Gold nanoparticle-based biosensors for the assay of tumor marker proteins with clinical applications

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Abstract

The detection of tumor markers plays an important role in clinical diagnosis and evaluation of therapeutic effect. Early detection of tumor markers, which are usually proteins, can greatly facilitate effective treatment with different modalities and even increase cure rate of patients. Currently, nanoparticle-based methods for cancer diagnostics are becoming an increasingly relevant alternative to traditional techniques. Gold nanoparticles (AuNPs) are one of the most extensively studied nanomaterials due to their remarkable physical and chemical properties. With the recent advances in nanotechnology, AuNPs have offered new ways to detect tumor markers at low concentrations and to target cancer cells in very deep sites. The use of AuNPs may increase the sensitivity of a biosensor and generate higher accuracy and precision of the assays. So, AuNPs have greatly facilitated the development of nanomaterials-based technology for clinic diagnostics and therapy. In this review paper, we have summarized different kinds of AuNPs-based biosensors for the detection of tumor marker proteins with a particular focus on optical and electrochemical techniques, which may provide valuable perspective for the colleagues in the related communities.

Keywords: Gold nanoparticles, biosensors, tumor marker protein, colorimetric analysis, electrochemical analysis.

Introduction

Cancer is still one of the leading causes of death in the world. Of those who are died of cancer, more than 30% of people can be saved if the cases are detected and treated earlier **[1-3]**. Although many advances in therapeutics have been made for cancer in the past decade, the early and quick diagnosis continues to be an extremely important part for cancer treatment. Therefore, quick diagnose and early preventions are critical for patient survival, which are also beneficial to reducing financial and social pressure on governments and families.

As tumors develop, tumor cells and other related non-tumor cells will release specific tumor markers into the circulation system in response to cancer growth, which are usually proteins **[1, 4]**. Tumor makers are commonly indicative of a normal biological or pathogenic process, which can be detected in the blood, urine, or body tissues **[5-7]**. So, the detection of tumor markers plays an important role in clinical diagnoses and evaluation of treatment for patients.

Tumor markers are usually present at very low concentration in first stages of cancer and are difficult to detect **[8, 9]**. It is therefore essential to develop biosensors which can detect tumor markers sensitively and specially. With the recent advances in nanotechnology, nanomaterials have offered new ways to detect tumor markers at low concentrations and to target cancer cells in very deep sites. The use of nanomaterials may increase the sensitivity of a biosensor and generate higher accuracy and precision, due to their size and shape-dependent physical, chemical and electrical properties, such as large surface area-to-volume ratio, excellent electrical conductivity, and good thermostability **[10-14]**.

Among the various nanomaterials employed for the development of biosensors, metal nanoparticles like gold nanoparticles are the most commonly used for clinical diagnostics **[15-18]** AuNPs have gained increasing interest due to their special features, e.g., easy surface

functionalization, high stability and biological compatibility, and especially extraordinary optical and electrical properties **[19-25]**. AuNPs can be prepared easily via citrate synthesis method, and their size can be varied by changing the concentration of sodium citrate. Meanwhile, the prepared AuNPs can be easily functionalized with recognizing biomolecules, such as lectins, antibodies, DNA, aptamers and cell surface receptors. Since the first immune gold labeling in 1970s **[26]**, AuNPs have been greatly facilitated the development of nanomaterials-based technology for clinic diagnostics and therapy.

Owing to the high importance of the detection of tumor marker proteins in the early diagnosis of cancer, a variety of novel biosensors have been reported for the development of these assays **[4]**. Therefore, more and more AuNPs-based biosensors have been reported in the field of tumor marker detection. In this paper, the use of AuNPs for the detection of tumor marker proteins are summarized, which may provide valuable perspective for the colleagues in the related communities. We will mainly focus on the latest achievements on the AuNPs-based biosensors for cancer diagnostics with different sensing strategies by making use of optical and electrochemical techniques, which will be more useful for clinical applications in the future.

AuNPs-based optical biosensors

AuNPs have unique optical properties, such as localized surface plasmon resonance (LSPR) absorption **[27, 28]**, resonance light scattering (RLS) **[29]**. These properties make AuNPs ideal for fast assay of a variety of substances including small molecules, bio-macromolecules, and even microbes **[30-34]**. Furthermore, many kinds of substances can be assembled onto the surface of AuNPs **[35-41]** to fabricate more optical biosensors for the assay of tumor markers **[42-45]**.

Colorimetric biosensors

The interparticle distance-dependent color change is a unique optical property of AuNPs, which can be easily distinguished by naked eye, thus it has been widely applied for the detection of proteins **[46-48]**, nucleic acids **[49-51]**, metal ions **[52]** and other molecules **[53-57]**.

Single stranded DNA (ssDNA) has raised a great interest in colorimetric assay, on account of its character that it can be easily immobilized onto AuNPs surface and prevents salt-induced AuNP aggregation by enhancing the electrostatic repulsion between ssDNA modified AuNPs. For instance, by combining this mechanism and the advantages of hyperbranched rolling circle amplification (HRCA), Liang et al. have developed a sensitive colorimetric biosensor for the detection of carcinoembryonic antigen (CEA) **[58]**. CEA is one of the most studied tumor markers associated with liver, colon, breast and colorectal cancers. A CEA aptamer can bind with its target and prevent the hybridizing of CDNA **(Fig. 1)**. Therefore, free CDNA can propagate the HRCA reaction to form a large number of ssDNA. ssDNA can prevent salt-induced AuNPs aggregation by adsorbing onto AuNPs, which results in the color change of the solution. Similarly, Chen et al. have reported a sensitive colorimetric biosensor for the detection of tumor marker protein based on rolling circle amplification (RCA) and ssRNA prevented salt-induced AuNP aggregation **[59]**. The established sensor allowed for the specific detection of alpha fetoprotein (AFP), a tumor marker of liver cancer, with a detection limit of 33.45 pg mL^{-1}.

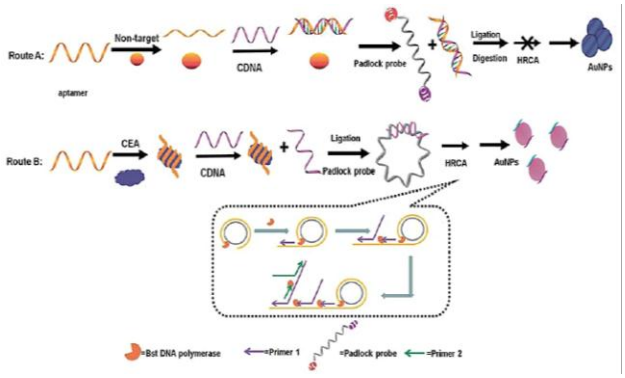

Fig. 1. Principle of the proposed AuNPs-based colorimetric biosensor combined with HRCA (reprinted from **Ref. 58** with the permission of Royal Society of Chemistry).

AuNP seed-mediated growth of AuNPs is also known as a promising colorimetric signal generation method. The extinction coefficient of 5 nm AuNPs is much lower than those of larger sized AuNPs, thus 5 nm AuNP solution is colorless compared with the larger sized AuNPs at the same concentration. Dependent on the glucose oxidase (GOx)-catalyzed 5nM AuNPs growth, Liu et al. have reported a quantitative colorimetric immunoassay that allows detection of attomolar tumor markers in clinical samples **[60]**. Based on the model sandwich structure, prostate-specific antigen (PSA) is captured and recognized by the capture antibody and GOx conjugated detection antibody. The immobilized GOx catalyzes the oxidation of glucose to generate H_2O_2. Then, the produced H_2O_2 induces the enlargement of 5 nm sized AuNPs in the presence of $AuCl_4^-$ and the solution turns red from colorless. By proposing alcohol dehydrogenase-catalyzed AuNP seed-mediated growth of AuNPs, Peng et al. have also designed a reliable colorimetric biosensor for the detection of disease biomarkers with naked eye **[61]**. In the presence of hepatitis B surface antigen (HBsAg), the labeled ADH catalyzes the reaction to produce NADH, which reduces $HAuC_{l4}$, thus the growth of AuNPs can be achieved.

The catalytic activity of AuNPs that gets less attention in biodetection assays has also been employed for the fabrication of colorimetric biosensors. Although gold is chemically inert, AuNPs are found to be extremely useful as artificial enzyme **[62]**. Therefore, Chang et al. have presented a simple, sensitive, label-free colorimetric biosensor for the assay of hCG based on peptide-regulated AuNPs catalysis **[63]**. The detection of hCG is associated with both the investigation of pregnancy-related changes

and the early diagnosis of testicular cancer, so assay of HCG is highly required. In the absence of target, the positively charged hCG-binding oligopeptide binds with AuNPs through electrostatic interaction, which decreases in the catalytic abilities of AuNPs **(Fig. 2)**. Once hCG presents and binds to the peptide aptamer, the AuNPs will restore their catalytic activity and catalytically convert a yellow-colored chemical compound (4-NP) to a colorless product [4-aminophenol (4-AP)].

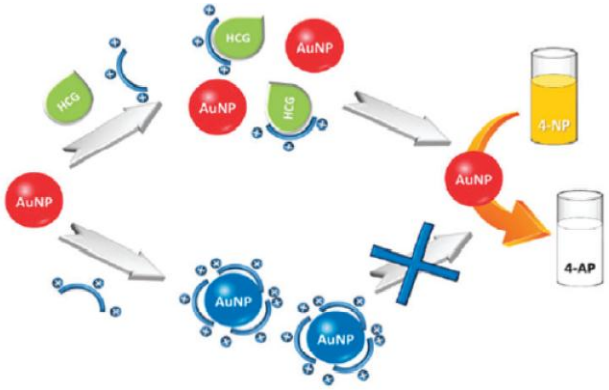

Fig.2. Schematic illustration of the proposed colorimetric biosensor for the detection of human chorionic gonadotropin (hCG) based on peptide-regulated AuNPs catalysis (reprinted from **Ref. 63** with the permission of Royal Society of Chemistry).

Light scattering biosensor

Metal nanoparticles such as AuNPs may exhibit exceptionally strong light scattering properties at the surface plasmon wavelength region. The light scattering ability of metal nanoparticles (gold, silver, etc.) is much higher than those of most biological samples **[64]**. Moreover, functionalized AuNPs can bind specific targets and emit an optical signal depending on their size, composition and the degree of surface plasmon resonance (SPR). So, AuNPs are excellent optical probes for the development of light scattering-based bioimaging as well as biomolecular detection **[65]**.

Our group has reported a dynamic light scattering (DLS)-based immunoassay for ultra-sensitive detection of tumor marker protein **[66]**. In the presence of the target, the antibody–MnO2–GNPs conjugates are pulled down onto the substrate, where GNPs is the abbreviation of AuNPs **(Fig. 3)**. When MnO_2 nanosheets are decomposed, a number of wrapped GNPs in the nanosheet is released to the solution, which can be directly used for the assay of the target protein by DLS measurement. In the meantime, Zheng et al. report a two-step AuNP-enabled DLS strategy for the detection of human immunoglobulin G (IgG) in blood serum samples of prostate cancer patients **[67]**. In the first step of the assay, a small amount of serum is directly mixed with a citrate-AuNP solution. Normal blood proteins and tumor specific antigens from the serum compete to adsorb to the citrate-AuNPs to form a "protein corona". The average particle size, D1, of the mixed solution is measured. In a second step of the assay, a rabbit antihuman IgG is added to the assay solution to analyze the relative amount of human IgG present in the protein corona. The binding of antihuman IgG with IgG present in the protein corona causes nanoparticle cluster formation. Then, the average particle size of the assay solution, D2, is measured. D2 versus D1 is calculated and expressed as the test score. Higher ratio corresponds to more IgG present in the protein corona.

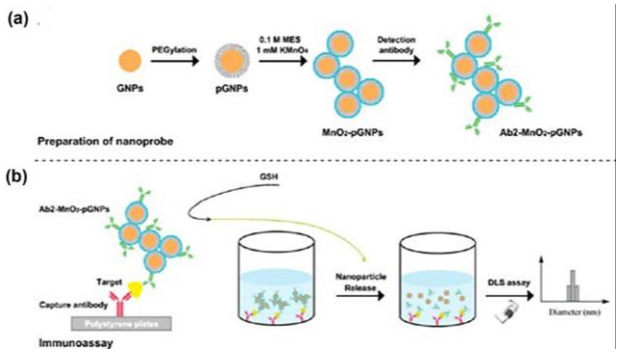

Fig. 3. Representation of the DLS-linked immunosorbent assay (DLS-LISA) for protein detection performed in one of the 96-well polystyrene (PS) plates (reprinted from **Ref. 66** with the permission of Royal Society of Chemistry).

Surface-enhanced Raman scattering (SERS) spectroscopy provides a high potential technology for immunoassay readouts. As a result of the near-field coupling between individual localized SPR of adjacent nanoparticles, assembly of plasmonic nanoparticles can generate nanostructures with enormously enhanced SERS signals **[68]**. Therefore, SERS spectroscopy has received great attention for the assay of tumor markers by using AuNPs. For instance, Wang *et al.* have reported a rapid, homogeneous and multiplexed immunoassay platform for quantification of protein targets based on SERS signal enhancement by controlled assembly of SERS nanoparticles **[69]**. Based on plasmonic coupling enhancement (highlighted as pink corona) via sandwiched antibody−antigen assembly, the single-step SERS immunoassay is achieved **(Fig. 4)**. The SERS nanoparticles is the key of the platform, which use nonfluorescent Raman-active dyes, AuNPs (or nanorods) codecorated with antibody half-fragments, and passivating proteins.

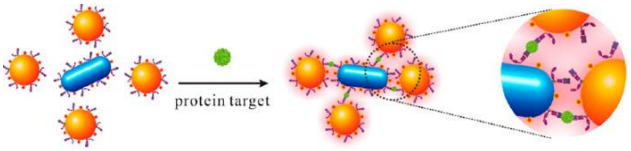

Fig. 4. Schematic illustration of TCA-SERS immunoassay (reprinted from **Ref. 69** with the permission of American Chemical Society).

Another interesting work is the combination of biobarcode probe with HCR amplification method, thus Ye *et al.* have proposed an asymmetric signal amplification method for simultaneous SERS detection of multiple tumor makers with significantly different levels **[70]**.

A colorimetry and SERS dual-mode system has also been presented, which has been used for the detection of

telomerase activity **[71]**. The combination of colorimetry and SERS into one detection system can achieve both fast screening by naked eye and high detection sensitivity. In this system, Raman molecules and telomeric repeat complementary oligonucleotide functionalized AuNPs are used as the reporting nanotag, while telomerase substrate oligonucleotide modified magnetic nanoparticles are employed as the capturing substrate. In the presence of target, telomeric repeats are synthesized and elongated onto the capturing substrate, which subsequently facilitate capturing of the reporting nanotag via hybridization. The captured nanotags can cause color change and SERS intensity difference of the magnetically separated sediments, so both colorimetry and SERS spectroscopy can be employed for the measurement.

AuNPs-based electrochemical biosensors

Electrochemical techniques, such as electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), square wave voltammetry (SWV), cyclic voltammetry (CV), allow rapid biosensing of key analytes for different types of cancer. Application of AuNPs in biosensors has significantly facilitated the development of electrochemical immunoassays due to their inherent advantages, especially high conductivity, ease of preparation, good biocompatibility, etc. Nowadays, AuNPs are mainly utilized in electrochemical immunosensors for two purposes: (i) as modifiers of the electrotransducer surfaces to create nanostructured surfaces with improved electrochemical response, since AuNPs possess good conductivity and high surface areas **[72]**; and (ii) as the carriers of labels to exert the effect of amplifiers for electrochemical signals **[73]**. So, we will summarize the advances of the AuNPs-based electrochemical biosensors by the sub-titles of AuNPs functionalized electrodes and AuNPs labels.

AuNPs functionalized electrodes

AuNPs functionalized electrode may provide high surface area, high adsorptivity of antibody and enzyme to enhance the conductivity and the affinity to bioactive materials **[74]**. Based on the use of gold nanostructured screen-printed graphite as a sensor platform and affibody as bioreceptor, Ravalli *et al.* have reported a label-free impedimetric biosensor for the detection of human epidermal growth factor receptor 2 (HER2), which is an indicator of normal biologic processes, pathogenic processes, or of pharmacologic responses to therapeutic intervention **[75]**. Cui *et al.* have synthesized a highly biocompatible polymer composite through electrochemical polymerization of the conducting polymer poly (3,4-ethylenedioxythiophene) and a polyethylene glycol (PEG) derivative, 4-arm PEG terminated with thiol groups. Through the unique interaction between AuNPs and the thiol groups, AuNPs are introduced to the composite surface to further immobilize antibodies for AFP assay **[76]**. Moreover, Shamsipur *et al.* **[77]** have developed a highly sensitive label free electrochemical biosensor for the detection of vascular endothelial growth factor 165 (VEGF165) based on differential pulse voltammetric "signal off" and impedimetri "signal on" methods. In this work, the BSA-gold nanoclusters/ionicliquid (BSA-AuNCs/IL) is used as a suitable nanocomposite platform for immobilization of anti-VEGF165 aptamer on a glassy carbon electrode. In "signal on" mechanism, the charge transfer resistance (Rct) of the probe can be linearly proportional with increasing concentration of VEGF165 in the range of 2.5–250 pM with a limit of detection of 0.48 pM.

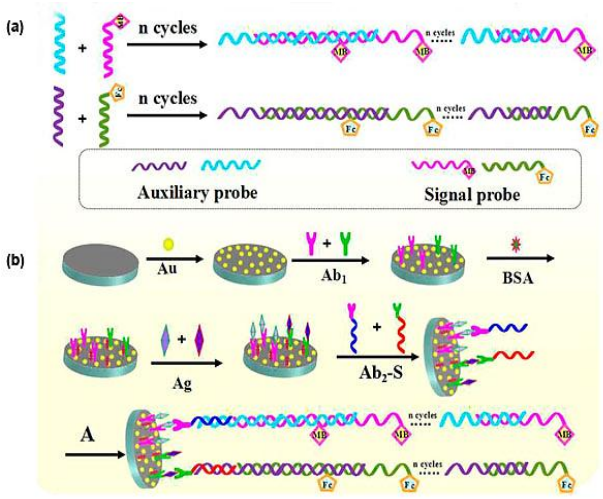

Fig. 5(a) Formation of the DNA concatemer; (b) Schematic representation of the fabrication procedure of the immunosensor (reprinted from **Ref. 78** with the permission of American Chemical Society).

As a matter of fact, AuNPs functionalized electrodes have been widely used for the development of electrochemical biosensors. So, there are many more reports on the AuNPs-based assay of tumor markers. For instance, by employing DPV to record the sensing signals, Li *et al.* **[78]** have reported an electrochemical immunoassay protocol for simultaneous determination of multiple tumor biomarkers. In this work, primary antibodies (Ab1) are immobilized on the surface of AuNP modified glassy carbon electrode (GCE) and secondary antibodies (Ab2) conjugated with primer are employed to hybridize with auxiliary probe and signal probe labeled with signal molecules **(Fig. 5)**. The reason for depositing AuNPs is that AuNPs possess good conductivity and high surface areas which can improve the conductivity of the electrode interface and load large amount of primary antibody. Meanwhile, DNA hybridization chain reaction (HCR) is employed to further improve the performance of this sensor. In the presence of target biomarkers, the sandwich immuocomplex is formed between the Ab1 and Ab2 bioconjugates carrying numerous signal molecules, and the electrochemical signal is greatly increased. Similarly, Wang *et al.* **[79]** have proposed a sensitive electrochemical biosensor for the assay of cytokeratins antigen 21-1, a kind of biomarker of lung cancer, with polyhydroquinone-graphene composite (rGO & PHQ) as a new redox species. In the meantime, electrodeposited AuNPs are used to further increase the specific surface

area as well as to improve the capability of electron transfer. The proposed immunosensor can display a good stability and selectivity, and the detection limit can be as low as 2.3 pg mL^{-1}.

By taking AuNPs functionalized electrode as a signal enhancement strategy, our group has also developed some electrochemical biosensors to assay tumor marker protein **[80]**. For instance, we once employed cluster of differentiation 147 (CD147) as the model protein for one study. CD 147 is also known as extracellular matrix metalloproteinase inducer (EMMPRIN), which plays an essential role in tumor progression and metastasis. In this work, a "sandwich'' structure can be formed on the electrode surface only when the CD147/EMMPRIN protein exists, yielding detectable electrochemical signals from the oxidation of 3, 3', 5, 5'-tetramethylbenzidine (TMB) catalyzed by horseradish peroxidase (HRP).

AuNPs labels

AuNPs labels are ideal in biotechnological systems due to their inherent advantages, such as ease of preparation, good biocompatibility, and so on. Up to now, AuNPs have been successfully used to label different biological receptors, such as enzymes, DNA **[81, 82]**, antigen–antibody and other biomolecules **[83, 84]** for preparing many useful immunoassays. For instance, by using polyamidoamine dendrimer-encapsulated AuNPs (AuNPs–PAMAM) loaded with enzyme linked aptamer as a new signal amplification strategy, Kavosi *et al.* **[85]** have presented an ultrasensitive electrochemical immunosensor for PSA detection in prostate cancer cells. The detection limit of as low as 5pg/ml can be obtained using EIS as measuring technique. Our group has developed a sensitive and easily operated electrochemical method for protein assay using AuNPs to amplify readout signal **[86]**. Cysteine-rich intestinal protein 1 (CRIP1) is employed as the model protein for the study, which has been identified as a biomarker for staging of breast cancer. The CRIP1-binding cyclic peptides are self-assembled onto the electrode surface. In the presence of CRIP1, a fraction of the peptides become protein-bound. Then, in turn, the protein-bound electrode is treated with zinc chloride and AT DNA modified AuNPs. The conformational change of CRIP1 for AT DNA binding will be triggered by Zinc ion. Consequently, some of the AT DNA molecules modified onto the surface of AuNPs are bound with the captured CRIP1, while most of the strands can serve to generate signal by recruiting methylene blue (MB), so vast signal amplification can be achieved **(Fig. 6)**. Furthermore, by taking advantage of Zr^{4+} mediated signal amplification using AuNP/DNA/MB nanocomposites, we once reported a novel method to assay the level of phosphorylated cAMP-response element binding protein (CREB) **[87]**. In addition, our group has proposed a novel method to detect human glypican-3 (GPC3) by using a peptide-based nano-label. GPC3 is a valuable biomarker for hepatocellular carcinoma **[88]**. A streptavidin-specific peptide is immobilized on a streptavidin-coated AuNPs to form a nano-label. In the meantime, the nano-label is used in combination with a capture probe.

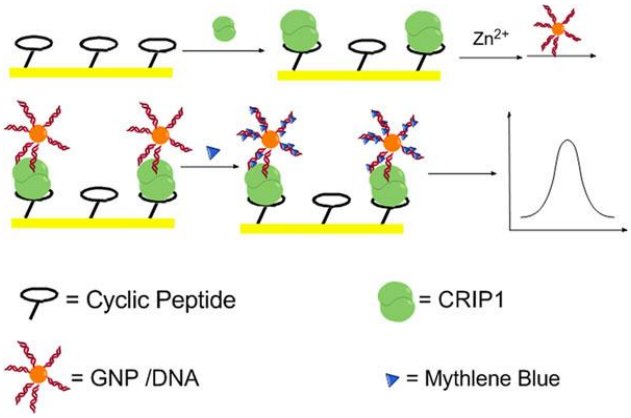

Fig. 6. Principle of the CRIP1 Assay (reprinted from **Ref. 86** with the permission of American Chemical Society).

This nano-label can efficiently amplify signal readout, owing to the large number of ferrocenyl-peptides immobilized on the electrode surface.

Recent researches have shown that some enzyme labels can be used to induce the deposition of noble-metal nanoparticles via the enzymatically catalytic reaction **[89]**. Additionally, AuNPs can be conveniently measured by electrochemical stripping analysis at relatively positive potential range **[90]**. Based on the combination of the electrochemical stripping analysis of nanoparticles and enzymatically catalytic deposition, Cheng *et al.* **[91]** have prepared an electrochemical immunosensor based on glucose oxidase (GOD) catalyzed AuNPs deposition for the quantitative measurement of CEA. The GOD-induced deposition of AuNPs by the nanoprobe can greatly amplify the signal response and lead to the high sensitivity of this sensor.

Conclusion and future perspectives

In summary, AuNPs have received more and more attention and they have been considerately used for the development of colorimetric and electrochemical biosensors so as to assay tumor maker proteins due to their superior properties, such as large surface-to-volume ratio, rapid and simple synthesis, strong adsorption ability, excellent optical and electronic characteristics. The efficient roles of AuNPs have greatly aided in enhancing the performance of the AuNPs-based biosensors for the detection of tumor maker proteins. Therefore, the well-constructed biosensors may exhibit good stability, high sensitivity and satisfactory selectivity, which can even be employed for the analysis from clinical samples. In the meantime, more and more AuNPs-based biosensors will be fabricated and reported in the future owing to their unique physical and chemical properties of the nanomaterial. Moreover, more researches will be conducted and more developments will be achieved by coupling AuNPs with some molecular biological techniques, such as RCA, hybridization chain reaction,

target-induced repeated primer extension and loop-mediated amplification, etc. Certainly, greater efforts are still required to be made to demonstrate the applicability of the reported sensors for clinical diagnosis and evaluation of therapeutic effect. Hopefully, AuNPs-based biosensors may play an important role for the early detection of tumor marker proteins, which will greatly facilitate the effective treatment of cancer.

Acknowledgements

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