Chapter 6
Nucleic Acid Amplification Strategies in Surface Plasmon Resonance Technologies

Xueming Li

Abstract Surface plasmon resonance (SPR) is a real-time and label-free technology for molecular interactions, chemical detection, and immunoassays. In this chapter, the application of nucleic acid amplification strategies, such as PCR, HCR, RCA, and SDA, in SPR technologies was summarized, providing an insight into the nucleic acid amplification strategies in SPR technologies.

6.1 Introduction

SPR-based biosensors are very powerful tools for real-time and label-free study of interactions between various biological and chemical analytes and have been widely applied in therapeutics, pharmacy, food safety, environmental monitoring, and homeland security (Wijaya et al. 2011; Homola 2008). The surface plasmons were first observed by R. W. Wood in 1902, when anomalous narrow dark bands were discovered in the diffraction spectrum of a metal grating illuminated with polychromatic light (Wood 1902). This anomaly was completely explained in 1968, when Otto (Otto 1968) and the same year Kretschmann (Kretschmann and Raether 1968), reported the optical excitation of surface plasmons by attenuated total reflection. In 1983, Liedberg et al. demonstrated the application of SPR-based sensors in gas detection and biosensing (Fig. 6.1) (Liedberg et al. 1983). Since then, the enormous potential of SPR sensor technology for detecting chemical and biological substances has received increasing attention from the scientific community. Today, SPR-based biosensors are used more and more not only in gas sensing, but in many other important applications, such as food safety, biology, and medical diagnostics. And numerous research papers have been published about the exploitation of SPR biosensors.

Biosensor is an analytical device, which composed of biological elements such as tissues, microorganisms, organelles, cell receptors, enzymes, antibodies, and physicochemical sensors. A physicochemical change, which can be detected by the trans-

X. Li (✉)
Shandong Provincial Key Laboratory of Detection Technology for Tumour Markers, College of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, People's Republic of China
e-mail: lixueming1988@163.com

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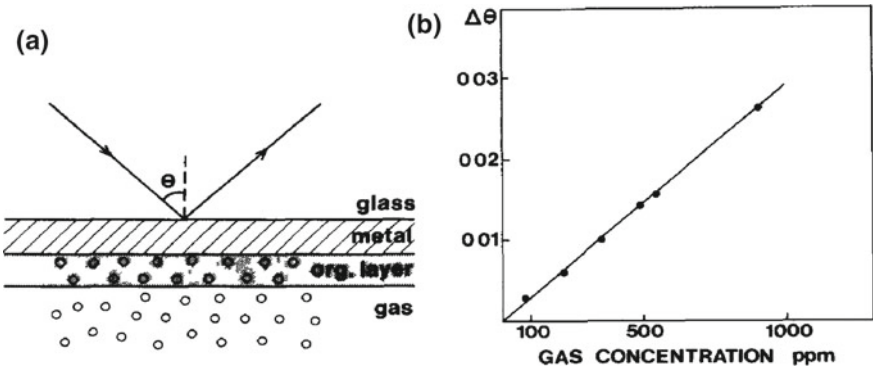

Fig. 6.1 **a** Schematic view of the layer system used in the gas sensing application. **b** Measured response to halothane gas exposure in the ppm range. Reprinted from Liedberg et al. (1983) Copyright (1983), with permission from Elsevier

ducer, is produced by the biological element when it specifically interacts with the target analyte. The transducer transforms the physicochemical change to an analog electronic signal which is relevant to the amount or concentration of the specific analyte (Tudos and Schasfoort 2008; Saha et al. 2012). The biosensor based on SPR technology is a type of optical refractometer, by which the change of the refractive index of the medium resulting from the binding and dissociation of the analyte molecules on the SPR sensor surface can be measured (Zeng et al. 2014).

6.2 Type of Surface Plasmon resonance

Surface plasmons (SPs) are coherent oscillations of free electrons at the boundaries between metal and dielectric which are often categorized into two classes: propagating surface plasmons (PSPs) and localized surface plasmons (LSPs). PSPs can be excited on the metallic films which have several approaches as the Kretschman and Otto prism coupler, optical waveguides coupler, diffraction gratings, and optical fiber coupler, whereas LSPs can be excited on metallic nanoparticles, which both can induce a strong enhancement of electromagnetic filed in the near-field region (resonance amplification), leading to an extensive application in surface enhanced raman scattering (SERS), fluorescence enhancement, refractive index (RI) measurement, biomolecular interaction detection, and so on (Chen and Ming 2012).

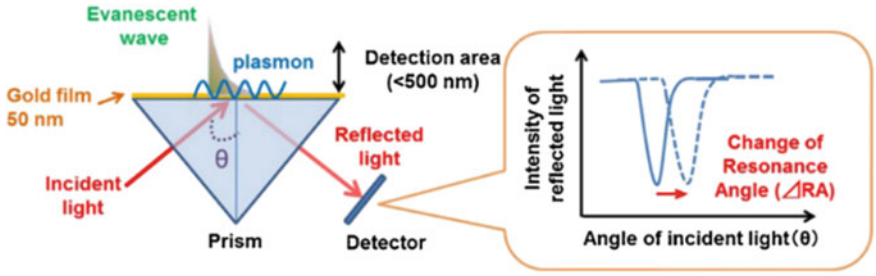

Fig. 6.2 Schematic description of conventional SPR sensor (Yanase et al. 2014)

6.2.1 Conventional SPR

In a conventional prism based SPR, a flat gold film is illuminated at a steep angle in total internal reflection mode, whereas the gold films can initiate SPR directly via polarized light. As shown in Fig. 6.2, the incident light is introduced to and reflected by the gold film through a prism. The intensity of the reflected light will change with the angle of the incident light and monitored by the detector. At a certain angle of the incident light, the intensity of the reflected light has a maximum loss, at which the light will excite surface plasmon resonance. The angle is called resonance angle or SPR angle. The adsorption of the accumulated mass, such as proteins or nucleic acids, will result in the changing of the refractive index of the media near the metal surface which will lead to the shift of the SPR angle (Yanase et al. 2014). The methodology based on SPR technology requires no labels for detection preventing the disruptive chemistry modification for labeling (Linman and Cheng 2009). For a more comprehensive description of SPR theory, there are a number of reviews available (Hinman et al. 2018; Linman et al. 2010; Singh 2016).

6.2.2 SPR Imaging

Generally, the standard SPR biosensor instrument has 1–4 flow cells or channels on a single sensor chip which limits its application in high-throughput screening and multiplex analysis. To overcome this limitation, an excellent alternative method is the combination of SPR biosensor with arrays. SPR imaging (SPRi), a modified version of SPR design, has been developed, which can measure hundreds or thousands of samples simultaneously. An array is prepared on the SPR sensor chip, and the whole of the chip can be visualized via a video CCD camera through a label-free approach (Scarano et al. 2010). Interacting with the specific analyte, each active site (spot) of the array on the chip provides SPR signal information simultaneously, where the data can be collected in a fixed angle/angle scanning/wavelength scanning format. Detailed information of the biomolecular interactions in the array, such as the affinity

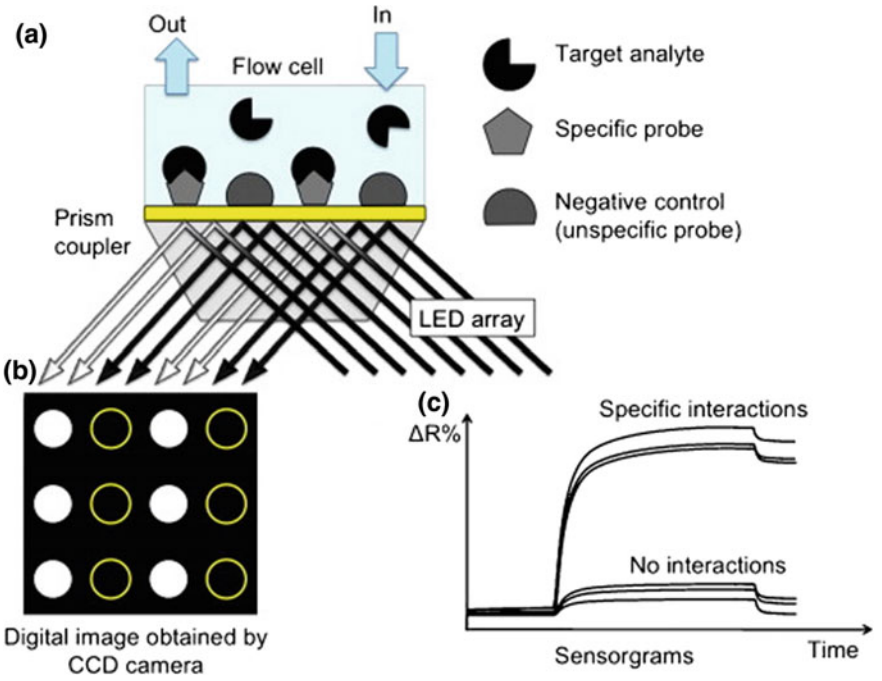

Fig. 6.3 General principle of SPRi. Reprinted from Scarano et al. (2010) Copyright (2010), with permission from Elsevier

and kinetic data, can be provided simultaneously. SPRi is faster and consumes lesser reagent than conventional techniques, providing a robust and sensitive manner for high-throughput screening of biomolecular interactions (Nguyen et al. 2015; Scarano et al. 2010) (Fig. 6.3).

6.2.3 Localized Surface Plasmon Resonance (LSPR)

Localized surface plasmon resonance (LSPR) is another optical phenomenon type of surface plasmon resonance. Without the metal film and the prism, the LSPR is generated by light near the conductive nanoparticles (NPs) or nanowires, the size of the which are smaller than the wavelength of the incident light. The interaction between the incident light and the surface electrons of the metal nanostructure produces the resonance of the localized plasmon. During the interaction, a portion of the incident light was absorbed and another portion was scattered in different directions. When the LSPR excited, the intensity of the absorption and scattering is dramatically enhanced and correlated with the composition, size, and shape of the

NPs as well as on the refractive index of the surrounding dielectric medium (Willets and Van Duyne 2007). Typically, the materials used for plasmonic applications are noble metals, such as gold or silver, which exhibit LSPR during the visible range of the spectrum. Among them, gold is preferred for biological applications due to its inert nature and biocompatibility, and thiol–gold association for immobilization of biomolecules (Mayer and Hafner 2011).

6.3 Nucleic Acid Amplification Strategies in SPR Technologies

Generally, the low signal intensity of the SPR assays severely impedes their widespread applications in disease diagnostics, especially in the high-sensitive and high-selective detection of the trace amount of targets in complex biological samples. To enhance the signal intensity and obtain efficient SPR-based assays, the development of amplification strategies is a straightforward and efficient way. Most of the present strategies are based on the nanomaterials, such as Au, Ag, SiO_2, magnetic or carbon-based nanoparticle, nanorod, nanoflower, or nanosheet. There are a number of publications about the nanomaterial-enhanced SPR sensors (Lou et al. 2017). Among these nanomaterials, Au NPs are one of the mostly and extensively used for the enhancement of SPR signals through the electronic coupling interaction between the localized surface plasmon of the Au NPs and the surface plasmon wave associated with the SPR gold film (Springer et al. 2014). For example, the amplification strategies for SPR assays using secondary antibodies labeled with Au NPs (Huang et al. 2005) aggregation of a network of Au NPs, Au NPs–polymer growth, and DNA-functionalized Au NPs amplification all have demonstrated the enhancement of the SPR signal.

Nevertheless, the signal enhancement can be further improved by combining the novel and powerful nucleic acid amplification strategies, such as PCR, HCR, RCA, and SDA amplification (Zhao et al. 2008; Bi et al. 2017; Zhou et al. 2018). There have been some reviews summarized the nanomaterial-based amplification strategies for SPR assays (Zhang et al. 2017; Fong and Yung 2013; Šípová and Homola 2013). However, a systematical and comprehensive review of the nucleic acid amplification strategies for SPR assays is needed. In this chapter, the application of nucleic acid amplification strategies in SPR technologies was summarized, providing an insight into the nucleic acid amplification strategies in surface plasmon resonance technologies.

6.3.1 Rolling Circle Amplification (RCA)

RCA, with unique characteristics, such as mild reaction conditions, ease of operation, high efficiency, excellent sensitivity, and specificity, has been attracting much attention as an advanced molecular amplification technique. In RCA reaction, the primer can be amplified isothermally by a circular template in the presence of certain DNA polymerases, producing a long single-stranded DNA. The thousands of tandem repeats of the RCA product, as the detection sites, can hybridize with a great deal of complementary DNA labeled with signal molecules to enhance the signals. The RCA strategies have been widely applied to the detection of various target molecules by coupling with electrochemistry, chemiluminescence, fluorescence, surface-enhanced Raman spectroscopy, colorimetry, and SPR (Ali et al. 2014).

Recently, Shusheng Zhang et al. combined with RCA and bio-bar-coded Au NP enhancement for ultrasensitive detection of the human thrombin using a SPR aptasensor. The sensor platform exhibited a broad dynamic range, excellent selectivity, and ultrahigh sensitivity. The assay platform is represented in Fig. 6.4, where the sensor surface was initially modified with the hairpin aptamer probe and mercaptohexanol (MCH) through an Au–S affinity binding. A hairpin structure was used to partially cage the primary sequence of the thrombin aptamer to reduce the nonspecific binding. When the thrombin was introduced to the sensor surface, the interaction of the thrombin and the aptamer opened the hairpin structure and a stable thrombin–aptamer complex was formed. The aptamer–primer complex, in which the secondary aptamer for thrombin was linked with a RCA primer, was extended through the RCA reaction producing a linear sequence with thousands of tandem repeats. Then the RCA products were introduced onto the SPR sensor surface and bound to the immobilized thrombin through the interaction of the secondary aptamer and the thrombin, in a classic sandwich assay format. Finally, a large amount of bio-bar-coded Au NPs were introduced onto sensor surface and assembled on the linear RCA products for the enhancement of the SPR signals by the increase of the surface mass and the refractive index of clustered Au NP conjugates. The detection limit was as low as 0.78 aM, which was almost 9 orders of magnitude lower compared with the direct SPR detection. Furthermore, the SPR sensor surface could be regenerated by 1 M HCl for the reusability of the sensor chip (He et al. 2014a).

6.3.2 Polymerase Chain Reaction (PCR)

PCR amplification is useful technology in molecular biology to amplify DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. This strategy is very useful for the detection of diseases caused by genetic factors. Generally, the detection of the PCR product is carried out using electrophoresis, in which a carcinogenic chemical such as an ethidium bromide is used as the developing agent (Fakruddin et al. 2013). Although this method is simple

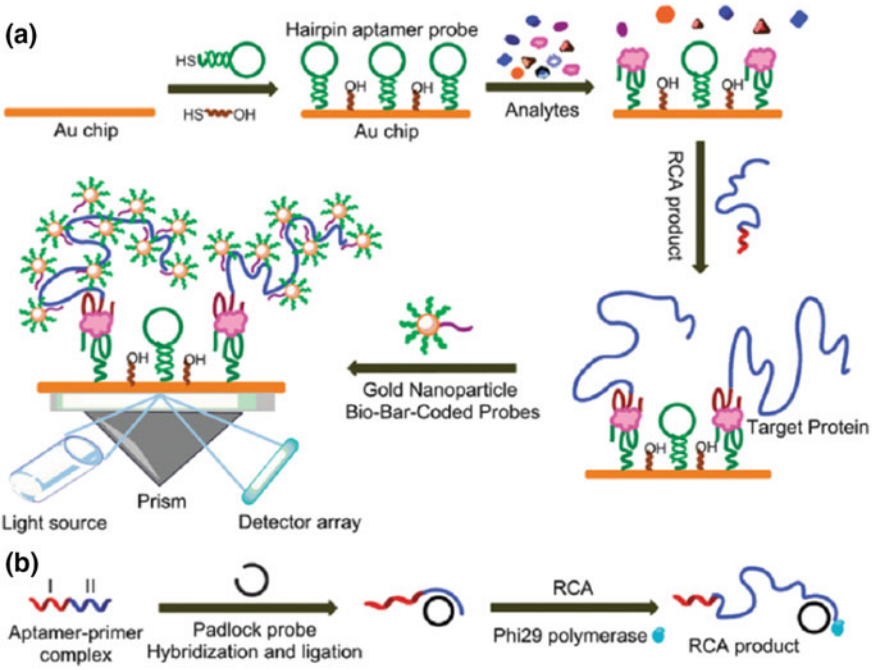

Fig. 6.4 Illustration of the SPR assay for protein detection using signal amplification by aptamer-based RCA and bio-bar-coded aunp enhancement. Reproduced from He et al. (2014a) with permission from The Royal Society of Chemistry

and effective to detect the PCR products, it cannot determine the sequence of the product DNA is the same as expected. Be aimed at hereat, Isao Karube demonstrated an asymmetric PCR strategy for DNA detection using SPR sensor. The target DNA sequence was amplified through asymmetric PCR, producing the PCR product with a single-stranded probe binding site, located in the 3′-terminus. A flow injection SPR sensor was used for the detection of the PCR product, by which the formation of intra- and intermolecular complexes was avoided (Kai et al. 1999). Although PCR can provide high sensitivity of detection, the highly precise temperature cycling, complex sample preparation, and strict laboratory conditions impede its widespread use for various analysis.

6.3.3 Hybridization Chain Reaction (HCR)

HCR is another type of nucleic acid amplification reaction triggered by a target DNA fragment, in which two stable species of DNA hairpins, as the monomers, self-assemble into complex structures through a cascade of hybridization events.

The HCR product, which is a type of nicked double helices analogous to alternating copolymers, can be functionalized act as an amplifying transducer for biosensing applications. Without the participation of enzymes, this strategy avoids the limitation of the thermostability of enzymes and the specific recognition sites for nicking endonucleases (Bi et al. 2017).

Normally, the detection of low molecular weight chemical and biological analytes under extremely dilute conditions is one of the main challenges for all electrical, mechanical, and optical sensors. SPR sensors have the unique ability for real-time detection of the molecular binding events. However, the sensitivities of SPR sensors are insufficient to detect trace amounts of small molecular weight molecules due to the extremely low signal intensity of small molecules. Therefore, signal amplification strategies are necessary for more sensitive detection of them. Adenosine triphosphate (ATP, 507 Da) plays fundamental roles in the regulation and integration of cellular processes and has also been used as substrate for cell viability and cell injury. Xuemei Li et al. developed a SPR detection system coupled with HCR for amplified detection of DNA and ATP with high sensitivity. Figure 6.5 represents the HCR-based SPR assay for ATP detections. The magnetic beads (MB) were modified with the ATP aptamer S_1, which partly hybridized with the trigger DNA S_2. In the presence of ATP, the trigger DNA was released from the MB due to the formation of the complex structure between ATP and the aptamer. After magnetic separation, the released trigger DNA S_2 was introduced onto the SPR sensor chip surface and hybridized with the capture DNA. The unpaired fragment of the trigger DNA worked as a trigger to initiate the linear assembly of Fc-modified hairpin H_1 and H_2 through HCR onto the SPR sensor surface. The assemblies of the large amount of Fc-modified H_1 and H_2 significantly enhance the SPR signals by increasing the refractive index of the surface. For DNA detection, the trigger DNA S_2 would work as the analyte, capture by the capture DNA S_3, and trigger the HCR event. The detection limit was 0.3 fM for DNA detection, which is 6 orders lower than a LSPR amplified with gold nanoparticles (Spadavecchia et al. 2013) and 0.48 nM for ATP detection. The authors also applied the HCR-based SPR system, with high sensitivity and selectivity, to ATP analysis in complicated biological samples, including human serum and lysates of HeLa cells and K562 leukemia cells. In addition, the biosensor surface can be reused through the regeneration step by the injection of 1 M HCl to remove the hybridized double-stranded DNA, which would make the assay more cost-effective and efficient (Li et al. 2014).

Different from the traditional HCR, in which the hairpin DNA is sequentially opened and assembled into a linear structure, nonlinear HCR is a hairpin-free and dendritically assembled system. In the nonlinear HCR amplification system, double-stranded DNA monomers are activated by a trigger sequence and dendritically assembled into highly branched nanostructure. Since the hairpin-free strands have few secondary structures, the reaction could be completed faster, and dendritic DNA nanostructures with high molecular weights can be formed (Xuan and Hsing 2014). Therefore, the nonlinear HCR could dramatically improve the sensitivity of SPR assays than traditional HCR. By employing the nonlinear HCR amplification, Shijia Ding et al. developed a label-free SPR biosensor for DNA and ATP detection. As

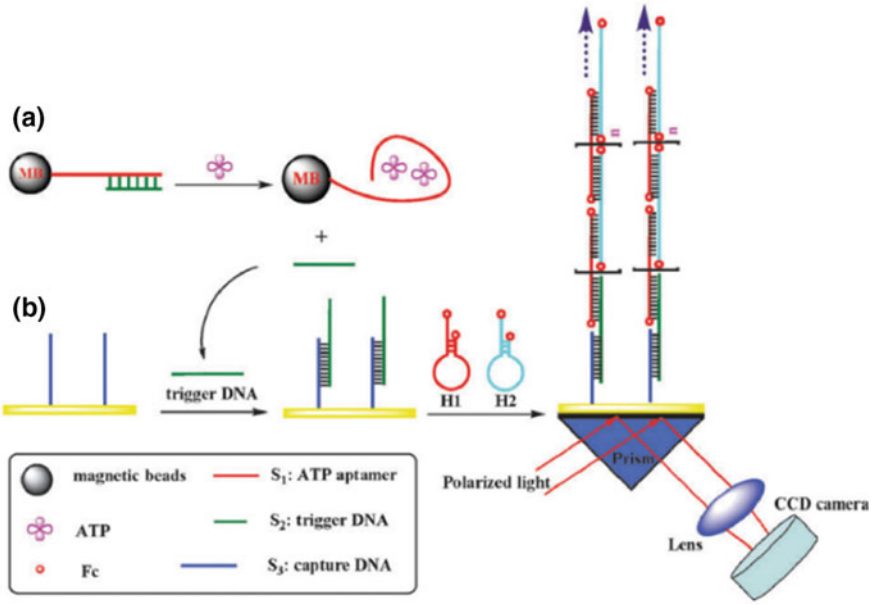

Fig. 6.5 Illustration of the SPR assay for ATP detection with the DNA-based HCR. Reproduced from Li et al. (2014) with permission from The Royal Society of Chemistry

shown in Fig. 6.6, the capture probe is immobilized on the SPR sensor surface for capturing the target DNA. The target DNA had two sections: One terminal of the target DNA hybridizes with the capture probe and the other terminal works as the trigger of the nonlinear HCR. When the double-stranded DNA monomers are introduced onto the sensor surface, the nonlinear HCR is initiated and a dendritic growth of DNA dendrimer is self-assembled on the sensor surface causing the greatly amplified SPR signal. This biosensing strategy, with a detection limit down to 0.85 pM, showed good reproducibility and precision and had been successfully applied for detection of target DNA in complex sample matrices (Ding et al. 2017).

6.3.4 Strand Displacement Amplification (SDA)

Strand displacement amplification (SDA), which can provide exponential amplification of a trace of DNA or RNA, has attracted more and more attention due to its fast, efficient, and no special equipment requirement (Walker et al. 1992). Therefore, the SDA technology is believed to be a suitable strategy to implement simply determination of miRNA combined with SPR technology to produce high sensitivity. Combining with a SDA strategy and Au NP enhancement for improving the sensitivity of the SPR sensor, Yinyin Peng et al. developed a simple and sensitive

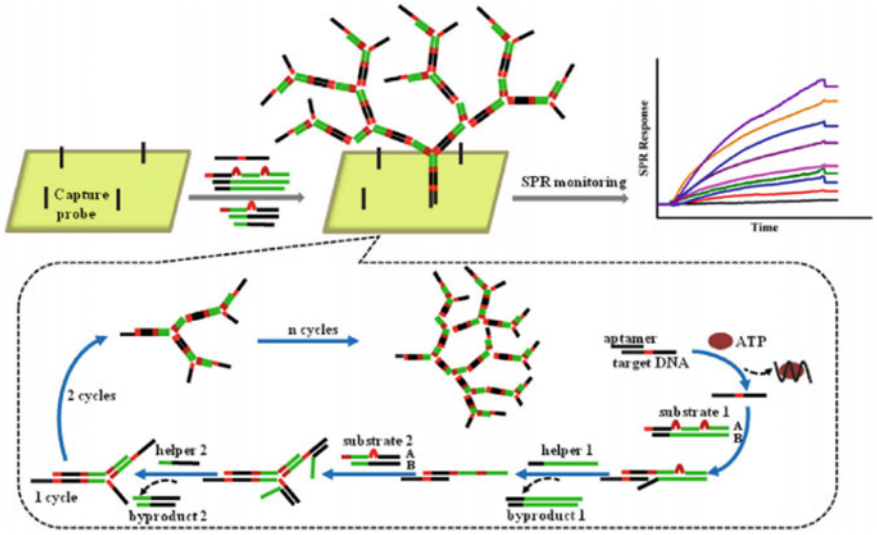

Fig. 6.6 Schematic representation of SPR biosensing srategy for DNA and ATP detection. Reprinted from Ding et al. (2017). Copyright (2016), with permission from Elsevier

SPRi biosensor for the detection of miRNA-155. As shown in Fig. 6.7, initiated by the miRNA, a large number of the DNA triggers are produced for further analysis through a SDA and enzymatic amplification cycle. The DNA triggers are captured onto the gold sensor chip surface by the capture probe. Then the DNA–Au NP is introduced onto the sensor surface through the hybridization with the unpaired fragment of the DNA trigger. The sandwich assembly of the DNA–Au NP results in a large increase in the SPR signal. The detection limit of this method for miRNA-155 detection is down to 45 pM (Zeng et al. 2017).

6.3.5 DNAzyme Signal Amplification

Recently, catalytic nucleic acids (DNAzymes) have been the focus of growing interest as amplifying units for the detection of DNA or aptamer–substrate complexes (Pelossof et al. 2011). Due to the good stability, low costs, and easy preparation, DNAzymes have been widely employed in various biosensor based assays. Among them, the horseradish peroxidase- (HRP-) mimicking DNAzyme, consisting of G-quadruplex and hemin, is one of the most frequently applied DNAzymes. The HRP-mimicking DNAzyme has been widely applied in electrochemical, chemiluminescent, and colorimetric assays due to its excellent properties. Utilizing the efficient catalytic activity of HRP-mimicking DNAzyme on the oxidative polymerization of aniline to polyaniline, Li et al. reported a ultrasensitive SPR biosensor for detection

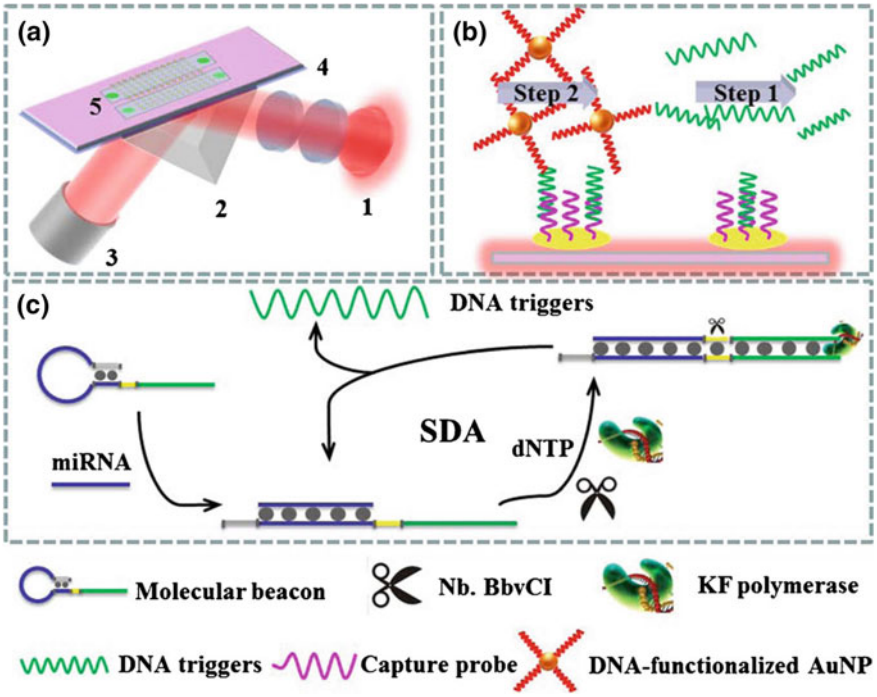

Fig. 6.7 Schematic of SPRi system combined with a SDA strategy and AuNP enhancement for improving the sensitivity of the SPR sensor for miRNA-155 detection. Reprinted from Zeng et al. (2017) with kind permission from Springer Science + Business Media

of bleomycin (BLM). As shown in Fig. 6.8, in the present of BLM, the DNA probe P1 on the sensor surface is cleaved and switches off the hybridization of the G-rich DNA probe P2 onto the sensor surface. As a result, less HRP-mimicking DNAzymes are formed, and less amount of polyaniline is deposited on the sensor surface (Li et al. 2017). Because of the high refractive index of the polyaniline, the SPR response is significantly enhanced (Gong et al. 2015) and the detection limit of BLM was down to 0.35 pM.

In addition, the hemin, with high extinction coefficient, can form the hemin/G-quadruplex on the gold surface of SPR sensor, would dramatically alter the dielectric properties of the surface. Thus, the hemin/G-quadruplex can be used as an amplifying label for SPR detection of different sensing events. Itamar Willner et al. developed a sensitive SPR sensor for Pb^{2+} detection employing the hemin/G-quadruplex nanostructure and the Pb^{2+}-dependent DNAzyme. As shown in Fig. 6.9, the sensor surface was coated with AuNPs to enhance the sensitivity of the SPR sensor. A G-rich DNA, containing the substrate of the Pb^{2+}-dependent DNAzyme, was assembled onto the Au NPs in which the G-rich domain was caged by the Pb^{2+}-dependent DNAzyme. In the presence of Pb^{2+}, the substrate was cleaved, leading to the release of the complex. The uncaged G-rich DNA self-assembled into the hemin/G-quadruplex

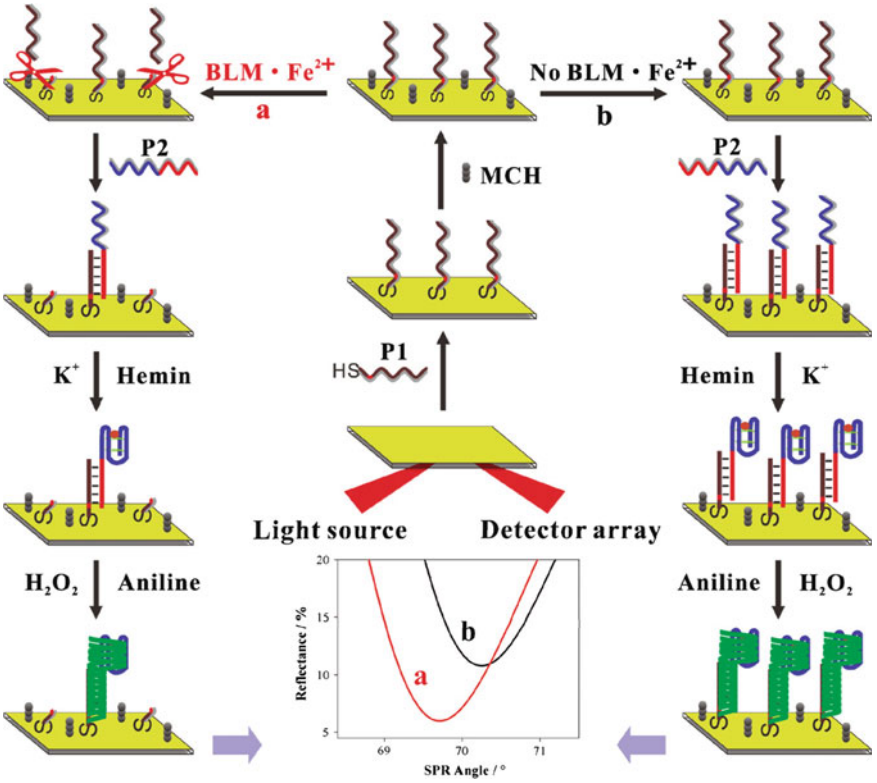

Fig. 6.8 Schematic of the SPR biosensor based on the HRP-mimicking DNAzyme catalyzed deposition of polyaniline. Reprinted with the permission from Li et al. (2017). Copyright 2016 American Chemical Society

structure resulting in the dielectric changes at the sensor surface detected by the SPR sensor. This sensing platform enabled the detection of Pb^{2+} down to 5 fM and revealed an impressive sensitivity and selectivity (Pelossof et al. 2012).

6.3.6 Multiple Signal Amplification Strategy

Although various amplification strategies have been devoted to SPR technology for sensitively detecting target analytes to meet the requirements of clinical diagnosis and medical treatment of diseases, the signal enhancement efficiency of single amplification strategy is insufficient. The signal enhancement can be further improved by combining multiple signal amplification strategies. For example, by combining the target-triggered isothermal exponential amplification with the magnetic nanoparticle-

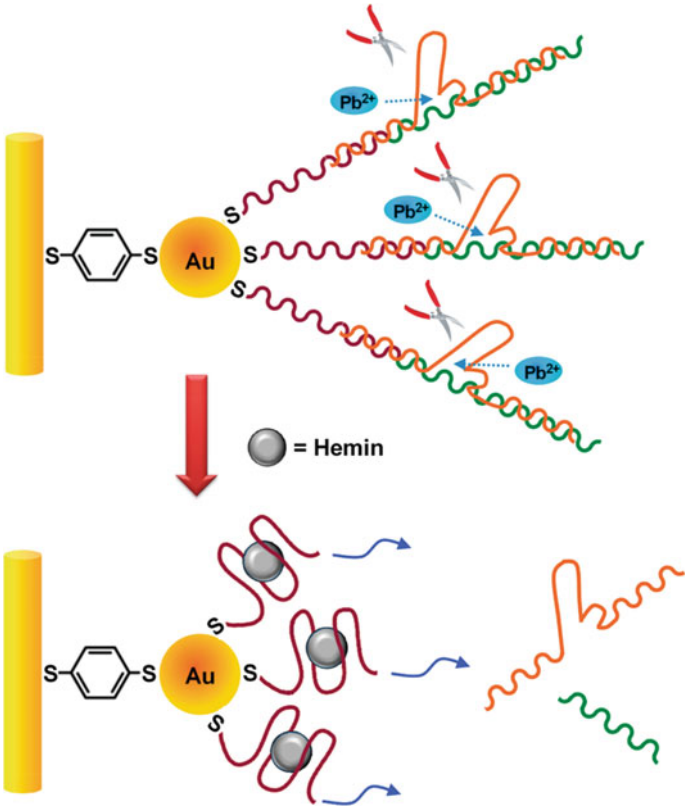

Fig. 6.9 Schematic presentation for the nucleic acid 1/2-functionalized Au NP monolayer-modified surface for the SPR detection of Pb^{2+} ions by the Pb^{2+}-dependent DNAzyme and the Hemin/G-quadruplex label. Reprinted with permission from Pelossof et al. (2012). Copyright 2012 American Chemical Society

based RCA, Shusheng Zhang et al. proposed a sensitive and versatile SPR sensor for the detection of DNA and Ramos cells. Although this methodology is relatively complex, the integration of multiple signal amplification strategy exactly produces a remarkable amplification efficiency. As shown in Fig. 6.10, the strategy includes the following three steps: (1) target-triggered isothermal exponential amplification reaction (T-EXPAR), (2) magnetic nanoparticle-based RCA reaction, and (3) AuNPs-enhanced SPR assay. The LOD for the multiple amplification strategy is 9.3 aM for DNA detection and 10 cells for Ramos cells (He et al. 2014b).

Jian-Ding Qiu et al. reported an ultrasensitive strategy for SPR detection of adenosine, in which an aptamer-based target-triggering cascade multiple cycle amplification strategy combined with Au NPs enhancement was designed to enhance the SPR signals. The amplification strategy was composed of the aptamer-based target-triggering nicking enzyme signaling amplification (T-NESA), the nicking enzyme

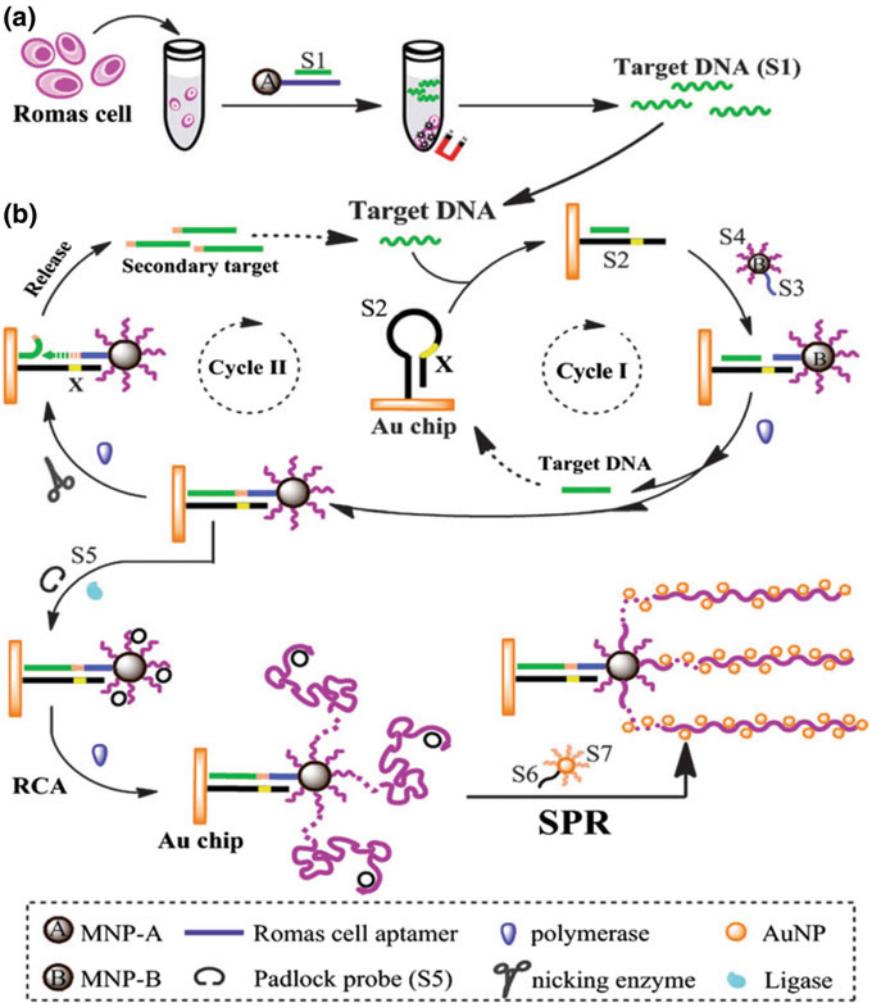

Fig. 6.10 Schematic representation of the multiple signal amplification SPR assay for DNA and cancer cells. Reproduced from He et al. (2014b) with permission from The Royal Society of Chemistry

signaling amplification (NESA) and HCR, which was triggered by the adenosine. The multiple cycle amplification strategy significantly enhanced the SPR signals and exhibited a detection limit of 4 fM for adenosine (Yao et al. 2015) (Fig. 6.11).

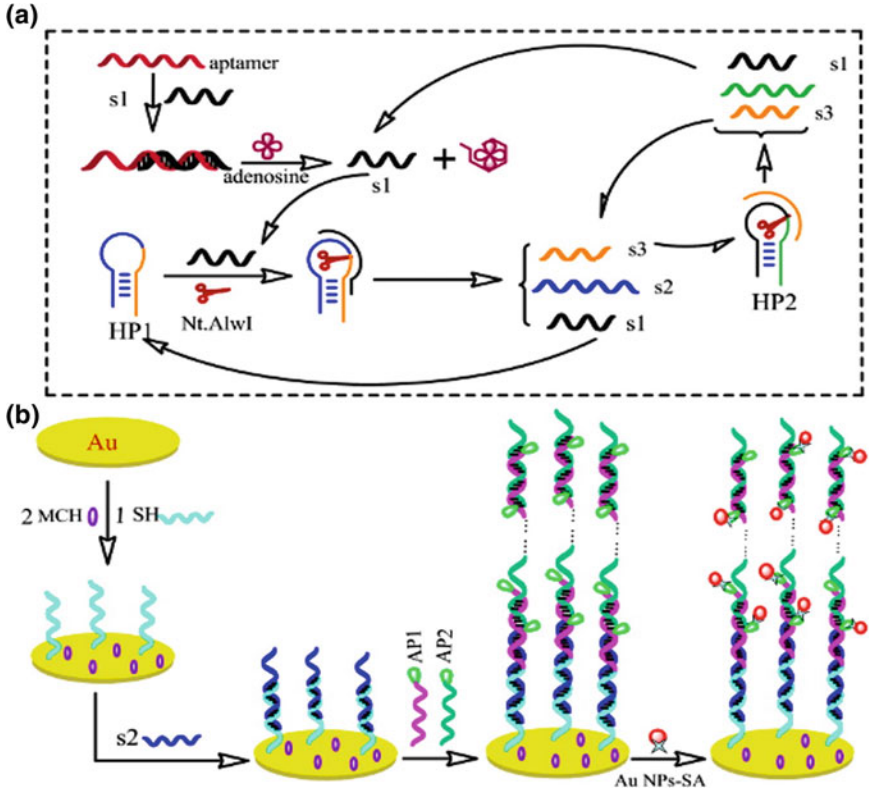

Fig. 6.11 Schematic illustration of (A) dual NESA and (B) HCR and Au NPs signal amplification-based SPR assay for the detection of adenosine. Reprinted with permission from Yao et al. (2015). Copyright 2015 American Chemical Society

6.4 Conclusions and Perspectives

In this chapter, we have summarized the remarkable advances in the development of nucleic acid amplification strategies for novel ultrasensitive SPR biosensors. Further construction of nucleic acid amplification reactions on smartly designed modern SPR biosensor interfaces will be widely applied in the recent biosensing field due to their excellent designability and stability, and demonstrates great promise for clinical application. Since the limited signal enhancement efficiency of single amplification strategy, the development of multiple signal amplification strategies, in which the nucleic acid amplification strategies, nanomaterials, enzyme amplification, and so on is combined, would be a trend in the future. In addition, the combination of SPR sensors with other sensor techniques, such as electrochemical analysis techniques, fluorescence spectroscopy or SERS, to achieve more sensitive and specific detection would also be a trend of SPR techniques.

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