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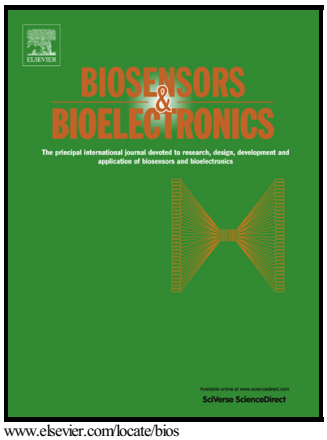

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Application of nanomaterials in the bioanalytical detection of disease-related genes

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ABSTRACT: In the diagnosis of genetic diseases and disorders, nanomaterials-based gene detection systems have significant advantages over conventional diagnostic systems in terms of simplicity, sensitivity, specificity, and portability. In this review, we describe the application of nanomaterials for disease-related genes detection in different methods excluding PCR-related method, such as colorimetry, fluorescence-based methods, electrochemistry, microarray methods, surface-enhanced Raman spectroscopy (SERS), quartz crystal microbalance (QCM) methods, and dynamic light scattering (DLS). The most commonly used nanomaterials are gold, silver, carbon and semiconducting nanoparticles. Various nanomaterials-based gene detection methods

are introduced, their respective advantages are discussed, and selected examples are provided to illustrate the properties of these nanomaterials and their emerging applications for the detection of specific nucleic acid sequences.

KEYWORDS: Nanomaterials, Gene detection, biosensor, DNA, disease

Contents

1. Introduction

The understanding of the association between genetic factors and the development of disease has contributed to the discovery and sequencing of the genome. Interest in the detection of specific nucleotide sequences has grown tremendously in the last decade, particularly in the areas of medicine, biology, and chemical biology. Gene detection plays an important role in the diagnosis of genetic diseases and conditions and is particularly useful for early-stage treatment and monitoring.

Conventional DNA (oligonucleotides) detection systems primarily rely on Fluorescence and radioactive detection readout methods such as polymerase chain reaction (PCR), RT-PCR, Northern blotting, Southern blotting, and enzyme-linked immune sorbent assay (Lien and Lee, 2010; Marti et al., 2007; Sassolas et al., 2008; Kolpashchikov, 2010). For example, careful primer design and accurate temperature control allow PCR to obtain sensitivities in the fM range with single-base mismatch specificity (Roberts et al., 2009; Tost and Gut, 2005). Many related PCR detection methods have been established based on labeling techniques using enzymes, radioactive isotopes, fluorescence, chemiluminescence, and other probes. Although these conventional technologies have provided the golden standard for laboratory-based DNA diagnostics, they cannot meet the different requirements of various analyses, including clinical diagnostics, and analyses at battlefield, Third World, and first responder sites, as required for bioterrorism defense application. Consequently, more convenient and effective DNA sequencing methods are required.

Nanomaterials have recently attracted significant interest from chemists, biochemists, physicists, medical experts, and environment researchers, reflecting the extraordinary functional properties and increasingly numerous applications in biomedical technology and the manufacturing industry. Nanoparticles of different sizes and shapes possess distinct optical properties, and nanomaterials exhibit unusual magnetic, electric, and catalytic characteristics. Researchers around the world have devised a plethora of methods for preparing nanoparticles of different compositions (such as metallic, carbon-based, magnetic, semiconducting, and polymeric nanoparticles) in high yield in solid, solution, and gas phases and on surfaces. Nanoparticles offer many applications in a range of fields from chemistry to biology and biomedicine.

Biomolecular components have typical size dimensions from 5 to 200 nM, which meet with nanomaterials at the same length scale (Niemeyer, 2001). To improve sensitivity, simplicity and rapid, and to bring biomolecular-based detection to point-of-care settings such as doctor's office, Third World, and first responder sites etc., nanoparticle technology has been encouraged in the development of molecular diagnostics such as gene detection. Systems based on nanoparticle technology potentially offer significant advantages over conventional diagnostic systems in terms of sensitivity, selectivity, and practicality (e.g., simplicity, sensitivity, specificity, portability, and reliability). The most commonly used nanomaterials are gold, silver, carbon and semiconducting nanomaterials.

In this review, we will highlight the applications of nanomaterials for probe-labeling, target-labeling and signal-reporting in the bioanalytical detection of specific nucleic acid sequences, associated with diseases, such as tumours. Herein, excluding PCR-related methods, we describe the application of nanomaterials in various disease-related gene detection methods including colorimetric method, fluorescence-based methods, electrochemical method, surface enhanced ramen spectroscopy, surface plasmon resonance, microarray detection, quartz crystal microbalances, dynamic light scattering, single particle mode inductively coupled plasma mass spectrometry, and electrochemiluminescence. The conclusions drawn from the literature reveal the advantages and disadvantages of these techniques as well as the future trends of the bioanalytical detection of genes based on nanomaterials, particularly for real clinical samples.

2. Disease-related genes

Human genome sequencing has revealed that the most common form of genetic variation is single nucleotide polymorphisms (SNPs); however, these anomalies are is extremely difficult to

detect (Yang et al., 2004). SNPs have been closely associated with tumor development and progression, and the analysis of these polymorphisms can provide a tool for the early diagnosis and risk assessment of malignancies (Perkel, 2008; Engle et al., 2006; Carlton et al., 2006; McCarthy and Hilfiker, 2000). The detection of SNPs, particularly those responsible for certain types of disease risk and individual variations in response to therapeutics, have become central topics in cancer research and studies on the prevention, and diagnosis of genetic diseases (Nakatani et al., 2005). Recently, advancements in modern biotechnologies have offered a wide variety of choices for SNP detection. These approaches can be based on three strategies in general (Li et al., 2010): analysis of the single-base mismatch induced difference in local chemical environments or secondary structures (Okamoto et al., 2003a, 2003b; Masato et al., 1989), thermodynamic properties, such as hybridization-free energy (Tyagi and Kramer, 1996; Wei et al., 2005) and melting temperature (Gerion et al., 2003; Russom et al., 2006; Ross et al., 1997) via precise temperature control, the enzymatic reactions, such as allele-specific extension by polymerase (Vreeland et al., 2002; Chen and Kwok, 1997), endonucleases (Ross et al., 19998; Nie et al., 2005) and allele-specific ligation by ligase (Tobe et al., 1996; Landegren et al., 1988; Barany, 1991). While each strategy has its distinct advantages, each technique also presents a unique set of limitations. The continuous development of alternative SNP screening technologies is currently underway.

For example, a diagnostic application for the detection of an SNP associated with a mutation in the breast cancer gene “Breast cancer 1” (BRCA1) has been carefully studied. BRCA1 is a human caretaker gene expressed in the cells of breast and other tissues. Certain variations of the BRCA1 gene can lead to an increased risk for breast cancer with hereditary breast-ovarian cancer syndrome. Mutations in BRCA1 gene are responsible for more than 80%

of inherited breast and ovarian cancers and ~40% of inherited breast cancers (Li et al., 2012; Tutt and Ashworth, 2002). Therefore, the sensitive and rapid detection of BRCA1 gene mutant in disease-related gene fragments is critical for genetic research and the clinical diagnosis of breast cancer. Currently, BRCA1 gene can be distinguished using multiple techniques, such as PCR, gel electrophoresis, colorimetric methods, fluorescence-based method, electrochemical method and other methods (Xu et al., 2012; Oh and Lee, 2011; Rasheed and Sandhyarani, 2014).

Furthermore, sequence-specific detection has been of great importance in various medical and scientific applications because of their potential application for the diagnosis of diseases and indentification of pathogenic genes, such as in pandemic surveillances (Trifonov et al., 2009). The tumour suppressor gene TP53 is mutated in most type of human cancers, and is one of the most popular genes in cancer research and an important early diagnostic cancer marker (Falette et al., 1998; Walker et al., 1999; Esteban-Fernández de Ávila et al., 2015). The TP53 gene in human tumors frequently undergoes missense mutations, in which a single nucleotide is substituted for another. Mutated TP53 genes typically occur with increased frequency in patients with late-stage cancer, at sites of metastatic disease and in undifferentiated tumors (Nemunaitis et al., 2000). Some of these mutations are associated with poor prognosis and are significant predictors of resistance to chemotherapy or radiotherapy (Bhatt et al., 2010; Balogh et al., 2010). Therefore, the sensitive and rapid detection of the TP53 gene and its mutations is of great value for predicting the prognosis and outcome of patients with various types of cancer. A variety of methods for measuring the TP53 gene have been reported, including reverse transcription polymerase chain reaction (RT-PCR) (Will et al., 1995), traditional nucleic acid probes (Wang et al., 1997b), electrochemical sensors (Esteban-Fernández de Ávila et al., 2015), fluorescence-based techniques (Behn and Schuermann, 1998), immunostaining (Noguchi et al.,

1998; Pardo et al., 2004), immunohistochemistry (Barnes et al., 2006; Henke et al., 1994).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen, as it is responsible for health problems worldwide (Moellering Jr, 2006). The early detection of MRSA facilitates infection control and improves the outcomes of individual patients (Harbarth et al., 2006). Currently, the two major strategies for the laboratory detection of MRSA are bacterial culture-based phenotypic methods (Cockerill et al., 2012) and nucleic acid detection assays. The former requires one to two days for the confirmation of MRSA infection, whereas the latter allow the rapid and sensitive detection of MRSA-specific sequences directly from clinical specimens. However, nucleic acid detection assays may not be feasible for use in some laboratories because these techniques are largely limited to specific types of clinical specimens and are very expensive (Arbefeville et al., 2011; Bischof et al., 2009).

The Kirsten rat sarcoma viral oncogene homolog (KRAS), an important diagnostic and prognostic marker in cancer, is commonly detected in different types of cancers, such as pancreatic cancer, non-small cell lung cancer (NSCLC) (Adjei, 2001) and metastatic colorectal cancer (mCRC) (Neumann et al., 2009). It has been estimated that 17-25% of all cancers harbor mutations in this gene (Arrington et al., 2012). The detection of point mutations in human genomic DNA using simple methodologies is challenging. Currently, these tests can be classified into three categories: those based on real-time PCR, those based on DNA hybridization, and those based on DNA sequencing (Domagala et al., 2012). The major limitation of these methods is the high cost per sample and the requirement of expensive instrumentation. Therefore, a cost-effective, rapid, and large-scale means of KRAS mutation screening is attractive for the personalized therapy of cancer patients (Valentini et al., 2013).

A number of neurodegenerative diseases were caused by the expansion of trinucleotide repeats (Davies et al., 1998; Campuzano et al., 1996; Sanchez-Ramos et al., 1996; Kang et al., 1996; Steinlein et al., 1995), prompting a considerable amount of researches regarding the structure, biology and sequence detection of trinucleotide repeats. To date, more than 12 human genetic diseases including Myotonic dystrophy, Fragile X syndrome, Huntington disease, several spinocerebellar ataxias, and Friedreich ataxia, have been found to be associated with the expansion of trinucleotide repetitive sequences such as CTG, CGG, or GAA repeats (Campuzano et al., 1996). To more effectively combat these diseases in the medical area and accelerate responses to bioterrorism threats, the early and accurate detection of DNA markers is crucial. Thus, the global use of DNA-based molecular diagnostics has become increasingly important. For these reasons, it is essential that detection is sensitive, specific, rapid, easy-to-use and preferably low-cost.

In addition, there are other disease-related genes commonly such as G-quadruplex (Xu et al., 2010; Deng, et al., 2008), specific point mutation of apolipoprotein E gene (ApoE) related to Alzheimer's disease (Chen, et al., 2014). The identification of the relationships between diseases and genes is important in biology and medicine (Kim et al., 2015), which facilate a large number of gene-based studies and generated vast amounts of gene data. These data are stored in data bases such as the Online Mendelian Inheritance in Man (OMIM) database (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute of Genetics Medicine, Johns Hopkins University (Baltimore, MD) <http://omim.org/>). Thus, the detection of these disease-related genes is important in the diagnosis of diseases and conditions and is particularly useful for early stage treatment and monitoring.

3. Colorimetric methods

The rational utilization of nanomaterials such as metal nanoparticles (Au, Ag and Pt), carbon materials (carbon nanotubes, graphene and carbon quantum dots) and magnetic nanomaterials (Fe_3O_4) to construct nucleic acid detection methods has been intensely studied achieving for early disease diagnosis, monitoring the responses to the treatment and assessing the prognosis. Therefore, the detection of disease-related gene has been widely developed through colorimetric methods using different types of nanomaterials.

3.1 AuNPs-based colorimetric methods

Gold nanoparticles have shown more significant advantages than other nanomaterials in colorimetric methods because of their unique optical properties, electrical properties, biocompatibility, chemical activity, etc. Thus, colorimetric methods based on AuNPs are a highly attractive candidates because of their simple operation and their use of ordinary optical instruments, including the naked eye (Yu et al., 2015; Zou et al., 2015; Qiu et al., 2015; Su et al., 2015; Quintela et al., 2015).

The aggregation of AuNPs of appropriate sizes (d > 3.5 nm) induces interparticle surface plasmon coupling, resulting in a visible color change from red to blue at nanomolar concentrations (Srivastava et al., 2005; Brust et al., 1995). The color changes during AuNPs aggregation (or redispersion of an aggregate) provides a practical platform for absorption-based colorimetric sensing of any target that directly or indirectly triggers AuNPs aggregation or redispersion (Rosi and Mirkin, 2005; Chen et al., 2009; Ding et al., 2010; Saha et al., 2012). Different types of AuNPs -based colorimetric analysis were developed, which can be classed into two main types according to whether AuNPs are modified by oligonucleotide probe. One is

modified AuNPs-based colorimetric method, including aggregation system by utilizing color change of AuNPs (Elghanian et al., 1997; Sato et al., 2003; Sato et al., 2005) and silver staining enhancement system by utilizing redox ability of AuNPs (Taton et al., 2000). Another is non-modified AuNPs-based colorimetric method (Li and Rothberg, 2004a; Li and Rothberg, 2004b; Han et al., 2006).

3.1.1 Modified AuNPs-based colorimetric methods

In a milestone discovery, Mirkin group (Mirkin et al., 1996; Elghanian et al., 1997; Mucic et al., 1998; Rosi and Mirkin, 2005) reported unique optical and melting properties of an aggregate of AuNPs and oligonucleotides with thiol group modified oligonucleotides. The detection limit of the approach was about 1.0×10^{-8} mol·L^{-1}. This method is "quick and easy" and its optical read-out does not require expensive, sophisticated instrumentation. AuNP-based colorimetric assays have been demonstrated to be a highly competitive technology for oligonucleotide targets. Since then, nanoparticle probes have shown to be superior over conventional probes in many detection systems (Rosi and Mirkin, 2005; Storhoff et al., 2000; Stoeva et al., 2006; Kazmaier and Stolz, 2006; Nam et al., 2003; Georganopoulou et al., 2005; Nam et al., 2004;).

Rolling circle amplification (RCA), an isothermal DNA replication technique, has been proven very useful for the highly sensitive detection of target nucleic acids and proteins (Fire and Xu, 1995; Schweitzer et al., 2000). In addition, DNA-functionalized AuNPs have been used for both chemical and biological detections based on the discriminated effects of different DNA structures on the aggregations of AuNPs (Li et al., 2005; Wang et al., 2007a; Wang et al., 2008). Although these approaches have the advantage of providing an easy read out with naked eye, their application for SNP detection is restricted by the limited sensitivity of colorimetry. Yu

group (Li et al., 2010) developed a rapid, sensitive, and specific SNP detection method for point mutation in clinical diagnosis through the combination of RCA and AuNPs-based visual colorimetry. Simple and sensitive detection and quantification of target gene was demonstrated using a model system for the identification of point mutations in β-thalassemia gene at position -28 (AAA mutated to AGA). The results revealed that both the wild-type and mutant were successfully scored. Neither complicated fluorescence or radioisotope-labeled techniques nor sophisticated instruments were needed in the assay. Indeed, this method could achieve a detection limit as low as 70 fM compared with the nanomolar detection limit of traditional AuNPs-based colorimetric DNA assays. This new RCA-based colorimetric method might prove useful for clinical diagnosis of genetic diseases containing single nucleotide mutations.

A diagnostic application for the colorimetric detection of an SNP associated with a mutation in the breast cancer gene BRCA1 has been carefully designed and demonstrated. Oh and Lee (Oh and Lee, 2011) investigated the chemical hybridization properties of DNA-AuNPs with respect to various parameters, such as ionic strength, [DNA-AuNPs], temperature, spacer length, and DNA loading. Importantly, a single-base mutation in the breast cancer gene BRCA1 can be very rapidly and accurately detected without any temperature control using assembly-based colorimetric detection system based upon these hybridization properties. The extremely amplified discrimination of a single-base mismatch after increasing DNA loading and controlling the hybridization properties could be considered a kinetic stringency condition.

Importantly, this color change strategy can be further developed and integrated with other emerging technologies. For example, this method could be based on an isothermal DNA replication technique and DNAzyme amplification. In an example of a successful integration, Chan et al. combined DNAzyme amplification with colorimetric coupling of surface plasmons of

AuNPs for point-of-care detection of infectious diseases (Zagorovsky and Chan, 2013). These authors performed the assay multicomponent nucleic acid enzyme (MNAzyme) outlined in Figure 1 to detect AF-1, based on a model genetic target designed to facilitate the assembly of a catalytically active MNAzyme complex. Subsequently, four additional targets were selected to investigate whether the colorimetric assay could detect multiple targets in parallel (Fig. 1D). The four targets corresponded to genetic sequences for gonorrhea (Gon) (Rossau et al., 1998) and syphilis (Syph) (Burstain et al., 1991) bacteria, malaria parasite (Mal) (Bharti et al., 2007), and hepatitis B virus (HBV) (Paraskevis et al., 2010). These infectious diseases are prevalent in both developing and developed countries, cause severe health problems and death in patients who are not properly diagnosed and treated. This method inherently requires no complex equipment, expensive reagents, or complicated operations, making it suitable for point-of-care diagnosis. Finally, synthetic genetic targets are commonly used as the first step in the assessment of new POC devices. In the next step, further evaluation using clinical samples is required to assess clinical specificity and sensitivity prior to real-world use.

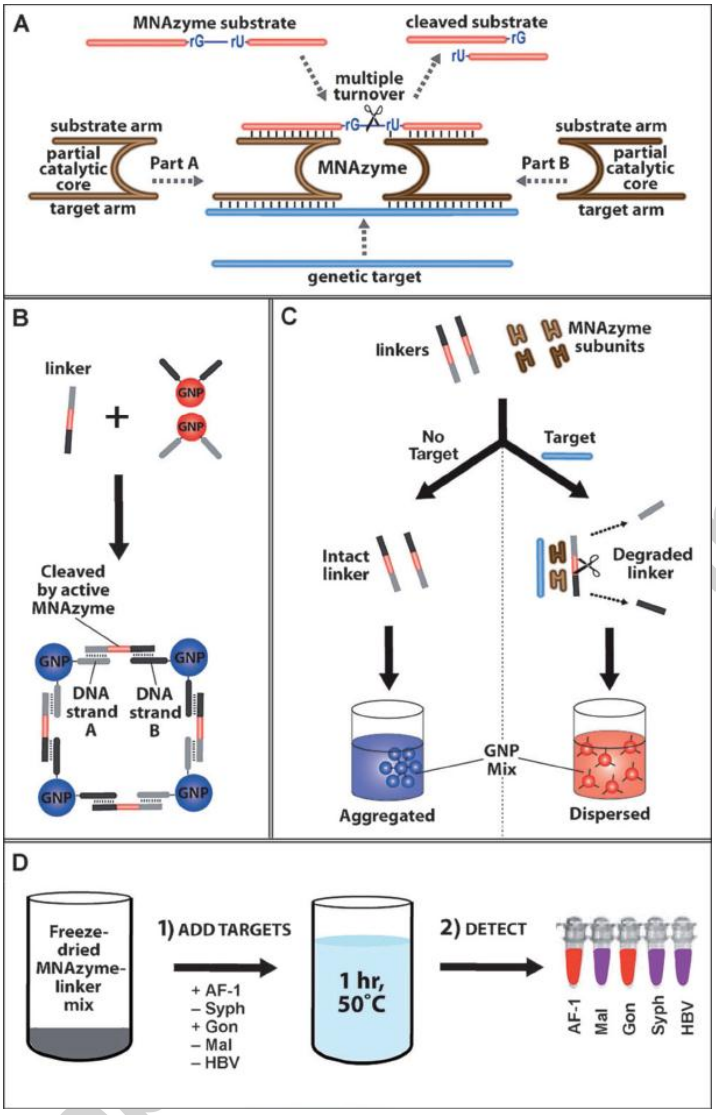

Fig. 1. Scheme showing the integration of AuNPs and DNAzyme technologies for multiplexed detection (Zagorovsky and Chan, 2013).

The application of AuNPs for the colorimetric PCR detection of MRSA, an important pathogen causing health problems worldwide, was reported in clinical specimens in 2014 (Chan et al., 2014). In this study, the authors have directly detected MRSA in a variety of clinical specimens, including blood culture broth, urine, and respiratory specimens as well as wound

swabs, pus and body fluids. The colorimetric assay comprised of two AuNPs probes functionalized with Staphylococcus aureus 23S rRNA-and mecA-specific oligonucleotides. The overall detection limit was 500 ng target amplicon. Moreover, this study was the first report of the use of AuNPs colorimetric assay for direct MRSA detection in various clinical specimens with decreased detection time. Its clinical performance was comparable to real-time PCR-based commercial kits, but the AuNPs colorimetric assay can be applied to a broader variety of specimens and has lower cost per reaction. Expensive instrumentation was not needed and the results could be detected qualitatively though visual examination or quantitatively by spectrophotometry. These data revealed the potential relationship between AuNPs probe-target hybridization site and assay performance, which may inform design of AuNPs biosensors for nucleic acid detection.

3.1.2 Non-modified AuNPs-based colorimetric methods

The detection of specific DNA sequences, DNA mutations, and SNPs was achieved though the direct binding of unmodified-nanoparticles to DNA (Fig. 2a) (Kanjanawarut and Su, 2009). When nanoparticles bind to ss-DNA, the hybridization of testing oligonucleotides might result in the formation of aggregation of nanoparticles, which in turn changes the color of the solution (Fig. 2a. (A)). In DNA colorimetric detection methods, DNA can be changed by peptide nucleic acids (PNA). In the absence of complementary target DNA, free PNA molecules in solution induce aggressive particle aggregation because of the removal of charge repulsion as a result of nanoparticle–PNA binding. The presence of the complementary DNA results in the formation of PNA–DNA complexes. Thus, the particles remain stable due to sufficient charge repulsions (Fig. 2a. (B)). The colorimetric detection of DNA was also achieved using unmodified gold nanoparticles and conjugated polyelectrolytes or conjugated polyelectrolytes (Fig. 2a. (C)). They

also validated the two-component assay using a mixture of AuNPs and AgNPs, to improve the method accuracy using of the multiple aggregation signatures from the two types of particles.

The colorimetric detection of the cancer-related point mutation in KRAS, an important diagnostic and prognostic marker for cancer, was demonstrated using AuNPs combined with signal enhancement on the surface of paramagnetic microparticles (Valentini et al., 2013). Upon hybridization with the universal AuNPs probe, the sample turns red in the presence of a specific mutation (Fig. 2b), providing a rapid indication of the disease progression and facilitating therapeutic decisions. This simple colorimetric assay is sensitive (with a limit of detection in the low picomolar range) and instrument-free. Its naked-eye detection, small sample volumes, and isothermal (PCR-free) amplification, make the assay suitable for point-of-care applications.

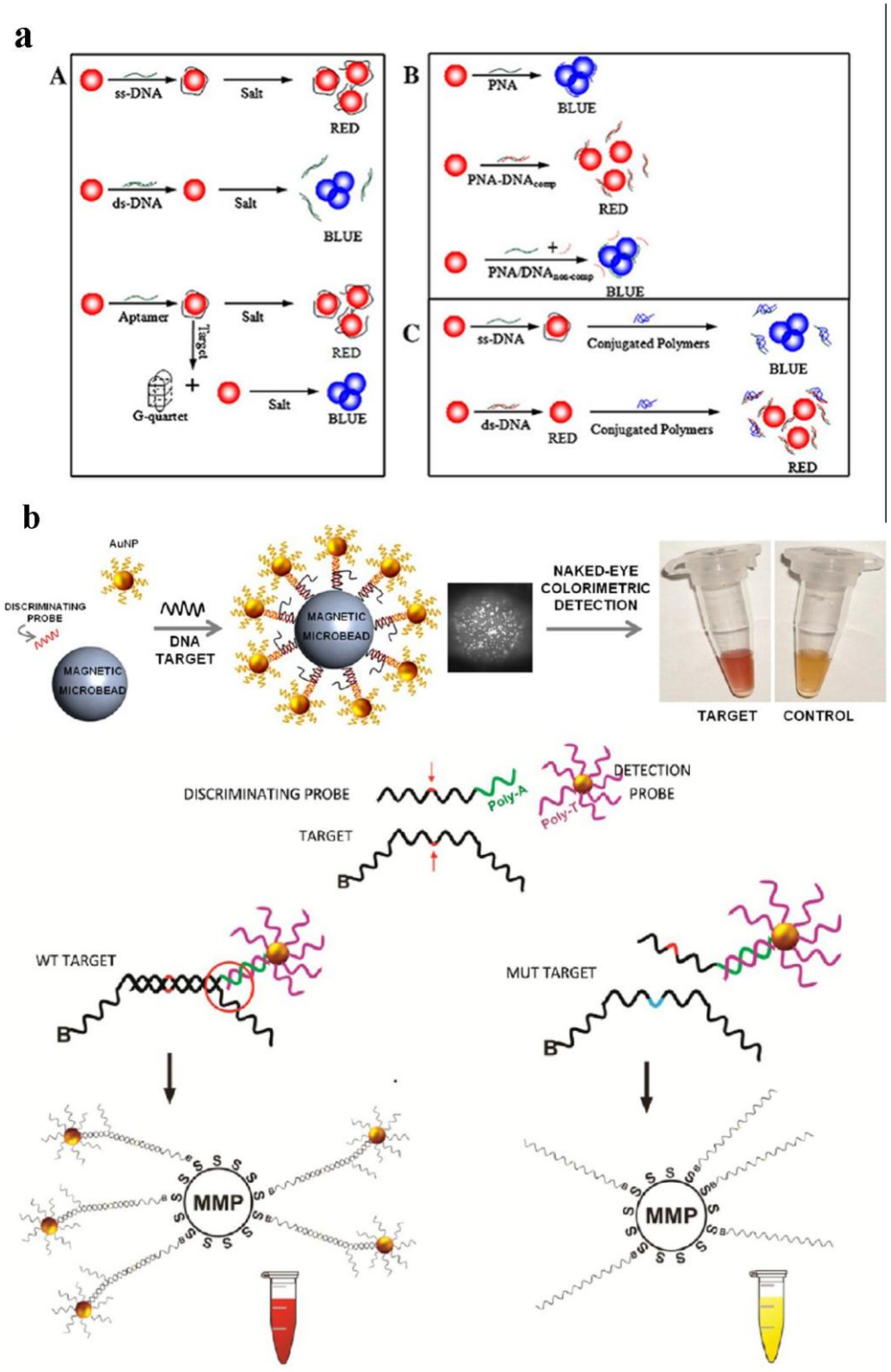

Fig. 2. (a) Schematic diagrams of colorimetric detection associated with Au/Ag-NP aggregations. (Kanjanawarut and Su, 2009).; (b) Reaction scheme of the KRAS genotyping assay (Valentini

et al., 2013).

NSCLC is a major histologic type of lung cancer (To et al., 2008). Targeting the epidermal growth factor receptor (EGFR) in NSCLC patients provides one of the most promising strategies for NSCLC therapy, as the tyrosine kinase inhibitor has emerged as an effective curing agent for some patients (Lynch et al., 2004; Kimura et al., 2006; Paez et al., 2004; Pao et al., 2007; Hoshi et al., 2007; Takano et al., 2007).

Although the direct sequencing of EGFR exons 18–21 has been widely used to detect EGFR mutations in cancer specimens, this method requires high cost of amplification and is time-consuming. Mutations in the EGFR of NSCLC were detected colorimetrically based on the selective aggregation of unmodified gold nanoparticles (Lee et al., 2010). Under optimal conditions, mutant type DNA was detected based on the color changes of a gold nanoparticles suspension. In this analysis, selective aggregation occurrs upon the addition of dsDNA with a perfectly matched sequence (mutant type), while the AuNPs remain colloidally stable in presence of mismatched dsDNA (wild type). The simplicity, rapid and low cost of this method make it superior to conventional methods.

3.2 Other nanomaterials-based colorimetric methods

Almost all research on colorimetric method for target DNA detection utilizes AuNPs rather than other nanomaterials, such as AgNPs, due to the difficulties in the synthesis of AgNPs with repeatable size and optical characteristics problematic compared to AuNPs synthesis. Hence, exploiting the advantages of other nanomaterials may open new research avenues for colorimetric assays.

Graham's group (Thompson et al., 2008) reported the synthesis of oligonucleotide silver

nanoparticle (OSN) probes, which align onto target polynucleotides in head-to-head, head-to-tail, and tail-to-tail sandwich formats. The use of these OSN probes for the colorimetric detection of target with a single base mismatch was demonstrated. The properties of OSN probes are almost identical to those of their gold analogs. However, due to much higher extinction coefficient of these probes, both visual and absorption analyses can occur at 50-fold lower concentrations. These probes are sequence-specific and offer improved sensitivity, which is of interest to many scientists.

Graphene oxide (GO) has also been widely used for detection of DNA targets due to its higher specific surface area, sensitivity to single base mismatch groups, water dispersibility, broad emission under UV light and ability to quench the fluorescence of nearby fluorophores. Recently, Kang et al. (Kang et al., 2014) reported the PNA-mediated aggregation of reduced graphene oxide (rGO) sheets for the label-free detection of DNA mutations. This method has been used to detect EGFR gene mutations that recurrently occur in lung cancer patients. As shown in Figure 3, the addition of PNA to rGOs suspension resulted in aggregation, which could be easily detected with the naked eye. Moreover, neither fluorescent dye labeling or chemical modification of oligonucleotides nor expensive analytical instrument are required, unlike in other GO-based detection methods. Visual detection of DNA hybridization was also found successfully achieved using DNA-conjugated dual nanoparticle platforms consisting of AuNPs and GO. The optimum detection was achieved at 63 nM of the DNA target with a limit of 8 nM (Thavanathan et al., 2014). These results showed that PNA-mediated rGO aggregation could be successfully used to detect EGFR mutations with high selectivity and sensitivity. To date, this method has been used to detect somatic mutations of KRAS, BRAF, and EGFR genes, which have been identified as crucial mutations for targeted treatment in cancer patients (Beau-Faller et

al., 2009; Jeong et al., 2013; Kim et al., 2012). In addition, this method could be applied for rapid screening of specific DNA sequences with high sensitivity and selectivity.

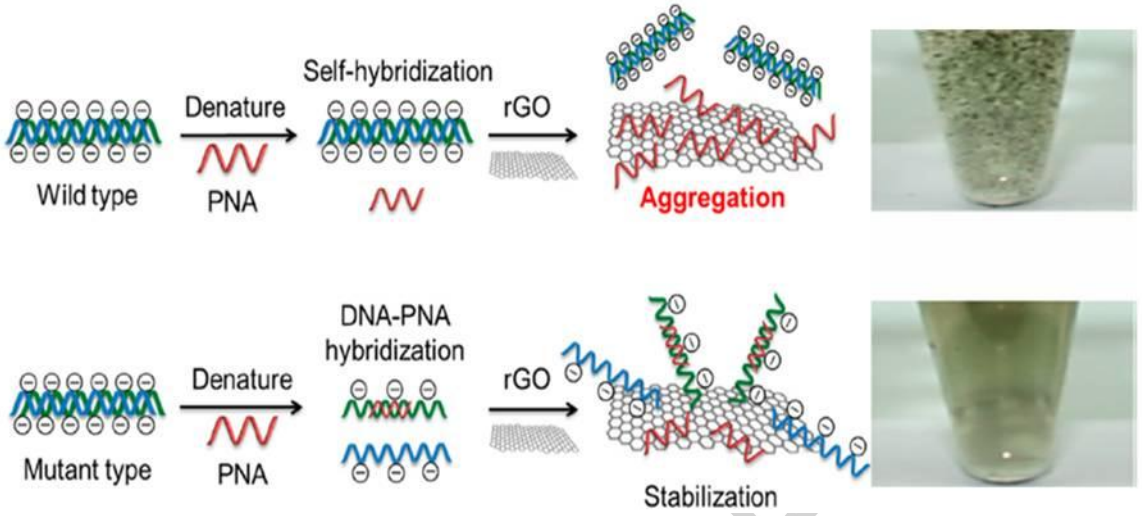

Fig. 3. Schematic for detection of DNA mutation (Kang et al., 2014).

Herein, we have summarized the present colorimetric methods for the detection of target DNA. Nanomaterial-based colorimetric methods have been rapidly developed and are constantly being improved, with additional applications in clinical settings. To open new research avenues in colorimetric assays, some practical problems must be solved, such as the control of the repeatable size of nanoparticles, the impact in the detection procedure and the presence of complex samples. The advances made in other research avenues, such as lateral flow strips technology, may be translated to colorimetric assays. Thus, colorimetric methods are directly applicable to or extremely promising for the rapid detection of actual clinical samples.

4. Fluorescence-based methods

Compared with organic fluorescent probes, semiconductor nanoparticles as fluorescent biological probes for the detection of biological molecules will greatly improve the sensitivity and selectivity due to broad excitation spectra, better symmetry and photochemical stability of

these particles (Saha et al., 2012).

The emission lifetime of fluorophores depends on the distance from the surface of the metal (Drexhage, 1970). Several spectroscopy techniques can be enhanced by the local field, generating diverse phenomena, such as surface-enhanced fluorescence (SEF), SERS and surface-enhanced infrared absorbance (SEIRA) (Le Ru and Etchegoin, 2008; Aroca, 2006; Moskovits, 1985). The emergence of nanotechnology increased interest in applying fluorescence resonance energy transfer (FRET)-based systems in bio-diagnostics.

4.1 AuNPs-based fluorescent detection

AuNPs can serve as excellent fluorescence quenchers for FRET-based assays due to their extraordinarily high molar extinction coefficients and broad energy bandwidth (Saha et al., 2012). The molecular beacon (MB), a particularly attractive molecular tool, is dual-labeled stem-loop-structured oligonucleotide that is internally quenched as a result of the close contact between the dye-quencher pair attached at either end (Song et al., 2009). For example, hairpin FRET-based systems for single-mismatch detection have been set up by labeling molecular beacons with AuNPs (Dubertret et al., 2001). The nucleic acid probe conjugated with the dye can form a hairpin structure on the AuNPs, inducing effective FRET fluorescence quenching. In the presence of the target DNA, the dye reporter would be displaced or released by construction of a more stable duplex between the target DNA and the oligonucleotide on AuNPs, resulting increasing dye fluorescence (Fig. 4A).

Mirkin group developed probes to detect and quantify intracellular analytes, such as mRNA in cells (Prigodich et al., 2009; Seferos et al., 2007; Zheng et al., 2009). Gu et al. (Xue et al., 2013) used a special hairpin deoxyribonucleic acid (DNA) for human signal transducer and

activator of transcription 5B (STAT5B) mRNA to functionalize AuNPs for detecting human STAT5B expression (Fig. 4B). STAT5B is a crucial transcription factor involved in the proliferative and survival signaling in a number of solid tumors, including breast cancer and prostate cancer. Up to 90% quenching efficiency was achieved. This beacon revealed the presence of target STAT5B mRNA through the specific hybridization of complementary oligonucleotides and was also used to visualize STAT5B gene expression in real time. The method could facilitate the rapid identification of different stages of tumor progression and assess treatment outcomes.

Furthermore, AuNPs can be used in conjunction with other nanomaterials for fluorescent detection. Fan et al. (Li et al., 2013) reported a comparative study of the nano-quenching effects for sequence specific DNA hybridization detection using a series of nanomaterials with different dimensions, such as AuNPs, carbon nanotubes (CNTs), and GO. Interestingly, the quenching ability of GO was superior to those of the other materials in terms of both efficiency and kinetics. Thus, a GO-based fluorescent sensor, designed in a simple mix-and-detect format could be used to detect concentrations of DNA as low as 0.2 nM, surpassing the detection capabilities of CNTs and AuNPs by an order of magnitude. This sensor can also differentiate single-base mismatches much better than either CNT- or AuNPs- based sensors.

4.2 AgNPs-based fluorescent detection

In 2014, Annink, C. and R. Gill (Annink and Gill, 2014) showed that fluorescence enhancement can be observed from dye-labeled DNA when mixed with silver nanoparticles in the presence of the polycation spermine in 2014. This cheap and simple method can be used as an amplification step to detect and quantify DNA at sub picomolar concentrations without any

enzymatic enhancement. To demonstrate the applicability of silver nanoparticle aggregation-based SEF for DNA detection, these authors assessed the effect of SEF amplification on the detection limit of dye-labeled DNA using a fluorescence microplate reader. The measured concentration ranged from 600×10^{-12} M down to less than 0.1×10^{-12} M, and the limit of detection (LOD) was below 50×10^{-15} M.

Recently, polysaccharide-functionalized silver nanoparticles (Oc-AgNPs) with a mean diameter of 15 nm were utilized as a novel and effective fluorescence-sensing platform for nucleic acid detection (Yan et al., 2015). Tests on the oligonucleotide sequences associated with the human immunodeficiency virus (HIV) as a model system showed that the Oc-AgNPs effectively absorbed and quenched dye-labeled single-stranded DNA through strong hydrogen bonding and week electrostatic attractive interactions (Fig. 4C). HIV interferes with host-cell immune effectors functions by these proteins as well as genetic variability. The proposed system efficiently differentiated between complementary and mismatched nucleic acid sequences with high selectivity and good reproducibility at room temperature. Thus, the proposed system has a great potential for practical and useful mismatch detection in future applications.

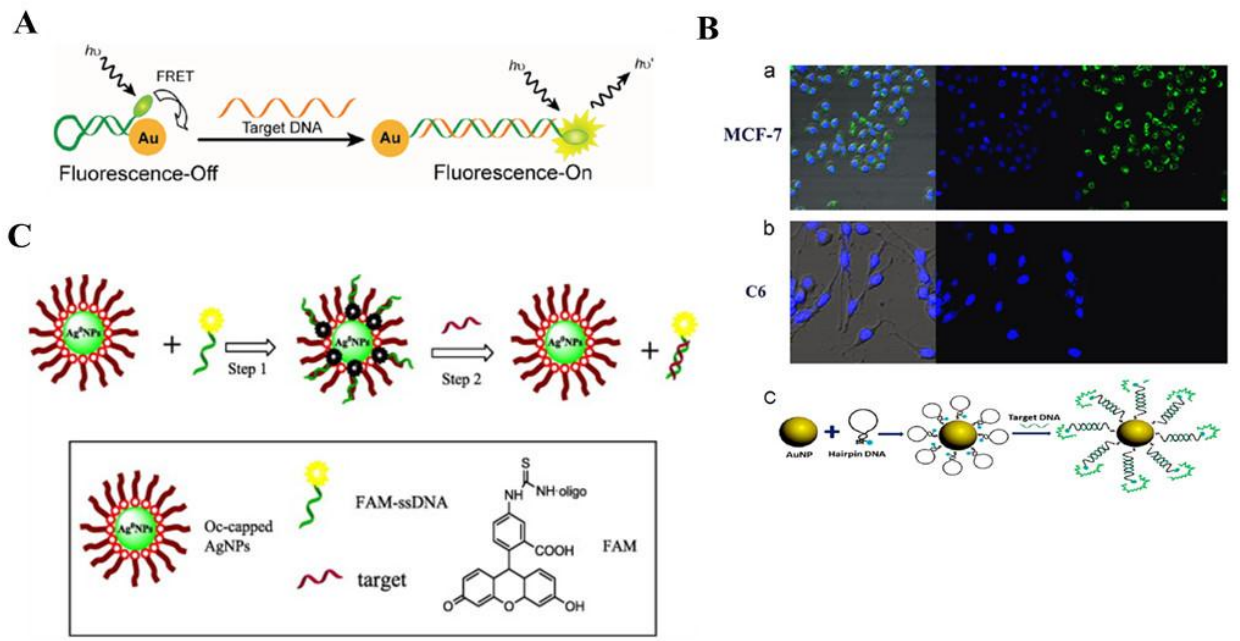

Fig. 4. (A) Schematic illustration of DNA detection showing the conformational changes of dye-oligonucleotide-AuNPs conjugates before and after hybridization with the target DNA (Dubertret et al., 2001).; (B) Fluorescence images of human STAT5B-expressing MCF-7cells, non-human STAT5B-expressing C6cells and schematic diagram of hairpin DNA assembling to AuNPs (Xue et al., 2013).; (C) A schematic diagram to illustrate the fluorescence-enhanced nucleic acid detection using Oc-AgNPs as a sensing platform (Yan et al., 2015).

4.3 Quantum dots-based fluorescent detection

Because of their unique photophysical properties such as their narrow and size-tunable emission spectra, robust signal intensity, broad excitation bandwidth, and high photochemical stability against photobleaching, semiconductor nanocrystals (quantum dots, QDs) have been shown to be excellent spectral labels for multiplexed bioanalysis (Peng et al., 2007; Gerion et al., 2003; Ho et al., 2005; Wang et al., 2009d). Although assays for DNA analysis using QDs must be

designed with care, QDs provide advantages over fluorophores for many emerging DNA analysis methods in which low copy numbers of DNA are present. Many of the more traditional DNA assay methods using fluorophore-labeled probes either cannot be translated or do not benefit from using QDs as lumophores.

The detection of single base mismatches using unmodified CdS quantum dots can be realized through fluorescence quenching due to the aggregation of quantum dots (Kim et al., 2009). In this study, target DNA sequences of interest were breast cancer 2 (BRCA2) and signal-induced proliferation-associated gene 1 (Sipa1) sequences. BRCA2 polymorphism is known to be associated with prenatal viability and breast cancer risk, and Sipa1 polymorphism with metastatic process. These results indicated that double-stranded DNA with a perfectly matched sequence can be easily discriminated from the single base mismatched DNA using the naked eye. This easy detection reflects the fluorescence-quenching phenomenon resulting from the aggregation of CdS quantum dots.

4.4 Nanoclusters-based fluorescent detection

Metal atoms can organize into well-defined structures (so called nanclusters) with molecular energy levels that yield strong fluorescence emission. Recently, the application of fluorescent metal nanocluster, particularly gold and silver nanolusters, has attracted interest for DNA detection (Liu et al., 2013a; Guo et al., 2010).

For example, a rapid, homogeneous, and in-situ label turn-on format for SNPs detection was designed by modulating intrinsic fluorescence of DNA-templated silver nanoclusters (DNA–AgNCs) (Park et al., 2013). As probes for SNPs, fluorescent AgNCs produced following hydride-mediated reduction of Ag^{+}, which can bound to a partial double-stranded

oligodeoxynucleotide. The sensing mechanism is based on the fluorescence enhancement of silver nanoclusters through an irreversible cluster transfer upon hybridization. Dramatically, the method could be toward practical applications in SNP genotyping.

Besides, based on the observation that the hemin/G-quadruplex complex could quench silver nanolusters (AgNCs) emission, Wang and co-workers (Zhang et al., 2013) incorporated a DNA hairpin with the probe DNA sequence in the loop region. Because a fraction of the hemin-binding sequence is locked in the hairpin, hemin cannot bind and the fluorescence remains high. In the presence of the target, the hairpin opens, freeing the hemin-binding sequence and the fluorescence drops (Fig. 5a). Qu and co-workers (Tao et al., 2012) also reported AgNCs fluorescence quenching upon the adsorption of the entire complex using GO. The addition of the target DNA resulted in probe desorption and fluorescence recovery.

Luminescent nucleic acid-stabilized AgNCs have been applied for the optical detection of DNA and the multiplexed analysis of genes (Enkin et al., 2014). The sensing module involves the assembly of a three-component sensing module composing of a nucleic acid-stabilized AgNCs, and a quencher-modified nucleic acid hybridized with a nucleic acid scaffold complementary to the target DNA (Fig. 5b). In addition, implementation of two-sized AgNCs allows the multiplexed analysis of two genes (the Werner Syndrome gene and the human immunodeficiency gene) using the hairpin sensing module approach.

4.5 Other nanoparticles-based fluorescent detection

Other nanoparticles, such as α- Fe_2O_3 nanoparticles (Song et al., 2015), silica nanoparticles (Santra et al., 2001a, 2001b; Zhao et al., 2003) and silica-coated magnetic nanoparticles (Thomson et al., 2012) have also been applied for gene detection. For example, α-Fe_2O_3 NPs

have the merits of a relatively simple, cost-effective and environment-friendly preparation compared to some of the other nanomaterials used in nucleic acid detection, such as AuNPs, GO and AgNPs. Thus, the application of α-Fe_2O_3 NPs as a sensing platform for DNA detection is worthy of further investigation.

Recently, Kong et al. (Song et al., 2015) investigated the interactions of α-Fe_2O_3 nanoparticles with different structural nucleic acids and the fluorescence quenching ability of these molecules towards fluorophore-labeled nucleic acid probes (Fig. 5c). Unlike bulk α-Fe_2O_3 samples, nanoscale α-Fe_2O_3 particles exhibit strong adsorption and fluorescence quenching of fluorophore-labeled single-stranded DNA probes. Based on these findings, a facile fluorescence detection was developed for the versatile quantification of nucleic acids. The targets of micro-RNA, ssDNA and double-stranded DNA are sensitively detected with detection limits of 0.8 nM, 1.1 nM and 0.64 nM, respectively. Thus, even a single nucleotide or single base-pair mismatch can be effectively discriminated.

In 2001, W. H. Tan group (Santra et al., 2001; Santra et al., 2001) synthesized inorganic dye-doped and organic dye-doped silica nanoparticles for detection of DNA. Based on a previous work, they developed an ultrasensitive DNA assay to detect gene products with an 0.8 fM detection limit using highly fluorescent and photostable bioconjugated dye-doped silica nanoparticles (Zhao et al., 2003). In addition, the NP-based DNA bioanalysis assay could effectively discriminate one-base mismatched DNA sequences. This sandwich assay is expected to be widely useful in a number of biomedical applications requiring accurate and ultrasensitive gene analysis.

The analysis of nucleic acids from viruses has greatly improved the pathologist's ability to

define infectious agents and thereby make informed decisions concerning disease etiology. Oligonucleotide and copolymer functionalized nanoparticles have been fabricated for the detection of herpes simplex virus (HSV)-specific oligonucleotides (Thomson et al., 2012). Herpes zoster is a viral infection and a common disorder of the eyes caused by herpes simplex virus. They detected a single-stranded DNA molecule derived from the UL30 DNA polymerase gene of HSV. As shown in Figure 5d, random copolymer brushes were grown from the surface of silica-coated magnetic nanoparticles using azide-modified and hydroxyl oligo ethylene glycol methacrylate monomers. The azide-functionalized polymer brush was first conjugated, via copper-catalyzed azide/alkyne cycloaddition, with HSV-specific oligonucleotides and subsequently with alkyne-substituted polyethylene glycol to eliminate all residual azide groups. The method can control the brush thickness and probe density, and enable multiple consecutive coupling reactions on the particle-grafted brush. These multi-structured brushes show potential for sensitivity improvements and clinically viable amplification-free DNA detection.

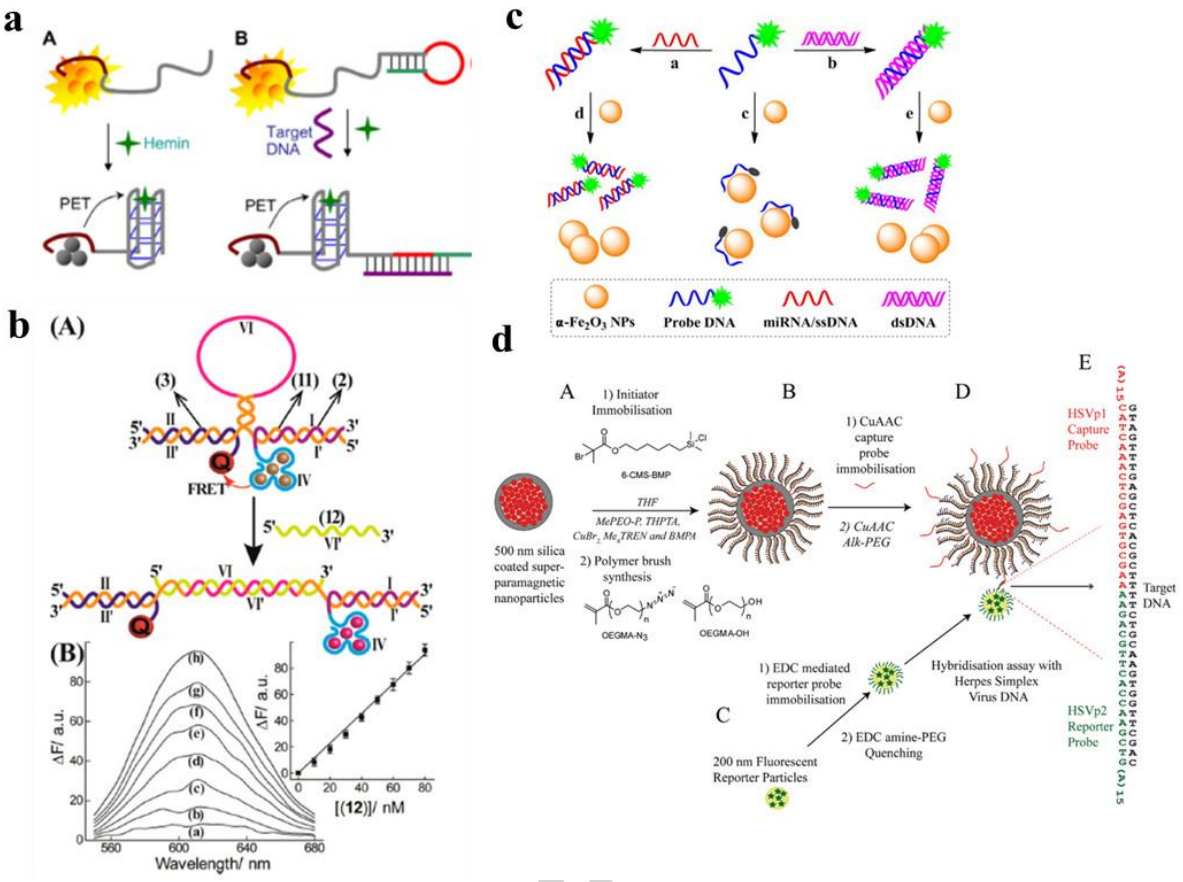

Fig. 5. (a) Molecular beacon type of signaling method involving an external quencher. (A) Hemin/DNA complex can quench AgNCs. (B) DNA detection using hemin as a quencher (Zhang et al., 2013).; (b) (A) Schematic sensing module for the analysis of the BRCA1 gene (12) using a hairpin DNA scaffold functionalized with a nucleic acid 2-functionalized AgNCs and a quencher-modified nucleic acid 3. (B) Luminescence spectra changes upon analyzing different concentrations of the BRCA1 gene (12) (Enkin et al., 2014).; (c) Schematic illustration of α-Fe_2O_3 NP-based sensing platform for miRNA and DNA detection (Song et al., 2015).; (d) Scheme for magnetic particle polymer modification and subsequent coupling of DNA oligonucleotides (Thomson et al., 2012).

5. Electrochemical methods

Electrochemical methods for detecting nucleic acids have become a promising area of research and development due to their rapid response, low cost, simple operation and portable and affordable instrumentation (Ulianas et al., 2014; Torres-Chavolla and Alocilja, 2011; Wang et al., 2003; Zhang et al., 2010; He et al., 2013a, 2013b, 2014; Drummond et al., 2003; Gooding, 2002; Palecek and Fojta, 2005). The development of an electrochemical sensor for DNA diagnostics is currently a popular topic among not only for electrochemists, but also for chemical biologists, molecular biologists and medical physicians involved in modern biomedicine. In particular, the detection of specific nucleic acids sequences is necessary in various fields including the molecular diagnosis of certain bacterial and viral infections and human genetic diseases.

5.1 The principles of electrochemical methods

Label-free and label-based methods have often been used in electrochemical DNA sensing. Electrochemical DNA sensing has the following foundations: direct oxidation/reduction of nucleotide bases, detection of intercalating molecules for single/duplex strands, detection of specific DNA with labeled reporting DNA, detection of specific DNA with integrated capture probes and reporter probes, direct detection after specific DNA enzymatic processes (cleavage of DNA), and detection of extra-labeled reporter after specific DNA enzymatic processes (cleavage of DNA). With the emergence of nanomaterials and nanotechnologies, electrochemical sensors offered a perfect interface for these technologies. Various nanomaterials (gold, carbon, magnetic nanoparticle, semiconducting, etc.) can play different roles in the electrochemical detection system, such as capture probes, reporting molecules, target labels, electrode fabrication, and

electrode coatings (Lord and Kelley, 2009; Radwan et al., 2009; Wei et al., 2009a; Pandey et al., 2008; Kerman et al., 2004; Liao and Ho, 2009; Ozsoz et al., 2003; Castaneda et al., 2007; Sun et al., 2015; Zhou et al., 2015). Ideally, the application of these nanomaterials would offer improved biocompatibility, additional binding sites and higher signal intensities (via enhanced electrical properties) compared with traditional electrochemical sensor materials. Pandey et al., 2008; Castaneda et al., 2007; Mao and Liu, 2008; Wang and Hu, 2009; Xu et al., 2009; Esteban-Fernández de Ávila et al., 2015).

5.2 AuNPs-based electrochemical methods

Several groups have developed powerful nanoparticle-based electrochemical DNA hybridization assays. In combination with AuNPs, an electrical method for the detection of DNA based on changes in electrical current or resistance between the two electrodes for sensitive and selective multiple DNA detection has been developed (Zhang and Zhang, 2010; Zhang et al., 2011a; Du et al., 2011; Li et al., 2012; Saha et al., 2012; Park et al., 2002). A simple, label-free and sensitive DNA sensing method was reported based on the interaction between AuNPs and ssDNA immobilized on an electrode surface, which can detect as low as 1 pM breast cancer gene BRCA1 in a 10 μL sample volume (Yang et al., 2014). Specifically, ssDNA-modified gold electrodes can absorb AuNPs via interactions between the gold and nitrogen-containing bases; thus, the negative electrochemical species $[Fe(CN)_6]^{3-/4-}$ can transfer electrons to the electrode through the adsorbed AuNPs, resulting in low impedance. In the presence of the target DNA, the formed double-stranded DNA (dsDNA) cannot capture AuNPs onto the electrode surface, potentially resulting in a high charge-transfer resistance due to the negatively charged phosphate backbone of DNA (Fig. 6A). Furthermore, the proposed method may also be generalized for detecting a spectrum of targets using functional DNA (aptamer, metalspecific oligonucleotide, or

DNAzyme), which may provide an alternative for cancer DNA detection in the future.

Normally, the specific electrochemical properties of the labeling molecules are selected based on the state of the target DNA on the electrode. Ozsoz et al. (Ozsoz et al., 2003) detected Leiden mutations with a sensitivity of as low as 0.78 fmol through the incorporation of AuNPs in DNA probes. Liao and Ho (Liao and Ho, 2009) detected the gene, specific to E. coli O157, with a low sensitivity of 0.75 aM, based on the competition between the target genes and reporter DNA-tagged liposomes. Enterohemorrhagic Escherichia coli O157, a verocytotoxin (VT1/2)-producing pathogen, can be deadly because it can induce acute or chronic renal failure. In this method, the electrodes were modified with AuNPs and a self-assembled monolayer of thiol-capped single-stranded DNA (capture probe). Conformational changes in the DNA molecules altered the distance between the labeling molecules and the electrode, affecting the EC signal.

Simpler, label-free electrochemical methods using nanomaterials have attracted increasing attention (Chua et al., 2009; Maehashi and Matsumoto, 2009). This approach does not require additional sample separation processes or reporting molecules and is promising for the real-time monitoring of DNA levels within biological systems. This simple label-free method involves the direct oxidation/reduction detection of nucleotide bases and the detection of intercalating molecules for single/duplex strands. One type of label-free electrochemical sensor is a single/duplex-specific intercalating complex, such as Co (2,2'-bipyridy1)$_3^{3+}$, Co(1,10-phenanthroline)$_3^{3+}$, methylene blue (MB), daunomycin, and aromatic amines (Millan and Mikkelsen, 1993; Wang et al., 1997a; Erdem et al., 1999; Mascini et al., 2001; Belluzo et al., 2008). This label-free method is based on a sandwich mode, which does not require target DNA labeling and only reporter molecules are labeled (Hajdukiewicz et al., 2009; Wang et al., 2009b;

Zuo et al., 2009). Rasheed and Sandhyarani (Rasheed and Sandhyarani, 2014) fabricated a sensitive and simple DNA biosensor for the low concentration detection of BRCA1 DNA sequences based on a "sandwich" detection strategy, involving the immobilization of DNA-c on the surface of a gold electrode surface. One half of the target DNA is hybridized to the immobilized DNA-c and the other half is hybridized to reporter probe DNA, which is labeled with AuNPs. In the absence of target DNA, the sandwich complexes do not form, leaving the surface-confined capture probe DNA unhybridized. The DNA sensor could detect up to 1 fM DNA target (5.896 fg of 1 gene/ml BRCA1) and exhibited excellent selectivity against non-complementary sequences and three base mismatch complementary targets. This DNA sensor showed good reproducibility, stability and reusability. Thus, it is expected that the sensor could be applied in cancer biomarker detection in the early stages of cancer, where the concentration of biomarkers is extremely low.

Recently, Liu et al. (Peng et al., 2015) reported a novel label-free electrochemical DNA biosensor for the simple, effective and convenient determination of the MDR1 (multidrug resistance) gene based on Au nanoparticles/toluidine blue–graphene oxide (AuNPs/TB–GO) modified electrodes (Fig. 6B). In human cells, expression of the MDR1 gene leads to decreased intracellular accumulation and resistance to a variety of lipophilic drugs, which has become a major obstacle to the successful performance of chemotherapy for cancer patients. The developed method has an ability to discriminate the MDR1 related DNA sequence from single-base mismatched DNA sequence, to assay the MDR1 related gene level precisely in the MDR1 DNA samples. Differential pulse voltammetry was employed to monitor the hybridization of DNA based on the changes in the peak currents of TB. The decline of the peak current was linearly related to the logarithm of the target DNA concentration from 1.0×10^{-11} M to 1.0×10^{-9}

M, with a detection limit of 2.95×10^{-12} M. In addition, the biosensor exhibited good selectivity and acceptable stability and reproducibility. In order to fully assess the application potential and added value of the electrochemical DNA biosensor, their future research should focus on other different target sequences and sample types, such as the samples derived from cells.

Besides, Fang et al. (Wang et al., 2015) developed a super-sandwich-type electrochemical biosensor for sequence-specific DNA detection. In design, single-strand DNA labeled with MB was used as a signal probe and an auxiliary probe designed to hybridize with two different regions of the signal probe (Fig. 6C). Finally, the biosensor exhibited high sensitivity and selectivity.

Electrochemical impedance detection is a label-free method desirable for POC DNA sensors (He et al., 2014; Daniels and Pourmand, 2007). Although obtaining high sensitivities remains challenging for impedance detection, impedance-based detection has received much attention, particularly due to the recent strides in the development of nanotechnology. Nanomaterials are important elements in impedance-based DNA sensors, especially those with semiconductor properties (Cheng, et al., 2014; Jin, 2009; Zhou et al., 2009; Suni, 2008; Tiwari and Gong, 2009). The most widely used nanomaterials in impedance sensors are AuNPs and CNTs, as these molecules can amplify the impedance signals by forming nanoparticle-biomolecule conjugates in solution. For example, electrochemical impedance spectroscopy (EIS) was performed on AuNPs-modified indium tin oxide (ITO)-coated glass surfaces to detect a specific point mutation in apolipoprotein E gene (ApoE) associated with the progression of Alzheimer's disease. In addition, this sensor was also used to detect DNA using another platform, localized surface plasmon resonance (LSPR) (Cheng et al., 2014).

At the same time, the sensor was also used to detect DNA by another platform, localized surface plasmon resonance (LSPR). Besides, an EIS sensor for the sequence-specific DNA detection of the phosphinothricin acetyltransferase (PAT) gene showed excellent dynamic detection range, from 1 pM to 1 μM with a detection limit of 0.3 pM (Feng et al., 2008). Furthermore, Fayazfar et al. (Fayazfar et al., 2014) developed a new platform based on the electrochemical growth of AuNPs on aligned multi-walled CNTs for the sensitive label-free DNA detection of the TP53 gene (Fig. 6D). EIS was used to monitor the sequence-specific DNA hybridization events related to TP53 gene, with a wide range of 1×10^{-15} ~ 1×10^{-7} M, and a detection limit of 1.0×10^{-17} M (S/N = 3).

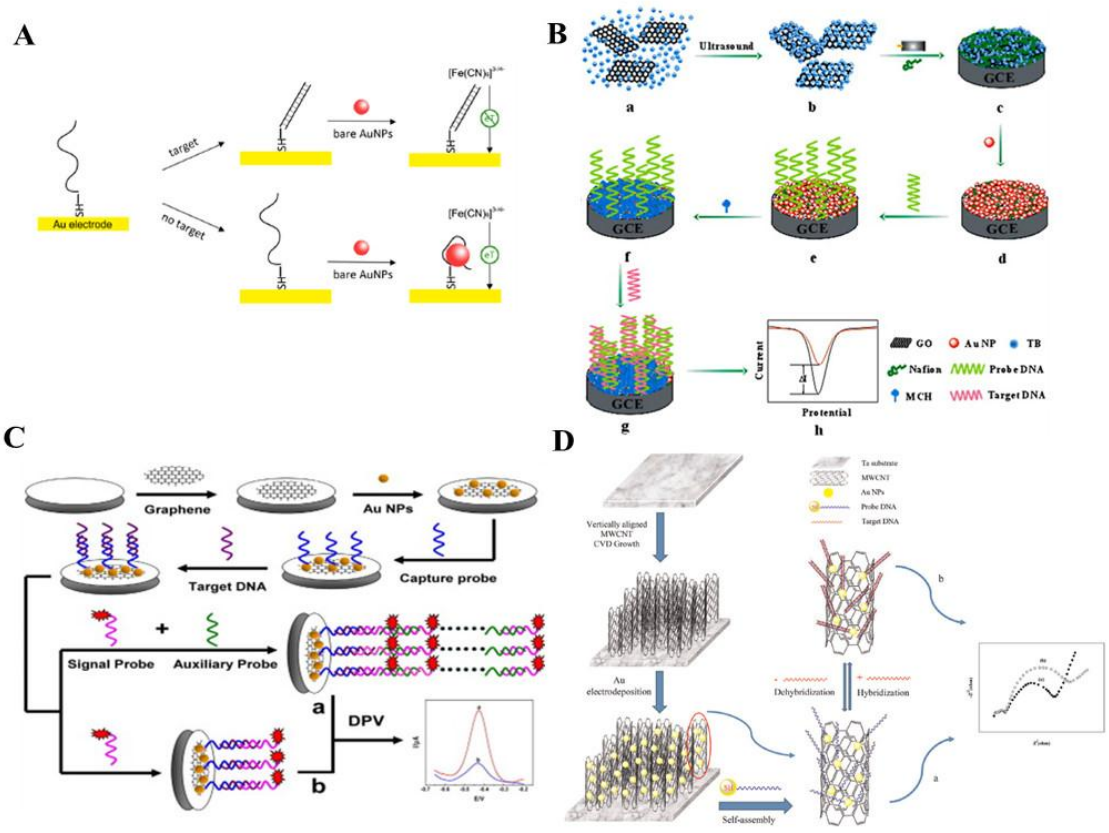

Fig. 6. (A) Interaction between AuNPs and DNA Immobilized on an Electrode Surface and Its Sensing Ability for Target DNA Detection (Yang et al., 2014).; (B) Fabrication and detection

process of the DNA biosensor (Peng et al., 2015).; (C) Schematic representation of the fabrication procedure of the DNA biosensor. (a) DPV cures from the supersandwich biosensor; (b) DPV cures from the sandwich biosensor (Wang et al., 2015).; (D) Schematic representations of the DNA electrochemical biosensor (Fayazfar et al., 2014).

To improve the sensitivity of the electrochemical DNA sensor, research should proceed in three directions: towards the amplification of the target hybridization event, improvement of the electron transfer efficiency, and suppression of the background. Yu and coworkers (Qiu et al., 2013) reported a signal-on E-DNA senor for the highly sensitive detection of specific p53 gene sequences, based on the ingenious combination of AuNPs-dependent enrichment of redox-active moieties, electron-transfer-determined signal amplification, and background suppression (Fig. 7 b). To achieve this goal, streptavidin-conjugated AuNPs capped with multiple ferrocene-signaling probes (Fc-AuNPs-SA) were used as reporters to signal and amplify the target DNA hybridization event. Using cooperative integration of multiple elements to increase the signal-to-noise ratio, the sensor achieves an ultrahigh sensitivity (a detection limit of 5 zmol) and a wide linear response range (from 1 nM to 1 fM). Wei et al. (Wei et al., 2008, 2009b) reported the detection of a cancer mRNA gene with low sensitivities of 3.9 fM in whole saliva by combining this folding process with another amplification process. Besides, Pang and coworkers (Wang et al., 2003) reported a voltammetric assay using Fc-capped AuNPs/streptavidin conjugates. The large number of Fc moieties present at each gold nanoparticle greatly amplified the voltammetric signals, resulting in a low detection level and a desirable selectivity for both oligonucleotide and polynucleotide analyses.

Recently, Rasheed and Sandhyarani (Rasheed and Sandhyarani, 2015) reported a simple strategy for signal amplification. These authors utilized appropriately functionalized AuNPs in

an electrochemical genosensor, resulting in the attomolar detection of the BRCA1 gene (Fig. 7a). The sensor was developed through the layer-by-layer assembly of mercaptopropionic acid (MPA), polyethylene glycol (PEG) functionalized AuNPs, capture DNA, target BRCA1 DNA and AuNPs labeled reporter DNA on a gold electrode. PEG-functionalized AuNPs on the MPA surface provided an adequate electron conduction path, nullifying the insulating effect of MPA. The results showed that the sensitivity could be significantly enhanced.

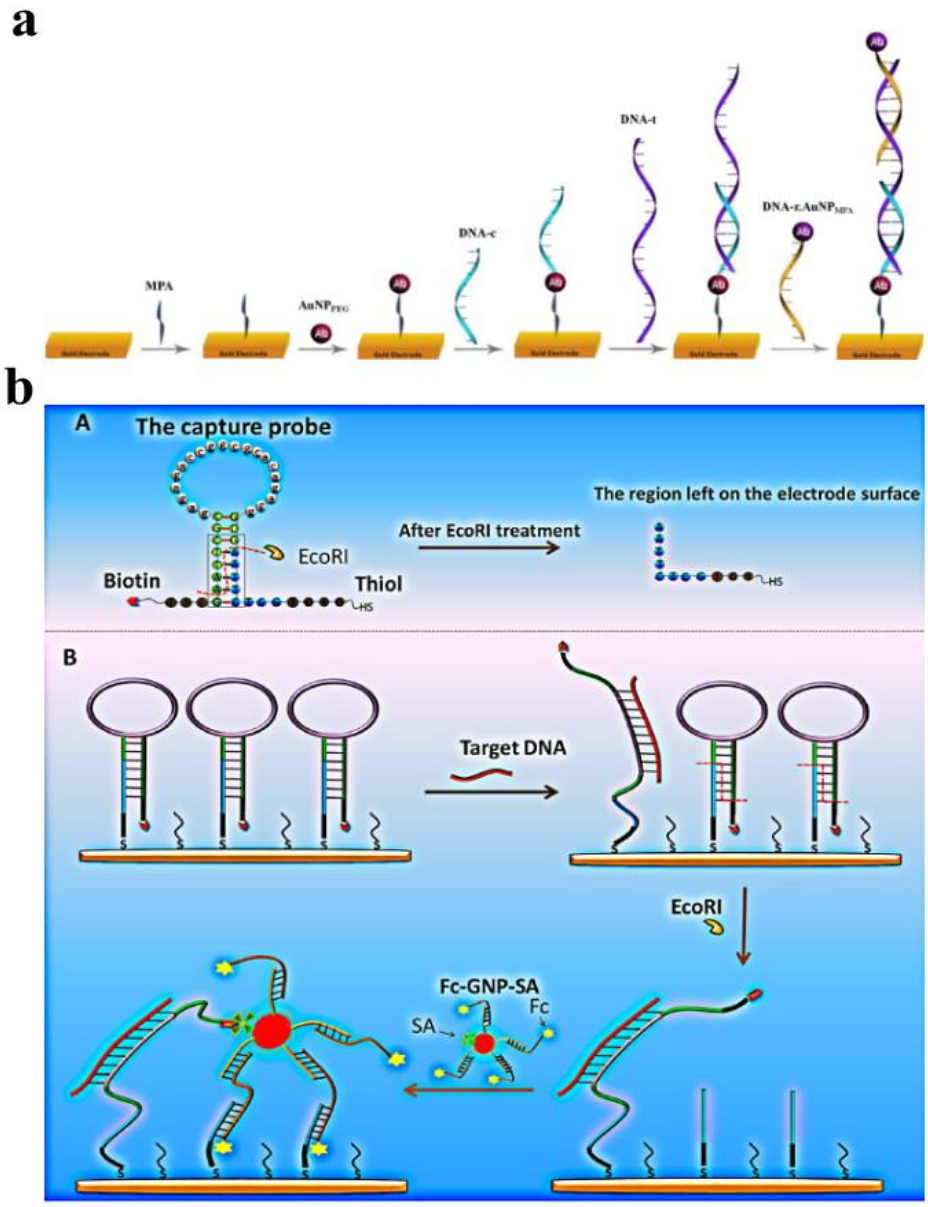

Fig. 7. (a) The schematic representations of the various stages of sensor fabrication and DNA hybridization related to specific BRCA1 sequences. (Rasheed and Sandhyarani, 2015).; (b) (A) Design of the Capture Probe Which Can Recognize the Target DNAa and (B) Schematic Illustration of This Sensor (Qiu et al., 2013).

5.3 Carbon nanotubes -based electrochemical methods

Carbon materials, such as CNTs, have shown great potential as an electrode materials for electrochemical DNA detection due to the superior mechanical and electrical properties of these molecules (Baughman et al., 2002; He and Dai, 2004; Wang et al., 2004; Heller et al., 2005; Koehne et al., 2003; Ye and Ju, 2005).

Specifically, CNTs demonstrate rapid electron transport and detection signal amplification, suggesting that these particles are effective transducers. For example, Zhang, et al. (Zhang et al., 2011b) reported a label-free, reagentless electrochemical DNA hybridization sensing strategy for grafting both redox and DNA probes onto carbon nanotubes. Oxidized single-walled carbon nanotubes (SWNTs) were first immobilized on a self-assembled monolayer of cysteamine; then the redox probes were grafted onto the free carboxylic groups of the nanotubes. Finally, a DNA probe was grafted onto the new carboxylic acids and hybridization was studied with different DNA targets.

Besides, an ultrasensitive electrochemical nucleic acids assay amplified by CNTs-based labels for the detection of human acute lymphocytic leukemia (ALL)-related p185 BCR-ABL fusion transcript was developed (Lee et al., 2014). The carboxylated CNTs were functionalized with horseradish peroxidase molecules and target-specific detection probes via diimide-activated amidation and used to label and amplify the target hybridization signal. Finally, the assay allowed robust discrimination between the perfect match and a three-base mismatch sequence, which was simple, low-cost and ultrasensitive in early disease diagnostic applications.

5.4 Other nanomaterials-based electrochemical methods

In the present study, an electrochemical DNA biosensor was successfully developed based on an ionic liquid, ZnO nanoparticles and a chitosan nanocomposite membrane on a modified

gold electrode (AuE) (Siddiquee et al., 2014). A single-stranded DNA probe was immobilized on this electrode. MB was used as the hybridization indicator to monitor the hybridization reaction of the target DNA. Finally, the target DNA sequences were detectable using differential pulse voltammetry at concentrations of 1.0×10^{-18} to 1.82×10^{-4} mol·L^{-1}, and the LOD was 1.0×10^{-19} mol·L^{-1}. The developed DNA biosensor facilitated the study of hybridization with crude DNA fragments and provided a rapid, sensitive and convenient method for the species-level identification of *Trichoderma harzianum.*

Besides, Miao et al. (Cheng et al., 2015) developed a novel and simple electrochemical miRNA biosensor by using Cd^{2+}-modified titanium phosphate nanoparticles as signal units, two DNA as capture probes, and $Ru(NH_3)_6^{3+}$ as an electron transfer mediator (Fig. 8a). Large quantities of cadmium ions were mounted in titanium phosphate spheres to output the electrochemical signal. This approach achieved a wide dynamic linear range from 1.0 aM to 10.0 pM with an ultralow LOD of 0.76 aM. The proposed biosensor was also sufficiently selective for rapid and direct analysis of miRNAs in human serum. So this strategy can provide a new and ultrasensitive platform for miRNA expression profiling in biomedical research and clinical diagnosis.

Recently, metal nanoclusters have also been applied to electrochemical analysis. Liu et al. (Liu et al., 2013b) developed a simple and specific DNA sensor that operates through analysis of the Au cluster ions formed from AuNPs under laser desorption/ionization (LDI) conditions. The signals for the Au cluster ions in the mass spectra allow the quantitation of the coverage and the exploration of the structure of the DNA on the AuNPs. These authors used pDNA (probe DNA)−AuNPs as a selective probe for the detection of SNPs of the Arg249Ser unit in the TP53 gene. These results provided the first example of DNA sensing based on the analysis of cluster

ions from metallic NPs under LDI. This high-throughput LDI-MS-based DNA detecting system shows excellent potential for DNA array analysis. Moreover, this system may also be used to amplify other biological signals after attaching different DNA or RNA aptamers onto the AuNPs surfaces.

5.5 Multi-single biomarker in electrochemical methods

Currently, a single biomarker is not sufficient for high specificity detection in clinical samples. Combining several biomarkers can greatly improved the detection accuracy, making multiplex detection for DNA diagnostics extremely important. For example, an electrochemical coding technology labeled with ZnS, CdS and PbS QDs was developed for the simultaneous detection of multiple DNA targets (Fig. 8b) (Liu et al., 2005). Three encoding QDs were used to differentiate the signals of three DNA targets, along with a sandwich hybridization assay and stripping voltammetry of the corresponding heavy metals.

Besides, Alocilja and coworkers (Zhang et al., 2010) developed a highly amplified, nanoparticle-based, bio-barcoded electrochemical biosensor for the simultaneous multiple detection of the protective antigen A gene of Bacillus anthracis and the insertion element gene of Salmonella enteritidis. This biosensor utilized nanoparticle tracers (NTs: PbS and CdS) terminating the ssDNA bio-barcode conjugated to AuNPs as electrochemical indicators as well as magnetic nanoparticles for easy and clean separation from the sample (Fig. 8c). Finally, the biosensor has a good specificity and the sensitivity for the detection of target genes and a potential application in multiple detection.

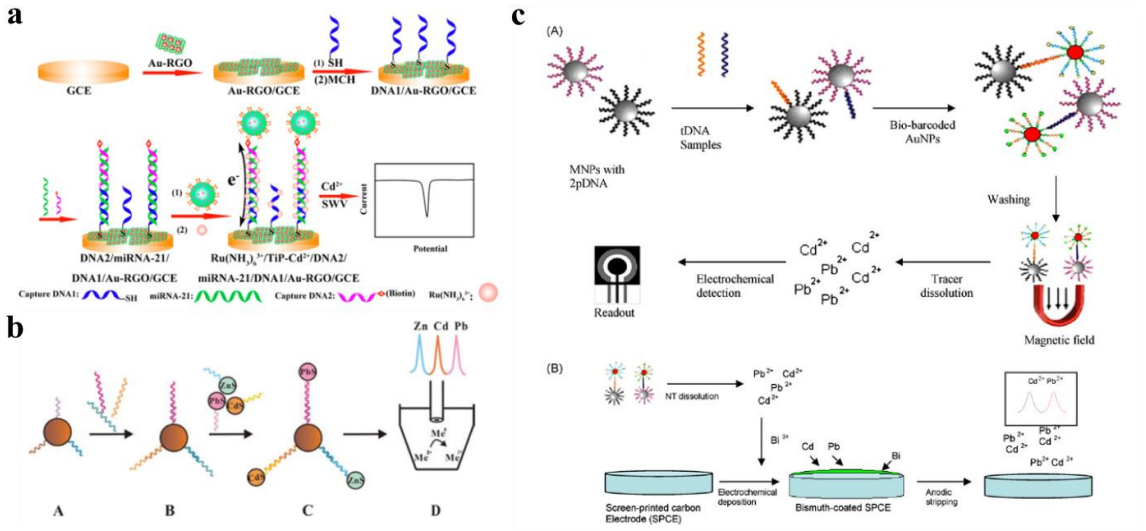

Fig. 8. (a) Schematic Illustration of the Stepwise Sensor Construction Process (Cheng et al., 2015).; (b) Multi-target electrical DNA detection protocol based on different inorganic colloid nanocrystal tracers. (Liu et al., 2005).; (c) Schematic of the multiple bio-barcode biosensor: (A) bio-barcode assay and (B) SWASV measurement (Zhang et al., 2010).

Briefly summarize the present electrochemical methods. With contributions from microfluidics and microelectromechanical system technologies, electrochemical sensors can be integrated onto portable platforms incorporating all the necessary preparation and fluidic processes to generate commercial devices for clinical diagnostics. Ultimately, the end goal of electrochemical sensor development is to construct a total analysis system for rapid DNA biosensing that incorporates sample pretreatment, sample delivery, and detection. Furthermore, the future direction of electrochemical DNA sensor development is primarily focused on the creation of POC systems that integrate sample handling, fluidic processing, and portable platforms. However, clinical applications for electrochemical DNA sensors are far from realization due to several important issues, including their insufficiently sensitive, specific, and simple processes. Electrochemical sensors show great potential for DNA biosensing and have

been improved by nanotechnology in particular, including new nanomaterials and fabrication techniques.

6. Surface-enhanced Raman spectroscopy

SERS has attracted considerable attention due to its molecular specificity, single-molecule sensitivity, and insensitivity to quenching. A large number of SERS substrates with consistent local field enhancements for improved chemical detection have been designed and fabricated, such as metal nanoparticles (Kelly et al., 2003; Gopinath et al., 2009; Emory and Nie, 1998) and nanoplasmonic resonators (Wang et al., 2007b; Su et al., 2006; Chen et al., 2012). As a result, SERS has long been anticipated as a label-free identification probe for nucleic acid sequence detection. SERS is also one of the most sensitive diagnostic approaches for gene detection (Fang et al., 2008).

For example, Cao et al. (Cao et al., 2002) developed a technique for the multiplexed detection of oligonucleotide targets using AuNPs probes labeled with oligonucleotides and Raman-active dyes. The AuNPs can facilitate the formation of a silver coating that acts as a SERS promoter for the dye-labeled particles that have been captured by the target molecules and an underlying chip in microarray format. Besides, Y. Fainman and coworkers (Freeman et al., 2014) created nucleic acid-silver systems with new electronic states, leading to different visible range absorption for different nucleic acid metal systems. The resulting SERS measurements were consistent with the simulated values. These findings paved the way for the future single molecule detection of nucleic acids other than adenine.

Additionally, Ju and coworkers (Gao et al., 2013) designed a label-free strategy for the SERS detection of the target DNA on a peptide nucleic acid (PNA)-modified glass slide. Upon

hybridization of the PNA with target DNA, the surface becomes negatively charged, facilitating the adsorption of silver ions onto the DNA skeleton. After chemical reduction using hydroquinone, the formed AgNPs could be further grown in a silver enhancement step to amplify the detectable SERS signal by absorbing rhodamine 6G as a SERS reporter on a silver nanoparticle surface (as shown in Fig. 9). The method was able to detect DNA with a linear range of 1.0×10^{-10} to 1.0×10^{-6} M and a LOD of 45 pM. By introducing a hybridization chain reaction to increase the DNA length, the Raman signal was further amplified, leading to a detection limit of 3.4 pM. The proposed label-free SERS strategy showed high selectivity against a single-base mismatch sequence in a cost-effective manner, which has a promising application in clinical diagnosis.

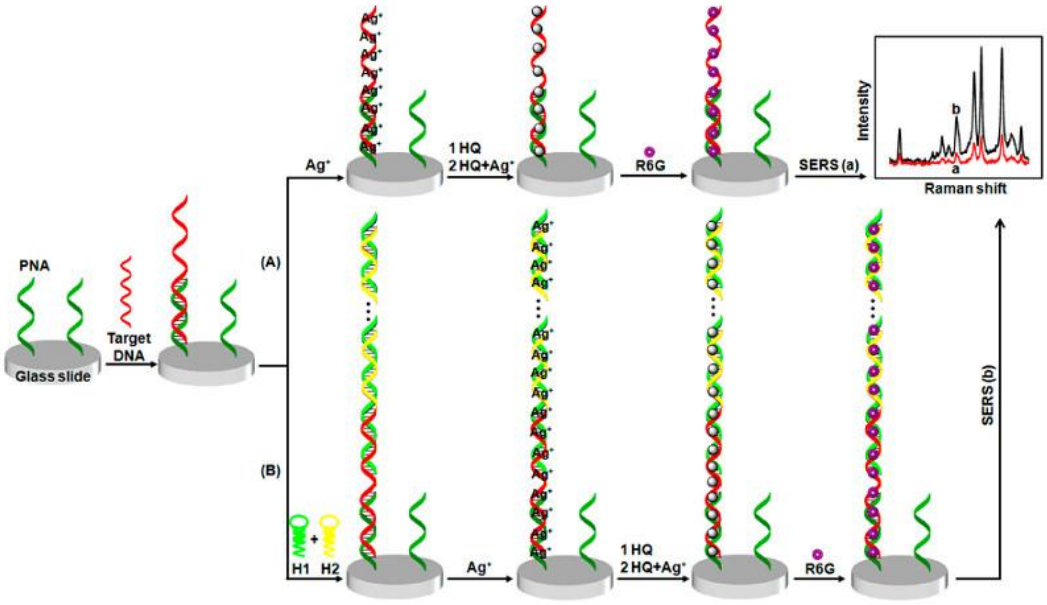

Fig. 9. Illustration of Label-Free SERS Strategy for DNA Detection without (A) and with (B) HCR Amplification (Gao et al., 2013).

7. Surface plasmon resonance

SPR is a popular surface label-free analytical technique for the detection of changes in the refractive index resulting from variations of the mass on the sensor chip surface. SPR has become a common tool in biochemistry and bioanalytical chemistry, particularly for determining the on(off)-rates and equilibrium binding constants that describe interactions between proteins, DNA or RNA and a wide variety of other biomolecules or ligands to investigate the effects of various cofactors or inhibitors on this binding constant. In most SPR studies, macromolecules, such as proteins and DNA, are immobilized onto the gold surface to detect protein-protein (Wang et al., 2006; Williams, 2000; Quinn et al., 2000) and protein-DNA interactions (Jayarajah and Thompson, 2002). Some researchers have immobilized low molecular weight ligands onto the gold surface to specifically detect DNA and other targets (Hagihara et al., 2004; Kobori et al., 2004; He et al., 2009).

To amplify the signal and increase the sensitivity, other strategies, such as AuNPs enhanced methods, PCR and enzymatic amplification, have been used. Among these methods, AuNPs have been widely adopted as popular and useful labels for amplifying weak signals (Luan et al., 2011; Yamaguchi et al., 2002; Fang et al., 2006; He et al., 2000; Yao et al., 2006; Bailey et al., 2003; Matsui et al., 2005; Riskin et al., 2009; Wang et al., 2009a). The AuNPs-tagged surface can generate angle shift concomitant increase 10-fold increases with a 1000-fold improvement in sensitivity and a ~10 pM detection limit for the target 24-mer oligonucleotide (He et al., 2000). For example, Zhou et al. (Yao et al., 2006) showed that an intermediate carboxylated dextran layer between a gold film and immobilized DNA molecules effectively eliminates the nonspecific adsorption of oligonucleotide functionalized AuNPs, resulting in a femtomolar level of detection for 39-mer DNA. Besides, the target-primed RCA in the liquid phase can be

monitored in real-time using the AuNPs-embedded SPR sensing method as well as the specificity of padlock probes, the simplicity of target-primed RCA, and the combination of RCA and digestion reactions (Xiang et al., 2015).

Surface plasmon resonance imaging (SPRI) is also a rapid, multiplexed method for the label-free detection of surface bioaffinity interactions (Shumaker-Parry and Campbell, 2004; Kanda et al., 2004; Wilkop et al., 2004) and has been used extensively with DNA microarrays (Wolf et al., 2005; Lee et al., 2005; Nelson et al., 2001). For example, an enzymatically amplified SPRI-based analytical approach was used to detect 7 fM genomic DNA (Goodrich et al., 2004). Based on nanoparticle-enhanced SPRI detection and PNA probes, the ultrasensitive detection of non-amplified genomic DNA solutions containing a target sequence as a minor component was achieved with a 41 zM sensitivity in targeting genomic DNA even in the presence of a large excess of non-complementary DNA (D'Agata et al., 2010). Besides, Corn and coworkers (Li et al., 2006) reported a SPRI methodology for SNP genotyping that uses a unique combination of a surface enzymatic ligation reaction in conjunction with AuNPs enhanced hybridization adsorption on DNA microarrays.

8. Other methods

8.1 Microarray detection

The application of microarrays is rapidly advancing high-throughput gene analysis technology (Fu et al., 2012). These applications include the quantitative analysis of gene expression (Schena et al., 1995; Karimi. And Farrokhnia, 2014), DNA sequencing and gene discovery (Hacia, 1990), disease diagnosis (Huang et al., 2001), drug discovery (Marton et al., 1998), and toxicogenomic research (Liu et al., 2004a). DNA microarrays are prepared in a single

experiment by employing the hybridization of target genes to probe molecules immobilized onto a solid surface (Schena et al., 1995; Fodor et al., 1991; Brown and Botstein, 1999; Lenz et al., 2015). DNA microarrays are typically small glass, plastic, silicon substrate or nylon membranes onto which probe molecules are tethered in the arrays of spots. Each spot carries probe molecules specific to one particular gene. The target molecules contained in a sample solution are captured on the array through hybridization to the corresponding homologous probe molecules on the array surface (Sun et al., 2007).

AuNPs are attracting increasing interest for microarray detection due to their unique electronic and optical properties (Shipway et al., 2000; Wang et al., 2002). Taton et al. (Taton, et al., 2000, 2001) reported the detection of DNA arrays in a three-component sandwich format using oligonucleotide-functionalized gold nanoparticles complementary to the target DNAs. The attained sensitivity is equivalent to that of conventional fluorescent detection, but it requires the incorporation of reporter molecules must be incorporated in the target DNA, which is costly and time-consuming.

McCann M P, et al. (Sun et al., 2005) developed a microarray system for gene expression analysis using cationic AuNPs with diameters of 250 nm as a target detection reagent. This approach utilizes non-labeled target molecules hybridized with complementary probes on the array as a simpler and more cost effective method than traditional microarray detection. The gold labeling signals can be detected using an inexpensive optical scanner, which will significantly reducing setup costs and making this technique available to a broader range of research and clinical communities. The sensitivity is estimated as less than 2 pg of DNA molecules captured on the array surface. The signal from hybridized spots quantitatively represents the amount of captured target DNA. Thus, this technique has the potential for quantitative gene expression

analysis. This new AuNPs-based detection technology is a promising gene expression analysis tool with low cost and ease of use as important characteristics.

In addition to AuNPs, carbon nanoparticles (CNPs) can also be used as labels in microarrays. CNPs have many advantages as labels in diagnostic assays, such as low price, good sensitivity, and a high signal-to-noise ratio (Gordon and Michel, 2008). Noguera, P.S., et al. (Noguera et al., 2011) used CNPs in nucleic acid microarray immunoassays (NAMIAs) for the detection of different Shiga toxin-producing *Escherichia coli* (STEC) virulence factors. STEC strains are highly pathogenic and can cause severe illness. NAMIA using CNPs was tested with DNA from 48 field strains originating from cattle feces, and the performance was evaluated by comparing the results with those obtained using the reference method, q-PCR (Fig. 10a). The incubation reaction between the CNPs and the label (here NA) can be performed under simple laboratory settings. The system is generic because the specificity of the assay is achieved by the combination of discriminating tags with specific primers. This technique is promising as a NAMIA with specificity, which can be readily developed after changing primer sequences to amplify genes of other targets (e.g., other pathogenic microorganisms).

Besides, Alivisatos and co-workers (Gerion et al., 2003) reported a microarray-based assay to detect SNP mutation in the human p53 tumor suppressor gene and single base-pair mutations in DNA using CdSe/ZnS nanocrystals at room temperature. Moreover, this method has the feasibility of simultaneous detection of multiple biological pathogens using hepatitis B virus (HBV) and hepatitis C virus (HCV), two common viral pathogens in the developing countries worldwide. Though their model system has been used only synthetic targets, it can be extended to real samples such as blood after appropriate extraction and purification of the genetic material in the future.

8.2 Quartz crystal microbalance methods

QCMs are ultrasensitive piezoelectric devices that use frequency changes on a quartz crystal resonator to monitor changes in mass arising from analyte binding. This method has been widely used to sense DNA because of its high sensitivity and label-free detection. The introduction of nanoparticles, particularly AuNPs, can enhance its sensitivity due to the high surface area of the molecules, and nanoparticles can serve as a "mass enhancer" by amplifying the frequency changes (Lin et al., 2000; Hong et al., 2010). Sandwich-based approaches using ssDNA-modified AuNPs might improve the detection limit (Zhou et al., 2000; Patolsky et al., 2000; Willner et al., 2002; Nie et al., 2007; Liu et al., 2003; Han et al., 2007; Liu et al., 2004b).

Jiang J H, et al. (Pang et al., 2007) proposed a simple and feasible piezoelectric approach for the detection of DNA point mutations using QCM based on DNA ligase reactions and Fe_3O_4/Au core/shell nanoparticle probes. This approach has been applied to the identification of a single-base mutation in the –28 site of an artificial β-thalassemia gene, and the wild type and mutant genes were successfully discriminated. These authors employed the synthesized core/shell nanoparticles to realize the isolation of DNA probes and amplification of detection signal. After the DNA ligase reaction and denaturing at an elevated temperature, the biotin-modified probe reacts with avidin on the electrode surface for the perfect match target, changing of the crystal frequency. In contrast, no frequency change is recorded for mismatch target. Point mutation discrimination could be achieved successfully, and a detection limit of 4.6×10^{-10} mol/L of oligonucleotides was achieved. Due to its ease of operation and low detection limit, the proposed procedure is promising for the potential use in the medical diagnosis of diseases associated with SNPs.

8.3 Dynamic light scattering

In 2011, Gao, D. et al. (Gao et al., 2011) developed a simple, rapid and ultrasensitive method for the detection of sequence-specific nopaline synthase (NOS) genes from the transgenic plants using a label-free AuNPs probe and DLS technology (Fig. 10b). AuNPs are stable in NaCl solution in the presence of NOS gene probe but aggregate when the probe sequence hybridizes with the target sequence. The change in the size of the AuNPs can be detected with high sensitivity using DLS. Under the optimal conditions, the average hydrodynamic diameter of gold NPs was linearly related to the concentration of the target sequence from 1.0×10^{-13} mol·L^{-1} to 5.0×10^{-9} mol·L^{-1}, with a detection limit of 3.0×10^{-14} mol·L^{-1} (S/N=3). These results showed that the AuNPs-based DLS method has great potential in the analysis of transgenic products.

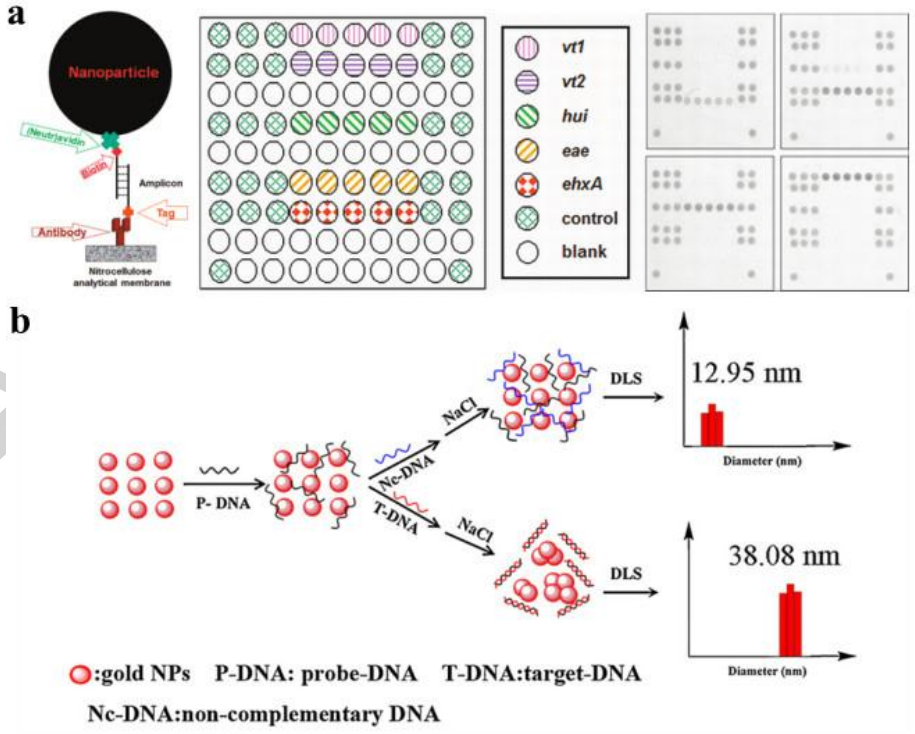

Fig. 10 (a) Scheme of the detection strategy (Noguera et al., 2011).; (b) Schematic diagram of the

detection of transgenic sequences using AuNPs and DLS (Gao et al., 2011).

8.4 Single particle mode inductively coupled plasma mass spectrometry

A multiplex DNA assay based on nanoparticle tags detection utilizing SP-ICP-MS for ultra-sensitive readout has been demonstrated (Zhang et al., 2014). Three DNA targets associated with clinical diseases (HIV, HAV, and HBV) were detected down to 1 pM using DNA probes labeled with AuNPs, AgNPs, and PtNPs via DNA sandwich assay (Fig. 11a). SNPs in genes could also be effectively discriminated. Because this method is not affected by the sample matrix, it is well suited for diagnostic applications. Moreover, due to the high sensitivity of SP-ICP-MS and the variety of NPs detectable using this technique, a high-throughput DNA assay could be achieved without signal amplification or chain reaction amplification.

Notably, the ultrasensitive detection could be further improved by combination with PCR. Compared with other techniques for multiplex NP detection in homogeneous solutions, such as the colorimetric method, ICP-MS detects NPs without any special requirements in terms of optical and electrochemical properties; thus, a wide range of NPs, such as TiO_2, Al_2O_3, ZrO_2, and ThO_2, could be applied for multiplex assay using SP-ICP-MS (Degueldre and Favarger, 2004; Degueldre et al., 2004, 2006a, 2006b). Therefore, this method could be further extended to the high-throughput assay of DNA with multiplex NP tags. As the signal of SP-ICP-MS is not affected by the sample matrix, this method has the potential to allow the accurate determination of DNA in real samples.

8.5 Electrochemiluminescence methods

Electrochemiluminescence (ECL) has been extensively investigated because it can be electrochemically controlled and displays sensitivity comparable to that of chemiluminescence

(Ding et al., 2015; Wang et al., 2009c; Zou and Ju, 2004). Quantum dots (QDs) have since received considerable attention due to their high fluorescence quantum yield, simultaneous excitation, and stability against photo-bleaching (Yin and Alivisatos, 2004; Burda et al., 2015; Myung et al., 2002; Wanget al., 2011). QD ECL sensors based on energy transfer have been used extensively as a sensitive analytical technique in DNA analysis (Shan et al., 2009).

Li M, et al. (Lu et al., 2015) fabricated an ECL sensor to detect DNA damage based on the distance between QDs and AuNPs. This damage was induced by perfluorooctanoic acid (PFOA) after measuring the electrochemiluminescence of a layer-by-layer electrostatic assembly (Fig. 11b). DNA damage was determined after measuring the ECL of the QDs, which decreases after exposure to PFOA, showing that DNA damage decreases the distance between the QDs and AuNPs, eventually leading to ECL energy transfer. This process is highly sensitive to distance. ECL intensity is logarithmically related to the concentration of PFOA in the range from 10 $\mu mol \cdot L^{-1}$ to 10 $pmol \cdot L^{-1}$, and the detection limit is 10^{-12} $mol \cdot L^{-1}$.

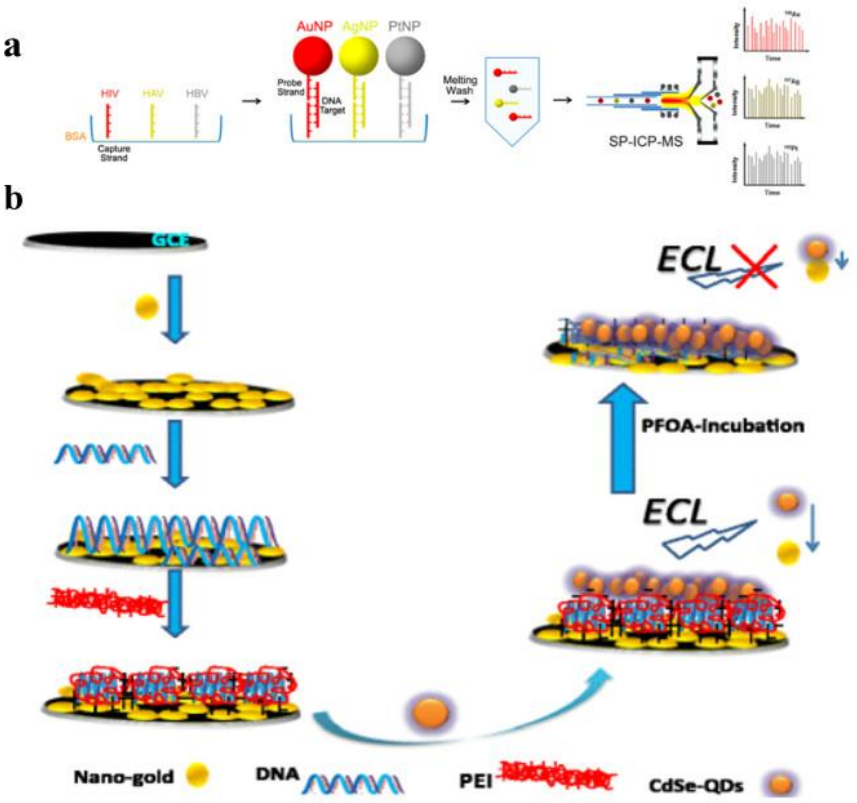

Fig. 11. (a) Schematic of Multiplex DNA Assay Based on NP Probes by SP-ICP-MS (Zhang et al., 2014)); (b) Schematic illustration of QDs/PEI/ctDNA/AuNPs/GCE ECL sensor (Lu et al., 2015).

9. Conclusions and perspectives

The physical properties and functionalization of nanomaterials suggests the use of these molecules as a versatile platform in chemical and biological fields. This technology represents a young and rapidly expanding research field at the crossroads of materials science, nanoscience, chemistry and biology. Because nanoparticles and biomolecules typically have the same nanometer length scale, advanced medical diagnosis, including the detection of genes, will be largely dependent on the successful development and application of new nanomaterials and

nanotechnologies in biology in the near future.

The detection of disease-related genes, such as SNPs and trinucleotide repeats, is important in the diagnosis of diseases and conditions and is particularly useful for early stage treatment and monitoring. Specifically, the BRCA1 and TP53 genes are commonly studied. Thus, nucleic acid-based detection techniques have attracted significant research attention particularly. For all these needs, it is the most critical issue to develop the detection methods that are sensitive, specific, rapid, easy-to-use and preferably low-cost.

This review describes the status of the development of gene detection approaches based on nanomaterials with the following bases (excluding PCR-related methods): colorimetry, fluorescence-based methods, electrochemistry, microarray methods, SERS, QCM methods, DLS and other methods. Gold, silver, carbon and magnetic nanomaterials can be used in a variety of applications for nucleic acid analysis and detection. Compared with conventional methods, nanomaterial-based gene detection has many practical merits. For example, colorimetric methods based on AuNPs are simple, rapid and feature a visible color change from red to blue at nanomolar concentrations. The Mirkin group reported that the detection limit of this approach was approximately 1.0×10^{-8} mol·L^{-1} DNA (Stoeva et al., 2006). However, the structure, stability and reactivity nucleic acids both in solution and at the interface are strongly affected by salt-dependent electrostatic effects, hindering nucleic acid detection using colorimetric methods. Electrochemical methods are also becoming increasingly attractive, reflecting their low cost, simplicity and portability as well as extremely high detection sensitivity. For example, Ozsoz et al. detected Leiden mutations with a sensitivity of 0.78 fmol through the incorporation of Au nanoparticles in DNA probes. Electrochemical DNA sensors show potential for point-of-care clinical diagnostics. Moreover, research concerning the effects of interfering substances is

conducted in pure, synthetic model-DNA sequences rather than complex real samples; thus selective molecular recognition is important and necessary. Clinical samples are complex mixtures containing a multitude of components and biological species. Even the in vitro detection of bodily fluids (i.e., blood) presents great challenges for simple detection. In addition to an excellent selectivity, the use of these techniques requires several pretreatment processes before detection, such as separation, purification, accumulation, and amplification. Importantly, the pretreatment processes can be greatly simplified and improved by the appropriate use of nanomaterials for gene detection.

Indeed, some nanomaterial-based DNA detection methods outperform conventional assays in terms of selectivity, sensitivity and practicality. Some current methods are simple and yield outputs in less than 1 h. However, a truly simple, rapid and low-cost assay for routine detection has yet to be developed for clinical applications. It will be necessary to optimize the parameters, including selectivity, sensitivity, operation, simplification, rapid, repeatability, for application in clinical and POC systems. DNA also has higher chemical stability than other bio-recognition elements (e.g., enzymes and antibodies). Nucleic acids from different strains have excellent discrimination abilities and are highly correlated with genetic factors and the development of disease, representing potential disease markers. Taken together, these findings show great promise for future disease-related gene detection methods based on nanomaterials for clinical and other purposes.

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.**Highlights**

For

Application of nanomaterials in the bioanalytical detection of disease-related genes

- The current and future trends about application of nanomaterials in disease-related gene were reviewed.
- The most used nanomaterials, such as gold, silver, carbon and semiconducting nanoparticles, were discussed mainly.
- Different bioanalytical methods excluding PCR-related method were discussed.