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Mini review

Boronic Acid-functionalized Nanomaterials for the Design of Electrochemical Biosensors

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Boronic acid can selectively bind with 1,2- or 1,3-diol to form boronate ester in neutral media. Various boronic acid-based derivatives and boronic acid-functionalized materials have been used to develop biosensors. Electrochemical biosensors have been extensively applied in various fields due to their high sensitivity, relative simplicity and easy operation. This review focused on the design and application of electrochemical biosensors with boronic acid-functionalized nanomaterials as the electrode modifiers or signal labels.

Keywords: Electrochemical biosensor; boronic acid; nanomaterials; electrode modifiers; signal labels

1. INTRODUCTION

Boronic acid can specifically and reversibly react with α-hydroxycarboxylate and diol-containing species by the formation of five or six-membered cyclic boronate ester bond, including citrate, sugar, catechol, saccharide, ribonucleotide, glycoprotein and RNA. This specific binding has attracted extensive attention for the synthesis of versatile boronic acid-containing compounds for the detection of biomolecules [1-5]. With the development of proteomics and glycomics research, boronic acid-modified materials have been successfully used for the separation and enrichment of biomolecules [6-10]. For example, 3-carboxybenzoboroxole (CBX)-modified monolithic columns have been utilized for the enrichment and separation of diol-containing molecules [11-13]. Kong et al. synthesized boronic acid-functionalized mesoporous graphene-silica composite to isolate intact glycopeptides from human serums

[14]. Glycoproteins have been recognized as the believable biomarkers for disease diagnosis including cancers and COVID-9. Thus, besides the prevalent antibody-based immunoassays, boronate-affinity methods have been extensively used in sensing fields of disease diagnosis and food control.

A variety of nanomaterials have been modified with boronic acids for the introduction of recognition sites and the development of sensing platforms [15]. Moreover, a large number of boronic acid groups on the nano-surface can interact with targets in a multivalent way, improving the affinity and specificity. The combination of boronic acids and porous nanomaterials such as mesoporous silca nanoparticles and metal organic frameworks (MOFs) can effectively improve the loading capacity. Fluorescent nanomaterials, such as quantum dots (QDs), carbon dots (CDs), metal nanoclusters and upconversion nanoparticles, can effectively convert the boronate binding event into the optical signal [16-22]. For instance, Shen et al. developed a one-step strategy to prepare fluorescent boronic acid-modified carbon dots (C-dots) for glucose sensing [23]. Glucose with two pairs of diols could induce the assembly of adjacent C-dots, leading to the significant quenching of the fluorescence of C-dots. Besides, diols species produced from the enzyme reaction can be determined by boronic acid-functionalized nanomaterials [24, 25]. For example, Wang et al. reported a ratiometric fluorescence sensor for the detection of tyrosinase (TYR) activity with the use of 3-aminophenyl boronic acid (3-APBA)-labeled quantum dots (QDs) [26]. TYR catalyzed the transformation of 6-hydroxycoumarin (6-HC) into 6,7-dihydroxycoumarin with enhanced fluorescence. The produced o-hydroxy compounds were then reacted with 3-APBA on the surface of QDs to form five-membered cyclic boronate esters, which quenched the fluorescence of QDs through the photoinduced electron transfer (PET) process. Huang et al. proposed a cleancap-regulated aggregation-induced emission (AIE) strategy for specific detection and cell imaging of alkaline phosphatase (ALP) activity [27]. D-glucose 6-phosphate (P-Glu) was hydrolyzed by ALP into Glu and the exposed 5,6-diol of Glu can bind with the free *p*-MPBA on the surface of CuNCs, resulting in the generation of red AIE luminescence. Moreover, boronic acid-derivatived nanomaterials have also been employed to develop colorimetry, surface plasmon resonance and surface enhanced Raman spectroscopy (SERS) biosensors [28]. However, most of these methods suffer from the shortcomings such as complicated operation, low sensitivity and large or expensive instrument.

Owing to the high sensitivity, relative simplicity and easy operation, electrochemical biosensors have been extensively applied in various fields, including disease diagnosis, environmental safety, food monitoring and so on. Since the common boronic acid derivatives are not electroactive, the redox-active moieties (e.g. ferrocene (Fc) and bipyridine Fe(II) complex) and electrochemiluminescent (ECL) moieties (tris-(2,2'-bipyridyl) ruthenium (II) can be integrated into the structure to provide a stable signal for the detection of glycated molecules [29-32]. Typically, Dechtrirat et al. developed an electrochemical displacement sensor for the detection of saccharide binding proteins and E. coli using a Fc-boronic acid derivative [33]. Xia et al. reported the magnetic bead-based electrochemical assays of circulating tumor cells using ferroceneboronic acid (FcBA) to recognize the sugar units over-expressed on the cell surface [34]. In this review, we mainly summarized the current progress in electrochemical biosensors by using boronic acid-functionalized nanomaterials as the electrode modifiers or signal labels.

2. BORONIC ACID-MODIFIED NANOMATERIALS AS THE ELECTRODE SUBSTRATES

In electrochemical biosensors, the binding of biorecognition element to the target with high affinity is the basis for the construction of sensing systems. Compared to the random physical adsorption and covalent-binding technique, oriented immobilization of biomolecules can retain the binding capacity and enhance the performances of bioassays. According to the surface characteristics of nanomaterials, boronic acids with additional groups and structures can form self-assembled monolayers (SAMs) and endow nanomaterials with boronate affinity capability to immobilize diol-containing biomolecules such as antibodies and enzymes. Typically, taking the advantage of the N-glycans in antibody, boronic acid derivative has been be utilized as the bridge to facilitate the attachment of antibody on the solid support [35-40]. Hashemi et al. proposed a well-orientation strategy for direct immobilization of antibody on the boronic acid-modified magnetic graphene nanoribbons (MGNRs) to detect cancer cells [41]. As shown in Figure 1A, 3-APBA was covalently conjugated on MGNRs without the introduction of long and flexible spacers and anti-CD20 antibodies were immobilized on 3-APBA-modified MGNRs with good orientation. After the immune-recognition and capture lymphoma cancer cell receptors in whole blood samples, the immune-complex was transferred on a screen-printed carbon electrode (SPCE) to further measure the impedimetric signal. In enzyme-based biosensor, enzyme immobilized on the electrode possess high catalytic activity and good stability. Thus, it is a general and important strategy to immobilize enzymes by boronic acid SAMs. For example, Dai et al. reported a H_2O_2 biosensor by using porous boronic acid-functionalized MOF as the matrix to immobilize horseradish peroxidase (HRP) with high loading efficiency [42].

Boronic acid SAMs-modified electrode can also be used to directly detect diol-containing molecules and organism [43-49]. Nallal et al. developed a photoelectrochemical-electrochemical dual-mode biosensors for the detection of glucose and glycated hemoglobin (HbA1c) based on poly[3-aminophenylboronic acid] (PAPBA)-modified nanohybrids (Figure 1B) [50]. In this study, graphene (G)-embedded titanium dioxide (TiO_2) nanowires were prepared by a simple electrospinning method and then in-situ decorated with Au NP-dispersed PAPBA nanocomposites. The synthesized heterojunction nanohybrid (HJNH) excellently combined the photocatalytic property of TiO_2, electrocatalytic ability of Au NPs, efficient electro transport capacity of G, and glucose binding ability of PAPBA. The synergistic effect ensure the sensor with good selectivity, high sensitivity and wide concentration range. Moreover, peptidoglycan on bacteria cell wall can be utilized as the targeting site for the development of boronic acid-assisted platforms [51]. Yang et al. developed an integrated multifunctional boronic acid-based PEC platform for simultaneous capture, detection, and inactivation of pathogenic bacteria [52]. To enhance the detection accuracy, Su et al. reported a pH-adjusted boronic acid-aptamer conjugate-based electrochemical biosensor for the conjugated N-glycolylneuraminic acid (CNeu5Gc) detection [53]. As shown in Figure 1C, the CNeu5Gc aptamer was modified with BA as the capture group and immobilized on the gold electrode surface via the formation of Au-S bond. Under acidic medium, a lot of CNeu5Gc molecules were captured by the boronic acid moiety on the electrode to form the stable boronic acid-CNeu5Gc complex through the formation of the boronate ester bond. When the pH value of solution was changed from 6 to 9, CNeu5Gc could be released from the complex and then quickly grasped by the aptamer under the proximity effect. Next, p-sulfonatocalix[4]arene

(pSC4)-modified silver nanoparticles (AgNPs) were introduced to recognize the aromatic amino acid residues of CNeu5Gc through the host-guest interaction, thus producing a strong electrochemical signal from AgNPs through the highly characteristic solid-state Ag/AgCl process.

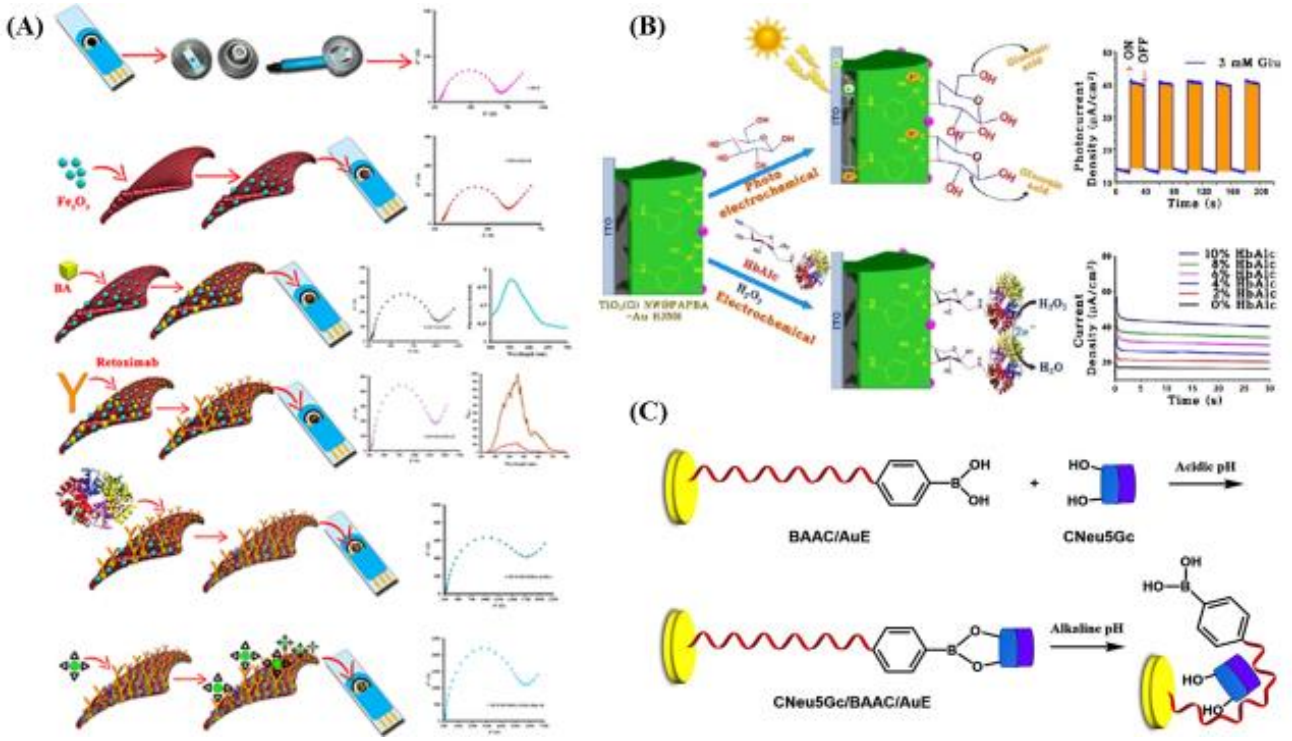

Figure 1. (A) Schematic illustration of well-orientation strategy for direct immobilization of antibodies on the BA-modified MGNRs for cancer cell detection [41]. Copyright 2020 American Chemical Society. **(B)** Schematic illustration of the photoelectrochemical-electrochemical dual-mode sensors based on TiO_2(G) NW@PAPBA-Au HJNH [50]. Copyright 2017 American Chemical Society. **(C)** Schematic illustration of the mechanism of CNeu5Gc analysis using boronic acid−aptamer conjugate [53]. Copyright 2020 American Chemical Society.

As an artificial molecular recognition technology, molecular imprinting (MIP) technology with preformed imprinted cavities allows for the analysis of targets with strong affinity and high specificity [37]. Boronic acids can be assembled on the surface of materials to establish glycosyl imprinting sensors with double recognition (3D molecular imprinted cavities and boronate affinity) [54]. For instance, Ma et al. reported an ECL sensor for CD44v6 determination by using magnetic glycosyl-imprinted microspheres to capture oligomeric hyaluronic acid on CD44v6 [55].

3. BORONIC ACID-MODIFIED NANOMATERIALS AS THE SIGNAL LABELS

2.1 Boronic acid-modified nanomaterials as the nanocarriers

Nanomaterials with high surface-to-volume ratio can load a large number of both recognition elements and signal reporters, thus transducing detection event with an amplified signal. To reduce the detection limit, numerous electroactive molecules and recognition elements have been loaded on nanomaterials as the signal tags [56]. You et al. developed a multiple signal-amplified electrochemical

platform for the detection of glycoprotein based on boronate-affinity sandwich assay with 6-ferrocenylhexanethiol (FcHT) and 4-MPBA-labeled SiO_2@Au nanocomposites [57]. As displayed in Figure 2, AuNPs was in-situ assembled on the surface of amino-functionalized SiO_2 NPs and then simultaneously labeled with FcHT and 4-MPBA. Meanwhile, boronate-affinity-based MIP films were prepared on the AuNPs-GO-immobilized GCE surface. In the presence of HRP, FcHT and 4-MPBA-labeled SiO_2@Au nanocomposites on the electrode surface produced a strong electrochemical signal.

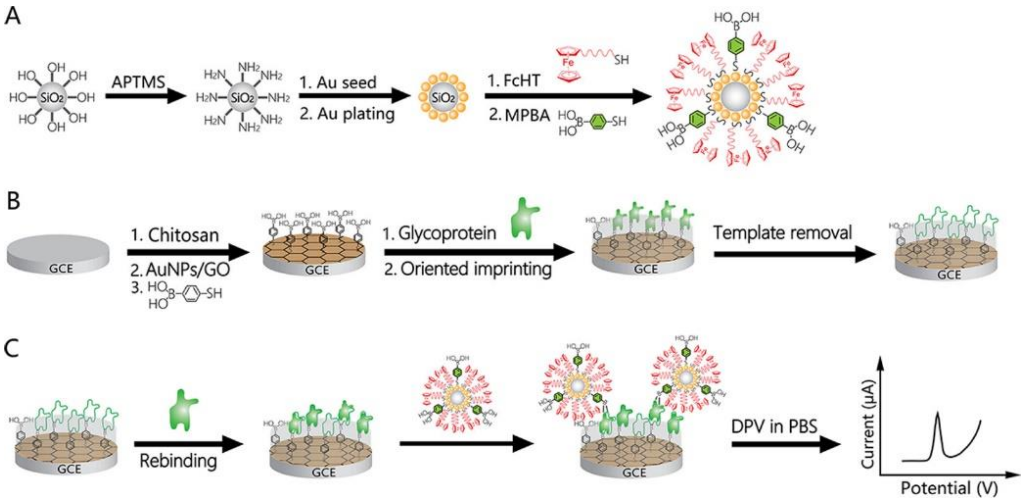

Figure 2. Illustration of the proposed approach: **(A)** preparation of SiO_2@Au/FcHT/MPBA; **(B)** Preparation process of the boronate affinity-based glycoprotein-imprinted electrode; **(C)** Fabrication of boronate affinity sandwich assay and the electrochemical detection of glycoproteins procedure [57]. Copyright 2017 American Chemical Society.

However, hydrophobic or charged molecules always decrease the dispersion and solubility of AuNPs in aqueous. To solve this problem, Xing et al. reported an electrochemical biosensor for the detection of glycoprotein avidin by using Fc-functionalized peptide to modify 4-MPBA-labeled AuNPs [58]. The biotin-modified peptide was immobilized on the gold electrode for the capture of avidin. The Fc/4-MPBA-modified bifunctional AuNPs were bound to oligosaccharide chains of avidin. Fc molecules on AuNPs with high loading efficiency could produce an amplified signal. Dopamine (DA) can not only interact with boronic acid, but also possess pH-dependent electrochemical properties. For this view, Xia and co-workers developed a sandwich-type electrochemical biosensor for glycoprotein detection based on dual-amplification of 4-MPBA-AuNPs and DA-AuNPs [59]. As displayed in Figure 3, when avidin was captured by biotin-modified electrode, 4-MPBA-AuNPs were bound to the electrode via the covalent interaction between the boronic acid group of 4-MPBA and the diol moiety of glycoprotein. Then, DA-AuNPs were tethered by the anchored 4-MPBA-AuNPs through the formation of boronic ester bonds between boronic acid and catechol. The free catechol groups on AuNPs produced an amplified voltammetric signal. Different from DNA, the ribose sugar in RNA has a hydroxyl group at the 2’ position. Thus, RNA strand with diol at the end can be recognized by boronic acid. The dual-amplification method based on 4-MPBA-AuNPs and DA-AuNPs has also been used to detect microRNA with high sensitivity [60].

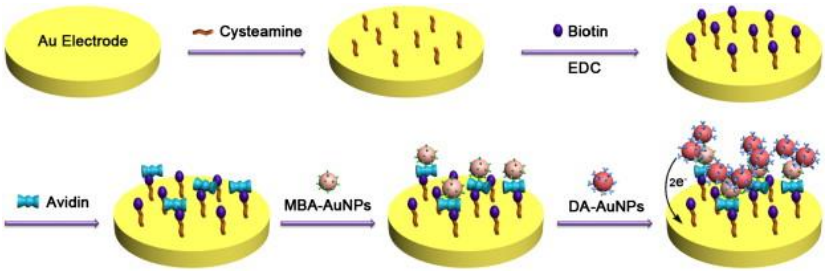

Figure 3. Schematic illustration of the strategy of avidin detection using MBA-AuNPs and DA-AuNPs [59]. Copyright 2013 Elsevier B.V.

Traditionally, redox enzyme was conjugated with antibody to develop immunoassays. However, one enzyme in per immunoreaction event was bound to the matrix, dramatically limiting the sensitivity. It is an important alternative to load abundant enzymes on nanomaterials for improving the number of enzyme involved in one immunoreaction. Liu's group reported the electrochemical detection of miRNAs based on triple signal amplification of biotin/4-MPBA-modified AuNPs, enzyme catalysis and redox-cycling reaction [61]. As displayed in Figure 4, after miRNAs were hybridized with DNA probes on the gold electrode, biotin/4-MPBA-modified AuNPs were attached onto the electrode surface through the formation of boronate ester covalent bonds between diol groups of ribose sugars at the end of the miRNAs chain and boronic acid groups on NPs. Then, many streptavidin (SA)-conjugated alkaline phosphatase (ALP, SA-ALP) were bound to the nanoparticle surface via the biotin-SA interactions. ALP catalyzed the hydrolysis of 4-aminophenylphosphate (p-APP) into electroactive p-AP. The produced p-AP could be oxidized and then re-cycled by the reduction of TCEP, subsequently generating an increased anodic current. The method can also be used to detect glycoproteins [62].

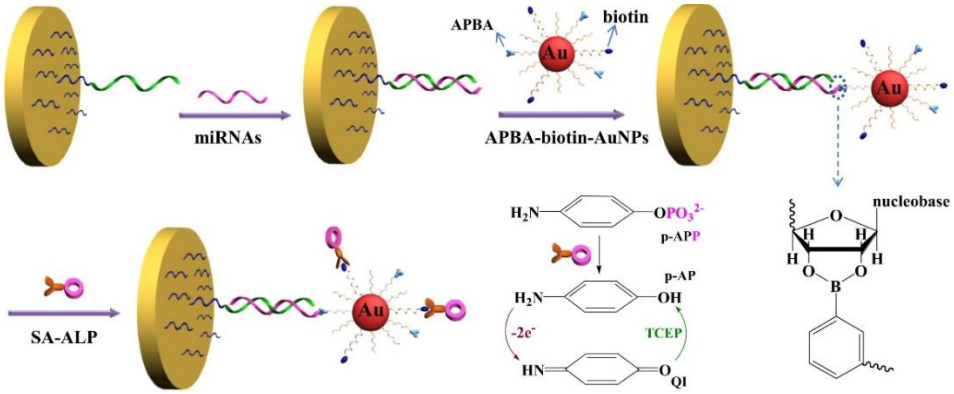

Figure 4. Schematic representation of the label-free detection of miRNAs based on the triple signal amplification of APBA-biotin-AuNPs, SA-ALP and the p-AP redox-cycling reaction [61]. Copyright 2014 Elsevier B.V.

2.2 Boronic acid-modified nanomaterials as the signal reporters

Nanomaterials with electrocatalytic ability can be directly modified with boronic acid groups. Fu et al. developed a sandwich-like electrochemical biosensor for the detection of cerebral DA by using 4-MPBA and dithiobis (succinimidyl propionate) (DSP) as the recognizer [63]. The catechol and amine

groups of DA were reacted with the boronic acid group of 4-MPBA and the succinimide residue of DSP, respectively, which confirmed the efficiency and accuracy of the proposed sensor. Son et al. developed boronate-affinity sandwich assay for the determination of glycated albumin using a 3-aminophenylboronic acid (APBA)-modified Prussian blue nanozyme to catalyze the oxidation of 3,5,3',5'-tetramethylbenzidine (TMB) by H_2O_2 [64]. To further improve the sensitivity, Sun et al. combined the MIP with hybridization chain reaction (HCR) in microfluidic paper-based analytical devices for glycoprotein detection [65]. As shown in Figure 5, AuNPs were in-situ grown on the surface of SiO_2 NPs and further modified with more 4-MPBA molecules and capture DNA stands. The dsDNA hybrids were formed on the nanocomposite surface through HCR reaction. CeO_2 NPs were coupled to the nicked dsDNA through the amidation reaction. In this work, Au nanorod (NR) layer was in-situ synthesized on the μPAD surface and 4-MPBA was immobilized on the surface through the Au-S bond. After the target glycoprotein ovalbumin (OVA) was captured, SiO_2@Au/dsDNA/CeO_2 was bound to the electrode surface as a signal tag and generated an amplified electrochemical signal by catalyzing the redox reaction.

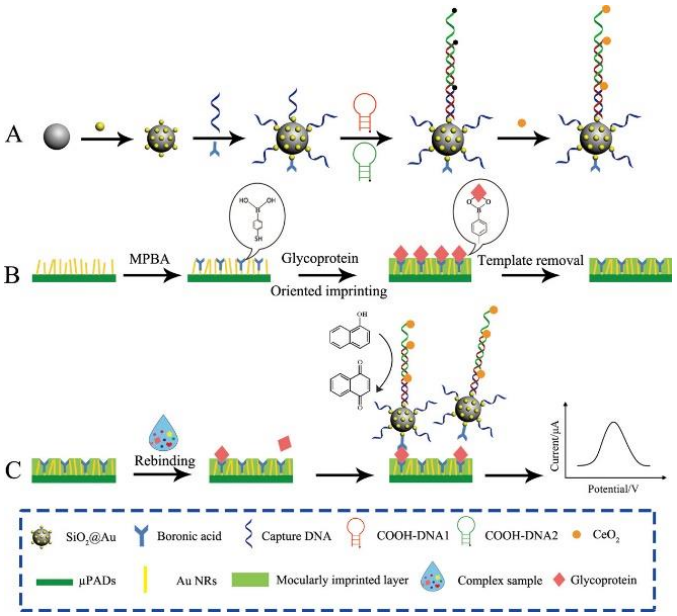

Figure 5. Schematic illustration of the proposed approach for the detection of glycoprotein: **(A)** preparation of the SiO_2@Au/dsDNA/CeO_2 signal tag; **(B)** Synthesis of the boronate affinity-based glycoprotein imprinted film; and **(C)** Fabrication procedure of the sensing platform and the electrochemical detection of OVA [65]. Copyright 2019 American Chemical Society.

After being treated by acid dissolution, metal nanomaterials and QDs can release a large number of metal ions that can be quantified with voltammetry. For this view, Song et al. utilized boronic acid-functionalized AuNPs-coated polyaniline microspheres to label the captured glycoprotein (carcinoembryonic antigen) on hollow magnetic silica coated nickel/carbon nanocomposites [66]. After magnetic separation, the attached AuNPs were oxidized to Au ions that could be sensitively determined by differential pulse voltammetry (DPV).

T4-β-Glucosyltransferase (β-GT) can act as the specific recognition unit and transfer a glucose moiety from a glucose donor to the hydroxyl group of 5-hydroxymethylcytosine (5hmC). Sui et al. reported a PEC biosensor for 5hmC and β-GT activity detection based on boronic acid-functionalized CDs [67]. In this work, WS_2 nanosheets and AuNPs were immobilized on an indium tin oxide (ITO) electrode and further labeled with double-strand DNA containing 5hmC. After glycosylation by β-GT, boronic acid-functionalized CDs were employed to label 1,2-diol groups of the carbohydrates and enhanced spatial charge separation, resulting in the increase of the photocurrent response. Besides, they also utilized 4-carboxyphenylboronic acid (4-CPBA) and 4-formylphenylboronic acid (FPBA) to label 5hmC and amino-functionalized MOF (Fe-MIL-88NH_2). CuO NPs were tethered on the surface of the electrode through the interactions between the active formyl and carboxyl groups in two boronic acid derivates and amino groups, respectively [68, 69].

4. BORONIC ACID-INDUCED AGGREGATION OF NANOPARTICLES

Owing to the distinguished local surface plasmon resonance (LSPR) property, AuNPs and AgNPs show different optical characteristics when their state changed from disperse to aggregation. Meanwhile, the color of AuNPs solution changed from ruby red to blue. The crosslinked reaction between diol-containing molecules and boronic acid-containing molecules can be integrated into the target-triggered aggregation in which the two molecules act as the aggregation-trigger and surface-functionalizing molecule, respectively [70-73]. For example, Yang et al. demonstrated that 1,4-benzenediboronic acid (BDBA) could act as the linker to induce the aggregation of citrate-capped AuNPs and H_2O_2 generated from enzyme catalysis reaction could oxidize boronic acid groups in BDBA to phenol groups, subsequently hampering the aggregation [74]. Nair et al. reported non-enzymatic colorimetric method for glucose detection by using glucose to induce the aggregation of 4-cyanophenyl boronic acid (4-CPBA)-functionalized AuNPs [75]. BDBA-AgNPs could bind with the diol groups in saccharides of the bacteria cell surface for colorimetric bacteria detection [76]. Besides, 4-mercaptophenylboronic acid (4-MPBA) with boronic acid group and thiol groups at two ends can trigger the aggregation of diol-containing AuNPs. In this aggregation format, thiol groups bind to AuNPs through the Au-S interactions and boronic acid groups reacted with citrate on other AuNPs. Boronic acid monomers can react with each other to form a boroxine ring [77, 78]. Jiang et al. demonstrated that 4-MPBA could bind to AuNPs through Au-S interaction and undergo condensation to form a boroxine ring between three boronic acid groups, leading to the aggregation of AuNPs [79]. ATP with 2', 3'-hydroxy group could interact with 4-MPBA to form a stable boronate ester and prevented the AuNPs aggregation.

Despite the simplicity and visualization, most colorimetric assays confront of poor sensitivity. To amplify the signal intensity and improve the sensitivity, the target-induced aggregation in solution was transferred into a solid (electrode)-liquid (electrolyte) surface [80-82]. Liu's group reported the electrochemical detection of miRNAs based on the *in situ* formation of 4-MPBA-induced AgNPs aggregates for signal amplification [83]. As illustrated in Figure 6, miRNAs were captured by DNA-modified electrode and then labeled with 4-MPBA molecules through the boronate ester covalent interactions. The exposed thiol groups could bind with AgNPs via the formation of Ag-S bonds. Then,

free 4-MPBA molecules in solution induced the in-situ assembly of AgNPs into networks on electrode surface through the covalent interactions between α-hydroxycarboxylate of citrate and boronate of 4-MPBA and the formation of Ag-S bonds. This strategy was also applied to detect glycoprotein prostate specific antigen (PSA) [84].

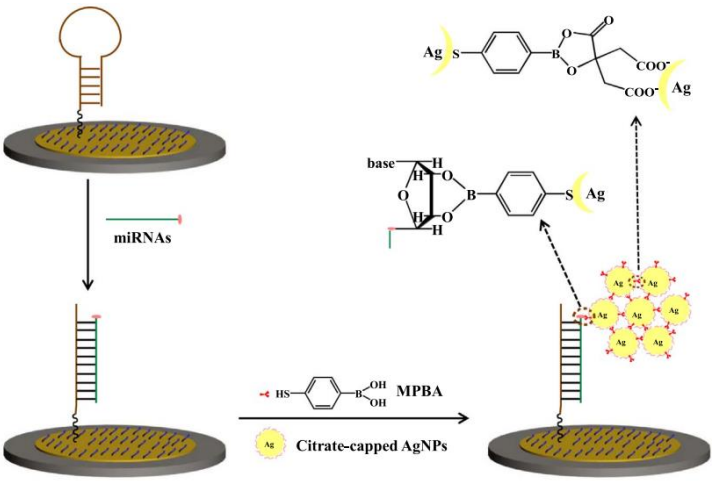

Figure 6. Schematic representation of the proposed electrochemical strategy for miRNAs detection based on MPBA-induced in situ formation of AgNPs aggregates as labels [83]. Copyright 2017 Elsevier B.V.

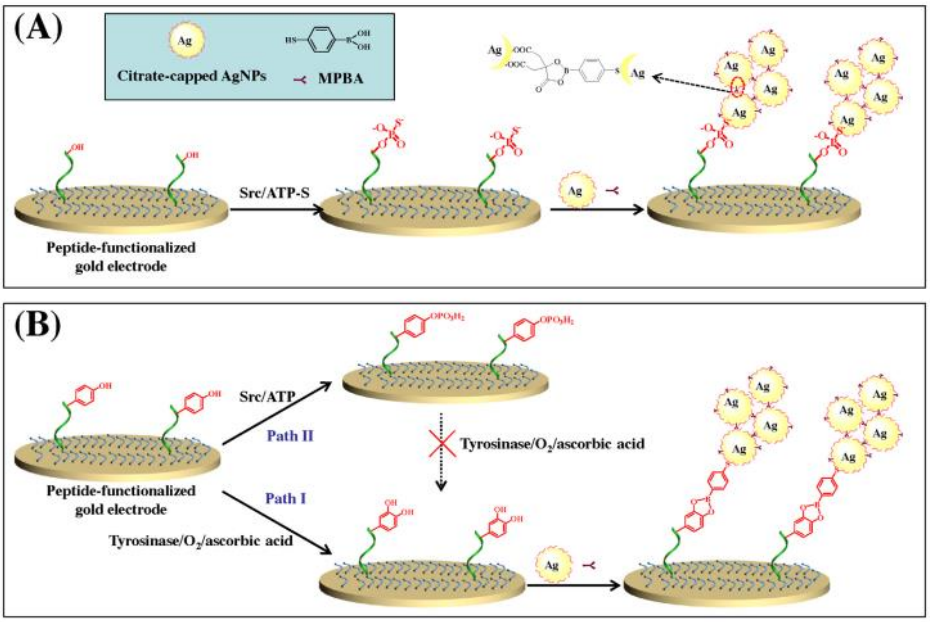

Figure 7. Schematic representation of the proposed electrochemical strategies for protein kinase detection based on the in situ formation of the AgNPs aggregates as labels. In the first design **(A)**, ATP-S was used as the co-substrate. In the second design **(B)**, ATP was used as the co-substrate and tyrosinase was used to convert monophenol into o-diphenol [85]. Copyright 2017 Elsevier B.V.

Besides, Liu and co-workers developed two sensitive electrochemical strategies for the detection of protein kinase activity based on the 4-MPBA-induced in-situ assembly of AgNPs [85]. As shown in Figure 7, after the peptide was phosphorylated with thiophosphate group, AgNPs were conjugated specifically to the peptides via the Ag-S interaction and the 4-MPBA-induced in-situ assembly of AgNPs was initiated on the electrode. In the second strategy, once the tyrosine residues in the peptide substrates were oxidized by tyrosinase into o-diphenol moieties, MPBA molecules were tethered on the electrode surface through the boronate ester bonds and triggered the in-situ assembly of AgNPs. However, when the tyrosine residues were phosphorylated by tyrosine kinase, the oxidation of phosphorylated tyrosine by tyrosinase was impossible and the 4-MPBA-induced in-situ assembly of AgNPs was prevented.

5. CONCLUSION

Diol-containing biomolecules are a type of important biomarkers for medical diagnosis. Abnormal expression of some diol-containing biomolecules is associated with many diseases, such as cancers, cardiovascular disease, diabetes and other diseases. As a diol-binding reagent, boronic acid has the advantages of high efficiency, stability and specificity. Boronic acid-based sensing materials have good application prospects in glucose monitoring, drug release, analysis and detection of polysaccharides and glycoproteins and so on. With the continuous emergence of novel synthesis technologies and new materials, boronic acid-based multiple functional materials have attracted more and more attention and show broad application prospects in the preparation of highly sensitive and diverse biosensors for the analysis of diol-containing biomolecules.

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