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Mini review

Recent Progress in Electrochemical Detection of Beta-amyloid Peptides and Their Aggregates

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Beta-amyloid (Aβ) peptides and their aggregates are regarded as the promising biomarkers for Alzheimer's disease (AD) diagnosis. In this work, we focused on the recent progress in the development of electrochemical methods for the detection of Aβ monomers and their aggregates. In particular, the receptors for the selective capture of Aβ monomers and oligomers were summarized.

Keywords: Alzheimer's disease; beta-amyloid; electrochemical detection; bioreceptors

1. INTRODUCTION

As a severe neurological disorder, Alzheimer's disease (AD) leads to progressive memory loss and cognitive impairment [1]. Although many details associated with AD pathology are still unknown, the deposition of extracellular beta-amyloid (Aβ) peptides containing 39 – 42 amino acid residues was regarded as one of the main hallmarks in the brain [2]. After the cleavage of the amyloid precursor protein (APP) induced by β- and γ-secretases, Aβ forms with a largely hydrophilic N-terminal domain (1–16) and a C-terminal hydrophobic domain [3]. Of these Aβ species, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ composed 40 and 42 amino acid residues dominate. In contrast to $A\beta_{1\text{-}40}$, the amount of $A\beta_{1\text{-}42}$ decreases significantly but it displays more neurotoxic resulting from the presence of the two additional hydrophobic amino acids [3]. Thus, the low-level $A\beta_{1\text{-}42}$ is always considered as a promising biomarker for AD diagnosis. $A\beta_{1\text{-}42}$ monomers tend to aggregate into the oligomers or fibrils under

different external stimuli including oxidation stress and metal ions. The aggregated ones of $A\beta_{1-42}$, especially $A\beta_{1-42}$ oligomers, are believed as major toxic effects in AD which could be considered as promising biomarkers or aggregation inhibitor-based drug targets for AD therapy [4, 5]. Therefore, the development of reliable methods for the detection of $A\beta_{1-42}$ species including monomers and oligomer/fibril aggregates will be vital of importance of early diagnosis, disease progression and drug delivery.

So far, many attempts have been made for detection of Aβ, such as mass spectrometry, enzyme-linked immunosorbent assay (ELISA), capillary electrophoresis, surface plasmon resonance (SPR) and so on [3,6-8]. However, most of these methods suffered from expensive, time-consuming, labor intensive. Thus, developing simple, cost-effective and sensitive methods for Aβ assay has been preferred. Due to their sensitivity, simple in operation, rapid response, and compatibility with miniaturization, various electrochemical biosensors have been fabricated for Aβ detection. In the field of electrochemical detection of Aβ, promising sensing platforms with synthetic bioreceptors for highly sensitive and selective recognition of Aβ analysts in real matrices are still required. In this review, we focused on the recent progress in the development of electrochemical methods for the detection of Aβ monomers and their aggregates. In particular, the receptors for the capture of Aβ monomers and oligomers are discussed.

2. DIRECT DETECTION BY MONITORING THE TYROSINE RESIDUE OXIDATION

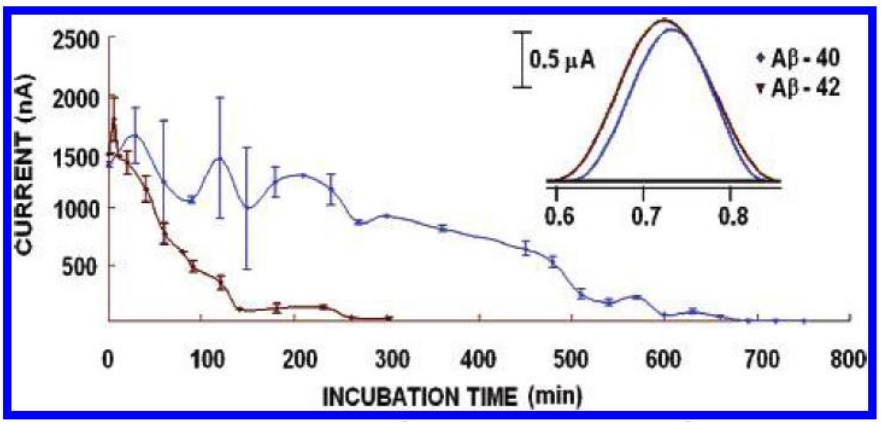

Figure 1. Kinetic study of Aβ-42 (red line) and Aβ-40 (blue line) aggregation after incubation at 80 *i*M in TBS at 37 ± 1 °C; detected at 8 and 4 μM, respectively, using SWV, at room temperature. Inset: Voltammograms of native Aβ-42 (red line) and Aβ-40 (blue line); detected at 8 and 4 μM, respectively, using SWV, at room temperature. Reprinted with permission from reference [9]. Copyright 2005 American Chemical Society.

The Aβ detection and their aggregation kinetics could be characterized by measuring the electrochemical oxidation of single tyrosine (Tyr) residue located at position 10 of the Aβ polypeptide (Figure 1) [9,10]. In the course of progressive Aβ aggregation, Aβ peptides turned into fibrillar or non-fibrillar oligomers aggregates. These conformational changes gave rise to a decrease of oxidation current resulting from the decreasing exposure degree of Tyr residue to the electrode surface, thereby

less accessible for oxidation. Thus, peptide monomers were responsible for a strong oxidation signal while a decreasing signal could be observed due to inclusion of Aβ molecules into aggregates. Detecting the amount of $A\beta_{1-40}$ or $A\beta_{1-42}$ and monitoring their aggregation process in real time could be achieved by measuring the relative change of the oxidation current.

3. BIOMIMETIC RECEPTORS-BASED DETECTION

3.1 α-Cyclodextrin

Recently, a novel electrochemical biosensor was fabricated using a sugar monomer (α-cyclodextrin, α-Cd) as recognition elements for a peptide biomarker $A\beta_{1-42}$ [11]. For the sensor fabrication, a conductive polyaniline (PANI) film was firstly electropolymerized on the Au-working electrode, followed by plastic antibody medication by co-electropolymerization of α-Cd and $A\beta_{1-42}$. Then, an acid treatment process was taken to remove the $A\beta_{1-42}$ molecules from the polymer film matrix, yielding an imprinting layer on the surface of PANI film and being capable of recognition of target peptides. It should be noted that the PANI conductive layer obtained from the electropolymerization of aniline was of vital importance for the sensor fabrication. The reasons were as follows: (i) the target capturing layer of α-Cd molecules was a non-conductive polymer, (ii) the PANI conductive polymer contributed to electrical stimulus for the subsequent α-Cd polymerization process, and (ii) the positively charged character of the conducting PANI layer was conductive for chemical recognition of the negative charged $A\beta_{1-42}$. The antibody-like imprint polymer film showed excellent performance for $A\beta_{1-42}$ detection and the limit of was 420.25 ng/mL and 0.20 ng/mL for electrochemical impedance spectroscopy (EIS) assay and square wave voltammetry (SWV) assay, respectively.

3.2 Peptides

Functional oligopeptides with amino acid sequence RGTWEGKWK could recognize $A\beta_{1-42}$ soluble oligomer in a ridge-notch matching style, which could be utilized for the fabrication of a peptide-based signal-on electrochemical biosensor for quantitative determination of $A\beta_{1-42}$ soluble oligomers [12]. The functional oligopeptides containing an Fc moiety at one terminal and an alkyl thiol moiety at the other terminal were assembled on the surface of gold electrode. In the absence of target, the peptide chains at an optimal density could bend or curb freely. Upon capturing the target through hydrophobic interactions between amino acid side chains of the oligopeptides and $A\beta_{1-42}$ oligomers, the linear peptide probe, a significant change of surface ET rate and the tune of the frequency of SWV could be observed.

The $A\beta_{1-42}$ oligomers was could be detected via noncovalent coupling between the protein-binding peptides and electrochemical reporter (MV) with the aid of cucurbituril CB[8] (Figure 2) [13]. The human tumor necrosis factor-α (TNF-α) peptides containing some aromatic amino acids maintained the binding ability of $A\beta_{1-42}$ oligomers and could form supermolecules through the inclusion of MV molecules and the aromatic side chain group of the peptide into cucurbituril. In the

presence of $A\beta_{1-42}$ oligomers, the protein-binding peptides bound to target protein, leading to the reporter molecules isolating with the peptides because of stronger binding affinity of peptide with its target protein than with CB[8], yielding a relatively low signal readout. According to the peak current of reporter inversely proportional to the amount of captured $A\beta_{1-42}$ oligomers, a low limit of detection of 0.048 nM was achieved.

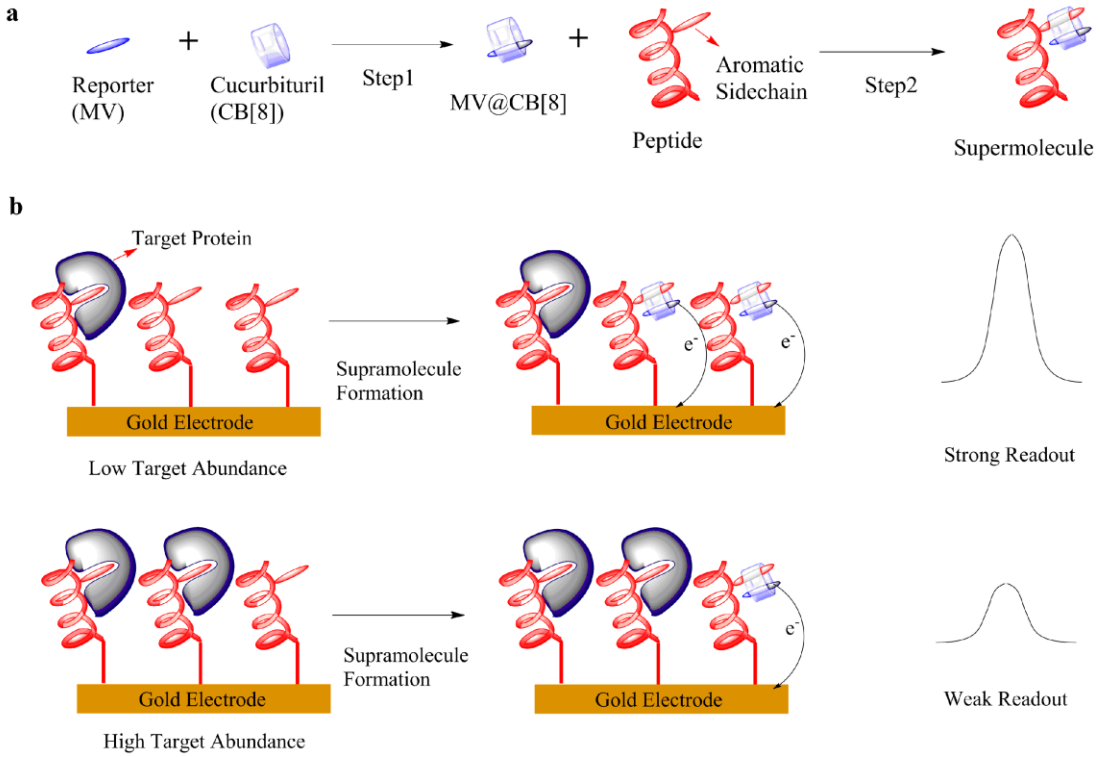

Figure 2. (a) Coupling peptide with reporter via supermolecule formation and (b) assay for protein detection. Reprinted with permission from reference [13]. Copyright 2013 American Chemical Society.

Prion protein (PrP^C), a natural and neuronal synaptic protein, was also demonstrated for binding specifically to soluble Aβ oligomers not to Aβ monomers or fibrils since the amino acid residues PrP_{95-110} with a sequence THSQWNKPSKPKTNMK of PrP^C displayed strong affinity to the core region of Aβ oligomers. This specific interactions between Aβ oligomers and PrP_{95-110} segments was applicable to develop an impedimetric biosensor for ultrasensitive detection of soluble Aβ oligomers [14]. For the fabrication of the impedimetric sensor, biotinylated PrP_{95-110} peptide were assembled on surface of polymer-coated screen-printed gold electrodes via the specific recognition of NeutrAvidin by biotin. A reasonable selectivity of the sensor for AβO was obtained using electrochemical impedance spectroscopy. The specific interaction provides us a hint that $PrP_{95–110}$ would be a good receptor for the design of novel electrochemical and optical biosensors for Aβ oligomers detection [15-20]. For example, metal nanomaterials, in particular gold and silver nanoparticles (AuNPs and AgNPs), have been widely used for creating effective recognition and transduction processes in chem/bio-sensing due to their unique physicochemical attributes. Based on

the specific Aβ oligomer/$PrP_{95–110}$ interaction and the well-defined and signal-amplified electrochemical signal of AgNPs aggregates, we have developed an electrochemical biosensor for the determination of Aβ oligomers by employing adamantine (Ad)-labeled $PrP_{95–110}$ (Ad-$PrP_{95–110}$) as the receptor and AgNPs aggregates as the redox reporters (Figure 3) [17]. In this work, the network architecture of Ad-$PrP_{95–110}$/AgNPs nanocomposites produced in solution were introduced onto the β-cyclodextrin (β-CD)-modified electrode surface through the host−guest interaction. The specific Aβ oligomer/$PrP_{95–110}$ interaction made the Ad-$PrP_{95–110}$ in solution losing its capability to trigger the formation of AgNPs-based network architecture. This work presents a concept for converting the colorimetric assay into a sensitive electrochemical analysis by simply incorporating the colorimetric principle into the electrochemical platform.

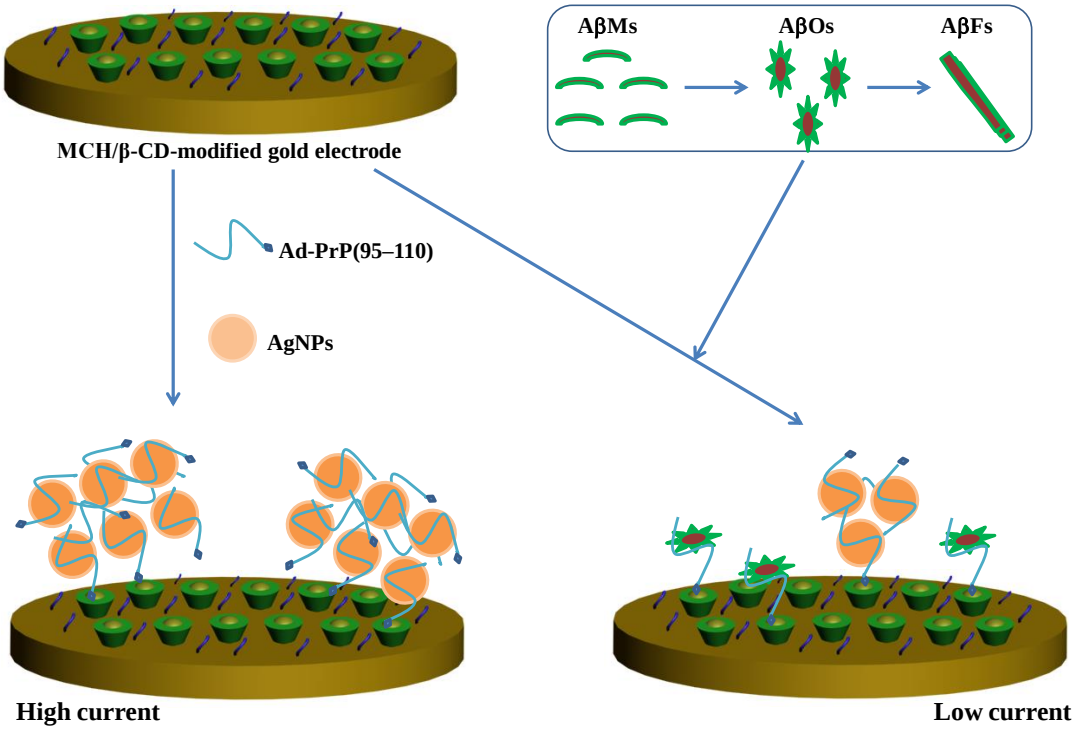

Figure 3. Schematic illustration of the electrochemical method for the selective detection of AβOs using AgNPs as the redox reporters and Ad-PrP(95–110) as the receptor. Reprinted with permission from reference [17]. Copyright 2016 American Chemical Society.

3.3 Gelsolin

Based on the specific binding of gelsolin and Aβ, somewhat like the strong recognition interaction of an antibody by an antigen, Shi and co-workers developed a novel electrochemical sensor for sensitive and selective detection of the soluble Aβ monomers, even the ones within the complex real systems of cerebrospinal fluid (CSF) or various brain regions including normal and AD rats (Figure 4) [21]. A common “sandwich” method was utilized for the fabrication of the sensors, where the $Aβ_{1-40}$/$Aβ_{1-42}$ peptides were captured by the gelsolin tethered on the biosensor, followed by the recognition of gelsolin-Au-Th bioconjugates. Due to the signal amplification of multiwalled carbon nanotubes (MWCNTs) and AuNPs and the specific interaction between gelsolin and $Aβ_{1-40}$/$Aβ_{1-42}$, a

reasonable sensitive and selective electrochemical analysis of the total amount of $A\beta_{1-40}/A\beta_{1-42}$ was acquired by monitoring thionine (Th) reduction signal. Further amplification was observed when Th was replaced by horseradish peroxidase (HRP) [22]. HRP attached on the surface of AuNPs could catalyze effectively 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2, leading to significant reduction current signals at 0.35 V. Due to the signal amplification of AuNPs and HRP, the electrochemical sensor could measure the soluble $A\beta_{1-40}/_{1-42}$ peptides in both CSF and brain tissues of normal and AD rats.

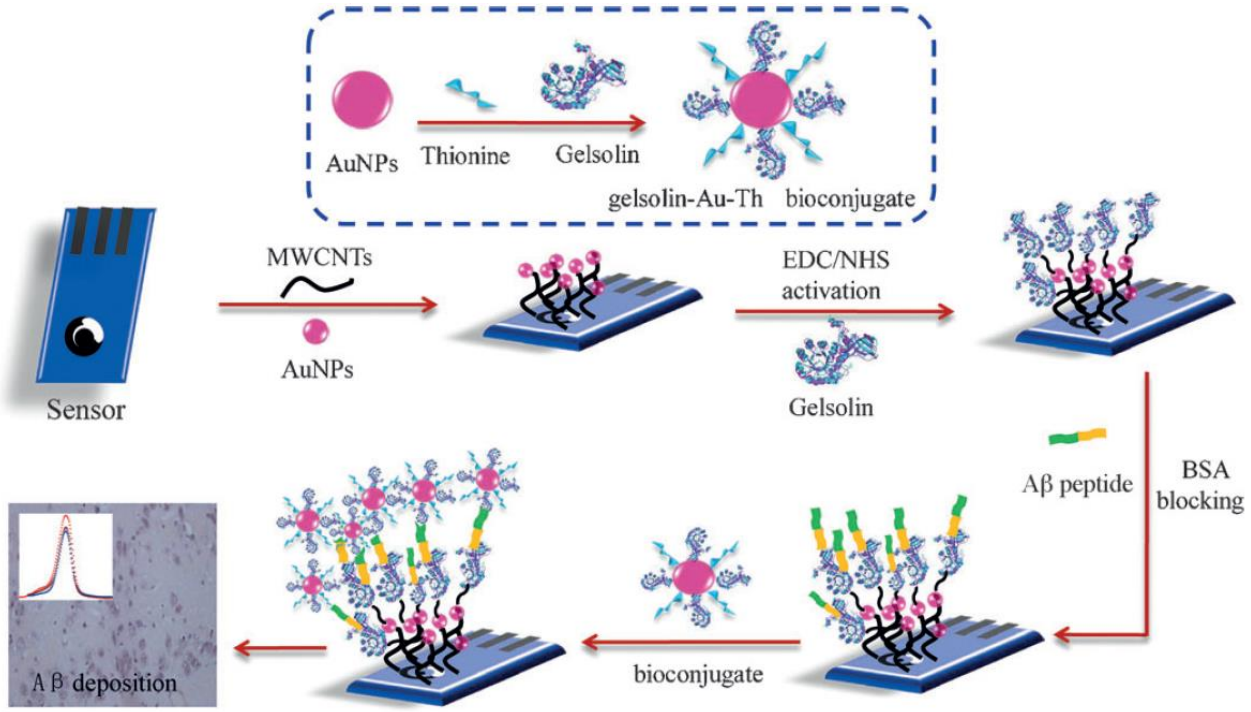

Figure 4. A schematic illustration of the electrochemical detection of Aβ(1–40/1–42) by using a gelsolin-Au-Th bioconjugate as a probe. Reprinted with permission from reference [21]. Copyright 2014 John Wiley and Sons.

3.4 Antibodies

Conformation-specific Aβ antibodies show strong affinity to Aβ monomers and their neurotoxic oligomeric intermediates or fibrils [23, 24]. Kerman and coworkers selected anti-oligomer (A11) and anti-fibril (OC) antibodies for the specific capture of the oligomeric or fibrillar states to evaluate the $A\beta_{1-42}$ aggregation processes (Figure 5) [23]. Conformation-specific antibodies were firstly covalently immobilized onto the surface of separate gold CD electrodes. Then, oligomeric or fibrillar aggregates of Aβ could be captured by their corresponding antibodies (A11 or OC), leading to the charge-transfer resistance signal change of $[Fe(CN)_6]^{3-/4-}$. EIS was applied for determining the binding amount of oligomers or fibrils, which enabled time-dependent analysis of $A\beta_{1-42}$ aggregate formation. The inhibition activity of symtriazine-derived aggregation modulators TAE-1 and TAE-2 were further evaluated by monitoring the reduction of total fibrils content.

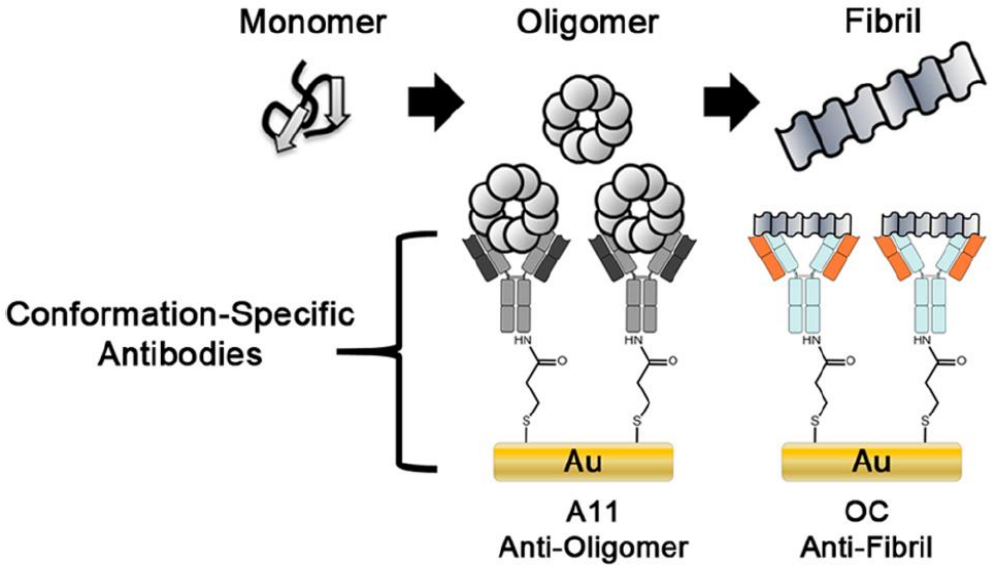

Figure 5. Detection principle for monitoring Aβ(1−42) fibrils and toxic oligomers using conformation specific antibodies in conjunction with EIS. Reprinted with permission from reference [23]. Copyright 2014 American Chemical Society.

Yang and co-workers presented a reusable biosensor for the detection of $A\beta_{42}$ using the antibodies of $A\beta_{1-28}$ ($A\beta_{A\beta}$) as the specific recognition molecules (Figure 6) [25]. The $A\beta_{1-28}$ antibodies were labeled on the magnetic nitrogen-doped graphene (MNG), followed by trapped on the surface of Au electrode due to the magnetic field provided by an external magnet located at the underside of the electrode. Without any drying process, the fabricated biosensor could be quickly constructed and conveniently regenerated just by switching off the magnetic field, providing a useful platform for simple and quick electrochemical detection of $A\beta_{1-42}$. In addition, an electrochemical immunsensor based on carbon nanotube (CNT) film with a metal semiconductor field effect transistor structure (MESFET) was developed by Yoo and co--workers [26]. For this CNT-based biosensor, probe antibodies were covalently attached on the surface of gold (Au) modified semiconducting CNT film channel via an antibody-binding protein. Upon binding of target analysts Aβ to the Au top gate with autodisplayed Z-domains of protein A as recognition regions, the conductance of the CNT channel responded quickly because of a potential change of the CNT channel. The fabricated CNT-MESFET biosensor offered a reproducible performance for Aβ detection due to the narrow distribution in the threshold voltage (Vth) and the on/off ratio. Furthermore, in comparison with a chemical linker, a higher sensitivity was obtained for the CNT-MESFET biosensor with probe antibodies immobilization using Escherichia coli outer membrane (OM), allowing for detection of Aβ of 1 pg/mL in human blood samples.

Costa-García's group designed a competitive immunosensor based on gold nanostructure modified screen-printed electrode for $A\beta_{1-42}$ detection [27]. The working electrode coated with streptavidin was incubated with biotin linked $A\beta_{1-42}$ for the formation of the sensing part of the immunosensor. Then, a competitive reaction for binding anti-$A\beta_{1-42}$ was initiated between the previously assembled biotin linked $A\beta_{1-42}$ and the analysts when a mixed solution of $A\beta_{1-42}$ and

antibody anti-$A\beta_{1–42}$ was added. Finally, anti-rabbit IgG linked with alkaline phosphatase (anti-IgG-AP) was incubated with the treated immunosensor, which could catalyze the reaction of 3-indoxyl phosphate and silver ions. Thus, the anodic stripping signal of enzymatically generated silver decreased with the increase of concentration of the analyst using cyclic voltammetry. The competitive electrochemical immunesensor displayed a very low detection limit of 0.1 ng/mL and a wide linear range between 0.5 and 500 ng/mL. Recently, Morais's group developed a label-free immunosensor for the detection of $A\beta_{1–42}$ with a monoclonal antibody mAb DE2B4 as the bioreceptor [28]. The antibodies were immobilized onto the surface of AuNPs electrodeposited on a self-assembled monolayer (SAM)-covered gold electrode. Under the optimized experimental conditions, $A\beta_{1–42}$ monomers could be determined within a linear range of 10–1000 pg/mL by square-wave voltammetry. The detection limit and quantification limit were found to be 5.2 pg/mL and 17.4 pg/mL, respectively.

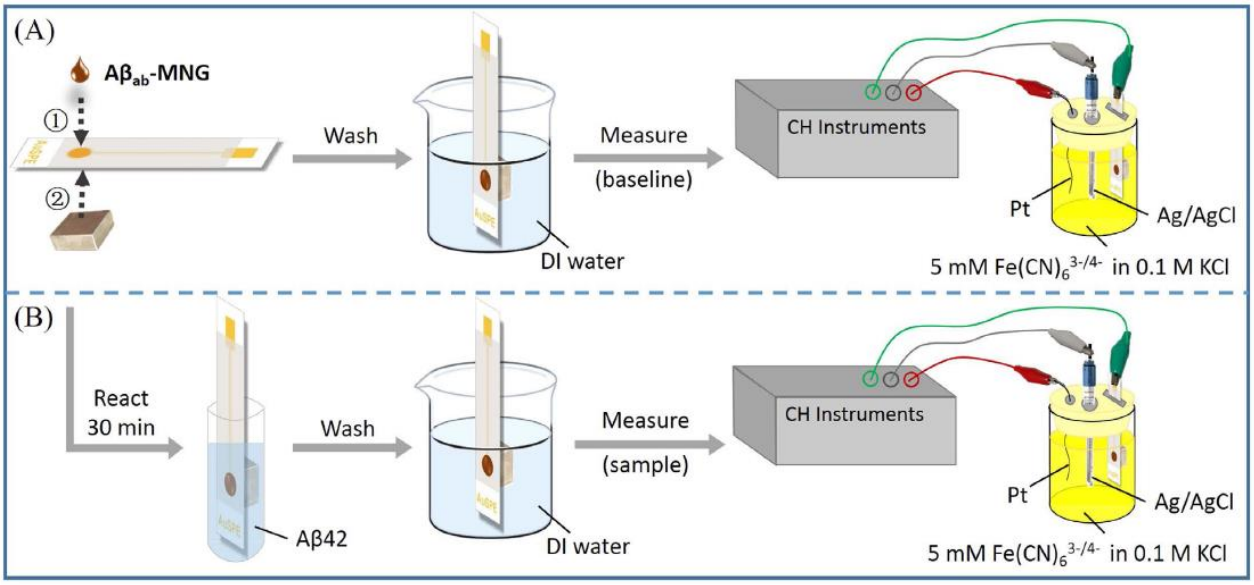

Figure 6. Schematic representation of the electrochemical detection by Aβab-MNG modified AuSPE (A) and the electrochemical detection of Aβ 42 using Aβ ab-MNG modified AuSPE (B). Reprinted with permission from reference [25]. Copyright 2016 Nature.

We reported two electrochemical immunosensors for the determination of the $A\beta_{1–42}$ and $A\beta_{1–40/1–42}$ monomers. The antibody of $A\beta_{1–16}$ (anti-$A\beta_{1-16}$) immobilized on a gold electrode captured the AuNPs modified with $A\beta_{1–16}$ and heme ($A\beta_{1–16}$-heme-AuNPs) [29]. The captured $A\beta_{1–16}$-heme-AuNPs allowed for the electrocatalytic reduction of O_2 to H_2O_2. The competition between the total $A\beta_{1–40/1–42}$ monomers and $A\beta_{1–16}$-heme-AuNPs to bind with the anchored anti-$A\beta_{1-16}$ on the electrode surface led to a decrease in the electrocatalytic current. Thus, this method could be used to detect total $A\beta_{1–40/1–42}$ from 0.02 to 1.50 nM. Furthermore, we reported the competitive electrochemical immunoassay of $A\beta_{1–42}$ and $A\beta_{1–40/1–42}$ monomers based on the signal amplification of redox cycling [30]. In this method, the antibodies specific to the N-terminus of Aβ and the C-terminus of $A\beta_{1–42}$ were immobilized on the electrode surface for the capture of $A\beta_{1–40/1–42}$ and $A\beta_{1–42}$, respectively. The competitive interaction of

$A\beta_{1-40/1-42}$ or $A\beta_{1-42}$ and the alkaline phosphatase-conjugated Aβ peptide facilitated the detection of the relative level of $A\beta_{1-42}$ with a detection limit of 5 pM.

3.5 Aptamer

An antibody-aptamer sandwich structure-based electrochemical platform was constructed for A*β* oligomers assays (Figure 7) [31]. For the sensor construction, the antibodies of A*β* oligomers were assembled on the electrode surface for specifically capturing A*β* targets. In the presence of A*β* oligomers, the electrochemical reporter of a nanocomposite of aptamer-Au-Th could link with target analysts *via* the interaction of A*β* oligomers and their specific aptamer. Thus, an amplifying signal of Th reduction could be obtained due to high loading of Th on the AuNPs. Combining the specific recognition ability of antibodies and DNA aptamers, a reasonable high specificity for A*β* oligomers assay could be achieved. The detection limit of ~100 pM estimated from 3σ of the baseline signals was obtained for the A*β* oligomers detection.

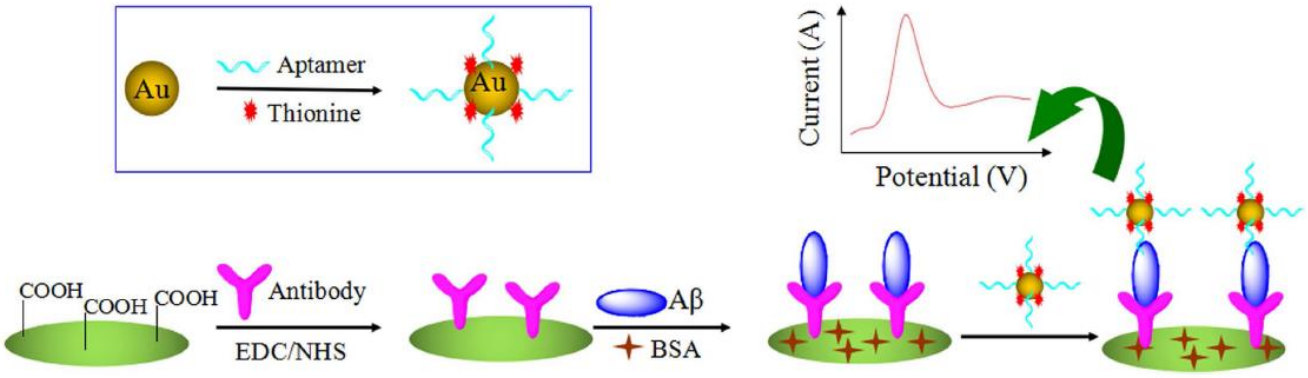

Figure 7. A schematic illustration of the electrochemical detection of A*β* oligomers using an antibodyaptamer sandwich assay. Reprinted with permission from reference [31]. Copyright 2016 Nature.

4. CONCLUSION

In summary, great efforts have been made toward innovative receptors for recognition of Aβ molecules in the field of AD biomarkers detection or monitoring the aggregation process of Aβ monomers. Most of these biomimetic receptors shared the following advantages: ease of production or chemical modification, specific recognition of target, low-cost and relatively high stability in comparison to universal antibodies. Up to date, several classes of biomimetic receptors have been widely used for construction of biosensors for AD biomarkers detection. However, the amount of biomimetic receptors was still scarce. Considering the low-level concentration in real sample of CSF and blood, time-dependent fluctuations of Aβ levels, biomimetic receptors with higher sensitivity and selectivity toward Aβ recognition would be pursued. There are no doubt that aptamers, peptides or their analogues, and molecularly imprinted polymers (MIP) are the widely used biomimetic receptors

for protein analysis. For AD diagnosis, peptides or their analogues have been widely used as biomimetic receptors for the construction of biosensors for Aβ assay. The methods using DNA aptamers or MIPs biomimetic receptors are still limited and thus much work is still desired.

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