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Mini review

Progress in Hybridization Chain Reaction-Based Photoelectrochemical Biosensors

Yunxiao Feng[1], *Hao Xie*[2] *and Binbin Zhou*[2,*]

[1] College of Chemistry and Chemical Engineering, Pingdingshan University, Pingdingshan 467000, Henan, China
[2] College of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang, Hunan 414006, China
[*]E-mail: bbzhou1985@163.com

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Hybridization chain reaction (HCR), a one-dimensional DNA self-assembly process, is considered to be an enzyme-free isothermal amplification method. It shows great prospects and provides a wide range of applications for the construction of biosensors. In recent years, photoelectrochemistry (PEC) biosensor has attracted extensive attention as a new bioanalysis technology. In this work, we addressed the progress in the development of PEC biosensors in combination with the HCR-based signal amplification.

Keywords: Hybridization chain reaction; photoelectrochemical biosensors; signal amplification; immunoassays

1. INTRODUCTION

Hybridization chain reaction (HCR), a one-dimensional DNA polymerization process, was found by Dirks and Pierce in 2004 [1]. It has been considered to be an important method for the development of DNA nanosystems [2]. HCR system consists of two metastable DNA hairpins (H1 and H2). The two DNA hairpins do not hybridize with each other when they coexist in aqueous solution. However, they could be assembled to form long double-stranded DNA (dsDNA) products when the initiators were introduced. Specifically, this HCR process occurs when the initiator opens and activates the first hairpin H1, which immediately hybridizes partly with the second hairpin H2 to form a dsDNA strand. The exposed ssDNA at the terminal of H2 will lead to the opening of another H1 and trigger the continuation of HCR process. Therefore, the long dsDNA polymer is assembled using H1 and H2 hairpins as the monomers. The initiators to promote the formation of dsDNA polymers include single-stranded DNA (ssDNA), metabolites, proteins, metal ions and others for a variety of applications [3-7]. From the

perspective of biosensor development, HCR is considered to be an enzyme-free, isothermal amplification process that can be triggered by highly specific biological/chemical molecules [8].

With the rapid development of nanotechnology and material chemistry, photoelectrochemistry (PEC) biosensor has attracted extensive attention as a new bioanalysis technology. PEC bioanalysis is an advanced integration of photoelectric chemistry and electrochemical technique. It inherits many advantages of electrochemical bioanalysis, such as low price, simple equipment and high sensitivity [9]. The good detection performance of PEC biosensors benefits from two key factors: photoelectric active material and signal amplification strategy [10-12]. This paper mainly summarizes the relevant works about the design of PEC biosensors based on the signal amplification of HCR.

2. HCR-BASED PEC BIOSENSORS

In order to meet the need of target quantification at ultra-low concentration, signal amplification is a valuable integrant of PEC sensing principle. Nowadays, many powerful signal amplification strategies and materials have been integrated with HCR protocol for the detection of low-abundance targets. The long dsDNA structure produced by HCR can load a variety of functional substance for signal amplification through different interactions. According to the type of substance used in the analysis, the HCR-based signal amplification strategies can be divided into natural enzymes, artificial enzymes, photoactive molecules and nanomaterials. In addition, the application of HCR-based methods in PEC immunoassays was also discussed.

2.1 Enzymatic amplification

Due to its high catalytic activity and substrate specificity, different natural enzymes have been introduced into the HCR-based PEC bioassays for signal amplification with improving sensitivity. After HCR reaction, a large number of streptavidin (SA)-conjugated enzymes can bind to biotin-labeled dsDNA polymers through the SA-biotin interactions. They can catalyze the conversion of substrates into hole/electron sacrificial reagents, preventing the recombination of electron-hole pairs and improving the performance of PEC biosensors [13-15]. For example, alkaline phosphatase (ALP) can catalyze the hydrolysis of ascorbic acid 2-phosphate (AAP) into ascorbic acid (AA) as the electron donor [16-18].The enzymatic reaction product (AA) can etch cobalt oxyhydroxide (CoOOH) into Co^{2+} ions via a redox reaction, leading to the exposed photoactive substrate g-C_3N_4/$CuInS_2$ nanohybrids and the increased photocurrent [19]. Recently, Zhao et al. integrated the redox cycling amplification strategy into ALP-based PEC assay, in which the oxidized product of AA was reduced by extra reducing agent to regenerate AA, thus providing more electrons after the injection of electrons into holes [20]. Moreover, glucose oxidase (GOx) captured by the dsDNA polymers can catalyze the oxidation of glucose, and the generated enzymatic product (H_2O_2) can act as the electron acceptor to enhance the photocurrent [21]. However, the inherent disadvantages of natural enzymes including sensitivity to environmental conditions and easy denaturation as well as inactivation dramatically hamper the further applications.

2.2 DNAzymes-based amplification

DNAzymes are a class of DNA-based complexes with enzyme-like catalytic capabilities [22]. As a typical representative of DNAzymes, hemin/G-quadruplex with peroxidase-like activity have been widely used in the design of biosensors [23, 24]. For example, Zhang et al. proposed a hemin/G-quadruplex-based PEC immunoassay of prostate specific antigen (PSA), in which hemin/G-quadruplex as the HCR product (DNA concatamer) catalyzed the insoluble/insulating precipitate on the photoelectrode materials and resulted in the reduced photocurrent [25]. Hemin/G-quadruplex can also catalyze the in-situ deposition of photoactive polyaniline on the backbone of DNA polymers to improve the capture of visible light and increase the photocurrent response [26]. Li et al. reported a cathode PEC paper device for miRNA-141 detection based on hemin/Pt nanoparticle-modified DNA concatamers [27]. As presented in Figure 1, the cascaded sensitization nanostructures were formed on the AuNPs-decorated tangled cellulose fiber networks and then successively assembled with pyramid-like Cu_2O and trepang-like $BiVO_4$–Bi_2S_3 heterostructures. After the duplex-specific nuclease (DSN)-aided target recycling reaction and multiple branched HCR (MHCR), DNA dendrimers were formed on the sensing interface. PtNPs were then assembled on the DNA backbone through the electrostatic interactions and the branched single-stranded (ssDNA) with the G-rich sequence transformed into DNAzymes in the presence of hemin. Finally, under the synergetic catalyzed effect, PtNPs and hemin/G-quadruplex in the DNA dendrimers catalyzed the in-situ generation of O_2 as an electron acceptor to amplify the photocurrent signal.

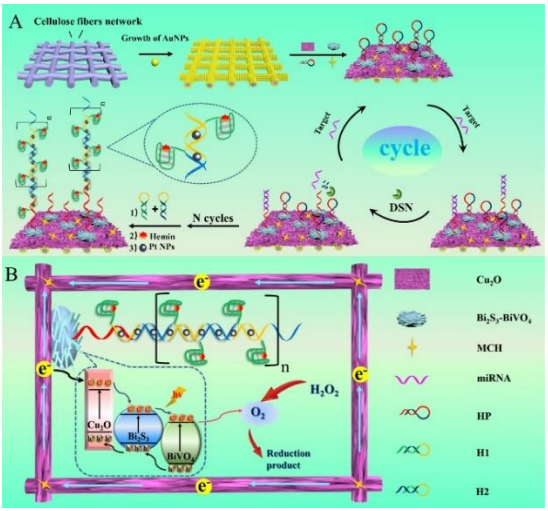

Figure 1. (**A**) Fabrication procedures of the paper-based cathode PEC sensing platform; (**B**) Schematic illustration of the PEC analytical principle [27]. Copyright 2020 American Chemical Society.

In traditional PEC assays, the external light source is always needed to excite the photoelectrode materials, which limits the realization of instrument miniaturization and operation simplification. Therefore, chemiluminescent emission is introduced into the system as an internal light source [28]. Lan et al. reported a chemiluminescence-driven three-dimentional reduced graphene oxide (3D-rGO)/cellolose device for the PEC detection of thrombin based on DNA amplification strategy and hemin/G-quadruplex [29]. As shown in Figure 2, 3D-rGO/Au flowers were assembled on the paper

cellulose fibers and then modified with the nitrogen-doped carbon dots-sensitized ZnO. Thrombin induced the cyclic generation of many target-analog chains through T7Exo-catalyzed target cycling amplification. Then, the produced N_3-containing target-analog chains were bound on the surface of the test tab based on the metal catalyst-free click chemistry, triggering the HCR reaction. With the formation of hemin/G-quadruplex, the luminol-H_2O_2 system led to the formation of chemiluminescence emission as internal light source to produce the photocurrent signal.

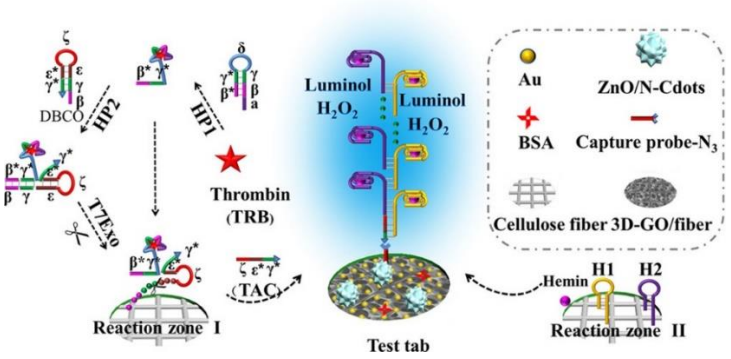

Figure 2. Schematic illustration of a chemiluminescence-driven PEC 3D-rGO/cellolose device coupling with DNA amplification strategy and DNAzyme [29]. Copyright 2017 American Chemical Society.

2.3 Photoactive molecules-based amplification

In order to amplify the photocurrent signal, photoactive molecules can be introduced into the dsDNA polymers as the labels, thus improving the utilization of light and the efficiency of charge separation. Metal complexes, such as ruthenium complexes and iridium complexes, are the most commonly used molecules in PEC analysis because of their excellent photochemical stability and good redox properties. Li et al. designed a cationic Ir(III) complex, $[(ppy)_2Ir(dppz)]^+PF_6^-$ (ppy = 2-phenylpyridine and dppz = dipyrido[3,2-a:2',3'-c]phenazine), and used it to develop a HCR-based PEC biosensor for the detection of DNA [30]. As illustrated in Figure 3, the molecules with dppz as the planar ancillary ligand facilitated their intercalation into the base pairs of the generated dsDNA polymers. By adjusting the introduction of the sacrificial electron donor triethanolamine (TEOA) or acceptor (dissolved O_2), cathodic and anodic photocurrent signals were recorded, respectively. To improve the absorption in the visible region, coumarin 6 was used as the cyclometalated ligand to synthesize the Ir(III) complex instead of ppy [31, 32].

Perylene-based compounds with a large π-conjugated system have been widely used in the development of organic solar cells and PEC analysis platforms. Li et al. reported a red-light-driven ultrasensitive PEC biosensor by using perylene-based photoactive polymer (PTC-NH_2) as a dsDNA-intercalating probe [33]. As displayed in Figure 4, probe HA was cleaved to release ssDNA s1 in the presence of DNA adenine methyltransferase (Dam MTase). The released s1 was then hybridized with the capture probe (CP) DNA to form the s1@CP dsDNA hybrid on the electrode surface. The unhybridized segment of s1 could trigger the HCR to generate a plenty of dsDNA concatamers. Then, the abundant PTC-NH_2 molecules were specifically intercalated into the grooves of the dsDNA

concatamers via the electrostatic interactions. Under the radiation of 600 nm light, the photoexcited electrons from the reaction between PTC-NH_2 and AA were transferred to the electrode, thus generating a significantly increased PEC current.

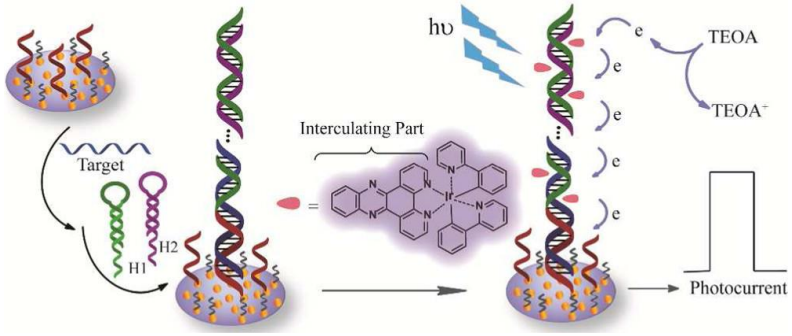

Figure 3. Schematic illustration of assembling of intercalated $[(ppy)_2Ir(dppz)]^+PF_6^-$ modified ITO electrode [30]. Copyright 2015 American Chemical Society.

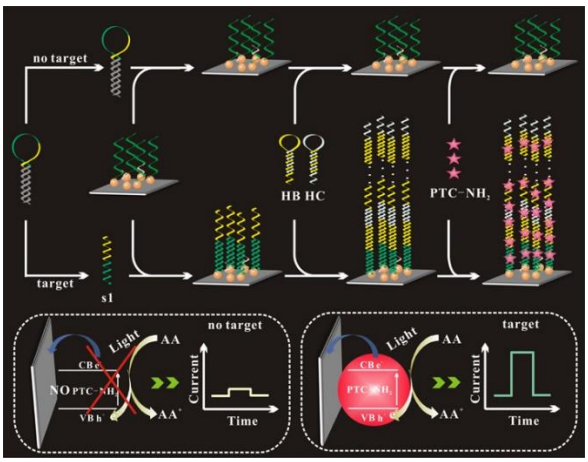

Figure 4. Schematic illustration of the perylene-based PEC sensor for Dam MTase sensitive detection based on target-triggered HCR [33]. Copyright 2019 American Chemical Society.

Generally, the single response-based analysis may be subject to internal and external interference. Therefore, the dual-signal strategy with self-calibration ability has great potential in the quantitative detection of complex samples [34]. Lu et al. reported a dual-signal biosensor for the determination of uracil-DNA glycosylase (UDG) activity based on the integration of PEC and electrochemical strategies [35]. In this work, substrate DNA on the electrode were cleaved under the catalysis of UDG and endonuclease IV (Endo. IV), resulting in the departure of PEC labels (AgInS2 QDs) and the decrease in the PEC signal. Meanwhile, DNA segments leaved on the electrode surface could initiate the HCR and a large amount of ferrocene (Fc) molecules bound to dsDNA, causing a "signal-on" electrochemical signal. Moreover, long dsDNA polymers further blocked photogenerated electron transfer to the electrode and decreased the PEC signal due to the steric hindrance. In addition, Liu et al. developed a dual-ratiometric aptasensor for streptomycin detection with methylene blue (MB) as the bifunctional probe [36]. In this strategy, MB molecules intercalated in the dsDNA could simultaneously generate photocurrent ($I_{MB\text{-}PEC}$) and redox current ($I_{MB\text{-}EC}$). The redox current of Fc ($I_{Fc\text{-}}$

$_{EC}$) was used as the reference signal, and two ratiometric signals ($I_{MB\text{-}PEC}/I_{Fc\text{-}EC}$ and $I_{MB\text{-}EC}/I_{Fc\text{-}EC}$) were calculated for sensitive bioassays.

2.4 Nanomaterials-based amplification

When the size of nanomaterials is reduced to the nanometer level, nanomaterials show excellent physical and chemical properties different from bulk materials [37]. So far, a variety of photovoltaic nanomaterials, including quantum dots, metal nanoparticles and carbon-based nanostructures, have been successfully used to construct HCR-based PEC biosensors. In addition, in order to further improve the photocatalytic performance and photocurrent conversion efficiency, hybrid nanomaterials have been explored to develop co-sensitized systems, including inorganic-organic and inorganic-inorganic heterostructures. Ye et al. reported a "signal-on" PEC biosensor for the detection of microRNA (miRNA) by using Bi_2S_3@MoS_2 nanoflowers as the photoexcited materials [38]. As shown in Figure 5, Bi_2S_3@MoS_2 nanoflowers were synthesized and used to modify the electrode, which could significantly increase the photocurrent response due to the suppressed electron-hole recombination and the prolonged lifetime of electron-hole pairs. Electron donor dopamine (DA) was linked with ligation probe (probe-N_3) via amidation reaction to form DA-labeled signal probe (P_{DA}-N_3). After the completion of HCR, the P_{DA}-N_3 probe was introduced to label the dsDNA ploymer through metal catalyst-free click chemistry, inhibiting the electron-hole recombinant and leading to the signal amplification.

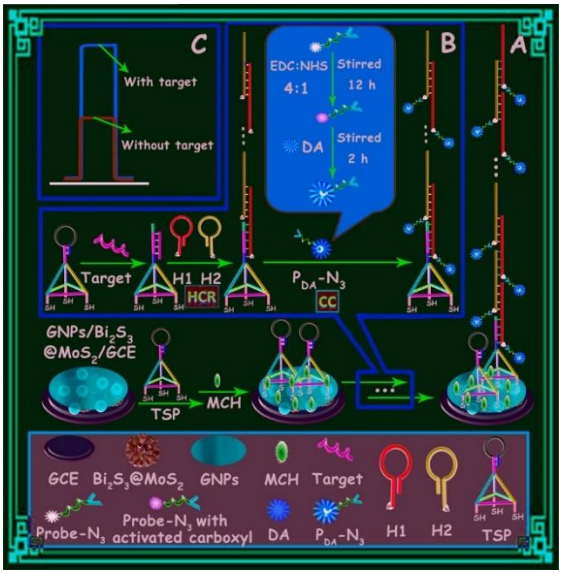

Figure 5. Schematic illustration of (**A**) PEC platform for miRNA assay; (**B**) Principle of metal-catalyst-free click chemical signal amplification via hybridization chain reaction (the blue box presents the fabrication process of P_{DA}-N_3; and (**C**) PEC response in the absence and presence of target [38]. Copyright 2016 American Chemical Society.

Nanomaterials as photoquenchers can compete with photoelectrode nanomaterials to consume light sources and electron donors, resulting in the reduction of photocurrent intensity. Li et al. developed an addressable TiO_2 nanotubes-functionalized paper-based cytosensor for the detection of cell-surface-expressing protein [39]. As illustrated in Figure 6, paper fibers were successively modified with neat

TiO_2 nanotubes arrays, PtNPs, and nitrogen-carbon dots. In the presence of carcinoembryonic antigen (CEA) at the MCF-7 cell surface, primer strands (PS) released from the surface of $ZnFe_2O_4$ could initiate the HCR and facilitate the immobilization of numerous CuS NPs on the working zones. CuS NPs competitively consume the light source and electron donor (H_2O_2) with the photoactive interface, realizing the dual signal amplification. Besides, photoactive nanomaterials can also be used as photosensitizers to amplify the detection response. Meng et al. reported a DNA-linked CdSe QDs/aminofunctionalized graphene quantum dots (AGQDs) "Z-scheme" system to sensitively analyze DNA with a negative background signal [40]. After the insertion of AGQDs into the long dsDNA strands, the "Z-scheme" heterojunctions formed and the anodic photocurrent switched into the cathodic photocurrent. When energy level was matched up with photoelectrode materials, the photoactive labels can act as the sensitizers to produce a sensitized photocurrent. For example, Yuan et al. developed a sensitive PEC biosensor for miRNA-21 detection based on HCR and QDs-sensitizing Bi_2Te_3 nanosheets (Figure 7) [41]. In this study, two HCR primers (H1 and H2) were bound with CdTe QDs. Through strand displacement reaction and HCR, long DNA polymers were formed on the electrode, allowing for the loading of a large number of QDs. The captured QDs can not only expand the absorption of sunlight and significantly reduce the destructive effect of ultraviolet light, but also match the energy level of Bi_2Te_3 and promote charge separation, so as to effectively amplify the photocurrent response of Bi_2Te_3.

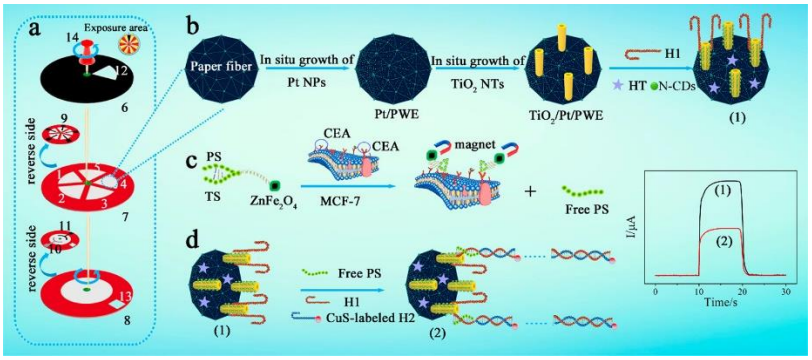

Figure 6. Schematic illustration of the addressable paper PEC chip (a) and construction process for the paper-based PEC cyto-sensor (b−d) [39]. Copyright 2018 American Chemical Society.

Photoactive nanomaterials directly deposited on the electrode through the drop coating method may result in high background signal. It is a promising strategy to generate electroactive species in-situ without the pre-synthesis procedure. To achieve zero background noise, Zeng et al. reported a PEC biosensor for organophosphorus pesticide detection by using the long dsDNA chain produced from HCR process as the template to in-situ produce photoactive Ag_2S NPs [42]. The resulting nanoparticles are far away from the electrode surface, causing a zero background noise. To avoid the use of ultraviolet light as the light source, Qiu et al. reported a near-infrared (NIR) light responsive HCR-based PEC aptasensor for the detection of carcinoembryonic antigen (CEA) based on unconversion nanoparticles (UCNPs) and the in-situ performed Ag_2S NPs [43]. As displayed in Figure 8, poly(ethylenimine)-functionalized UCNPs were dropped on the ITO electrode and further modified with the CEA aptamer. In the presence of CEA, a sandwich-type structure was formed and the trigger stand on the CEA aptamer initiated the HCR. The DNA polymer with cytosine-rich repeats acted as the template for the in-situ

generation of Ag_2S NPs. The UCNPs concerted with NIR light into visible light which was absorbed by Ag_2S NPs for amplifying the photocurrent signal.

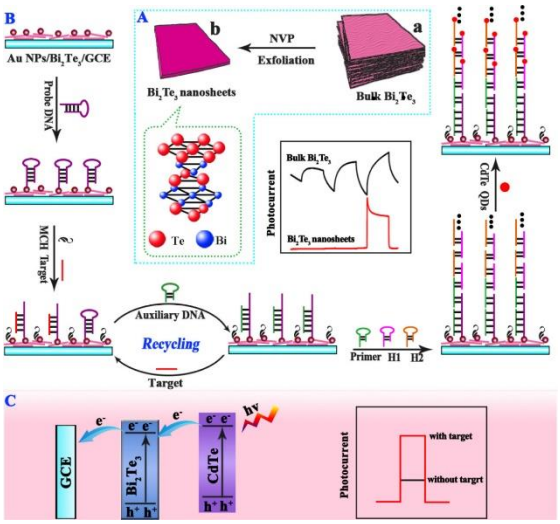

Figure 7. Schematic illustration of (**A**) synthesis of Bi_2Te_3 nanosheets, (**B**) assembly process of the PEC biosensor, and (**C**) mechanism of photocurrent generation [41]. Copyright 2020 American Chemical Society.

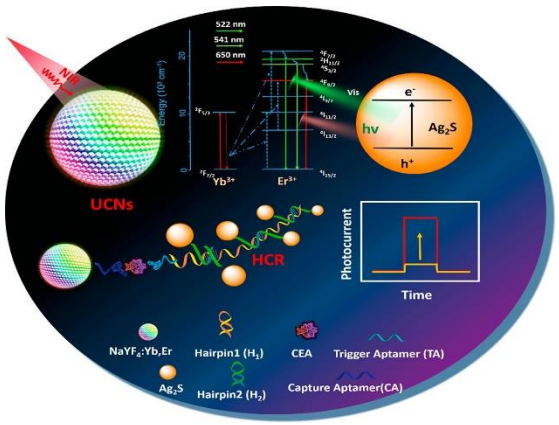

Figure 8. Schematic illustration of $NaYF_4$:Yb,Er UCNPs-based NIR light responsive PEC aptasensor to detect CEA by coupling with target-triggered HCR, accompanying in situ formation of Ag_2S NPs on the basis of typical C−Ag^+−C chelation reaction for the signal amplification [43]. Copyright 2018 American Chemical Society.

Nanomaterials with enzyme-like catalytic ability have also been utilized to develop PEC biosensors. For example, Yang et al. reported a donor/acceptor-induced ratiometric HCR-based PEC paper analytical device with GOx-mimicking AuNPs [44]. As presented in Figure 9, when miRNA-141 triggered the duplex-specific nuclease (DSN)-based target recycling reaction and a cascade of HCR, the trunk and branch dsDNA polymers were in-situ formed on the electrode to capture many AuNPs through the electrostatic interactions. Through the AuNPs-catalyzed glucose oxidation, the dissolved O_2 as an electron acceptor was reduced into H_2O_2 as an electron donor. Then, the cathodic photocurrent from

Ag_2S/Cu_2O nanocomposites was reduced while the anodic photocurrent from graphene quantum dots (GQDs) and Ag_2Se QDs co-sensitized ZnO nanosheets was increased.

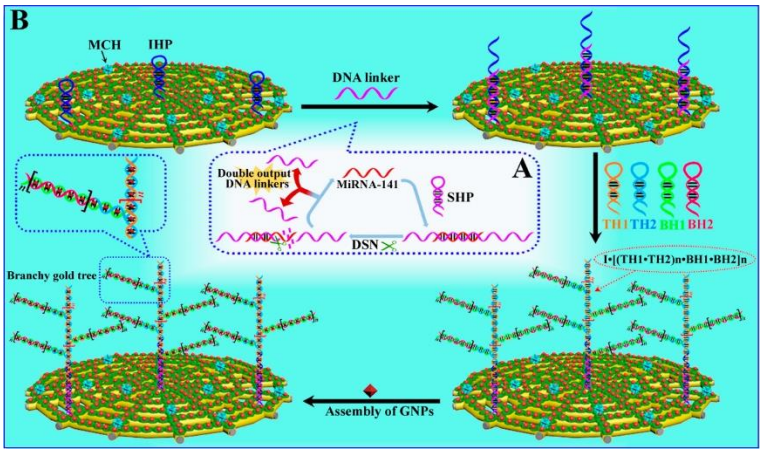

Figure 9. Schematic illustration of (**A**) DSN-induced target recycling reaction with the output of a double amount of DNA linkers and (**B**) Construction procedure for the photocathode sensing zone [44]. Copyright 2019 American Chemical Society.

2.5 HCR-based immunoassays

Immunoassay is the most frequently used approach for the determination of a wide range of targets in disease diagnosis, environment monitoring, and food safety [45]. To improve the sensitivity of immunoassays, different DNA-based signal amplification strategies have been developed. For example, Zang et al. reported a HCR-based PEC immunoassay for CEA detection. In this work, biotin-labeled trigger DNA was conjugated with biotin-labeled detection antibody with SA as the linker. The HCR occurred in the presence of H1 and H2 [46]. In addition, the simultaneous immobilization of trigger DNA and antibody on the NPs can facilitate the HCR amplification strategy into immunoassays [47, 48]. For example, Wei et al. reported a PEC detection of microcystin-LR (MC-LR) based on HCR-assisted exciton-plasmon interaction and enzymatic biocatalytic precipitation [49]. In this study, numerous SA molecules and ALP-modified AuNPs were captured by biotin-labeled dsDNA polymers after the immunoreaction and HCR progress. In the presence of 4-aminophenyl phosphate monosodium salt hydrate (4-APP), ALP catalyzed the in-situ silver metallization on AuNPs and the generation of insoluble biocatalytic precipitation (BCP) of benzoquinone, leading to the decrease of the photocurrent signal. To further increase the accuracy and sensitivity of the method, they reported a dual-modal split-type PEC immunosensor for the detection of MC-LR with complementary colorimetry [50]. As shown in Figure 10, mesoporous silica nanospheres (MSNs) were utilized as the nanocarriers to immobilize secondary antibody and trigger DNA. Numerous ALP molecules were bound on the HCR products to catalyze the generation of AA acting as the electron donor to increase the photocurrent and as the reducing agent to produce silver shells on Au nanobipyramids, thus resulting in the multiple color changes.

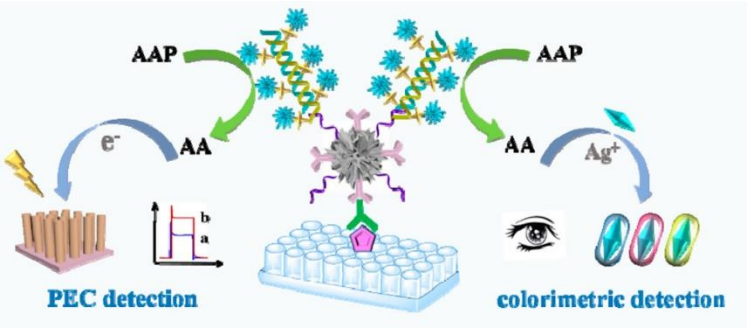

Figure 10. Schematic illustration of dual-modal PEC and colorimetric immunosensor [50]. Copyright 2018 American Chemical Society.

3. CONCLUSION

In this review, we summarized the progress in PEC biosensors to detect various targets in combination with HCR nanotechnology. Although the accuracy of target quantification should be improved due to the difference size of HCR products, the signal amplification strategy provides great prospects for the construction of biosensors. In addition, PEC bioanalysis has been widely concerned because of its low background signal, high sensitivity and low cost. However, compared with traditional detection methods such as electrochemical method, PEC bioanalysis is still in the primary stage of development and faces many problems and challenges. In order to further improve the applications of PEC bioanalysis, photosensitive materials with high performance should be developed. It is also important to realize the miniaturization, automation and high-throughput detection of PEC bioanalysis.

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