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REVIEW ARTICLE

Electrochemical DNA sensors based on the use of gold nanoparticles: a review on recent developments

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Abstract Electrochemical DNA sensors represent a simple, accurate and economical platform for DNA detection. Gold nanoparticles are known to be efficient labels in electrochemical sensors and to be viable materials to modify the surface of electrodes thereby to enhance the detection limit of the sensor. For surface modification, gold nanoparticles are used in combination with nanomaterials like graphene, graphene oxide, or carbon nanotubes to improve electrochemical performance in general. This review (with 116 refs.) mainly covers the advances made in recent years in the use of gold nanoparticles in DNA sensing. It is divided into the following main sections: (a) An introduction covers aspects of electrochemical sensing of DNA and of appropriate nanomaterials in general. (b) The use of gold nanoparticles in DNA is specifically addressed next, with subsections on AuNPs acting as electrochemical labels, electron transfer mediators, signal amplifiers, carriers of electroactive molecules, catalysts, immobilization platforms, on silver enhancement strategies, on AuNPs modified with carbonaceous materials (such as graphenes and nanotubes), and on multiple amplification schemes. The review concludes with a discussion of current challenges and trends in terms of highly sensitive DNA based sensing using AuNPs.

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Introduction

Development of a cost-effective, sensitive and sequence specific DNA detection method is in high demand for the early stage diagnosis of genetic diseases [1]. DNA biosensors, also referred to as genosensors, are analytical devices created from the integration of a sequence-specific probe and a transducer [2, 3]. Electrochemical DNA detection methods offer significant advancement in DNA diagnostics as it provide simple, accurate and inexpensive platform in addition to the generation of direct electronic signal without the introduction of expensive signal transduction equipments [4–10]. Besides, the single stranded DNA (ssDNA) probe sequences can be easily immobilized to a wide range of electrode substrates [11–14]. A range of nanomaterials such as metal nanoparticles (MNPs), carbon based nanomaterials, quantum dots (QDs), magnetic nanoparticles (MNPs) and polymeric NPs [15–22] have been introduced in the sensor design to enhance the limit of detection and selectivity of electrochemical DNA sensor.

Various detection strategies used in electrochemical DNA sensing are direct and indirect DNA electrochemistry, DNA specific redox indicator detection, electron mediated charge transport and nanoparticle based electrochemical signal amplification [8]. The most commonly used techniques for electrochemical analysis are cyclic voltammetry, chronoamperometry, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS),

etc. In cyclic voltammetry, the DNA hybridization can be detected quantitatively from the specific peaks of the nanoparticle or labelling molecule in the cyclic voltammogram. DPV gives an enhanced version of cyclic voltammetry with well-defined peaks. The current value at different time interval is given by chronoamperometry and impedimetry measures the impedance of the system over a range of frequencies.

The irreversible nature of reduction and oxidation reactions of nucleic acids at electrodes limits the analytically useful signals in electrochemical detection of DNA. To overcome this issue, various labels have been used to produce electrochemical signal which in turn reflect the DNA concentration [9]. Nanomaterials based labels attracted significant attention in the recent past in view of the enhanced attained. Among the variety of nanomaterials, gold nanoparticles (AuNPs) are one of the most widely used labels in electrochemical DNA sensors [23]. AuNPs are commonly used as oligonucleotide labels in DNA hybridization detection [24–26]. The substrate used for the immobilization of DNA strands also greatly influence the response of the electrochemical DNA sensor. Glassy carbon electrodes (GCE) and gold electrodes are the most commonly used substrates for electrochemical DNA sensors [1, 3]. Different kinds of nanomaterials such as AuNPs, carbon nanotubes (CNTs), graphene and graphene derivatives have been used for modification of these electrodes to improve the detection limit and stability of the sensor [13, 27–29]. This review discusses the use of AuNPs as labels as well as substrate material in various electrochemical DNA sensors.

Gold nanoparticles in DNA sensors

AuNPs are used for several biomedical applications owing to their narrow size distribution, efficient surface modification, conductivity, attractive biocompatibility and electrochemical properties [30–33]. AuNPs can be easily conjugated with biomolecules without altering the biochemical activity of the conjugated biomolecules and used in different kind of DNA sensors [34–37]. AuNPs which contain thousands of atoms, can be either oxidized or reduced electrochemically and it act as electrochemical mediators to improve electron transfer. These properties led AuNPs to be excellent candidate for several biorecognition applications [38].

AuNPs can be functionalized with thiolated oligonucleotides (DNA-AuNPs) and stabilized in aqueous biological buffers [39]. Based on this finding, researchers tailored DNA-nanoparticle probes with varying properties suitable for a range of applications [40–44]. Modification of AuNP with probe DNA offers high stability, strong binding and electrochemical activity which leads to DNA sensors with high sensitivity and selectivity [44, 45]. DNA hybridization detection is based on the recognition of AuNPs either by using any of the AuNP properties or by the identification of electrochemical tags attached to the gold or gold-DNA complex [30]. Possibilities of AuNPs in electrochemical DNA sensors are the 1) use of oligonucleotide modified AuNPs to enhance the signal, 2) use of AuNP modified electrodes to increase the amount of DNA which is adsorbed onto the electrode surface by appropriate modification, 3) silver deposition on AuNPs in electrochemical detection, 4) utilization of AuNP labels for electrochemical detection of DNA hybridization and 5) use of signal amplification of oligonucleotide-functionalized AuNPs by carrying the signal element such as other AuNPs.

A schematic of the different detection strategies for AuNPs in electrochemical DNA detection are given in Fig. 1.

AuNPs as label

AuNPs have been used as an efficient electron transfer mediator in which the electrons are transferred to the electrode through the AuNPs which in turn leads to signal amplification. The characteristic oxidation/reduction of Au also have been exploited for the DNA detection. The signal is found to be greatly enhanced as a result of the availability of large number of oxidizable or reducible gold atoms in each nanoparticle label. In these cases by increasing the number of AuNPs on the surface the signal and hence the detection limit can be enhanced. With this strategy the limit of detection down to attomolar concentration of target DNA has been achieved. Various strategies used are outlined below.

AuNPs as an efficient electron transfer mediator

AuNPs without any modification have been used as label in electrochemical detection of breast cancer gene by Yang et al. [46]. The sensor works on the fact that the ssDNA on the gold electrode bind to AuNPs so that the electrons from the redox mediators are transferred to the electrode through the AuNPs efficiently as in Fig. 2. In the presence of target DNA, the hybridization occurs and the AuNPs are detached by the double stranded hybridized DNA which reduces the electron transfer to the electrode. On monitoring the signal difference, the presence of target DNA was identified with a detection limit of 1 pM. By using the same principle, Gao et al. developed a DNA sensor in which AuNPs were displaced by target DNA and it was used for the impedimetric detection of DNA hybridization with high sensitivity [47]. The displacement of AuNPs after hybridization led to a change in electron transfer resistance and this was used for the sensitive DNA detection. The detection limit achieved was 50 fM. These DNA sensors consist of simple structural designs of the capture probes with minimum steps of pretreatment of the electrodes which are the advantages of the sensor. However, in these sensors the DNA-gold binding is through the interaction between gold and bases of DNA through the electrostatic interaction and hence the

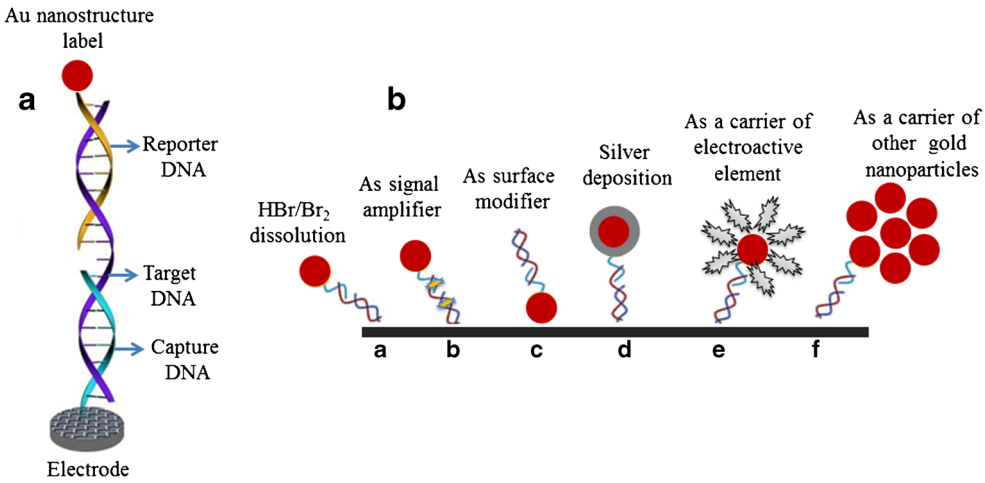

Fig. 1 **a** Schematic representation of sandwich hybridization used in DNA sensors with AuNPs as label. **b** The integration of AuNPs into electrochemical DNA sensor-Different strategies: **a** Au(III) ions detection after dissolving AuNP by using HBr/Br_2 mixture or direct detection of AuNPs; **b** as signal amplifier, **c** as surface modifier, **d** enhancement with silver or gold; **e** AuNPs as carriers of other electroactive labels, **f** AuNPs as carriers of other AuNPs

success of binding between AuNPs and DNA is important during the sensor fabrication where the charge on the AuNP, and size of Au nanoparticle become significant.

Functionalized AuNPs were used to solve the above issues and achieved successful binding between AuNP and DNA. Towards this direction, dithiothreitol modified AuNPs were used as tag for impedimetric electrochemical DNA sensor by Wang et al. [48]. In this sensor, the thiol modified single-stranded DNA provides good contact between AuNP tag and 11-mercaptoundecanoic acid modified gold electrode in the absence of target DNA. After hybridization with target DNA, the AuNP tag pushed away from the electrode, thereby increasing the electron transfer resistance of the DNA sensor. The detection limit was found to be 0.3 fM. with the advantage of simple design of the sensor and the fast assay without the use of label-containing reagent.

Another aspect reported for the electrochemical DNA detection was using positively charged AuNPs along with hybridization chain reaction [49]. In this work, capture DNA was immobilized on the gold electrode and sandwich DNA complex is formed between the capture DNA and the target DNA. The other exposed part of the target DNA opened two alternating DNA hairpins and initiated the hybridization chain reaction. The electrostatic adsorption of positively charged AuNPs onto the double helix was used to amplify the electrochemical signal. In this type of sensors however, the presence of Ag^+ is crucial for the formation of the parallel triplex under biological pH. The hybridization chain reaction increases the assay time for the DNA detection and the detection limit of the sensor is only in the picomolar range which are the major disadvantages.

In our research group, we explored various possibilities of AuNP label for reporter probe DNA in the development of electrochemical genosensors. The AuNPs were functionalized with polyethylene glycol (PEG) and the reporter DNA was conjugated to the functionalized AuNP (DNAr-AuNP) and was used as label. Sandwich hybridization was used for the detection of target DNA with reporter DNA conjugated AuNPs. The sandwich hybridization strategy increases the sensor response by reducing the signal to noise ratio though it takes more time for hybridization compared to normal hybridization. The capture DNA was immobilized on the gold electrode which undergoes hybridization with one half of the target DNA and the remaining half of target DNA was hybridized with AuNP conjugated reporter DNA. Femtomolar level detection limit was achieved using a AuNP labeled DNA

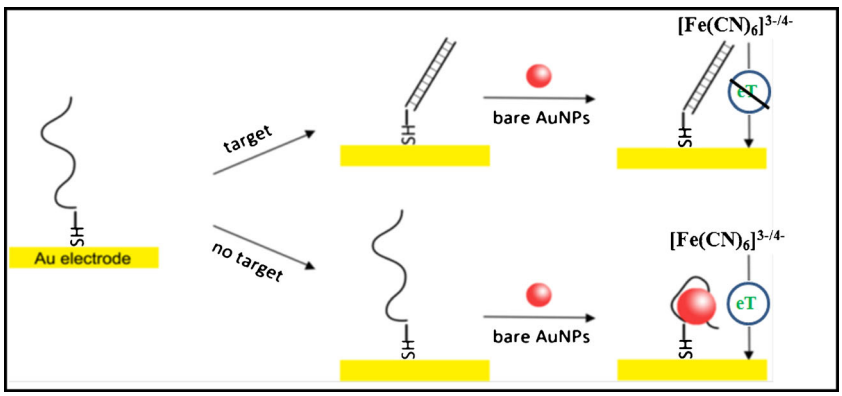

Fig. 2 Schematic representation of sensor which uses interaction between AuNPs and DNA immobilized on an electrode surface. Reprinted with permission [46]

sensor [50]. However, since the immobilization of capture DNA was done by the self-assembly of the ssDNA on the gold electrode the immobilization will be with defects and some of the DNAs would be in non preferred orientation for hybridization. In the next experiment, a self assembled monolayer (SAM) of mercaptopropionic acid (MPA) was used for preventing the non-specific binding of DNA on gold surface. To surmount the insulating nature of SAM, differently functionalized AuNPs were immobilized on the surface of SAM. Cyclicbisureas (CBU) modified AuNPs were immobilized on MPA SAM and this was used as the immobilization platform for capture ssDNA. In addition, MPA functionalized AuNPs were used as the label and this sensor showed a detection limit of 100 aM. Citrate stabilized AuNPs were also used as immobilization platform which showed the detection limit down to 100aM. Then sensitivity of the sensor was enhanced by using PEG functionalized AuNPs instead of citrate modified AuNPs. It was evident from the quartz crystal microscopy analysis, the immobilization of DNA on the PEG functionalized AuNPs is higher compared to MPA functionalized AuNPs which enhances the sensitivity of the sensor. This sensor showed a detection limit of 50 aM and excellent selectivity against three base mismatch sequences as well as non-complementary sequences [51]. The sensitivity enhancement is due to the incorporation of AuNPs as electrochemical label and as the immobilization platform for capture probe DNA owing to the efficient electron conduction of AuNPs. Here, the chain length of molecule used for the functionalization affects the electron conduction. Molecules with least chain length have shown to possess more electron conduction [52] thereby an increased sensitivity. The schematic of the sensor which uses PEG functionalized AuNPs and MPA functionalized AuNPs is given in Fig. 3a.

AuNP mediated electron transfer across a self-assembled monolayer (SAM) on gold electrode was used for sequence-specific and highly sensitive DNA detection [53]. An alkanethiol monolayer was self-assembled on the thiolated hairpin structured DNA probe modified electrode. The self-assembled monolayer blocks the $[Fe(CN)_6]^{3-/4-}$ in a solution from the electrode. After hybridization with target DNA, the stem duplex of DNA probe breaks and it was made accessible for hybridization with the reporter DNA-AuNPs conjugates. Further growth of AuNPs was carried out by dipping the electrode in a growth solution containing $HAuCl_4$ and H_2O_2.The redox reaction of the ferrocyanide redox couple was measured by coulometric measurement (Fig. 3b). The developed DNA sensor showed a detection limit of 1 fM. with good selectivity and reproducibility. It should be noted that the length of the SAM is an important factor in the electrochemical response due to its electron transfer resistance. Normally, SAMs block the electrons from solution-surface interface to reach the electrode surface. To overcome this hurdle smaller chain alkanethiols are used for biomolecule immobilization and also AuNPs are used either in between the SAM and biomolecule or at the solution-surface interface [52]. Use of smaller chain length SAM and addition of AuNPs on SAM will enhance the sensor response.

Recent report showed that the use of highly conductive AuNPs assembled on the free terminal of hairpin-structured probe DNA will enhance the detection limit of the sensor towards attomolar range [54]. Owing to the excellent specificity of the hairpin-like DNA stem-loop structure for detecting nucleic acid targets, it has been widely used for specific DNA detection. The sensor fabrication involves multiple procedures. First the hairpin probe DNA was immobilized on a gold electrode surface through Au-S bond. Thioglycolic acid (TGA) was attached on the 3′-end of hairpin DNA by carboxylic-amino condensation reaction. Then, the AuNPs were attached to the hairpin DNA through reaction with the thiol-group of TGA. Finally, a highly conductive bio interface with ultra-low charge transfer resistance was obtained. When the hybridization with target DNA occurs, the AuNP displaces from the electrode surface. A detection limit of 1.7 attomolar was found with a linear range of 10 pM to 10 aM. One drawback of the system is the relatively large hybridization time than that of linear DNA on account of the more time taken by the breaking of stem structure in hairpin DNA. However, the detection limit achieved is excellent and can lead to the development of point of care devices.

Factors affecting sensitivity on using gold nanoparticles as electron transfer mediators are the size of the gold nanoparticle, charge on the surface, nature of the protective layer on the AuNP, chain length of the protective layer, etc. Upon taking proper care and considerations a highly sensitive DNA sensor can be fabricated with the use of AuNPs as electron transfer mediators.

AuNPs as tracer or electrochemical signal generators

AuNPs are also used as electrochemical signal generators in DNA sensors as AuNPs contain thousands of gold atoms, which can be either oxidized or reduced electrochemically. Stripping voltammetry have been used for the sensitive detection of target DNAs since it is a powerful technique used for the detection of metal ions in trace levels [55–57]. An electrochemical DNA sensor using AuNPs coated on multiple layers of latex for the sensitive DNA detection was fabricated by Kuan et al. [58]. After the hybridization, the AuNPs were detected by differential pulse anodic stripping voltammetry. Generally, in stripping voltammetry, the AuNPs are detected after HBr/Br_2 dissolution. One of the major limitations in this method is the toxicity of HBr/Br_2 solution.

Our research group used the electrochemical oxidation of AuNPs for sensitive detection of BRCA1 gene in a sandwich type assay. On using AuNPs as reporter probe DNA label and graphene as the immobilization platform in the sensor it

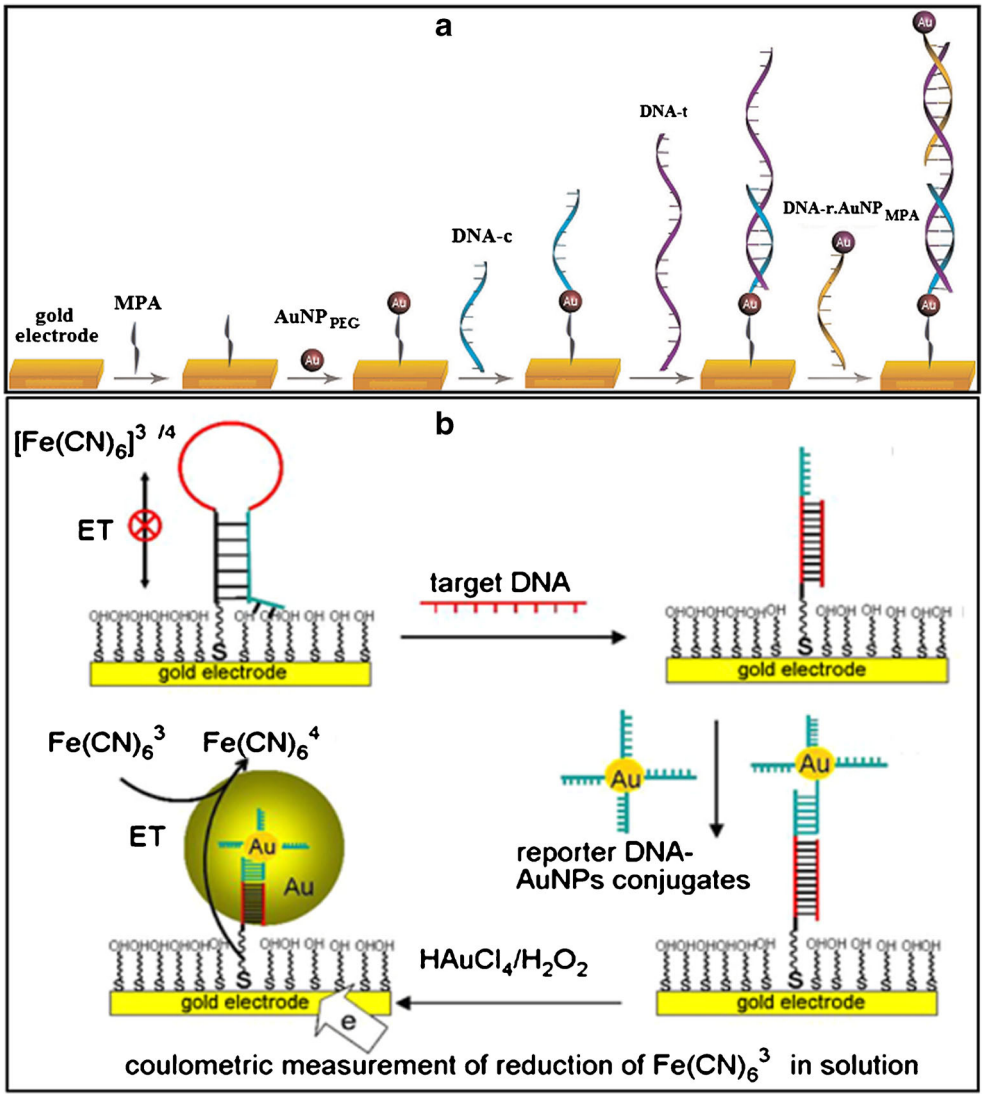

Fig. 3 **a** Schematic of the sensor which uses PEG functionalized AuNPs as the immobilization platform and MPA functionalized AuNPs as electrochemical label. Reprinted with permission [51]. **b** Schematic illustration of the DNA sensor with AuNP-mediated electron transfer across a SAM. Reprinted with permission [53]

showed detection down to femtomolar of target DNA [59]. Advantage of using the electrochemical signal of AuNP is that it is possible to enhance the signal merely by increasing the number of gold atoms. However, we observed that the size effect on sensitivity is marginal. Thus, to increase the sensitivity, we synthesized clusters of AuNPs (AuNPCs) such that the reporter DNAs are conjugated on the outer nanoparticles. The schematics and the SEM images of the AuNPC is given in Fig. 4. The sensor was then modified by replacing AuNP with clusters of AuNPs. The cyclic voltammogram showed a higher sensor response for AuNPC in comparison with AuNP. The schematic of the sensor is given in Fig. 4. The detection limit was enhanced to 50 aM by the synergic effect of AuNPC label and the conducting immobilization platform like graphene [60]. Recently we reported an enhancement in the detection limit of the genosensor to 10 aM by the use reduced graphene oxide-yttria nanocomposite instead of graphene [61]. All of these sensors use the electrochemical oxidation peak of AuNPs bound to the electrode surface and the peak varies depending on the number of AuNPs on the surface. The detection limit of the developed sensors was in the attomolar range and even approximately 50 molecules of DNA can be detected using this sensor. Drawback of the sensor lies in its repeated use. These are not regenerative since the carbon nanostructure is detached from the electrode after few electrochemical measurements hence is suggested as a single use genosensor.

Different categories of functionalized AuNPs have been used as a label in electrochemical DNA sensing. For example, cyclodextrin functionalized AuNP (Au-CD) is used as label in electrochemical detection of Hepatitis B virus (HBV) sequences [62]. In this sensor, the molecular beacon probe conformation changed to double stranded DNA (dsDNA) only in presence of target DNA and as a result, the Au-CDs were conjugated to ds DNA through host-guest recognition. With this the hybridization event can be easily monitored by

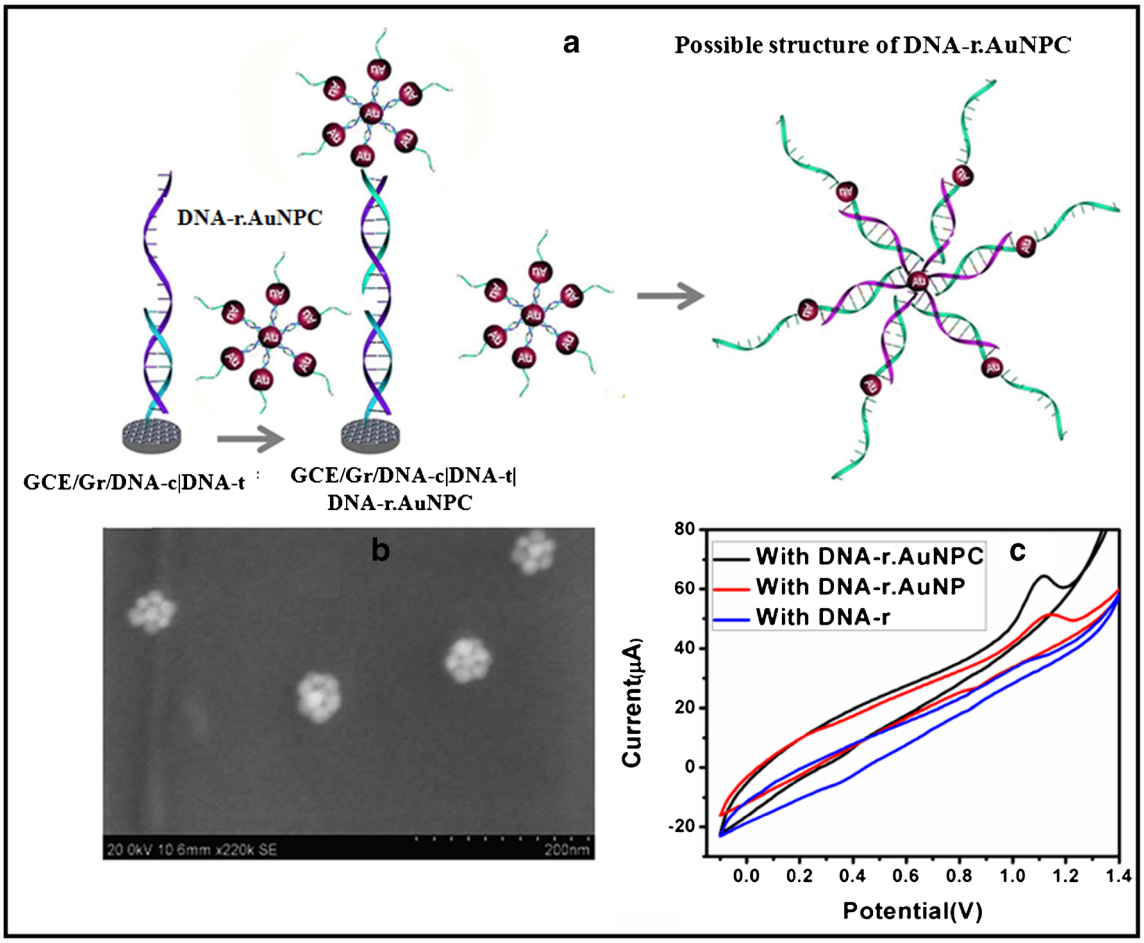

Fig. 4 **a** The schematic of sensor which uses AuNPCs as labels. The schematic of AuNPCs is also given. **b** SEM images of AuNPCs **c** cyclic voltammogram of the sensor with AuNPCs, AuNPs and without AuNPs. Reprinted with permission [60]

electrochemical signal provided by the AuNPs. This sensor showed a detection limit of 30 pM for HBV DNA sequence. The specificity of the sensor is very high since the binding of mismatched sequence with molecular beacon is much weaker which results in lower response. Au quantum dots are also used as tracer for DNA hybridization detection. Pumera et al. used Au_{67} quantum dot as electrical tracer for direct electrochemical detection of DNA hybridization [63]. They used 1.4 nm Au_{67} quantum dot tag linked to the target DNA and after the DNA hybridization, this quantum dot was directly detected without any acidic (HBr/Br_2) dissolution.

The advantage of these kinds of sensors is that there is no need for additional labels such as electroactive tags or redox indicators. The response of the AuNPs is measured directly to detect the DNA. Electrochemical Impedance Spectroscopy (EIS) is suggested as one of the best methods to detect the DNA-gold binding event since EIS is sensitive to changes in interfacial impedance in the transducer surface. The main points to be considered in this type of sensors are the slow electron transfer kinetics of sDNA-AuNPs and the distance between AuNP and DNA bioelectrode. If the distance is long, slow electron tunneling happens between them even in the presence of labels with high electrocatalytic activity. This in turn affects the sensitivity of the sensor. A comparison of different DNA sensors which used AuNP as label is given in Table 1.

AuNPs as signal amplifiers

Taking advantage of the unique conducting properties of AuNPs, these are used as signal amplifiers in various electrochemcial DNA sensors to increase the sensitivity. There are several reports that use AuNPs for amplifying the transduction of hybridization event in electrochemical DNA sensors. Here, the amplification have been done by incorporating redox labels in a single hybridization event. Kawde et al. in 2004 employed an amplifying platform based on polymeric carrier spheres loaded with AuNP tags in electrochemical DNA detection [64]. The polystyrene beads are coated with streptavidin to bind biotinylated AuNPs. This platform combined with catalytic amplification of the multiple AuNP tags resulted in drastic enhancement of the sensitivity

Table 1 A comparison of different DNA sensors which used AuNPs as label

Electrode modification	Label	Technique used	Detection limit	Ref
DNA modified gold electrode	AuNP	EIS	1 pM	[46]
DNA modified gold electrode	Positively charged AuNP	EIS	28 fM	[47]
Functionalized gold electrode	AuNP	EIS	0.3 fM	[48]
Functionalized gold electrode	DNA-AuNP	Chronoamperometry	1 fM	[50]
AuNP functionalized gold electrode	DNA-AuNP	Chronoamperometry	50 aM	[51]
DNA modified gold electrode	Positively charged AuNPs	DPV	2.6 pM	[49]
Gold electrode	AuNP	EIS	1.7 aM	[54]
GCE/Graphene	DNA-AuNP	Chronoamperometry	1 fM	[59]
GCE/Graphene	Clusters of AuNPs	Chronoamperometry	50 aM	[60]
GCE/ graphene oxide-yttria nanocomposite	Clusters of AuNPs	Chronoamperometry	10 aM	[61]
o-aminobenzoic acid (ABA) modified GCE	β-cyclodextrins functionalized AuNP	DPV	30 pM	[62]

of the sensor in the femtomolar level. The signal is observed using stripping voltammetry after HBr/Br_2 dissolution.

Zhang et al. used DNA-AuNPs to carry the redox molecules $[Ru(NH_3)_6]^{3+}$ (RuHex) for the electrochemical DNA detection [65]. Electrochemical signals are generated by RuHex molecule which was bound to the surface-confined capture probe DNA via electrostatic interactions as seen in Fig. 5a. So the redox charge of RuHex is a direct function of the amounts of DNA strands adsorbed on electrode surface. The integration of AuNPs significantly improved the sensitivity of the sensor in the femtomolar range (detection limit of 10 fM) and the sensor showed single base mismatch selectivity. The sensitivity of the sensor is greatly enhanced with sandwich hybridization however, the time of assay will be more on using sandwich hybridization assembly compared to linear hybridization. One major disadvantage of the sensor is the increased background signal resulting from the nonspecific adsorption of small amount of AuNPs. Wang et al. developed a DNA sensor with DNA-AuNP as signal amplifier with a detection limit of 50 fM. [44]. The sensor fabrication involves the immobilization of capture probe on the surface of the Au electrode, then hybridization with the corresponding target DNA, and further hybridization with DNA-AuNP conjugate containing methylene blue. The amplified methylene blue signal is used for the detection of DNA. The main advantage of this method is the modification of AuNPs using two types of signaling reporter DNA molecules. The use of two signaling molecules reduced the cross-reaction between target DNA and DNA-AuNP conjugates compared to the single signaling molecules. The detection limit of the sensor was 50 fM.

In addition to the oligonucleotides modified with AuNPs, other AuNP conjugates are also used for the signal amplification. AuNP-streptavidin conjugates have been used as signal amplifier in several electrochemical DNA sensors. The property of strong interaction between streptavidin-biotin is used in the sensor development. Bonnani et al. developed a DNA sensor with streptavidin-coated AuNPs (strept-AuNPs), which was used to enhance the impedance signal generated by the DNA sensor [67]. The biotin modified target DNA is used for hybridization. The binding between the strept-AuNPs and the biotin-target DNA led to increase in the sensor response. The Rct value was enhanced by the addition of strept-AuNPs indicating the increased resistance due to the presence of Au-streptavidin conjugates. Slight negative charge of streptavidin at the working pH also contributed to the resistance enhancement in consequence of electrostatic repulsion with redox marker. In another report, Fang et al. used biotin labeled molecular beacon for binding with DNA capped AuNP-streptavidin conjugate which was used for signal amplification [66]. The biotin labeled molecular beacon was immobilized on the electrode surface. After hybridization with target DNA, the molecular beacon opens its loop structure so that biotin will bind with the DNA capped AuNP-streptavidin conjugates as seen in Fig. 5**b**. As a result of this binding, the electron transfer resistance increases and the hybridization event was measured by electrochemical impedance spectroscopy. The impedance signal amplification was due to the AuNP-streptavidin conjugate having negative charge. The hybridization time of the molecular beacon will be larger than that of traditional linear DNA and the detection limit of this sensor was 0.35 fM. The detection limit is further enhanced by 10 times using horseradish peroxidase (HRP)-streptavidin capped AuNPs conjugates by the same group using the same sensing principle [68]. In addition they have used AuNPs to modify the electrode surface also. In the presence of target, the stem-loop structure is changed to a rigid linear structure, resulting in the biotin being exposed to the HRP-streptavidin AuNP conjugates. Here the conjugate is attached on the electrode surface via biotin-streptavidin interaction. The signal amplification was due to more numbers of HRP present on the electrode surface. The detection limit of

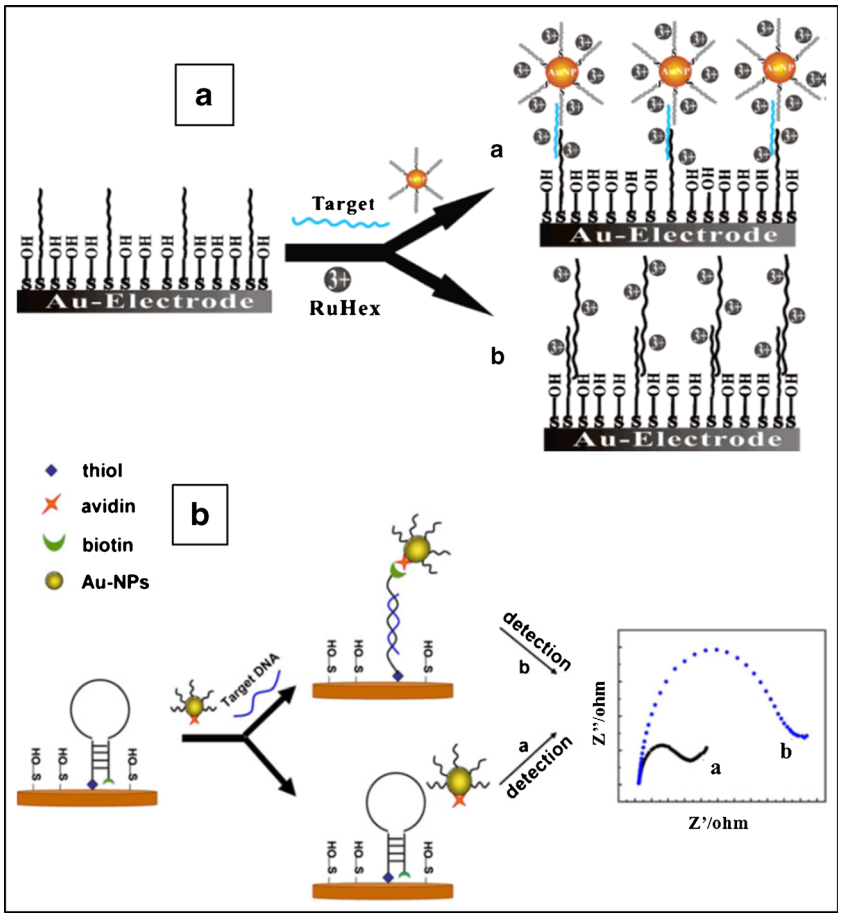

Fig. 5 (**A**) **a** The illustration of AuNPs amplified detection in presence of target DNA. A sandwich complex is formed between three probes. **b** Non-amplified detection without AuNPs: target DNA is attached to the electrode surface after hybridization. The increased RuHex redox charge is due to the hybridized targets. Reprinted with permission from [65]. Copyright (2006) American Chemical Society. (**B**) Schematic representation of the sensor which uses DNA capped AuNPs-streptavidin conjugate for signal amplification. Reprinted with Permission [66]

the sensor was 0.035 fM. and it also shows excellent selectivity towards mismatched DNA sequences.

An ultra-sensitive electrochemical DNA biosensor has been developed based on a signal amplification strategy using horseradish peroxidase (HRP) functionalized AuNPs as signal amplifier on AuNPs/MoS_2/Graphene/chitosan composite modified electrode [69]. The nanocomposite on the surface provides good electrical conductivity and the HRP modified AuNPs gives the signal amplification. Hybridization with the target DNA was evaluated by measuring the electrochemical signal response of HRP using DPV. The streptavidin-HRP-AuNPs tracer permits the hybridization event and providing amplified signal for ultrasensitive DNA detection. The detection limit of the sensor was 2.2 fM. but the sensor fabrication step seems to be complex process and the enzymatic reaction of HRP was used for the detection.

In another work, hairpin DNA (hpDNA) conjugated with AuNPs has been used for the electrochemical DNA detection [70]. Here, hairpin DNA (hpDNA) was used as a novel bio barcode and it was conjugated with AuNPs. The detection is based on simple direct intercalative binding of the $[Ru(NH_3)_5L]^{2+}$ complex with hpDNA/dsDNA as seen in Fig. 6. High content of hpDNA on the AuNPs, and multiple $[Ru(NH_3)_5L]^{2+}$ complex molecules intercalated with one hpDNA/dsDNA molecule are the reason for enhanced electrochemical response. Detection was based on the binding of the complex molecule with the hpDNA and the dsDNA. The detection limit was 1 fM. and use of gold electrode modified with some nanostructures may enhance the sensitivity of this sensor further.

Advantage of the sensors using AuNPs based signal amplification is the enhanced sensitivity in DNA detection. But it needs extra label molecules for the detection and some of the sensors need complex fabrication procedures. The use of molecular beacons provide the better selectivity results however it consume more time for hybridization procedures which is one of the major drawback of the system.

AuNPs as carriers of electroactive molecules

AuNPs are used as carriers for electrochemical tags in electrochemical DNA sensors taking advantage of the large surface area possessed by the nanoparticles, which lead to more binding sites and hence immobilizations of large number of biorecognition elements or electrochemical tags on AuNPs. This method is attractive due to the amplification of the analyte signal in a single recognition reaction. Ferrocene [71–73], methylene blue [74, 75] and thionine [76–79] are the most

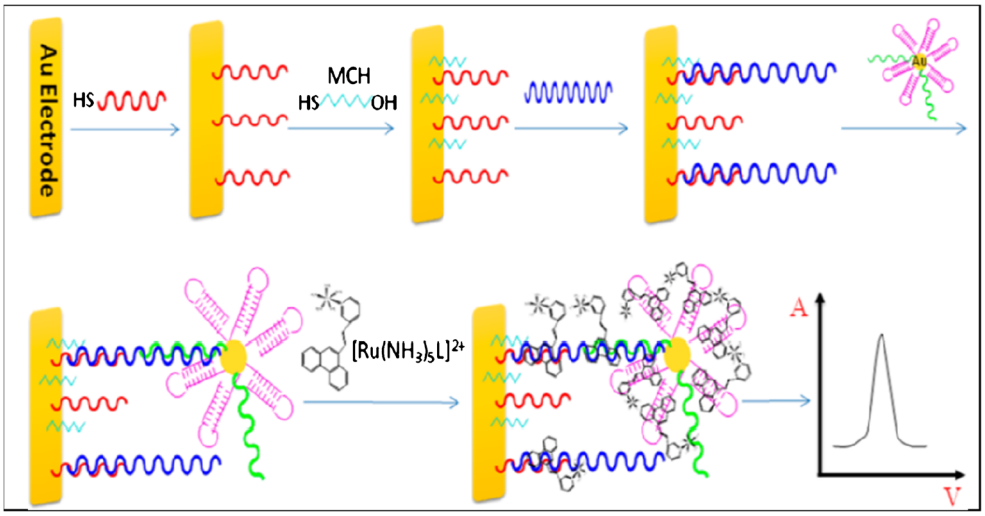

Fig. 6 Sensor fabrication procedures for the hpDNA based sandwich DNA sensor and its detection strategy. Reprinted with permission [70]. Copy right 2015 American Chemical Society

commonly used electroactive molecules in electrochemical DNA sensors.

In order to hold the electroactive molecules on AuNPs, appropriate functionalization with specific molecules is required. AuNP/streptavidin conjugate was used for carrying large number of ferrocene molecules towards the development of a sensitive electrochemical DNA sensor [80]. The conjugate was attached to the DNA by biotin-streptavidin interaction. A detection limit of 2.0 pM was achieved and the amplification of the signal was due to the addition of a large number of ferrocene molecules on the AuNP/streptavidin conjugates. A similar strategy was used by Baca et al. [81] by modifying the electrode surface and the detection limit of this sensor was found to 0.25 pM. They have also studied the voltammetric behavior of the ferrocene groups based on the effect of the DNA probe and target strand lengths. The result showed that the shorter duplexes would lead to a more reversible voltammetric signal and the formation of duplexes with a larger number of base pairs will provide the lower detection limit. Both the sensors work on an assumption that the ferrocene moieties are located in close proximity to the underlying electrode due to the large mass of nanoparticle conjugate and the elasticity of the DNA strands. Similarly Qiu et al. developed two sensors for two different gene sequences with the detection limits in femtomolar level [82, 83]. They have used AuNP-streptavidin conjugate to carry ferrocene molecules and the amplified signal was obtained due to the AuNP-based enrichment of redox-active moieties. One of the sensors used a restriction site of endonuclease enzyme (EcoRI) for breaking the capture probe and the capture probe residues after cleavage treatment also promoted the interfacial electron transfer which led to further enhancement in the signal [82]. In these sensors, the ferrocene tags are dragged in close proximity to the electrode surface after hybridization to increase the sensor response. The detection limits of the sensors are in the femtomolar range and also the modification of AuNPs is a complex process.

Methylene blue (MB) is another commonly used redox indicator in DNA sensors which gives very good signal in differential pulse voltammetry. If the signal of MB is increased a highly sensitive detection would be possible. Such attempts were reported by many research groups. DNA-AuNPs were used for the amplification of electrochemical signal of MB which is attached to the AuNP through single stranded DNA [84]. The DNA-AuNP contained two kinds of DNA, one biotin modified hairpin probe and other is MB labeled signal probe. After hybridization with target DNA the hairpin was opened and the DNA-AuNPs are brought towards the electrode surface through biotin-avidin interaction as shown in Fig. 7. Electrochemical signals of MB bound to signal DNA were measured by DPV. The detection limit is only in the picomolar range and the AuNP modification seems to be a complex process. Kong et al. showed that the detection limit of this type of sensor can be enhanced by combining the AuNP based signal amplification and enzymatic recycling reaction [85]. Here a hairpin structured probe was used to hybridize with target DNA and an exonuclease ExoIII was selected for the homogeneous enzymatic cleaving amplification. The enzymatic product is then hybridizes with the hairpin structured capture probe and the introduction of DNA-AuNPs along with enzymatic recycling reaction enhances the sensor response. The success of the sensor depends on the product after the enzymatic reaction and the sensor achieved a detection limit only in the picomolar range. The sensor exhibited high specificity for target DNA which attributes to the use of two hairpin probes but the use of hairpin probes increases the assay time.

In a similar way, DNA-AuNP was used to load thionine, an electroactive molecule for sensitive electrochemical DNA sensing [85]. The AuNPs were attached with reporter DNA, which increases the number of adsorbed thionine

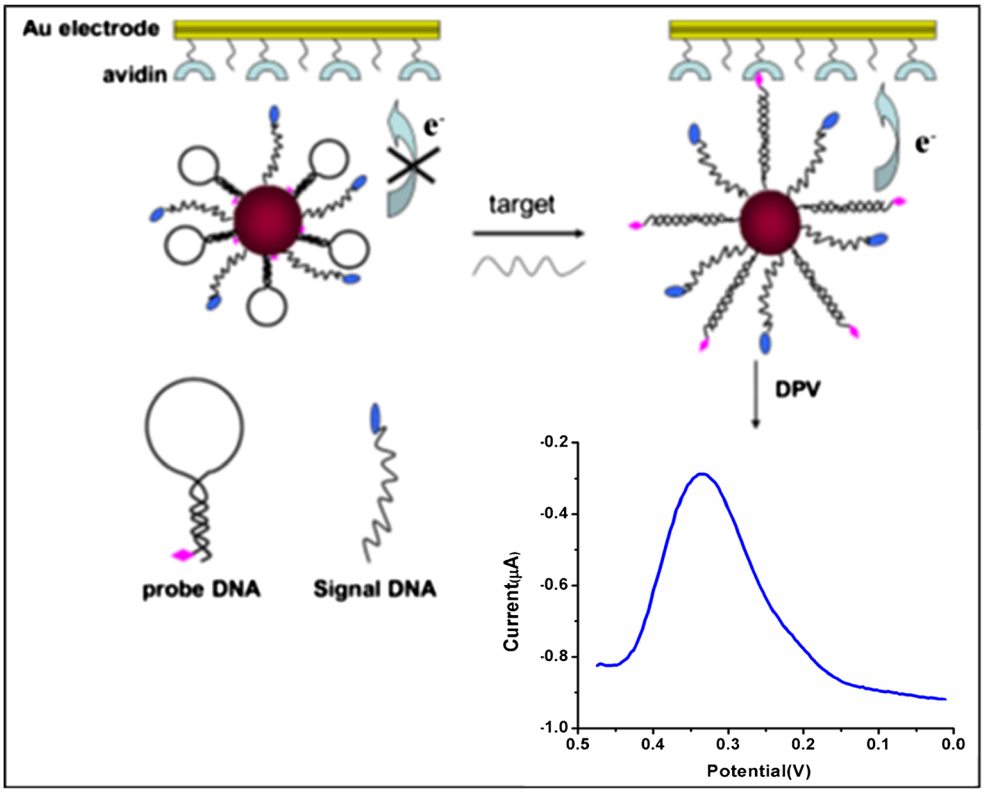

Fig. 7 Scheme for DNA sensor based on DNA-functionalized AuNPs for the amplification of electrochemical signal of MB. Reprinted with Permission [84]

molecules onto the AuNPs as well as the stability of AuNPs. After hybridization with target DNA, a rigid probe-target duplex is formed, which pushes the thionine capped DNA-AuNP away from the electrode which resulted in decrease in the current of the DNA sensor. The high sensitivity of this sensor made by Wang et al. was due to large number of electroactive thionine molecules present on the AuNPs/reporter DNA conjugates [86]. The detection limit was 0.5 pM and since they have used dsDNA-AuNP conjugate as the signal tag which is larger than ferrocene, the distance between the immobilized DNA may affect the characterizations of the sensor. The detection limit of this type of sensor was further enhanced to 0.05 fM. by modifying the electrode surface with AuNPs by Liu et al. [87]. The enhancement in the sensitivity is due to the combined effect of AuNPs on the electrode surface as well as large number of electroactive thionine molecules present on the AuNPs/reporter DNA conjugates. A comparison of the DNA sensors using AuNPs as carriers of electroactive molecules is given in Table 2. The advantages of using AuNPs as carriers for electroactive molecules are the high sensitivity and no requirement of labeling of the DNA targets. But the binding between the electroactive molecule and AuNPs in the analytical environment is a concern during the analysis. The steric hindrance between electroactive molecules and the sensing interface is another important issue. To avoid this issue, proper dragging strategy can be used to drag the electroactive molecule towards the electrode surface. Moreover, the length of DNA sequences will also affect the sensitivity.

AuNPs acting as a catalyst

AuNPs are also used as electrocatalyst due to its high catalytic behavior. Due to the large surface area and surface energy of the nanoparticle they have numerous catalytically active sites on the surfaces. When AuNPs are used as catalysts, signal generation from the active sites of nanoparticle possibly will allow higher amplification [88]. For example, Liu et al. used electrocatalytic amplification of AuNPs to increase the sensitivity of the sensor with DNA-AuNP as label [89]. The electrocatalytic response of the sensor is lowered when AuNPs conjugated DNA is used owing to the slow electron transfer kinetics on DNA-modified AuNPs compared to the unmodified AuNPs. In this work, $NaBH_4$ treatment was used to increase the electrocatalytic activity of AuNPs for hydrazine electrooxidation. The combined effect of high electro-catalytic activity of AuNPs and enhanced activity of DNA-modified AuNPs by $NaBH_4$ treatment significantly increases the sensitivity of the sensor. Nevertheless the addition of chemicals like $NaBH_4$ and hydrazine limits the repeated use of the sensor.

Silver enhancement strategy

Silver enhancement strategy of AuNPs is a widely explored method for enhancing the sensitivity of electrochemical DNA sensors. In this method, silver ions are reduced to silver metal at the surface of AuNPs (which is used as label) and it grows on the surface. Bonnani et al. used silver enhancement

Table 2 A comparison of the DNA sensor using AuNPs carriers of electroactive molecules

Modification of AuNPs	Electroactive molecule	Technique used	Detection limit	Ref
AuNPs /streptavidin conjugate	Ferrocene	Cyclic voltammetry	2.0 pM	[80]
AuNPs /streptavidin conjugate	Ferrocene	Cyclic voltammetry	0.25 pM	[81]
AuNPs /streptavidin conjugate	Ferrocene	DPV	0.5 fM	[82]
AuNPs /streptavidin conjugate	Ferrocene	DPV	4 fM	[83]
DNA functionalized AuNPs with special molecules	Methylene blue	DPV	1 pM	[84]
DNA functionalized AuNPs	Methylene blue	DPV	0.6 pM	[85]
DNA functionalized AuNPs	Thionine	DPV	0.5 pM	[86]
DNA functionalized AuNPs	Thionine	DPV	0.05 fM	[87]

treatment applied to electrodes which is already modified with DNA-nanoparticle conjugates. A significant increment of Rct value was observed in impedance measurement, attributable to silver deposition on gold [67]. Lin et al. reported an electrochemical DNA sensor by the combination of graphene and DNA-conjugated AuNPs along with silver enhancement strategy [90]. The captured DNA was immobilized on graphene and target DNA and AuNP labeled probes are hybridized in sandwich assay organization followed by AuNP catalyzed silver deposition. Owing to the high DNA loading capacity of graphene and signal amplification done by AuNPs-catalyzed silver staining, the resultant biosensor gave a good sensitivity with a detection limit of 72 pM and with single base mismatch discrimination. In another work, a highly sensitive method was developed by combining circular strand-displacement polymerization (CSRP) and AuNP catalyzed silver enhancement to achieve dual signal amplification by Gao et al. [91]. This dual signal amplification provides a better enhancement in the sequence specific detection in sub femtomolar level with single base mismatch discrimination. The detection limit obtained was 2.88 fM. but it consisted of complex sensor fabrication steps. In a different approach, Ye et al. used silver enhancement strategy to enhance the sensor response by increasing the blockage efficiency of the nanopore [92]. Impedance sensing was used to measure the blockage in the nanopore which is induced by the DNA hybridization. AuNP tags on DNA as well as catalytic silver deposition increases the blockage efficiency of the nanopore and as a result the impedance signal is amplified. The sensor used a different approach for DNA sensing but the detection limit obtained was 50 pM. Here the concentration of silver enhancer solution and the deposition time are very important to get the high sensitivity. High concentration of silver enhancer solution and deposition time should produce high background current. This may be due to the growth of silver particles through polyanionic DNA chains, because of the formation of ion-pair complex to the bases by cation exchange between Ag^+ and Na^+.

AuNPs as immobilization platform

Taking advantage of the excellent electronic conductivity offered by the AuNPs these are used as the immobilization platform in DNA sensors. In addition to this, DNA sequences can be easily immobilized on the surface of the AuNPs mainly using Au-S bond. There are three different strategies for using AuNPs as immobilization platform. 1) Functionalized AuNPs, 2) AuNP composites and 3) AuNPs in combination with some other nanomaterials.

Ensafi et al. used AuNPs to modify the gold electrode towards impedimetric detection of DNA related to chronic lymphocytic leukemia [25]. The AuNPs are electrodeposited on the gold electrode followed by the immobilization of capture probe DNA and hybridization with target DNA. It was found that the AuNP-modified electrode can improve the density of the probe DNA attachment and hence the sensitivity of the DNA sensor significantly. Though the sensor fabrication was simple, the detection limit achieved was only 1 pM whcih will limit its applications. AuNP electrodeposited glassy carbon electrode was used for DNA hybridization detection as well as DNA damage [93]. Using this sensor, the DNA damage caused by the Cd^{2+} ions have been detected by measuring the peak current in differential pulse voltammetry. This type of sensor can also be used to study the mechanism of DNA damage caused by oxidative pathways. Radhakrishnan et al. used AuNPs functionalized poly(3,4-ethylenedioxythiophene) (PEDOT) film on a glassy carbon electrode for sensitive DNA detection. The incorporation of the AuNPs in the PEDOT matrix enhances the conductivity and eliminates the direct functionalization of the polymer for DNA attachment. The high sensitivity is ascribed to the large surface area of the nanoparticles and the AuNP assisted charge transfer kinetics of PEDOT matrix [28]. The detection limit was observed as 0.26 fM. using ruthenium complex as redox probe.

Gold nanoparticles modified with carbonaceous materials as immobilization platform

AuNPs have been used in conjunction with other nanomaterials to improve the sensitivity. The most commonly used materials in combination with AuNPs are graphene, graphene oxide and CNTs. Carbon nanostructures act as a skeleton support for AuNPs as well as this increases the surface area and AuNPs are used for carrying the DNA probe through Au-S bond.

AuNPs modified with graphene or graphene oxides

Zhang et al. used AuNPs, polythionine and graphene for modifying the glassy carbon electrode towards the fabrication of label free DNA sensor. Thionine is a kind of phenothiazine dye and has good electron transfer ability [94–97]. They have combined the advantage of thionine polymer with properties of graphene to fabricate a label-free electrochemical DNA sensor. The use of graphene and thionine increase the electrode surface area and electrical conductivity, and AuNPs act as the immobilization platform for the probe DNA [29]. A detection limit of 35 fM. was observed for this sensor.

Graphene sheets together with PANI and AuNPs [98] were used for electrochemical detection of BCR/ABL fusion gene in picomolar level with functional hairpin probe. Graphene/Au nanorod/polythionine composite modified GCE was used for the detection of human papilloma virus (HPV) DNA with a detection limit of 40.3 fM. [99]. Here Au nanorods enhances the immobilization of the probe DNA and the ability for hybridization. But it is difficult to synthesize combinations of Au nanorods onto the graphene sheets with good water dispersibility. The sensor fabrication seems to be complex procedure and it used ruthenium complex as redox probe.

Layered CuS-graphene composite and AuNPs [100], tungsten sulfide (WS_2)-graphene composites and AuNPs [101] were also have been investigated for the development of electrochemical DNA sensors. The schematic of the sensor which uses layered CuS-graphene composite and AuNPs is given in Fig. 8. In both the cases, graphene served as a two dimensional conductive skeleton support to CuS as well as WS_2 and the composite provides a stable and conductive interface for the electrochemical DNA detection. The detection limit of the sensor which used layered CuS-graphene composite was 0.1 pM and that of WS_2-graphene composite was 2.3 fM. Both the sensors were used without any redox label. The sensor is found to be promising and can be modified to achieve higher sensitivity after evaluating the stability of the composite during the electrochemical measurement in detail.

Similar to graphene, the graphene oxide is also used as a substrate material to increase electroactive surface area. Yola et al. used Fe@AuNPs-aminoethanethiol functionalized graphene oxide for modifying the glassy carbon electrode for DNA detection [102]. The use of 2-aminoethanethiol functionalized graphene oxide in the nanocomposite may load more Fe@AuNPs and therefore increase the electrode active area, which can support a higher amount of DNA probe on the surface. This will result in enhanced the detection limit down to 2 fM. with the use of basic blue 41 as electrochemical indicator. The sensor fabrication was very simple and provided high selectivity and stability. Zhang et al. used reduced graphene oxide (rGO) sheets decorated with AuNPs as the immobilization platform for sequence-specific DNA detection [103]. The electrode surface area is improved by using reduced graphene oxide (rGO) and Au NPs. The detection limit was found to be 35 fM. and the reduction peak current of adriamycin is used for the DNA detection. They found that the peak currents of adriamycin were higher than the sensor which uses MWCNT and GO as immobilization platform. This may be due to the larger amount of probe DNA immobilized on the electrode surface because of the larger surface area of rGO. This biosensor provided high selectivity since complementary DNA was detected by this sensor in presence of a large amount of mismatched DNA (1000:1).

Another interesting work have been reported with graphene oxide sheet decorated with AuNPs on glassy carbon electrode for developing a supersandwich type electrochemical DNA biosensor with a detection limit of 0.35 fM. [104]. The target DNA hybridizes with signal probe and capture probe by sandwich type hybridization. Continuous hybridization reaction occurred between target DNA and signal probe when the target DNA concentration is increased, leading to long DNA concatamers formation. Thus, the response of the sensor was enhanced by the loading of multiple signal probes on the electrode surface. The sensor fabrication seems to complex procedure and it used methylene blue labeled DNA for the detection. Recently, nitrogen doped graphene nanosheets functionalized with AuNPs was investigated for sensitive and selective DNA detection by Chen et al. [105] with a detection limit of 3.12 fM. The nitrogen doped graphene/AuNP modified GCE exhibited high electrochemical activity as well as high electron transfer, significantly enhancing the signal detection. Nitrogen doping in graphene can introduce some defective sites on the graphene surface to alter its conducting properties. A hybrid nanomaterial of nitrogen doped graphene and AuNP for the modification of the electrode and methylene blue as electrochemical label has been used to construct the sensor. AuNPs-aminothiophenol functionalized graphene oxide nanocomposite [106] and Au nanoparticles/toluidine blue - graphene oxide nanocomposites [107] were also used as immobilization platform with picomolar detection limit. The detection limits obtained were 11 fM. and 2.95 pM respectively. The sensor fabrication steps were simple but the detection limit has to be improved for the sensor to make it useful for the real sample analysis for early stage diagnosis.

Recently, Benvidi et al. developed a highly sensitive electrochemical biosensor for BRCA1 mutation detection based on rGO and AuNPs modified GCE [108]. The enhanced sensitivity and selectivity of the sensor was due to the occurrence of DNA synthesis at the surface of the electrode. This label free DNA sensor showed simple fabrication steps and a wide dynamic detection range with a very low detection limit of 10 zeptomol. The detection limit of 10 zeptomolar is the lowest among this type of DNA sensors. In another recent report, oracet blue is used an electroactive label for sensitive detection of DNA with a detection limit of 27 pM by using graphene oxide and AuNPs as the immobilization platform [109]. They

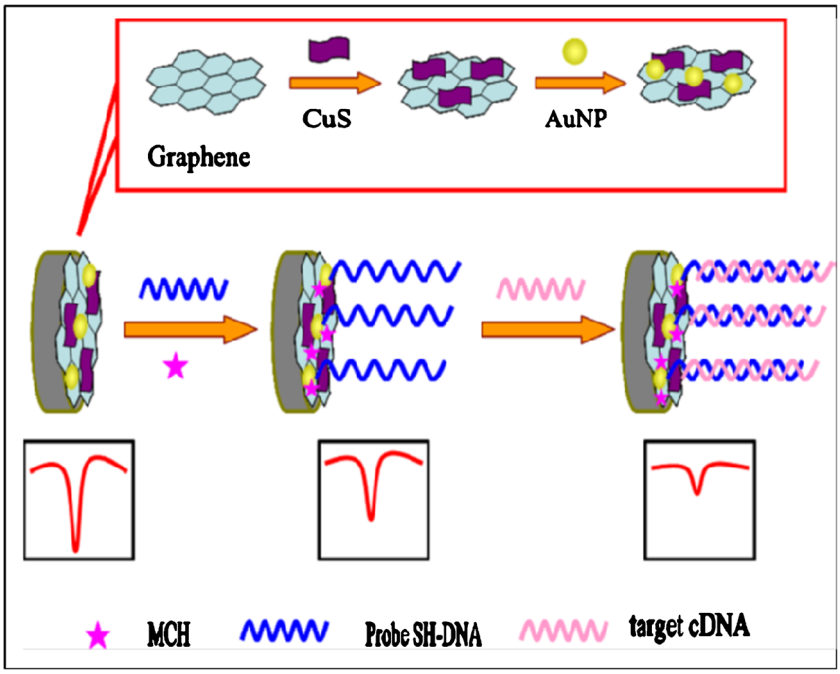

Fig. 8 Schematic of ultra-sensitive electrochemical DNA biosensor made from a composite of molybdenum disulfide, graphene, chitosan and AuNPs to modify the glassy carbon electrode. Reprinted with permission [100]

have introduced a new label for DNA sensing but the detection limit has to be improved for this sensor. Very recently, hemin functionalized rGO sheets along with AuNPs were used for the modification of glassy carbon electrode towards the construction of electrochemical DNA sensor [110]. DPV was used for the detection by measuring the change in current of the oxidation-reduction reaction of hemin before and after DNA hybridization. A promising detection limit of 0.14 aM was achieved.

Main advantages of these types of electrochemical DNA sensors are the low detection limit and the label free detection. The DNA immobilization on the carbon nanostructure is based on π interaction and there is chance for non-uniform assembly of DNA especially when short DNA strands are used. The stability of binding between graphene or graphene oxide and the electrode should also be considered during the measurement in presence of liquids. There is a chance of detaching carbon nanostructures from the electrodes after immersing the electrode in solutions which can be solved with the use of a binder such as nafion, though it leads to some resistance. Also the repeatability of the sensor modification of the electrode is also needs to be taken care of especially in drop casting method. Electrochemical deposition can be used to minimize the non-uniform modification of the surface.

AuNPs modified with carbon nanotubes

CNTs in combination with AuNPs can be used for DNA sensing taking advantage of the high surface area as well as good conductivity of CNT and AuNPs possibility of stable immobilization of DNA on AuNP. Functionalized CNTs are also used as the substrate material in electrochemical DNA sensors. Zhang et al. used multi-walled carbon nanotubes (MWCNT) with carboxyl groups on GCE for the sensitive detection of target DNA [111]. Aminobenzoic acid (ABA) was electropolymerized on the surface of the functionalized MWCNT which was immobilized on the glassy carbon electrode and then the surface was modified with AuNPs. Probe DNA was immobilized on the surface of AuNPs through Au-S bond and the detection of DNA hybridization was done by the measurement of the intercalated adriamycin by using DPV. The detection limit was 0.35 pM with the help of electroactive label adriamycin.

A sensitive label-free platform based on electrochemical growth of Au nanoparticles onto vertically aligned MWCNT arrays has been reported to detect specific sequence of TP53 gene [112]. In this, thiol modified probe DNA was adsorbed on the aligned MWCNTs and then AuNPs were adsorbed through self-assembly process. EIS was used to evaluate the sequence-specific DNA hybridization events related to TP53 gene in attomolar level and it exhibited outstanding response to target DNA related to TP53 mutation detection. The detection limit was 10 aM and the sensitivity enhancement was due to the synergistic interactions of aligned MWCNT array and AuNPs.

An electrochemically grown AuNPs on horizontally aligned single walled carbon nanotube (SWCNT) array was reported [113]. The gold-coated SWCNT acts as an isolated microelectrode as seen in Fig. 9**a** and it can detect complimentary 10-base DNA in zeptomolar level concentration. The

charge transfer resistance of the sensor was reported to be varying with respect to the target DNA concentration. The major enhancement in the sensitivity of this sensor was due to synergistic interactions of horizontal SWCNT array and AuNPs. By using this methodology, the authors achieved one of the lowest detection limits of 10 zeptomolar. Also the sensor gave a detection limit of 100 aM for single base mismatch DNA. Dong et al. used glassy carbon electrode modified with AuNPs, poly(dopamine) and CNTs for sequence specific detection of target DNA with a detection limit of 3.5 fM. [114]. The ruthenium(III)hexammine complex acts as the electrochemical indicator in this sensor and they verified that the combination of substrate material increases the conductivity of the electrode interface and improves the electron transfer rate. The schematics of the sensor fabrication are given in fig. 9b.

A very high sensitivity with zeptomolar detection limit is achieved by the sensors with CNT and AuNPs combination as the immobilization platform. As in the previous case, the stability of the binding between CNT and the electrode and the repeatability of the sensor modification are the major concerns.

Other than carbon nanostructures, CuS nanosheets have been used along with AuNPs for sensor fabrication by Huang et al. [115]. Here, acetylene black incorporated two-dimensional CuS nanosheets and AuNPs were used as the immobilization platform. This DNA sensor exhibits a detection limit as low as 20 fM. with an excellent selectivity. The high sensitivity of the sensor can be attributed to the synergistic effect of acetylene black and the unique nanostructure of CuS nanosheets as well as AuNPs. The detection limit of the sensor was 20 fM. but the synthesis of composite was a complex procedure. A comparison of sensor which uses AuNPs as immobilization platform is given in Table 3.

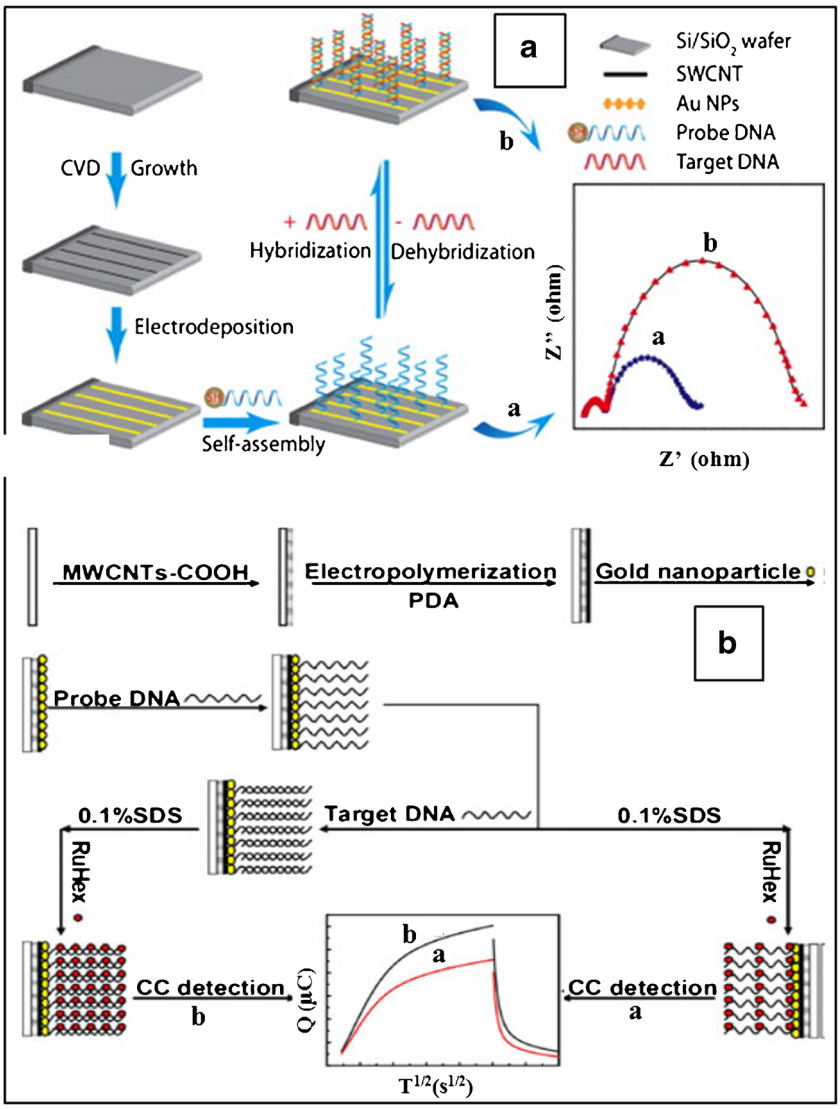

Fig. 9 **a** Illustration of sensor fabrication which uses electrochemically grown AuNPs on horizontally aligned SWCNT array. Reprinted with permission [112]. **b**. Schematic illustrations of the sensor which uses MWCNTs functionalized with carboxylic acid groups along with poly dopamine and AuNPs on GCE. Reprinted with permission [114]

Table 3 A comparison of sensor which uses AuNPs as immobilization platform

Electrode used	Technique used	Detection limit	Ref
GCE/AuNPs functionalized PEDOT film	Chronocoulometry	0.26 fM	[28]
GCE/ Graphene/polythionine/AuNP	DPV	35 fM	[29]
GCE/graphene-chitosan solution/polyaniline/AuNP	DPV	2.11 pM	[98]
GCE/graphene/Au nanorod/polythionine	DPV	40 fM	[99]
GCE/graphene/CuS/AuNP	DPV	0.1 pM	[100]
GCE/WS_2-graphene/AuNP	DPV	2.3 fM	[101]
GCE/Fe@AuNPs-aminoethanethiol GO	DPV	2 fM	[102]
GCE/graphene/AuNP	DPV	35 fM	[103]
GCE/rGO/AuNP	DPV	0.35 fM	[104]
GCE/nitrogen doped graphene-Au nanocomposite	DPV	3.12 fM	[105]
GCE/ AuNPs-aminothiophenol functionalized GO	EIS	11.3 fM	[106]
GCE/rGO/AuNP	DPV	1 fM	[107]
GCE/rGO/AuNP	EIS	10 zM	[108]
GCE/GO/AuNP	DPV	27 pM	[109]
GCE/hemin-rGO/AuNP	DPV	0.14 aM	[110]
GCE/ MWCNT/polyaminobenzoic acid/ AuNPs	DPV	0.35 pM	[111]
Ta substrate/ MWCNTs / electrochemically grown AuNP	EIS	10 aM	[112]
Si/SiO_2 wafer/SWCNT/electrodeposited AuNP	EIS	100 aM	[113]
GCE/MWCNT/polydopamine/AuNP	Chronocoulometry	3.5 fM	[114]
GCE/CuS-acetylene black/AuNP	DPV	20 fM	[115]

Multiple amplification using AuNPs as label as well as immobilization platform

There some reports which uses two different AuNPs in the sensor design. Here, the immobilization platform is modified with AuNPs or nanocomposite and the label is also specially modified AuNPs or AuNPs in conjugation with some other nanomaterials. The synergic effect of these two AuNPs combined with other nanomaterials will enhance the sensitivity of the sensor. For example, we have developed an electrochemical DNA sensor in which poly ethylene glycol functionalized AuNPs as immobilization platform and MPA functionalized AuNPs as label [50]. It was evident from the experiments that the attomolar sensitivity of the developed sensor was due to the high electron conduction by the use of two appropriately functionalized AuNPs.

In one another report, gold nanorod (AuNR) decorated with reduced graphene oxide (rGO) sheets were used as immobilization platform and AuNR as a carrier of redox molecule for the detection of the specific-sequence target DNAs [27]. The sensor uses"sandwich-type" detection strategy, which utilizes three different probes as seen in Fig. 10. The capture probe was immobilized on the AuNRs decorated reduced graphene oxide (rGO) sheets. The reporter probe DNA is labeled with AuNPs hybridizes with the target DNA and the electrochemical signal of adriamycin was measured to detect the DNA hybridization. The combination of AuNPs labeled reporter probe DNA and AuNR decorated rGO sheets as immobilization platform for capture probe DNA significantly enhanced the sensing performance of this sensor. The detection limit of the sensor was found to be 35 aM with the help of electroactive label adriamycin and it also showed good selectivity.

Liu et al. used gold particle-modified screen-printed carbon electrode (SPCE) and thionine-capped AuNP/reporter DNA conjugate tags together to enhance the sensitivity of the sensor [87]. A detection limit of 0.05 fM. is obtained based on this dual amplification procedure. Another DNA biosensor has been developed based on a signal amplification strategy using AuNPs/MoS_2/graphene/chitosan composite modified electrode and horseradish peroxidase (HRP) functionalized AuNPs as tracer [69]. The detection limit achieved is 2.2 fM. Graphene-AuNPs composite together with AuNP-polyaniline (Au-PANI) nanocomposite label [116] were also used for the electrochemical DNA detection in femtomolar level. The good electron transfer ability of polyaniline along with the good biocompatibility and excellent electrochemical activity Au-PANI is effectively utilized here to achieve the high sensitivity. These types of sensors are having high sensitivity but the fabrication steps are complex compared to other sensors. A comparison of different sensors which uses AuNPs as immobilization platform as well as label is given in Table 4.

Conclusion and future prospective

In view of the integral role of DNA/RNA sensors in medical and clinical field, development of highly sensitive and

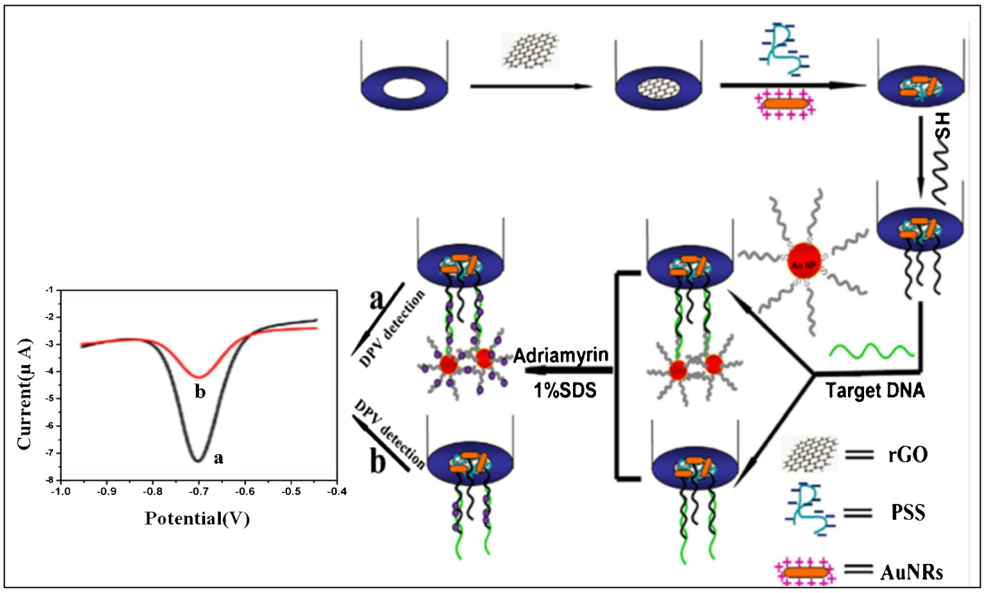

Fig. 10 Schematic represents the fabrication procedure of DNA biosensor which uses AuNRs decorated reduced graphene oxide (rGO) sheets as the immobilization platform [27]

selective DNA sensors are a highly explored area which receives considerable attention presently with the scope of improving the sensitivity and selectivity of sensors. Of the various types of DNA sensors the electrochemical sensors offer sensitivity, selectivity and low cost for the specific DNA sequence detection. The electrochemical sensors take advantage of the nanoscale interactions between the target sequences, the recognition layers and the electrode surface. Sensitivity of the sensors essentially depends on how well the electrochemical label transports the electrons from the analyte to the electrode surface. The immobilization platform plays a crucial role in both sensitivity and stability of the sensors. Another important factor is the electrochemical label. Gold nanoparticles play a major role in enhancing the sensitivity of DNA sensors and it can be used either as immobilization platform or as electrochemical label. In this review, we have highlighted the use of AuNPs which contributed significantly in improving the limit of detection and stability of the electrochemical sensors.

The multiple amplification strategies, which exploit two or more types of nanomaterials, can further improve the sensing performance of the sensor as suggested with the combination of rGO and AuNP or CNT and AuNP. From the existing literature the most suitable architectures to achieve lower detection limits are the combination of carbon nanostructures and AuNPs as the sensor surface and this type of sensors provides zeptomolar level detection. However, for multiple detection this system may not be the ideal one, rather the self assembled monolayer strategy to immobilize nanoparticles will be the best choice. In addition to this, novel nanomaterials with more defined and controllable structure as well as superior properties will be very beneficial for DNA-based electrochemical sensing systems. The graphene like 2D layered materials in conjunction with Au nanoparticles will definitely improve the sensitivity of the sensor. Upon realizing the lowest detection limit to the level of single molecule detection the selectivity should be focused on. The appropriate electrochemical mediators which selectively bind to the bases should be used in conjunction with the nanosystems.

There are two main strategies of the use of AuNPs in electrochemical DNA sensing. One is the adsorption of AuNPs

Table 4 Comparison of different sensors which uses AuNPs as immobilization platform as well as label

Immobilization platform	Label	Technique used	Detection limit	Ref
PEG functionalized AuNPs	DNA-AuNP	Chronoamperometry	50 aM	[50]
GoldNRs/rGO sheets	DNA-AuNP	DPV	35 aM	[27]
AuNP modified screen-printed carbon electrode (SPCE)	Thionine capped DNA-AuNP	DPV	0.05 fM	[87]
AuNPs/MoS_2/Graphene/chitosan composite	Horseradish peroxidase (HRP) functionalized AuNPs	DPV	2.2 fM	[69]
Graphene-AuNP composite	Au-PANI nanocomposite	DPV	1fM	[116]

onto the surface of electrode by appropriate chemical modification to increase the surface area and electrical conductivity of an electrode. Second is to use properly functionalized AuNPs as labels to increase the loading of electroactive species for signal amplification. The main points to be considered for further development are, to ensure the chemical interaction between the nanomaterial and the electrode as well as the conjugation of nanomaterial with DNA and the conjugation of labeling molecule on DNA-AuNPs. Also care should be taken to minimize the complex fabrication procedure, use of expensive and toxic materials and complex detection mechanisms.

Major challenge rests in the ability to detect the target sequence from a mixed population of complementary target and mismatched target. Therefore, attempts should be focused for the detection of mutation if the mutated gene is seen as 1 part mutated DNA in 10,000 wild type sequences. Such a selective sensor will make the dream of early stage detection of genetic diseases comes true. The sensor arrays by integrating with individual nanomaterial-based DNA electrochemical sensors for bio-detection at single cell and molecule level as well as the point-of-care diagnosis will be the most attractive aspect in the future of sensor devices. It is also fascinating to develop suitable nanomaterial-based DNA sensors for in vivo studies. Furthermore great efforts should be made for improving electrochemical DNA biosensor by enhancing its sensitivity, selectivity, miniaturization, and speed with the persistent development of nanotechnology for easy to use and portable devices.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

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