Thus, the peaks become broad as the resonant frequency shifts downwards and the motion of the cantilever decreases. The energy lost in one period of vibration of a cantilever can be measured by a quality factor which is expressed by the following equation:

$$Q = \frac{E_{drive}}{E_{lost}} = \frac{f_o}{\Delta f_{\frac{1}{2}}}$$

Where Q = Quality factor

$E_{drive}$ = Energy required to drive the cantilever in one cycle

$E_{lost}$= Energy lost during driving of the cantilever in one cycle

$f_o$= Resonant frequency

$\Delta f_{\frac{1}{2}}$= Peak width at half frequency

### 2.4.2. THE DESIGN OF QUARTZ CRYSTAL MICROBALANCE (QCM)

A single quartz crystal is cut to a thin disc in order to design a quartz crystal microbalance. Two gold electrodes are placed on either side of the wafer which produces an electric field normal to the surface of the crystal. The orientation of the crystal lattice in the electric field dictates the direction of the oscillation (Deakin et al, 1989). The most common quartz crystal microbalance device is the AT-cut induces shear mode and generates wafer with the vibrations which are orthogonal to the electric field. The resonant frequency of the wafer lies within the range of 2 to 20 MHz. The resonant frequency is a function of the thickness of the wafer. The frequency counters can calculate the resonant frequency differences having small values like 0.1 Hz because of the sharp resonant frequency peak. The resonant frequency and the sensitivity can be augmented by reducing the thickness of the wafer. The Sauerbrey equation gives the change in the resonant frequency in gas phase as shown in the following equation (Lucklum et al, 2000).

$$\Delta f_f = \frac{2f_o^2}{Z_{cq}} \frac{\Delta m}{A}$$

Where $\Delta f_i$ = Function of initial resonant frequency

$f_o$ = Initial resonant frequency

$\frac{\Delta m}{A}$= Mass per unit area

$Z_{cq}$ = Characteristic impedance of the crystal

The mechanical and electrical properties of the crystal dictate the characteristic impedance. The relation between the Newtonian fluid and resonant frequency of the quartz crystal microbalance is given by the following equation:

$$\Delta f_l = \frac{2f_o^2}{Z_{cq}} \sqrt{\frac{\rho_1 \eta_1}{4\pi f_o}}$$

Thus, the decrease in resonant frequency is directly proportional to the square root of viscosity and density of the liquid of the liquid. The loading of the viscous fluid and the inertial effects of the adsorbed film is given by the following equation:

$$\Delta f_l = \frac{2f_o^2}{Z_{cq}} \left( \sqrt{\frac{\rho_1 \eta_1}{4\pi f_o}} + \frac{\Delta m}{A} \right)$$

The analysis of the resonant frequency shifts is very complex when viscoelastic properties are present in the adsorbed film layer (Lucklum et al, 2000). The Newtonian behaviour of the fluid is taken into consideration for approximations as the target is generally present in aqueous fluid for most of the biological assays. The high sensitivity can be obtained by amplification. Additionally, the frequency response as well as the sensitivity can be highly enhanced by binding of the nucleotide tagged gold nanoparticles like a sandwich (Chen et al, 2008). The detection of DNA by quartz crystal microbalance is shown in figure 6.

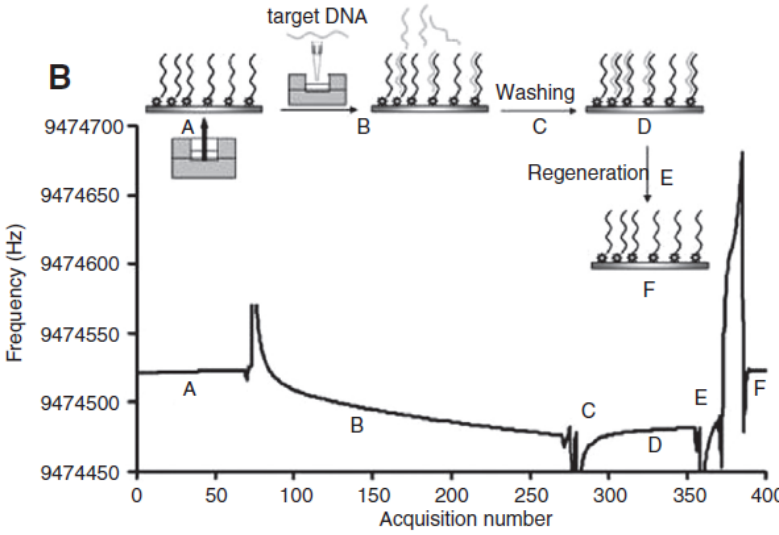


Figure 6: The temporal responses for different phases of hybridisation and the QCM detection of DNA (Wu et al, 2007)

### 2.5. SENSING PRINCIPLE OF ELECTROCHEMICAL BIOSENSORS

The hybridisation of nucleic acids is transduced by two general methods by electrochemical biosensors. The adsorption of insulating molecules is transduced as enhancement of electrical impedance by the first method. The process of oxidation or reduction of electroactive species are transduced as electrical current by the electrochemical sensor in the second method. The sensitive detection of unlabelled nucleic acids are not possible as they are not enough electrochemically potent. Thus, the unlabelled nucleic acids are detected mainly with the aid of intercalating redox indicators and sandwich binding which is a two-step procedure. An electrochemically active molecule is chosen which is allowed to intercalate with the target nucleotides and the hybridised capture. The redox indicators are the intercalating molecules or the labelled reported probes. The concentration as well as the alteration in the mass transport of the redox indicator is identified by the biosensors. The recognition molecule present on the electrode binds to the target molecule thereby reducing the rate of transportation. The redox indicator may either be intercalated within the hybridised DNA strands, fixed to the electrode or may be in solution based on the washing and reacting procedures. The potential of the working electrode is controlled during the detection of the current from the redox indicators by the electrochemical biosensors. An increase in the concentration of the redox indicator increases the current according to the voltammetry technique.

In the sandwich method, the target nucleotides hybridise to its complementary capture probe which is immobilised. The available sites on the nucleotide combine with the labelled reporter probe which creates the top layer of the sandwich. The capture probe immobilised on the surface of the electrode

forms the bottom layer. An enzyme or a metal nanoparticle is chosen as the electrochemically active species which is used to covalently label the reporter strand.

### 2.5.1. EXPERIMENTAL DESIGN OF ELECTROCHEMICAL BIOSENSORS

The electrochemical biosensors constitute of a cell equipped with three electrodes in which the cell is thermostated since the reference electrode potential is a function of temperature. The condition inside the glass container is anaerobic since the oxygen takes place at the electrodes. The electrolyte used in inert in order to avoid movement of the electroactive ions. The working electrode is made of a screen printed electrode or a simple piece of metal which has the ability for detecting the hybridisation of the nucleotide either by galvanostatic or potentiostatic control. In the case of galvanostatic control the current is maintained at a constant value while for the potentiostatic control the potential is maintained at a constant value. A platinum wire is used as an auxillary electrode for completing the circuit. The reference electrode commonly used is the silver-silver chloride electrod (Figure 7).

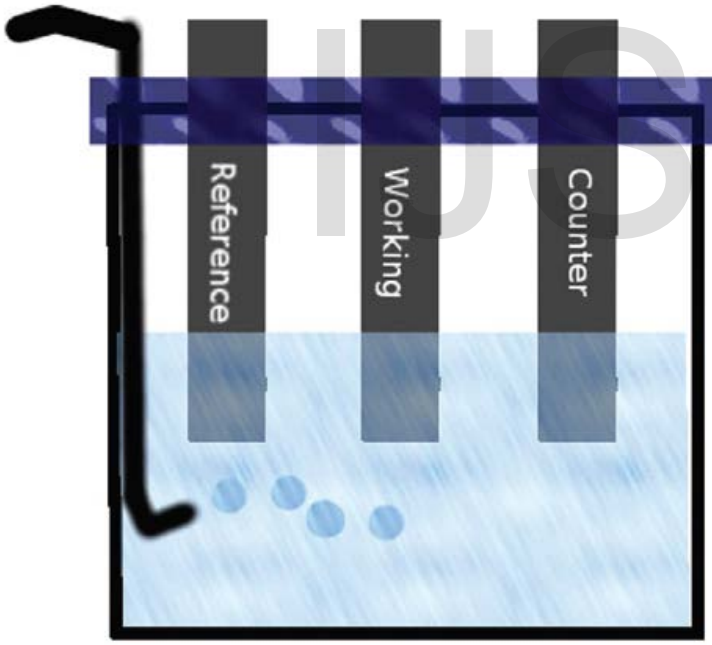


Figure 7: A schematic representation of an electrochemical biosensor (Rosario et al, 2014)

### 2.5.2. CYCLIC VOLTAMMETRY

The saw tooth wave form is used to control the applied potential in cyclic voltammetry and analyse the resultant current. A redox couple is used for measuring the current in solution. The potential is continuously moved and the current is a combination of substantial capacitive current as well as faradaic current. The oxidation and reduction of the redox couple leads to two peaks for current. The characterisation of the electrode can be conducted based on the magnitude and distance between the peak currents. The peak current reduces when the availability of the electrode surface decreases due to subsequent modifications of the electrodes. The reduction of electrode transfer reaction takes place with the increase in the value of □Ep. There is a possibility of different forms of modifications with or without the labelled species may take place at the electrodes of this type of voltammetry.

### 2.5.3. PULSE VOLTAMMETRY

The applied pulse in the working electrode potential is altered and the current is measured in pulse voltammetry. There are two common techniques of voltammetry – differential pulse voltammetry (DPV) and square wave voltammetry (SWV) where the applied working electrode potential is either decreased or increased in small amounts. The target nucleotides can be identified at femtomole level by both the techniques (Liao et al, 2009). The potential is altered in steps and considerable time gap is kept between the steps. The current is sampled in order to decay the capacitive current to zero in both differential pulse voltammetry and square wave voltammetry. The difference in the sampled current before and after each step change is denoted by □I. Thus, □I is plotted as a function against temperature. As the value of potential becomes equal to that of the redox potential of the redox indicator there occurs an enhancement of the current signal. The increase in current signal is directly proportional to the potential of the concentration of the indicator. The labels of the reporter probe should be an electrochemically active species where the nucleotide is allowed to bind to metal ions bound within a layer of liposomes or horseradish peroxides. The main principle of differential pulse voltammetry is that the current is proportional to the rate of oxidation thereby the concentration of the label at the surface of electrode is directly proportional to the generated current.

### 2.5.4 CHRONOAMPEROMETRY

The technique in which current is analysed as a function of time when potential is altered in steps at the working electrode is termed as chronoamperometry. The potential takes the form of a wave in chronoamperometry which is conducted in two steps. The alteration of the potential either reduces or oxidises the redox indicator which generates current and can be measured. The case where the reaction is controlled by diffusion and is reversible the calculation of the bulk concentration is possible (Bard et al, 2001).

### 2.5.5 IMPEDIMETRIC GEOSENSING

The hybridisation of the nucleic acid can be identified by electrochemical impedance spectroscopy. A constant ratio of the redox couple is maintained by managing the DC potential at a constant value. Under this condition, a small AC potential is applied at different frequencies. Small changes in the AC current causes linear current over potential relations which makes

the current to propagate only at the similar frequency of the applied potential. The resistance to charge transfer is denoted as $R_{ct}$ which is monitored at the interface of the electrode by analysing the capacitance and the equivalent resistance of the cell at each frequency with the aid of a potentiostat. The sensing signal is based on the resistance to charge transfer parameter making it a very significant parameter. The rate of the reaction decreases during sensing which causes an increase in the resistance to charge transfer parameter. The mass transport nature of the cell can be manipulated by modification of the electrodes. The charge transfer resistance is enhanced when the transport of the redox couple is reduced due to the adsorption of a species. Thus, the resistance to charge transfer parameter is used as a transduction signal for sensing.

## 2.6. BIOSENSORS BASED ON DNA HYBRIDISATION

The process of bio recognition is based on the base pairing of complementary DNA bases. The electrode surface is immobilised with short 20 – 40 base pair of single stranded DNA which are highly target selective. The process of immobilisation should have the reactivity, stability as well as accessibility to the target analyte. An electrochemical signal is generated when electrochemical indicator like ferrocenyl naphthalene diimide (FND) binds selectively to the DNA duplex. Other commonly used enzyme labels are alkaline phosphates, colloidal gold as well as horseradish peroxide which are used to analyse hybridisation. The high level of sensitivity and selectivity can be obtained by controlling the parameters like ionic strength, temperature and time required for hybridisation as in the case of complex biological macromolecules.
Aptamers are sequences of single stranded DNA or RNA oligonucleotides consisting of 15 – 40 lon bases which can be identified by the process of systematic evolution of ligands by exponential enrichment (SELEX). The aptamers can bind selectively to targets consisting of low molecular weight proteins, organic and inorganic compounds. The aptamer molecules have a three dimensional shape in solution due to the intra molecular interaction between the chain of the synthetic nucleotides. The aptamers possessing three dimensional shapes can bind selectively and strongly with the target molecule. They have the capacity to surround a small molecular target or bind to a small part of a macromolecule. The affinity and the stability of the aptamers can be enhanced after several cycles of systematic evolution of ligands by exponential enrichment by chemical modification of the aptamers. However, the aptamers should not be exposed to high temperature and DNAase enzymes based on its sensitivity to the environmental change. A sandwich type assay is used in the capture probe and insulator component of the biosensor. An alkane thiol linker is used to immobilise the DNA capture probe on the gold. The chemical layer on the gold surface constitutes of shorter alkane thiols which is placed by self assembled monolayer (SAM) technology (Figure 8). Each layer is composed of a sequence specific surface between the capture probes of the DNA and the layers can be termed as mixed self-assembly monolayers. Thus, the nonspecific adsorption as well as the reaction with the active materials present in the sample can be inhibited.

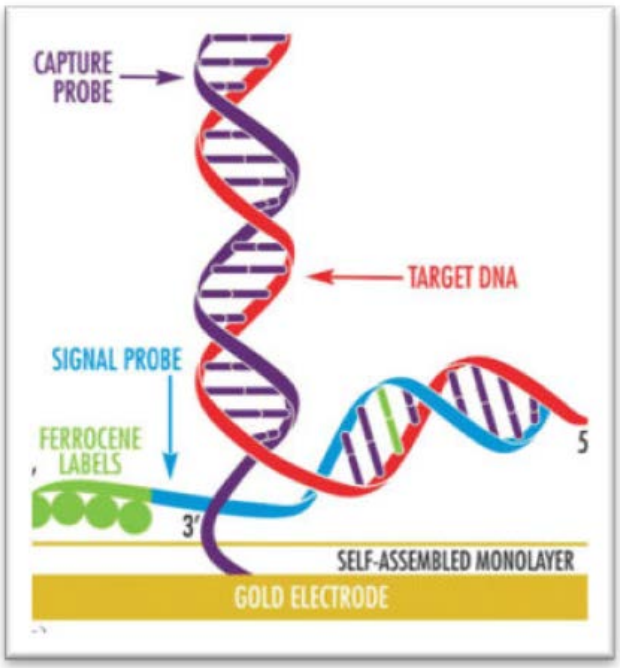


Figure 8: Self assembled monolayer (Kavita, 2017)

A Surface Plasmon Resonance (SPR)-DNA biosensor has been reported which has screened DNA aptamers which can bind specifically to micro-cystin. Thus, the in vitro identification of the micro-cystin was conducted effectively. However, the sensitivity of the detection was comparatively less and required further improvement.

### 2.6.1. ELECTROCHEMICAL DNA BIOSENSORS

The technique of detection plays a significant role in the fabrication of a biosensor. The electrochemical DNA biosensors are most popular as they are highly sensitive and exhibit rapid response. These devices play a significant role in the sequence specific biosensing of the DNA. They are useful for DNA diagnostics as the devices can be designed to a miniature size with the help of advanced technology. The current is analysed by keeping the potential fixed in the case of identification of the hybridisation of DNA electrochemically. The labelled as well as unlabelled objects were detected by various electrical modes (Lucarelli et al, 2008). The efficiency of the DNA biosensors depends completely on the technique of immobilisation of the nucleic acid probe on the surface of the transducer. The orientation of the probe should be well defined along with proper access to the target are two major requirements during the process of immobilisation. The different methods for the attachment of the solid surface to the DNA probe are dictated by the nature of the physical transducer. Apart from self assembled monolayer technology, the attachment of DNA probe can be conducted by interaction of biotin and avidin on the electrode surface which can be used for detection of E. coli (Arora et al, 2007b).
The DNA biosensors are also based on the knowledge of peptide nucleic acids (PNA). The peptide nucleic acids are a

mimic of deoxyribonucleic acids where the backbone composed of sugar phosphate is substituted by a pseudophosphate. The solution phase peptide nucleic acids exhibits specific hybridisation, structural and recognition features. These characteristics can be utilised for the fabrication of highly sensitive and selective DNA biosensors. The single base mismatches can be effectively detected with the application of surface confined peptide nucleic acid recognition layers. Thus, the device can be upgraded to sequence specific DNA biosensors (Marrazza et al, 1999).

The hybridisation is identified by alteration of electrochemical parameters like current, capacitance and conductivity induced by the process of hybridisation. The new redox indicators have the capacity to distinguish between single stranded and double stranded DNA. Ferrocenyl naphthalene diimide is an effective inter calator which has the capacity to bind strongly to DNA hybrids as compared to other intercalators. Additionally they do not exhibit affinity towards single stranded probes (Tombelli et al, 2000).

The electrochemical DNA biosensor detection may either be labelled or label free. The target molecule is not required to be labelled for direct detection which makes the process of nucleic acid assays simple and less time consuming. The complementary sequence in the target is recognised by the immobilised probe. The transducer transforms the process of recognition into a signal. The amount of hybridisation is proportional to the amount of target molecule in the sample. This technique does not exhibit unwanted effects like steric hindrance and instability which are present in labelled systems (Owino et al, 2007). The oxidation peak of hybridisation of guanine can be used for exploiting the alteration in the intrinsic electroactivity of DNA. The inosine residues were used to substitute the guanines in the probe sequence. The identification of the hybridisation was done through the DNA guanine signal of the target (Prabhakar et al, 2007). Thus, the chemical and physical damage can be identified by the changes in the guanine oxidation and other types of intrinsic redox signals. The application of $Ru(bpy)_3$ as a redox mediator enhanced the hybridisation response and finally the guanine signal. The transformation of the conductivity values of the molecular interfaces of conducting polymer can be electrically monitored for recognition of the DNA hybridisation by label free methods. The DNA-modified poly pyrrole films are used as the molecular interfaces of the conducting polymer. The rate of electron transfer between the single stranded DNA and double stranded DNA is different which can also be used as a technique for detecting hybridisation by label free methods. The identification of mutations are also carried out with the use of redox indicator by electrochemical DNA biosensors. However, the requirement of high potential for the redox probes damage the structure of the hybrid double strand and cause interference as well. Thus, the metal enhanced detection technique based biosensor was developed for the identification of micro cystis spp which was designed by immobilising 17-mer DNA on gold electrode with the reaction of avidin-biotin chemistry.

The process of hybridisation is recognised by the use of biosensor specific metal complexes, enzymes or organic dyes. Among these the probe labelled with enzyme is widely used where the enzyme is immobilised to the surface of the probe. The process of hybridisation can be identified in a simple manner based on the electrochemical activity (Yurish, 2005). The signal is obtained by placing redox active molecules like methylene blue and dauno mycin between the double stranded DNA for recognition of the hybridisation.

## 2.7. BIOSENSORS BASED ON APTAMERS

The nucleic acid based biosensors became capable of recognising proteins and whole cells apart from oligonucleotides after the advent of aptamers in the field of analytical science. The apatamers constitute of single stranded RNA or DNA molecule which has high affinity to bind to specific targets (Ellington et al, 1990). They are similar to the antibodies in the field of biorecognition in biosensors and their use as biorecognition elements has several advantages over antibodies. The in vitro process called systematic evolution of ligands by exponential enrichment by the use of apatamers does not require animals or cell lines. The modification of the chemical structure of apatamers is simpler as compared to that of antibodies. The apatamers can be used in extreme conditions as they are highly stable when compared to the antibodies. The conformation of the aptamers is altered during the analyte binding process which is the basis of the aptamers based biosensors. The binding of the aptamers to the analyte induces the change in conformation and thereby alters the position of the redox reporter with respect to the surface of the electrode. The movement of the redox reporter towards the surface of the electrode generates one on signal. The design where the binding of the analyte reduces the signal are classified as signal off apatamers. The dynamic monitoring of the analytes can be conducted as the steps like washing and labelling can be avoided and the aptamers signal can be obtained directly. The release of the inflammatory cytokinesis IFN-□ and TNF-□ from the cultured immune cells within the microfluidic devices are carried out by the biosensors using aptamers (Liu et al, 2012). The microfluidic devices which are reconfigurable are small aptasensor devices which can be integrated into the cells. These can be used as on chip aptasensor regeneration and to control the communication among the cells (Kwa et al, 2014) (Figure 9).

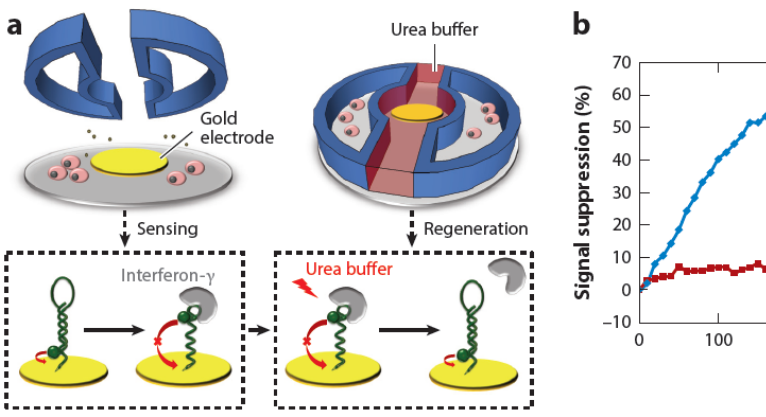

Figure 9: Aptasensors for in vivo and in vitro sensing (Zhou et al, 2014)

The aptamers based upon on chip regeneration technology enhances the affinity lifetime of the biosensors and the experiment on detection of cell multiday will be made possible. The aptasensors can be effectively used for in vivo monitoring. A report indicated the insertion of signal on aptasensors into the bloodstream of humans and rats and monitored the effect of an antibiotic called kanamycin and an anti-cancer drug called doxorubicin (Fergusson et al, 2013). The development of aptasensors using the phenomenon of fluorescence is still in the stage of development.

## 2.8. DNA BASED NANOBIOSENSORS

The biosensor which operates at the nanometric level on a region of nanoscale is termed as nanobiosensor. A variety of nanobiosensors based on optical, electronic, nanowires and nanotubes based and electrical properties have been proposed for clinical and laboratory applications. The detection of genetic materials as well as infectious diseases are conducted by DNA based nanobiosensors. The labelling of the DNA probes are carried out either enzymatically or chemically with ligands like biotin. The sensitivity of the nano based transducers is increased by more than one magnitude as compared to that of the transducers which are devoid of nanoparticles. Additionally, the loading of the recognition element can be increased along with the electrical communications than the conventional electrodes. The nanomaterials used in the nanobiosensors are semiconductor nanoparticles which are called quantum dots, fluorescent lanthanide nanorods or surface enhance Raman spectroscopy (SERS) nanotags.

The fluorescent nanostructures are used for the design of DNA-based nanobiosensors which is based on the technology of fluorescence resonance energy transfer (FRET). In this technology, the bio assays are carried out for quantitative polymerase chain reaction (qPCR). The overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor triggers the transfer of the energy from the donor fluorophore to the acceptor fluorophore. The mutations of gene along with the calculation of the gene expression have been carried out with the aid of this technology (Tang et al, 2008). The sensing of proteins and DNA is done with the help of semiconductor quantum dots. The identification of DNA is done by a fluorescence competition assay with quantum dots and gold nanoparticles as acceptor donor couple. The fluorescence of the quantum dots is regenerated by the release of gold nanoparticles from the quantum dots triggered by the complementary oligonucleotides (Figure 10). Quantum dots functionalised by a multicolour single stranded DNA is used as a nanobiosensor for the identification of hybridisation of single molecule. A list of sequence specific spectral codes can be produced due to independent hybridisation reaction in presence of different sequences of targets. This type of simultaneous detection of multiple sequences is conducted for bacteria like anthrax. The chemiluminescence of bacteria can be analysed with the use of luminal functionalised silver nanoparticles.

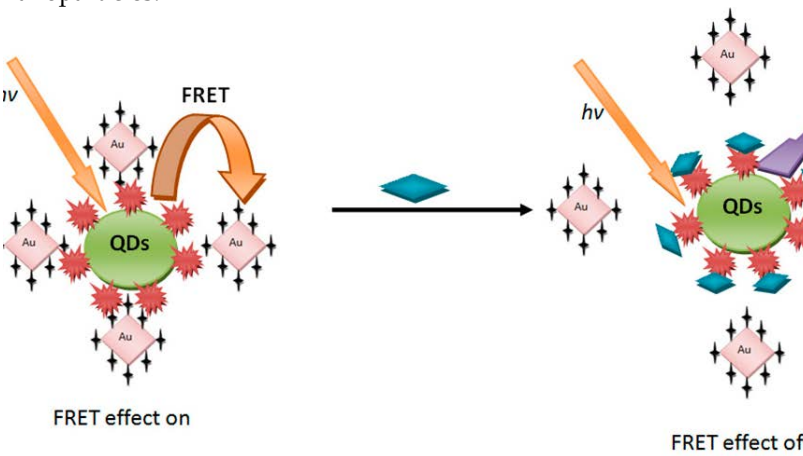


Figure 10: Schematic representation of operating principles based on FRET (Abu-Salah *et al*, 2015)

Additionally, the specific and rapid identification of viruses is carried out through a spectroscopic assay by surface enhance Raman spectroscopy silver nanorods. The alteration of the frequency of the near infrared laser is calculated during the scattering of the RNA or the DNA of the virus and the spectral differences are recorded rapidly. The sample should always be transparent for optical detection since the colour of the solution may interfere with the signal from the sensor. The samples which are not transparent can be analysed by electrochemical detection methods.

### 2.8.1. NANO PARTICLES AS CATALYTIC LABELS IN NUCLEIC ACID BIOSENSORS

Some metal nanoparticles can convert the events of biorecognition into amplified signals by the process of catalysing a specific chemical reaction. As the enzymes are not stable at high temperature, the use of nanoparticles is an asset due to its high environmental and thermal stability. The nanoparticles catalysts have innumerable active sites on its surface as compared to enzyme surface. Thus, nanoparticles as catalytic labels induce large electrocatalytic signals as compared to enzymes which in turn enhance its sensitivity of detection. The generation of a sandwich complex between the electrode immobilised capture probe and metal nano particle linked reported probe produces an amplified signal by arresting the nano particles. The oxidation and reduction reaction is catalysed by the captured nanoparticles which are used for producing an amplified peak during the detection of the biomolecular targets (Hun et al, 2015). The structural switching of a hairpin DNA probe which is immobilised on an electrode surface and conjugated to a gold nanoparticles at its free end is induced by hybridisation which has been used for detection of hepatitis C virus (Li et al, 2012). The gold nanoparticles remains close to the surface of the electrode by the stem loop structure of the hairpin DNA when the target DNA is absent. Thus, electron transfer with the electrode takes place effectively allowing reduction of the dissolved oxygen electrocata-

lytically. In presence of the target DNA hybridisation takes place which induces the opening of the hair loop structure to form a double stranded DNA. Thus, the double stranded DNA carries the metal nanoparticles away from the electrode surface thereby hindering the electron transfer reaction.

Another report summarised the isothermal amplification of Leishmania DNA (de la Escosura et al, 2016). The particular DNA was labelled with gold nanoparticles and magnetic beads. The double labelled DNA was collected on a screen printed carbon electrode with the help of the magnetic beads. The gold nano particles electrocatalytically carry out the hydrogen evolution reaction which aided in recognising parasites at very low concentration.

Another strategy for amplification of the signal avoids the functionalisation of the DNA with nano particles. The DNA template synthesis of the metal nano particles in situ can also amplify the signals due to the electrocatalytic activity of the nano particles towards specific chemical reactions. The DNA/RNA heteroduplex template synthesis of copper nano materials have been used for the design of a reusable micro RNA sensor. The copper nano particles electrocatalyse the reduction reaction of hydrogen peroxide.

### 2.8.2. NANO PARTICLES AS CARRIERS OF SIGNALS IN NUCLEIC ACID BIOSENSORS

The presence of the specific surface area of nano particles makes it an effective loading site for different signal elements which in turn improves the identification of biomolecular targets. The functionalisation of the nano particles dictates the routes from transduction of biorecognition to the electrochemical signals for the sandwich type detection. The target detection is done by electrochemical analysis of the binding of the electroactive molecules in solution to the DNA, in the case where functionalisation of nucleic acid probes are done by nano particles in absence of redox labels. However, functionalisation of the nucleic acid probes by nano particles in presence of redox labels can produce electrochemical signals even in absence of additional reagents. Thus, the binding of the single probe functionalised nano particle is carried out with a variety of DNA targets. The above drawback can be modified by the introduction of molecular spacers between the reporters probes immobilised with nano particles. The spacers are DNA strands which are not complementary to the targets. The cross reaction of the functionalised nano particles with the different target biomolecules is inhibited due to the dilution of the reporter probe on the nano particles by the molecular spacer. The sensitivity of the biosensor is enhanced due to the inhibition of the cross reaction since excess of nano particles can move towards the surface of the electrode to form sandwich complexes. The gold nano particles which were functionalised with redox labelled DNA strands complementary to the target DNA exhibited the detection limit of 1 pM while the gold nano particles which were functionalised with two different kinds of redox labelled DNA strands exhibited detection limit of 50 fM (Wang et al, 2014).

Another study reports the development of a highly efficient aptasensor for detection of cardiac troponin I by using silica nano particles on ferrocene (Jo et al, 2015). In absence of the target, the ferrocene modified silica nano particles could easily move to the surface of the electrode aided by the apatamers, thus generating ferrocene current response. However, in presence of cardiac troponin I as target molecule, the aptamers form a complex with the target molecule which makes the access of the nano particles to the electrode surface difficult.

The enzymes and DNAzymes can also be loaded to the nano particles which amplify the biorecognition signal. It was reported (Wan et al, 2015) that sandwich complex was formed between the reporter probe linked to gold nano particle and electrode immobilised capture probes which was induced in presence of the target DNA. The reporter probes increased in length on the surface of the gold nano particles. The process of elongation was catalysed by enzyme called deoxynucleotidyl transferase. The synthesized long DNA strands formed bond with biotin molecules during the process of elongation. The conjugation between the functionalised gold nano particles with the avidin modified horseradish peroxide molecules took place with the help of biotin. The electrocatalytic signal was enhanced due to the catalysis of the oxidation reactions by enzymes arrested on the surface of the gold nano particles

### 2.8.3. DNA NANOSTRUCTURES

The self-assembly of four single stranded DNA sequences are immobilised on the gold electrodes through thiol linkage in the shape of a tetrahedron for designing tetrahedral DNA nanostructures (TDN). The vertex of the tetrahedron which moves outwards has a pendant DNA strand which can either act as a binding site for the immobilisation of the biorecognition element or as a capture probe. Biosensors developed on sandwich based hybridisation using tetrahedral DNA nanostructures have been reported (Dong et al, 2015). The sandwich complex is initiated by the targets like DNA or miRNA between the DNA probes and the biotinylated reporter strands. The DNA probe is placed at the vertex of the tetrahedron formed by the nano particles. The interaction between the biotin and avidin induces the conjugation with the sandwich complex. The process of bond formation catalyses the oxidation of the substrate in presence of hydrogen peroxides. An electrocatalytic current is produced due to the redox activity of the product of oxidation reaction which can be used for the identification of target nucleic acids at femtomolar level (Figure 11).

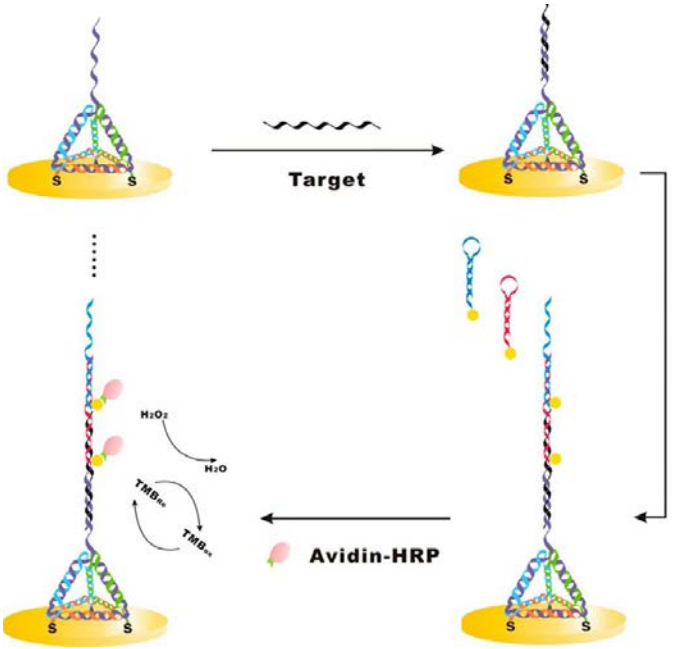


Figure 11: Schematic representation of detection of nucleic acids by tetrahedral DNA nanostructures (Ge et al, 2014)

The immobilisation of the antibodies or aptamers which are target specific are immobilised on the vertex of the tetrahedral DNA nanostructures and used for the analysis of proteins (Chen et al, 2014). The antibodies can be immobilised with the desired orientation and intermolecular spacing of the DNA probes with the aid of tetrahedral DNA nanostructures which considerably enhanced the efficiency of binding and sensitivity of detection. A comparative study indicates that the efficiency of these biosensors are much higher for the identification of antigens which are specific to prostrates than the surface confined double stranded DNA used for the immobilisation of antibodies.

Apart from the tetrahedral nanostructures, electrode tethered DNA nanostructure was reported having the shape of a triangular pyramid for the recognition of IFN-□□ (Sheng et al, 2013). The assembly of the DNA immobilised on the electrode gets restructured from a closed to the open state in presence of the target. This phenomenon reduced the resistance the interfacial electron transfer. In another report the DNA was analysed by using a three-way DNA junction which was designed by formation of a hemin/G-quadruplex complex on the surface of the electrode (Zhang et al, 2015). The structural rearrangement of a DNA nano structure to form an aptamer /DNAzyme conjugate was initiated by the events of hybridisation which was utilised for the process of homogeneous electrochemical sensing. The ATP at very low concentration was identified with the aid of the electrochemical signal generated due to the oxidation of o-phenylenediamine by hydrogen peroxide. The oxidation reaction was catalysed by the in situ synthesis of DNAzyme. Additionally, the nucleic acids have also been analysed by the process in which the short electrode tethered DNA strands have been elongated to DNA nano structures in a single dimension.

A detection process which avoid immobilisation of bio recognition elements was put forth by Xuan et al (Xuan et al, 2015). The self-assembly of ferrocene labelled peptide nucleic acids and short DNA was induced by the DNA strands of the target molecule. Thus, nanostructure formation of dendritic DNA/peptide nucleic acids are initiated by toehold initiated strand displacement reaction (Figure 12). The transfer of electron between the negatively charged surface of the electrode and the ferrocene molecule is hindered due to the merging of the neutral ferrocene labelled peptide nucleic acids into the negatively charged nanostructures formed by DNA/PNA. A solution devoid of targets would generate a stronger signal due diffusion of the ferrocene labelled peptide nucleic acids o the surface of the electrode as compared to a solution containing targets. Thus, the perfectly matched and single-nucleotide polymorphism containing sequences can be separately identified with this advanced technology.

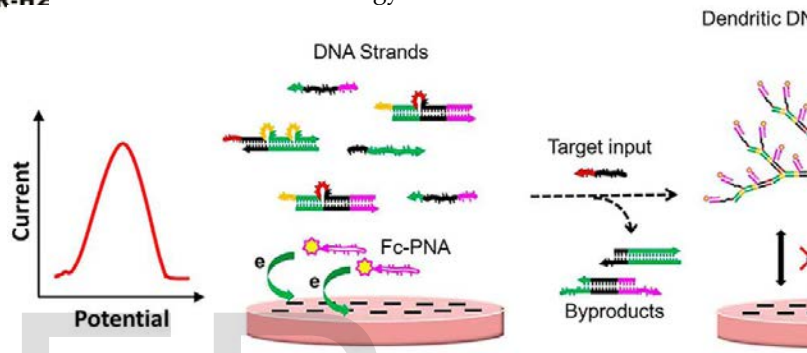


Figure 12: Schematic representation of the sensing strategy if immobilisation free nucleic acid (Xuan et al, 2015).

## 2.9. DNAZYME BASED BIOSENSORS

It has been a complex problem for choosing an aptamer for metal ions as there are no improved methods for the immobilisation of the metal ions. The DNAzyme can be produced by in vitro selection process which can be functionalised with metal ions and exhibit superb specificity towards metal ions (Wang et al, 2014). They can break specific substrates in presence of cofactors like metal ions and amino acids. The DNAzymes consist of two strands namely an enzyme strand and a substrate strand. The substrate strand consists of a single RNA linkage where cleavage can occur while a catalytic core with two arms is present in the enzyme strand. The enzyme strand separates the substrate strand into two parts in presence of the cofactor. Thus, various kinds of cofactor responsive biosensors can be designed. In the case of G-quadruplex DNAzyme, generation of anti-parallel or parallel G-quadruplex takes place in presence of metal ions like potassium ion or ammonium ion. This particular DNAzyme exhibits peroxidise activity in presence of hemin which can be used as biosensor.

## 2.10. PEPTIDE NUCLEIC ACID (PNA) BASED BIOSENSORS

The backbone of peptide nucleic acid is made of N-(2-aminoethyl) glycine units which are connected to each other by peptide linkages (Nielsen et al, 1999). The pure peptide nucleic acids are neutral, partially soluble in water and have a

tendency for self-aggregation. The tendency of the aggregation of PNA can be considerably reduced by the introduction of a charged group to the structure. The complexes formed by PNA/RNA and PNA/DNA have more stability than that of the complexes formed by DNA/DNA since the backbone of the PNA do not have any charge. The thermal and chemical stability of PNA is more and thus inhibits easy degradation (Uhlmann et al, 1998). Additionally, the PNA also retards the enzymatic cleavage within the living cells and are dormant to change in pH. The selectivity of the PNA is more than that of DNA. The disparity between a single PNA/DNA heteroduplexes reduces the melting temperature to a large extent (Park et al, 2006). The standard methods of peptide solid phase synthesis are employed for the chemical synthesis of PNA (Sharma et al, 2017). The synthesized oligomers of PNA are removed from the solid state support by standard chemical procedures. The oligomers are finally purified by reverse phase high performance liquid chromatography and analysed by spectroscopic techniques.

The PNA can be used in the capture probes for biosensing based on their biophysical properties (Gambari, 2014). The sensitivity of the PNA probe is much higher than that of DNA probe. According to the Watson-Crick base pairing rules, the PNA hybridises to the complementary oligonucleotide sequences forming hydrogen bonds between the complementary nucleobases. A wide range of chemical signatures can be identified after the hybridisation as the PNA depicts excellent hybridisation characteristics. The application of the neutral PNA probes along with the combination of redox indicators, oligomer polymerisation and functionalised nanoparticles can design novel biosensors with high sensitivity (Singh et al, 2010). A triplex structure is formed when the double stranded DNA are attacked by the PNA oligomers through a mechanism called strand invasion (Demidov et al, 2002). The above characteristics is applied or antigene or antisense strategy. These biosensors are used for drug delivery and the identification of tumours (Juliano, 2016). The main disadvantage of PNA is its low water solubility which requires proper modification like introduction of charged peptides to the PNA backbone or substitution othe glycine present in the backbone with a chiral amino acid. The modification of the PNA structures leads to the designing of novel nucleic acid biosensors (Manicardi et al, 2017). The application of PNA clamps hinders the PCR amplification of the wild type DNA templates as the PNA is not a substrate for the DNA polymerase. Thus, the separation of the alleles which differ by one single nucleotide polymorphism can be done by the process of PNA assisted PCR clamping.

### 2.10.1. PNA BASED BIOSENSORS FOR DETECTION OF RNA

The RNA has been considered as a potent target for the molecular recognition based on the non coding RNA species which take part in the different biological functions (Nam et al, 2016). The different types of RNAs which are involved in various cancers are messenger RNA (mRNA), micro RNA (miR), long non coding RNA (lnc RNA) and circulating RNA. The micro RNAs are present in the eukaryotic cells which have short RNA sequences constituting of 19 – 25 nucleotides which can behave as regulators (Nana-Sinkam et al, 2013). The micro RNAs take part in translational repression as well as transcription and silencing of gene. The micro RNA binds to the enzyme to form a complex called RNA induced silencing complex which is capable of interacting with the complementary sequences of messenger RNA which leads to the silencing of messenger RNA. The Messenger RNA is silenced due to the breaking of the bond induced by enzymes. A wide range of disease are caused due mutations of the micro RNAs which leads to dysfunction of their biogenesis (Lu et al, 2008). Although there exists various methods for the detection of micro RNAs, the factors like small size, low concentration and sequence homology make it a difficult task for identification. The different micro RNAs are either downregulated or upregulated in specific kinds of cancers while certain micro RNAs are connected to various cytogenetic abnormalities. The physical as well as the chemical properties of the nanomaterials have been used for modification of the sensitivity of the micro RNAs with the aid of various types of nanomaterials like magnetic nanoparticles, carbon based nanoparticles and quantum dots (Degliangeli et al, 2014). The biosensing is carried out using graphene and graphene like two dimensional nano materials which are generally used in fluorescence resonance energy transfer assays. The fluorescence signals are generated by the labelled probes which are used for optical detection. The basis of the detection of micro RNAs in the living cell is by the use of nano graphene oxide along with dye labelled PNA where the fluorescence emitted by the labelled PNA is quenched by the nano graphene oxide. The PNA probe is more competent than that of the DNA probe as the background produced by the PNA has low fluorescence and the bond formed with the nano graphene oxide is stronger. The addition of the micro RNAs improves the fluorescence property of the labelled PNA. Three different PNA probes could be used simultaneously for the recognition of three different micro RNAs which are expressed in the cell lines of cancer cells. Additionally, the micro RNAs can also be detected with the nanoporous metal organic framework which also has the property of fluorescence quenching. The target micro RNA induces the release of labelled PNA linked to the nano metal organic framework (Zhu et al, 2013). The fluorescence is recovered due to the hybridisation between the complementary target micro RNA and the PNA probe. The complementary PNA probes can be labelled with different fluorophores for the identification of different micro RNAs. The carbon nitride nanosheet and graphitic carbon nitride can be also used for the identification of the micro RNAs.

## 2.11. APPLICATION OF DNA-BIOSENSORS

The signature pattern of a variety of DNA mutations and different proteins can be recognised with the aid of biosensors (Wang, 2006). The interactions between the industries and academic sector plays an important role in the exposure of a biosensor into the market. The treatment of cancer is mainly based on the DNA analysis which gives an insight into the

relation between genetics and cancer. The different kinds of techniques like amplification methods which are condute in vitro, electrophoresi and hybridisation of the probes with DNA have been utilised for the detection of disease like cancer. The bioanalysis of environmental pollution and the reactions between the DNA and drug can be detected with the DNA biosensors (Bagni et al, 2006). The recent use of biosensors is point of care testing (POCT) which has potential for a rapid development of the healthcare (Rasooli, 2006). The clinical samples for different biomarkers can be tested in diverse settings according to the point of care clinical programmes. The development of the point of care methods will have a variety of assets like improved monitoring of the treatment, stringent screening of the patients and monitoring the recurrence of diseases. The technologies associated with point of care are cheap which enables screening in a large scale for the prevention of diseases. The technology requires designing of biomarker assays which are reproducible. The biosensors should be able to identify non-antibody recognition ligands. The multi-channel biosensors should be designed along with proper integration and miniaturisation. Additionally, the sensitivity of the transducers should be increased with the aid of advanced manufacturing techniques. The environmental screening and the clinical diagnostics are not based on data gathering which indicates flexibility in the designing of the biosensors.

## CONCLUSION

The electrochemistry of the nucleic acid was discovered by Palecek towards the end of 1950. Since then, huge development has taken place in the design of biosensors where nucleic acids are used as bio recognition element. These biosensors can replace the traditional methods of detecting different biological agents. The amperometric electrochemical biosensors are more sensitive than that of the potentiometric biosensors. Additionally, these types of biosensors are cheap, simple to operate and smaller in size than the optical devices. The electrochemical sensors incorporated with nanomaterials are more competent as it can induce reversibility to certain redox reactions which are irreversible at general electrodes. Additionally, they become capable of multidetection of different biologically active molecules.

The complementary target sequence can be identified with the help of various electrodes immobilised with specific probes with the aid of hybridisation technique. There are a variety of methods of immobilisation along with different electroactive indicators for hybridisation. Additionally, nano composites behaving as conducting polymer have also been used for the improvement of nucleic acid biosensors. Different types of improved biosensors for the molecular diagnosis are based on surface Plasmon resonance, piezoelectric and quantum dot principle. The application of opto sensors has improved the sensitivity of the DNA biosensors.

The performance of the electrodes using nanomaterials in the DNA biosensor has been improved considerably. Still, the further modification process using nanomaterials is very complex. The presence of organic molecules like polymer matrix or surfactant during the preparation of the nanomaterials hinders the identification of the DNA. The gold based nanomaterials are commonly used for the design of electrochemical biosensors based on its excellent biocompatibility property and surface function strategies. The integrated biosensors as well as implanted biosensors are the subject of recent research to date. However, the application of DNA biosensors in different arenas is still limited and has a wide scope for continuous improvement.

The polymerase chain reaction can be accomplished by replacing it with biochip and DNA biostrip technologies. Thus, there is a requirement for the development of biosensors based on nucleic acid having higher amplification, transduction, processing and transformation of the biological signals. Nano particles and nano tubes have been effectively used in biosensors for the detection of toxin like micro cystins. The research is continuing for the commercial development of nucleic acid biosensors in the field of analytical chemistry having improved sensor technologies.

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