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Mini review

Application of Oxygen Reduction Reaction, Oxygen Evolution Reaction and Hydrogen Evolution Reaction in Electrochemical Biosensing

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Signal amplification based on catalytic reactions is one of the effective strategies to improve the sensitivity of electrochemical biosensors in which additional chemicals as the substrates are acquired. Water and oxygen are two simple and natural small molecules existed in the reaction medium of electrochemical biosensors. In this work, we reviewed the progress in the design of electrochemical biosensors with water or oxygen as the reaction substrate by oxygen reduction reaction (ORR), oxygen evolution reaction (OER) or hydrogen evolution reaction (HER) for signal enhancement.

Keywords: Electrochemical biosensors; oxygen reduction reaction; oxygen evolution reaction; hydrogen evolution reaction

1. INTRODUCTION

Catalytic reactions for small molecules such as oxygen reduction reaction (ORR), oxygen evolution reaction (OER) and hydrogen evolution reaction (HER) are the core of natural and artificial energy conversion schemes [1]. For example, the reduction of oxygen to water mediated by cytochrome c oxidase is the basic process of cell respiration [2]. Photosynthetic water oxidation for oxygen evolution in photosystem II achieves the conversion from light energy to chemical energy [3]. In addition, the

conversion between hydrogen and proton catalyzed by hydrogenase is the energy metabolism process of many microorganisms [4]. Thus, the current researches for the conversion of artificial energy mainly focus on the effective catalysts of ORR, OER and HER [5].

Electrochemical biosensors have broad application prospects in environmental monitoring, biological analysis, clinical diagnosis and so on. Signal amplification based on catalytic reactions is one of the effective strategies to improve the sensitivity of electrochemical biosensors [6]. At present, the materials used for signal amplification of catalytic reactions mainly include enzymes, carbon nanomaterials, metal nanoparticles, metal oxides and sulfides, and metal organic frameworks (MOFs). They can catalyze the electrochemical reactions of small molecules including hydrogen peroxide, glucose, thionine and nicotinamide adenine. Moreover, almost all the electrochemical biosensors are carried out in aqueous medium. Water and oxygen are two simple and natural small molecules existed in the reaction medium. Electrochemical biosensors with water or oxygen as the substrate of catalytic reactions exhibit the advantages of simple operation and environmental friendliness. In this work, we review the progress in the design of electrochemical biosensors with ORR, OER and HER catalysis for signal enhancement.

2. ORR-BASED ELECTROCHEMICAL BIOSENSORS

ORR is one of the basic reactions for designing different electrochemical devices in the fields of sensing and fuel cell [7]. Noble metals such as Au, Ag and Pt exhibit high catalytic activity toward ORR. Among them, Pt-based materials are the commonly studied catalysts for electrocatalytic oxygen reduction. Moreover, in contrast to the single counterpart, bimetallic nanomaterials exhibit high catalytic ability, long-term stability and enhanced electrical conductivity due to the synergistic effect. PtCo alloyed nanodendrites (PtCo NDs) can be synthesized with L-carnosine as the co-structure-directing agent [8]. Using PtCo NDs-modified electrode, Feng and co-workers developed an electrochemical immunosensor for the detection of carbohydrate antigen 15-3 (CA15-3). The modified electrode exhibited high electrocatalytic ability for ORR. The interaction between CA15-3 and its antibody decreased the electrocatalytic activity of PtCo NDs, thus allowing the detection of CA15-3 in the linear range of 0.1 ~ 200 U/mL (Table 1). The detection limit was found to be 0.0114 U/mL. Wang et al. prepared rhombic dodecahedral Cu_3Pt nanoframes (Cu_3Pt NFs) with solvothermal method [9], in which theobromine was used as the reductant and structure-directing agent and cetyltrimethylammonium chloride (CTAC) was used as the co-structure-director. The Cu_3Pt NFs showed high electrocatalytic performance for ORR. Using Cu_3Pt NFs as the electrode materials to catalyze oxygen reduction, an electrochemical immunosensor was developed for the determination of alpha-fetoprotein (AFP) with a detection limit of 0.033pg/mL. Wang et al. reported an immunosensor for prostate specific antigen (PSA) detection using bimetallic core-shell Au@Pt nanocrystals (Au@Pt NCs)-modified electrode [10]. 2-Pyrrolidone-5-carboxylic acid sodium salt (PCA-Na) was used as the growth-directing agent for the preparation of Au@Pt NCs. The Au@Pt NCs-modified electrode exhibited excellent catalytic activity for ORR. The interaction between PSA and anti-PSA immobilized on the electrode surface on decreased the ORR current, thus allowing for the analysis of PSA in a linear range of 0.1 ~ 50 ng/mL. Li et al. suggested that the Pt-Sn@TiO_2 composite showed an enhanced electrocatalytic property for ORR due

to the synergistic effect between Pt and Sn [11]. The composite was prepared by decoration of Pt and Sn nanoparticles on the surface of TiO_2 nanorods. Through the dual signal amplification of Pt-Sn@TiO_2 and exonuclease-assisted target recycling, streptomycin had been determined with a detection limit of 20 pM. In this method, the double-stranded DNA hybrids on the Pt-Sn@TiO_2-modified electrode prevented the ORR catalysis. In the presence of streptomycin and exonuclease, the aptamer and streptomycin-aptamer were digested by exonuclease, which made the Pt-Sn surface available for ORR. Photocathodic bioassay shows great potential in practical application because of its intrinsic ability against the interferences from reducing reagents in biological samples. Fan et al. developed a photocathodic immunosensor for CA19-9 detection using Pt/graphene nanocatalysts as the signal amplifiers for oxygen reduction [12]. The Pt/graphene nanocatalysts were prepared by loading Pt nanoparticles on the surface of graphene nanosheets. The nanocatalysts can accelerate the ORR and cause the enhancement of cathodic photocurrent signal.

Moreover, Wang et al. prepared AuAg hollow nanocrystals (AuAg HNCs) by a one-pot aqueous strategy with polycytidysic acid (PCA) as the growth-directing agent [13]. The AuAg HNCs-modified electrode was used to develop immunosensors for carbohydrate antigen 199 (CA199) detection. The capture of CA199 by the antibody on the electrode surface decreased the catalytic current of ORR by AuAg HNCs. The electrochemcial signal linearly reduced with the increase of CA199 concentration from 1 to 30 U/mL. Toyos-Rodríguez et al. suggested that the bimetallic Pd-AuNPs with an optimum Pd:Au ratio showed excellent electrocatalytic activity for ORR [14]. The Pd-AuNPs were then used as the signal tags to develop a magnetic electrochemical immunosensor for hyaluronidase detection. Hyaluronidase at the concentration down to 50 ng/mL was readily determined.

Table 1. Analytical performances of ORR-based electrochemical biosensors.

Electrode materials or signal reporter	**Analyte**	**Detection limit**	**Linear range**	**Ref.**
PtCo NDs	CA15-3	0.0114 U/mL	0.1 ~ 200 U/mL	[8]
Cu_3Pt NFs	AFP	0.033 pg/mL	0.1 ~ 10^4 pg/mL	[9]
Au@Pt NCs	PSA	0.018 ng/mL	0.1 ~ 50 ng/mL	[10]
Pt-Sn@TiO_2	streptomycin	20 pM	0.05 ~ 1500 nM	[11]
Pt/graphene	CA19-9	0.036 pg/mL	0.1 ~ 10^4 pg/mL	[12]
AuAg HNCs	CA199	0.228 U/mL	1 ~ 30 U/mL	[13]
Pd-AuNPs	hyaluronidase	50 ng/mL	125 ~ 4600 ng/mL	[14]
Ag@C	S. aureus	10 CFU/mL	10^2 ~ 10^6 CFU/mL	[15]
heme-peptide/AuNP	β-Amyloid	10 pM	0.02 ~ 1.5 nM	[16]
Au_{25} NCs/Cu_2O@Cu	CEA	0.43 pg/mL	1 ~ 10^3 pg/mL	[17]
	MUC1	5.8 fg/mL	1 ~ 10^3 pg/mL	
Fe-N-C nanosheet	tetracycline	3.88 nM	Not reported	[18]
Ag_3BiO_3 NCs	miR-21	7.1 aM	10 ~ 10^8 aM	[19]
AuNP/peptide-Cu^{2+}	PSA	0.40 pg/mL	1 ~ 500 pg/mL	[20]
AgPt/PCN-223-Fe	OTA	14 fg/mL	20 fg/mL ~ 2 ng/mL	[21]
Pt/Sn-In_2O_3	miRNA	1.92 fM	5 pM ~ 0.5 fM	[22]
DNAzyme	thrombin	1 fM	1 ~ 10^3 fM	[23]

Since the first electrochemiluminescence (ECL) sensor was reported in 2004, various novel nanomaterials have been developed for the design of ECL sensing devices due to the advantages of high sensitivity, low background and wide detection range [24, 25]. Dissolved O_2 is a common coreaction for ECL sensing platforms [26]. Chen et al. proposed an ECL biosensor for the determination of Staphylococcus aureus (S. aureus) using Ag@C hybrid bipolar electrodes [15]. The Ag@C could quench the ECL emission of luminol. However, the captured Staphylococcus aureus could catalyzed the electrochemical reduction of O_2 at the cathode, thus leading to the ECL emission recovery from luminol. The method showed a detection limit as low as 10 CFU/mL due to the merits of anodic dissolution of Ag and cathodic biocatalysis of O_2 reduction. The synchronous ECL detection of multi-targets is challenged by the cross-reaction from dual-luminophors. Zhou et al. reported the detection of carcinoembryonic antigen (CEA) and mucin 1 (MUC1) using serum albumin-stabilized Au_{25} nanoclusters (Au_{25} NCs) as the bipolar ECL probes (Figure 1) [17]. In this method, TiO_2 nanosheet was used as the cathodic coreaction accelerator to catalyze the reduction of O_2, thus improving the cathodic ECL emission. Meanwhile, Cu_2O@Cu was used as the anodic coreaction accelerator to stimulate the oxidation of N,N-diethylethylenediamine (DEDA), thus promoting the anodic ECL emission. The method solves the problem of cross reaction of double luminophors for the synchronous determination of two biomarkers. Consequently, CEA and MUC1 at the concentrations as low as 0.43 pg/mL and 5.8 fg/mL can be readily determined, respectively.

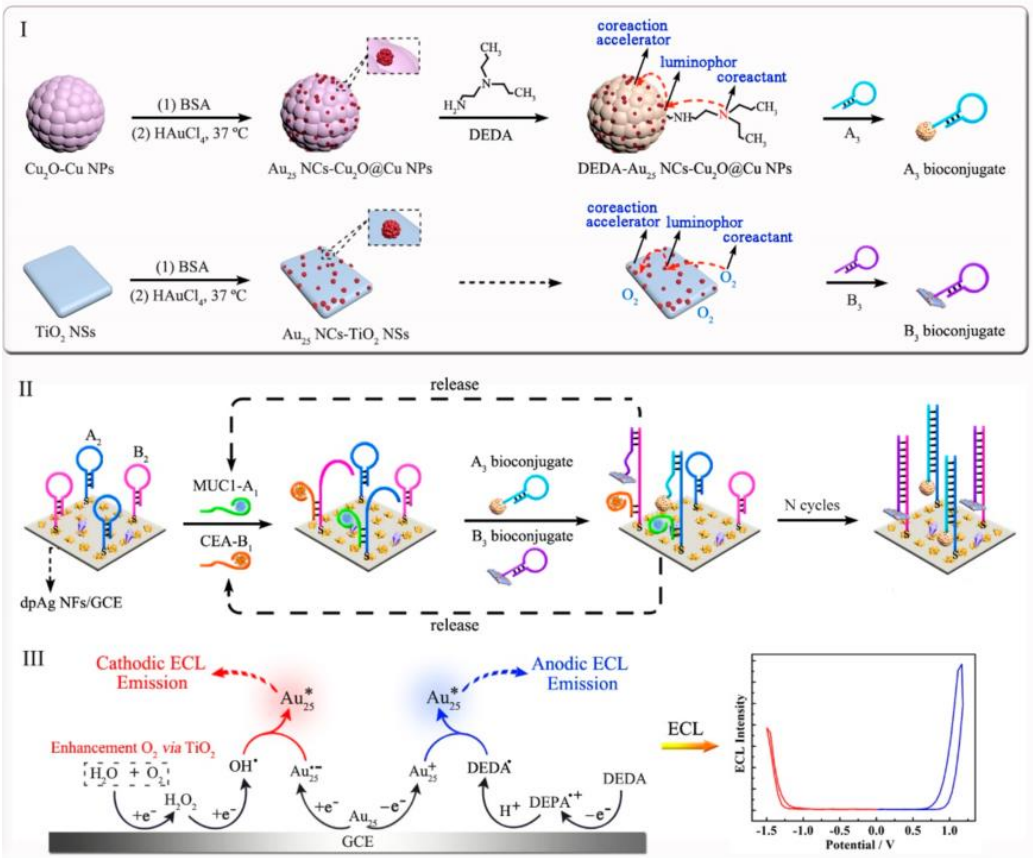

Figure 1. Schematic diagram showing fabrication of the ECL aptasensor. Copyright 2019 American Chemical Society [17].

The doping of iron and nitrogen in nanomaterials can improve the catalytic activity of ORR electrocatalysts. Recently, Zong et al. prepared an electrocatalyst for O_2 reduction by using a pre-organized ligand of 1,10-phenanthroline-2,9-dicarboxylic acid (PDA) to destroy the saturated coordination sites [18]. Using the Fe-N-C/luminol-modified electrodes, a signal-amplified ECL system was proposed for the immunoassay of tetracycline with a low detection limit. Silver bismuth oxide nanocrystals (Ag_3BiO_3 NCs) can promote the reduction of O_2 by generating abundant superoxide anion radicals. Liao et al. developed a ternary ECL strategy for miR-21 detection with N-(aminobutyl)-N-(ethylisoluminol) (ABEI) as the emitter, O_2 as the coreactant, and Ag_3BiO_3 NCs as the coreaction accelerators (Figure 2) [19]. In this work, a target miR-21 induced the formation of DNA nanonet by DNA self-assembly. Then, a large number of ABEI-doxorubicin (Dox) conjugates were embedded in the DNA nanonet. The resulting nanonet-Dox-ABEI was then captured by the cDNA-covered TiO_2-Ag_3BiO_3@Au-modified electrode via hybridization, thus achieving the signal-enhanced ECL detection. The method can determine miR-21 with a detection limit of 7.1 aM.

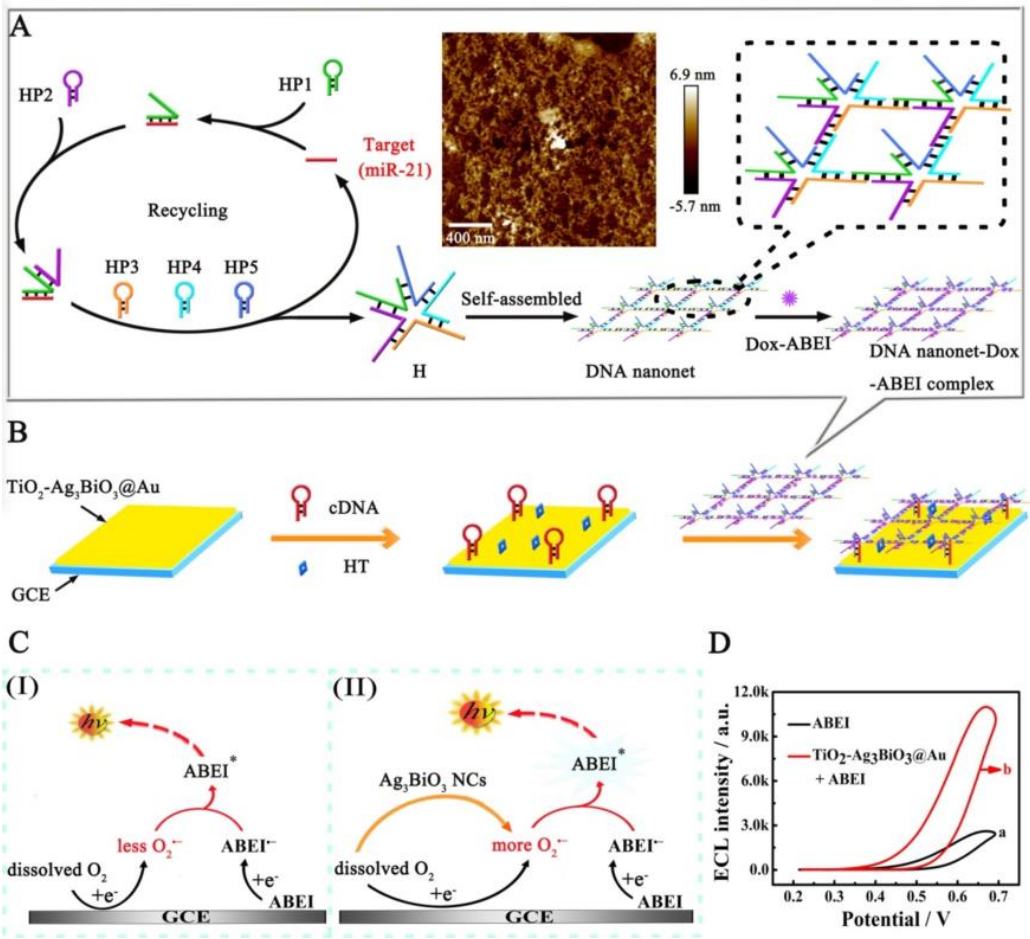

Figure 2. (A) Schematic diagram of the self-assembled fabrication process of DNA nanonet triggered by the target miR-21; (B) establishment process of the ECL biosensor for detection of miR-21; (C) Possible ECL emitting mechanism without (I) and with (II) the Ag_3BiO_3 NCs as coreaction accelerator; (D) ECL intensity of without (a) and with (b) Ag_3BiO_3 NCs@Au. Copyright 2019 American Chemical Society [19].

Nanomaterials can be used as the electrode modifiers to increase the surface area and accelerate

the electron-transfer rate, thus improving the detection sensitivity. Besides, nanomaterials can also be used as the signal probes or nanocatalysts for electrochemical bioassays [27]. Xia and coworkers have investigated the catalytic performance of peptide-Cu^{2+} and found that the complex reveals oxidase-mimicking activity for ORR [20, 28]. The peptide is featured with a histidine residue in the N-terminal with a free amine. With gold nanoparticle (AuNP) as the carrier, the metal-peptide complex has been readily modified on the AuNP surface to produce a nanocatalyst for the design of electrochemical biosensors [20]. With PSA as the example analyte, the electrochemical immunoassay showed a linear range of 1 ~ 500 pg/mL with a detection limit of 0.40 pg/mL. Zhang et al. developed an electrochemical ochratoxin A (OTA) biosensor with bimetallic AgPt nanoparticles-decorated iron-porphyrinic metal-organic framework (PCN-223-Fe) as the signal reporter [21]. The streptavidin (SA)-modified AgPt/PCN-223-Fe can be captured by the biotinylated OTA aptamer on electrode surface, thus producing a strong current from ORR. In the presence of OTA, the aptamer was reacted with OTA to induce the release of AgPt/PCN-223-Fe composite, thus leading to the decrease in the catalytic current. The method exhibited a linear range of 20 fg/mL ~ 2 ng/mL with a detection limit of 14 fg/mL. Based on the same principle, other toxins could be determined with a sequence-specific aptamer. Zhang et al. designed an electrochemical miRNA biosensor using Pt/Sn-In_2O_3 as the signal label to promote the ORR (Figure 3) [22]. The biotinylated hairpin capture probe immobilized on the electrode surface showed no interaction with SA-functionalized Pt/Sn-In_2O_3 because of the large steric effect. The binding of target miRNA to the capture probe opened the hairpin structure and made the biotin group be away from the electrode surface. Consequently, the SA-functionalized Pt/Sn-In_2O_3 was captured by the sensor electrode, thus producing a strong electrochemical signal by ORR. The method achieved the detection of miRNA in the linear range of 5 pM ~ 0.5 fM with a detection limit of 1.92 fM.

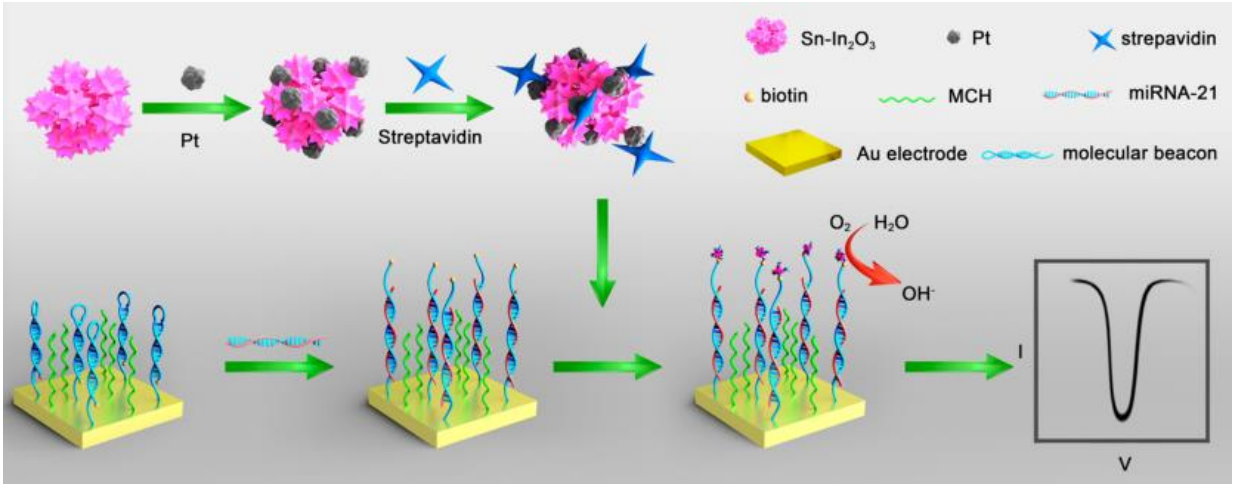

Figure 3. Illustration of electrochemical detection of miRNA using SA/Pt/Sn-In_2O_3 as electrochemical tracer. Copyright 2017 American Chemical Society [22].

Recently, Malecka et al. reported an interesting electrochemical aptasensor for thrombin detection through the electrocatalytic reduction of O_2 by G4-hemin DNAzyme (Figure 4) [23]. The formation of DNAzyme was triggered by the interaction of thrombin and a hemin-labeled 29-mer DNA aptamer on a gold disk electrode. The method achieved the detection of femtomolar thrombin without

the use of additional electron transfer mediator. At the same time, the O_2-dependent detection principle was used prepare a thrombin aptasensor on a gold screen-printed electrode for point-of-care testing (POCT) [29].

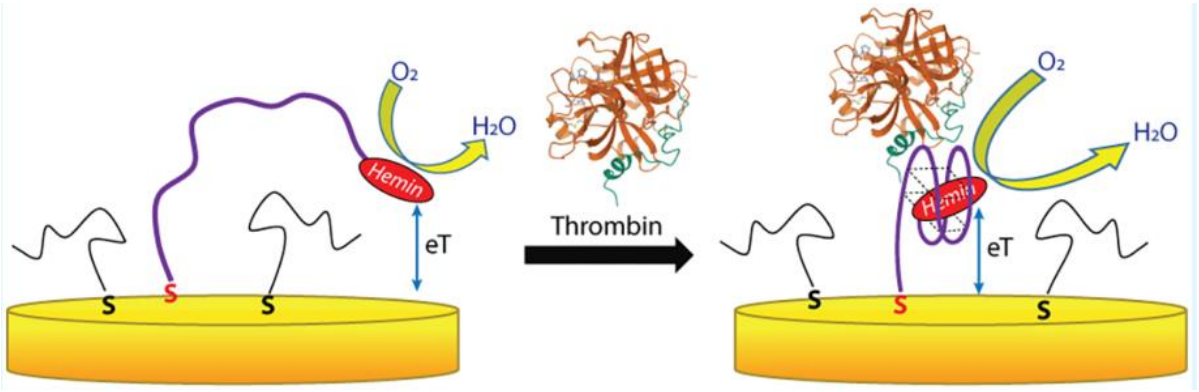

Figure 4. (A) Schematic representation of the O_2-dependent electrocatalytic detection of α-thrombin: the hemin-modified thiolated aptamer is immobilized on the gold electrode altogether with thiolated PEG and directly (with no mediators) electrocatalyzes the O_2 reduction; binding of thrombin (image from the RCSB PDB (rcsb.org) of PDB ID: 1PPB) triggers G4-hemin self-assembly with enhanced electrocatalytic properties for O_2 reduction, which is electrochemically detected. Copyright 2021 American Chemical Society [23].

3. OER-BASED ELECTROCHEMICAL BIOSENSORS

Since the first homogeneous water oxidation catalyst (binuclear bipyridine ruthenium complex) was reported in 1982 [30], metal complexes containing Ru, IR, Mn, Fe, Co, Ni and Cu have been able to be used for electrocatalytic water oxidation for OER [31, 32]. Copper is a relatively rich and cheap transition metal element in nature. Compared with other metal ions, copper ions have a lower oxidation potential. In 2012, Mayer's group reported the first case of water oxidation electrocatalyst, a copper ion complex [33]. After the divalent copper ion is oxidized to trivalent copper ion, the resulting trivalent copper ion can quickly oxidize water molecule to release oxygen. Subsequently, a series of electrocatalysts for water oxidation based on copper ion complexes were reported [34, 35]. Compared with noble metals and other non-noble metals with low catalytic rate, these catalysts have better stability and higher catalytic efficiency. These catalysts often work at higher pH (> 11) and potential (> 1 V vs. Ag/AgCl). However, the configuration of biomolecules will be destroyed in high pH environment, which is not conducive to the biomolecular interaction. Moreover, high oxidation potential will produce large background current, which has a great impact on the accuracy and sensitivity of the biosensor. Liu's group found that the Cu(II) complexes formed with ATCUN peptides exhibit good electrocatalytic ability towards water oxidation at neutral pH and low oxidation potential [36-38]. The ATCUN-Cu(II) complexes have been successfully used as the signal reporters of electrochemical biosensors (Table 2). Moreover, the metal complexes can be readily modified on the surface of nanomaterials by introducing specific amino acid residues in the peptide sequence, such as cysteine and phenylalanine. For example, by modifying ATCUN-Cu(II) complexes on the surface of AuNPs through Au-S interactions, the

resulting nanocatalysts exhibiting good electrocatalytic ability towards water oxidation have been used to determine DNA as low as down to 0.1 pM [37]. Based on the production of ATCUN-Cu(II) complexes by proteolytic hydrolysis on electrode surface, caspase-3 has been determined using graphene electrode [36]. The substrate peptide was anchored on the graphene surface via the hydrophobic and π-stacking interaction. The proteolytic hydrolysis of peptide led to the formation of ATCUN-Cu(II) for water oxidation. The signal enhanced linearly in the concentration range of 0.5 pg/mL ~ 2 ng/mL. Moreover, Liu's group proposed an electrochemical immunosensor by using protease-modified nanomaterial as the signal label to catalyze the production of ATCUN-Cu(II) complexes for water oxidation (Figure 5) [38]. In this work, trypsin was used as the model protease and carbon nanotube (CNT) was employed as the carrier of detection antibody and trypsin. Based on the electrocatalytic water oxidation by the produced ATCUN-Cu(II) complexes, PSA has been determined with a concentration down to 10 pg/mL.

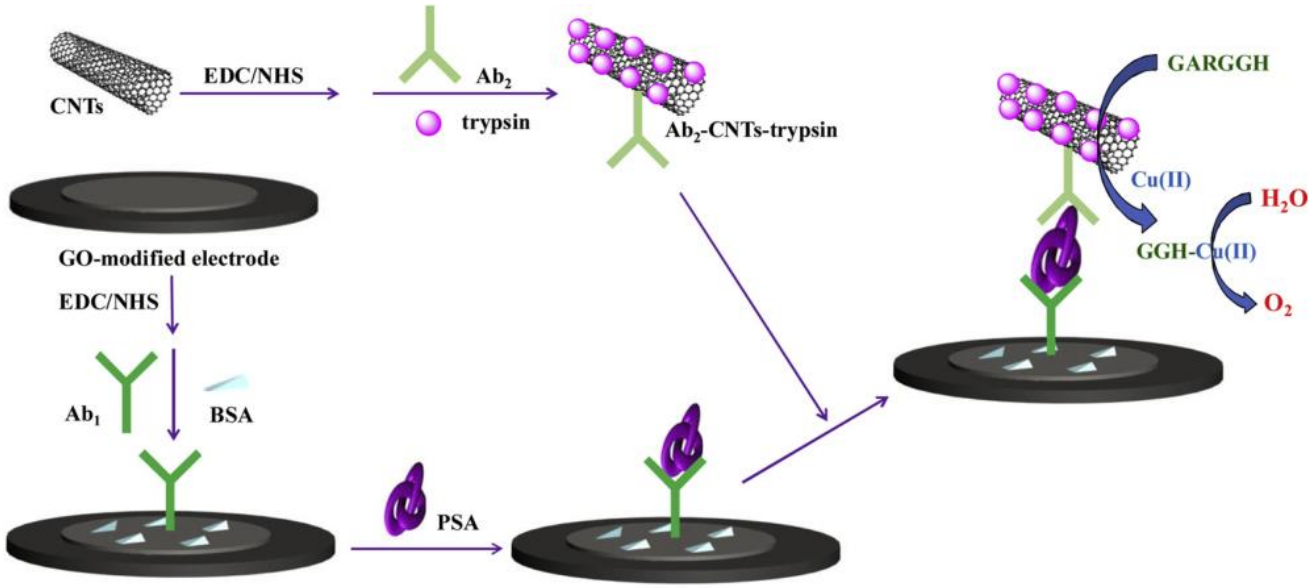

Figure 5. Scheme representation of the proposed electrochemical immunosensor by the generation of ATCUN-Cu(II) metallopeptides as the electrocatalysts toward water oxidation. Copyright 2019 Elsevier B.V. [38].

4. HER-BASED ELECTROCHEMICAL BIOSENSORS

Decreasing the overpotential is the main challenge in the field of electrocatalytic HER at present. Pt-based noble metals have low overpotential and excellent catalytic performance, but their low reserves and high price limit the wide application of such materials in industrial hydrogen production. Therefore, the research and development of non-noble metal catalysts is an important research direction of HER catalysis. Due to their high catalytic activity towards HER, transition metal dichalcogenides (TMDs) have been regarded as the signal-enhanced labels of biosensors (Table 2). Pumera's group fabricated WS_2, $MoSe_2$ and black phosphorus (BP) nanoparticles (NPs) by solution-based electrochemical exfoliation technique with bipolar electrodes [39-41]. These nanoparticles, exhibiting high activity towards HER catalysis, have been successfully used as the nanolabels of magneto-immunosensors for

competitive or sandwich detection of rabbit IgG (Figure 6) [39-41]. All the electrochemical measurements in these methods were performed in 0.5 M H_2SO_4. However, the low pH environment may promote the dissolution of magnetic beads and decrease the antigen-antibody interaction. Recently, Cao et al. developed a double-signal immunosensor with multi-wall carbon nanotube-cobalt phosphide (MWCNTs-CoP) as the signal label for carcinoembryonic antigen (CEA) detection [42]. The MWCNTs-CoP exhibits excellent electrocatalytic activity in neutral medium for both HER and OER. The double-signal biosensor shows a linear range of 0.0001 ~ 100 ng/mL by HER or 0.0001 ~ 10 ng/mL by OER. The detection limits were found to be 10 and 12 fg/mL, respectively.

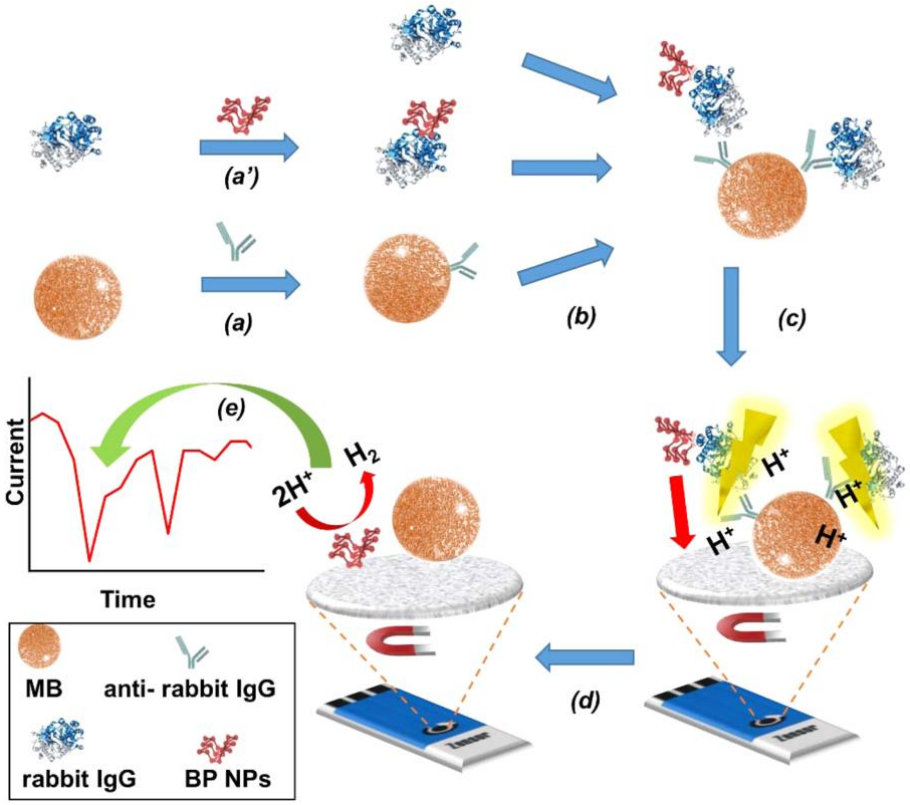

Figure 6. Schematic of the competitive magneto-immunoassay for protein using BP NPs as a tag and HER electrocatalysis (proton reduction) by impact electrochemistry (spikes count) as a detection technique. Copyright 2016 American Chemical Society [41].

Table 2. Analytical performances of OER and HER-based electrochemical biosensors.

Electrode materials or signal reporter	**Analyte**	**Detection limit**	**Linear range**	**Ref.**
Graphene	caspase-3	0.2 pg/mL	0.5 ~ 2000 pg/mL	[36]
AuNPs/ATCUN-Cu^{2+}	DNA	0.1 pM	0.1 ~ 2.5 pM	[37]
CNT/trypsin	PSA	10 pg/mL	10 ~ 2000 pg/mL	[38]
WS_2 NPs	IgG	2 ng/mL	2 ~ 500 pg/mL	[39]
$MoSe_2$ NPs	IgG	1.23 ng/mL	2 ~ 500 pg/mL	[40]
BP NPs	IgG	0.98ng/mL	2 ~ 100 pg/mL	[41]

MWCNTs-CoP (OER)	CEA	12 fg/mL	0.1 ~ 10^4 pg/mL	[42]
MWCNTs-CoP (HER)	CEA	10 fg/mL	0.1 ~ 10^5 pg/mL	[42]

4. CONCLUSION

In summary, the electrochemical biosensors with water or oxygen as the substrate of electrocatalytic reaction obviate the addition of additional substrates and have the advantages of simple operation procedure, environment friendliness and low background current. However, the electrocatalysts exhibiting the advantages of uniform size, good water solubility, easy synthesis and fast electron transfer rate are still desired. This work provides important information for the design of biosensors, which is of great significance for the wide applications of ORR, OER and HER catalysis.

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